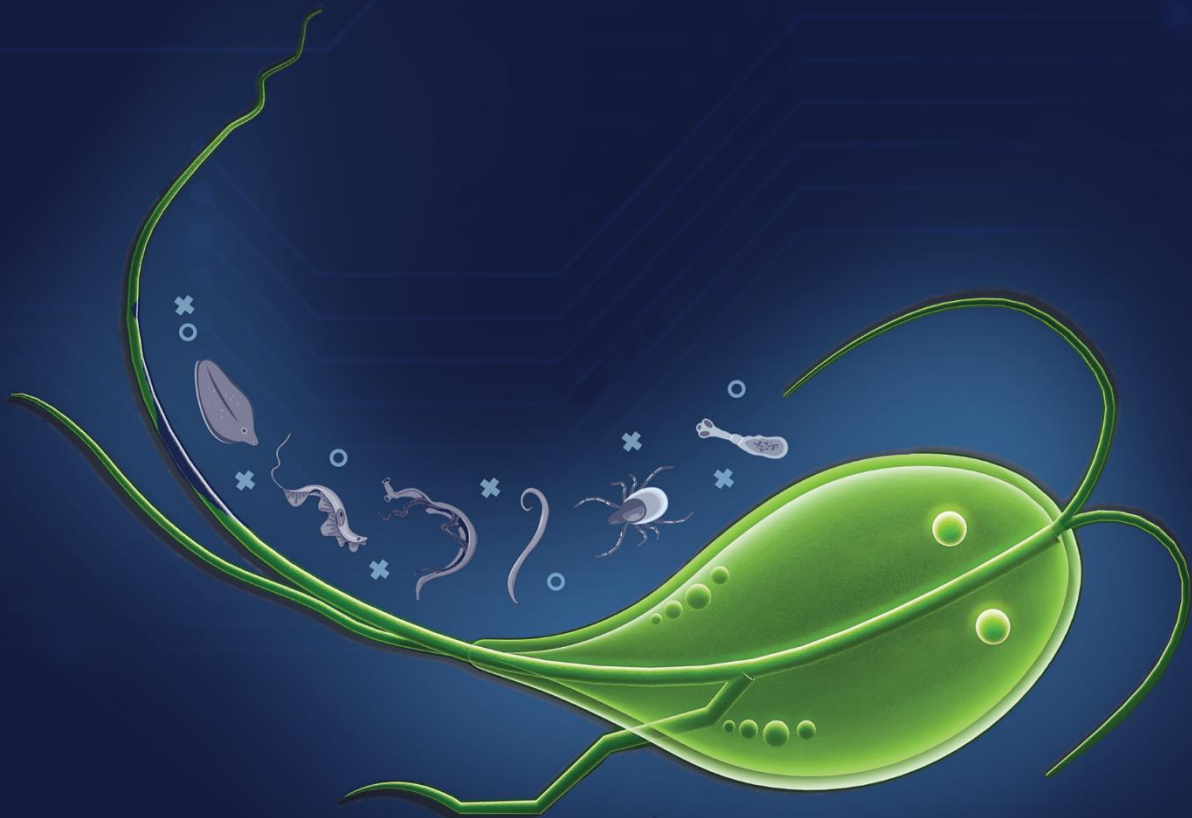




3rd National Congress Of Animal parasitic Diseases and Zoonoses

PROCEEDING

17-18 september 2025, Mashhad, iran
Ferdowsi University of Mashhad





سومین کنگره ملی بیماری‌های انگلی دام⁹ بیماری‌های انگلی مشترک انسان و حیوان⁹



دانشگاه تهران



دانشگاه علوم پزشکی
مشهد



استاندارد خراسان رضوی
اسکالر ری سازمان رفاهی



بنیاد حامیان دانشگاه
فردوسی مشهد



سازمان نظام پزشکی
چ. ا. ایران



سازمان دامپزشکی کشور
ایران



دانشگاه شیراز



موسسه تحقیقات واکسن
و سرم سازی زاری



سازمان نظام دامپزشکی
چ. ا. ایران



دانشگاه ارومیه



انجمن گنه شناسی
ایران



شرکت صنایع دامپزشکی و
لبی رضوی



مرکز تحقیقات کنه ها و
بیماری های منتقله از کنه
ایران



سازمان جهاد کشاورزی

دانشگاه فردوسی مشهد
دانشکده دامپزشکی
شهریور ماه ۱۴۰۴



National Congress of
Animal Parasitic Diseases and Zoonoses

Ferdowsi University of Mashhad - September 2025



سومین کنگره ملی بیماری‌های انگلی دام و انگل‌های مشترک انسان و حیوان

The 3rd National Congress Of Animal Parasitic Diseases and Zoonoses

دانشگاه فردوسی مشهد، ۲۶ و ۲۷ شهریور ۱۴۰۴

مدیریت نوین بیماری‌های انگلی. تأمین سلامت یکپارچه و امنیت غذایی

ثبت نام از اول اردیبهشت ۱۴۰۴

دارای امتیاز باز آموزی



۰۴۲۵-۵۸۱۷۳

مهمورهای کنگره

بیماری‌های انگلی مشترک انسان و میوهان

بیماری‌های انگلی نوپدید و بازپدید

برهمکنش‌های انگل و میزبان

اپیدمیولوژی بیماری‌های انگلی، تغییر اقلیم و فسادات اقتصادی

ایمنی‌شناسی و آسیب‌شناسی در بیماری‌های انگلی

بندپایان و بیماری‌های عفونی منتقل شونده توسط آن‌ها

انگل‌های میات و منش، اکزوتیک، آبزیان، زنبور عسل و کرم ابریشم

انگل‌های منتقل شونده از طریق آب و غذا

داروها، سموم ضدانگلی و مقاومت‌های دارویی

هوش مصنوعی و سایر فناوری‌های نوین در تشخیص و درمان بیماری‌های انگلی



آدرس دبیرخانه: ایران، مشهد، میدان آزادی، دانشگاه فردوسی مشهد، دانشکده دامپزشکی.

تلفن دبیرخانه: ۰۵۱-۳۸۸۰۳۷۵۰ ۰۹۳۳۶۵۷۸۰۶۳ فکس: ۰۵۱-۳۸۸۰۷۰۷۶

ایمیل: Paravet3@um.ac.ir تارنما: Paravet3.um.ac.ir



مولتی کافه





3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



The 3rd National Congress of Animal Parasitic Diseases and Zoonoses

17-18 September 2025

Ferdowsi University of Mashhad, Mashhad, Iran

Organizer:

Ferdowsi University of Mashhad

Scientific Society of Animal Parasitic Diseases and Zoonoses

Congress President:

Dr. Seyed Hossein Hosseini

Vice President of the Congress:

Dr. Hassan Borji

Congress Scientific Secretary:

Dr. Elahe Ebrahimzadeh

Congress Executive Secretary:

Dr. Ali Moshaverinia



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



“In the name of God”

With greetings and respect to the presence of professors, colleagues, and students, I am pleased that the “**3rd National Congress of Animal Parasitic Diseases and Zoonoses**” will be held at the Faculty of Veterinary Medicine of Ferdowsi University of Mashhad on September 17 and 18, 2025. This congress offers a valuable opportunity for researchers to share their ideas and the latest scientific achievements in a collaborative and engaging environment on key topics, including parasitic diseases of animals and humans, emerging and re-emerging diseases, epidemiology, the economic impact of parasitic diseases, parasites of wildlife.

The successful hosting of two conferences on Animal Parasitic Diseases and Zoonoses has been a valuable experience, both scientifically and practically. We hope that this conference, with the valuable presence of researchers, will also be a practical step towards scientific advancement and addressing related issues in the country.

I sincerely invite all professors, researchers, and students to participate in this scientific congress, thereby enhancing its scientific value by sharing their latest and most significant scientific work achievements.

We hope that the participants in this conference will achieve the maximum scientific benefit through enthusiastic participation. I would like to express my sincere appreciation and gratitude to the members of the scientific and executive committees, as well as to the respected dean of the Faculty of Veterinary Medicine at Ferdowsi University of Mashhad, for their cooperation and efforts in organizing this congress.

Seyed Hossein Hosseini

President of the Congress



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



“In the name of God”

We are honored to host the “**3rd National Congress of Animal Parasitic Diseases and Zoonoses**” at Ferdowsi University of Mashhad. We thank the professors, students, and all specialists who participated in the congress. We hope that, through mutual collaboration, we can exchange innovative scientific ideas and jointly promote scientific progress in the country.

This congress is organized through the efforts of the Scientific Society of Animal Parasitic Diseases and Zoonoses, in collaboration with Ferdowsi University of Mashhad, with support and assistance from various universities and organizations. I want to thank and appreciate all the sponsors.

Parasitic infections in veterinary medicine are particularly important not only for economic losses, but also for health concerns and zoonotic diseases.

This congress, with the slogan "Modern Management of Parasitic Diseases, Ensuring One Health and Food Security," aimed to convey that handling parasitic infections in veterinary and human medicine can only be achieved through collaboration among all stakeholders, from universities to government agencies. This is a critical issue that is being actively addressed and planned worldwide under the One Health program.

In this regard, alongside this congress, a specialized meeting program on zoonotic parasitic diseases for physicians was organized by the Faculty of Medicine and the Mashad Medical Council, under the scientific and executive secretaryship of Dr. Bibi Razieh Hosseini Farrash.

A total of 421 articles were sent to this congress, of which 406 were accepted. Of these, 72 lectures were included in 10 panels, and 321 posters were included in 11 panels. We sincerely appreciate the presence of all participants at this scientific congress.

Elahe Ebrahimzadeh

Scientific Secretary of the Congress



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



کمیته علمی سومین کنگره ملی بیماری‌های انگلی دام و بیماری‌های مشترک انسان و دام، ۲۶ و ۲۷ شهریور ماه ۱۴۰۴، مشهد

۱. دکتر الهه ابراهیم زاده، هیأت علمی دانشگاه فردوسی مشهد
۲. دکتر رضا اسدی، مدیر کل دفتر بهداشت و بیماری‌های دام سازمان دامپزشکی کشور
۳. دکتر علیرضا باهنر، هیأت علمی دانشگاه تهران
۴. دکتر حسن برجی، هیأت علمی دانشگاه فردوسی مشهد
۵. دکتر فریبا برنجی، هیأت علمی دانشگاه علوم پزشکی مشهد
۶. دکتر سمیه بهرامی، هیأت علمی دانشگاه شهید چمران اهواز
۷. دکتر حبیب الله پایکاری، رئیس بخش تحقیقات بیماری‌های انگلی دام موسسه تحقیقات واکسن و سرم سازی رازی کرج
۸. دکتر یاسر پیرعلی، هیأت علمی دانشگاه شهرکرد
۹. دکتر موسی توسلی، هیأت علمی دانشگاه ارومیه
۱۰. دکتر فاطمه جالوسیان، هیأت علمی دانشگاه تهران
۱۱. دکتر عماد چنگیزی، هیأت علمی دانشگاه سمنان
۱۲. دکتر سیدحسین حسینی، رئیس کنگره و رئیس انجمن بیماری‌های انگلی دام و انگل‌های مشترک انسان و حیوان
۱۳. دکتر حمیدرضا حسینی، مدیر کل محترم دفتر امور دارو و درمان سازمان دامپزشکی کشور
۱۴. دکتر بی‌بی راضیه حسینی فراش، هیأت علمی دانشگاه علوم پزشکی مشهد و دبیر علمی-اجرایی نشست تخصصی بیماری‌های انگلی مشترک انسان و حیوان
۱۵. دکتر مهدی خلیج، سازمان دامپزشکی کشور و عضو هیات مدیره انجمن بیماری‌های انگلی دام و انگل‌های مشترک انسان و حیوان
۱۶. دکتر غلامرضا رزمی، هیأت علمی دانشگاه فردوسی مشهد



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



۱۷. دکتر سیدحسین رضوانی، مدیرکل حوزه ریاست، حقوقی و ارزیابی عملکرد سازمان دامپزشکی کشور
۱۸. دکتر مصطفی رضوی دینانی، هیأت علمی دانشگاه شیراز
۱۹. دکتر صادق رهبری، استاد پیشکسوت دانشگاه تهران
۲۰. دکتر حمید زینالی، مدیر کل محترم دفتر بهداشت و مدیریت بیماریهای دامی سازمان دامپزشکی کشور
۲۱. دکتر علیرضا سازمند، هیأت علمی دانشگاه بوعلی سینا همدان
۲۲. دکتر پرویز شایان، هیأت علمی دانشگاه تهران
۲۳. دکتر حمیدرضا شکرانی، هیأت علمی دانشگاه لرستان
۲۴. دکتر سید علی اکبر شمسیان، هیأت علمی دانشگاه علوم پزشکی مشهد
۲۵. دکتر سالومه شیرعلی، هیأت علمی دانشگاه علوم و تحقیقات تهران
۲۶. دکتر فاطمه عرب خزائلی، هیأت علمی دانشگاه تهران
۲۷. دکتر عبدالمجید فتی، هیأت علمی دانشگاه علوم پزشکی مشهد
۲۸. دکتر مهدی محبعلی، هیأت علمی دانشگاه علوم پزشکی تهران
۲۹. دکتر بهنام مشگی، هیأت علمی دانشگاه تهران
۳۰. دکتر محمد موذنی، هیأت علمی دانشگاه شیراز
۳۱. دکتر کامران میرزایی، رئیس محترم مرکز تحقیقات بیماری های منتقله از دام و فراورده های دامی سازمان دامپزشکی
۳۲. دکتر صدیقه نییان، هیأت علمی دانشگاه تهران
۳۳. دکتر سعیدرضا نوراللهی فرد، هیأت علمی دانشگاه شهید باهنر کرمان
۳۴. دکتر علی نیک پی، هیأت علمی دانشگاه فناوری های نوین آمل
۳۵. دکتر الهام هوشمند، هیأت علمی دانشگاه آزاد اسلامی واحد رشت



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Congress President:

Dr. Seyed Hossein Hosseini, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Vice President of the Congress:

Dr. Hassan Borji, Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Congress Scientific Secretary:

Dr. Elahe Ebrahimzadeh, Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Executive Secretary:

Dr. Ali Moshaverinia, Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

IT & Media:

Mohammad Sajed Sarvari, Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Congress Secretariat:

Dr. Asma Keyvanlou Shahrestanaki, Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Suppling Affairs:

Dr. Nooshinmehr Soleymani, Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Transportation:

Dr. Jamal Boros Ghoncheh, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Public Relations Affairs:

Dr. Abbas Jadidoleslami, Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Sponsorshp and Exhibition Affairs Coordinator:

Dr. Bahareh Meftahi, Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Publication:

Mohammad Sajed Sarvari and **Dr. Asma Keyvanlou Shahrestanaki**, Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Registration Affairs:

Dr. Arezoo Firoozeh, Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Contents

Evaluation of the Efficacy and Economic Viability of Targeted Selective Treatment for Parasite Control in Afshari Sheep.....	1
Ali Sallehi ¹ , Gholamreza Mohammadi ²	1
Effects of Some Parasitic Infections on the Physiology of Central Feeding Behavior and Energy Balance in Birds.....	6
Farshid Hamidi ¹	6
Development of A Triplex Nested PCR for Simultaneous Diagnosis of Three Important Zoonoses Agents <i>Plasmodium</i> , <i>Babesia</i> , and <i>Toxoplasma</i> in Blood Samples of Humans and Animals.....	12
Abbasali Eskandarian ¹ , Alireza Najafi Rad ¹	12
Cryptosporidiosis in Newborn Calves: Prevalence and Control Strategies in Dairy Herds of Mashhad, Iran	20
Hassan Esmailjani ¹ , Gholamreza Mohammadi ¹	20
Describing the Distribution Pattern in Canine and Feline Parasitic Dermatoses	24
Javad Khoshnegah ¹	24
Investigation of Leucocytozoonosis in Poultry: Epidemiological Analysis, Biology, Economic Consequences, and Control Strategies	30
Seyed Reza Hosseini ¹	30
In vitro Evaluation of Susceptibility of <i>Dermanyssus gallinae</i> to Phoxim, Sevin and Cypermethrin ..	36
Maryam Keyghobadi ¹ , Ali Moshaverinia ²	36
Integration of Next-Generation Sequencing and Metagenomics for Detection of <i>Cryptosporidium</i> and <i>Giardia</i> in Ready-to-Eat Fresh Produce.....	40
Hediyeh karbasi ^{1,2} , Mohammad Mahdi Teymori ^{1,2} , Parnian Pezeshki ¹ , Atefeh Sarafan Sadeghi ¹	40
The application of Artificial Intelligence to Combat Leishmaniasis: A Comprehensive Review of Innovations in Diagnosis, Therapeutics, and Epidemiological Control.....	46
Afsaneh Dolatkah ¹ , Adel potin ¹	46
Introduction to Molecular Detection Tools for Differential Diagnosis of Hemoprotozoan Parasites <i>Theileria sp.</i> and <i>Babesia sp.</i>	51
Parviz Shayan ¹	51
Introduction of RT-Nested Multiplex for Easy Detection of Dengue Virus and Its Serotypes in Biological Samples	56
David Shayan ^{1,2} , Reza Heidari ³ , Parviz Shayan ^{1,4}	56



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



A 10-year Report of <i>Neospora</i> Infection in Aborted Bovine Fetuses Referred to the Faculty of Veterinary Medicine Hospital, Ferdowsi University of Mashhad.....	60
Narges Khaleghnia ¹ , Gholamreza Razmi ² , Pezhman Mirshokraei ³	60
A Review of the Epidemiology, Prevalence, and Genetics of <i>Varroa destructor</i> , the Parasitic Mite of <i>Apis mellifera</i> in Iran	67
Sedigheh Nabian ^{1,2} , Mahsa Shahbakhsh ³	67
<i>Dicrocoelium dendriticum</i> in Iran: A Neglected Threat, Emerging Challenge or an Escalating Crisis?	74
Behnam Meshgi ¹	74
Applications of Machine Learning Algorithms in Veterinary Medicine: From Parasite Detection to Disease Outbreak Prediction.....	79
Shadi Shahvand ¹ , Arash Omid ¹	79
Radiologic Evaluation of Visceral Changes in BALB/c Mice Infected with Cutaneous Leishmaniasis Caused by <i>Leishmania major</i>	85
Ayna Azimzadeh Tabrizi ¹ , Sedigheh Nabian ² , Majid Masoudifard ³ , Parviz Shayan ² , Mehdi Mohebbali ⁴ , David Shayan ⁵ , Amineh Pouyandeh ¹	85
Stage-Specific cDNA Profiling of Helminth Parasites with a Focus on <i>Fasciola hepatica</i> and Computational Modeling of <i>Fasciola hepatica</i> HDM-1 Protein Internalization	93
Fateme Jalousian ¹	93
Food- and Water- Borne Parasites, One Health Strategy, and Their Role in Achieving Sustainable Development Goals.....	100
Seyed Hossein Hosseini ^{1,2} , Fateme Jalousian ¹	100
Epidemiological Status of Cutaneous Leishmaniasis in Iran.....	109
Mohammad Zeinali ¹ , Faham Khamesipour ¹	109
Applications of Artificial Intelligence and Deep Learning in the Diagnosis of Parasitic Diseases: A Case Study on <i>Dirofilaria immitis</i> Microfilariae	115
Fatemeh Haji Agha Khiyabani ¹ , Fateme Jalousian ¹	115
One Health and Parasitology: From Historical Foundations to Practical Challenges in Iran	123
Mohammad Majid Johari ¹ , Elahe Ebrahimzadeh ¹	123
Evaluation of the Larvicidal Efficacy of <i>Mentha piperita</i> Alcoholic Extract on <i>Linguatula serrata</i> Nymphs under Laboratory Conditions.....	131
Nasser Hajipour ¹	131
Three Technical Challenges for AI in Veterinary Parasitology	138
Mohammad sajed Sarvari ¹	138
Artificial Intelligence and Parasitology: Recent Advances and Future Prospects	144



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Soren Mohammadi ¹ , Elahe Ebrahimzade ¹ , Mohammad Majid Johari ¹	144
One Health Approaches to the Control of <i>Cryptosporidium</i> infection in livestock, Humans, and the Environment.....	152
Elahe Ebrahimzadeh ¹	152
Artificial Intelligence and Computer Vision in the Diagnosis of Helminths and Parasite Eggs: A Systematic Review and Practical Outlook with Emphasis on Zoonotic Potential.....	159
Mohammad Mahdi Darvishi ¹	159
Investigation of Soil Contamination in Mashhad Public Park with <i>Toxocara</i> Species Eggs During the Years 1402-1403	166
Hassan Yazdanfar ¹ , Mohadesh Sabori darbandi ¹	166
Comparative Frequency of <i>Varroa</i> , <i>Nosema</i> , and <i>Acarapis</i> Infection in Honeybee (<i>Apis mellifera</i>) Colonies of Khorasan Razavi Province: A Two-Year Study	174
Bahareh Meftahi ¹ , Samaneh Rezaei ¹ , Mohammad Reza Shariati ² , Gholamreza Razmi ¹	174
Post Anticoccidial Growth Promoters Era: Coccidiosis in Iranian Poultry Production over the Last Ten Years.....	179
Fatemeh Arabkhazaeli ¹	179
Molecular Mimicry as a Common Factor in the Immune Response of Rheumatic Fever and Toxoplasmosis.....	184
Ahmed Saadi khalaf, Teeba faris hadi, Ghazwan talal Mahdi	184
Crimean-Congo Hemorrhagic Fever, Transfer Patterns, Vectors, and History in Iran and Neighboring Countries	192
Mousa Tavassoli ¹ , Salar Zarrabi Ahrabi ² , Gurkan Akyildiz ² , Aysen Gargili Keles ²	192
Frequency of <i>Sarcocystis</i> spp Infection in Cattle Slaughtered in Birjand Slaughterhouse Using Digestion and Molecular Methods in 2024.....	193
Amir Tavakkoli Kareshk ¹ , Mahmoodreza Behravan ¹ , Ali Khazaei ² , Rahmat Solgi ¹	193
Molecular Detection of <i>Anaplasma</i> , <i>Babesia</i> , and <i>Theileria</i> Species from Sheep in the West of Iran.....	194
Maryam Nazarbeigy ¹ , Roohollah Fattahi ¹ , Fazel Pourahmad ²	194
Copro-PCR based Determination of <i>Toxoplasma gondii</i> Oocysts in the Cats Referred to Veterinary Clinics in Ardabil City	195
Abbas Imani Baran ¹	195
<i>Simulium turgaicum</i> Complex (Diptera: Simuliidae): Potential Vectors of Filarial Parasites in the Aras River Basin – Evidence from Epidemiological, Molecular, and Phenological Studies	196
Naseh Maleki-Ravasan ¹ , Fariba Khanzadeh ²	196
Using PCR and High- Resolution Melting (HRM) Analysis for Dirofilariasis Diagnosis	197
Niayesh Mohammadi ¹ , Fateme Jalousian ²	197



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Comparative Evaluation of the HALP Score between PCR-confirmed <i>Theileria</i> -infected and Healthy Sheep in Semnan, Iran	199
Vahid Dokhaei ¹ , Mahmood Ahmadi-hamedani ² , Mohammad Reza Salimi Bejestani ³	199
A Study on the Frequency of <i>Anaplasma platys</i> Infection in Shelter Dogs in the Mashhad area, Iran, Using Microscopic and Molecular Methods	201
Seyyed AmirSohrab Tabatabaie Hosseini ¹ , Amin Bakhshani ¹ , Gholamreza Razmi ¹	201
IL-33 and Its Association with Visceral Leishmaniasis in the Hamsters Infected with <i>Leishmania infantum</i>	202
Shamsi Noorpisheh Ghadimi ¹	202
Phylogenetic Analysis of Avian Schistosomes from <i>Melanoides tuberculata</i> in Southwest Iran, and Evaluation of their Pathogenicity for Mice.....	203
Mehdi Karamian ^{1,2} , Seyed Mahmoud Sadjjadi ¹ , Ahmad Oryan ³ , Jitka Aldhoun ⁴ , Sharif Maraghi ⁵	203
An Outbreak of Clinical Coccidiosis in Lambs in a Sheep Farm in Chenaran Area, Khorasan Razavi Province	205
Gholamreza Razmi ¹ , Hossien Nourani ² , Mostafa Torkaman ³	205
Investigation of the Possibility of <i>Theileria annulata</i> Resistance to Buparvaquone in Treated Cows Against Theileriosis in Roshtkhar Area, Khorasan Razavi Province.....	206
Jamil Arab Banishibani ¹ , Ali Moshaverinia ¹ , Asma Keyvanlou ¹ , Gholamreza Razmi ¹	206
Molecular and Morphological Characterization of a New <i>Eimeria</i> Species in the Domestic Pigeon (<i>Columba livia domestica</i>) in Northwest Iran.....	207
Parisa Shahbazi ¹ , Monireh Khordadmehr ¹ , Hosein Hashemzadeh Farhang ²	207
Laboratory Diagnosis of Cutaneous Leishmaniasis Based on an In-House ELISA Using a New Antigen.....	209
Zohreh Askarypour ¹ , Sahel Hamzehpour ¹ , Negin Elahirad ¹ , Mahmoud Nateghi-Rostami ¹	209
Molecular Identification of <i>Toxoplasma gondii</i> in Domesticated and Industrial Chickens in Isfahan Region, Iran, 2022.....	210
Nader Pestehchian ¹ , Mahnaz Sami ¹ , Reza Kalantari ¹ , Hossainali Yousefi ¹ , Shima Toghyani ³	210
Biological Control of Fasciolosis: An Integrated Review of Emerging Solutions	212
Mohammad Moazeni ¹ , Artin Sheibani ²	212
Molecular Cloning and Heterologous Expression of the <i>GroEL</i> Gene Encoding a Chaperonin Protein from <i>Wolbachia</i> symbiont of <i>Phlebotomus papatasi</i> (Diptera: Psychodidae)	214
Donya Moayedi Moafagh ^{1,2} , Naseh Maleki-Ravasan ¹ , Soheila Ajdary ³ , Asal Katebi ³ , Fateh Karimian ¹ , Jalal Babaie ¹	214
Acanthamoeba-Bacteria Interactions: A Review of Experimental Findings and Implications	216
Somayeh Bahrami ¹ , Mehdi Zarei ²	216



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Investigation of <i>Toxoplasma gondii</i> Presence in Raw Milk of Sheep, Goats, Cows, and Camels in the Sistan Region	218
Mohammad Mohebi ¹ , Mohammad Mirzaei ¹ , Bagher Amirheidari ² , Maryam Nooshadokht ³	218
Prevalence of <i>Dirofilaria immitis</i> and <i>Dirofilaria repens</i> in Dogs from Gilan Province, Iran: A GIS-Based Epidemiological Study	219
Fatemeh Manshouri ¹ , Fatemeh Jalousian ² , Seyed Hossein Hosseini ² , Parviz Shayan ² , Saeed Bakaei ³	219
Comparison of Surgical Approaches to Hepatic Hydatid Cyst Management in Humans and Animals: A Cross-Disciplinary Surgical Review	221
Samaneh Ghasemi ¹ , Nima Movasseghi ² , Paniz Azadpeima ²	221
Comparison of Ziehl Neelsen Staining, ELISA and PCR in Diagnosis of Human <i>Cryptosporidium</i> Infection	222
Mohamad Molazadeh ¹ , Hossein Yousofi Darani ¹	222
Detection and Genotyping of <i>Toxoplasma gondii</i> in Aborted Fetuses of Ewes and Goats from South-west of Iran	223
Hossein Hamidinejat ¹ , Morteza Noormahmadi ² , Morad Beyranvand ² , Somayeh Bahrami ¹ , Saad Goraninezhad ³ , Mohammad Reza Tabandeh ⁴ , Meysam Maki ³	223
Immunomodulatory Dynamics and Interleukin-Mediated Therapeutic Strategies in Cystic Echinococcosis: Advancing Immune-Targeted Interventions through Parasite Secretome Profiling	224
Zahra Gahvechi Amiri ¹ , Mohadeseh Ramzi ² , Sima RoshanFar ³ , Fatemeh Ramzi ²	224
The Systematic Review Application of Artificial Intelligence in Identifying Foodborne Zoonotic Parasites: Exploring Challenges and Opportunities in Food Safety and Veterinary Parasitology	226
Fatemeh Karimi Kesheh ¹ , Shakiba Mazaheri ² , Mohaddeseh Karimi Kesheh ³ , Leyli Shabani Vieh ⁴ , Zahra Nabizadeh ⁵ , Niknaz Rajaei ⁶ , Mohammad Arad Zandieh ⁷	226
Comparison of Attenuated and Killed Vaccines Against <i>Neospora caninum</i> in Mice	228
Zahra khabazan ¹ , Reza rasti ¹ , Mehdi Namavari ¹	228
A Preliminary Study of Canine Leishmaniasis in Tehran Province Were Referred to Iranian Pet Hospital from 2015-2025	229
Seeyamak Mashhady Rafie ¹ , Hamed Mansoor Lakooraj ² , Siamak Zarei ³ , Golsa Saadati ⁴	229
Canine leishmaniosis in Iran: It Is not Only <i>Leishmania infantum</i>	230
Alireza Sazmand ¹	230
Induction of a Th1 Immune Response Against Leishmaniasis Infection by a Kinetoplast-associated Gene Knockout <i>Leishmania major</i> Strain	231
Fatemeh Darzi ¹ , Mahmoud Nateghi-Rostami ¹	231
Investigation of the Seroprevalence of <i>Fasciola</i> spp. Infection in Pregnant Women Referring to Al-Zahra Hospital, Rasht, Northern Iran	233



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Mahdieh Sorouri Majd ^{1,2} , Mojgan Aryaeipour ¹ , Eshrat Beigom Kia ¹ , Seyedeh Hajar Sharami ³ , Mehdi Mohebbali ^{1,4} , Akram Sepahvand ¹ , Azadeh Jafari ³ , Zohreh Fakhrieh-Kashan ^{1,2}	233
Prevalence and Subtype Distribution of Blastocystis in Humans and Animals in Eastern Iran	235
Fariba Berenji ¹ , Saeid Zoghdar Moghadam ¹ , Ali Gholizadeh ³ , Mohammad Ali mohaghegh ² , Mehdi Zarean ¹ , Lida Jarahi ⁴ , Bibi Razieh Hosseini Farash ¹ , Seyed Ali Akbar Shamsian ¹ , Ghodratollah Salehi Sangani ¹	235
Molecular Identification and Comparative Analysis of <i>Echinococcus granulosus</i> Genotypes in Human and Livestock Hydatidosis in Chaharmahal and Bakhtiari Province, Iran	237
Mohammad javad Pourmaram ¹ , Dr. Ebrahim Saedi Dezaki ² , Mahdieh Valibegi ³	237
Effects of <i>Toxocara cati</i> Larvae and Somatic Antigens of Adult Worms in Experimental Asthmatic Mice	239
Amin Bakhshani ¹ , Sima Parandeh ¹ , Mohsen Maleki ² , Alireza Haghpars ² , Hassan Borji ²	239
Diagnostic Challenges in Echinococcosis.....	241
Abdolhossein Dalimi ¹	241
Genotyping of <i>Neospora caninum</i> Using Microsatellite Markers: Recent Advances	242
Asma Keyvanlou Shahrestanaki ¹ , Mohammad Taghi Vajed Ebrahimi ² , Gholamreza Razmi ¹	242
Detection and Characterization of <i>Trichinella</i> Species in Wild Carnivores Across Northern Regions of Iran	244
Mohammad Reza Khoshvaght ¹ , Elahe Ebrahimzadeh ¹ , Nooshinmehr Soleymani ¹ , Moein Abolhasani Daroukola ¹ , Hassan Borji ¹	244
Using Salivary anti- CarLA IgA as a Tool to Manage Gastrointestinal Nematode in Ruminants	245
Hassan Borji ¹	245
Nanomedicine in the Treatment of Parasitic Diseases: Advances, Challenges, and Future Directions	246
Nooshinmehr Soleymani ¹ , Hassan Borji ¹	246
A Retrospective Analysis of Hydatid Cyst Management and Associated Risk Factors: A 10-Year Study in Mashhad, Iran	248
Zahra Nehbandani ¹ , Hassan Borji ² , Amir sadra Keyvani ³	248
First Molecular and Morphological Characterization of <i>Echinococcus equinus</i> in Iranian Donkeys.	250
Seyed Hossein Hosseini ^{1,2} , Fateme Jalousian ¹ , Mohammad Bolandmartabe ¹ , Shirin Khodabakhsh ¹	250
Present Status of Visceral Leishmaniasis in the Islamic Republic of Iran	251
Mehdi Mohebbali ^{1,2}	251
Biosynthesis of Zinc Oxide Nanoparticles Mediated by Probiotic and Their Bioactivity Against <i>Giardia</i> Species in Dogs	252
Ammar R. Algburi ¹ , Munaf Al-Dulaimi ²	252
Rethinking <i>Cryptosporidium</i> Biology: Novel Insights and Intervention Opportunities	253



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Seyed Ali Mousavi Rad ¹ , Mahdi Naseri ¹	253
A One Health-Based Comparative Study of Public Awareness, Attitudes, and Perceptions Toward Food and Waterborne Zoonotic Parasitic Threats in Western Iran: An Immunological Approach to Improving Biosafety	255
Mehrdad Pooyanmehr ¹	255
Assessment of Knowledge and Attitudes of Industrial and Traditional Livestock Farmers Toward the Role of Vaccination and Management of Zoonotic, Emerging, and Re-emerging Parasitic Infectious Diseases: An Essential Immunological and Biosafety Approach	261
Mehrdad Pooyanmehr ¹	261
Comparative Evaluation of Farmers' Awareness and Preventive Measures Against Arthropod-Borne Protozoan Infections in Western Iran, with a Focus on Immunopotentiating Vaccine Use	268
Mehrdad Pooyanmehr ¹	268
Copro-microscopic Study on Helminthic Infections of the Digestive and Respiratory Tracts of Goats in East Azerbaijan Province	275
Abbas Imani Baran ¹ , Ahmad Nematollahi ¹ , Mohammad Kashani ¹ , Parisa Shahbazi ¹	275
Study on Digestive Tract Helminthic Infection of Wild Rabbits in Northwest of Iran	280
Abbas Imani Baran ¹	280
Emerging Perspectives on Foodborne and Waterborne Parasites: From Epidemiology to Carcinogenesis	286
Fatemeh Rasekhnia ¹	286
A Rare Case of Sarcoptic Mange Transmission from a Pet Rabbit to Humans	291
Ali Samemaleki ¹	291
Infestation of <i>Ctenocephalides felis</i> in a Sheep Herd: A Case Report	295
Seyed Kian Mohseni Mofidi ¹ , Mousa Tavassoli ¹ , Saleh Sharifi ¹ , Alireza Ghorbani ¹	295
Comparing the Effects of Cryotherapy and Conventional Injectable Treatments on the Healing of <i>Leishmania major</i> Skin Wounds in Mice	300
Seyed Kamyab Sajadi ¹ , Ghazaaleh Adhami ¹ , Yahya Maroufi ²	300
<i>Hepatozoon canis</i> Infection in a Rottweiler Dog: A Case Report from Ahvaz, Khuzestan Province, Iran	309
Zahra Mashayekhi ¹ , Somayeh Bahrami ¹ , Mahnush Kaamyabnia ²	309
Study of the Synergistic Effects of Combined Essential Oils from <i>Lavandula angustifolia</i> and <i>Artemisia absinthium</i> Shoots on <i>Leishmania infantum</i> Promastigotes	316
Morteza Eshagh Nimori ¹ , Sohrab Rasouli ² , Samin Julai Sani ³	316
CRISPR-based Rapid Diagnosis of Zoonotic Parasites: Advances and Prospects	321
Reza Soltani ¹ , Bahareh Meftahi ^{2,3}	321



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Evaluation of the Impact of Anthelmintic Treatment of Herding Dogs with Praziquantel and Their Nutritional Hygiene Management on Reducing Cerebral Coenurosis in Female Afshari Lambs Under Two Years of Age.....	326
Ali Sallehi ¹ , Gholamreza Mohammadi ²	326
Investigation of <i>Dipylidium Caninium</i> infection in Domestic Dogs and Cats.....	331
Sarah Emamdadi ¹ , Mohammad Sayah Layin ² , Sadaf PakNafas ¹ , Shiba Yousefvand ³	331
First Report of Molecular Detection of Leishmaniasis in a Domestic Persian Cat (<i>Felis catus</i>) from North of Iran: A Case Report	335
Mostafa Kami ^{1, 2, 3} , Najva Zivdari ⁴ , Morteza Valinezhad ⁵ , Mahla Homayounzade ² , Ali Safari ² , Seyed Mohammad Hosseini ^{2, 6}	335
Metal-based Nano Particle Against Protozoa: The Role of Nanoparticles in Treatment and Diagnosis	342
Sarvenaz Sadeghi Mahoonak ¹ , Parsa Farzam ¹	342
Physiological Modeling of the Autonomic Nervous System Response to Parasitic Infections in Animals.....	354
Farshid Hamidi ¹	354
Physiological Responses of the Host Body to Parasitic Infections: Focus on the HPA Neurohormonal Axis.....	359
Farshid Hamidi ¹	359
Comparative Evaluation of Molecular Methods for Differential Diagnosis of <i>Leishmania</i> Infection: A Narrative Review	364
Mobina Mohammadi ¹ , Parviz Shayan ¹	364
<i>Aedes albopictus</i> as a High-Risk Invasive Species in Arbovirus Transmission: Taxonomy, Ecology, and Implications.....	371
Parsa Farzam ¹ , Sarvenaz Sadeghi Mahoonak ¹	371
Molecular Prevalence of <i>Toxoplasma gondii</i> in Zahedan County Using Nested-PCR Method.....	378
Mohammad Mohebi ¹ , Mohammad Mirzaei ¹ , Bagher Amirheidari ² , Maryam Nooshadokht ³	378
Review of the Impact of External Parasites on Hematological Parameters in Domestic and Wild Animals.....	384
Mahsa Sadeghi ¹ , Moosa Javdani ¹ , Yaser Pirali ² , Negin Hajian ¹ , Reza Abedizade ¹	384
A Review of the Immune Response and Surgical Wound Healing in Dogs Infected with Endoparasites	389
Mahsa Sadeghi ¹ , Moosa Javdani ¹ , Yaser Pirali ² , Negin Hajian ¹ , Reza Abedizade ¹	389
Parasitic Infections in Small Animals and Anesthetic Complications	395
Mahsa Sadeghi ¹ , Moosa Javdani ¹ , Yaser Pirali ² , Negin Hajian ¹ , Reza Abedizade ¹	395



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Do Parasitic Infections Negatively Affect Surgical Wound Healing in Veterinary Medicine? Mechanism of Action and Clinical Significance.....	400
Reza Abedizade ¹ , Moosa Javdani ¹ , Negin Hajian ¹ , Mahsa Sadeghi ¹ , Mohammad Salimi ¹	400
Detection of Feline Hemotropic <i>Mycoplasmas</i> : Implications of Arthropod Vectors in Diseases Transmission	405
Afsaneh Nazarpour Kalaei ¹ , Somayeh Bahrami ¹ , Mahyar Vazifeshenas Javan ²	405
Assessment of <i>Anaplasma platys</i> Prevalence and Hematological Effects in Canines from Ahvaz	410
Sajjad Alishiri ¹ , Somayeh Bahrami ¹ , Dorin Motamedi ¹ , Hossein Hamidinejat ¹ , Bahman Mosallanejad ² , Misagh Jalali ²	410
In Vitro Evaluation of a Nanoemulsified Blend of Clove, Lemongrass, and Tea Tree Essential Oils Against Poultry Lice (<i>Lipeurus caponis</i>).....	415
Elaheh Roohbakhsh Amoli Moghadam ¹ , MohammadReza Khajehdaluei ² , Somayeh Alamdar ³	415
First Report of <i>Plasmodium</i> spp. Infection in a Cattle Egret (<i>Bubulcus ibis</i>) in Ahvaz, Khuzestan, Iran	420
Arman Nourishirazi ¹ , Yassamin Mahmoudian ² , Poorya Soleymani ³ , Somayeh Bahrami ¹	420
Prevalence of <i>Babesia canis</i> Infection in Dogs in Ahvaz, Southwest Iran: A Molecular Epidemiological Study.....	425
Dorin Motamedi ¹ , Somayeh Bahrami ¹ , Sajjad Alishiri ¹ , Bahman Mosallanejad ²	425
First Evidence of <i>Anaplasma platys</i> and <i>Hepatozoon canis</i> Co-infection in a Dog from Ahvaz, Khuzestan Province	430
Zahra Mashayekhi ¹ , Somayeh Bahrami ¹ , Sajjad Alishiri ¹ , Dorin Motamedi ¹ , Bahman Mosalla Nejad ²	430
Alternative Anti-parasitic Approaches: The Role of Probiotics in Helminth and Protozoan Infections	436
Sarvenaz Sadeghi Mahoonak ¹ , Seyedeh Yasna Hosseini ¹	436
A Review of <i>Trichostrongylus axei</i> Infection in Cattle	445
Ali Soleimanzadeh ¹ , Soroush Heidari ² , Ali Mosayebzadeh ²	445
Drug Resistance in Zoonotic Parasites: Challenges and Strategies	451
Yasaman Shadvar ¹ , Shayesteh Mosleh ¹	451
Symbiotic Bacterial Organism <i>Wolbachia pipientis</i> as a Detection Marker for Molecular Diagnosis of <i>Dirofilaria immitis</i> Infection	459
Melika Mohammadi ¹ , Parviz Shayan ¹	459
Parasitic Extracellular Vesicles: Emerging Mediators in Host-Parasite Communication and Targets for Therapeutic Innovation	464
Siyamak Kakekhani ¹ , Arshia Dousti ² , Behnaz Momeni ²	464
First Report of <i>Giardia psittaci</i> in Captive Psittacines in Tehran, Iran.....	472



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Sahar Mirbagheri ¹ , Salomeh Shirali ^{1,2} , Bahar Shemshadi ¹ , Mohammad parsa Bayatpoor ¹	472
A Review on In Vitro Studies on Plant Extracts and Metal Nanoparticles Effectiveness on Domesticated Ruminant Liver Flukes.....	478
Farnaz Malekifard ¹ , Seyyed Kian Mohseni Mofidi ¹ , Alireza Ghorbani ¹	478
Cryptosporidial Diarrhea in Neonatal Lambs: A Clinical Investigation in an Industrial Flock in Iran	485
Mohadese Loukhi ¹ , Gholamreza Mohammadi ¹	485
A Review of <i>Varroa</i> Mite Infestation and Plant-Based Control Strategies: Current Insights and Future Perspectives.....	490
Farnaz Malekifard ¹ , Alireza Ghorbani ¹ , Seyyed Kian Mohseni Mofidi ¹	490
Clinical Infection of a Female Najdi Goat with <i>Anaplasma ovis</i> in Ahvaz City	497
Hossein Hamidinejat ¹ , Ali Abbas Nikvand ² , Seyedeh Missagh Jalali ² , Somayeh Bahrami ¹ , Maryam Abbaszadeh ³	497
Diectophymosis in a Well-cared-for Pitbull Dog with no History of Paratenic Host Ingestion	506
Javad Jafari ¹ , Mostafa Rahimof ¹ , Mohadese Hosseinzadeh ¹	506
<i>Toxoplasma gondii</i> and Schizophrenia: A Neurobiological Review of the Evidence	512
Sara Kazemi ¹ , Seyyed Mohammad Aghapour ¹ , Farnaz Seilanian Toosi ¹ , Fatemeh Zahra Gharib ²	512
Parasitic Infections in Goldfish (<i>Carassius auratus</i>) Sold at Ornamental Fish Markets in Ahvaz.....	520
Abdolhossein Jangaran Nejad ¹ , Maryam Sadin ² , Ali Yarahmadi ²	520
Review on Equine Protozoal Myeloencephalitis and Possibility of Occurrence Disease in Iran	526
Milad Partovi ¹ , Fereydon Rezazadeh ²	526
Third-generation Pesticides: A Novel Approach for Improving Food Safety and Controlling Parasitic Contamination.....	531
Mohammad Mahdi Fahmideh ¹ , Mina Gozal ¹ , Parnian Pezeshki ¹ , Atefeh Sarafan Sadeghi ¹	531
Foodborne Toxoplasmosis: Transmission from Animal Products.....	539
Parisa Behnam ¹ , Alireza Esmailzadeh Marandiz ¹ , Parnian Pezeshki ¹ , Atefeh Sarafan Sadeghi ¹	539
Systematic Review: <i>Cryptosporidium</i> in Pet Reptiles, Human Risks, Prevention, and Iran-Specific Species Analysis.....	547
Reza Ameri ¹ , Siamak Mashhadi Rafiei ¹	547
Evaluation of the Larvicidal Effects of Aqueous-alcoholic Extracts of <i>Glycyrrhiza glabra</i> , <i>Calotropis procera</i> and <i>Moringa oleifera</i> on Pre-Parasitic Larval Stages of Gastrointestinal Strongyles in Monogastric Animals	553
Sara Larki ¹ , Fatetmeh Madankan ¹ , Gholamreza Pirmoradi ²	553
Investigation of Contamination of Zoonotic Common Parasites in Slaughtered Animals at Semnan Industrial Slaughterhouse in the Period 2024-2025	560



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Amin DehghanTezerjani ¹ , Sabike Bakhtiyari ²	560
Cyathostomins in Horses: Life Cycle, Pathogenesis, Resistance	566
Mohammad mobin Sabri ¹ , Arman Valipour ¹ , Marzieh Kefayat ¹	566
A Review of <i>Neospora caninum</i> in Sheep: Epidemiology, Pathogenesis, and Control Strategies	571
Javad Qaneie ¹ , Pezhman Mirshokraei ¹	571
Evaluation of Hydatidosis Status in Slaughtered Livestock at Shahrud, in 2023	577
Ali Ghandali ¹ , Amin Dehghan Tezerjani ² , Reza Momeni ³	577
The Prevalence of Hydatidosis and Dicrocoeliasis in Slaughtered Animals in Damghan, 2024-2025	583
Reza Momeni ¹ , Amin Dehghan Tezerjani ² , Ali Ghandali ³	583
Prevalence and Molecular Characteristics of <i>Linguatula serrata</i> in Mesenteric Glands of Animals (sheep, goats and cows) in Slaughterhouses: A Cross-Sectional Study in Ilam Province, Iran	590
Shahab Falahi ¹ , Razi Naserifar ¹ , Hossien Ebrahimpour ² , Somayeh Chamanara ² , Azra Kenarkoohi ^{3,4} , Ebrahim Babaahmadi ⁵ , AliMohammad Bahrami ⁵ , Amir Abdoli ⁶ , Morteza Shams ¹	590
A Review on the Role of <i>Varroa destructor</i> in Transmitting Viruses and Bacteria to Honey Bees....	596
Sara Gholami ^{1,2} , Sedigheh Nabian ^{1,2} , Parviz Shayan ^{1,2} , Narges Amininia ^{1,2}	596
Challenges and Opportunities of Data Mining and Artificial Intelligence in the Detection and Management of Parasitic Zoonotic Diseases in Iran.....	601
Zahra Mohammadi ¹ , Arash Omid ¹	601
Rising Drug Resistance in Animal Parasites: Epidemiological Insights and Sustainable Control Measures	607
Shadi Shahvand ¹ , Arash Omid ¹	607
Prevalence of Toxocara Egg in the Parks Soil in Karaj	613
Sina Arabi ¹ , Nadia Taiefi Nasrabadi ² , Marzieh Kefayat ²	613
Prevalence and Risk Factors of Intestinal Parasitic Infection in Children Referred to Private Clinics in Yazd, Iran, 2018-2020.....	622
Nasser Hajipour ¹	622
Impact of Climate Change on the Prevalence of Zoonotic Parasitic Diseases in Aquatic Animals...	629
Zahra Mohammadi ¹ , shadi shahvand ¹ , Amir Ali Heidari ¹	629
A Review of the Effects of <i>Neospora caninum</i> in Cattle and Humans	634
Ali Mosayyebzadeh ¹ , Abolfazl Hajibemani ² , AmirMohammad NaghsheJavaheri ³ , Samin Heydarzadeh ⁴	634
The Role of Pathology in the Identification and Confirmation of Parasitic Diseases in Animals: A Systematic Review of Scientific Evidence	641
Sahar Adibinia ¹	641



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Prevalence of Taeniidae and <i>Toxocara</i> spp. Infections in Shepherd Dogs in Alborz Province, Iran	647
Ali Jebeli Eshratabadi ¹ , Babak Mogadasi ² , Mona Hamed ²	647
Seven-year Molecular Surveillance of <i>Toxoplasma gondii</i> Infection in Aborted Ovine Fetuses from Northeastern: Infection Trends and Epidemiological Insights	653
Kiana Irandoosti ¹ , Pezhman Mirshokraei ² , Narges Khaleghnia ³ , Gholamreza Razmi ⁴	653
<i>Aedes</i> spp. and Its Potential Risks in Iran; Past, Present and Future	661
Mohammad Majid Johari ¹ , Ali Moshaverinia ¹ , Seyedeh Zahra Bayegan ²	661
Parasitological Examination of Liver and Lung Infections in Slaughtered Livestock in Garmsar Slaughterhouse, in the Period 2023-2024	669
Sabike Bakhtiyari ¹ , Amin DehghanTezerjani ²	669
Comparing the Effectiveness of Two Conventional Anthelmintics, Albendazole and Triclabendazole, in Sheep	675
Gholamali Kojouri ¹ , Nazanin Gharib ² , Hamidreza Azizi ³ , Tarkan Shokohi ⁴	675
Comparative Evaluation of the Susceptibility of <i>Dicrocoelium</i> and <i>Fasciola</i> Parasites Following Oral Administration of Triclabendazole in Sheep	683
Gholamali Kojouri ¹ , Nazanin Gharib ²	683
Epidemiological Status and Surveillance of Zoonotic Parasitic Diseases in Iran	688
Mohammad Zeinali ¹	688
The Impact of <i>Toxoplasma gondii</i> on Reproductive Disorders in Ruminants	695
Fazel Sadeghian ¹ , Arash Kheradmand ² , Roohollah Dehghani Tafti ²	695
Food-borne Zoonotic Parasites: Control and Management Methods and role of Veterinary Medicine	703
Ata Kaboudari ¹ , Abbas Hashemi ²	703
Role of <i>Trichomonas foetus</i> Infection in the Pathogenesis of Repeat Breeding Syndrome in Cattle: A Review	710
Fazel Sadeghian ¹ , Arash Kheradmand ² , Roohollah Dehghani Tafti ²	710
Molecular Detection of <i>Toxoplasma gondii</i> in Domestic Pigeons (<i>Columba livia domestica</i>) and Assessment of Its Zoonotic Potential in Urban Areas of Lorestan Province	718
Homayun Rajabipour ¹ , Majid Taati ²	718
Toxoplasmosis in Iranian Dogs: A Review of Prevalence and Risk Factors	723
Abolfazl Hajibemani ¹ , Ali Mosayyebzadeh ² , AmirMohammad NaghsheJavaheri ³	723
Herbal Medicines and Nanotechnology-Based Drug Delivery in the Treatment of <i>Leishmania</i> Infections: A Focus on Iran	728
Azin Salehe ¹	728



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



<i>Tritrichomonas foetus</i> in Iran and Nearby Countries: Absence or Not Reported	734
Sattar Zolfaghari Daghighan ¹ , Seyedeh Yasaman Ghanoon ¹ , Elahe Ebrahimzadeh ²	734
The Public Health Significance of the Pigeon Tick, <i>Argas reflexus</i> : A Comprehensive Review.....	741
Mohammad Sajed Sarvari ¹ , Ali Moshaverinia ¹	741
Advances in <i>Toxoplasma gondii</i> Vaccines for Ovine Health	746
Seyedeh Yasaman Ghanoon ¹ , Sattar Zolfaghari Daghighan ¹ , Elahe Ebrahimzadeh ²	746
The Role of the Gut Microbiome Ecosystem in Susceptibility to <i>Cryptosporidium parvum</i> Infection in Cattle and Humans	753
Morteza Rajabi ¹ , Hossein Nouri Avaljahan ² , Zahra Dolati ² , Narges Safari ² , Amirreza Khoda Karami ²	753
<i>Trichuris suis</i> Ova Therapy in Inflammatory Bowel Disease: Immunological Mechanisms, Clinical Efficacy, and Safety Considerations	758
Ali Sardarzadeh Majd ¹ , Mostafa Kami ^{2, 3} , Najva Zivdari ⁴ , Ali Abbasi ³ , Mahdi Fakhar ^{5, 6, 7}	758
A Review of the Antiparasitic Effects of <i>Mentha longifolia</i> Extracts on Arthropod Parasites: An Analysis of Experimental and Clinical Studies.....	763
Amirabbas Motaharnezhad ¹ , Ali Moshaverinia ²	763
Control of Parasitic Diseases in the Iranian Horse Population	768
Mehrnaz Gorgani ¹ , Gholamreza Mohammadi ¹	768
Microscopic Investigation of Soil Contamination of Public Places with <i>Toxocara</i> Species Eggs in Semnan Province	774
Ali Ali-Mohammadi ¹ , Mohammadreza Salimi-Bejestani ²	774
The Application of Artificial Intelligence in Diagnosing Arthropod Vectors of Parasitic Diseases: Toward Region-Specific Preventive Strategies in Veterinary Medicine	782
Mohammad Mahdi Darvishi ¹	782
Clinical Impact, Epidemiology, and Prevention of Major Zoonotic Canine Parasites: <i>Dirofilaria immitis</i> and <i>Toxocara canis</i>	788
Ali Abbasi ¹ , Farnaz Seilanian Toosi ¹ , Mostafa Kami ^{1,2} , Najva Zivdari ³ , Emad Godazi Langeroudi ¹ , Mahdi Fakhar ^{4,5,6}	788
Cytokine Signaling in Host Defense Against Toxoplasmosis and Leishmaniasis: Focus on IFN- γ , IL-12, and IL-10.....	794
Ali Abbasi ¹ , Mostafa Kami ^{1, 2} , Emad Godazi Langeroudi ¹ , Najva Zivdari ³ , Mahdi Fakhar ^{4, 5, 6}	794
Presentation of an Uncommon Infestation of Toxoplasmosis: Pleural Effusion in a Domestic Cat... 801	
Golnaz Babaki ¹ , Soroush Ghadaksaz ² , Seyed Mahdi Nassiri ³	801
Report of Poultry Cyst Mite (<i>Laminosioptes cysticola</i>) Contamination of Carcasses of Broiler Chickens in Poultry Slaughterhouses in Qazvin Province in 2013 and 2014	803



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Shahabaldin Sohraby ¹ , Vahid Ebrahimnejadhamidi ² , Hamid Moradi ³ , Saiedali Mirmoeini ⁴ , Amirhosin Fadaee ⁵	803
<i>Hypodermosis</i> in Livestock from Dasht-e Bare and Ziaran Slaughterhouses in Qazvin in 1402 and 1403	805
Shahabaldin Sohraby ¹ , Mohsen Imandar ² , Amirhosin Fadaee ³ , Sajad Godsi ⁴	805
Determining the Level of <i>Linguatula serrata</i> Infestation in Livestock Animals at the Dasht Bareh Slaughterhouse in Qazvin	806
Shahabaldin Sohraby ¹ , Mohsen Imandar ² , Amirhosin Fadaee ³ , Sajad Godsi ⁴	806
The Role of Helminth–Microbiota Interactions in Modulating Host Immunity and Disease Outcomes	808
Mohadese Amiri ¹ , Hamidreza Farzin ² , Majid Jamshidian-Mojaver ³	808
Prevalence of Ectoparasites in Ornamental Birds in Tehran Province.....	810
Jabber davoodi ¹ , Mohammad Reza Maadina ² , Reza Norian ³	810
Chewing Lice Infesting the Common Buzzard (<i>Buteo buteo</i>) and Eastern Imperial Eagle (<i>Aquila heliaca</i>) in the West of Iran	811
Maryam Nazarbeigy ¹ , Mehdi Rashnavadi ¹ , Sajad Mami ¹	811
Investigation of the Anti-Leishmanial Effects of Essential Oils from <i>Zataria multiflora</i> and <i>Thymus vulgaris</i> Against <i>Leishmania major</i> : A Detailed Analysis of Phytochemical Composition and Therapeutic Efficacy	812
Kambiz Karimi ¹ , Mahmoodreza Moein ² , Qasem Asgari ¹ , Mohammad Hossein Motazedian ¹	812
Recumbency and Anemia associated with Severe <i>Linognathus vituli</i> Infestation in a Calf in East Azerbaijan Province, Iran: A Case Report	813
Yaghoub Firouzivand ¹	813
Morphomolecular Characterization of <i>Babesia</i> spp. in Camels in Southeastern Iran	815
Reza Shafiei ¹ , Hadi Mirahmadi ²	815
Diversity and Prevalence of Parasites in Domestic Pigeons in North-west of Iran: Insights and Implications.....	816
Atousa Mohseni ¹ , Mansour Ebrahimi ² , Emad Changizi ³	816
The Parasitic Profile of Indigenous Poultry: Prevalence Rates and Species Diversity in West-Azerbaijan	817
Atousa Mohseni ¹ , Emad Changizi ² , Mansour Ebrahimi ³	817
Prevalence of <i>Theileria equi</i> Infection in Piranshahr in 2024 Using Microscopy and PCR Approaches	818
Atousa Mohseni ¹ , Mansour Ebrahimi ² , Emad Changizi ³	818
Canine Anaplasmosis in a Ghahderijani Dog (Shahrekord, Iran): A Case Report	819



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Soroush Ghasemi ¹ , Abbas Ghorbani ¹ , Ali Maddahi Nejad ¹ , Mohammad Ali Mohammadi ¹	819
<i>Trypanosoma evansi</i> Infection in Camels Slaughtered in Zahedan, in the Summer of 2023.....	820
Seyed Reza Hosseini ¹ , Saeed Dadkhah Tehrani ¹ , Ali Maddahi Nejad ² , Mohadeseh Soltanian Dehkordi ³	820
Drug Resistance and Novel Therapeutic Strategies in Babesiosis: An Integrated Review of Epidemiological, Clinical, and Molecular Data.....	822
Siamak Kakehkhani ¹ , Erfan Eftekhari ² , Negin Shahri ²	822
<i>Leishmania</i> and the Challenges of Skin Repair: The Role of the Immune Response.....	824
Yaser Pirali ¹ , Moosa Javdani ² , Shayan Akhavan ² , Moein Mirzajani ²	824
Acaricide Resistance in Biological Tests and Molecular Mechanisms	825
Amir Arshia Lotfi ¹ , Zahra Jafari-Ardakan ² , Amir Mahdi Amjadi ³ , Mohammad Alinezhad-Taheri ³ , Sedigheh Nabian ⁴	825
Case report: Simultaneous Outbreak of <i>Microsporum canis</i> and <i>Ixodes ricinus</i> Infections in a Persian Chinchilla Cat in Tehran, Iran.....	827
Amir Arshia Lotfi ¹ , Zahra Jafari-Ardakan ² , Sedigheh Nabian ³	827
Role of Probiotics in the Prevention and Reduction of Coccidiosis Effects in Poultry	828
Hamidreza Farzin ¹ , Mohadesse Amiri ² , Majid Jamshidian-Mojaver ³	828
Serosurvey of <i>Neospora caninum</i> and <i>Toxoplasma gondii</i> in Stray Dogs of Fars Province, Southern Iran: Implications for Epidemiology and Control.....	830
Bahador Shahriari ^{1,2} , Negin Abbasi ³ , Niksa Adibso ³ , Mostafa Omidian ¹ , Qasem Asgari ¹ , Kourosh Azizi ⁴ ..	830
Evaluation of the Effect of 7- <i>alpha</i> -Acetoxyroyleanone, an Abietane Diterpenoid Derivative, on <i>Leishmania Major</i> in <i>In Vitro</i> Conditions	832
Kambiz Karimi ¹ , Asma Mousivand ² , Erfan Ahmadi ³ , Gholamreza Hatam ¹	832
The Effect of Climate Conditions on the Distribution Pattern of Sheep Gastrointestinal Nematodiasis in Three Different Climate Zone of Iran Based on Geographical Information System (GIS)	833
Mohammad Shadman ¹ , Behnam meshgi ²	833
Molecular Identification of Medicinal Leeches and Assessment of Microbial Contamination in Mashhad.....	835
Mohsen Najjari ¹ , Parisa Eysi ² , Hossien Zarrinfar ³ , Hadi Farsiani ⁴ , Arash Pourdard ⁵ , Rahmat Solgi ⁶	835
Renal Lesions Associated with <i>Theileria annulata</i> in a Cow from Northwest Iran	837
Melika Hasani ¹ , Bijan Esmaeilnejad ² , Seyede Soraya Mahmoudi ² , Asghar Afshari ³	837
Raman Spectroscopy and Confocal Imaging for the Identification of <i>Parascaris</i> Eggs	839
Ehsan Rakhshandehroo ¹ , Ali Owliaee ¹ , Tina Yaghoobpour ²	839
Case Report: Identification of <i>Rhipicephalus sanguineus</i> Ticks on a Domestic Rabbit (Black and White Mini Lop)	840
Amir Arshia Lotfi ¹ , Niayesh Mohammadi ¹ , Sedigheh Nabiyan ² , Amir Rostami ³	840



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



A Review of <i>Neospora caninum</i> and Current Therapeutic Approaches in Cattle.....	842
Soroush Heidari ¹ , Mohammad Taha Heidari ² , Ali Maddahi Nejad ³	842
An Investigation on the Infection with <i>Hepatozoon</i> sp. in Stray Dogs of Shiraz City and Its Suburbs	843
Tina Yaghoobpour ¹ , Hassan Sharifiyazdi ¹ , Ehsan Rakhshandehroo ² , Mahsa Rouhana ¹	843
Application of Artificial Intelligence in Diagnosis and Control of Parasitic Zoonoses in Iran	844
Reza Soltani ¹ , Bahareh Meftahi ^{2,3}	844
Emerging Trends in Tick-Borne Parasitic Diseases of Iranian Livestock.....	846
Reza Soltani ¹ , Bahareh Meftahi ^{2,3}	846
Morphological Characterization and Complete Sequence Determination of the ITS2 Region of <i>Ostertagia occidantalis</i> in Sheep and Goat Isolates in Iran.....	847
Meisam Sabour-Darbandi ¹ , Behnam Meshgi ² , Fateme Jalousian ²	847
The Survey of Morphological Characterization and Determination Complete Sequence of ITS2 Region of <i>Ostertagia (Teladorsagia) circumcincta</i> in Goat Isolate in Iran.....	848
Meisam Sabour darbandi ¹ , Behnam Meshgi ² , Fateme Jalousian ²	848
Infection of a Persian Cat with <i>Otodectes cynotis</i> Mite in Khorramabad.....	849
Amir Hossein Ghiasvand ¹ , Mobina Torabi Feijani ¹ , Mohammadhossein Abbasi ¹ , Shahriar Yavari ¹ , Hassan Nayebzadeh ¹	849
Effects In Vitro and In Vivo Antihydatid Activity of a Nano Emulsion of Zataria Multiflora Essential Oil on the Hydatid Cysts in Laboratory Mice.....	850
Meisam Sabour Darbandi ¹ , Hassan Borji ² , Mohammad Moazeni ¹	850
Study of <i>Leishmania</i> Species of Human Cutaneous Leishmaniasis in Fasa City, Southeast Iran (2022-2024).....	851
Shamsi Noorpisheh Ghadimi ¹ , Shiva Kazemi ²	851
Investigation of the Anti-Leishmanial Potential of <i>Lapachol</i> on Promastigote Forms of <i>Leishmania major</i> : An In Vitro Study	852
Kambiz Karimi ¹ , Erfan Ahmadi ² , Gholamreza Hatam ¹	852
Molecular Identification of <i>Anaplasma phagocytophilum</i> in Sheep in East Azarbaijan Province	853
Ahmad Nematollahi ¹ , Abbas Imani ¹ , Behzad Ghorbanzadeh ¹ , Elahe Jahanifard ¹	853
Microscopic Investigation of Haemosporidian Parasites in Raptors from Kerman Province, Iran	854
Hemad Shafiei ¹ , Mohammadjavad Jahedi ² , Mohammad Zamani Ahmadm Mahmudi ¹	854
Scaly Face Disease in Budgerigars: Clinical and Microscopic Confirmation of <i>Knemidocoptes pilae</i> Infestation	856
Mohammadjavad Jahedi ¹ , Hemad Shafiei ²	856



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



<i>Theileria</i> Infection in Camels in Kashan, Central Iran	858
Zainab Sadeghi-Dehkordi ¹ , Somayeh Moradi ¹ , Alireza Sazmand ¹	858
A Report on the Ectopic Presence of a Hookworm Belonging to the Family Ancylostomatidae in the Urinary Bladder of a Male Domestic Rabbit (<i>Oryctolagus cuniculus</i>)	859
Sadegh Motamedi ¹ , Saeed Dadkhah Tehrani ² , Parinaz Heydari ³	859
Hydatid Cyst, <i>Dicrocoelium dendriticum</i> , and <i>Fasciola</i> spp. Infections of Slaughtered Animals in Hamedan, Iran (2024)	861
Zainab Sadeghi Dehkordi ¹ , Khadigeh Rahmati ¹	861
The First Report of <i>Setaria labiatopapillosa</i> Isolated from the Abdominal Cavity of a Goat in Ahvaz, Iran	862
Alireza Alborzi ¹ , Saleh Esmailzadeh ² , Afsaneh Nazarpour Kalaei ¹ , Boshra Khajehian ³ , Hossein Hosseini ³ , Seyedeh Zahra Bayegan ³	862
Detection and Phylogenetic Analysis of LRV2 in <i>Leishmania</i> Isolates from Qom Province, Iran.....	863
Seyedeh Tara Mosavi Zadeh ^{1,2} , Elham Gholami ² , Negar Seyed ² , Shima Hadifar ² , Homa Hajjaran ³ , Sima Rafati ² , Tahereh Taheri ²	863
BCG Training Effects on Mice Peritoneal Macrophages Infection Caused by <i>Leishmania major</i> and <i>Leishmania tropica</i>	865
Negin Elahirad ¹ , Zohreh Askarypour ¹ , Mahmoud Nateghi-Rostami ¹	865
Nematode Infection of Terrestrial Slug <i>Drusia ibera</i> (Eichwald, 1841) from North of Iran	866
Sepideh Safarzadeh ¹ , Mousa Tavassoli ¹ , Ebrahim Ghale Kafai ¹ , Laya Shamsi ²	866
Case Report of <i>Cotugnia mohekarii</i> Infection in a Laughing Dove (<i>Spilopelia senegalensis</i>) from Ahvaz, Iran.....	867
Alireza Alborzi ¹ , Afsaneh Nazarpour Kalaei ¹	867
Prevalence of Metacestode Infections in Afshari Sheep Slaughtered in Bandar Abbas	868
Mohammad Mandegari ¹ , Mohammad Mazrouei Sebdani ¹ , Amin Ahmadi ^{2,3} , Zahra Nasr Esfahani ¹ , Soheila Bakhshayesh ¹	868
Evaluation of Ivermectin Efficacy Against Gastrointestinal Nematodes in Equines	869
Negar Askari ¹ , Mohammad Rahim Haji Hajikolaei ¹ , Alireza Alborzi ² , Afsaneh Nazarpour Kalaei ²	869
Investigation of Nematode Infection and Radula Structure in <i>Limacus flavus</i> (Linnaeus, 1758).....	870
Ebrahim Ghale Kafai ¹ , Mousa Tavassoli ¹ , Sepideh Safarzadeh ¹ , Laya Shamsi ²	870
Allergenic Effects of <i>Echinococcus granulosus</i>	871
Hadi Ebrahimnejad ¹ , Zahra Moayyed ²	871
Immunoenhancing Effects of Vitamin D3 on <i>Leishmania tropica</i> -Infected Macrophages.....	872
Razie Zeinali ¹ , Ali Afgar ² , Iraj Sharifi ³ , Zahra Babaei ³ , Amirhosein abbasi ⁴	872
Evaluation of Golden Eagle Lice Infestation in Northern Iran	873



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Seyedeh Mohanna Zarei ¹ , Behrad Asghari ¹ , Sara Sadat Pourmirafzali Firouzabadi ¹ , Maryam Hosseinpour Mohammadabadi ¹ , Seyed Javad Nourbakhsh Lamouki ¹	873
Identification of Gastrointestinal Parasites of Native Poultry in Mazandaran Province.....	874
Nasrollah Vahedi Noori ¹ , Javad Abbasi Harazi ² , Farnaz Seilanian Toosi ³ ,Mahmod Reza Rezaie Erami ⁴	874
Evaluation of an Herbal Anticoccidial Compound in Experimental Coccidiosis in Broiler Chickens.....	875
Negar Najafifard ¹ , Fatemeh Arabkhazaeli ² , Seyed Ahmad Madani ³ , Sarvenaz Azadnia ¹ , Fatemeh Babaei ⁴ , Negin Mahdavi Jafari ⁵ , Mohammad Saberi ⁶	875
A Report on Parasitic Contamination of Raw Edible Vegetables in Mazandaran Province	877
Nasrollah Vahedi Noori ¹ , Farnaz Seilanian Toosi ²	877
The Importance of Hard Ticks in Contamination and Transmission of Protozoan Pathogens in Domestic Livestock in Mazandaran Province	878
Nasrollah Vahedi Noori ¹ , Sayed Hamid Aghajanzadeh ¹ , Farnaz Seilanian Toosi ²	878
Occurrence of Cutaneous Leishmaniasis in an 8-Year-Old Male Poodle Dog: A Case Report	879
Behrad Asghari ¹ , Seyed Mohammad Hosseini ¹ , Amir Hossein Moshrefi ¹ , Samin Katuli ¹	879
An Outbreak of Acute Theileriosis in a Sheep Flock in Kerman, Iran	880
Mohammad Mazrouei Sebdani ¹ , Soheila Bakhshayesh ¹ , Zahra Nasre Esfahani ¹	880
Epigenetic Orchestration by MicroRNAs in <i>Toxocara canis</i> : Implications for Parasite Development, Host Immune Evasion, and Novel Diagnostic and Vaccination Strategies	881
Fatemeh Ramzi ¹ , Sima RoshanFar ² , Mohadeseh Ramzi ³ , Golnaz Mobayen ⁴ , Masoud Lahouty ⁵	881
NETosis-Mediated Immune Escape in Fascioliasis: A Narrative Review on Diagnostic Advances and Immunotherapeutic Prospects	883
Mohadeseh Ramzi ¹ , Sima RoshanFar ² , Golnaz Mobayen ³ , Fatemeh Ramzi ⁴ , Masoud Lahouty ⁵	883
Coincidence of <i>Babesia canis</i> and <i>Candida</i> spp. Infections in a German Shepherd Dog	885
Dorin Motamedi ¹ , Somayeh Bahrami ¹ , Sajjad Alishiri ¹ , Reza Naghi ha Najaf Abadi ² , Bahman Mosallanejad ³	885
Study of the Prevalence of Gastrointestinal Parasitic Worms in Domestic Dogs in Tabriz City	887
Elyar Azimi Zang Abad ¹ , Saba Sabbaghkar ² ,Mahdi Farshbaf Nobarian ³ , Salar Sartipi ⁴	887
Epidemiology and Prevalence of <i>Neospora caninum</i> Infection and Its Impact on Abortion in Cattle in Hormozgan Province, Iran	889
Seyyed Amirali Foroughi ¹ , Fateme Azizipour ²	889
Seroprevalence of Cystic Echinococcosis among the Population with Substance Use Disorders in Fars Province, Southern Iran: A Cross-sectional Study	890
Sara Shahriarirad ¹ , Seyed Abolfazl Soltani ¹ , Mina Zarrabi ¹ , Sahel Rohampour ¹ , Ghazal Shayan ¹ , Mostafa Omidian ¹ , Jamal Sarvari ² , Bahador Shahriari ^{3,4}	890



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



The Role of Artificial Intelligence in Revolutionizing Parasitology Education in Veterinary Medicine	892
Kimia Taheri Kartiji ¹ , Maedeh sadat Moghaddasi sani ¹ , Mahsa Shahbakhsh ¹	892
Molecular Detection of <i>Babesia</i> and <i>Theileria</i> Species in Sheep and Goats with a Recent Abortion Aistory in East Azerbaijan Province, Northwest Iran	894
Parisa Shahbazi ¹ , Reza Ayoubi ¹ , Ahmad Nematollahi ¹ , Monireh Khordadmehr ¹ , Soheila Aghaalizadeh ¹ , Maedeh Nazadi ¹ , Alireza Zandna ¹	894
Application of Artificial Intelligence in Diagnosis and Epidemiological Surveillance of <i>Toxoplasma gondii</i> : A Narrative Review	895
Farnaz Seilanian Toosi ¹ , Nasrollah Vahedi Noori ²	895
A Comprehensive Review of YOLOv9 and Deep Learning Approaches for Automated Malaria Parasite Detection in Blood Smears with Rouleaux Formation	897
Ali Motamedi ¹	897
The Impact of Latent Toxoplasmosis on Human Mental Health and Neurological Disorders: A Review	899
Maedeh Bakhshandeh ¹ , Simin Jalili Abjazi ¹ , Mahsa Khademi Kalantari ¹ , Somayeh Bahrami ¹	899
Comparison of Diagnostic Accuracy between Wet Mount and PCR Methods for Trichomoniasis Detection in Pigeons from Tehran	901
Ali Naser ¹ , Zahra Davanloo ¹ , Seyedeh Ommolbanin Ghasemian ² , Leyla Derakhshan ³ , Behrad Asghari ¹	901
Assessment of <i>Cytauxzoon felis</i> Prevalence and Its Impact on Oxidative Stress and Antioxidant Defense in Feline Populations	903
Ali Naser ¹ , Behrad Asghari ¹ , Sedighe Sharifpour ¹ , Seyedeh Ommolbanin Ghasemian ²	903
Prevalence of Hemoparasites and Ectoparasites of Horses in Gonbad-e Kavus, Iran: A Cross-Sectional Study	904
Ali Naser ¹ , Sedighe Sharifpour ¹ , Seyedeh Ommolbanin Ghasemian ² , Leyla Derakhshan ³	904
Recombinant expression of a <i>Wolbachia</i> WSP from <i>Phlebotomus sergenti</i> (Diptera: Psychodidae).	905
Fateme Bamshad ^{1,2} , Naseh Maleki-Ravasan ¹ , Jalal Babaie ¹ , Fateh Karimian ¹ , Asal Katebi ³ , Soheila Ajdary ³	905
Application of Artificial Intelligence in Diagnosis of Protozoan Parasites in Veterinary Medicine: A Review of Innovative Technologies and Global Studies	907
Mohammad Mahdi Darvishi ¹ , Parinaz Hanafi ² , Parnia Shams ² , Mohammad Yazdan Ashouri ² , Behrad Asghari ³	907
Molecular Investigation of <i>Cytauxzoon</i> Infection in Owned and Stray Cats in Tehran City	909
Hadis Boujarzadeh ¹ , Hamidreza Shokrani ² , Alireza Rocky ³ , Ali Forouharmehr ⁴	909
The Prevalence of Avian Trichomoniasis in Domestic Pigeons (<i>Columba livia domestica</i>) in Khorramabad	910



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Mazyar Rashidi ¹ , Hamidreza Shokrani ²	910
Neosporosis in Dogs from Kermanshah, Iran	911
Mohammad Hossein Abbasi ¹ , Hamidreza Shokrani ² , Saman Salmani ³ , Bahman Mosallanejad ⁴	911
The Prevalence Rate of Infection to <i>Toxoplasma gondii</i> in Domestic and Industrial Breeding Poultry in Isfahan City, Iran, 2020 by Modified Agglutination Test	912
Nader Pestehchian ^{1,2} , Mahnaz Sami ¹ , Hossain Yousofi-Darani ¹ , Hosseinali Yousefi ¹ , Reza Kalantari ¹	912
A Review of Drug Resistance Mechanisms in <i>Acanthamoeba</i> : Challenges and Opportunities for Veterinary Therapeutics	913
Maedeh Bakhshandeh ¹ , Simin Jalili Abjazi ¹ , Mahsa Khademi Kalantari ¹ , Somayeh Bahrami ¹	913
Pigeon Fly (<i>Pseudolynchia canariensis</i>) Infestation in Urban Pigeons(<i>Columba livia</i>): A Systemic Review	915
Ali Safari ¹ , Saman Moradian ²	915
Prevalence of <i>Cryptosporidium</i> in Free-range Cattle in the Eastern Part of Golestan Province.....	916
Mohades Chitband ¹ , Elahe Ebrahimzadeh ¹	916
Molecular Detection of Protozoan Parasites in the Semen of Dogs in Iran	917
Pouria Rafiei ¹ , Baharak Akhtardanesh ² , Seyed Morteza Aghamiri ² , Seyed-Mahshid Mostafavi ² , Alireza Sazmand ¹	917
Recent Advances in Multivalent and Targeted Vaccine Design Against Babesiosis: From Antigen Selection to Immunological Efficacy.....	918
Zahra gahvechi amiri ¹ , Mohadeseh Ramzi ² , Fatemeh ramzi ²	918
Biological Control of Nematodes: A Review of <i>Bacillus thuringiensis</i> and Its Nematicidal Compounds	920
Azar Motamedi Boroojeni ¹ , Kazem Hajati Bardmili ¹	920
Molecular Detection of Protozoan Parasites in Bottled Drinking Water from Different Cities of Iran	921
Paniz Khodakarami ¹ , Zahra Qeysari ¹ , Zahra Kharaghani ¹ , Pouria Rafiei ¹ , Somayeh Bahrami ² , Alireza Sazmand ¹	921
Chewing Lice in a Common Myna (<i>Acridotheres tristis</i>): A Case Report.....	922
Shirin Aliakbari ¹ , Fatemeh Arabkhazaeli ^{2,3} , Mohammad Bagher Ahoo ⁴ , Mohammad Reza Haddadmarandi ⁵	922
Helminthic infection in a Rhesus Macaque (<i>Macaca mulatta</i>): A Case Report.....	923
Mohammad Hossein Asadi ¹ , Tannaz Eghdami ¹ , Seyed Kian Mohseni Mofidi ² , Alireza Ghorbani ²	923
Helminth Infections in a Deer: A Case Report.....	924
Tannaz Eghdami ¹ , Mohammad Hossein Asadi ¹ , Seyed Kian Mohseni Mofidi ² , Alireza Ghorbani ²	924



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Prevalence of Canine Heartworm (<i>Dirofilaria immitis</i>) in Golestan Province Shelters: A Zoonotic Risk	925
Arvin Azadeh ¹ , Ali Safari ² , Saman Moradian ³	925
Application of Artificial Intelligence in Leishmaniasis Diagnosis	927
Mohammadreza Motezarre ¹ , Yasaman Hosseini ¹ , Anahita Gharagozlou ¹ , Armina Jalali ¹ , Sedigheh Nabian ²	927
Visceral leishmaniosis in dogs from Hamedan, Iran.....	929
Alireza Sazmand ¹ , Georgiana Deak ² , Ali Kalantari-Hessari ³ , Pouria Rafiei ¹ , Navid Etemadi ⁴ , Hessam Hajilou ⁴ , Mina Malekmohammadi ⁵ , Farhad Mahra ⁶ , Leili Moradi ¹ , Monireh Khordadmehr ⁷ , Seyedmousa Motavallihaghi ⁸ , Ali Fattahi-Bafghi ⁹ , Mariaelisa Carbonara ¹⁰ , Stephanie Oren ¹¹ , Domenico Otranto ^{10,12}	929
A Cross-sectional Study of Helminth Parasites in Stray Dogs in Iran Using a One Health Approach.....	931
Mohammad Bagher Ahoo ^{1,2} , Fateme Jalousian ¹ , Seyed Hossein Hosseini ^{1,2}	931
Comparative Analysis of Native and Denaturing Conditions for the Extraction and Purification of Recombinant Proteins: Focus on <i>Toxocara canis</i> Recombinant C-Type Lectin.....	933
Narges Amininia ¹ , Parviz Shayan ¹ , Seyed Hossein Hosseini ^{1,2} , Fateme Jalousian ¹	933
Antitrematodal Effects of <i>Allium sativum</i> (Garlic) Extract Against <i>Fasciola</i> spp.	935
Yasaman Hosseini ¹ , Mohammadreza Motezarre ¹ , Anahita Gharagozlou ¹ , Armina Jalali ¹ , Behnam Meshgi ²	935
Human Dirofilariasis Due to <i>Dirofilaria</i> spp. in Iran.....	937
Armina Jalali ¹ , Yasaman Hosseini ¹ , Mohammadreza Motezarre ¹ , Anahita Gharagozlou ¹ , Behnam Meshgi ²	937
Emerging Parasitic Zoonoses in Iran: An Overlooked One Health Challenge	939
Fatemeh Sayareh ¹ , Hesameddin Akbarein ²	939
Molecular Detection of Tick-Borne Parasites in Ardabil Province.....	940
Zahra Heidari ¹ , Faraz Malek Bagali ²	940
Logistic Regression Modeling for the Prediction of <i>Leishmania major</i> PTR1 Inhibition.....	941
Yasamin Raziee ^{1,2} , Sima Rafati ² , Negar Seyed ²	941
Investigation of Drug Resistance in Gastrointestinal Parasites of Ruminant Livestock.....	943
Setayesh Sadat Naseri ¹ , Mobina Sadat Naseri ²	943
Evaluating Serum Carotenoid in Poultry Experimental Coccidiosis	944
Ali Naseri ¹ , Fatemeh Arabkhazaeli ^{2,3} , Seyed Ahmad Madani ⁴ , Negar Najafifard ¹ , Sarvenaz Azadnia ¹	944
Bovine Theileriosis as a Model for Studying Human Leukemia	946
Sina Nazifi ¹ , Mohammadreza Aslani ¹ , Nazanin Gharib ¹	946
Emerging Biotherapeutic Strategies for Wound Management in Companion Animals: A Review of Efficacy and Practical Considerations	947



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Sogand Fallahfar ¹ , Mahdi Shojae ¹ , Mahsa Shahbakhsh ²	947
Molecular Investigation and Genotyping of <i>Cryptosporidium</i> in Fecal Samples of Parrots in Tehran City.....	949
Alireza Rastegar Moghaddam ¹ , Saloomesh Shirali ^{1,2} , Kaveh Parvandar ¹ , Melika Mohammadi ¹	949
Investigating the Prevalence of <i>Hepatozoon canis</i> in Dogs of Gilan Province by PCR.....	950
Parvin Mojerloo ¹ , Saloomesh Shirali ^{1,2} , Hooman Rounaghi ¹ , Melika Mohammadi ¹	950
Survey of Gastrointestinal Protozoa in Dogs in the Capital of Mazandaran Province	951
Case Report of Infestation of a Steppe Eagle (<i>Aquila sp.</i>) by Three Genera of Chewing Lice in Ahvaz, Iran	952
Maryam Abbaszadeh ¹ , Somayeh Bahrami ¹ , Mansour Mayahi ²	952
Sensitive Detection of Specific Cell-free DNA in Serum Samples of Sheep Infected with Hydatid Cyst	954
Mahboubeh Hadipour ¹ , Hossein Yousofi Darani ¹	954
Investigation of Bacterial and Parasitic Agents Associated with Neonatal Calf Diarrhea in Fars Province	955
Atefeh Shirazi ¹ , Yahya Tahamtan ¹ , Masoumeh Hayati ¹ , Hajar Molae ¹ , Sona Amiri ¹	955
Case Report of Parasitic Infection in a Stray Cat from Ahvaz, Iran	956
Alireza Alborzi ¹ , Saleh Esmailzadeh ² , Sara Larki ¹ , Afsaneh Nazarpour Kalaei ¹ , Maryam Abbaszadeh ¹ , Fatemeh Madankan ¹	956
Evaluation of Published Primer Sets for Identification of <i>Cryptosporidium</i> and <i>Giardia</i> spp. by Nested PCR.....	957
Masoumeh Hayati ¹ , Yahya Tahamtan ¹ , Atefeh Shirazi ¹ , Ali Shirazinezhad ¹	957
Species Distribution and Seasonal Dynamics of Tick Infestation in Dromedary Camels (<i>Camelus Dromedarius</i>) in Marand Suburbs	958
Farnaz Malekifard ¹ , Mousa Tavassoli ¹ , Adel Jafari ¹	958
Major Symbiotic Bacteria Associated with Nematodes.....	959
Mohadese Amiri ¹ , Majid Jamshidian-Mojaver ² , Hamidreza Farzin ³	959
Canine Diotrophymosis: A Case of Renal Parasitism in a Pitbull Dog	961
Javad Jafari ¹ , Mostafa Rahimaf ¹ , and Mohadeseh Hosseinzadeh ¹	961
The Transformative Impact of Artificial Intelligence on Diagnostic and Management Strategies in Veterinary Parasitology.....	962
Mahdi Shojae ¹ , Sogand fallahfar ¹ , Mahsa Shahbakhsh ²	962
The Role of Parasites in Modulating Intestinal Bacterial Populations.....	963
Anahita Gharagozlou ¹ , Armin Gharagozlou ² , Yasaman Hosseini ¹ , Armina Jalali ¹ , Mohammadreza Mottezarre ³ , Fatemeh Moradalian ¹ , Behnam Meshgi ⁴	963



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Study of the Prevalence of <i>Cryptosporidium</i> in Dogs in Southeastern Iran Using the Concentration Method	965
Mahdi Khoshshima Shahraki ¹ , Saeid Reza NourollehiFard ² , Mansour Dabirzadeh ¹	965
A Study on the Prevalence of Theileriosis Among Ruminants in Three Selected Regions of South Khorasan	966
Afsane Hoseini ¹ , Maryam Arbabi Dastgerd ¹ , Amir Nasiraei Tabas ¹ , Atiye Etebari ¹	966
Artificial Intelligence in the Diagnosis of <i>Babesia</i> : A Step Toward Smarter Veterinary Hemoparasitology	967
Amir Yosef Momenzadeh ¹ , Shayeste Noshadian ¹ , Saber Rahmati ²	967
Prevalence of Tick-Borne Haemoparasites in Clinically Suspected Cattle in Northern Iran.....	969
Saman Moradian ¹ , Ali Safari ²	969
Cerebral Hydatid Cyst in Humans from an Endemic Region in Northeastern Iran (Mashhad): A Case Report.....	971
Farnaz Seilanian Toosi ¹ , Sara Kazemi ¹ , Nasrollah Vahedi Noori ² , Seyyed Mohammad Aghapour ¹	971
Epidemiology of Toxocariasis by Concentration Method in Dogs of Southeast Iran.....	972
Mansour Dabirzadeh ¹ , Saeidreza NourollahiFard ² , Mansour Aminzadeh Gowhar ³ , Mahdi Khoshshima shahraki ⁴	972
Morphological Identification of Ticks on Afshari Sheep in Kerman City	974
Zanko Rahmani ¹ , Mohammad Mazrouei Sebdani ¹ , Amin Ahmadi ^{2,3} , Hossein Iravani ¹ , Amirhosein Shekari ¹	974
Detection of <i>Theileria</i> spp. and <i>Babesia ovis</i> in Sheep Using Molecular Methods in Taybad, Iran...	975
Erfan Sepahvand-Mohammadi ¹ , Seyed Omid Naghibi Torbat ² , Zahra Davoodian ³ , Seyed Mohamadreza Bolandi ⁴	975
Nasopharyngeal Myiasis in a Motor Vehicle Accident Victim: A Case Report from Shushtar, Khuzestan Province, Iran	977
Seyede Manizhe Heidar Nejadi ¹ , Pegah Golafshan ²	977
Emerging Recombinant Antigen Strategies and Immune Modulation for Effective Control of <i>Neospora caninum</i> : A Narrative Review	979
Maryam Hassan Nejad ¹ , Amirhossein Ramzi ² , Sima RoshanFar ¹ , Mohadeseh Ramzi ³	979
Increasing Drug Resistance among Zoonotic Protozoan Parasites: A Worldwide Review	981
Amir Hessam Khani Sarhoozaki ¹ Mahyar Yarahmadi Khorasani ² , Ali Fatahi ² , Mehran Arian Shokooh ³	981
Retrospective Review of Toxocara spp. Prevalence in Domestic Dogs and Cats: A Global and Local Perspective	982
Amir Hessam Khani Sarhoozaki ¹ , Ali Fatahi ² , Mahyar Yarahmadi Khorasani ² , Mehran Arian Shokooh ³	982



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Microbial Symbiosis Between <i>Trichomonas vaginalis</i> and HPV in Cervical Carcinogenesis: A Narrative Review of Pathological Interactions and Clinical Implications	983
Maryam Hassan Nejad ¹ , Sima RoshanFar ² , Golnaz mobayen ³	983
Overview of Parasitic Diseases in Ostriches.....	985
Mohammadjavad Aghasizadeh ¹ , Hamideh Zeynali ¹ , Mostafa Sadeghidokht ¹	985
Applications of Artificial Intelligence in the Diagnosis of Canine Babesiosis: A Mini Review.....	986
Sara Kazemi ¹ , Seyyed Mohammad Aghapour ¹ , Farnaz Seilanian Toosi ¹ , AmirMohammad Malekpour ¹	986
Reengineering Serodiagnosis of Strongyloidiasis: A Narrative Review of Recombinant and Chimeric Antigen Innovation	988
Fatemeh Ramzi ¹ , Amirhossein Ramzi ² , Zahra gahvechi amiri ³ , Sima RoshanFar ⁴ , maryam Hassan Nejad ⁴ , Mohadeseh Ramzi ¹	988
A Study on Gastrointestinal Parasites in Captive Reptiles from Tehran and Alborz Provinces.....	990
Mehrassa SheikhEsmaili ¹ , Fateme Jalousian ^{1,2} , Amir Rostami ¹	990
Medicinal Plants as a Source of Antiparasitics: A Review	991
Fatemeh Forouzanfar ¹	991
Ectoparasite Prevalence on Dogs in Western Iran: Epidemiological Patterns in Shepherd and Stray Populations.....	992
Alireza Ghorbani ¹ , Maryam Hataminejad ^{2,3} , Amirreza Ghorbani ⁴ , Erfan Darmanloo ⁵	992
Morphological Identification of <i>Rhipicephalus</i> spp. in Small Asian Mongooses (<i>Herpestes javanicus</i>) in Sistan and Baluchestan Province, Southeastern Iran	994
Seyede Mahshid Mostafavi ¹ , Mahdiye Mollashahi ² , Seyede Mahnoosh Mostafavi ³ , Kian Mosaali ¹	994
Evaluation of the Diversity of Freshwater Snail Species in Borujen City	996
Yaser Pirali ¹ , Hamidreza Yoonesi Borujeni ¹ , Behnam Meshgi ² , Fatemeh Nasiri Dashtaki ¹ , Seyed Reza Hosseini ¹	996
Diphyllobothriasis: A Zoonotic Infection Affecting Humans and Livestock and Its Role in Causing Anemia.....	998
Seyed Reza Hosseini ¹	998
Tick Infestation in Free-Ranging Tortoises (<i>Testudo graeca</i>) from Sirch and Lalehzar Regions of Kerman Province, Southeastern Iran	999
Seyede Mahshid Mostafavi ¹ , Kian Mosaali ¹ , Mahdiye Mollashahi ² , Seyede Mahnoosh Mostafavi ³	999
Investigation of Parasitic Diseases in Koi Fish (<i>Cyprinus carpio koi</i>) from an Aquaculture Farm in Rasht City, Gilan Province.....	1001
Fatemeh Madankan ¹ , Mohammad Hossein Razi Jalali ^{1,3} , Mehrzad Mesbah ^{2,3} , Hossein HamidiNejat ¹ , Mohammad Vali Mohammadpour Choshali ²	1001



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Emerging Role of Artificial Intelligence in Advancing Parasitology: Current Applications and Future Perspectives.....	1003
Mahsa Shahbakhsh ¹ , Maryam Shahbakhsh ²	1003
Mesenchymal Stem Cells and CAR-T Therapy for <i>Toxoplasma gondii</i> -Associated Miscarriage: A Cell-Based Approach for Maternal–Fetal Protection.....	1005
AmirReza Kanani ^{1,4} , Hossein Javid ^{1,2,3}	1005
The Link Between Parasitic Infections and Inflammatory Bowel Disease (IBD)	1007
Elahe Mousavi ¹ , Sanaz Dehbashi ^{2,3} , AmirReza Kanani ^{2,3}	1007
The Role of Artificial Intelligence and Novel Techniques in Overcoming Zoonotic Parasite Detection and Outbreak Management in Wildlife	1009
Zahra Nabizadeh ¹ , Leyli Shabani Vieh ² , Fatemeh Karimi Kesheh ³ , Mehdi Farjoud ⁴ , Edris Motamedi ⁴ , Sahar AriaeeNasab ⁵ , Mohamad Arad Zandieh ⁶	1009
The Impact of Parasites on Neurobiology and Mental Health (Review)	1011
Shiva Hafezi Ahmadi ^{1,2} , Sanaz Dehbashi ³	1011
Muelleriosis in a Traditional Sheep Flock: A Histopathological Case Report from Northwestern Iran	1013
Rastin Bakhtiari Lafmejani ¹ , Donya Pourbagher ¹ , Seyedeh Soraya Mahmoudi ²	1013
Application of Artificial Intelligence in Diagnosis and Epidemiological Control of <i>Neospora caninum</i> : A Narrative Review.....	1014
Farnaz Seilanian Toosi ¹ , Nasrollah Vahedi Noori ² , Sara Kazemi ¹ , Seyyed Mohammad Aghapour ¹	1014
Revolutionizing Parasitic Disease Diagnostics: The Rise of AI and Molecular Technologies in Human and Veterinary Health.....	1016
Mostafa Kami ^{1,2,3} , Ali abbasi ¹ , Najva Zivdari ⁴ , Mahdi Fakhar ^{5,6,7}	1016
Regulatory Immune Pathways Underlying the Persistence of <i>Echinococcus granulosus</i> : Mechanistic Insights from Experimental Models.....	1018
Hannaneh Mirzaei Rad ¹ , Amirhossein Gohari ² , Maryam Mirzaei ³	1018
Addressing Therapeutic Challenges in Giardiasis Management in a Household Samoyed.....	1020
Tina Daneshrad ¹ , Siamak Mashhadi Rafiee ²	1020
Parasitic Infections in Companion Animals: Implications for Lameness and Neuromuscular Disorders	1022
Leyli Shabani Vieh ¹ , Zahra Nabizadeh ² , Edris Motamedi ³ , Niknaz Rajaei ⁴ , Parsa Amoozadeh ¹ , Parham Rahmanian ⁵	1022
Faunistic Study of Ectoparasitic Arthropods in Dogs of Sanandaj County, Iran	1024
Zahra Ghashghaei ¹ , Ghazaaleh Adhami ² , Pouria Rafiei ³	1024



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



The Role of CO ₂ in Attracting Soft Ticks (Argasidae) and Its Practical Use in Poultry Management	1025
Shirin Aliakbari ¹ , Parviz Shayan ²	1025
Enhancing Anti-Acanthamoeba Potential of <i>Satureja khuzistanica</i> Essential Oil through Ultrasonic-Nanoemulsification Approach	1026
Somayeh Bahrami ¹ , Fatemeh Navidi ¹ , Maryam Ghaderi-Ghahfarokhi ² , Mehdi Zarei ²	1026
Systematic Review of the Effect of Prolonged In Vitro Passage on the Immunopathogenicity of <i>Theileria</i> spp.	1028
Mohaddeseh Karimi Kesheh ¹ , Parviz Shayan ² , Fatemeh Karimi Kesheh ³	1028
Survey of Gastrointestinal Helminth Fauna in Native Dogs in Eastern Gilan Province.....	1030
Elahe Noorollahi ¹ , Fatemeh zahra Gharib ² , Shohreh Alian ³	1030
Microsatellite Analysis as a Tool for Revealing the Genetic Diversity and Origin of <i>Leishmania tropica</i>	1031
Mobina Mohammadi ¹ , Parviz Shayan ¹	1031
Investigation on <i>Toxocara</i> spp. Eggs in Breeding Dogs in Tehran Province, Iran.....	1032
Ali Ahmadi ¹ , Fateme Jalousian ¹	1032
Acute Bilateral Hyphema and Sudden Blindness Associated with Ehrlichiosis in a German Shepherd Dog: A Case Report	1033
Reza Amjadi ¹ , Alireza Ghaffari Cherati ² , Parviz Noushivani ³	1033
Study of the Prevalence of <i>Taenia</i> spp. Isolated from Dogs in Southeastern Iran Using the Concentration Method	1034
Maziar Jajarmi ¹ , Saeidreza NourollahiFard ¹ , Mansour Dabirzadeh ² , Mahdi Khoshshima shahraki ^{1,2}	1034
A Study on the Frequency and Diversity of Ectoparasites in Stray Dogs in Shiraz	1036
Shadi Shahvand ¹ , Zahra Mohammadi ¹ , Mohammadsalar Tasdighi ¹ , Seyed Mostafa Razavi ¹	1036
Global Research Trends on Re-emerging Parasitic Diseases: A Bibliometric Analysis (2000-2025)	1037
Masoud Imani ¹ , Sina Etminan ²	1037
Revolutionizing Parasitic Disease Control: The Role of Artificial Intelligence in Diagnosis and Public Health.....	1039
Fatemeh Valikhani ¹ , Yaser Habibi ¹ , Mohammed Reza Anani ¹ , Mobina Rajabzadeh ¹ , Tohid Javaheri ²	1039
A Case Report of Severe <i>Hepatozoon canis</i> Infection in a Mixed-breed Puppy in Sari.....	1041
Reza Amjadi ¹ , Siavash Faramarz ² , Parviz Noushivani ³	1041
A Comparative Review of Amphotericin B, Meglumine Antimoniate (Glucantime), and Curcumin as Treatment Agents for Cutaneous Leishmaniasis Caused by <i>Leishmania major</i>	1042
Mobina Mohammadi ¹ , Parviz Shayan ¹	1042



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



A Survey of Gastrointestinal Parasite Infection in Horses and Donkeys in the Dargaz Area, Khorasan Razavi Province	1044
Mohammad Hosein Gholinezhadnokhandan ¹ , Abbas Jadidoleslami ² , Zahra Poorejbari ³ , Ali Parsafar ⁴ , Gholamreza Razmi ⁵	1044
Diversity and Prevalence of Cestode Infections in Three Lizard Species from the Arid Regions of Sistan and Nehbandan, Southeastern Iran.....	1045
Kian Mosaali ¹ , Mahdiye Mollashahi ² , Seyede Mahnoosh Mostafavi ³ , Seyede Mahshid Mostafavi ¹	1045
Tick Diversity and Distribution in Iranian Reptiles: A Narrative Review (1922–2025).....	1047
Mehrasa SheikhEsmaili ¹ , Parviz Shayan ²	1047
Possible Contribution of Migratory Birds to Dengue Virus Spread: A Narrative Review	1048
Mehrasa SheikhEsmaili ¹ , Parviz Shayan ²	1048
Survey of Filariasis in Shelter Dogs from Selected Cities of Khorasan Razavi Province, Iran.....	1050
Amin Bakhshani ¹ , Safa Moghadam ² , Neda Sasan Nejad ³ , Alireza Ghodrati ⁴ , Hassan Borji ⁵	1050
Dermatitis Caused by <i>Ornithonyssus bacoti</i> in Laboratory Rats	1052
Mohammad Farshad Golestanifar ¹ , Niknaz Rajaei ² , Mobina Ghorbaninasab ³ , Seddighe Nabian ⁴	1052
Explainable AI in Zoonotic Parasite Diagnosis: Enhancing Real-Time Clinical Trust and Transparency across One Health Systems	1054
AmirReza Kanani ^{1,4} , Sanaz Dehbashi ^{1,2,3}	1054
The Study of the Effect of Ethanolic <i>Citrullus colocynthis</i> Extract on <i>Leishmania major</i> In Vitro .	1056
Nadia Taiefi Nasrabadi ¹	1056
<i>Hepatozoon felis</i> Infection in a Cat Undergoing FIP Treatment: A Case Report	1057
Reza Amjadi ¹ , Parviz Noushervani ² , Reza Javadzadeh ³ , Alireza Ghaffari Cherati ⁴	1057
Detection of <i>Moniliformis moniliformis</i> in Long-eared Hedgehogs (<i>Hemiechinus auritus</i>) in Nehbandan in South Khorasan Iran	1059
Kian Mosaali ¹ , Mahdiye Mollashahi ² , Seyede Mahnoosh Mostafavi ³ , Seyede Mahshid Mostafavi ¹	1059
Prevalence and Genetic Diversity of <i>Blastocystis</i> sp. in Human and Animal Fecal Samples Identified by High-Resolution Melting Analysis.....	1061
Ghodratollah Salehi Sangani ¹ , Ahmad Hosseini-Safa ² , Bibi Razieh Hosseini Farash ¹ , Pooria Salehi Sangani ¹ , Mehdi Zarean ¹ , Elham Moghaddas ¹ , Saeid Zoghdar Moghadam ¹ , Lida Jarahi ³ , Ali Gholizadeh ⁴ , Fariba Berenji ¹	1061
A Survey of <i>Acarapis woodi</i> in Festaion in Apiaries in Iran.....	1063
Golnush Bolvardi ¹ , Sedigheh Nabian ^{1,2} , Alireza Arab ³ , Kamyar Ahmadi ³ , Abbas gerami Sadeghian ¹ , Ali Ahmadi ¹	1063
The First Report of Infestation of Small Asian Mongoose (<i>Herpestes javanicus auropunctatus</i>) with <i>Ctenocephalides canis</i> Flea in Iran	1064



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Saeed Dadkhah Tehrani ¹ , Seyed Reza Hosseini ¹ , Mehran Riahi ²	1064
Equine Leishmaniasis: Overview of Etiology, Clinical Manifestations, Diagnosis, and Management	1065
Mohammad Hekmatmansh ¹ , Mehdi Hekmatmanesh ² , Elahe Ghazi Shehni Zad ³ , Somayeh Bahrami ^{1,5} ...	1065
Scabies in Pet Rabbits in 2025: A Case Report.....	1067
Abbas Jadidoleslami ¹ , Fatemeh Jadidoleslami ² , Mostafa Imani ³ , Ali Moshaverinia ¹	1067
A Review on the Role of Climate Change on the Occurrence of Parasitic Diseases	1069
Ali Naseri ¹ , Mirabi.S ¹ , Akbarein.H ²	1069
The Application of Artificial Intelligence in the Prediction, Diagnosis and Treatment of Parasitic Diseases	1071
Mohammadsaeed Azamian ¹ , Hesameddin Akbarein ²	1071
The Necessity of Rational Prescribing of Anthelmintic and Antiprotozoal Drugs to Prevent Antimicrobial Resistance	1073
Mohammadsaeed Azamian ¹ , Hesameddin Akbarein ²	1073
Association Between Bovine Leukemia Virus Infection and Parasitic Susceptibility in Cattle: A Review	1075
Nikta Amiri ¹ , Mohammadreza Rezaeigolestani ²	1075
Host Spectrum and Impact of <i>Trypanosoma evansi</i> Infections Across Animal Species	1076
Elahe Ghazi Shehni Zad ¹ , Mohammad Yarmahmoudi ¹ , Mehdi Hekmatmanesh ² , Mohammad Hekmatmansh ³ , Somayeh Bahrami ^{3,5}	1076
Parasitology and Histopathology Investigation of Cryptosporidium in Industrial Poultry of Mashhad City.....	1078
Nima komeili ¹ , Elahe Ebrahimzadeh ¹ , Zahra Moosavi ¹	1078
Study of Helminth Parasites in the Digestive Tract of Donkeys in Farahan Villages of Markazi Province	1079
Abdolhossein Dalimi ¹ , Fatemeh Rezaee ¹ , Faezeh Kafi Mousavi ¹ , Majid Pirestani ¹	1079
Histopathologic Investigation of the Caffeic Acid Effects on the Liver and Kidney of Laboratory Mice Infected by Hydatid Cyst	1080
Mohammad Sajjad Ghorbani Parsa ¹ , Zahra Moosavi ¹ , Elahe Ebrahimzadeh	1080
Morphological Identification of <i>Rhipicephalus sanguineus</i> Infestation on Wild Canids (<i>Vulpes vulpes</i> and <i>Canis aureus</i>) in Northeastern Iran Including Golestan Forests	1082
Seyede Mahshid Mostafavi ¹ , Kian Mosaali ¹ , Shahab Shirzahi ² , Leila Saravani ³	1082
Molecular Detection of <i>Encephalitozoon</i> Species in Dogs and Cats in Tabriz City, Iran.....	1084
Parisa Shahbazi ¹ , Ali Seyed Zadeh ¹ , Ahmad Nematollahi ¹	1084



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Application of Artificial Intelligence in Predicting <i>Plasmodium</i> spp. Transmission under Climate Change Scenarios: A National and Global Perspective.....	1085
Azin Salehe ¹	1085
Leishmaniasis in Equids and Its Potential Role in the Epidemiology of the Disease in Iran: A Review	1086
Zahra Salehzadeh Kazerouni ¹ , Maedeh Beiki Zare ² , Mohammad Reza Norouzi ³ , Mahmoud Nateghi-Rostami ⁴	1086
Systematic Review of <i>Encephalitozoon cuniculi</i> Within a One Health Framework: Epidemiology, Diagnosis, and Prevention	1088
Amir Rostami ¹ , Reza Kalantari ^{2,3}	1088
Literature Review of Promising Antiparasitic Effects of Phytotherapy Against <i>Ctenocephalides felis</i>	1090
Mohammad Amrollahi-Sharifabadi ¹ , Mobina Torabi Feijani ² , Amirhossein Ghiasvand ² , Hamidreza Shokrani ³	1090
A Review of New Toxoplasmosis Control Approach in Urban and Domestic Cats: Public Health Impact and Zoonotic Implication.....	1092
Arian Esfandiary ¹ , Golnaz Azizimotlagh ² , Parva Abiar ³ , Fatemeh Ghaderi ⁴ , Mohammad Bahmanzadegan Jahromi ⁵ , Parham Rahmani ³	1092
Microscopical and Molecular Study of <i>Nosema</i> Infection in Some Apiaries in Iran.....	1094
Ali Ahmadi ¹ , Sedigheh Nabian ¹ , Ali Nikpay ² , Mahsa shahbakhsh ³ , Alireza Arab ⁴ , Kamyar Ahmadi ⁵ , Abbas Gerami Sadeghian ¹	1094
First Molecular Detection and Phylogenetic Analysis of <i>Menacanthus stramineus</i> and <i>Argas persicus</i> in Ornamental Birds from Iran.....	1096
Soroosh Edrisnia ¹ , Salomeh Shirali ^{1,2} , Bahar Shemshadi ¹	1096
Phenotypic and Molecular Detection of <i>Babesia</i> spp. in Domestic Dogs from <i>Guilan</i> Province, Northern Iran.....	1097
Ali asbaghi Jamalabad ¹ , Soroosh Edrisnia ¹ , Salomeh Shirali ^{1,2} , Kiumarsh Amini ³	1097
Frequency of Trichomoniasis in Domestic Pigeons in Aligudarz Region and Assessment of Its Sensitivity to Anti-protozoal Drugs	1098
Homayun Rajabipour ¹ , Majid Taati ²	1098
Comparison of Simple and Nested PCR Methods for the Detection of <i>Neospora caninum</i> in the Brain of Aborted Bovine Fetuses.....	1099
Asma Keyvanlou Shahrestanaki ¹ , Gholamreza Razmi ²	1099
A Review of CRISPR-Cas9-Driven Insights into the Molecular Basis of Antimalarial Drug Resistance in <i>Plasmodium falciparum</i>	1101
Mohammed Reza Anani ¹ , Yaser Habibi ¹ , Fatemeh Valikhani ¹ , Mobina Rajabzadeh ¹ , Tohid Javaheri ²	1101



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Neospora caninum and Bovine Reproductive Failure: A Review of Epidemiology, Diagnostics, and Control Strategies.....	1102
Fazel Sadeghian ¹ , Arash Kheradmand ² , Roohollah Dehghani Tafti ²	1102
Organophosphate Poisoning Following Misuse of Pesticide for Pediculosis Treatment in a Child: A Case Report.....	1103
Tarane Farhangi ¹ , Anahita Alizadeh ²	1103
The Role of One Health in the Sustainable Management and Surveillance of Parasitic Diseases in the Era of Climate Change.....	1105
Mohammad Arad Zandieh ¹ , Hesamedin Akbarein ¹	1105
The Role of Artificial Intelligence in the Diagnosis of Equine Parasitic Diseases: A Systematic Review	1107
Mohammad Taghi Vajed Ebrahimi ¹ , Asma Keyvanlou Shahrestanaki ²	1107
An Elderly Patient with a Hydatid Cyst in the Liver, Suspected of <i>Echinococcus multilocularis</i> ...	1109
Setareh Alizadeh ¹ , Elahe Ebrahimzadeh ¹	1109
Prevalence of <i>Eimeria</i> Infection in Free-Grazing Cattle and Calves in Eastern Golestan Province.	1110
Mohadese Chitband ¹ , Elahe Ebrahimzadeh ¹	1110
Cestode Infection in Eurasian Collared Dove (<i>Streptopelia decaocto</i>)	1111
Hannaneh Moghaddam ¹ , Setareh Alizadeh ¹ , Javad Khedri ¹ , Elahe Ebrahimzadeh ¹	1111
Geographic Distribution and Fauna of <i>Ixodidae</i> (Hard) Ticks in Northwest of Iran, in 2023-2024.	1112
Faraz Malek Bagali ^{1, 2} , Eslam Moradi-Asl ³	1112
Prevalence of Tick-Borne Bacteria in Livestock, in northwestern Iran (2024)	1114
Faraz Malek Bagali ^{1, 2} , Farzad Khademi ³	1114
Molecular Detection and Identification of <i>Crimean-Congo Hemorrhagic Fever Virus</i> (CCHFV) in Ixodid and Argasid Ticks from Northwestern Iran, 2024	1116
Faraz Malek Bagali ^{1, 2} , Chiman Karami ¹	1116
Seasonal Variation of <i>Toxocara</i> Egg Contamination in Park Soils of Gersar County, Iran	1118
Narges Safari ¹	1118
The Report of Acanthocephalan Eggs in a Long-eared Hedgehog in Isfahan Province, Iran.....	1119
Mohammadreza Ghorani ¹ , Mahdi Salimi ² , Nasser Hajipour ³	1119
In Vitro Evaluation of <i>Rhipicephalus sanguineus</i> Resistance to Commercial Formulations of Cypermethrin and Deltamethrin.....	1121
Seyed Amirreza Boskabadi ¹ , Ali Moshaverinia ²	1121
Meat and Corn By-Products as Feedstock for Enhanced Black Soldier Fly Larvae (<i>Hermetia illucens</i>) Growth	1122



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Maryam Ghaderi-Ghahfarokhi ¹ , Gholam Hussein Bagheri ² , Somayeh Bahrami ² , Alireza Alborzi ²	1122
Anemia Caused by Heavy <i>Ctenocephalides felis</i> Infestation in Young Holstein Calves under Poor Hygienic Conditions: A Case Series from Sirch, Kerman, Iran (2024)	1123
Seyede Mahnoosh Mostafavi ¹ , Seyede Mahshid Mostafavi ² , Kian Mosaali ² , Shahab Shirzahi ³ , Leila Saravani ⁴	1123
A Case Report of <i>Hyalomma spp.</i> Tick Infestation in 3 Free-Roaming Dogs in Southeastern Iran (Zabol), 2024.....	1125
Seyede Mahnoosh Mostafavi ¹ , Seyede Mahshid Mostafavi ² , Shahab Shirzahi ³ , Leila Saravani ⁴	1125
Prevalence of <i>Rickettsia</i> Species in Hard Ticks In northwest Iran (2025)	1127
Faraz Malek Bagali ^{1, 2} , Mahdi Rezaverdinejad ³ , Ahmad Enferadi ³	1127
Ticking Threat: Prevalence of <i>Hyalomma marginatum</i> in Cattle Farms of Hasan-Abad Fashafuyeh, Tehran Province	1129
Erfan Darmanloo ¹ , Alireza Ghorbani ² , Mehrdad Ghaledari ¹ , Negar Shaverdi ¹ , Amirreza Ghorbani ³	1129
Diagnosis of Ovine Aborted Fetuses Associated with <i>Toxoplasma gondii</i> in Mazandaran Province	1131
Afsaneh Amouei ¹ , Fatemeh Daraei ¹ , Fatemeh Parandin ² , Samira Dodangeh ³ , Elham Kia Lashaki ⁴ , Saeide Mir Yaghoobzadeh ¹ , Azadeh Mizani ⁵	1131
Detection of <i>Toxocara cati</i> in a Deceased Stray Cat Using Morphological and Molecular Approaches: A Case Study from Shiraz, Iran	1133
Erfan Darmanloo ¹ , Amirreza Ghorbani ² , Alireza Ghorbani ³	1133
Frequency and Associated Factors of <i>Sarcoptes scabiei</i> Infestation Among Suspected Patients Referred to Selected Medical Laboratories in Mashhad, Iran.....	1135
Amin Bakhshani ¹ , Ali Moshaverinia ² , Bahareh Meftahi ^{2,3} , Mohammad Reza Khakzad ^{3,4}	1135
Case Report: Internal and External Parasites in Crested and Eurasian Skylarks Collected from Environmental Mortalities in Khorasan Province and the Persian Gulf Bird Market in Tehran	1137
Mohammad Mahdi Darvishi ¹ , Jalil Pourhaji Motab ²	1137
Prevalence of Toxoplasmosis in Wild Cattle as Human Zoonotic Meat-borne Pathogens: A Systematic Review and Meta-analysis	1138
Azadeh Mizani ¹ , Afsaneh Amouei ² , Elham Kia Lashaki ³ , Samira Dodangeh ⁴ , Fatemeh Parandin ⁵	1138
Seroprevalence of <i>Neospora caninum</i> Infection in Cows with Abortion in Khorasan Razavi, Iran.	1140
Asma Keyvanlou Shahrestanaki ¹ , Bahareh Meftahi ¹ , Narges Khaleghnia ² , Ali Barati ¹ , Gholamreza Razmi ¹	1140
Study of the Practices of Rural Livestock Farmers in Saveh County Regarding Cystic Echinococcosis	1141
Amirhossein Kakaei ¹ , Elahe Ebrahimzadeh ² , Ehsanollah Afshari Safavi ³ , Arman Latifi ⁴	1141
Knowledge of Rural Livestock Farmers in Saveh County about Cystic Echinococcosis.....	1143



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad

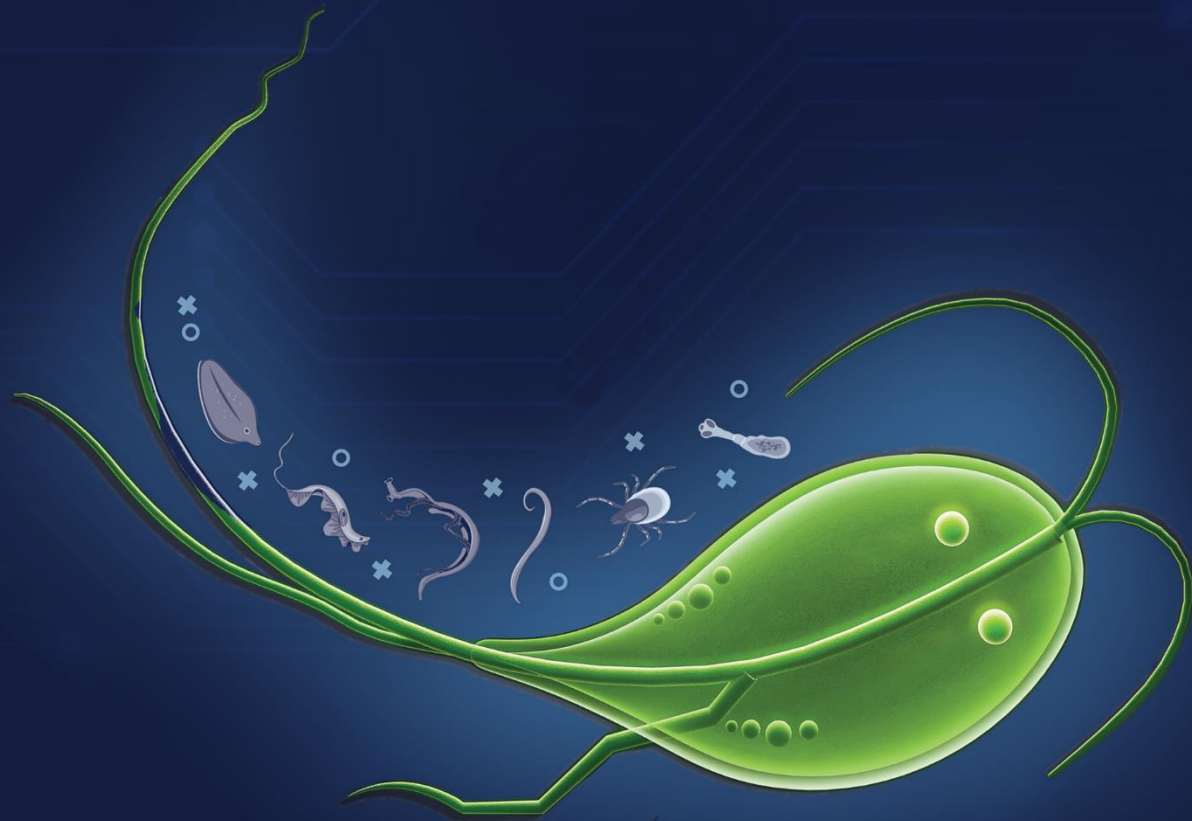


Amirhossein Kakaei ¹ , Elahe Ebrahimzadeh ² , Ehsanollah Afshari Safavi ³ , Arman Latifi ⁴	1143
In Vivo Evaluation of Acaricidal Effect of Seed Oil of <i>Melia azedarach</i> on <i>Dermanyssus gallinae</i>	1145
Mohammad Reza Khoshvaght ¹ , Ali Moshaverinia ² , Ehsan Khoshvaght ³	1145
Nano-Phytosomal Delivery of Phytochemicals: An Emerging Strategy for Controlling Zoonotic and Veterinary Parasitic Infections	1147
Seyed Mobin Aghilian ¹ , Mohammad Mohsenzadeh ² , Soodeh Alidadi ³	1147
Assessment of Cryptosporidium parvum Infection and Associated Risk Factors in Calves from Dairy Farms in Southern and Southwestern Iran	1149
Faezeh Falahatnezhad ¹ , Aria Rasooli ¹ , Mehdi Mohebbi Fani ¹ , Seyyed Mostafa Razavi Dinani ² , Saeed Hosseinzadeh ³	1149
A Case of Acute Toxoplasmosis in a Veterinarian: Potential Zoonotic Transmission from a Domestic Cat in Iran	1150
Heidarzadeh Saeid ¹ , Ghorbani Parsa Mohammad Sajjad ¹ , Ghorbani Parsa Mobina ¹	1150
The Study of the Prevalence of Hemoparasites in Camels of Northeastern Iran	1151
Fatemeh Rezazadeh ¹ , Hassan Borji ¹ , Gholamreza Razmi ¹ , Javad Khedri ¹	1151
Investigation of Blood Parasites of Columbidae in Mashhad City in the Summer of 1404	1152
Mohammad Hossein Ghafourian Abadi ¹	1152
Phylogenetic Relationships of <i>Giardia duodenalis</i> Isolates from Human and Animal Sources Based on <i>tpi</i> and <i>gdh</i> Genes.....	1153
Zeynab Khajegiri ¹ , Maryam Naderi Soorki ¹	1153
Epidemiological Analysis of Cutaneous Leishmaniasis in the Catchment Area of Mashhad University of Medical Sciences, Iran (2014-2024).....	1154
Zahra Nehbandani ¹ , Amir sadra Keyvani ²	1154



3rd National Congress Of Animal parasitic Diseases and Zoonoses

ORAL PRESENTATION





Evaluation of the Efficacy and Economic Viability of Targeted Selective Treatment for Parasite Control in Afshari Sheep

Ali Sallehi¹, Gholamreza Mohammadi²

Corresponding Author: Gholamreza Mohammadi, gmohamad@um.ac.ir

1. Private veterinarian, Mashhad, Iran.
2. Department of clinical sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article Code: 1282

Abstract

Background: Effective control of internal parasites is vital to sheep farming, as parasitic infections lead to substantial economic losses due to decreased weight gain, increased mortality, and compromised nutrient absorption. Targeted Selective Treatment (TST) has emerged as a strategy to optimize parasite control while mitigating drug resistance.

Methods: A comparative field study was conducted on a 20,000-head semi-industrial Afshari sheep farm in the Jalgheh Rokh–Torbat Heydarieh region. The efficacy and cost-effectiveness of TST were assessed against blanket (whole-flock) treatment. TST implementation relied on the Five Point Check (FPC) system and parasitological indicators, particularly fecal egg counts (EPG), to identify individuals requiring treatment.

Results: Using TST, only 10–15% of the flock received anthelmintic intervention, resulting in an 85–90% reduction in drug use. Parasite burden, as measured by EPG, remained statistically comparable between TST and blanket treatment groups. Economic analysis demonstrated significant cost savings without compromising parasite control.

Conclusion: TST represents a sustainable and economically viable alternative to blanket deworming in Afshari sheep. Its implementation minimizes drug usage and delays anthelmintic resistance development, while maintaining effective parasite suppression.

Keywords: Targeted Selective Treatment (TST); Afshari sheep; Internal parasites; Anthelmintic resistance; Economic efficiency; Five Point Check; EPG.

Introduction:

Controlling internal parasites in ruminant farming systems, particularly sheep, is a major challenge for maintaining animal health and economic productivity (1). Gastrointestinal parasites such as coccidia, nematodes, cestodes, and liver flukes can cause significant losses in the livestock industry, even in subclinical cases, by reducing weight gain, impairing body condition, and affecting reproduction (2,3). Traditional parasite control methods, which rely on



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



blanket treatment with broad-spectrum drugs, face limitations due to increasing anthelmintic resistance (4).

Targeted Selective Treatment (TST) is an innovative approach that selectively treats animals based on specific clinical criteria, reducing drug use and mitigating the risk of resistance. One practical TST method is the Five Point Check system, which assesses five clinical indicators (nose, eyes, jaw, back, and tail) to accurately identify infected animals (5). This study evaluates the efficacy and economic benefits of TST compared to blanket treatment in Afshari sheep.

Significance of Internal Parasites in Sheep Farming

Internal parasites, particularly nematodes, coccidia, and liver flukes, are major contributors to reduced sheep productivity. Clinical coccidiosis causes diarrhea and mortality, while subclinical cases lead to weight loss (6). Liver flukes, such as *Fasciola hepatica*, cause anemia, reduced wool quality, and ascites, endangering animal health (7). Even less harmful parasites, like tapeworms (*Moniezia expansa*), can reduce production under poor nutritional conditions due to competition for nutrients (8).

Parasite prevalence depends on environmental factors such as pasture quality, temperature, humidity, and grazing management (9). Given the negative impact of parasites on growth, milk production, and fertility (10), effective parasite management is critical to meet the food demands of a growing global population with limited resources (11).

Targeted Selective Treatment (TST)

TST involves selectively treating individual animals based on specific clinical criteria, targeting those most likely to benefit from treatment and avoiding unnecessary treatment of healthy animals. This approach reduces anthelmintic use, lowering the risk of resistance (4).

A successful TST tool is the FAMACHA system, which assesses conjunctival color to detect anemia caused by blood-feeding parasites like *Haemonchus contortus* (5). However, FAMACHA is less effective for non-blood-feeding parasites. The Five Point Check system, a more comprehensive method, evaluates five clinical criteria:

- **Nose:** Nasal discharge may indicate infection with nasal botflies (*Oestrus ovis*).
- **Eyes:** Conjunctival color is assessed for anemia (FAMACHA system).
- **Jaw:** Submandibular edema (bottle jaw) indicates hypoproteinemia caused by blood-feeding parasites.
- **Back:** Body Condition Score (BCS) identifies animals affected by gastrointestinal parasites.
- **Tail:** Diarrhea or visible soiling around the tail area may indicate gastrointestinal parasites.

This system is simple, cost-effective, and practical for field use, making it suitable for parasite management in diverse conditions.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Materials and Methods:

This study was conducted in a semi-industrial Afshari sheep farm with a population of 20,000 in the Jolgeh Rokh-Torbat Heydarieh region. Before 2023, the farm used blanket treatment with broad-spectrum drugs every 10 months. From 2023 to 2025, TST based on the Five Point Check system was implemented across all flock groups. Due to the hot, dry climate, which is unsuitable for *Haemonchus contortus*, only 30% of each group was evaluated using FAMACHA.

Data Collection

- Before implementing TST, fecal samples from 31 randomly selected lactating ewes were collected to measure eggs per gram (EPG).
- During the study, fecal samples (3 pellets per fresh fecal mass) were collected seasonally from 10% of each group and sent to a laboratory.
- The McMaster method was used to determine EPG, and appropriate drugs were selected based on the type of infection.

Results and Discussion:

EPG counts for lactating ewes before TST (Table 1) and after TST implementation across various groups (Table 2) are presented. With TST, only 10-15% of each group required treatment. Results showed no significant change in parasite infection levels (primarily *Nematodirus* spp.) after switching from blanket treatment to TST.

Table 1. EPG Results for Lactating Ewes Before TST (Blanket Treatment)

Row	Tag Number	Infection Type	EPG
1	29466	...	...
2	29886	...	...
3	...	...	...
4	29327	Nematodirus	3
5	...	...	...
6	33051	...	...

Notes: Only one ewe (Row 12) showed *Nematodirus* spp. infection with EPG = 3. The average EPG was very low (~0.1), indicating minimal infection during blanket treatment.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 2. EPG Results for Various Groups After TST

Row	Season	Group	Infection Type	EPG
1	Spring 2023	Fattening 4-6 mo	Nematodirus	4
2	Spring 2023	Fattening 4-6 mo	0	0
4	Spring 2023	Fresh Ewes	0	0
7	Spring 2023	Heavy Pregnant	Nematodirus	1
19	Summer 2023	Fresh Ewes	Nematodirus	2
33	Summer 2023	Lactating Ewes	Nematodirus	6
42	Autumn 2023	Fresh Ewes	Nematodirus	5
70	Winter 2024	Fresh Ewes	Nematodirus	7
88	Winter 2024	Lactating Ewes	Nematodirus	2
89	Winter 2024	Lactating Ewes	Trichuris	2

Notes: Table 2 includes seasonal data from various groups (fattening, fresh ewes, heavy pregnant, lactating ewes, etc.) in 2023 and 2024. Infections were primarily *Nematodirus spp.*, with one case of *Trichuris spp.* Only samples with infections or representative groups are listed for brevity.

The study demonstrated that TST maintained similar parasite control levels as blanket treatment while reducing treatment costs by 85-90%, primarily by limiting treatment to animals in need. Reduced anthelmintic use also lowers the risk of resistance, a major challenge in parasite management (Charlier et al., 2020). The low prevalence of *Nematodirus spp.* in this flock, likely due to the region's climate, was effectively managed by TST. The Five Point Check system proved practical and effective, especially in resource-limited settings.

TST in Afshari sheep was economically viable and reduced the risk of anthelmintic resistance by minimizing drug use. This approach is a sustainable strategy for managing internal parasites in sheep farms, particularly in regions with similar climates. Further studies are recommended to optimize diagnostic criteria and assess the long-term effects of TST.

Acknowledgments:

We express our gratitude to the esteemed management of Khazaei Rafie Agro-Industry, Mr. Eng. Abdulreza Khazaei, and the livestock unit staff for their sincere cooperation in conducting this research.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Junkuszew A, Milerski M, Bojar W, et al. Effect of various antiparasitic treatments on lamb growth and mortality. *Small Ruminant Res.* 2015;123(2-3):306-13. doi: 10.1016/j.smallrumres.2014.11.019
2. Gadahi JA, Arshed MJ, Ali Q, et al. Prevalence of gastrointestinal parasites of sheep and goat in and around Rawalpindi and Islamabad, Pakistan. *Vet World.* 2009;2(2):51.
3. Charlier J, Rinaldi L, Musella V, et al. Initial assessment of the economic burden of major parasitic helminth infections to the ruminant livestock industry in Europe. *Prev Vet Med.* 2020; 182:105103. doi: 10.1016/j.prevetmed.2020.105103.
4. Sutherland IA, Leathwick DM. Anthelmintic resistance in nematode parasites of sheep: a review. *N Z Vet J.* 2011;59(1):8-14. doi:10.1016/j.pt.2010.11.008.
5. Bath GF, Van Wyk JA. The Five Point Check© for targeted selective treatment of internal parasites in small ruminants. *Small Ruminant Res.* 2009;86(1-3):6-13. doi: 10.1016/j.smallrumres.2009.09.009.
6. Chartier C, Paraud C. Coccidiosis due to *Eimeria* in sheep and goats, a review. *Small Ruminant Res.* 2012;103(1):84-92. doi: 10.1016/j.smallrumres.2011.10.022.
7. Mitchell G. Update on fasciolosis in cattle and sheep. *In Pract.* 2002;24(7):378-85.
8. Jankovská I, Lukešová D, Száková J, et al. Competition for minerals (Zn, Mn, Fe, Cu) and Cd between sheep tapeworm (*Moniezia expansa*) and its definitive host sheep (*Ovis aries*). *Helminthologia.* 2011; 48:237-43. doi:10.2478/s11687-011-0033-3.
9. Pal RA, Qayyum M. Prevalence of gastrointestinal nematodes of sheep and goats in upper Punjab, Pakistan. *Pak J Zool.* 1993; 25:353-5.
10. Fitzpatrick JL. Global food security: the impact of veterinary parasites and parasitologists. *Vet Parasitol.* 2013;195(3-4):233-48. doi: 10.1016/j.vetpar.2013.04.005.
11. Vercruysse J, Charlier J, Van Dijk J, et al. Control of helminth ruminant infections by 2030. *Parasitology.* 2018;145(13):1655-64. doi:10.1017/S003118201700227X.



Effects of Some Parasitic Infections on the Physiology of Central Feeding Behavior and Energy Balance in Birds

Farshid Hamidi¹

Corresponding author: Farshid Hamidi, Farshidhamidi@um.ac.ir

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article Code: 1296

Abstract

Background: Intestinal parasitic infections are among the important factors that disrupt energy balance and feeding behavior in birds. These parasites disrupt the physiological function of the digestive tract by damaging the intestinal mucosa, reducing absorption, and stimulating immune responses. Reduced nutrient digestibility, changes in enzyme secretion, and increased energy expenditure to combat inflammation are common consequences of these infections, which directly affect energy balance.

Methods: Several sources were reviewed on this subject. Animal behavior refers to any action, reaction or activity that an animal performs in response to various stimuli or for various reasons. Of course, these internal changes are accompanied by the appearance of specific behavioral signs, including decreased appetite, altered feeding patterns, aggression and reduced motivation to search for food.

Results: Studies have also shown that parasitic infections can alter the composition of the gut microbiota, thereby affecting the neural and hormonal pathways associated with food intake and metabolism.

Conclusion: Focusing on avian feeding behavior, this article demonstrates the physiological and behavioral aspects of how gastrointestinal parasites affect physiological feeding behavior and how local disturbances in the gut can lead to widespread changes in behavior and energy balance. The aim of this review is to highlight the importance of physiological interpretation of behavioral findings in veterinary studies of avian animals, especially in situations where changes in feed intake or feeding behavior may be only a secondary sign of an underlying pathology.

Keywords: Physiological effects, Feeding behavior, Birds, Energy balance, Parasitic infections.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Introduction:

Parasitic infections, especially in farmed species such as poultry, can be an important challenge in maintaining health and growth and productivity. These parasites not only directly attack tissues, but also affect basic behaviors such as feeding, drinking, and locomotion by causing physiological disorders and stimulating immune responses.

In birds, the hypothalamus functions as a central hub where feeding behavior is orchestrated through the activity of its specialized nuclei. The arcuate, lateral, ventromedial, paraventricular, and dorsomedial regions act in concert to sense metabolic cues and translate them into signals of hunger or satiety. These nuclei are not passive relay stations; rather, they are dynamic centers where orexigenic and anorexigenic pathways converge and are finely tuned. For poultry, especially broiler chickens, this regulation directly shapes growth, feed efficiency, and even responses to environmental challenges (1).

One of the first signs of parasites in birds is a decrease in food consumption and a change in feeding patterns. This is particularly common in gastrointestinal infections such as coccidiosis (caused by *Eimeria* spp.). In this disease, direct damage to the intestinal mucosa reduces the ability to absorb nutrients and provokes pain during digestion. Such a reaction leads to a decrease in the bird's desire to eat, even in conditions of starvation. Also, in infections with roundworms such as *Ascaridia galli*, the physical presence of the parasite in the small intestine can cause partial obstruction, a feeling of fullness, and subsequently a decrease in the frequency of visits to the feeder to eat (2). In infections such as *Heterakis gallinarum* (nematode parasite that lives in the cecum of some birds) or *Capillaria* spp. (around 30mm in length), although the severity of clinical signs may be less severe, birds experience a gradual decrease in weight gain and a decrease in feed conversion ratio. This decrease usually occurs in the absence of severe outward signs, and only by carefully assessing weight changes, recording feeding behavior, and ultimately performing a necropsy can the underlying cause be identified (3).

The present paper, focusing on the impact of common parasites on birds, examines the behavioral and metabolic changes caused by these factors.

Materials and Methods:

Numerous articles and sources were reviewed that had investigated and experimented on bird food intake and the function of hypothalamic nuclei, and on the other hand, some studies showed a decrease in this function in parasitic infections. These connections and dependencies were further investigated.



Results and Discussion:

Reduced feed intake, together with increased metabolic costs due to the activation of the immune system, ultimately leads to energy imbalance in infected birds. In many cases, birds not only lose weight but also lose their fat and protein reserves. Numerous studies have shown that in chronic parasitic infections, the daily growth rate is significantly reduced, in other words, the growth of these birds lags behind their age group.

Interestingly, sometimes infected birds not only eat less, but their eating patterns also change. For example, decreased concentration during feeding, frequent interruptions of eating, and restlessness have been observed. These behaviors may be due to the effects of inflammation on the central nervous system and appetite control pathways, which, have been discussed in other sections (4).

Functional Behaviors

Animal behavior refers to any action, reaction, or activity that an animal performs in response to various stimuli or for various reasons. For example, some birds migrate to tropical regions with the onset of the cold season. There is also an interesting behavior in the case of worker ants, in that they spend the spring and summer collecting food and carrying it to their nest. Even a turtle goes into its shell when it senses danger. Many animals, when faced with an enemy, assume a frightening appearance, raise their hair, and appear larger than they really are. These are examples of animal behavior.

Behavior is a set of actions that an animal performs or does not perform in response to a stimulus. There are two types of stimuli for behavior: internal stimuli and external stimuli. Hunger and thirst are internal stimuli. The smell of food, the sight of an enemy or another person are external stimuli for behavior. One of the most important behaviors in birds, which is also indirectly very important for humans, is feeding behavior (1,5).

In addition to decreased appetite and growth retardation, parasitic infections are often accompanied by changes in the bird's general behavioral pattern. Infected birds are usually less inclined to move, play, and even respond to environmental stimuli. Decreased level of consciousness, standing with a retracted neck, drooping wings, and sometimes half-open eyes are among the symptoms that, although not specific, can be used as early signs of subacute or chronic infection along with other indicators. This allows for timely diagnosis.

Some studies have shown that the time spent resting and sitting per day in infected birds increases by up to twofold. This behavior, which is also a kind of tendency to isolate, is probably due to a feeling of digestive discomfort, muscle weakness, or the immune system's attempt to save energy. In severe infections, birds may even selectively restrict their intake of certain compounds, such as high protein or minerals, experiencing a type of selective anorexia! This condition can be considered in interpreting the physiological state of the bird (5).



The link between hypothalamic inflammation and energy control

Currently, there is no direct and definitive research information on the effect of parasitic infections on the specific function of hypothalamic nuclei in energy balance and feeding control. However, there are some relevant pathways and preliminary studies that may shed light:

Inflammation in the hypothalamus, even in the absence of a parasitic agent, can lead to dysregulation of energy balance and appetite. For example, the hypothalamus plays a central role in the control of food intake and energy expenditure; and inflammatory disorders (such as activation of microglia and astrocytes) may contribute to decreased appetite, weight loss, or even obesity (5).

One study suggests that *Trypanosoma brucei* (the causative agent of sleeping sickness) can enter the arcuate nucleus of the hypothalamus through specific areas such as the median eminence. It has also been suggested that: Parasitic infections can alter behavioral or biological signals in brain centers; for example, *Toxoplasma gondii* can inhibit the host's anxiety response, possibly involving effects on the hypothalamus or limbic system (6).

Direct studies of the effects of parasites on the hypothalamus in the context of energy balance are still limited. However, the entry of some parasites such as *Trypanosoma brucei* into hypothalamic regions can have consequences on the central function of this region. On the other hand, hypothalamic inflammation, which can be caused by various infections, affects appetite and energy regulation. Nuclei such as the arcuate nucleus, VMH, DMH and LH play a critical role in the regulation of feeding and energy (7).

Areas such as the median eminence provide a relative permeability to blood signals, allowing cytokines to reach the environment surrounding the arcuate nucleus via various pathways. This access can alter the activity of neurons controlling appetite. Studies with *Trypanosoma brucei* and other parasites have shown that parasite invasion and local inflammation occur in ME/ARC and are associated with behavioral-metabolic changes (8).

Diagnostic applications of physiology in parasitology

In situations where laboratory tests or necropsy are expensive or time-consuming, feeding behavior and growth rate can be used as early, non-invasive and inexpensive indicators (9). This approach has high practical value, especially in industrial flocks with high volume and the need for rapid monitoring. In infections such as ascariasis or capillariasis, which are generally chronic and asymptomatic, the use of modeling changes in feed intake, feed conversion rate and bird alertness level can be useful in designing automated monitoring algorithm. This issue has presented a new path for researchers in the connection between physiological sciences , veterinary medicine and new technologies and their integration (3).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Parasitic infections affect avian physiology through various central and peripheral pathways, the most prominent of which include decreased appetite, impaired energy balance, reduced growth, and behavioral changes. Also parasitic infections can also release inflammatory or proinflammatory factors directly or indirectly (via host immune stimulation), and these factors can signal through specific structures around the arcuate nucleus, particularly the median eminence (ME) and other "periventricular structures", and affect the neuronal populations of the arcuate nucleus.

Finally, the link between behavioral physiology and parasitology is a dynamic and promising path for future research, field studies, and applied projects in the field of avian veterinary medicine.

Acknowledgment:

Author would like to thank the Ferdowsi University of Mashhad for their support.

References:

1. Zendehdel M, Mokhtarpouriani K, Babapour V, Pourrahimi M, Hamidi F. The role of 5-HT 2A and 5-HT 2C receptors on harmaline-induced eating behavior in 24-h food-deprived broiler cockerels. *Iranian Journal of Veterinary Research*. 2013 Apr 1;14(2).
2. Williams RB. Intercurrent coccidiosis and necrotic enteritis of chickens: rational, integrated disease management by maintenance of gut integrity. *Avian pathology*. 2005 Jun 1;34(3):159-80.
3. Cupo KL, Beckstead RB. *Heterakis gallinarum*, the cecal nematode of gallinaceous birds: a critical review. *Avian diseases*. 2019 Sep 1;63(3):381-8.
4. Kogut MH, Yin XiaoNan YX, Yuan JianMin YJ, Broom LJ. Gut health in poultry. *CABI Reviews*. 2017 Oct 25(2017):1-7.
5. Yousefvand S, Hamidi F, Parham A. Effect of lipopolysaccharide on body physiological responses. *Veterinaria México OA*. 2025;12.
6. Abdulai-Saiku S, Vyas A. *Toxoplasma gondii* infection causes an atypical abundance of oxytocin and its receptor in the female rat brain. *Pathogens*. 2021 Nov 17;10(11):1495.
7. König HE, Bragulla H. *Veterinary anatomy of domestic mammals: textbook and colour atlas*. Schattauer Verlag; 2007.
8. Olivera GC, Vetter L, Tesoriero C, Del Gallo F, Hedberg G, Basile J, Rottenberg ME. Role of T cells during the cerebral infection with *Trypanosoma brucei*. *PLOS Neglected Tropical Diseases*. 2021 Sep 29;15(9):e0009764.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



9. Wilcoxon TE, Horn DJ, Hogan BM, Hubble CN, Huber SJ, Flamm J, Knott M, Lundstrom L, Salik F, Wassenhove SJ, Wrobel ER. Effects of bird-feeding activities on the health of wild birds. *Conservation physiology*. 2015;3(1):cov058.



Development of A Triplex Nested PCR for Simultaneous Diagnosis of Three Important Zoonoses Agents *Plasmodium*, *Babesia*, and *Toxoplasma* in Blood Samples of Humans and Animals

Abbasali Eskandarian¹, Alireza Najafi Rad¹

Corresponding Author: Abbasali Eskandarian, aeskandarian@med.mui.ac.ir

1. Department of Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Article Code: 1299

Abstract

Background: The risk of transmission of some infectious agents from domestic and wild animals to humans has always been an important health problem. This study aimed to develop a triplex nested PCR (tnPCR) to assess the presence of protozoan parasites *Plasmodium*, *Toxoplasma*, and *Babesia* in blood samples concurrently. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the developed tnPCR method compared to gold standard methods were determined for all three parasite genera.

Methods: After selecting the genus-level specific primers and setting up of tnPCR, blood samples were collected. Some different samples from humans and animals were tested by this method in comparison with the gold standard methods (microscopic method for *Plasmodium*, *Babesia*, and ELISA for *Toxoplasma*).

Results: This tnPCR works well, and the sensitivity, specificity, PPV, NPV and accuracy, of this molecular method were all 100% for *Plasmodium* spp., 93.33%, 99.16%, 93.33%, 99.16% and 99.25% for *Babesia* spp. and 100%, 98.5%, 85.72%, 98.5% and 100% for *Toxoplasma gondii* respectively compared to standard methods. On average, there were 100%, 99.22%, 95.24%, 99.5% and 99.75% contingency for all three parasites ($\alpha < 0.05$). The designed and provided method can detect one, two, or all three potentially dangerous pathogens simultaneously in one tube and one step, in biological specimens as well as blood.

Conclusion: The developed tnPCR works well. We recommend it for facilitating tests, saving time, reducing the expense and cross-contamination, and subsequently the promotion of health levels in humans and animals as well.

Keywords: Plasmodium, Babesia, Toxoplasma, Nested PCR, Diagnosis.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Introduction:

Some important parasitic protozoa are common to mankind and some domestic and even wild animals. We developed a triplex nested PCR (tnPCR) for the simultaneous detection of three important common pathogens: Plasmodium, Babesia, and Toxoplasma in human and animal blood samples(1-5).

Plasmodium is a protozoan infection that is almost transmitted by *Anopheles* mosquito bites.

In some cases, infection is also caused by direct injection of infected blood, such as blood transfusions (6). It is generally assumed that asymptomatic infections are frequent in areas of high malaria transmission, where people rapidly receive immunity (7-10).

Another parasite is *T. gondii*. Toxoplasmosis is a zoonotic disease caused by this parasite in a variety of warm-blooded vertebrates such as humans, livestock, birds, marine mammals, etc. (11-14).

Another parasite that can be transmitted by blood is *Babesia*. In the United States, induced babesiosis has been reported by blood transfusions (15). The elderly are at risk for the disease and may cause severe infections in these individuals that may even lead to death (16, 17).

Materials and Methods:

Parasite sources and preparation: All three parasites were obtained from department of parasitology, Isfahan university of medical sciences.

DNA extraction: DNA from each blood sample containing a determined parasite number was extracted separately according to the instructions Genetbio kit (South Korea).

Nested PCR setting up: The multiplex nested PCR set up for all three parasites. The genus specific primers are mentioned in Table 1. The PCR program was used for simultaneous detection of 3 protozoa. Five minutes (min) at 95°C and then 30 cycles (for first step) 1 min at 95°C, 1 min at 56.5°C and 1min at 72°C, as well as 26 cycles (second phase), the samples were incubated for 45s at 95°C 70s at 61°C and 45s at 72°C, and then the specimen was incubated for 5 min at 72°C.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 1. Genus specific primers, which were used in tnPCR.

<i>Parasite</i>	<i>Stage</i>	<i>Primers assigns and sequences</i>	<i>Reference</i>
<i>Plasmodium</i>	Nested	Rplu1 (tcaaagattaagccatgcaagtga)	(18)
	one	Rplu5 (cctgttggctcctaaacttc)	
<i>Babesia</i>	Nested	Rplu3 (ttttataaggataactacggaaaagctgt)	(18)
	two	Rplu4 (taccgctcatagccatgttaggccaatacc	
<i>Babesia</i>	Nested	Bab5 (5'-aattacccaatcctgacacagg-3')	(19)
	one	Bab8 (5'-tttggcagtagt tcgtctttaaca-3')	
<i>Babesia</i>	Nested	Bab6 (5'-gacacaggg aggtagtgacaaga-3')	(19)
	two	Bab7(5'-cccaactgctcctattaaccattac-3')	
<i>Toxoplasma</i>	Nested	F1 (ggaggactggcaacctggtgtcg)	(20)
	one	R1 (ttgtttcaccggaccgttttagcag)	
<i>Toxoplasma gondii</i>	Nested	Tox1(cggaaatagaaagccatgaggcactcc)	(20)
	two	Tox2(acgggagtagtagcacctgaggagat)	

Sample collection: Normal human blood was used to make the 50, 5 and 1 parasites per microliter.

After setting up the tnPCR, 133 blood samples belonging to different origins were used for comparing the results of this method against gold standard tests (Table 2).

Table 2. comparative results of developed nested PCR against standards (microscopy for *Plasmodium* and *Babesia* and the ELISA for *Toxoplasma gondii*) on 133 samples.

<i>Row</i>	<i>Sample type</i>	<i>Sample size</i>	<i>Parasites were diagnosed</i>		
			<i>Plasmodium</i>	<i>Babesia</i>	<i>Toxoplasma gondii</i>
1	Donors' blood	60	-	-	2
2	Birds' blood	3	1	-	-
3	Cows' blood	4	-	2	-
4	Human patients' blood/serum	24	-	-	12
5	Animal patients' serum	22	10	-	-
6	Disease sheep, horse, goats' blood	20	-	14	-
Sum		133	11	16	14



Analysis method: The data were analyzed using a contingency table for comparing the accuracy of the new method with the gold standards method ($\alpha \leq 0.05$).

Results and discussion:

The results are shown in Figs 1 and 2. Because the primers were genus-specific for each parasite, 133 blood samples from humans and animals origin were tested using our developed method as well as by standard methods, double-blindly (Table 2). The sensitivity, specificity, PPV, NPV, and accuracy were determined by contingency table analysis.

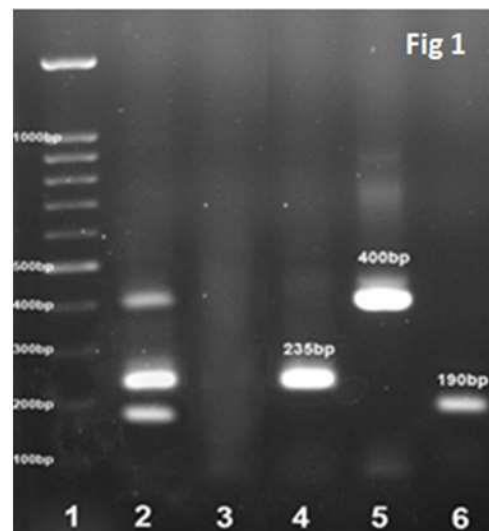


Figure 1. agarose gel electrophoresis showing PCR products obtained by triplex nested PCR (tnPCR).
1. 100bp DNA size marker ladder 2. positive control (three bands of parasitic agents at the same line),
3. Negative control, 4. *Plasmodium*, 5. *Babesia*, 6. *Toxoplasma* specific bands separately.

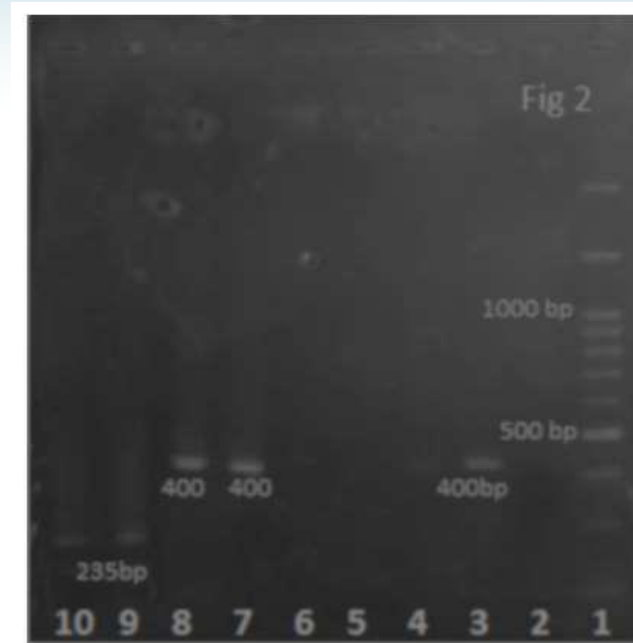


Figure 2. Agarose gel electrophoresis showing triplic nested PCR (tnPCR) products in some human and animals' samples. 1. 100bp DNA size marker ladder 2. Bird blood sample (Negative), 3-6: cow blood samples from slaughterhouses (3 and 4 positives for *Babesia*), 7 and 8: sheep blood samples (positive for *Babesia*), 9 and 10: human blood samples (positive for malaria).

		True class		Measures
		Positive	Negative	
Predicted class	Positive	True positive <i>TP</i>	False positive <i>FP</i>	Positive predictive value (PPV) $\frac{TP}{TP+FP}$
	Negative	False negative <i>FN</i>	True negative <i>TN</i>	Negative predictive value (NPV) $\frac{TN}{FN+TN}$
Measures		Sensitivity $\frac{TP}{TP+FN}$	Specificity $\frac{TN}{FP+TN}$	Accuracy $\frac{TP+TN}{TP+FP+FN+TN}$

Figure 3. contingency table and each character formula (Vihinen BMC Genomics2012,13 (Suppl 4):S2.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



The data of this study showed that this **tnPCR** worked well. The sensitivity, specificity, PPV, NPV, and accuracy of this molecular method compared to gold standard methods determined using contingency are shown in Tables 3-5. The designed and provided method can detect one, two, or all three potentially dangerous pathogens simultaneously in one tube and one step, in biological specimens as well as blood.

Table 3. contingency table of **tnPCR** method against microscopy as gold standard for *Plasmodium* spp.

<i>tnPCR</i> test	Positive	Negative	Positive predictive value
Positive	15	0	15/15=100%
Negative	0	118	neg. predictive value 118/118=100%
Parameters	Sensitivity 15/15=100%	Specificity 118/118=100%	Accuracy 133/133=100%

Table 4. contingency table of **tnPCR** method against microscopy as gold standard for *Babesia* spp.

<i>tnPCR</i>	Positive	Negative	Parameters
Positive	14	1	Pos. predictive value 14/15=93.33%
Negative	1	118	Neg. predictive value 118/119=99.16%
Parameters	Sensitivity 14/15=93.33%	Specificity 118/119=99.16%	Accuracy 132/133=99.25%

There was a considerable agreement between our developed **tnPCR** and gold standard methods. As a matter of fact, the newly developed **tnPCR** showed the same or higher accuracy in sensitivity, specificity, positive and negative predictive values, and accuracy compared to the current gold standards. It worked with a high performance on human and different animals' blood and/or serum samples.

The sensitivity of **PCR** for detection was higher than that of microscopically. The accuracy and efficiency of this method is also much higher than that of the microscopic method, which increases the diagnostic value of this method (18,19). On the other hand, it is very important to design a method that can detect all three parasites simultaneously with high accuracy and sensitivity at the same time and save time and cost (20).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Acknowledgements:

My deepest thanks to the Deputy of Research of Isfahan University of Medical Sciences.

References:

1. World Health Organization (2021). Guidance on increasing supplies of plasma-derived medicinal products in low-and middle-income countries through fractionation of domestic plasma. WHO 2021.
2. McCullough J. Transfusion-transmitted diseases. Transfusion-Transmitted Diseases. 2021;422-452.
3. Busch MP, Bloch EM, Kleinman S. Prevention of transfusion-transmitted infections. Blood. 2019;133(17):1854-1864.
4. Sarmah P, Dan M, Adapa D, et al. A review on common pathogenic microorganisms and their impact on human health. Electronic Journal of Biology. 2018;14(1):50-58.
5. Brouwer EE, van Hellemond JJ, van Genderen PJ, et al. A case report of transfusiontransmitted *Plasmodium malariae* from an asymptomatic non-immune traveller. Malaria J. 2013;12(1):1-6.
6. Lima GF, Sanchez MCA, Levi JE, et al. Asymptomatic infections in blood donors harbouring *Plasmodium*: An invisible risk detected by molecular and serological tools. Blood Transfus. 2018;16:17-25.
7. Mardani A. Prevention strategies of transfusion-transmitted parasitic infections (ttpis): Strengths and challenges of current approaches, and evaluation of the strategies implemented in Iran. Parasite Epidemiology Control. 2020;9:e00141.
8. Schnittger L, Rodriguez AE, Florin-Christensen M, et al. Babesia: A world emerging. Infect Genet Evol. 2012;12(8):1788-809.
9. Hill DE, Dubey JP. *Toxoplasma gondii*. Foodborne Parasites. Springer; 2018:119-138.
10. Sarkari B, Shafiei R, Zare M, et al. Seroprevalence and molecular diagnosis of *Toxoplasma gondii* infection among blood donors in southern Iran. J Infect Dev Ctries. 2014;8(4):543-7.
11. Derouin F, Pelloux H, Parasitology ESGoC. Prevention of toxoplasmosis in transplant patients. Clin Microbiol Infec. 2008;14:1089- 101.
12. Siegel SE, Lunde MN, Gelderman AH, et al. Transmission of toxoplasmosis by leukocyte transfusion. Blood. 1971;37(4):388-94.
13. Tonnetti L, Townsend RL, Deisting BM, et al. The impact of *Babesia microti* blood donation screening. Transfusion. 2019;59:593-600.
14. Leiby D. Babesiosis and blood transfusion: Flying under the radar. Vox sang. 2006;90(3):157-65.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



15. Bloch EM, Herwaldt BL, Leiby DA, et al. The third described case of transfusion-transmitted *Babesia duncani*. *Transfusi*. 2012;52:1517-22.
16. Doni NY, Zeyrek FY, Seyrek A. Detection of Plasmodium using filter paper and nested pcr for patients with malaria in sanliurfa, in turkey. *Malar J*. 2016; 15(1):299.
17. Kim JY, Cho SH, Joo HN, et al. First case of human babesiosis in korea: Detection and characterization of a novel type of Babesia sp.(ko1) similar to ovine *Babesia*. *J Clin Microbiol*. 2007;45(6):2084-7.
18. Hierl T, Reischl U, Lang P, et al. Preliminary evaluation of one conventional nested and two real-time pcr assays for the detection of *Toxoplasma gondii* in immunocompromised patients. *J Med Microbiol*. 2004;53:629-632.
19. Bankole Henry O, Richard O, Eguagie Osareniro O, et al. Asymptomatic malaria among blood donors in Benin city Nigeria. *Iran J Parasitol*. 2014 9(3):415-422
20. Eskandarian A. Seroepidemiology of toxoplasmosis in admitted pregnant women in maternity ward of kowsar teaching and cure center in qazvin-2006. *Iran J Med Microbiol*. 2009;3:73-79.



Cryptosporidiosis in Newborn Calves: Prevalence and Control Strategies in Dairy Herds of Mashhad, Iran

Hassan Esmailjani¹, Gholamreza Mohammadi¹

Corresponding Author: Gholamreza Mohammadi, gmohamad@um.ac.ir

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article Code: 1304

Abstract

Background: *Cryptosporidium parvum* is a protozoan parasite causing severe diarrhea in neonatal calves, impacting dairy farm productivity. This study investigates the prevalence and management of this infection in dairy herds in Mashhad, Khorasan Razavi, Iran.

Methods: From October 2023 to July 2025, fecal samples were collected from 70 diarrheic Holstein-Friesian calves (aged 5–21 days) across seven dairy farms in Mashhad. Calves were housed individually post-colostrum feeding. Samples, obtained rectally, were analyzed using modified Ziehl-Neelsen staining to detect *Cryptosporidium* oocysts under microscopy ($\times 400$ – $\times 1,000$ magnification).

Results: *Cryptosporidium* spp. were detected in 56 of 70 (80%) diarrheic calves, confirming its role as a major pathogen. Clinical signs included watery diarrhea, dehydration, lethargy, weight loss, and abdominal pain. All farms reported oocyst shedding, with higher prevalence in moist, crowded conditions. Environmental persistence of oocysts and inadequate sanitation were key risk factors. Control measures, including 3% hydrogen peroxide disinfection and isolation of sick calves, were effective in reducing transmission. No curative treatments exist, but supportive fluid therapy improved outcomes.

Conclusion: *Cryptosporidium* is a significant cause of neonatal calf diarrhea in Mashhad, with an 80% prevalence. Robust sanitation, isolation, and water management are critical for control. The zoonotic potential underscores the need for worker protection. Further research on seasonal trends and prophylactic treatments is warranted to enhance dairy herd health in Iran.

Keywords: *Cryptosporidium*, Neonatal calves, Diarrhea, Dairy farms.

Introduction:

Cryptosporidium parvum is a protozoan parasite causing significant morbidity in neonatal calves, particularly in dairy systems, due to its high infectivity and environmental resilience (1). It is a leading cause of diarrhea in calves aged 5–21 days, leading to dehydration, malabsorption, and reduced growth (2). The parasite spreads via the fecal-oral route through ingestion of oocysts in contaminated feces, water, or feed (3). Oocysts are shed in high numbers



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



(up to 10^7 per gram of feces) and remain viable for months in cool, moist environments (4). Their resistance to common disinfectants complicates control (5). In Mashhad, Khorasan Razavi, Iran, dairy farms face challenges from *Cryptosporidium* due to intensive management practices. This study hypothesizes that *Cryptosporidium* is a primary cause of neonatal calf diarrhea in Mashhad's dairy herds and aims to assess its prevalence and propose control strategies.

Materials and Methods:

Study Population: Seven dairy farms in Mashhad, Khorasan Razavi, Iran, were studied from October 2023 to July 2025. All farms raised Holstein-Friesian calves, separated from dams after colostrum feeding and housed in individual pens until weaning at 3 months.

Sampling Technique: Fecal samples were collected from 70 calves under 1 month of age with diarrhea, untreated with antibiotics. Samples were obtained rectally by manual stimulation, transported on ice to the parasitology laboratory at Ferdowsi University of Mashhad, and stored at 4°C until processing.

Parasitological Examination: Fecal smears were air-dried, fixed with methanol, and stained using modified Ziehl-Neelsen. Smears were examined at $\times 400$ – $\times 1,000$ magnification. *Cryptosporidium* oocysts appeared as bright red, round bodies (4–6 μm) with sporozoites against a pale green background (4). Samples were scored positive if oocysts with characteristic morphology were detected.

Results and Discussion:

Of 70 diarrheic calves, 56 (80%) tested positive for *Cryptosporidium* spp. Affected calves, aged 5–21 days, exhibited watery diarrhea (yellow or pale, occasionally with mucus or blood), dehydration, lethargy, weight loss, and abdominal pain. All seven farms reported oocyst shedding, indicating widespread infection. Environmental factors, such as moist bedding and crowded pens, correlated with higher prevalence.

The 80% prevalence confirms *Cryptosporidium* as a dominant cause of neonatal calf diarrhea in Mashhad, consistent with global trends (2). Epidemics are driven by oocyst resilience, surviving in cool, humid conditions prevalent in winter (1,6). Poor sanitation and contaminated water sources exacerbate transmission (6).

Oocysts resist chlorine and iodine but are inactivated by 3% hydrogen peroxide or boiling water (72°C for 3 minutes) (7). Pens should be cleaned with 3% hydrogen peroxide, rinsed after 10 minutes to prevent corrosion, and dried, as oocysts are susceptible to desiccation (5).

Isolating sick calves reduces transmission, as one tablespoon of diarrheic feces can infect multiple calves (8). Testing and filtering water sources mitigate oocyst contamination (6).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



No curative treatments exist, but fluid therapy corrects dehydration (9). Halofuginone lactate (7 µg/kg daily for 7 days) reduces oocyst shedding (10).

The study did not assess seasonal variations or other diarrhea-causing pathogens (e.g., rotavirus, *E. coli*), which may co-infect calves. Molecular typing to confirm *C. parvum* was not performed.

The high prevalence underscores the need for rigorous biosecurity. The zoonotic potential of *C. parvum* necessitates protective measures for farm workers (11). Future research should explore seasonal trends and the prophylactic efficacy of halofuginone in Iran's dairy systems.

Acknowledgment:

We thank the staff of the parasitology laboratory at Ferdowsi University of Mashhad for their technical support and the dairy farm managers for their cooperation.

References:

1. Thomson S, Hamilton CA, Hope JC, Katzer F, Mabbott NA, Morrison LJ, et al. Bovine cryptosporidiosis: impact, host-parasite interaction and control strategies. *Vet Res.* 2017; 48:42. doi:10.1186/s13567-017-0447-0
2. Urie NJ, Lombard JE, Shivley CB, Kopral CA, Adams AE, Earleywine TJ, et al. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. *J Dairy Sci.* 2018;101(10):9229–44. doi:10.3168/jds.2017-14019
3. Cho Y, Yoon KJ. An overview of calf diarrhea – infectious etiology, diagnosis, and intervention. *J Vet Sci.* 2014;15(1):1–17. doi:10.4142/jvs.2014.15.1.1
4. Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium*: transmission, detection, and identification. *Int J Parasitol.* 2000;30(12-13):1305–22.
5. Harp JA, Goff JP. Strategies for the control of *Cryptosporidium parvum* infection in calves. *J Dairy Sci.* 1998;81(11):289–94. doi:10.3168/jds.S0022-0302(98)75578-X
6. Smith HV, Caccio SM, Cook N, Nichols RA, Tait A. *Cryptosporidium* and *Giardia* as agents of waterborne disease. *Annu Rev Microbiol.* 2005; 59:247–77.
7. Fayer R, Trout J, Nerad T. Effects of a wide range of temperatures on infectivity of *Cryptosporidium parvum* oocysts. *J Eukaryot Microbiol.* 1996; 43:64S. doi:10.1111/j.1550-7408.1996.tb04995.x
8. Nydam DV, Wade SE, Schaaf SL, Mohammed HO. Number of *Cryptosporidium parvum* oocysts or *Giardia spp* cysts shed by dairy calves after natural infection. *Am J Vet Res.* 2001;62(10):1612–5. doi:10.2460/ajvr.2001.62.1612



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



9. Berchtold J. Treatment of calf diarrhea: intravenous fluid therapy. *Vet Clin North Am Food Anim Pract.* 2009;25(2):73–99. doi: 10.1016/j.cvfa.2008.10.001
10. Brainard J, Hammer CC, Hunter PR, Katzer F, Hurle G, Tyler K. Efficacy of halofuginone products to prevent or treat cryptosporidiosis in bovine calves: a systematic review and meta-analyses. *Parasitology.* 2021; 148:408–19. doi:10.1017/S0031182020002267
11. Chalmers RM, Giles M. Zoonotic cryptosporidiosis in the UK – challenges for control. *J Appl Microbiol.* 2010; 109:1487–97. doi:10.1111/j.1365-2672.2010. 04764.x



Describing the Distribution Pattern in Canine and Feline Parasitic Dermatoses

Javad Khoshnegah¹

Corresponding Author: Javad Khoshnegah, khoshnegah@um.ac.ir

1. Department of Veterinary Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article Code: 1394

Abstract

Background: In dermatology, the distribution of the lesions is a major clue for the diagnosis of skin changes in canine and feline patients.

Methods: In this review, we focus on the importance of distribution pattern of different types of skin lesions in the diagnosis of canine and feline parasitic and non-parasitic dermatoses. This study thoroughly investigated and analyzed the pattern of injuries caused by parasitic and non-parasitic dermatoses, using schematic images to display the different stages of injuries accurately. We, therefore evaluate the skin and hair coat, identify primary and secondary lesions, note distribution pattern and configuration of lesions, evidence of pruritus and the presence of ectoparasites or debris.

Results: In cats, *Demodex* mites are often missed because they can be tricky to diagnose. *Otodectes cynotis* (not only cause itching inside the ears but can also cause crusty or inflamed skin around the face. In *Pelodera* dermatitis, skin problems usually appear on legs, belly, chest, tail, and around the rear end. Most species of fleas move freely around their host's body and can be found virtually anywhere. In dogs with scabies (*Sarcoptes scabiei*), the rash usually starts on the belly, chest, elbows, hocks, and feet. Itching is often severe. In more than 70% of dogs, the face and ears are also affected. Feline scabies, caused by *Notoedres cati*, tends to start at the inside edge of the ears. It spreads quickly to the rest of the ears, face, eyelids, and neck. Canine demodicosis can show up in two main forms. In the localized form, dogs get small bald patches, usually on the face, around the eyes, lips, and sometimes the front legs.

Conclusion: Looking carefully at where and how skin lesions appear is very important for correctly diagnosing skin diseases in dogs and cats.

Keywords: Parasitic dermatoses, Dermatitis, Pattern, Distribution pattern.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Introduction:

In dermatology, the distribution of the lesions is a major clue for the diagnosis of skin changes in canine and feline patients. One should also try to identify the main reaction pattern and to consider all diagnostic hypotheses usually associated with this pattern.

The distribution of lesions often helps in prioritizing differential diagnoses. The clinician should note whether the lesions are localized, multifocal (multiple discrete regions affected such as head, feet, and ventral abdomen) or generalized, and also if the lesions are symmetric or asymmetric. Symmetric lesions usually reflect an internal cause—for example, allergies or an endocrine, metabolic, or immune-mediated disease. Asymmetric lesions are usually a result of infections, certain ectoparasites, or neoplasia. The region(s) of the body affected may provide an additional clue regarding the underlying cause of the skin problem. Terms such as otitis externa, pododermatitis, and nasal dermatitis merely describe the locations of lesions and are not specific diagnoses. For example, the distribution pattern of canine scabies typically involves the ventral portions of the abdomen, the chest, elbows, hocks, and feet. The face and pinna are involved in over 70% of the cases.

Therefore, the purpose of this review study is to investigate and analyze the pattern of injuries caused by parasitic and non-parasitic dermatoses, using schematic images to display the different stages of injuries accurately.

Materials and Methods:

This study thoroughly investigated and analyzed the pattern of injuries caused by demodicosis, using schematic images to display the different stages of injuries accurately. This schematic model helps veterinarians and researchers better understand the patterns in various stages of demodicosis and completely follow the process of skin changes by viewing related images and explanations. These visual and schematic maps offer a suitable method for examining and identifying affected areas, as well as analyzing cellular changes during disease progression. This approach is also efficient for evaluating treatments and formulating disease management plans.

1- Dermatologic examination

To properly diagnose parasitic skin diseases, we need to take a close, hands-on look at the patient. Start by examining the skin and coat carefully — look for both primary lesions (like papules or pustules) and secondary ones (like crusts or lichenification). Pay attention to where the lesions are on the body and what pattern they form. Is the animal itchy? Can you see fleas, mites, or any suspicious debris? Don't forget to check the ears with an otoscope, inspect the feet, mucocutaneous areas, and even the mouth — parasites can show up in all sorts of places.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



1- The process of diagnosing feline and canine dermatoses:

- Evaluation of skin and hair coat,
- Identification of primary and secondary lesions,
- Determination of distribution pattern and configuration of lesions,
- Determination of evidence of pruritus, presence of ectoparasites or debris,
- Checking ears (otoscopic examination), feet, mucocutaneous junctions, and oral cavity.

2- When Localization Suggests the Diagnosis

Facial skin lesions in pets can be caused by a number of parasites. In cats, *Demodex* mites are often missed because they can be tricky to diagnose. *Otodectes cynotis* (ear mites) not only cause itching inside the ears but can also cause crusty or inflamed skin around the face. Another important parasite is *Notoedres cati*, which causes feline scabies and spreads quickly if not treated.

In Pelodera dermatitis, skin problems usually appear on parts of the body that touch the ground—like the feet, legs, belly, chest, tail, and around the rear end. The skin in these areas often looks red and may lose its hair, either in patches or completely. Small red bumps can turn into scabs and flakes, and if the animal keeps scratching, it may develop a skin infection. The itching can be mild or very intense, depending on the case.

Most species of fleas move freely around their host's body and can be found virtually anywhere. In general, *C. felis* seems to prefer the rump and inguinal regions. *E. gallinacean* has a preference for the facial region. During feeding, the flea releases material into the dermis to prevent blood clotting. *S. cuniculi*, the rabbit flea, has a preference for the pinna and periauricular areas. *Tunga* penetrans burrows into the skin and produces significant damage at the site of attachment.

In dogs with scabies (*Sarcoptes scabiei*), the rash usually starts on the belly, chest, elbows, hocks, and feet. Itching is often severe. In more than 70% of dogs, the face and ears are also affected.

Feline scabies, caused by *Notoedres cati*, tends to start at the inside edge of the ears. It spreads quickly to the rest of the ears, face, eyelids, and neck. It can also reach the feet and rear end. This pattern may be due to the cat's habits—like grooming itself and curling up to sleep, which helps spread the mites.

Canine demodicosis (caused by *Demodex* mites) can show up in two main forms. In the localized form, dogs get small bald patches, usually on the face—around the eyes, lips, and sometimes the front legs. These spots might look a bit red or flaky but usually don't itch much.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



In the generalized form, the problem is more serious. Hair loss can spread across large areas of the body, and the skin may become oily, thickened, or infected. The paws, especially between the toes, are often involved. Dogs with weakened immune systems or genetic risk are more likely to develop this severe form.

Results and Discussion:

In cats, *Demodex* mites are often missed because they can be tricky to diagnose. *Otodectes cynotis* (ear mites) not only cause itching inside the ears but can also cause crusty or inflamed skin around the face. In *Pelodera dermatitis*, skin problems usually appear on parts of the body that touch the ground—like the feet, legs, belly, chest, tail, and around the rear end. Most species of fleas move freely around their host's body and can be found virtually anywhere. In general, *C. felis* seems to prefer the rump and inguinal regions. *E. gallinacean* has a preference for the facial region. In dogs with scabies (*Sarcoptes scabiei*), the rash usually starts on the belly, chest, elbows, hocks, and feet. Itching is often severe. In more than 70% of dogs, the face and ears are also affected. Feline scabies, caused by *Notoedres cati*, tends to start at the inside edge of the ears. It spreads quickly to the rest of the ears, face, eyelids, and neck. It can also reach the feet and rear end. Canine demodicosis (caused by *Demodex* mites) can show up in two main forms. In the localized form, dogs get small bald patches, usually on the face, around the eyes, lips, and sometimes the front legs. In the generalized form, the problem is more serious.

The distribution pattern of lesions in dogs with non-demodectic parasitic skin diseases is marked in the schematic images (Figure 1).

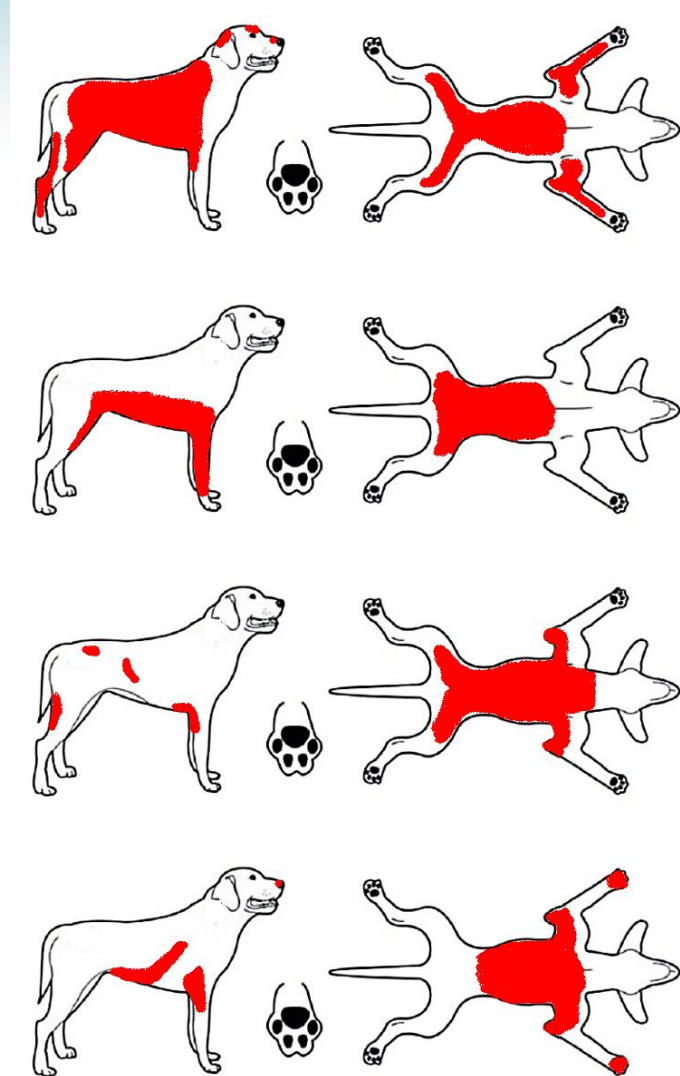


Figure 1. The distribution pattern of lesions in dogs with non-demodectic parasitic skin diseases.

Looking carefully at where and how skin lesions appear is very important for correctly diagnosing some of hair and skin diseases in dogs and cats. However, The results of the present our recently study (Unpublished data) showed that the distribution pattern of skin lesions is not a suitable diagnostic criterion for differentiating dogs suspected of demodicosis dermatosis from other parasitic and non-skin diseases.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Miller WH, Griffin CE, Campbel KA. Diagnostic methods. In: Miller WH, Griffin CE, Campbel KA, eds. Muller and Kirk's Small Animal Dermatology. 7th ed. Elsevier; 2013:68-70.
2. Alizadeh A, Sadr S, Azizzadeh M, Khoshnegah J. Feline dermatoses at Ferdowsi University of Mashhad (Iran): 154 cases (2009–2020) Vet Dermatol. 2024; 35(4):450-452.
3. Khoshnegah J, Movassaghi AR, Rad M. Vet Res Forum, 2013; 4 (2): 99-103.
4. Aslan J, Burrows M, Crothers S, Hoolahan D, Sipstone M, Vogelnest L. Advancing veterinary practice in dermatology. Australian veterinary dermatology advisory panel (AVDAP guidelines for the diagnosis and management of pruritus in dogs. Third edition, December 2024.
5. Favrot C and Habil D. Skin lesions and their distribution in the cat: lessons to be drawn World Small Animal Veterinary Association World Congress Proceedings, 2010.



Investigation of Leucocytozoonosis in Poultry: Epidemiological Analysis, Biology, Economic Consequences, and Control Strategies

Seyed Reza Hosseini¹

Corresponding Author: Seyed Reza Hosseini, rezahosseini2912@gmail.com

1. Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran.

Article Code: 1404

Abstract

Background: *Leucocytozoon* spp. are protozoan parasites infecting domestic and wild birds, transmitted mainly by blood-feeding insects such as black flies (*Simuliidae*) and biting midges (*Ceratopogonidae*). More than 100 species have been identified worldwide, all causing leucocytozoonosis, a disease that affects poultry health and productivity by destroying blood cells, damaging organs, and modulating the immune response. Key species include *L. caulleryi* in chickens, *L. smithi* in turkeys, and *L. simondi* in ducks. The parasite's complex life cycle involves sexual reproduction in insect vectors and asexual reproduction in avian hosts. Understanding the disease's epidemiology, diagnosis, and control is vital to reducing its impact on the poultry industry. The aim of this article is to investigate the epidemiology, biology, economic impact, and control strategies of leucocytozoonosis in poultry.

Methods: In this review article, relevant sources were retrieved from reputable scientific databases such as PubMed, Science Direct, and Google Scholar. The literature search was conducted using keywords related to the topic of the study.

Results: Studies have demonstrated that *Leucocytozoon* infections cause significant cellular damage by destroying blood cells, injuring tissues, and altering the immune system. Clinical signs commonly observed include anemia, weight loss, greenish diarrhea, and increased mortality rates. Diagnosis primarily depends on microscopic identification of gametocytes, with molecular techniques such as PCR offering enhanced accuracy. Environmental factors and climate change contribute to the increased survival and activity of vectors, thereby facilitating the spread of the disease. Pharmacological interventions, including primaquine and pyrimethamine, combined with supportive care, have demonstrated efficacy in improving clinical outcomes.

Conclusion: Leucocytozoonosis is a major threat to global poultry health and productivity. Early diagnosis, vector control, treatment, and supportive care are key to managing the disease. Ongoing research and addressing environmental factors are vital to lessen its economic and health impacts.

Keywords: *Leucocytozoon* spp, Poultry, Blood cells, Blood-feeding insects, PCR.



Introduction:

Leucocytozoon spp. are protozoan parasites belonging to the order Haemosporida within the phylum Apicomplexa, that infect both domestic and wild birds, including chickens (*Gallus gallus domesticus*), ducks (*Anas platyrhynchos domesticus*), turkeys (*Meleagris gallopavo*), and geese (*Anser anser domesticus*) (1). The disease caused by these parasites, known as leucocytozoonosis, is primarily transmitted through blood-feeding vectors such as black flies (*Simuliidae*) and biting midges (*Ceratopogonidae*). To date, more than 100 species of these parasites have been identified worldwide, which vary in their morphological characteristics, genetic sequences, and host preferences (2, 3). These parasites exert significant impacts on poultry health and production performance through complex mechanisms at the cellular level (destruction of white blood cells), tissue level (damage to the liver and spleen), and molecular level (alteration of immune gene expression) (4). A thorough understanding of the biology, epidemiology, and control methods of this disease is essential for optimal poultry management.

Multiple species of *Leucocytozoon* parasites infect poultry. Among them, *L. caulleryi* is primarily found in chickens; *L. smithi* commonly infects turkeys; and *L. simondi* is considered the most prevalent causative agent of disease in ducks (5). These parasites have a complex life cycle that includes sexual stages in the insect vector and asexual stages in the avian host. Schizogony in *Leucocytozoon* consists of two generations. After the sporozoites enter the avian host, these parasites first invade the endothelial cells of the organs' vessels and form the first generation of schizonts. Approximately 5 to 6 days later, the resulting merozoites form the second generation of schizonts in the endothelium of other vessels, causing tissue destruction, hemorrhage, edema, and granulomatous inflammation. Merozoites released from the second generation infect red blood cells around day 14 after infection and enter the gametogony stage. Gametocytes form in red blood cells and cause their deformation and destruction by day 18. Subsequently, when biting mosquitoes feed on infected blood, the gametes are transferred to the mosquito's digestive tract, fertilized, and undergo the stages of sexual reproduction, including the formation of zygotes, ookinetes, and oocysts. After the oocysts burst, the new sporozoites migrate to the mosquito's salivary glands and enter the body of the new host bird when it bites (6).

The aim of this article is to provide a comprehensive investigation of leucocytozoonosis in poultry, encompassing its epidemiology, biology, economic impact, and control strategies. This study seeks to offer valuable insights for effective management of the disease in the poultry industry.



Materials and Methods:

In this review article, relevant scientific sources were systematically retrieved from several reputable and widely recognized academic databases, including PubMed, ScienceDirect, and Google Scholar. These platforms were selected for their comprehensive coverage of peer-reviewed literature and their high reliability in providing access to scientific publications.

A structured search strategy was employed, utilizing a carefully selected set of keywords and search terms that aligned closely with the central theme and objectives of the study. To refine the search results and ensure relevance, additional filters were applied based on publication date, language, and type of study (e.g., review articles, clinical trials, or original research).

This rigorous and methodical approach enabled the collection of a broad yet focused body of evidence, which forms the foundation for the discussions and analyses presented throughout this article.

Results and Discussion:

The present study highlights that infection with *Leucocytozoon* parasites is a significant cause of clinical disease in poultry, characterized by anorexia, weight loss, decreased feed conversion ratio, anemia, greenish feces, and high mortality rates. Pathological findings in infected birds include pneumonia, pulmonary congestion, alveolar capillary occlusion (especially in turkeys), liver necrosis, ovarian follicular atrophy, oviduct edema, splenomegaly, lymphocytic infiltration of the liver and heart, hemosiderosis, and subcutaneous hemorrhages (7-9). These findings underscore the substantial clinical impact and broad pathological consequences of this parasite in growing birds. Hematological analysis reveals a decrease in mature red blood cells and an increase in immature (polychromatophilic) Red blood cells in chicks infected with *Leucocytozoon* as well as helminths (10).

Globally, Leucocytozoonosis is widely distributed, particularly in areas with abundant vector populations such as rivers, wetlands, and other moist environments. The prevalence of the disease is influenced by environmental factors including climate and landscape, as well as host-related variables (11). In Iran, particularly in Lorestan Province, studies have demonstrated notable infection rates among domestic poultry species, with prevalence reported as 5.1% in chickens, 4.3% in geese, 3.6% in ducks, and 2.9% in turkeys. Co-infection with multiple *Leucocytozoon* species within flocks further complicates disease management and control efforts (12).

From an economic perspective, *Leucocytozoon* infections cause considerable losses in the poultry industry by directly and indirectly reducing production performance. Key impacts include decreased growth rates, up to 30% reduction in egg production, increased incidence of eggs with soft or brittle shells, mortality rates exceeding 50% in chicks and young birds, and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



increased feed conversion ratios. Additionally, the costs associated with treatment, prevention, and sanitation contribute to the financial burden on producers (13-15).

Climate change, including gradual increases in global temperatures, altered precipitation patterns, and higher humidity levels, plays a critical role in altering vector behavior and distribution. These environmental shifts create more favorable conditions for vectors by extending their activity periods and expanding their geographic ranges. Consequently, increased vector-host interactions, particularly involving poultry, promote the geographical spread and higher incidence of vector-borne diseases like leucocytozoonosis. Regions experiencing significant warming and increased rainfall are particularly vulnerable to heightened disease risks. Moreover, these environmental changes present new challenges for disease control and prevention strategies (16).

Definitive diagnosis of *Leucocytozoon* infection is made by detecting gametocytes in blood and tissue samples. In *L. caulleryi* infections, pigmentless gametogony occurs exclusively within red blood cells (17). Histologically, second-generation schizonts in blood vessel endothelial cells, thrombus formation, hemorrhage, and inflammation are characteristic. Hemosiderin deposits in the liver and spleen, resulting from red blood cell destruction, are also observed. Large second-generation schizonts (megaloschizontes) are commonly found in field cases. Molecular methods such as PCR and immunological assays like ELISA provide higher sensitivity and specificity than microscopy, with PCR being particularly sensitive for detecting *Leucocytozoon* (7).

Vector control through drainage, insecticides, and hygiene is key for prevention. Treatment primarily involves primaquine, effective against gametocytes, and combination therapy of pyrimethamine with sulfadimethoxine, which has shown partial success (18, 19). Supportive care also enhances treatment outcomes.

Recent studies report that daily administration of 0.5 g *Artemisia annua* powder in chickens improved weight gain and reduced parasite load, lowering mortality and symptoms (20). Additionally, immunization with the oil-adjuvanted recombinant R7 vaccine targeting *L. caulleryi* schizonts shows promising protective effects (21, 22).

Leucocytozoonosis is one of the most significant and prevalent diseases affecting poultry, with considerable economic and health impacts. This disease can cause significant reduction in productivity, loss of life, and disruption of poultry flock performance, which in turn affects the poultry industry and its related economy. Therefore, rapid and accurate diagnosis of this disease is of great importance in order to implement timely and effective treatment and control measures. Additionally, the development and implementation of comprehensive strategies for vector control, including environmental management and the targeted use of antiparasitic drugs, play a crucial role in reducing the prevalence and spread of this disease.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Acknowledgment:

With utmost respect and gratitude, I hereby sincerely thank all the esteemed professors and colleagues who assisted me in conducting this study. Their valuable guidance, support, and encouragement played an indispensable role in the advancement of this research, for which I am deeply grateful.

References:

1. Bennett, G. F., Peirce, M. A., & Ashford, R. W. (1993). Avian haematozoa: mortality and pathogenicity. *Journal of Natural History*, 27(5), 993-1001.
2. Adler, P. H., & McCreddie, J. W. (2019). Black flies (Simuliidae). In *Medical and veterinary entomology* (pp. 237-259). Academic Press.
3. Jumpato, W., Wannasingha, W., Jaroenchaiwattanachote, C., Mintara, R., Wongpakam, K., Adler, P. H., & Pramual, P. (2024). Diversity and prevalence of Leucocytozoon in black flies (Diptera: Simuliidae) of Thailand. *Parasites & Vectors*, 17(1), 475.
4. Zhou, Z., Shen, B., & Bi, D. (2020). Management of pathogens in poultry. In *Animal agriculture* (pp. 515-530). Academic Press.
5. Nakamura, K. (2022). Leucocytozoon caulleryi infection in chickens: etiology, pathology, and diagnosis. *Japan Agricultural Research Quarterly: JARQ*, 56(2), 121-127.
6. Akiba, K. (1970). Leucocytozoonosis of chickens.
7. Lee, H. R., Koo, B. S., Jeon, E. O., Han, M. S., Min, K. C., Lee, S. B., ... & Mo, I. P. (2016). Pathology and molecular characterization of recent Leucocytozoon caulleryi cases in layer flocks. *Journal of biomedical research*, 30(6), 517.
8. Adamu, A. (2017). Epidemiology of haemoparasite infection and their effects on hematological parameters in scavenging chickens of west Gojjam administrative zone, Amhara region, Ethiopia (Doctoral dissertation, MSc. Thesis. University of Gondar, Ethiopia).
9. . Atkinson, C. T., & Van Atkinson, C. (1991). Pathogenicity and epizootiology of avian haematozoa: Plasmodium, Leucocytozoon, and Haemoproteus. *Bird-parasite interactions*, 19-48.
10. Jin, K., Homma, S., & Omi, Y. (1976). Occurrence of leucocytozoonosis in southern part of Hokkaido [chickens, Japan].
11. Sehgal, R. N. (2015). Manifold habitat effects on the prevalence and diversity of avian blood parasites. *International Journal for Parasitology: Parasites and Wildlife*, 4(3), 421-430.
12. AN Emami, R. (2013). Leucocytozoonosis in domestic birds in southwestern Iran: an ultrastructural study.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



13. Alabadi, I. K., ABBASS, Z. A. A., ALKHUZAIE, S. S., Khayoon, H. A., & Alsaadawi, M. (2024). Detection and prevalence of *Leucocytozoon* spp. in Local chicken breeds in Al Muthanna province of Iraq. *Online J. Anim. Feed Res*, 14(5), 321-329.
14. Jones, P. J., Niemi, J., Christensen, J. P., Tranter, R. B., & Bennett, R. M. (2018). A review of the financial impact of production diseases in poultry production systems. *Animal production science*, 59(9), 1585-1597.
15. Nakamura, K. (2022). *Leucocytozoon caulleryi* infection in chickens: etiology, pathology, and diagnosis. *Japan Agricultural Research Quarterly: JARQ*, 56(2), 121-127.
16. Santiago-Alarcon, D., Palinauskas, V., & Schaefer, H. M. (2012). Diptera vectors of avian Haemosporidian parasites: untangling parasite life cycles and their taxonomy. *Biological Reviews*, 87(4), 928-964.
17. Hidaka, R., Nakajima, S., Kuriwaki, K., Goto, Y., Matsumoto, M., & Sakotsubo, T. (2015). Broiler chicken malaria caused by *Plasmodium juxtanucleare* detected during poultry inspection. *Journal of the Japanese Society on Poultry Diseases (Japan)*, 51(3).
18. Alabadi, I. K., ABBASS, Z. A. A., ALKHUZAIE, S. S., Khayoon, H. A., & Alsaadawi, M. (2024). Detection and prevalence of *Leucocytozoon* spp. in Local chicken breeds in Al Muthanna province of Iraq. *Online J. Anim. Feed Res*, 14(5), 321-329.
19. Zhao, W., Pang, Q., Xu, R., Liu, J., Liu, S., Li, J., & Su, X. Z. (2016). Monitoring the prevalence of *Leucocytozoon sabraezesi* in southern China and testing tricyclic compounds against gametocytes. *PLoS One*, 11(8), e0161869.
20. Chiang, Y. H., Lin, Y. C., Wang, S. Y., Lee, Y. P., & Chen, C. F. (2022). Effects of *Artemisia annua* on experimentally induced leucocytozoonosis in chickens. *Poultry Science*, 101(4), 101690.
21. Saeed, Z. F., Kadhim, B. A., Al-Yasari, A. M. R., Kheder, R. K., Abdulhussein, S. A., James, H., & Alsaadawi, M. A. (2022). Proinflammatory activation of osteoclasts due to high prolactin level. *Iraqi Journal of Science*, 5166-5185.
22. Ito, A., & Gotanda, T. (2004). Field efficacy of recombinant R7 vaccine against chicken leucocytozoonosis. *Journal of veterinary medical science*, 66(5), 483-487.



In vitro Evaluation of Susceptibility of *Dermanyssus gallinae* to Phoxim, Sevin and Cypermethrin

Maryam Keyghobadi¹, Ali Moshaverinia²

Corresponding Author: Ali Moshaverinia, moshaverinia@um.ac.ir

1. M.Sc. in Veterinary Parasitology. Graduated from Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article Code: 1443

Abstract

Background: The poultry red mite, *Dermanyssus gallinae* known as the most important blood-feeding parasite of poultry in the world affecting egg layer and breeder flocks. This mite represents a significant threat to egg production industry in many parts of the world including Iran. The extensive and improper use of chemical compounds in infected poultry houses can lead to developing resistance of this parasite. The aim of this study was to evaluate the susceptibility of *D. gallinae* to Phoxim, Sevin and Cypermethrin under laboratory conditions.

Methods: Five infested poultry farms were sampled and collected *D. gallinae* mites transferred to parasitology laboratory. Samples from each poultry farm (each batch including 25 mites) were individually exposed to the concentrations of 0.001, 0.01, 0.1 and 1v/v of Phoxim, Cypermethrin and Sevin, according to contact test invented by Thind and Muggleton 1998 for 24 h; after which the test cells were opened and live and dead mites were counted.

Results: The effectiveness of cypermethrin in all studied poultry farms was very low and the highest mean efficacy of this acaricide was 20.19% in poultry farm A. In other sampled poultry farms, determined efficacy for Cypermethrin was less than 10%. Phoxim in one sampled poultry farm (B) had an effectiveness of 79.61%, but in other farms, its efficiency was more than 90%. Sevin also had a relatively high efficacy and the lowest efficacy was observed for poultry farm B (55.04%) and in other poultry farms its efficiency was more than 70%.

Conclusion: The results of this study showed that *D. gallinae* sampled from five studied farms was significantly resistant to Cypermethrin. The results also revealed that Sevin in low concentrations did not has proper efficacy but the most of *D. gallinae* mites sampled from five poultry farms were susceptible to Phoxim.

Keywords: Cypermethrin, Phoxim, Sevin, Susceptibility, *Dermanyssus gallinae*, Iran.



Introduction:

The poultry red mite (PRM), *Dermanyssus gallinae* parasitizes laying hens and wild birds and it can cause anemia, weakness, reduction in egg production and growth. Heavy Infestation with this mite may lead to death in affected birds. These mites not only can adversely affect egg production, but also act as vectors of some pathogenic agents such as chicken pox virus, Newcastle virus, fowl spirochaetosis, pullorum and typhoid agents (1). Chemical acaricides have conventionally been used for mite control in infested avicultures (1). The extensive and improper usage of chemical compounds in infested poultry houses can lead to developing resistance of this parasite (2). Therefore, the toxicity and effectiveness of common acaricide should be checked periodically. The aim of this study was to evaluate the susceptibility of *D. gallinae* to Phoxim, Sevin and Cypermethrin under laboratory conditions.

Materials and Methods:

Five infested poultry farms located in Khorasan Razavi province were sampled and collected *D. gallinae* mites transferred to parasitology laboratory. Samples from each poultry farm (each batch including 25 mites) were exposed to the concentrations of 0.001, 0.01, 0.1 and 1v/v of Phoxim, Cypermethrin and Sevin, according to contact test invented by Thind and Muggleton 1998 (3). The filter paper of control group was treated with water. The mites were confined in the test cell for 24 h, after which the test cells were opened and live and dead mites were counted. The filter experiments were repeated at five concentrations.

Results and Discussion:

The results showed that the effectiveness of cypermethrin against *D. gallinae* in all studied poultry farms was very low and the highest mean efficacy of this acaricide was 20.19% in poultry farm A. In other sampled poultry farms, determined efficacy for Cypermethrin was less than 10%. Phoxim in one sampled poultry farm (B) had an effectiveness of 79.61%, but in other poultry farms, its efficiency was more than 90%. Sevin also had a relatively high efficacy and the lowest efficiency was observed for poultry farm B (55.04%) and in other poultry farms its efficiency was more than 70%. The results of this study showed that *D. gallinae* sampled from five studied farms was significantly resistant to Cypermethrin. Reports of resistance to cypermethrin date back to 1990, when Abo-Taka documented resistance in *Dermanyssus* in Egypt (4). In subsequent years, reduced sensitivity or resistance to various pyrethroids—particularly cypermethrin—was reported by Fiddess et al. (2005) and Thind et al. (2007) (5, 6). In recent years, molecular and biochemical techniques have largely replaced bioassay



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



methods for detecting resistance. Koc et al. (2022) demonstrated severe resistance of *Dermanyssus gallinae* to cypermethrin in Turkey using bioassay, molecular, and biochemical approaches (7). Wang et al. (2021) reported high and very high resistance of poultry red mite to cypermethrin and permethrin in China (8). Both bioassay and molecular studies have shown strong resistance of *Dermanyssus* to cypermethrin (8).

The results also revealed that Sevin in low concentrations did not have proper efficacy but the most of *D. gallinae* mites sampled from five poultry farms were susceptible to Phoxim. Since 2010, phoxim has been one of the few acaricides approved for the control of PRM in poultry houses across most of Europe. Pugliese et al. (2019), in a seven-year study on the efficacy of the acaricide phoxim, demonstrated that since 2015, its effectiveness has declined in certain poultry farms in Italy (9). A decrease in sensitivity to phoxim was reported in mites collected from an ornamental bird farm in Italy (10). Another report of diminished sensitivity to phoxim in *Dermanyssus* mites emerged from Germany, France, and Spain (11). Koc et al. (2022), through bioassay studies, reported resistance of *Dermanyssus* to phoxim in Turkey (7). Given that phoxim is one of the few acaricides approved for use in poultry farms in many European countries—and considering that this compound is produced and utilized in Iran—the observed decrease in sensitivity may serve as a warning sign for the emergence of resistant populations and their potential spread in laying hen farms across Iran.

The findings of the present study indicate that the acaricides cypermethrin and sevin do not exhibit effective performance against *Dermanyssus* mites collected from sampled poultry farms, suggesting that these mites have developed resistance to these compounds. Regarding phoxim, reduced efficacy has been observed in certain farms, which may serve as a warning sign for the emergence of resistance.

Acknowledgment:

We would like to thank Ferdowsi University of Mashhad for financial support of this project. We also thank Mr. Hamid Eshrati for his technical assistance.

References:

1. Sigognault Flochlay, A., Thomas, E., Sparagano, O. Poultry red mite (*Dermanyssus gallinae*) infestation: a broad impact parasitological disease that still remains a significant challenge for the egg-laying industry in Europe. *Parasites & Vectors*. 2017; 10(1): 357.
2. Marangi, M., Cafiero, M. A., Capelli, G., Camarda, A., Sparagano, O. A. E., & Giangaspero, A. (2009). Evaluation of the poultry red mite, *Dermanyssus gallinae*



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- (Acari: Dermanyssidae) susceptibility to some acaricides in field populations from Italy. *Experimental and Applied Acarology*. 2009; 48(1): 11-18.
3. Thind, BB, & Muggleton, J. A new bioassay method for the detection of resistance to pesticides in the stored product mite *Acarus siro* (Acari: Acaridae). *Experimental & applied acarology*. 1998; 22(9): 543-552.
 4. Abo-Taka S. Toxicity of certain pesticides against the chicken mite, *Dermanyssus gallinae*. *Medical and Veterinary Entomology*. 1990; 4(1):125-6.
 5. Fiddes, M. D., et al. Prevalence of the poultry red mite (*Dermanyssus gallinae*) in England. *Veterinary Record*. 2005; 157: 233.
 6. Thind B, Ford H .Assessment of susceptibility of the poultry red mite *Dermanyssus gallinae* (Acari: Dermanyssidae) to some acaricides using an adapted filter paper based bioassay. *Veterinary Parasitology*. 2007; 144(3-4):344-8.
 7. KOÇ, Nafiye, et al. Biochemical and molecular mechanisms of acaricide resistance in *Dermanyssus gallinae* populations from Turkey. *Pesticide biochemistry and physiology*. 2022; 180: 104985.
 8. Wang F, Wang M, Xu F, Liang D, Pan B. Survey of prevalence and control of ectoparasites in caged poultry in China. *Veterinary record*. 2010; 167(24):934-7.
 9. Pugliese N, Circella E, Cocciolo G, Giangaspero A, Horvatek Tomic D, Kika TS, et al. Efficacy of λ -cyhalothrin, amitraz, and phoxim against the poultry red mite *Dermanyssus gallinae* De Geer, 1778 (Mesostigmata: Dermanyssidae): an eight-year survey. *Avian Pathology*. 2019; 48(1): 35-43.
 10. Guerrini, A., et al. Evaluation of the Acaricidal Effectiveness of Fipronil and Phoxim in Field Populations of *Dermanyssus gallinae* (De Geer, 1778) from Ornamental Poultry Farms in Italy. *Veterinary sciences*. 2022, 9.9: 486.
 11. Thomas E., Zoller H., Liebisch G., et al. In vitro activity of fluralaner and commonly used acaricides against *Dermanyssus gallinae* isolates from Europe and Brazil. *Parasites & vectors*. 2018; (11): 1-10.



Integration of Next-Generation Sequencing and Metagenomics for Detection of *Cryptosporidium* and *Giardia* in Ready-to-Eat Fresh Produce

Hediyeh karbasi^{1,2}, Mohammad Mahdi Teymori^{1,2}, Parnian Pezeshki¹, Atefeh Sarafan Sadeghi¹

Corresponding Author: Atefeh Sarafan Sadeghi, sarafana@varastegan.ac.ir

1. Department of Food Science and Technology (Quality Control and Hygiene), Varastegan Institute for Medical Sciences, Mashhad, Iran.
2. Student Research Committee, Varastegan Institute for Medical Sciences, Mashhad, Iran.

Article Code: 1449

Abstract

Background: *Cryptosporidium* and *Giardia* are significant protozoan parasites responsible for waterborne and foodborne diseases globally. Their presence in ready-to-eat (RTE) fresh produce poses a critical public health concern, especially in regions with insufficient sanitation and agricultural hygiene. Traditional detection techniques, such as microscopy and immunoassays, often lack the sensitivity and specificity to detect low-level or mixed infections.

Methods: Next-generation sequencing (NGS) and metagenomics provide promising alternatives for the sensitive, high-throughput detection of protozoan pathogens in complex matrices such as fresh produce. This review explores the integration of NGS technologies, including amplicon-based and shotgun metagenomics, to detect *Cryptosporidium* and *Giardia* in RTE vegetables and fruits. A comprehensive literature search was conducted using PubMed, ScienceDirect, and Google Scholar for relevant peer-reviewed studies published from 2007 to 2025.

Results: Several recent studies have demonstrated that NGS-based approaches allow for the accurate identification of *Cryptosporidium* and *Giardia* species and genotypes, even in samples with low parasitic loads. Shotgun metagenomics, in particular, offers the advantage of untargeted detection, capturing not only protozoa but also bacterial and viral co-contaminants. Integration of molecular enrichment protocols, such as IMS or pre-amplification, further enhances NGS performance.

Conclusion: The combination of NGS and metagenomics offers a powerful and comprehensive strategy for monitoring protozoan contamination in RTE produce. While challenges such as cost, bioinformatic complexity, and the need for standardized protocols persist, these approaches represent the future of foodborne pathogen surveillance and outbreak prevention.

Keywords: *Cryptosporidium*, *Giardia*, Metagenomics, Next-generation sequencing, Ready-to-eat produce.



Introduction:

Foodborne protozoan infections continue to pose significant global public health threats, particularly in low- and middle-income countries where food hygiene and sanitation are often inadequate. Among the most concerning protozoan parasites in this context are *Cryptosporidium* and *Giardia*, both of which are responsible for widespread outbreaks of diarrheal diseases (1). These pathogens are commonly transmitted through contaminated water and food, with ready-to-eat (RTE) fresh produce being increasingly implicated as a vehicle for transmission (1,2).

RTE vegetables and fruits, such as lettuce, parsley, strawberries, and spinach, are minimally processed and typically consumed raw, providing a direct route for parasite ingestion[2]. Contamination can occur at multiple points along the production and supply chain, including during irrigation with contaminated water, contact with animal feces, handling by infected workers, or through the use of unclean packaging materials (3). Conventional methods for detecting *Cryptosporidium* and *Giardia* in fresh produce, including microscopy, immunofluorescence assays (IFA), and enzyme-linked immunosorbent assays (ELISA), while widely used, often fall short in sensitivity and specificity, especially when parasite loads are low or when mixed microbial communities are present (4).

In recent years, molecular methods have significantly advanced pathogen detection, with PCR-based techniques offering improved sensitivity and specificity (3,4). However, these methods typically require prior knowledge of the target organism and are limited in their ability to detect unknown or emerging genotypes (5). To overcome these limitations, next-generation sequencing (NGS) technologies and metagenomic approaches have emerged as powerful tools capable of providing a comprehensive overview of microbial communities in a given sample (6).

NGS allows for the sequencing of millions of DNA fragments simultaneously, enabling high-throughput, culture-independent identification of microorganisms, including protozoa (6,7). Metagenomics, which refers to the direct analysis of genetic material recovered from environmental samples, can uncover the presence of pathogens without the need for specific primers or probes (8). The integration of these technologies in food safety surveillance has revolutionized the detection of foodborne pathogens and holds great promise for monitoring protozoan contamination in RTE produce (9,10).

This review aims to explore the current advancements and applications of NGS and metagenomic techniques for the detection of *Cryptosporidium* and *Giardia* in ready-to-eat fresh produce (11,12). By summarizing recent scientific literature, identifying key technological developments, and discussing existing challenges, this article provides a comprehensive overview of how modern sequencing technologies can contribute to safer food production and more effective outbreak prevention (13).



Materials and Methods:

This review was based on a targeted search of peer-reviewed literature from 2010 to 2025, using databases including PubMed, ScienceDirect, and Google Scholar. Keywords such as *Cryptosporidium*, *Giardia*, NGS, metagenomics, and ready-to-eat produce were applied.

Relevant articles focusing on the use of NGS and metagenomic approaches for detecting protozoa in fresh produce were selected. Only English-language, peer-reviewed studies were included. The most significant and up-to-date findings were analyzed and summarized.

Results and Discussion:

The detection of protozoan parasites in food systems, particularly *Cryptosporidium* and *Giardia*, has long posed significant analytical challenges due to their low infectious dose, resilience in harsh environmental conditions, and the complexity of food matrices such as ready-to-eat (RTE) produce (3,5). Traditional techniques like light microscopy, immunoassays, and antigen-based detection are often hindered by low sensitivity, high labor intensity, and limited applicability to diverse food samples (14). Moreover, the inability of these methods to discriminate between species, genotypes, and viable versus non-viable organisms restricts their utility in comprehensive food safety surveillance (6-8).

In response to these limitations, molecular technologies have rapidly evolved, with next-generation sequencing (NGS) and metagenomics emerging as transformative approaches in parasitological diagnostics (11,13). These techniques enable high-throughput, culture-independent detection of pathogens based on the analysis of DNA directly extracted from environmental or food samples [15]. Their power lies in the ability to detect multiple pathogens simultaneously, identify novel or uncharacterized strains, and reveal co-infections or interactions with other microbiota in the sample (14,15). These features are especially valuable in RTE vegetables and fruits, which often harbor diverse and complex microbial communities (16).

Amplicon-based sequencing, typically targeting conserved genetic markers such as 18S rRNA, provides species-level resolution of known protozoa (10). While targeted sequencing offers high sensitivity, its dependency on specific primers limits its scope and risks overlooking unexpected or genetically divergent strains. In contrast, shotgun metagenomic sequencing provides an unbiased view of the entire genomic content within a sample. This approach not only facilitates broad-spectrum pathogen detection but also supports microbial source tracking, identification of antimicrobial resistance genes, and detection of microbial shifts associated with contamination events (12,14).

Implementing these tools in the context of RTE produce has revealed important insights into contamination patterns and risk factors (7). For instance, the presence of parasite DNA in



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



seemingly clean or prewashed vegetables suggests that current hygiene and decontamination protocols may be insufficient to eliminate microbial hazards (8,9). Furthermore, detection of protozoa alongside bacterial and viral contaminants points to a multifactorial risk scenario, especially in regions with limited control over agricultural water quality and post-harvest handling (13,14).

Despite their strengths, several technical and logistical challenges continue to limit the widespread application of NGS and metagenomics. Efficient and consistent extraction of high-quality nucleic acids from plant matrices remains a significant hurdle due to inhibitors such as polyphenols and polysaccharides. Additionally, the high cost of sequencing platforms, the need for trained personnel, and the complexity of bioinformatic data analysis restrict accessibility, particularly in routine food monitoring programs (6,9).

Another critical limitation is the interpretation of sequencing data (16). While the presence of protozoan DNA indicates contamination, it does not necessarily reflect organism viability or infectious potential (9). This distinction is essential for accurate risk assessment, as detection of DNA fragments from dead organisms could lead to overestimation of public health threats. Standardization of protocols for sample processing, sequencing, and data interpretation is urgently needed to support comparability of results across laboratories and geographic regions (13,14).

Nonetheless, ongoing advances in sample enrichment techniques, portable sequencing devices, and user-friendly bioinformatics pipelines are steadily reducing these barriers (8-10).

The potential for real-time, field-deployable diagnostics based on metagenomics offers a future where foodborne pathogen surveillance can be faster, more comprehensive, and more responsive to emerging threats. Integration of these technologies into regulatory frameworks and industry practices could dramatically enhance the detection of *Cryptosporidium*, *Giardia*, and other protozoa in the global food supply chain (5,7).

In conclusion, the integration of NGS and metagenomic approaches offers a paradigm shift in the detection of protozoan contamination in RTE fresh produce. These tools provide unparalleled resolution, sensitivity, and breadth of detection, allowing for a more complete understanding of foodborne parasitic risks. As technological, economic, and regulatory challenges are gradually addressed, the role of these methods in proactive food safety systems is expected to expand, ultimately contributing to the reduction of disease burden and strengthening of global food security (5).

Nonetheless, ongoing advances in sample enrichment techniques, portable sequencing devices, and user-friendly bioinformatics pipelines are steadily reducing these barriers. The potential for real-time, field-deployable diagnostics based on metagenomics offers a future where foodborne pathogen surveillance can be faster, more comprehensive, and more responsive to



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



emerging threats. Integration of these technologies into regulatory frameworks and industry practices could dramatically enhance the detection of *Cryptosporidium*, *Giardia*, and other protozoa in the global food supply chain (3).

References:

1. Naushad S, Gao S, Duceppe MP, Dupras V, Reiling SJ, Merks H, et al. Metagenomic detection of protozoan parasites on leafy greens aided by a rapid and efficient DNA extraction protocol. *Front Microbiol.* 2025;16:1566579.
2. Nichols H, Santin M, Maloney JG. Establishing the performance of next-generation amplicon sequencing for detection of *Giardia duodenalis* in ready-to-eat packaged leafy greens. *J Food Prot.* 2024;87(10):100355.
3. Faria CP, Pereira A, Almeida D, Pinto M, Lourenço Á, Sousa MC. Molecular investigation of ready-to-eat salads for *Giardia duodenalis* and *Cryptosporidium spp.* in Portugal. *Food Waterborne Parasitol.* 2023;30:e00190.
4. Nasser AM. Transmission of *Cryptosporidium* by fresh vegetables. *J Food Prot.* 2022;85(12):1737–1744.
5. Utaaker KS, Kumar A, Joshi H, Chaudhary S, Robertson LJ. Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in vegetables sold across different counters in Chandigarh, India. *Int J Food Microbiol.* 2017;263:1–8.
6. Berrouch S, Escotte-Binet S, Madline A, Aubert D, Nast E, La Carbona S, et al. Detection methods and prevalence of transmission stages of *Toxoplasma gondii*, *Giardia duodenalis* and *Cryptosporidium spp.* in fresh vegetables: a review. *Parasitology.* 2020;147(5):516–532.
7. Berrouch S, Escotte-Binet S, Harrak R, Huguenin A, Flori P, Favenec L, et al. Protozoan parasites and leafy greens in Marrakech: Study of occurrence using a molecular method. *Acta Parasitol.* 2022;67(1):546–554.
8. Caradonna T, Marangi M, Del Chierico F, Ferrari N, Reddel S, et al. Detection and prevalence of protozoan parasites in ready-to-eat packaged salads on sale in Italy. *Food Microbiol.* 2017;67:67–75.
9. Huvarova V, Kralik P, Vasickova P, Kubankova M, Verbikova V, Slany M, et al. Tracing of selected viral, bacterial, and parasitic agents on vegetables and herbs originating from farms and markets. *J Food Sci.* 2018;83(10):3044–3053.
10. Ferreira FP, Caldart ET, Freire RL, Mitsuka-Breganó R, de Freitas FM, Miura AC, et al. Effect of water source and soil supplementation on parasite contamination in organic vegetable gardens. *Rev Bras Parasitol Vet.* 2018;27(3):327–337.
11. Rzeżutka A, Nichols RAB, Connelly L, Kaupke A, Cook N, Smith HV. *Cryptosporidium* oocysts on fresh produce from areas of high livestock production in Poland. *Int J Food Microbiol.* 2010;139(1):96–101.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



12. Zahedi A, Gofton AW, Greay T, Monis P, Oskam C, Ball A, et al. Profiling the diversity of *Cryptosporidium* species and genotypes in wastewater treatment plants in Australia using next generation sequencing. *Sci Total Environ*. 2018;644:635–648.
13. Ma L, Sotiriadou I, Cai Q, Karanis G, Wang G, Lu Y, et al. Detection of *Cryptosporidium* and *Giardia* in agricultural and water environments in the Qinghai area of China by IFT and PCR. *Parasitol Res*. 2014;113(9):3177–3184.
14. Berrouch S, Escotte-Binet S, Biary A, Nast E, Laaouidi Y, Aubert D, et al. Investigation of *Toxoplasma gondii*, *Giardia duodenalis*, and *Cryptosporidium spp.* in drinking waters in Marrakech, Morocco. *J Food Prot*. 2023;86(8):100112.
15. Nguyen TT, et al. Prevalence and molecular characterization of *Giardia* and *Cryptosporidium* detected in fresh produce. *Food Waterborne Parasitol*. 2016;2–3:49–57.
16. Smith HV. *Cryptosporidium* and *Giardia* as foodborne zoonoses. *Int J Food Microbiol*. 2007;117(3):67–84.



The application of Artificial Intelligence to Combat Leishmaniasis: A Comprehensive Review of Innovations in Diagnosis, Therapeutics, and Epidemiological Control

Afsaneh Dolatkah¹, Adel potin¹

Corresponding Author: Afsaneh Dolatkah, afsaneh.dolatkah@gmail.com

1. Department of parasitology, entomology and mycology, Faculty of Medicine, Tabriz university of Medical sciences.

Article Code: 1463

Abstract

Background: Leishmaniasis remains a significant global health threat, particularly affecting vulnerable populations where conventional control strategies face persistent obstacles. Challenges include the low sensitivity and expertise-dependent nature of diagnostic methods, limited therapeutic efficacy due to growing drug resistance, and the reactive posture of public health surveillance. The emergence of Artificial Intelligence (AI), particularly its subfields of machine learning (ML) and deep learning (DL), offers a transformative toolkit to address these long-standing issues.

Methods: This comprehensive review article synthesizes and critically evaluates the burgeoning body of research on AI applications across the entire spectrum of leishmaniasis control. We delve deeply into the role of AI in revolutionizing “diagnosis”, examining the performance of specific deep learning architectures (e.g., CNNs like DenseNet201, ResNet, and VGG19) for the automated analysis of microscopic slides and clinical lesion photographs.

Results: The AI's burgeoning impact on “therapeutics”, from accelerating “in silico” drug discovery and repurposing efforts to developing predictive models for treatment response were explored, thereby paving the way for personalized medicine.

Conclusion: By consolidating current evidence and outlining future research trajectories, this article underscored the necessity of a synergistic collaboration between computational scientists, parasitologists, clinicians, and public health authorities to translate these powerful technological innovations into tangible, life-saving interventions in the global fight against leishmaniasis.

Keywords: Artificial Intelligence, Machine Learning, Deep Learning, Leishmaniasis, Convolutional Neural Networks (CNN).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Introduction:

Leishmaniasis, a parasitic disease prevalent in 98 countries (1), presents a major public health challenge with forms ranging from disfiguring cutaneous to fatal visceral leishmaniasis (2). With up to one million new cases yearly, it imposes a severe socioeconomic burden, particularly in resource-limited areas (3, 4).

Conventional control methods are failing; the diagnostic gold standard (microscopy) is labor-intensive with low sensitivity (2, 5), and mainstay therapies face issues of toxicity and emerging resistance (6). Artificial Intelligence (AI) offers a paradigm shift, leveraging machine and deep learning to analyze complex data and overcome these obstacles (4, 7). This review analyzes AI's transformative potential in enhancing diagnostics, accelerating drug discovery, and enabling predictive epidemiological surveillance for leishmaniasis, while also considering implementation challenges.

Materials and Methods:

Revolutionizing Diagnosis with Artificial Intelligence

Accurate and timely diagnosis is the critical first step in effective patient management and disease control. AI-driven image analysis is emerging as a powerful tool to augment or even automate this process, addressing the core limitations of traditional microscopy and clinical assessment.

Automated Detection of Amastigotes in Microscopic Images

Artificial intelligence, particularly Convolutional Neural Networks (CNNs), has demonstrated outstanding success in automating the diagnosis of leishmaniasis from microscopic images, with models achieving accuracy rates above 99%. These systems provide standardized, objective analysis that reduces human error and enables high-throughput screening and teleradiology for remote areas.

Automated Classification of Clinical Lesions

AI models trained on clinical photographs help overcome the frequent misdiagnosis of cutaneous leishmaniasis by accurately differentiating it from other skin conditions. Furthermore, advanced models can even predict a lesion's responsiveness to treatment with over 95% accuracy, enabling clinicians to select the most effective therapy from the start.

Accelerating Therapeutics: From Drug Discovery to Personalized Treatment

The pipeline for developing new anti-leishmanial drugs is notoriously slow, costly, and inefficient. AI offers a suite of computational methods to de-risk and accelerates this process at multiple stages.



In Silico Drug Discovery and Repurposing

AI can screen vast virtual libraries of chemical compounds far more efficiently than traditional methods. Machine learning models, such as Quantitative Structure-Activity Relationship (QSAR) models, learn the link between a molecule's structure and its biological activity against *Leishmania* (7). These models can predict the anti-leishmanial potential of novel compounds, prioritizing a smaller, more promising set for laboratory validation (4).

Furthermore, AI is a powerful engine for drug repurposing—finding new uses for existing drugs. This approach significantly shortens the development timeline, as the safety profiles of these drugs are already known. AI algorithms can mine interconnected biological databases (genomics, proteomics) to uncover non-obvious links between existing drugs and parasite-specific molecular targets, offering a faster path to new therapies (8).

Advancing Epidemiological Control and Surveillance

Effective control of leishmaniasis requires a proactive public health strategy that can predict where and when transmission is likely to occur. AI provides the analytical power to achieve this.

Geospatial AI for Risk Mapping and Hotspot Identification

By integrating Geographic Information Systems (GIS) with machine learning, researchers can develop predictive risk maps. These models analyze the interplay of environmental factors—such as temperature, humidity, and proximity to animal reservoirs—that influence sand fly populations and parasite transmission. Shabanpour et al. (2022) used a boosted regression tree (BRT) model in Iran to create a detailed map of CL transmission hotspots (9). Other studies have employed algorithms like MaxEnt to model the ecological niche of vector species, even under different climate change scenarios (1). Such tools empower public health officials to allocate resources and implement preventive measures with greater precision.

Predictive Modeling for Outbreak Forecasting

Beyond spatial mapping, AI can forecast the temporal dynamics of leishmaniasis. By analyzing historical time-series data on case incidence alongside climatic data (e.g., rainfall, temperature) and other variables, models like ARIMA (Auto-Regressive Integrated Moving Average) or more complex recurrent neural networks (RNNs) can predict future trends and provide early warnings of impending outbreaks (1).

Critical Challenges and Future Directions

Despite its immense potential, the widespread adoption of AI in leishmaniasis control faces significant hurdles:

- **Data Scarcity and Quality:** AI models require large, well-curated, and consistently annotated datasets. For leishmaniasis, such data are scarce. Establishing public,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



multi-center databases is crucial for developing robust and generalizable models (1).

- The "Black Box" Problem: Many advanced DL models are not easily interpretable, making it difficult to understand their decision-making process. This lack of transparency is a barrier to clinical trust. The development of Explainable AI (XAI) techniques are essential to build confidence and facilitate regulatory approval (2, 4).
- Ethical and Privacy Concerns: Training models on sensitive patient data raises significant ethical issues. Robust governance frameworks and privacy-preserving technologies like federated learning, where models are trained locally without centralizing raw data, are needed to address these concerns (8).
- Implementation and Infrastructure: Deploying AI solutions requires computational power and technical expertise. A key research priority is developing lightweight models optimized for mobile devices (mHealth) and low-cost hardware to ensure accessibility in endemic regions (10).

The ultimate goal is the rigorous clinical validation of these tools through prospective trials to prove their real-world utility and cost-effectiveness (11).

Artificial intelligence is now a tangible force capable of transforming the fight against leishmaniasis by solving key diagnostic, therapeutic, and epidemiological challenges. However, significant data, ethical, and infrastructural hurdles hinder its widespread use, making interdisciplinary collaboration essential to translate AI's promise into real-world impact for controlling this neglected disease.

Acknowledgment:

Special thanks for Dr. Adel Spotin, the head of Parasitology department of Tabriz university of medical Sciences.

References:

1. Bamorovat M, Sharifi I, Tahmouresi A, et al. Unlocking Responsive and Unresponsive Signatures: A Transfer Learning Approach for Automated Classification in Cutaneous Leishmaniasis Lesions. *Transboundary and Emerging Diseases*. 2025;2025(1):5018632.
2. Sadeghi A, Sadeghi M, Fakhari M, et al. A deep learning-based model for detecting *Leishmania* amastigotes in microscopic slides: a new approach to telemedicine. *BMC Infectious Diseases*. 2024;24(1):551.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



3. Leal JFdC, Barroso DH, Trindade NS, et al. Automated identification of cutaneous leishmaniasis lesions using deep-learning-based artificial intelligence. *Biomedicines*. 2023;12(1):12.
4. Parija SC, Poddar A. Artificial intelligence in parasitic disease control: A paradigm shift in health care. *Tropical Parasitology*. 2024;14(1):2-7.
5. Abdelmula AM, Mirzaei O, Güler E, Sür K. Assessment of deep learning models for cutaneous *Leishmania* parasite diagnosis using microscopic images. *Diagnostics*. 2023;14(1):12.
6. Bamorovat M, Sharifi I, Rashedi E, et al. A novel diagnostic and prognostic approach for unresponsive patients with anthroponotic cutaneous leishmaniasis using artificial neural networks. *PLoS One*. 2021;16(5):e0250904.
7. Harigua-Souiai E, Oualha R, Souiai O, et al. Applied machine learning toward drug discovery enhancement: Leishmaniasis as a case study. *Bioinformatics and Biology Insights*. 2022;16:11779322221090349.
8. Winkler DA. Use of artificial intelligence and machine learning for discovery of drugs for neglected tropical diseases. *Frontiers in Chemistry*. 2021;9:614073.
9. Shabanpour N, Razavi-Termeh SV, Sadeghi-Niaraki A, et al. Integration of machine learning algorithms and GIS-based approaches to cutaneous leishmaniasis prevalence risk mapping. *International Journal of Applied Earth Observation and Geoinformation*. 2022;112:102854.
10. Rubiano L, Alexander ND, Castillo RM, et al. Adaptation and performance of a mobile application for early detection of cutaneous leishmaniasis. *PLoS Neglected Tropical Diseases*. 2021;15(2):e0008989.
11. de Vries HJ, Schallig HD. Cutaneous leishmaniasis: a 2022 updated narrative review into diagnosis and management developments. *American Journal of Clinical Dermatology*. 2022;23(6):823-40.



Introduction to Molecular Detection Tools for Differential Diagnosis of Hemoprotozoan Parasites *Theileria sp.* and *Babesia sp.*

Parviz Shayan¹

Corresponding author: Parviz Shayan, pshayan@ut.ac.ir

1. Research Center Ticks and Tick-borne Diseases, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article Code: 1486

Abstract

Background: Obligate intracellular hemoprotozoan parasites cause diseases with high economic losses worldwide. Among these parasitic diseases, theileriosis and babesiosis play an important role in animal health management.

Methods: For serological diagnosis ELISA assay was recommended. At the antigen level, the genes encoding Tasp and Tosp proteins can be used. Whereas for molecular diagnosis based on PCR, Nested PCR, PCR-RLFP, and Reverse line blot techniques, primers and probes derived from the V4 hypervariable region of the 18S rRNA gene can be used.

Results: For serological diagnosis of malignant theileriosis in sheep, the data according to Tasp in *Theileria annulata* and *Theileria lestoquardi* and Tosp Protein in *Theileria ovis* can be used. For molecular diagnosis at nucleic acids, V4 hypervariable region of 18S rRNA gene can be considered as a suitable DNA region for designing of specific primers and probes for differential detection of *Theileria* or *Babesia* species using PCR, Nested PCR, PCR-RLFP and Reverse line blot techniques.

Conclusion: *Theileria* and *Babesia* species can be preferably differentiated and diagnosed by molecular methods based on Tasp and Tosp proteins in the ELISA technique and based on 18S rRNA gene in PCR, Nested PCR, PCR-RLFP, and Reverse line blot, respectively.

Keywords: *Theileria sp.*, *Babesia sp.*, PCR, nested PCR, PCR-RFLP, Reverse Line blot, ELISA.

Introduction:

The obligate intracellular hemoprotozoan, *Babesia spp.* and *Theileria spp.* can cause diseases in animals with high economic losses worldwide. Early diagnosis and rapid intervention can be observed as important measures to be taken into account for successful management of animal and public health. The most diagnostic method used for intracellular hemoprotozoan parasite infection is Giemsa staining. Thick-smear, poor staining, staining of artifacts and the need of extensive knowledge limit the use of this method. The methods based on the molecular level



can be considered as suitable methods for diagnosing of the mentioned parasites. Serological tests based on parasite surface proteins were previously described and used as successful parasite or antibody detection methods in different studies (1, 2, 3, 4). The molecular methods based on the nucleic acids, like PCR, PCR-RFLP, nested PCR, Real Time PCR, and Reverse line blot, were also intensively studied (5-13). In the present study, the results dealing with molecular methods based on protein and nucleic acid levels will be discussed.

Materials and Methods:

RNA was isolated from cell line *Theileria annulata* infected bovine cells (Research Center Borstel, Germany) and cDNA was synthesized. Subsequently, the cDNA was cloned in bacteriophage λ gt11 and screened with rabbit anti-macroschizont antibody generated from isolated macroschizont from *Theileria annulata* infected cells and immunization of a rabbit (5). The screening of recombinant λ gt11 resulted in 3 positive clones, which were named SA288 and SB288 and SD288. The recombinant proteins were later named HSP70, Tasp and TaD, respectively (6, 7, 8, 9). *Theileria ovis* surface protein (Tosp) was also determined as described by Shayan et al. (10).

PCR, Nested PCR, and PCR-RFLP were done as previously described (11, 12, 13). Reverse line blot was done as described by Bahadori-Ranjbar et al. (14). Since the V4 hypervariable region of 18S rRNA gene was suitable for designing species-specific primers and probes, developing of Reverse Line Blot Technique was then possible. In this method, the specific probe designed from the V4 hypervariable region of the 18S rRNA gene of each *Theileria* species or *Babesia* species was transferred on the Biodyne C blotting membrane. Subsequently, the membrane was screened with biotin-marked PCR product. The positive reaction was then visualized using Streptavidin-peroxidase by the chemiluminescence method (14, 15).

Results and Discussion:

Theileria annulata-infected animal was analyzed by Dot blot and ELISA techniques (9, 16). The analysis showed that Tasp as an antigen, belongs to the favorite antigens of *T. annulata* in comparison to other *T. annulata* antigens like TaD, TaSE and rTamHSP70 for serological diagnosis (17). Amino acid sequence analysis of *Theileria ovis* (ToSp) showed 96, 96, 99, and 86 % homology to the corresponding nucleotide sequence of TaSp genes by *T. annulata*, *Theileria China I*, *Theileria China II*, and *Theileria lestoquardi* (9). The corresponding protein was for the first time described by Shayan et al. 2016 (10). It was shown that TaSP is as a single-copy gene in genome and is expressed in the sporozoite and schizont life cycle stages of parasite. Homologues of Tasp gene is found also in other *Theileria* species, which makes this protein interesting as a detecting antigen for serological diagnosis. More interestingly, the



difference between amino acid sequence of Tasp and Tosp gene in *T. annulata*, *T. ovis* and *T. lestoquardi* makes it possible to design partial polypeptides for differentiation of *T. lestoquardi* from *T. annulata* and *T. ovis*, which can be used as a suitable antigen in serological diagnostic methods. Such methods can be used for discrimination of causative agents of malignant from benign theileriosis in small ruminants. Furthermore, since this gene (Tasp) occurs in different *Theileria* species genome, the Tap protein can be recommended as a suitable antigen for vaccine development against theileriosis.

There are many molecular methods based on nucleotide sequence that have been described for the differential diagnosis of theileriosis. For this aim, the nucleotide sequence of the rRNA gene is usually used. In one study, we showed that it is possible to extract DNA from a small area of stained blood smear for PCR analysis (11). In the mentioned study (11), Shayan and Rahbari (2005) described a primer pair for the simultaneous detection of *Theileria* spp. and *Babesia* spp. in one PCR reaction (11, 12). Interestingly, in the case of *Babesia* spp., Shayan et al. 2008 (13) could show that in contrast to the assumption that *Babesia ovis* belongs only to the small *Babesia*, *Babesia ovis* can have the size of typical paired pyriforms with an acute angle of $2.7 \times 0.4 \mu\text{m}$ ($n = 10$) and obtuse angle of $3.5 \times 0.6 \mu\text{m}$ ($n = 10$). The *Babesia* with the mentioned biometrical characteristics was also confirmed as *Babesia ovis* by nested PCR-RFLP (13). It seems that the 18S rRNA gene can be used for molecular differential detection of *Theileria* as well as *Babesia* at the species level. Interestingly, the 18S rRNA gene in *Theileria* spp. or *Babesia* spp. has a hypervariable region, which makes it possible to design species-specific probes. This finding led to the development of differential diagnostic methods based on 18S rRNA derived PCR between all *Theileria* species from each other and *Babesia* species from each other. Furthermore, the hypervariable region of 18S rRNA give the possibility to design suitable PCR-RFLP for discrimination of different piroplasma (*Theileria* /*Babesia*) at species level. Gubbels (1999) used the nucleotide sequences from mentioned hypervariable region of 18S rRNA gene for discrimination between *Theileria annulata*, *Theileria parva*, *Theileria mutans*, *Theileria Taurotragi*, *Theileria Velifera*, *Theileria Orientalis*, *Babesia Bovis*, *Babesia Bigemiona* and *Babesia divergens* (15) from each other by RLB method. Bahadori-Ranjbar et al. (2012) also used the hypervariable region of 18S rRNA gene in *Theileria lestoquardi*, *Theileria ovis*, *Theileria separate*, *Theileria (China I)*, *Theileria (ChinaII)*, *Babesia ovis*, *Babesia motasi*, *Babesia crassa*, *Babesia (Lintan) (China)* for discrimination of the mentioned agents from each other in small ruminant (14).

In conclusion, it is to mention that serological test based on Tasp antigen and PCR, nested PCR, PCR-RFLP and RLB based on 18S rRNA gene can be considered as suitable methods for diagnosis and epidemiological analysis of *Theileria* sp. and *Babesia* sp.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Ryskeldina A, Korobeinikov A, Tursunbay N, Berdikulov M, Shevtsov A, Bauer C, Mukhanbetkaliyev Y, Kuibagarov M. A novel recombinant *Theileria annulata* surface protein as an antigen in indirect enzyme-linked immunosorbent assay for the serological diagnosis of tropical theileriosis. *Veterinary World*. 2024; EISSN: 2231-0916. Available at www.veterinaryworld.org/Vol.17/August-2024/32.
2. Jaffer O, Abdishakur F, Hakimuddin F, Riya A, Wernery U, Schuster RK. A comparative study of serological tests and PCR for the diagnosis of equine piroplasmiasis. *Parasitol Res*. 2010; 106:709–713.
3. Shayan P, Nabian S. The use of immunostaining for determination of *Babesia* and *Theileria* and gene expression of proliferation associated nuclear protein in *Theileria* infected cells. *Iranian Veterinary Journal*. 2007; 3(3): 21-29.
4. Shayan P, Gerlach C, Huegel FU, Kay G, Campbell JDM, Gerdes J, Ahmed JS. The proliferation-associated nuclear protein Ki-67 in the bovine system: partial characterisation and its application for determination of the proliferation of *Theileria* -infected bovine cells. *Parasitol Res*. 1999; 85: 613-620.
5. Shayan P, Biermann R, Shayan E, Gerdes J, Ahmed JS. Detection and Differentiation of *Theileria annulata* and *Theileria parva* Using Macroschizont-derived DNA Probes. *Ann N Y Acad Sci*. 1998 Jun 29;849:88-95. doi: 10.1111/j.1749-6632.1998.tb11038.x.
6. Schnittger L, Katzer F, Biermann R, Shayan P, Boguslawski K, McKellar S, Beyer D, Shiel BR, Ahmed JS. Characterization of a polymorphic *T. annulata* surface protein (TaSp) closely related to PIM of *Theileria parva*: implications for use in diagnostic tests and subunit vaccines. *Mol Biochem Parasitol*. 2002; 120: 247– 256.
7. Schneider I, Haller D, Seitzer U, Beyer D, Ahmed J. Molecular genetic characterization and subcellular localization of a putative *T. annulata* membrane protein. *Parasitol Res*. 2004; 94: 405-415.
8. Schnittger L, Katzer F, Biermann R, Shayan P, Boguslawski K, McKellar S, Beyer D, Shiel BR, Ahmed JS. Characterization of a polymorphic *T. annulata* surface protein (TaSp) closely related to PIM of *Theileria parva*: implications for use in diagnostic tests and subunit vaccines. *Mol Biochem Parasitol*. 2002; 120: 247– 256.
9. Sadr-Shirazi N, Shayan P, Eckert B, Ebrahimzadeh E, Amininia N. Cloning, and Molecular Characterization of Polymorphic Iranian Isolate *Theileria annulata* Surface Protein (Tasp). *Iranian J Parasitol*. 2012; Vol. 7, No.2, 2012, pp.29-39.
10. Shayan P, Jafari S, Fattahi R, Ebrahimzade E, Amininia A, Changizi E. Identification and characterization of *Theileria ovis* surface protein (ToSp) resembled TaSp in *Theileria annulata*. *Parasitol Res*. 2016; 115(5):1893-9. doi: 10.1007/s00436-016-4929-1. Epub 2016 Feb 2.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



11. Shayan P, Rahbari S. Simultaneous differentiation between *Theileria* spp. and *Babesia* spp. on stained blood smear using PCR. *Parasitol. Res.* 2005; 97: 281-286.
12. Shayan P, Rahbari S. Differentiation of *Theileria* spp. and *Babesia* spp. by polymerase chain reaction. *Journal of veterinary Research.* 2007; 62 (2): 15-20.
13. Shayan P, Hooshmand E, Nabian S, Rahbari S. Biometrical and genetical characterization of large *Babesia Ovis* in Iran. *Parasitology Research.* 2008; 103: 217-221.
14. Bahadori-Ranjbar S, Eckert B, Omidian Z, Sadr Shirazi N, Shayan P. *Babesia ovis* as the main causative agent of sheep babesiosis in Iran. *Parasitol. Res.* 2012; 110(4):1531-6.
15. Gubbels JM, de Vos AP, van der Weide M, Viseras J, Schouls LM, de Vries E, Jongejan F. Simultaneous detection of bovine *Theileria* and *Babesia* species by reverse line blot hybridization. *J Clin Microbiol.* 1999; 37(6):1782-9. doi: 10.1128/JCM.37.6.1782-1789.1999.
16. Seitzer U, Beyer D, Kullmann B, Bakheit MA, Ahmed JS. Evaluation of *Theileria annulata* Recombinant Immunodominant Proteins for the Development of ELISA. *Transbound Emerg Dis.* 2008; 55(5-6):244-8. doi: 10.1111/j.1865-1682.
17. Seitzer U, Mohammed A, Bakheit MA, Salih DEA, Ali A, Haller D, Yin H, Schnittger L, Ahmed J. From molecule to diagnostic tool: *Theileria annulata* surface protein TaSP. *Parasitol Res.* 2007; 101 (Suppl 2):S217–S223. DOI 10.1007/s00436-007-0685-6.



Introduction of RT-Nested Multiplex for Easy Detection of Dengue Virus and Its Serotypes in Biological Samples

David Shayan^{1,2}, Reza Heidari³, Parviz Shayan^{1,4}

Corresponding Author: David Shayan, davidsh919@gmail.com

1. Institute of Molecular Biological System Transfer, Tehran, Iran.
2. Department of Biology, Kish International Campus, University of Tehran, Tehran, Iran.
3. Medical Biotechnology Research Center, AJA University of Medical Sciences, Tehran, Iran.
4. Research Center of Ticks and Tick-borne Diseases, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article Code: 1487

Abstract

Background: *Dengue* virus is a single positive RNA virus causing Dengue fever (break-bone fever), belonging to the *Flavivirus* in the family of *Flaviviridae*. Four serotypes were described for this virus world wide, which can infect animal and human through mosquitoes *Aedes aegypti* or *Aedes albopictus*.

Methods: primers were designed from 5' end of the genome of the virus and used for RT-PCR followed by multiplex PCR. For this aim 660 bp of 5' end of the genome of all four serotypes were separately cloned in pEX-A128 vector and the recombinant vectors were used as positive control for the establishment of the method.

Results: Our results showed that by Nested- Multiplex PCR it was possible to discriminate all four serotypes from each others.

Conclusion: Since the management of the public health and monitoring of the circulating *Dengue* virus has great importance, we recommend the presented method a suitable tool for diagnosis of *Dengue* virus and simultaneously its four serotypes in biological samples.

Keywords: *Dengue* virus, RT-PCR, Multiplex PCR, Antigenically distinct serotypes.

Introduction:

The causative agent of break-bone fever is *Dengue* virus belonging to genus *Flavivirus* in the family *Flaviviridae* with four serotypes (DEN-1, DEN-2, DEN-3 and DEN-4) (1). It is known that *Dengue* virus is transmitted by mosquitoes especially by *Aedes aegypti* or *Aedes albopictus*. Most of people show after infection with virus no remarkable symptoms, but in some patients, the infection can be accompanied with high fever, headache, body aches (therefore break-bone fever), nausea and rash (2). WHO has registered over 7.6 million of *Dengue* cases in 2024,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



from which 16000 with severe clinical signs and over 3000 death (3). The Dengue virus infection was also reported in Iran as transferred infection from abroad (4). Diagnosis of Dengue infection is performed by serological examination but also molecular techniques like RT-PCR and multiplex PCR (12). In the present study, we present a RT-PCR and nested-RT-multiplex PCR for simultaneously detection of *Dengue* virus serotypes and discrimination of serotypes from each other.

Material and Methods:

5' end of the four Dengue virus genome (660 bp) was cloned in pEX-A28 vector as a control for the four serotypes of the virus. The recombinant plasmids were transfected into *E. coli* H5- α and subsequently, the recombinant Dengue serotypes plasmids were isolated. Additionally, two Primer pair were designed to amplify simultaneously all Dengue serotype plasmids to achieve a PCR product of 657 bp in length. Subsequently, 5 primers were designed to amplify each Dengue serotype recombinant plasmid specifically in Multiplex PCR to achieve a PCR products of 480 bp, 119 bp, 290 bp and 389 bp for Dengue 1-, Dengue 2-, Dengue 3- and Dengue 4- serotype respectively. In the next approach, the PCR. Products in the first step (657 bp) were amplified by Nested Multiplex PCR to the specificity of the PCR reaction for all 4 serotypes. Finally, RNA was isolated from larvae of *Aedes* mosquito originated from Bandar Abbas. The purity of the RNA was controlled using primer pair derived from 18S rRNA gene. After that the RNA was used for cDNA synthesis. The cDNA was used as sample for RT-PCR and RT-Multiplex PCR.

Results and Discussion:

The recombinant pEX-A128 vector was performed using 660 bp in the 5'-end of the genome of all four Dengue serotypes separately. As expected, the recombinant Dengue serotype plasmids could be amplified separately using primer pair generating a PCR product of 657 bp for all four recombinant Dengue (D1, D2, D3 and D4) plasmids. This experiment showed that the *Dengue virus* infection can be easily diagnosed by PCR using the designed primer pair. The recombinant plasmids were also amplified using 5 serotype specific primers by Multiplex PCR. The results showed that each Dengue serotype could be amplified specifically in Multiplex PCR to achieve a PCR product of 480 bp, 119 bp, 290 bp and 389 bp for Dengue 1-, Dengue 2-, Dengue 3- and Dengue 4- serotype respectively. The specificity of the 5 serotypes was also performed using the PCR products (657 bp) in Nested Multiplex PCR. As expected, the 5 serotype specific primers could amplify specifically each serotype. In the next approach, *Aedes* larvae from Bandar Abbas was used as a sample. The isolated RNA from Larvae showed that the RNA was pure, which was confirmed using primer pair derived from 18S rRNA gene. The



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



generated cDNA was checked using the above mentioned primer pair and used for *Dengue* PCR analysis. Our results showed that the used larvae were negative (5).

European Centre for Disease Prevention and Control has reported that in 2025 until July this year 3.6 million were infected with *Dengue virus* worldwide. In this report is described that 1900 people have died with symptoms related to *Dengue virus* infection in 94 countries/territories in the WHO Region including Europe, Americas, SouthEast Asia, West Pacific, Eastern Mediterranean and Africa. Interestingly, four and three autochthonous cases has been also reported in France and Italy, respectively (6). Since the infection with Dengue virus is clinically in febrile phase difficult to differentiate from vector borne viral and parasitical infections like Malaria, *Chikungunya* and *Zika* fever, the early diagnosis at molecular level is favorable for successful treatment. Therefore, the management of this increased infection rate requires a safe method for early diagnosis. Virus isolation, specific PCR or antigen detection using ELISA technique can be considered as the safe diagnostic methods at acute stage of infection. ELISA method based on NS1 (non-structural protein) is recommended for acute phase of infection (7). Since serological methods can be used commonly five or six days after the clinical symptoms, the early diagnosis based on the specific PCR could have more benefits. Furthermore, since the management of febrile returned travelers infected with various *Dengue* viruses circulating globally is of great importance for public health, the characterization of serotype of virus is crucial not only for dengue endemic countries but for physicians in non-endemic countries, the method with capability to detect the early *Dengue* virus infection as well as to characterize simultaneously the circulating serotype of *Dengue* virus, is desirable.

In conclusion, it is to mention that the prevalence of *Dengue* virus has increased in Iran and public health management through the early diagnosis and determination of the circulating and introduced *Dengue* virus serotype is essential for monitoring the infection and rapid intervention. The presented RT-PCR followed by nested-multiplex PCR can help to realize this intention.

References:

1. Charles r. Anderson CR, Wilbur g. Downs WG, Hill AE. Isolation of Dengue Virus from a Human Being in Trinidad. Science. 1956; 124: 3214. 224-225. <https://doi.org/10.1126/science.124.4214.224>.
2. WHO, 23 April 2024. <https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue> .
3. <https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON518>. WHO 30 may 2024.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



4. Dabbagh Moghaddam A, Bakhtiari M. Dengue Fever in Iran, a Review of an Arrived Viral Disease with an Uninvited Guest. J M C Iri. 2024; 42(2): 6-13. <https://doi.org/10.22034/42.2.6>.
5. Shayan D, Heidari R, Shayan P. Simultaneous detection of Dengue virus serotypes by nested-multiplex PCR. Journal of Zoonotic Diseases, 2025 (in Press); pshayan@ut.ac.ir <https://doi.org/10.22034/jzd.2025.19864>.
6. <https://www.ecdc.europa.eu/en/dengue-monthly>
7. <https://www.who.int/activities/enhancing-dengue-diagnosis-and-case-management>



A 10-year Report of *Neospora* Infection in Aborted Bovine Fetuses Referred to the Faculty of Veterinary Medicine Hospital, Ferdowsi University of Mashhad

Narges Khaleghnia¹, Gholamreza Razmi², Pezhman Mirshokraei³

Corresponding Author: Pezhman Mirshokraei, mirshokraei@um.ac.ir

1. Centre of Excellence in Ruminant Abortion and Neonatal Mortality, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
3. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article Code: 1488

Abstract

Background: *Neospora caninum* is an intracellular protozoan parasite that infects cattle worldwide and is a leading cause of bovine abortion, resulting in significant economic losses. In Iran, initial reports highlighted its role in fetal loss; however, data on long-term trends remain limited. This study aimed to investigate the temporal trend of *N. caninum* infection in aborted bovine fetuses over the past decade in northeast Iran.

Methods: This retrospective study analyzed data from 1009 brain tissue samples of aborted bovine fetuses submitted to the Center of Excellence in Ruminant Abortion and Neonatal Mortality, Ferdowsi University of Mashhad, from 2015 to 2024. DNA was extracted, and a PCR assay targeting the *Nc-5* gene (337 bp fragment) was performed. PCR products were visualized by electrophoresis. Statistical analysis included Chi-square tests to compare annual positive rates, with Bonferroni correction for multiple pairwise comparisons.

Results: Among 1009 samples, 191 (18.9%) tested positive for *N. caninum*. Annual prevalence rates ranged from 10.7% to 44.2%, with a significant rise in 2024 ($p < 0.05$). The overall Chi-square test indicated significant differences between years ($\chi^2 \approx 35.5$, $df=9$, $p \approx 0.000047$). Pairwise comparisons confirmed 2024 had a significantly higher prevalence than several previous years.

Conclusion: The decade-long analysis revealed fluctuating infection rates, with a marked increase in the final year. Factors such as vertical transmission, increased farm size, and exposure to infected dogs may contribute to this trend. Despite nearly two decades since the first report in Iran, neosporosis remains underdiagnosed and unregistered, highlighting the urgent need for systematic surveillance, preventive strategies, and farmer education to reduce its reproductive and economic impact on the cattle industry.



Keywords: *Neospora caninum*, Bovine abortion, Prevalence, Iran, PCR.

Introduction:

Neospora caninum (Apicomplexa: Sarcocystidae) is a protozoan parasite that forms intracellular cysts and affects a wide range of hosts(1). Dogs and related canids act as definitive hosts, whereas many herbivores serve as intermediate hosts. Carnivores probably acquire infection by ingesting tissues containing bradyzoites, while herbivores typically become infected through consumption of food or water contaminated with sporulated oocysts of *N. caninum* (2). Furthermore, transplacental transmission can occur when tachyzoites are passed from an infected dam to the fetus during pregnancy(3). *Neospora caninum* causes abortion and subsequent economic losses in both dairy and beef cattle (2). Abortions may occur at any stage between three months of gestation and term, most commonly at 5–6 months. Outcomes include fetal death, resorption, mummification, or birth of calves, either clinically normal or displaying signs such as neurologic deficits or limb deformities(4). Abortions can occur sporadically or as epidemics, sometimes affecting up to 33% of cows(5). Epidemic abortion is defined as when more than 10% of cows at risk abort within a 6–8 week period; however, repeated abortions are rare(6).

Globally, neosporosis has been reported in most regions (4,7,8). In some areas, up to 42.5% of abortions are attributed to this infection. The economic impact depends not only on direct fetal losses but also on indirect costs such as veterinary care, diagnostic work, re-breeding, extended lactation intervals, reduced milk yield, and replacement of culled cows(9). Studies from the USA, New Zealand, the Netherlands, and Germany report that 12–42% of aborted fetuses in dairy herds are infected (10). Seroprevalence varies by region, test type, and cut-off thresholds; in some dairies, up to 87% of cows may be seropositive (4).

In Iran, the first detection of *N. caninum* in aborted fetuses was in Mashhad, showing parasite DNA in the brains of 13 cases by PCR (11). Subsequent data suggest that *N. caninum* is widespread among cattle in Iran, confirming its significance as a cause of abortion and highlighting the need for prevention and control (12). This study aimed to investigate the temporal trend of *Neospora caninum* infection rates in aborted bovine fetuses over the past decade in northeast Iran and to perform detailed statistical analyses.

Materials and Methods:

Sample Collection and Preparation

This retrospective study was conducted on data from aborted bovine fetuses referred to the Center of Excellence in ruminant abortion and Neonatal Mortality (COE-RAM), Faculty of



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Veterinary Medicine, Ferdowsi University of Mashhad, during the period 2015 to 2024. DNA samples were extracted from 1009 brain tissue specimens of aborted bovine fetuses.

PCR Design and Setup

To detect *Neospora caninum*, a pair of primers targeting the *Nc-5* gene was selected with sequences of *Np21plus* (5'-CCCAGTGCCTCAATCCTGTAAC-3') and *Np6plus* (5' CTCGCCAGTCCAACCTACGTCTTCT-3') (13) for amplifying the fragment of 337 bp. PCR reactions were carried out in 20 μ L mixtures containing 10 μ M of each primer (Metabion International AG, Planegg, Germany), 10 μ L of Taq 2x Master Mix Red (Ampliqon A/S, Odense, Denmark), 1 μ L of template DNA, and seven μ L of UltraPure™ DNase/RNase-Free Distilled water. Amplifications were performed using a thermocycler device (Gene Atlas 322®, Astec Co., Fukuoka, Japan) with the following conditions: initial denaturation at 94°C for 15 min, with 40 cycles of 94°C for 30 seconds, an annealing temperature of 63°C for 30 seconds, and an extension temperature of 72 °C for 30 seconds, followed by a final extension phase at 72 °C for 10 min. PCR product was subjected to electrophoresis on a 1% agarose gel in a Tris-acetate-acetic acid buffer at 90 V for 60 min and visualized with an ultraviolet transilluminator after staining with Green Viewer(0.01 v/v).

Statistical Analysis

The annual positive percentage was calculated. Initially, to compare the overall rate of positive cases across different years, a Chi-square test was used on a 2×10 contingency table (positive/negative vs. ten years). For a more detailed analysis, pairwise Chi-square tests were conducted between all pairs of years, resulting in 45 pairwise comparisons. To control Type I error from multiple testing, the Bonferroni correction was applied, and the final significance level after correction was set at a value below 0.05.

Results and Discussion:

The results showed that out of a total of 1,009 examined samples, 191 samples tested positive. The total prevalence of *N. caninum* over a decade was calculated to be 18.9%. Annual data are summarized below:

Year	Total Samples	Positive Cases	Positive Rate
2015	52	12	23.1%
2016	139	21	15.1%
2017	175	44	25.1%
2018	167	26	15.6%



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Year	Total Samples	Positive Cases	Positive Rate
2019	103	11	10.7%
2020	100	14	14.0%
2021	86	15	17.4%
2022	56	10	17.9%
2023	79	15	19.0%
2024	52	23	44.2%

Overall Chi-square: ≈ 35.5 (df=9, $p \approx 0.000047$). Pairwise comparisons showed 2024 significantly higher than 2016, 2018, 2019, and 2020.

Currently, *Neospora caninum* is a major cause of bovine abortion worldwide and is accompanied by considerable negative economic and reproductive impacts in the cattle industry(14,15). *N. caninum* was detected in the brain samples of 5%, 6.8%, and 8.6% of cattle fetuses in Brazil(16), aborted sheep fetuses in Spain, and aborted sheep fetuses in Italy, respectively (17,18), and 18.9% aborted bovine fetuses in England (19). These variances could be attributed to changes in climate, study design, detection techniques, farm management, sample size, and varying degrees of exposure to risk factors.

In Iran, neosporosis has been reported from 0.9% to 8.5% in *aborted ovine fetuses* by various PCR assays(20,21). In addition, the results of previous studies in Chahar Mahal Bakhtiari and Mashhad showed that the prevalence rates of *N. caninum* were 11%(22) and 33% in aborted bovine fetuses, respectively (23). A Study conducted by Salehi et al. (2021) on 7878 brain samples using nested PCR showed that the prevalence rate of *N. caninum* in the aborted cattle fetuses in North Iran was 20.5%. This difference could be related to the livestock rearing system and the number of dogs present in these regions(24). Furthermore, Ansari-Lari (2021) in a systematic review of studies in Iran reported that the prevalence of *N. caninum* infection in 1239 aborted bovine fetuses, of which 351 were positive, was 34.8%(25), while in her previous study (2020), the overall seroprevalence of *N. caninum* in 10,967 cattle with 2,619 positive ones was 23.6% (12). According to this conservative estimate, approximately one bovine aborted fetus from every four in the country is infected with *N. caninum* (12). An investigation on the detection of *N. caninum* from bovine aborted fetuses in Mashhad resulted in the prevalence of 33%, in the year 2007, although it is worth noting that this high result could be due to the small sample size(23). Moreover, the highest record for prevalence of *N. caninum* in Iran was recorded in Kamali's study, with 45% positivity in aborted bovine fetuses referred to COE-RAM between the years 2009 and 2013(26). In our study, the total prevalence of *N. caninum* in bovine aborted fetuses, referred to the same center, from 2015 to 2024, was



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



18.9 %. The reduction in the total prevalence of the disease could be due to the larger sample size, or control and prevention management appointed by the professionals and governments in the region. The results of the present study revealed that the prevalence of *N. caninum* in aborted bovine fetuses fluctuated over the ten years, with a significant increase in 2024. There could be some reason for this considerable trend in the rise in the prevalence of *N. caninum* in aborted fetuses. First of all, *N. caninum* is efficiently transmitted vertically in cattle, perhaps for several generations (27), and the presence of dogs in these farms can complete the life cycle of the parasite (4). In addition, studies have shown that frequent exposure to the sources of infection tends to increase the probability of animals being seropositive for *N. caninum* (28,29) while seropositive *N. caninum* cattle are more prone to abortion (2,30,31). On the other hand, most reports of neosporosis outbreaks have occurred in cows that are intensively managed; thus, the incidence increases as larger cattle farms with intensive feeding practices become more common (27). The results of our study may reflect changes in herd management, diagnostic awareness, or a failure in control of the disease and a true rise in infection.

Since our data were limited to referred cases, findings may not represent the entire cattle population. Nevertheless, results underline the need for regular surveillance to reduce economic losses from neosporosis.

In Iran, despite nearly 20 years since the first report of the disease, it remains overlooked as there is currently no established system for registering and reporting cases, nor are there effective diagnosis and prevention programs within the healthcare and veterinary systems. *Neospora caninum* is a critical cause of cattle abortion and an important reason for huge economic loss. Therefore, analyzing seroprevalence and, consequently, the exposure of dairy cattle populations to *N. caninum* is crucial for determining populations that may be susceptible to neosporosis and for investigating the probable modes of the parasite's transmission. Therefore, farmers should further become aware of maintaining hygienic conditions and keeping livestock food out of the reach of dogs.

References:

1. Asmare K, Regassa F, Robertson LJ, Martin AD SE. Reproductive disorders in relation to *Neospora caninum*, *Brucella* spp. and bovine viral diarrhoea virus serostatus in breeding and dairy farms of central and southern Ethiopia. *Epidemiology & Infection*. 2013;141(8):1772–80.
2. Dubey JP, Schares G OML. Epidemiology and control of neosporosis and *Neospora caninum*. 2007;20(2).
3. Smith MO, George LW. Diseases of the nervous system. *Large animal internal medicine*. 2002;1:972-1111.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



4. JP. D. Review of *Neospora caninum* and neosporosis in animals. The Korean journal of parasitology. 2003;41(1):1.
5. Wouda W, Bartels CJM MAR. Characteristics of *Neospora caninum*-associated abortion storms in dairy herds in the Netherlands (1995-1997). Theriogenology. 1999;52:233–245.
6. Anderson ML, Palmer CW, Thurmond MC et al. Evaluation of abortions in cattle attributable to neosporosis in selected dairy herds in California. J Am Vet Med Assoc. 1995;207:1206–1210.
7. Constable PD, Hincheliff KW, Done SH, Grünberg W. Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier Health Sciences; 2016 Oct 25.
8. Taylor MA, Coop RL, Wall RL. Veterinary Parasitology. Third Edn. Blackwell Publishing; 2007.
9. Thurmond MC, Hietala SK BPC. Herd-based diagnosis of *Neospora caninu* induced endemic and epidemic abortion in cows and evidence for congenital and postnatal transmission. J Vet Diag Invest. 1997;9:44–9.
10. Lindsay DS DJP. Neosporosis, toxoplasmosis, and sarcocystosis in ruminants: an update. Veterinary Clinics: Food Animal Practice. 2020 Mar 1;36(1):205-22.
11. Razmi G.R., Maleki M., Farzaneh N., Talebkhan Garoussi M. FAH. First report of *Neospora caninum*-associated bovine abortion in Mashhad area, Iran. Parasitology Research. 2007;100:755–7.
12. M. AL. Bovine neosporosis in Iran: a systematic review and meta-analysis. Preventive Veterinary Medicine. 2020 Mar 1;176:104913.
13. Müller N, Zimmermann V, Hentrich B GB. Diagnosis of *Neospora caninum* and *Toxoplasma gondii* infection by PCR and DNA hybridization immunoassay. Journal of Clinical Microbiology. 1996 Nov;34(11):2850-2.
14. Reichel MP, Ayanegui-Alcérreca MA, Gondim LF EJT. What is the global economic impact of *Neospora caninum* in cattle—the billion dollar question. International journal for parasitology. 2013 Feb 1;43(2):133-42.
15. MM M. Diagnosis and control of bovine neosporosis. Veterinary Clinics: Food Animal Practice. 2016 Jul 1;32(2):443-63.
16. Santos SL, de Souza Costa K, Gondim LQ, da Silva MS, Uzêda RS, Abe-Sandes K GLF. Investigation of *Neospora caninum*, *Hammondia* sp., and *Toxoplasma gondii* in tissues from slaughtered beef cattle in Bahia, Brazil. Parasitology Research. 2010 Jan;106(2).
17. Masala, G., Porcu, R., Daga, C., Denti, S., Canu, G., Patta, C. and Tola S. Detection of pathogens in ovine and caprine abortion samples from Sardinia, Italy, by PCR. Journal of Veterinary Diagnostic Investigation, 19(1), pp.96-98.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



18. Moreno B, Collantes-Fernández E, Villa A et al. Occurrence of *Neospora caninum* and *Toxoplasma gondii* infections in ovine and caprine abortions. *Vet Parasitol.* 2012;187(1–2): 312–8.
19. Hughes J, Williams R, Morley E et al. The prevalence of *Neospora caninum* and co-infection with *Toxoplasma gondii* by PCR analysis in naturally occurring mammal populations. *Parasitology.* 2006; 132(Pt 1):29–36.
20. Asadpour R, Jafari-Joozani R SN. Detection of *Neospora caninum* in ovine abortion in Iran. *J Parasit Dis.* 2013;37(1): 105–9.
21. Sasani F, Javanbakht J, Seifori P et al. *Neospora caninum* as causative agent of ovine encephalitis in Iran. *Pathol Discov.* 2013;1(1): 5.
22. Rafati N JM. The determination of prevalence of *Neospora caninum* in aborted fetuses in dairy cattle of Shahrekord area, Chahar Mahal Bakhtiari province, by Nested-PCR. *Journal of Veterinary Laboratory Research.* 2014;6(1): 45–50.
23. Sadrebazzaz A, Habibi G, Haddadzadeh H AJ. Evaluation of bovine abortion associated with *Neospora caninum* by different diagnostic techniques in Mashhad, Iran. *Parasitology research.* 2007 May;100(6):1257–60.
24. Salehi B, Amouei A, Dodangeh S, Daryani A, Sarvi S, Safari-Kharyeki MR, Salehi S, Hosseini SA HZ. Molecular identification of *Neospora caninum* infection in aborted fetuses of sheep, cattle, and goats in Mazandaran Province, Northern Iran. *Iranian Journal of Parasitology.* 2021;16(3):483.
25. M. AL. *Neospora caninum* in aborted bovine fetuses in Iran: a systematic review and meta-analysis. *Annals of Parasitology.* 2021;67(3):357–66.
26. Kamali A., Seifi H.A., Movassaghi A.R., Razmi G.R. NZ. Histopathological and molecular study of *Neospora caninum* infection in bovine aborted fetuses. *Asian Pacific Journal of Tropical Biomedicine.* 2014;4:990–4.
27. WO F. A current update on *Neospora caninum*. *Microbiol Res.* 2024;33(11–12):32–7.
28. Asmare, K., Regassa, F., Robertson, L. & Skjerve E. Seroprevalence of *Neospora caninum* and associated risk factors in intensive or semi-intensively managed dairy and breeding cattle of Ethiopia. *Vet Parasitol.* 2013;193:85–94.
29. Eiras C et al. *Neospora caninum* seroprevalence in dairy and beef cattle from the northwest region of Spain, Galicia. *Prev Vet Med.* 2011;98:128–132.
30. Llano, H. A. B., Guimarães, M. S., Soares, R. M., Polo, G. & da Silva AC. Seroprevalence and risk factors for *Neospora caninum* infection in cattle from the eastern Antioquia, Colombia. *Vet Anim Sci.* 2018;6:69–74.
31. Selim A, Alshammari A, Gattan HS, Marzok M, Salem M AJOA. *Neospora caninum* infection in dairy cattle in Egypt: a serosurvey and associated risk factors. *Scientific reports.* 2023;13(1):15489.



A Review of the Epidemiology, Prevalence, and Genetics of *Varroa destructor*, the Parasitic Mite of *Apis mellifera* in Iran

Sedigheh Nabian^{1,2}, Mahsa Shahbakhsh³

Corresponding Author: Sedigheh Nabian, nabian@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Center of Tick and Tick-borne Disease, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article Code: 1501

Abstract

Background: *Varroa destructor*, an ectoparasitic mite, poses a major threat to *Apis mellifera* populations worldwide by feeding on hemolymph and fat bodies, transmitting pathogens like deformed wing virus (DWV), and weakening immunity. Factors such as altitude, agro-ecological zones, presence of other diseases (e.g., *Nosema*), colony strength, seasonal variation, climate, and management practices influence its prevalence. This review consolidates current knowledge on the epidemiology, prevalence, and genetics of *V. destructor* in Iran.

Methods: Over the past five years, several studies have been conducted in various areas related to *Varroa* mites in our laboratory. This research has been conducted in various fields of prevalence, epidemiology of distribution, and morphological and molecular patterns of this mite in Iran, and its comparison with other investigations.

Results: The findings revealed higher *Varroa* infestation rates in the Hirkani Ecozone compared to the Zagros Ecozone. Seasonal variation was notable, with the highest infestation in winter and the lowest during warmer months. Additionally, morphological and molecular results showed 100% similarity among all mite samples, with the highest similarity to haplotype P1.

Conclusion: The study indicates that *Varroa* infestation peaks during late winter. Molecular findings showed that the samples are most similar to haplotypes P1 (Peshter1, accession JX970939) and S1 (Serbia, accession JX970938). Given the importance of accurate taxonomic and molecular identification, further research involving complete mitochondrial genome sequencing from various regions in Iran is recommended to accurately determine the circulating haplotypes.

Keywords: *Apis mellifera*, *Varroa destructor*, Epidemiology, prevalence, genetics, Iran.



Introduction:

The Western honeybee, *Apis mellifera*, is vital for pollination and food production (1, 2). Over recent decades, a decline in honeybee populations has been significantly attributed to *Varroa destructor*, an obligate ectoparasite originally native to *Apis cerana*. Its successful host shift to *A. mellifera* has resulted in widespread infestations and colony losses worldwide (3,4). Understanding its biology and interactions with its host is crucial for mitigating its impact on apiculture.

Varroa mite prevalence is influenced by various factors and exhibits fluctuations over time and across different locations. Studies have shown a significant impact of factors like altitude, agro-ecology, colony strength, and management practices on infestation levels (5). Furthermore, seasonal variations and the presence of other diseases can also affect *Varroa* prevalence. *Varroa destructor* has become ubiquitous across nearly all regions where *A. mellifera* is managed, except for some isolated areas like parts of Australia (6). Its rapid spread is facilitated by migratory beekeeping practices and global trade in bee products and colonies. The mite is a competent vector for several honeybee viruses, notably DWV, Israeli acute paralysis virus (IAPV), and acute bee paralysis virus (ABPV). The interaction between *V. destructor* and DWV has been shown to be synergistic, dramatically increasing viral virulence and mortality.

This mite originally was parasitizing *Apis cerana*, and then switched to *A. mellifera* in the early 20th century. Genetic studies suggest that only a few haplotypes (e.g., the Korean and Japanese types) successfully made this host switch, with the Korean haplotype being the most widespread globally (4). This review presents current knowledge on the epidemiology, prevalence, morphological and genetic data of *varroa* in Iran.

Materials and Methods:

Over the past 5 years, several studies have been conducted in various areas of *Varroa* mites in our laboratory. This research has been conducted in various fields of prevalence, epidemiology of distribution, and morphological and molecular patterns of this mite in Iran, and its comparison with other investigations.

Distribution and Prevalence

At first, A study was conducted to investigate *Varroa* infestation in two different ecozones, the Hyrcanian and Zagros forests, from February 2022 to August 2022. In this study, 184 apiaries were selected from six northern provinces along the Caspian Sea (Mazandaran, Gilan, Golestan, Ardabil, East Azerbaijan, and Semnan) and eight provinces within the Zagros Mountain range (Lorestan, Hamedan, Isfahan, Ilam, Khuzestan, Chaharmahal and Bakhtiari, Shiraz, and Kermanshah). A total of 798 adult bee samples were collected. From each apiary,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



five to eight colonies were selected, and from each colony, an average of 60 adult bees were collected based on clinical signs. The samples were transferred under cold chain conditions to the Parasitology Laboratory, Faculty of Veterinary Medicine, and University of Tehran.

Varroa detection was performed using boiling water and detergent methods, following the guidelines of the World Organisation for Animal Health (WOAH) (7). To identify *Varroa* mites on bees, the bees were placed in a glass container filled with boiling water, ensuring that the water level covered the bees' bodies. To facilitate the detachment of the mites from the bees' bodies, one to two drops of a detergent solution were added to the mixture, which was then stirred for 10 minutes. Subsequently, the bees, along with the boiling water, were poured through a sieve with mesh openings approximately two to three millimeters in size. The mature bees remained on the sieve, while the mites passed through. As a result, the mites were collected underneath the sieve and within a catch tray, where they could be observed and counted.

Morphological and molecular examination

Abdollahzadeh et al. performed a study to examine the morphology of *Varroa* mites and determine the nucleotide sequences of the COX1 gene in four provinces: Gilan, Isfahan, Tehran, and Alborz. For this purpose, at least 50 adult honeybees were collected from each hive, resulting in a total of 117 samples. The mites were separated using boiling water and a detergent solution. Subsequently, to record morphological characteristics, different body parts of the mites were photographed and measured using a stereo microscope. To observe the external features, the samples were stored overnight in a 10% Potassium hydroxide solution. Seven characters were measured on each mite from each population. These factors were length of dorsal shield (from anterior to posterior margins of the shield along midline (LDS), width of dorsal shield (WDS), trochanter, femur, genua, tibia, tarsus, and pretarsus. A one-way ANOVA test was done to check if the *V. destructor* populations varied in the measured characters, to determine the morphometric link between populations, and to analyze significant differences. DNA extraction was carried out using a kit from MBST Iran. The mitochondrial COX1 gene was then amplified via PCR using specifically designed primers. Out of the 117 samples, 12 were sent for sequencing to Sinaclon Company.

The morphological and molecular analyses of *Varroa* mites in the mentioned provinces were performed. The obtained sequences from each region were aligned using Clustal Omega software and compared with sequences from other regions and the GenBank database.



Results and Discussion:

Distribution and prevalence

In total, 178 apiaries from the Hyrcanian and Zagros regions were sampled, yielding 768 bee samples. Among these, 169 samples reported positive for *Varroa* presence, representing approximately 21.17%.

Regarding seasonal variation, during winter 2021, spring 2022, and summer 2022, from 51 apiaries in eight provinces of the Zagros region, a total of 210 adult bee samples were collected, with an infestation rate of 12.85%. The highest infestation in this region was observed during winter, with 25 positive cases (16.16%). The province with the highest infestation rate was Isfahan, with 13 cases, accounting for 43.33%.

In the Caspian Sea region, from six provinces (Mazandaran, Gilan, Golestan, Ardabil, Semnan, and East Azerbaijan), 127 apiaries were sampled, resulting in 558 samples, of which 142 reported positive for *Varroa* (25.42%). The highest infestation rate in this region was recorded during winter, with 134 positive cases (30.73%). Gilan province had the highest infestation rate, with 78 positive cases (34.78%).

Based on the obtained results, the highest infestation rate was observed during winter and the lowest during the warmer seasons. This pattern is likely due to the absence of capped larval stages during winter, which makes all developmental stages detectable on adult bees.

Morphological and molecular results

The morphological and molecular analyses of *Varroa* mites in the mentioned provinces were performed. The comparison of the sizes of various parts of the legs, including the coxa, trochanter, femur, tibia, tarsus, and pretarsus, in the statistical analysis of one-way ANOVA indicated no significant differences among the samples from different provinces. These morphometric characteristics were fully consistent with those of the *V. destructor*. Additionally, the length and width measurements of samples collected from different provinces showed no statistically significant differences. The mean length and width were 1195.833 micrometers and 1778.333 micrometers, respectively, which align with the measurements reported by Anderson and Truman (4). The standard deviations obtained in this study were 110.902 for length and 54.745 for width, which, when compared to those reported by Anderson and Truman (4), suggest that the variability in the measured mite lengths in our study was greater than that observed in their research. However, the standard deviations did not differ significantly from those reported in the study by Anderson and Truman (4).

The obtained sequences from each region were aligned using Clustal Omega software and compared with sequences from other regions and the GenBank database. The results showed 100% similarity among all comparable sequences. Only one sequence from Alborz Province differed from the others.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Based on the sequence alignment results, the highest similarity was observed with haplotypes P1 (Peshter1), accession number JX970939, and S1 (Serbia), accession number JX970938. In light of the importance of taxonomic identification and molecular characterization of *V. destructor* in Iran, it is recommended that complete mitochondrial genome sequences of this parasite from other regions of the country be studied further to determine the haplotypes present in Iran accurately.

Due to its plant and climate biodiversity, Iran has special conditions for beekeeping. Our country ranks fourth in terms of the number of beehives and fifth in terms of honey production in the world. Given the great importance of honeybees in preserving the country's ecosystem and economy, it is essential to pay special attention to this beneficial insect and the hazardous factors that endanger its life.

Varroa mites are among the most significant factors affecting the survival of honeybees, which play a crucial role in their life cycle due to their direct losses (deformities) and indirect losses (pathogen transmission). Therefore, in recent years, it has attracted the attention of many researchers around the world. In this article, an attempt has been made to examine various aspects of the *Varroa* mite in the country, including its seasonal distribution and abundance, morphology, and genetics.

In total, 178 apiaries from the Hyrcanian and Zagros regions were sampled, yielding 768 bee samples. Among these, 169 samples reported positive for *Varroa* presence, representing approximately 21.17%.

In the Caspian Sea region, from six provinces (Mazandaran, Gilan, Golestan, Ardabil, Semnan, and East Azerbaijan), 127 apiaries were sampled, resulting in 558 samples, of which 142 reported positive for *Varroa* (25.42%). The highest infestation rate in this region was recorded during winter, with 134 positive cases (30.73%). Gilan province had the highest infestation rate, with 78 positive cases (34.78%).

Based on the obtained results, the highest infestation rate was observed during winter and the lowest during the warmer seasons. This pattern is likely due to the absence of capped larval stages during winter, which makes all developmental stages detectable on adult bees.

Bokaie et al. determined the prevalence of varroosis in Golestan Province and evaluated some management factors in this province. They showed a high prevalence of the disease (92%) and recommended the new methods for *varroa* control (8).

The average width and length of the mites collected from apiaries in Gilan Province were 1.79 mm and 1.12 mm, respectively. In Tehran Province, these measurements were 1.75 mm and 1.08 mm; in Isfahan Province, 1.77 mm and 1.24 mm; and Alborz Province, 1.75 mm and 1.28 mm. Reports by Anderson and Truman (2000) also noted size variations in *V. destructor* mites collected from different regions (4). Anderson and Truman demonstrated that these mites differ



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



not only in size but also in nucleotide sequences based on the COX1 gene when compared to *Varroa jacobsoni* mites (4). A comparison of the results related to the length and width of the studied mites indicated no statistically significant differences among samples collected from different provinces. Additionally, these findings showed substantial discrepancies with the body measurements of *V. jacobsoni* reported by other researchers. Consequently, the presence of *V. destructor* mites in these four regions was confirmed.

V. destructor remains a formidable challenge to apiculture due to its complex biology, high adaptability, and interaction with honeybee viruses. A multidisciplinary approach encompassing epidemiology, genetics, behavior, and chemical ecology is essential for managing this parasite and protecting honeybee health. A long-term monitoring strategy is recommended to understand the dynamics of *Varroa* infestation in different environments and regular monitoring of mite populations, including sampling adult bees and drone brood, is essential for timely intervention.

References:

1. Ghazoul J. Buzziness as usual? Questioning the global pollination crisis. Trends in ecology & evolution. 2005 Jul 1;20(7):367-73.
2. Muli E, Patch H, Frazier M, Frazier J, Torto B, Baumgarten T, Kilonzo J, Kimani JN, Mumoki F, Masiga D, Tumlinson J. Evaluation of the distribution and impacts of parasites, pathogens, and pesticides on honey bee (*Apis mellifera*) populations in East Africa. PLoS one. 2014 Apr 16;9(4):e94459.
3. de Guzman LI, Rinderer TE. Identification and comparison of *Varroa* species infesting honey bees. Apidologie. 1999;30(2-3):85-95.
4. Anderson DL, Trueman JW. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Experimental & applied acarology. 2000 Mar;24(3):165-89.
5. Chemurot M, Akol AM, Masembe C, De Smet L, Descamps T, De Graaf DC. Factors influencing the prevalence and infestation levels of *Varroa destructor* in honeybee colonies in two highland agro-ecological zones of Uganda. Experimental and Applied Acarology. 2016 Apr;68(4):497-508.
6. Warner S, Pokhrel LR, Akula SM, Ubah CS, Richards SL, Jensen H, Kearney GD. A scoping review on the effects of *Varroa* mite (*Varroa destructor*) on global honey bee decline. Science of the total environment. 2024 Jan 1;906:167492.
7. Terrestrial Animal Health Code. (2024b) Chapter 9.5. Infestation of honey bees with *Tropilaelaps* spp. https://www.woah.org/en/what-we-do/standards/codesand-manuals/terrestrial-code-online-access/?id=169&L=1&htmlfile=chapitre_tropilaelaps_spp.htm



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



8. Bokaie S, Sharifi L, Mehrabadi M. .2014. Prevalence and epizootical aspects of varroasis in Golestan province, Northern Iran. J Arthropoda- Borne Dis, 102-107.



***Dicrocoelium dendriticum* in Iran: A Neglected Threat, Emerging Challenge or an Escalating Crisis?**

Behnam Meshgi¹

Corresponding Author: Behnam Meshgi, Bmeshgi@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. P.O.Box. 14155-6453 Tehran-Iran.

Article Code: 1506

Abstract

Background: Dicrocoeliasis, mainly caused by *Dicrocoelium dendriticum*, is a parasitic disease that affects the bile ducts of herbivorous mammals, especially ruminants. Although often considered a neglected parasite, growing evidence from different regions indicates that it presents significant veterinary, economic, and ecological challenges.

Methods: Distribution data of *D. dendriticum* (prevalence or frequency data) in Iran during recent years were gathered through searches in three search databases.

Results: This silent and complex expansion prompts the question: Is *D. dendriticum* simply a manageable issue in Iran, or is it evolving into a larger, more serious threat? To address this question, we conducted a literature review on *D. dendriticum* in Iran. The review included epidemiological surveys, molecular characterization, abattoir reports, ecological modeling, and diagnostics.

Conclusion: In Iran, where livestock farming is a key part of rural economies and food security, dicrocoeliasis is increasingly seen as more than just a local problem. Its subclinical nature in animals, combined with gaps in surveillance and diagnostic methods, has allowed the parasite to persist—and in some areas, spread—mostly unnoticed. These efforts aimed to define better prevalence, transmission patterns, parasite diversity, and the current gaps in detection and control within Iran's animal health infrastructure.

Keywords: *Dicrocoelium dendriticum*, Challenge, Threat, Emerging crisis, Iran.

Introduction:

In the northern provinces of Guilan, Mazandaran, and Golestan, where climatic and ecological conditions support intermediate host survival, a large-scale fecal survey was conducted on 2,688 ruminants. This effort revealed significant regional and species-specific differences in infection prevalence. In Guilan, the infection rate reached 36.72% in sheep and 6.09% in cattle. In Mazandaran, the corresponding rates were 22.4% for sheep and 3.91 for cattle (1). Across all regions, sheep were consistently more heavily infected than cattle, both in prevalence and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



egg per gram (EPG) counts, with EPG values four to five times higher in sheep. These findings strongly suggest that sheep are the primary host sustaining transmission cycles in northern Iran, likely due to traditional grazing practices and environmental exposure to intermediate hosts such as land snails.

Materials and Methods:

Distribution data of *D. dendriticum* (prevalence or frequency data) in Iran during recent years were gathered through searches in three search databases, Google Scholar, Pubmed, Scopus, and other sources referenced in the included articles.

Results and discussion:

In the provincial-level surveys, a broader national picture was developed through a meta-analysis of 15 published studies conducted between 2000 and 2015. This synthesis revealed pooled prevalence rates of 3.1% in sheep, 1.3% in goats, and 2.1% in cattle (2). However, these values may significantly underrepresent true infection rates. Many of the studies relied on slaughterhouse records, which often fail to detect subclinical infections. The underreporting is further exacerbated by inconsistencies in meat inspection protocols and the fact that chronic infections may cause only subtle or no gross liver changes. These limitations point to the need for more sensitive diagnostic tools and standardized reporting practices to inform national surveillance programs.

In a study aimed to determine the prevalence and intensity of *D. dendriticum* infection in sheep and cattle in the provinces of Guilan, Mazandaran, and Golestan along the Caspian Sea coast in northern Iran. For this purpose, four cities in each province, four villages in each city, and four districts in each village were selected for sampling. A total of 1344 fecal samples from sheep and cattle were examined. The results suggested that sheep were infected 4-5 times more than cattle in all three provinces. The average prevalence of *D. dendriticum* infection was 36.21% in sheep and 9.37% in cattle in Guilan province. In Mazandaran province, the prevalence was 21.35% in sheep and 4.16% in cattle. Notably, the highest infection rates were observed in Chalus and Noor in Mazandaran province. In Golestan province, the average infection rates were 6.87% in sheep and 1.87% in cattle (3). These findings emphasize the need for targeted parasite control strategies, particularly in sheep-dense areas.

In another study, a molecular analysis aimed to characterize genetic variation in *D. dendriticum* populations and identify any evidence of host-specific or regional strain divergence. Sequencing of the internal transcribed spacer 2 (ITS2) region revealed nine distinct haplotypes. However, most of the isolates clustered around a dominant haplotype that showed high similarity to sequences reported from Europe and Asia (4). This indicates a lack of strong



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



geographic or host specificity in Iranian *D. dendriticum* populations, supporting the hypothesis of a genetically stable, broadly adaptable parasite. A separate study focused on adult flukes collected from 45 infected livers. Using PCR-RFLP of the 28S rDNA gene, they confirmed all 180 adult worms as *D. dendriticum*, with no evidence of co-infection by other trematodes (5). Histopathological evaluation of affected liver tissues revealed classical features of chronic dicrocoeliasis, including bile duct hyperplasia, fibrosis, and glandular swelling. These findings reinforce the cumulative pathological burden associated with long-term infections, even when overt clinical signs are absent.

Abattoir data collected from 413 facilities across all 31 provinces of Iran were analyzed to determine the prevalence of *Dicrocoelium* spp. infections from 2015 to 2019. Infection prevalence at the provincial level was calculated annually, and spatial autocorrelation was assessed using Local Moran's I statistic. The overall prevalence of dicrocoeliasis was 4.63% (95% CI: 4.62–4.63%) in sheep and goats, and 3.08% in cattle. High infection rates were concentrated in northern provinces, particularly Zanzan, Gilan, Qazvin, and Tehran. The study also estimated direct economic losses due to dicrocoeliasis over the study period to be approximately US\$41,771,377 for sheep and goats, and US\$1,668,986 for cattle (6). These results highlight the significant economic impact and geographic clustering of *Dicrocoelium* infections

In another study to support targeted control efforts, ecological niche modeling was applied using MaxEnt software to estimate suitable habitats for *D. dendriticum* in the littoral regions of the Caspian Sea. Environmental variables such as precipitation during the driest quarter, temperature seasonality, and altitude were the most influential predictors in the final model (1). The habitat suitability map generated by this approach closely matched observed patterns of prevalence in Guilan and Mazandaran, validating the model's utility. This spatial risk prediction method offers a powerful tool for prioritizing high-risk areas for intervention, especially in regions where blanket deworming or surveillance is not feasible due to limited resources.

The diversity of diagnostic methods used across Iranian studies presents both a challenge and an opportunity. Field-level surveys primarily rely on fecal flotation, sedimentation, and quantitative egg counts. These methods are low-cost and accessible but often lack sensitivity in early or light infections. Slaughterhouse inspections remain a mainstay of surveillance but vary in rigor and are biased toward detecting only more advanced cases. The absence of standardized diagnostic protocols hinders meaningful comparisons across studies and impedes coordinated control efforts.

Economically, dicrocoeliasis imposes a considerable burden on the Iranian livestock sector. Infected animals exhibit reduced weight gain, lower feed conversion efficiency, and decreased milk production. These zoonotic cases typically result from accidental ingestion of infected



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



ants and present with nonspecific gastrointestinal symptoms, often leading to misdiagnosis or underdiagnosis.

Despite the accumulating evidence of its veterinary and economic significance, *D. dendriticum* remains underrecognized in Iran's national parasite control strategy. Another is the fragmented nature of the existing data, which spans different diagnostic techniques, host species, and geographic areas. Nonetheless, the consistent pattern of moderate to high prevalence in key livestock regions, combined with demonstrable economic losses and zoonotic potential, calls for a more unified and proactive approach to dicrocoeliasis control. Control of *D. dendriticum* is feasible, but it requires a multifaceted strategy. Regular anthelmintic treatment, especially when timed with transmission seasons, can reduce worm burden in animals. Improving grazing management to minimize contact with intermediate hosts is also important, particularly in high-risk ecological zones. At the national level, implementing standardized diagnostic protocols in abattoirs and field clinics would enable more accurate prevalence tracking. Incorporating molecular tools into surveillance programs could help detect emerging patterns of resistance or identify new transmission hotspots. Finally, spatial modeling offers a cost-effective mechanism to allocate limited resources strategically, focusing efforts where they are most needed.

The current evidence suggests that *D. dendriticum* in Iran is neither fully controlled nor simply a localized nuisance. It occupies a middle ground—prevalent, impactful, and expanding—yet still amenable to intervention if coordinated action is taken. If ignored, its geographic spread may increase further, aided by climate change and changes in land use that affect host distribution and environmental conditions. The situation may not yet constitute a national crisis, but the trajectory points toward one unless preventive steps are taken now. Our review underscores that dicrocoeliasis in Iran is a pressing yet manageable problem. Its persistence is mainly due to neglect rather than inevitability. With coordinated surveillance, targeted deworming, and environmental risk mapping, controlling this parasite is feasible. Recognizing it as more than a minor threat is the first step toward effective mitigation. In this context, *D. dendriticum* should no longer be seen as just a background parasite, it is an emerging concern that requires strategic focus and ongoing action Bottom of Form.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Meshgi, B., Majidi-Rad, M., Hanafi-Bojd, A.A., Fathi, S. (2019). Ecological niche modeling for predicting the habitat suitability of fascioliasis based on maximum entropy model in southern Caspian Sea littoral, Iran. *Acta Tropical*. 198, 105079. <https://doi.org/10.1016/j.actatropica.2019.105079>.
2. Bari, S., Sarvi, S., Daryani, A., Ziaei Hezarjaribi, H., Arbabi, M., Pirestani, M. (2016). *Dicrocoelium dendriticum* infection among domestic animals in iran: A Systematic Review and Meta-analysis. 25, 367-375.
3. Majidi-Rad, M., Meshgi, B., Bokaie, S. (2018). The prevalence and intensity rate of *Dicrocoelium dendriticum* infection in ruminants of 3 provinces in coastal regions of the Caspian Sea. *Iranian Journal of Veterinary Medicine*. 12(1), 27-33. DOI: 10.22059/ijvm.2017.236806.1004822.
4. Javanmard, E., Mohammad Rahimi, H., Nemati, S., Soleimani Jevinani, S., Mirjalali, H. (2022). Molecular analysis of internal transcribed spacer 2 of *Dicrocoelium dendriticum* isolated from cattle, sheep, and goat in Iran. *Veterinary Research*. 18(1):283. DOI: 10.1186/s12917-022-03386-2. PMID: 35864506.
5. Ebrahim Pour, M.H., Shemshadi, B., Bahrami, A.M., Shirali, S. (2020). Molecular identification of *Dicrocoelium dendriticum* using 28S rDNA genomic marker and its histopathologic features in domestic animals in western Iran. *Journal of Basic Research in Medical Sciences*. 7(4), 43–52.
6. Kiani, B., Budke, C.M., Shams Abadi, E., Hashtarkhani, S., Raouf Rahmati, A., AkbarPour, M., Zarean, M., Hosseini Farash, B.R., Kiani, F., Moghaddas, E. (2021). Evaluation of zoonotic platyhelminthe infections identified in slaughtered livestock in Iran. *Veterinary Research*. 17(1),185. DOI: 10.1186/s12917-021-02888-9. PMID: 33952280.



Applications of Machine Learning Algorithms in Veterinary Medicine: From Parasite Detection to Disease Outbreak Prediction

Shadi Shahvand¹, Arash Omid¹

Corresponding Author: Shadi Shahvand, shahvandshadi99@gmail.com

1. Department of Animal Health Management, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Article Code: 1509

Abstract

Background: This study investigates how machine learning algorithms enhance parasite detection and disease outbreak prediction in veterinary medicine, addressing limitations of traditional methods.

Methods: This review article systematically evaluates the application of machine learning algorithms, including supervised (e.g., CNN, SVM, Random Forest), unsupervised (e.g., clustering), and reinforcement learning, through literature analysis, focusing on their use in parasite detection and disease outbreak prediction in veterinary medicine, with an emphasis on challenges, opportunities, and proposed solutions for implementation in Iran.

Results: Machine learning algorithms, including CNNs (achieving up to 99.5% accuracy in parasite detection), SVMs (90.5% accuracy for *Trypanosoma cruzi*), Random Forest, and LSTM models, significantly improve rapid and accurate parasite identification and disease outbreak prediction in veterinary medicine. Systems like VETSCAN IMAGYST enhance automated fecal analysis, while IoT and tele-veterinary platforms improve diagnostic access. Challenges such as limited high-quality data, infrastructure constraints, and ethical concerns persist, with proposed solutions including portable devices, data integration, and specialized training for implementation in Iran.

Conclusion: Machine learning algorithms transform veterinary medicine by enabling precise parasite detection and accurate disease outbreak prediction, enhancing animal health, food security, and productivity. Despite challenges like data quality and infrastructure limitations, solutions such as portable devices, data integration, and training can facilitate implementation in Iran, with a One Health approach integrating human, animal, and environmental data to improve disease management and public health outcomes.

Keywords: Machine Learning, Artificial Intelligence, Veterinary Parasitology, Disease Outbreak Prediction, Animal Health.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Introduction:

The global surge in demand for livestock products, driven by population growth, underscores the critical need to enhance animal health management, particularly in combating parasitic diseases that pose significant challenges to the veterinary industry. Parasitic infections, prevalent in tropical and subtropical regions, cause substantial economic losses in livestock production and, in some cases, threaten human health through zoonotic transmission (1). Traditional diagnostic methods, such as microscopic examination and molecular techniques like PCR, are often time-consuming, prone to human error, and reliant on specialized expertise and costly equipment, limiting their scalability and accessibility, especially in resource-constrained settings like rural areas or developing countries such as Iran. Moreover, predicting the spread of parasitic diseases remains complex due to the interplay of environmental, climatic, and epidemiological factors. Machine learning (ML), a subset of artificial intelligence, offers transformative potential to address these limitations by analyzing large and complex datasets to uncover hidden patterns, enable rapid and accurate parasite detection, and predict disease outbreaks. ML algorithms, including supervised methods like convolutional neural networks (CNNs) and support vector machines (SVMs), unsupervised techniques like clustering, and reinforcement learning for optimizing treatment protocols, have shown promise in veterinary applications (2,3). These technologies can enhance diagnostic precision, reduce reliance on manual processes, and improve outbreak forecasting, thereby supporting proactive disease management. This article aims to comprehensively review the applications of ML algorithms in detecting parasitic infections and predicting their spread in livestock, with a focus on their performance, challenges, and opportunities. It also explores the integration of complementary technologies like IoT and tele-veterinary systems, emphasizing the One Health approach to improve animal and human health outcomes. Specifically, it addresses the research question: How can ML algorithms be effectively implemented in Iran to overcome diagnostic and predictive challenges in veterinary parasitology, enhancing health and productivity?

Materials and Methods:

This study is a comprehensive literature review designed to evaluate the application of machine learning (ML) algorithms in veterinary parasitology, specifically focusing on the detection of parasitic infections and the prediction of disease outbreaks in livestock. The research adopts a systematic approach to synthesize existing evidence, assess the performance of ML techniques, identify challenges, and propose practical solutions for their implementation, particularly in the context of Iran's veterinary infrastructure.

Study Design: A narrative review was conducted, integrating peer-reviewed studies, technical reports, and scientific articles published between 2012 and 2025. The review targeted research



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



on ML applications in veterinary science, with a focus on parasitic disease detection and outbreak prediction. The inclusion criteria encompassed studies that:

- utilized ML algorithms such as supervised (e.g., convolutional neural networks [CNNs], support vector machines [SVMs], Random Forest), unsupervised (e.g., clustering), or reinforcement learning;
- addressed parasitic infections in livestock;
- discussed challenges, opportunities, or implementation strategies. Exclusion criteria included studies focused solely on human parasitology or non-ML-based methods.

Data Collection: Relevant literature was sourced from databases including PubMed, Scopus, IEEE Xplore, and Web of Science using keywords such as machine learning, veterinary parasitology, parasite detection, disease outbreak prediction, and One Health. Additional sources were identified through snowballing techniques, examining references of key articles (e.g., 2,3). A total of 50 studies were selected for in-depth analysis based on relevance and quality, with 13 key references cited for their direct contributions to ML applications in veterinary parasitology (e.g., 4,5).

Analysis: The selected studies were categorized into three main areas:

- ML-based parasite detection (e.g., CNNs for image-based diagnosis, VETSCAN IMAGYST for automated fecal analysis);
- disease outbreak prediction (e.g., LSTM models, XGBoost for resistance prediction);
- complementary technologies (e.g., IoT, tele-veterinary systems). Performance metrics such as accuracy, sensitivity, and specificity were extracted to evaluate ML efficacy. Challenges (e.g., data quality, infrastructure) and proposed solutions (e.g., portable devices, data integration) were analyzed, with a focus on their applicability in Iran.

Contextualization for Iran: The review incorporated an analysis of Iran's veterinary infrastructure, emphasizing the need for localized solutions such as low-cost devices and training programs to address resource constraints and enhance ML adoption.

Results and Discussion:

This review highlights the transformative impact of machine learning (ML) algorithms in veterinary parasitology, particularly in the detection and prediction of parasitic diseases in livestock. Supervised ML algorithms, such as convolutional neural networks (CNNs), achieved up to 99.5% accuracy in identifying parasite eggs and larvae from microscopic images, outperforming traditional microscopy in speed and reliability (4). For instance, automated systems like VETSCAN IMAGYST, utilizing deep learning, demonstrated high sensitivity and specificity in analyzing fecal samples for strongyles and *Parascaris* spp. (5). Support vector



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



machines (SVMs) showed 90.5% accuracy and 88.6% specificity in classifying *Trypanosoma cruzi* from mobile phone-based blood smear images (6). Random Forest models effectively analyzed clinical data to predict infection risks, handling noisy datasets robustly (2). For disease outbreak prediction, Long Short-Term Memory (LSTM) models accurately forecasted epidemiological trends by analyzing time-series data (7), while XGBoost achieved 94.92% accuracy in predicting anthelmintic resistance in sheep flocks (8). Integration of Normalized Difference Vegetation Index (NDVI) data with ML models produced precise risk maps for diseases like fasciolosis (9). Complementary technologies, including Internet of Things (IoT) sensors and tele-veterinary platforms, enhanced real-time monitoring and diagnostic access in remote areas (10,11). However, challenges such as insufficient high-quality data, high development costs, ethical concerns regarding data privacy, and infrastructural limitations in regions like Iran were identified (2,12).

These findings align with existing literature demonstrating ML's superiority over traditional methods in veterinary diagnostics. CNNs and SVMs surpass human technicians in image-based parasite detection due to their ability to extract complex features from diverse datasets (4,6). LSTM and XGBoost models advance predictive capabilities by integrating environmental and clinical data, supporting proactive disease management (7,8). The integration of IoT and tele-veterinary systems addresses accessibility issues, particularly in resource-limited settings, aligning with the One Health approach that emphasizes interconnected human, animal, and environmental health (13). However, limitations such as data scarcity and infrastructural constraints, especially in Iran, hinder widespread adoption. For instance, Kumar et al. (2023) noted that incomplete datasets reduce model accuracy, necessitating comprehensive data repositories. High development costs and ethical concerns, such as data privacy, further complicate implementation (12). Proposed solutions, including portable diagnostic devices, edge computing, and training programs, are critical for overcoming these barriers, particularly in Iran where localized solutions are needed (11,14). These advancements have significant implications for improving animal health, reducing economic losses, and enhancing food security. Future research should focus on developing cost-effective, scalable ML tools and fostering interdisciplinary collaboration to integrate ML into Iran's veterinary framework, ensuring sustainable disease management and public health benefits.

Acknowledgment:

We are particularly grateful to the library of the School of Veterinary Medicine at Shiraz University for providing access to critical resources and databases that enabled a comprehensive analysis of the literature.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Fuhad KMF, Tuba JF, Sarker MRA, Momen S, Mohammed N, Rahman T. Deep learning based automatic malaria parasite detection from blood smear and its smartphone based application. *Diagnostics*. 2020;10(5):329. doi:10.3390/diagnostics10050329
2. Kumar S, Arif T, Alotaibi AS, Malik MB, Manhas J. Advances towards automatic detection and classification of parasites microscopic images using deep convolutional neural network: Methods, models and research directions. *Arch Comput Methods Eng*. 2023;30(3):2013-39. doi:10.1007/s11831-022-09858-w
3. Lee YW, Choi JW, Shin EH. Machine learning model for predicting malaria using clinical information. *Comput Biol Med*. 2021;129:104151. doi:10.1016/j.compbiomed.2020.104151
4. Ramos-Briceño DA, Flammia-D'Aleo A, Fernández-López G, Nascimento FSC, Pereira DAF. Deep learning-based malaria parasite detection: Convolutional neural networks model for accurate species identification of *Plasmodium falciparum* and *Plasmodium vivax*. *Sci Rep*. 2025;15(1):3746. doi:10.1038/s41598-025-53746-8
5. Steuer A, Fritzler J, Boggan S, Daniel I, Cowles B, Penn C, et al. Validation of Vetscan Imagyst®, a diagnostic test utilizing an artificial intelligence deep learning algorithm, for detecting strongyles and *Parascaris* spp. in equine fecal samples. *Parasit Vectors*. 2024;17(1):465. doi:10.1186/s13071-024-06553-2
6. Morais MCC, Silva D, Milagre MM, De Oliveira MT, Pereira T, Silva JS, et al. Automatic detection of the parasite *Trypanosoma cruzi* in blood smears using a machine learning approach applied to mobile phone images. *PeerJ*. 2022;10:e13470. doi:10.7717/peerj.13470
7. Revathi TK, Balasubramaniam S, Sureshkumar V, Dhanasekaran S. An improved long short-term memory algorithm for cardiovascular disease prediction. *Diagnostics*. 2024;14(3):239. doi:10.3390/diagnostics14030239
8. Niciura SCM, Sanches GM. Machine learning prediction of multiple anthelmintic resistance and gastrointestinal nematode control in sheep flocks. *Rev Bras Parasitol Vet*. 2024;33(1):e019023. doi:10.1590/S1984-29612024014
9. Krishna R, Prema KV. Constructing and optimizing RNN models to predict fruit rot disease incidence in areca nut crop based on weather parameters. *IEEE Access*. 2023;11:110582-95. doi:10.1109/ACCESS.2023.3320938



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



10. Farooq MS, Sohail OO, Abid A, Rasheed S. A survey on the role of IoT in agriculture for the implementation of smart livestock environment. *IEEE Access*. 2022;10:9483-505. doi:10.1109/ACCESS.2022.3142848
11. Watson K, Wells J, Sharma M, Robertson S, Dascanio J, Johnson JW, et al. A survey of knowledge and use of telehealth among veterinarians. *BMC Vet Res*. 2019;15(1):474. doi:10.1186/s12917-019-2220-1
12. Xiao S, Dhand NK, Wang Z, Hu K, Thomson PC, House JK, et al. Review of applications of deep learning in veterinary diagnostics and animal health. *Front Vet Sci*. 2025;12:1511522. doi:10.3389/fvets.2025.1511522
13. Bhavnani SP, Parakh K, Atreja A, Druz R, Graham GN, Hayek SS, et al. 2017 Roadmap for innovation—ACC health policy statement on healthcare transformation in the era of digital health, big data, and precision health: A report of the American College of Cardiology Task Force on Health Policy Statements and Systems of Care. *J Am Coll Cardiol*. 2017;70(21):2696-718. doi:10.1016/j.jacc.2017.10.018
14. Alonso RS, Sittón-Candanedo I, García Ó, Prieto J, Rodríguez-González S. An intelligent Edge-IoT platform for monitoring livestock and crops in a dairy farming scenario. *Ad Hoc Netw*. 2020;98:102047. doi:10.1016/j.adhoc.2019.102047



Radiologic Evaluation of Visceral Changes in BALB/c Mice Infected with Cutaneous Leishmaniasis Caused by *Leishmania major*

Ayna Azimzadeh Tabrizi¹, Sedigheh Nabian², Majid Masoudifard³, Parviz Shayan², Mehdi Mohebbali⁴, David Shayan⁵, Amineh Pouyandeh¹

Corresponding Author: Sedigheh Nabian, nabian@ut.ac.ir

1. Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Department of Surgery and Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
4. Department of medical mycology and parasitology, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
5. Department of molecular cellular biology, University of Tehran Kish International Campus.

Article Code: 1511

Abstract

Background: *Leishmania major* is a protozoan parasite in genus *Leishmania*, that causes cutaneous leishmaniasis (CL)— a self-limiting, non-fatal disease. However, it can also cause visceral forms. Most researchers use parasitological, sero-molecular, and histopathological methods to diagnose and treat CL. This study uniquely investigates the probability of visceral changes associated with CL in infected BALB/c mice and the applicability of imaging techniques in its diagnosis

Methods: 15 BALB/c mice were divided into 3 groups of five and experimentally infected with *L. major* promastigotes at the tail-base. After scar formation, they received topical treatments for a month. 7 mice were selected for ultrasonography before and after treatment to assess abdominal organ sizes. At the end, all were euthanized for organ measurements.

Results: Ultrasonography showed that in healthy BALB/c mice, the spleen, liver, and kidneys measure $1.16 \pm 0.14 \times 0.28 \pm 0.05$ cm, 1.01 ± 0.12 cm, and $0.96 \pm 0.05 \times 0.47 \pm 0.04$ cm, respectively. In infected mice, organomegaly was observed. The spleen measured $1.60\text{--}2.15 \times 0.34\text{--}0.46$ cm (mean: $1.80 \pm 0.19 \times 0.41 \pm 0.04$ cm; P-value=0.0017); with some mice showing slight, insignificant hypoechoic change in spleen echotexture by the end of the treatment. Splenomegaly was confirmed via necropsy. The liver measured $0.91\text{--}1.49$ cm (mean: 1.11 ± 0.21 cm, P-value= 0.15) and both kidneys sized $0.93\text{--}1.12 \times 0.44\text{--}0.57$ cm (mean: $1.01 \pm 0.06 \times 0.50 \pm 0.03$ cm, P-value= 0.007). P-value lower than 0.05 shows significant changes. Renomegaly was not confirmed during necropsy.

Conclusion: This study highlights that ultrasonography effectively detects visceral involvement in *L. major* infection in BALB/c mice. Also, ultrasonography is a simple, valuable,



powerful, non-invasive, and ethical tool for examining visceral changes, systemic disease, and drug efficacy in small exotic/laboratory animals.

Keywords: Cutaneous leishmaniasis, *Leishmania major*, BALB/c, Ultrasonography, Splenomegaly.

Introduction:

Leishmania major is a protozoan parasite from the genus *Leishmania* (1). These parasites are transmitted via the bite of the infected female sand-flies (genus *Phlebotomus*) (2). Among over 30 species causing leishmaniasis, *L. major* primarily causes a self-limiting, non-fatal cutaneous form, known as cutaneous leishmaniasis (CL), which leaves permanent scars (3,4). However, *L. major* can reportedly cause visceral form as well (5); which is the most fatal form (6,7).

This “Neglected Tropical Disease” is seen in Africa, Southern Asia and America and the Mediterranean region (8). Leishmaniasis is also endemic in Iran, with over 30,000 cutaneous and 100-300 visceral cases reported annually (9,10). This underscores the importance of conducting related studies within the country.

Rodents are considered as reservoirs of CL. In Iran, the main reservoirs of CL caused by *L. major* are *Rhombomys opimus*, *Psammomys obesus*, *Meriones libycus erythraeus* (11,12). In addition, in Fars province *L. major* has been isolated and detected by PCR assay in *Mus musculus* (13).

In most experimental studies on CL, parasitological, serological, molecular, and histopathological methods were used in animals; and among which BALB/c is a common lab animal. However, the use of imaging techniques to diagnose CL in BALB/c mice is rare.

Since ultrasonography is a non-invasive approach, this study was uniquely designed to investigate whether visceral changes occur in BALB/c mice with CL and whether radiological methods can be effective in detecting these changes.

Materials and Methods:

Fifteen BALB/c mice (Mean BW=20.07g) were randomly divided into three groups of five mice: Group 1) healthy Negative control, group 2) mice which received a placebo treatment (100μl) as Positive control, and group 3) mice which received a curcumin-based topical gel (100μl). Two weeks later, the lower back was shaved, and 2 groups were experimentally infected with *L. major* promastigotes at the tail-base region, with a dose of 2×10^6 promastigotes per mouse (14).



After scar formation, all mice were weighed; 7 mice (the heaviest mice in each group: 3 from the treatment, 3 from positive control and 1 from negative control) were selected for abdominal ultrasonography (AUS), via E-I Voluson and 50MHz-5cm-linear probe, to measure the size of the spleen, liver, and kidneys. Anesthesia is necessary for AUS in BALB/c. for this purpose, ketamine and xylazine were combined in a 2:1 ratio, and was injected intramuscularly into the quadriceps muscle at a dose of 1 unit of insulin per 10 grams of body weight; which induced anesthesia for 30-45 minutes. Subsequently all mice received topical treatment twice a day for a month. During this period, changes in the health statue and the lesion progression were continuously documented. On the final day of treatment, the same 6 mice underwent AUS once more to assess visceral organ sizes once again (one mice died due to cannibalism).

At the end of the study, all mice were euthanized and organ sizes were recorded. The data collected during the study were analyzed to evaluate potential organomegaly and determine the statistical significance of visceral changes.

Results and Discussion:

1. General and Ultrasonographic Observations:

A total of 15 mice (Mean BW= 20.07g) were used for our study. Body weight increased to 22.56g after 30 days. Skin scars were evident in infected mice, with the size increasing over the month.

Quantitative measurements via AUS showed significant enlargement in spleens of all infected groups compared to negative controls. Hepatomegaly was also observed. However, both increases and reductions have been reported for the kidneys (Table 1) (Figure 1)

Gross examination during necropsy revealed significant splenic enlargement in infected groups compared to healthy controls (Table 1). Liver enlargement was apparent in 3 infected mice. But no apparent renomegaly was confirmed via necropsy.

2. Statistical analysis:

Data was recorded in Excel to run average and t-test; P-value lower than 0.05 shows significant changes.

Ultrasonography showed that in healthy BALB/c, the spleen, liver, and kidneys measure $1.16 \pm 0.14 \times 0.28 \pm 0.05$ cm, 1.01 ± 0.12 cm, and $0.96 \pm 0.05 \times 0.47 \pm 0.04$ cm, respectively. In infected mice spleen measured $1.80 \pm 0.19 \times 0.41 \pm 0.04$ cm (P-value=0.0017); which indicates splenomegaly. By the end of the treatment, some mice exhibited, non-significant hypoechoic alterations in spleen echotexture. Splenomegaly was confirmed through necropsy (Table 1).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 1. Visceral organ size (cm) measured via AUS and necropsy, -Control = Negative Control, +Control=Positive Control. D/C= Discontinued/Dead

Mouse ID	Spleen				Liver		Left Kidney				Right Kidney			
	21.Apr.2025		25.May.2025		21.Apr.2025	25.May.2025	21.Apr.2025		25.May.2025		21.Apr.2025		25.May.2025	
	Length	Width	Length	Width			Length	width	Length	width	Length	width	Length	width
- Control 1	1.07	0.20	1.05	0.31	0.88	0.91	0.89	0.37	0.93	0.44	0.90	0.44	0.98	0.49
Curcumin 1	1.16	0.36	1.71	0.45	0.95	1.09	0.94	0.49	1.09	0.51	0.98	0.45	1.06	0.48
Curcumin 2	0.96	0.25	1.83	0.34	1.04	0.91	0.95	0.52	0.96	0.52	0.89	0.46	1.01	0.44
Curcumin 3	1.06	0.24	1.60	0.46	1.19	1.49	0.98	0.47	1.12	0.55	1.01	0.49	1.04	0.53
+ Control 1	1.13	0.24	D/C		0.92	D/C	0.92	0.46	D/C		0.96	0.41	D/C	
+ Control 3	1.22	0.25	1.72	0.39	0.84	0.92	0.95	0.50	1.04	0.53	0.99	0.44	1.01	0.49
+ Control 4	1.07	0.29	2.15	0.39	1.13	1.16	1.04	0.51	1.05	0.57	0.99	0.49	0.93	0.50

The liver measured 1.11 ± 0.21 cm (P-value=0.15). Both kidneys sized 1.024 ± 0.06 cm (P-value=0.0006) in 0.50 ± 0.03 cm (P-value=0.0001). This means that changes in liver were statistically non-significant; while based on AUS, kidneys, in general, underwent significant renomegaly. However, in necropsy, the overall renal size was estimated as normal (table1).

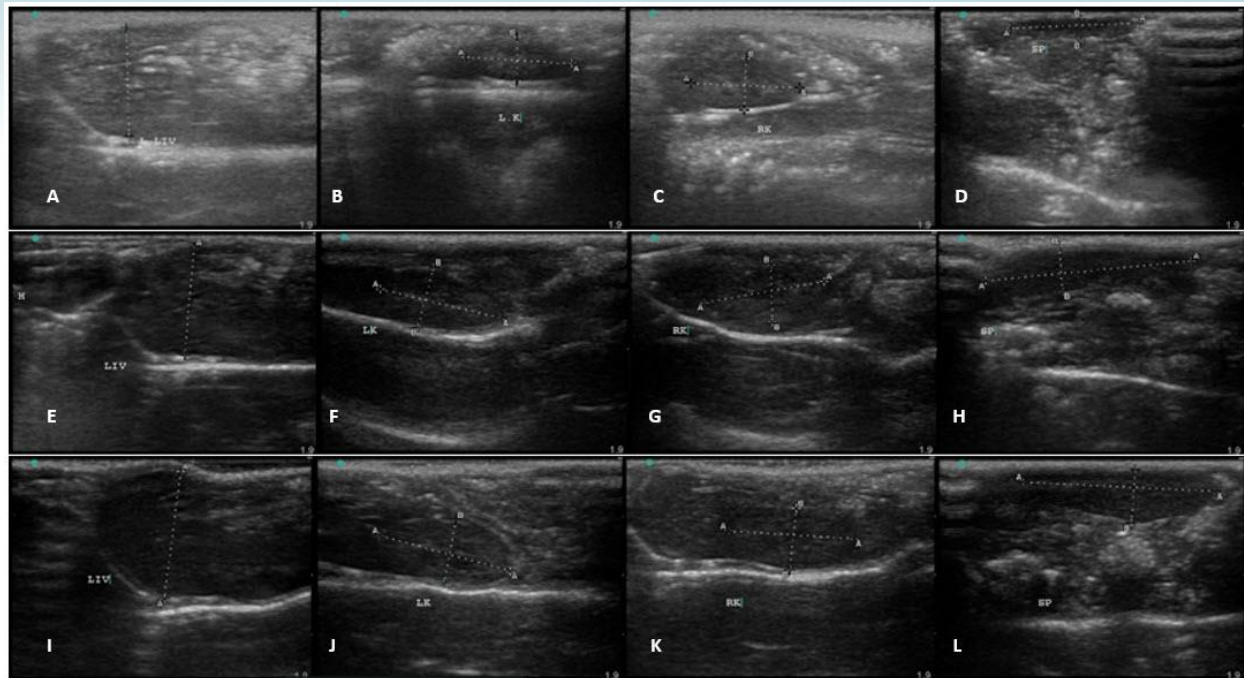


Figure 1. Representative images of the visceral organs from each group at the end of the study; A-D) Negative Control Group Mouse No.1: A) Normal liver, B) Normal Left Kidney, C) Normal Right Kidney, D) Normal Spleen; E-H) Positive Control Group Mouse No.3: E) Liver, F) Left Kidney, G) Right Kidney, H) Spleen; I-L) Curcumin-treated Group Mouse No.3: I) Liver, J) Left Kidney, K) Right Kidney, L) Spleen.

So far, the most commonly-used methods to diagnose cutaneous leishmaniasis and/or to evaluate drug efficacy in human and veterinary medicine are either based on clinical features or laboratory testing. These diagnostic techniques include *Leishmania Skin Test*, direct parasitological examination (microscopy, histopathology, and culture), serology (ELISA, IFA, and DAT) and molecular (PCR and LAMP) diagnostics (15,16,17). The lack of information regarding the potential use of ultrasound (despite its non-invasiveness and ability to instantly examine the involved layers) prompted us to conduct further research in this area.

In recent years, some researchers have utilized ultrasonography to diagnosis and follow-up CL, and confirmed its usefulness (18,19,20). However, all of them conducted dermatological ultrasound; and probable visceral changes caused by *L. major* have never been considered. Additionally, to our knowledge, neither dermatological nor abdominal ultrasonography has been never used in veterinary medicine for CL before.

Given that *L. major* can have visceral manifestations as well, we used AUS for the first time to assess visceral changes caused by *L. major*. We chose curcumin to evaluated the visceral



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



changes, because intralesional injection of Curcumin is proven to be useful in CL treatment, with less side effects in comparison with Glucantime (21).

Despite some reduction in the size of the dermal lesions during the final days of the study, severe significant splenomegaly was observed in the positive and treatment groups via AUS; which is either due to immunologic responses or parasite tissue migration; however, AUS cannot distinguish these two hypotheses and further studies must be carried out. If parasitic migration is the cause of splenomegaly in cutaneous leishmaniasis), despite successful dermal treatment, it is advisable to perform AUS to assess possible organomegaly. Additionally, AUS can help examining new drug formulation in preclinical animal studies.

Reference:

1. Vincent HM. William Boog Leishman: parasitologist and politician. *Parasitology*. 2017 Oct;144(12):1582-9.
2. Leonel JA, Vioti G, Alves ML, Spada JC, Yamaguchi AK, Pereira NW, Silva DT, Benassi JC, Galvis-Ovallos F, Galati EA, Starke-Buzetti WA. Species, natural *Leishmania* spp. detection and blood meal sources of phlebotomine sandflies (Diptera: Psychodidae: Phlebotominae) in peridomiciles from a leishmaniasis endemic area of Brazil. *Transboundary and Emerging Diseases*. 2024;2024(1):9932530.
3. Maurício IL. *Leishmania* taxonomy. In *The Leishmaniasis: Old Neglected Tropical Diseases* 2018 Jan 13 (pp. 15-30). Cham: Springer International Publishing.
4. Mann S, Frasca K, Scherrer S, Henao-Martínez AF, Newman S, Ramanan P, Suarez JA. A review of leishmaniasis: current knowledge and future directions. *Current tropical medicine reports*. 2021 Jun;8:121-32.
5. Thakur L, Singh KK, Shanker V, Negi A, Jain A, Matlashewski G, Jain M. Atypical leishmaniasis: A global perspective with emphasis on the Indian subcontinent. *PLoS Neglected Tropical Diseases*. 2018 Sep 27;12(9):e0006659.
6. van Griensven J, Diro E. Visceral leishmaniasis: recent advances in diagnostics and treatment regimens. *Infectious Disease Clinics of North America*. 2019 Mar 1;33(1):79-99.
7. Bélard S, Stratta E, Zhao A, Ritmeijer K, Moretó-Planas L, Fentress M, Nadimpalli A, Grobusch MP, Heller T, Heuvelings CC. Sonographic findings in visceral leishmaniasis—a narrative review. *Travel medicine and infectious disease*. 2021 Jan 1;39:101924.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



8. World Health Organization, Leishmaniasis, [internet], [place unknown: author unknown]; WHO; 2023 [cited 12 Jan 2023], Available from: <https://www.who.int/news-room/fact-sheets/detail/leishmaniasis>
9. Heidari A, Dashtaki NM, Mizbani S, Rejali M, Maracy MR. Residential environment, human behavior and socio-economic status in transmission of cutaneous leishmaniasis in central Iran. *Scientific Reports*. 2025 Mar 1;15(1):7271.
10. Mohebbali M. Visceral leishmaniasis in Iran: review of the epidemiological and clinical features. *Iranian journal of parasitology*. 2013 Jul;8(3):348.
11. Saliba EK, Oumeish OY. Reservoir hosts of cutaneous leishmaniasis. *Clinics in dermatology*. 1999 May 1;17(3):275-7.
12. Nasiri Z, Kalantari M, Mohammadi J, Daliri S, Mehrabani D, Azizi K. Cutaneous leishmaniasis in Iran: A review of epidemiological aspects, with emphasis on molecular findings. *Parasite*. 2022 Oct 21;29:47.
13. Parhizkari M, Motazedian MH, Asqari Q, Mehrabani D. The PCR-based detection of *Leishmania major* in *Mus musculus* and other rodents caught in southern Iran: a guide to sample selection. *Annals of tropical medicine & Parasitology*. 2011 Jun 1;105(4):319-23.
14. Lamour SD, Veselkov KA, Posma JM, Giraud E, Rogers ME, Croft S, Marchesi JR, Holmes E, Seifert K, Saric J. Metabolic, immune, and gut microbial signals mount a systems response to *Leishmania major* infection. *Journal of proteome research*. 2015 Jan 2;14(1):318-29.
15. Goto H, Lindoso JA. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. *Expert review of anti-infective therapy*. 2010 Apr 1;8(4):419-33.
16. Antonio LD, Fagundes A, Oliveira RV, Pinto PG, Bedoya-Pacheco SJ, Vasconcellos ÉD, Valet-Rosalino MC, Lyra MR, Passos SR, Pimentel MI, Schubach AD. Montenegro skin test and age of skin lesion as predictors of treatment failure in cutaneous leishmaniasis. *Revista do Instituto de Medicina Tropical de São Paulo*. 2014;56(5):375-80.
17. Adams ER, Schoone GJ, El Safi S, Schallig HD. Development of a reverse transcriptase loop-mediated isothermal amplification (LAMP) assay for the sensitive detection of *Leishmania* parasites in clinical samples. *The American journal of tropical medicine and hygiene*. 2010 Apr;82(4):591.
18. Saavedra AC, Valencia BM, Tueros P, Wortsman X, Llanos-Cuentas A, Lavarello RJ. Ultrasonographic characteristics of cutaneous leishmaniasis. *Journal of the European Academy of Dermatology and Venereology: JEADV*. 2020 Jan 22;34(4):e193.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



19. Vergara-de-la-Campa L, Cembrero-Saralegui H, Luna-Bastante L, Martínez-Lorenzo ER, Alfageme-Roldán F. Usefulness of ultrasound for treatment and follow-up of cutaneous leishmaniasis. *Journal of Ultrasound*. 2021 Dec;24(4):573-6.
20. Sechi A, Neri I, Patrizi A, Di Altobrando A, Clinca R, Caro RD, Leuzzi M, Misciali C, Gaspari V. Ultrasound patterns of localized cutaneous leishmaniasis and clinical correlations. *Journal of Ultrasound*. 2022 Jun;25(2):343-8.
21. Shirmohammad S, Mohebbali M, Arabkhazaeli F, Hassan J, Shayan D, Amininia N, Shayan P. Evaluation of Nanonanoliposomal Curcumin on Cutaneous Leishmaniasis Skin Lesions Caused by *Leishmania major* in BALB/c Mice. *Iranian Journal of Parasitology*. 2024 Apr;19(2):238.



Stage-Specific cDNA Profiling of Helminth Parasites with a Focus on *Fasciola hepatica* and Computational Modeling of *Fasciola hepatica* HDM-1 Protein Internalization

Fateme Jalousian¹

Corresponding Author: Fateme Jalousian, jalousian_f@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article Code: 1513

Abstract

Background: Helminth defense molecules such as FhHDM-1 mimic host antimicrobial peptides to suppress immune responses and prolong parasite survival. However, the stage-specific expression and membrane-interaction mechanisms of FhHDM-1 in *Fasciola hepatica* (*F. hepatica*) remain poorly understood. This study aimed to profile FhHDM-1 mRNA expression across developmental stages and computationally model its internalization by host membranes.

Methods: Adult flukes were morphologically characterized and confirmed as *F. hepatica* via mitochondrial COI PCR. Eggs harvested from adults were incubated at 27 °C for 21 days to yield miracidia. Total RNA from eggs, miracidia, and adults was extracted for cDNA synthesis, and FhHDM-1 transcript levels were assessed by RT-PCR. The peptide structure was predicted with I-TASSER and PEP-FOLD, and coarse-grained molecular dynamics simulations (MARTINI force field, 1000 ns) in GROMACS evaluated membrane interactions. Phylogenetic relationships were inferred using MEGA11's neighbor-joining method. Antigenicity (VaxiJen v2.0) and T cell epitope mapping (IEDB) assessed vaccine candidacy.

Results: RT-PCR detected FhHDM-1 mRNA exclusively in adult flukes, with no expression in eggs or miracidia; miracidial hatching rate was 48.8%. Simulations showed rapid peptide insertion into lipid bilayers, inducing curvature that favors endocytosis. Phylogenetic analysis grouped FhHDM-1 within a conserved trematode HDM clade featuring an amphipathic α -helix. Immunoinformatics yielded an antigenicity score of 0.5093 and identified multiple high-affinity MHC I/II epitopes with strong proteasomal cleavage and TAP transport predictions.

Conclusion: FhHDM-1 is an adult-stage-specific immunomodulator that actively interacts with host membranes, underscoring its role in chronic infection. Its predicted antigenic and epitope profiles support further in vitro and in vivo validation as a vaccine or immunotherapeutic candidate.

Keywords: *Fasciola hepatica*, FhHDM-1, Helminth defense molecule, cDNA profiling, Molecular dynamics, Immunoinformatics.



Introduction:

F. hepatica, commonly known as the liver fluke, is a parasitic trematode that infects a wide variety of mammalian hosts, including livestock and humans, causing the disease fasciolosis (1). The parasite's complex life cycle involves multiple developmental stages—ranging from the environmental metacercariae, which are ingested by the host, to juvenile and adult fluke stages that migrate through and ultimately reside in the liver. Each stage exhibits distinct biological features and interacts differently with the host environment (2).

Metacercariae, although encased within a protective cyst, are metabolically active and primed for infection. Upon excystment, newly excysted juveniles (NEJ) rapidly undergo metabolic and physiological changes, including hypoxic conditions and immune defenses. The excretory-secretory (E/S) products released by these stages contain a suite of proteases, antioxidants, and immunomodulators that are critical for disarming host innate immune responses and promoting parasite establishment (3). Despite existing treatments, concerns about drug resistance—especially to frontline drugs like triclabendazole—highlight the urgent need for novel control strategies such as effective vaccines (4). Advances in transcriptomics and proteomics have begun to elucidate stage-specific gene expression profiles and secreted molecules involved in pathogenesis and immune evasion, identifying candidate targets for therapeutic intervention (3). Among these, immunomodulatory molecules such as fatty acid-binding proteins and cathepsin L-like proteases have emerged as promising vaccine candidates due to their roles in altering host immune cell function and facilitating parasite survival. cDNA profiling of *F. hepatica* is a critical approach for understanding the molecular mechanisms underlying its development, pathogenicity, immune evasion, and drug resistance (1). Since cDNA is synthesized from expressed mRNA, it provides a snapshot of active gene expression at specific stages of the parasite's life cycle (2). Stage-specific gene expression mapping in each developmental stage of *F. hepatica*, from miracidium to sporocyst, redia, cercaria, metacercaria, and adult worm, has unique biological functions and interactions with the host or environment (5). cDNA profiling enables identification of genes that are specifically or differentially expressed at each stage, offering insights into host invasion mechanisms (6). These stage-specific proteins can serve as vaccine candidates, especially those involved in host penetration or immune modulation, Diagnostic markers, allowing early detection of active infections based on circulating or excreted proteins (4). Understanding the expression dynamics of the genes that alter host signaling pathways, allows for targeted immunological studies and may inform immunotherapy or adjuvant design (5). These can guide the development of new anthelmintic drugs and companion diagnostics in the field strains (1). The present study aimed to investigate the expression levels of FhHDM-1 in various developmental stages of *F. hepatica*, including adult flukes, eggs, miracidia, and to clarify how FhHDM-1 is taken up by macrophages through the process of endocytosis.



Materials and Methods:

This study investigated FhHDM-1 gene expression in various life stages of *F. hepatica* (egg, miracidium, and adult). Eight naturally infected sheep livers were collected from Tehran slaughterhouses and processed within 3 hours. Adult flukes were manually extracted, and eggs were obtained by compressing washed flukes between slides. Eggs were incubated at 27°C in sterile water, and miracidia were harvested 17–20 days later under light microscopy (7). Adult flukes were morphologically identified via stereo microscopy and confirmed by PCR amplification of the mitochondrial COI gene. DNA was extracted using MBST kits and amplified using universal primers with a Bio-Rad thermal cycler. Total RNA was isolated from all three life stages using SinaPure kits, and cDNA was synthesized using oligo (dT) primers. RT-PCR was conducted to amplify a 153 bp fragment of the FhHDM-1 gene, with COI as internal control. PCR products were visualized by agarose gel electrophoresis, sequenced by Bioneer (South Korea), and analyzed using NCBI-BLAST, ClustalW, and MEGA11 software. The FhHDM-1 peptide 3D modeling was done via I-TASSER. Structural validation used PROCHECK and Ramachandran plots; hydrophobicity and charge distribution were analyzed at pH 6.3 using Protein-sol. Peptide–membrane interaction was studied through coarse-grained molecular dynamics simulation using GROMACS 2022 with the MARTINI force field. Simulations included energy minimization, NVT/NPT equilibration, and a 1000 ns production run. Metrics included membrane insertion and lipid dynamics. Antigenicity was predicted using VaxiJen v2.0. T and B cell epitope predictions were performed using IEDB tools, focusing on proteasomal cleavage, TAP transport, and MHC binding (8).

Results and Discussion:

Morpho-molecular identification confirmed *F. hepatica* (132 worms, avg. size 30×13 mm) and excluded *F. gigantica* (53 worms, 75×12 mm). Species confirmation was done via COX1 gene PCR. Egg cultivation yielded a 48.75% miracidia development rate (1,425 out of 10,000 eggs), with peak hatching on day 21 (570 miracidia). RT-PCR analysis showed HDM-1 gene expression exclusively in adult flukes. No expression was detected in eggs or miracidia. COX1 was used as internal control. The adult HDM-1 sequence was submitted to GenBank (accession MN786462) (Figure 1). Membrane simulations confirmed stable lipid bilayer formation and peptide internalization into membranes via endocytosis, occurring within 1000 ns. Simulations showed peptide-membrane interaction caused membrane curvature and insertion (Figure 2). Antigenicity prediction scored FhHDM-1 at 0.5093, indicating immunogenic potential. Several strong MHC-I and MHC-II binding T cell epitopes were identified (Figure 3), based on proteasome cleavage, TAP transport, and binding affinity, suggesting vaccine relevance.

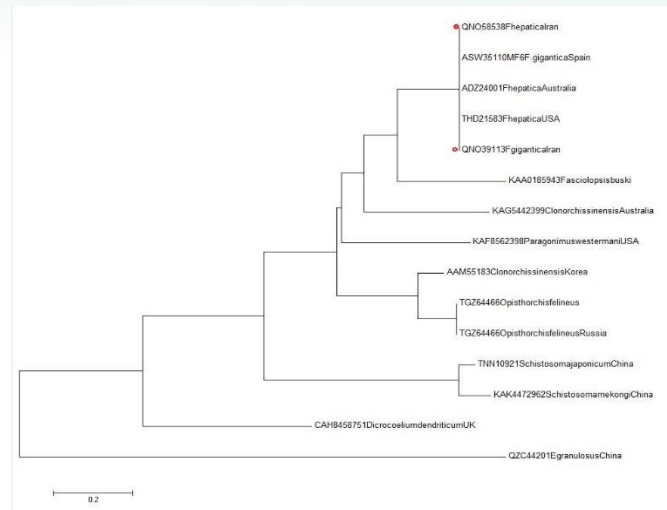


Figure 1. Phylogenetic tree of Helminths Defense Molecule 1(HDM-1) from *F. hepatica*

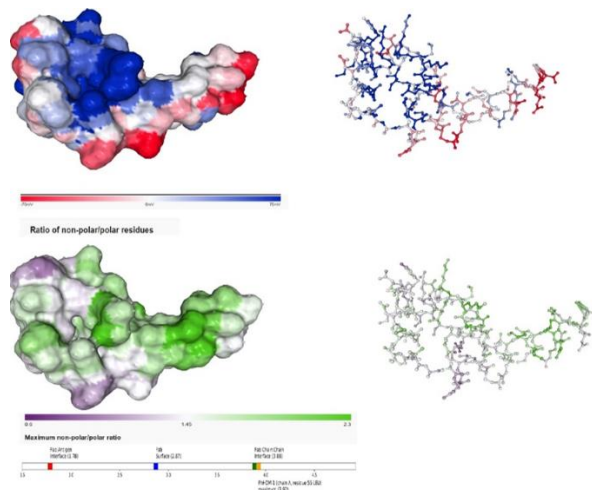


Figure 2. The patches of charge and hydrophobicity of *F.hepatica*HDM-1surface.The top Image visualizes charged surface patches from negative charge (color-coded: red regions) to positive charge (color-coded: blue regions). The bottom Image shows hydrophobic patches for the FhHDM-1, where the ratio of non-polar to polar solvent accessible surface area (NPP ratio) values was given as a scale from low NPP ratio (purple regions) to high NPP ratio (green regions).

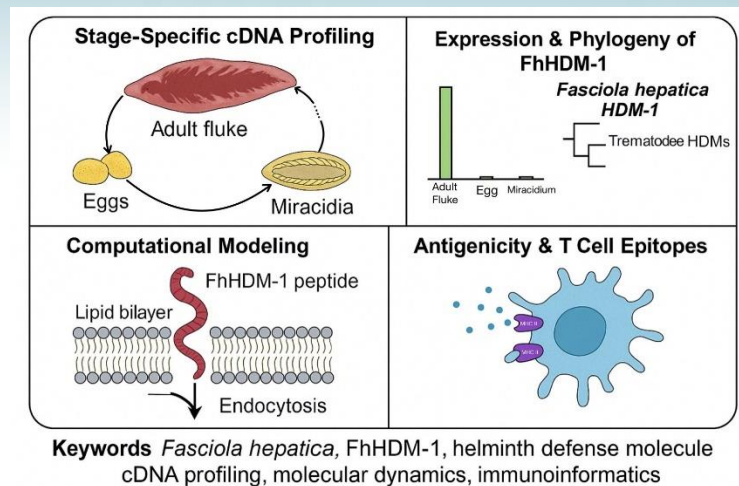


Figure 3. The graphical abstract of the results of the present study.

Investigating FhHDM-1 (*F. hepatica* Helminth Defense Molecule-1) is essential for understanding the pathogenesis of fascioliasis, especially regarding its immunological role (9). In this study, RT-PCR results showed no detectable expression of FhHDM-1 in eggs and miracidia of *F. hepatica*, while strong expression was observed in adult flukes. Previous studies have already identified MF6p/FhHDM-1 in the excretory/secretory (E/S) products of adults and in extracts from newly excysted juveniles (NEJ). However, current data emphasize expression primarily in adult stages, overlooking potentially critical stages like metacercariae. This discrepancy underlines the need for more consistent and sensitive methods to clarify expression dynamics and resolve conflicting findings across studies. To explore evolutionary relationships, the study performed phylogenetic analysis using the neighbor-joining method on HDM-related cDNA sequences from *F. hepatica* and other trematodes. The resulting tree grouped *Fasciola*, *Clonorchis*, *Schistosoma*, and *Paragonimus* HDMs into significant clades. Shared structural elements among them included a signal peptide at the N-terminus, a predicted α -helical region, and a conserved amphipathic helix at the C-terminus. This 34-amino acid region, which binds heme and lipopolysaccharide (LPS), was highly conserved across helminth species, and the sequences in this study matched known *F. hepatica* entries with 100% identity (Figure 1). Simulation modeling revealed the peptide penetrates host membranes by embedding among lipid chains, displacing water molecules, and associating with phospholipid head groups. This process causes membrane bending, facilitating internalization via endocytosis. Radial distribution analysis showed the peptide migrates from the aqueous environment into lipid-rich areas, interacting with hydrophobic and polar components. A 1-nm distance between the peptide and lipid phosphate groups was observed during membrane insertion. In simulations mimicking macrophage membranes, FhHDM-1 demonstrated rapid internalization, entering within 1000 ns. Additionally, peptide entry increased membrane



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



thickness and diffusion compared to control membranes. This behavior is consistent with previous findings that FhHDM-1 binds LPS and interacts directly with macrophage surfaces (Figure 2). It has been shown to counteract LPS-induced inflammation in mice, and its C-terminal domain has therapeutic potential in conditions like arthritis, by disrupting osteoclast formation and lysosomal acidification via modulation of p62 expression. Mechanistically, FhHDM-1 targets membrane lipid rafts, is internalized via endocytosis, and is processed by lysosomal enzymes like Cathepsin L. This leads to the release of its conserved amphipathic C-terminal fragment, which inhibits vacuolar ATPases, disrupts antigen processing, impairs MHC II presentation, and suppresses NLRP3 inflammasome activation. As a result, IL-1 β production and Th1 responses are reduced—facilitating immune modulation and prolonged parasite survival. Immunoinformatic analysis supports the antigenic potential of FhHDM-1, with an overall score of 0.5093. Using IEDB tools, both MHC-I and MHC-II binding T cell epitopes were predicted (Figure 3). These findings position FhHDM-1 as a strong vaccine or therapeutic candidate. Future research should aim to validate these computational insights through laboratory and in vivo studies, integrating both approaches for a more complete understanding.

Acknowledgement:

The authors express their sincere gratitude to the Association of Animal parasitic and zoonotic diseases for organizing the congress, as well as Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran for their invaluable support.

References:

1. Meshgi, B., Jalousian, F., Fathi, S., & Jahani, Z. (2018). Design and synthesis of a new peptide derived from *Fasciola gigantica* cathepsin L1 with potential application in serodiagnosis of fascioliasis. *Experimental parasitology*, 189, 76-86. <https://doi.org/10.1016/j.exppara.2018.04.013>
2. Etebar, F., Hosseini, S. H., Jalousian, F., & Ranjbar, M. M. (2018). Phylogenetic analysis of C type lectin from *Toxocara canis* infective larvae and comparison with the C type lectin family in the immune system of mouse and human. *Iranian journal of parasitology*, 13(1), 49. PMID: 29963085 PMCID: [PMC6019583](https://pubmed.ncbi.nlm.nih.gov/PMC6019583/)
3. Ricafrente, A., Nguyen, H., Tran, N., & Donnelly, S. (2021). An evaluation of the *Fasciola hepatica* miRnome predicts a targeted regulation of mammalian innate immune responses. *Frontiers in Immunology*, 11, 608686. <https://doi.org/10.3389/fimmu.2020.608686>
4. Das, K. C., Konhar, R., & Biswal, D. K. (2023). *Fasciola gigantica* vaccine construct: an in silico approach towards identification and design of a multi-epitope



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- subunit vaccine using calcium binding EF-hand proteins. *BMC Immunology*, 24(1), 1-17. <https://doi.org/10.1186/s12865-022-00535-y>
5. Etebar, F., Hosseini, S. H., Borhani Zarandi, M., Moghadasi, A. N., & Jalousian, F. (2023). The immunomodulatory effects of the C-type lectin protein of *Toxocara canis* on experimental autoimmune encephalomyelitis. *Parasite Immunology*, 45(11), e13010. <https://doi.org/10.1111/pim.13010>
 6. Shahbakhsh, M., Hosseini, S. H., Bonab, S. F., Malekzadeh, P., Moghadasi, A. N., Shayan, P., ... & Jalousian, F. (2022). Recombinant C-type lectin protein of *Toxocara canis* increases the population of spleen Foxp3+ regulatory T cells in BALB/c mice. *Annals of Parasitology*, 68(1). <https://doi.org/10.17420/ap6801.418>
 7. Valero, M. A., Pérez-Crespo, I., Periago, M. V., Khoubbane, M., & Mas-Coma, S. (2009). Fluke egg characteristics for the diagnosis of human and animal fascioliasis by *Fasciola hepatica* and *F. gigantica*. *Acta Tropica*, 111(1), 64–69. <https://doi.org/10.1016/j.actatropica.2009.04.005>
 8. Malekzadeh, P. , Hosseini, S. H. , Shahbakhsh, M. , Shayan, P. , Zibaei, M. , Jamshidi, S. , Rismani, E. , Naser Moghadasi, A. , Akrami, M. and Jalousian, F. (2024). Preliminary study of *Toxocara canis* Recombinant C- type Lectin as a suitable antigen for serodiagnosis of human toxocariasis. *Journal of Zoonotic Diseases*, 8(1), 422-435. <https://doi.org/10.22034/jzd.2024.17589>
 9. Shahbakhsh, M., Jalousian, F., Hosseini, S. H., Moghadasi, A. N., Shayan, P., Malekzade, P., & Vojgani, M. (2024). Amelioration of Clinical Scores in an Experimental Autoimmune Encephalomyelitis (EAE) Model through Expansion of Regulat. J Neuroimmunol. 547, 54772764. <https://doi.org/10.1016/j.jneuroim.2025.578569>



Food- and Water- Borne Parasites, One Health Strategy, and Their Role in Achieving Sustainable Development Goals

Seyed Hossein Hosseini^{1, 2}, Fateme Jalousian¹

Corresponding Author: Seyed Hossein Hosseini, hhoseini@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Parasitology Museum, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article Code: 1535

Abstract

Background: Water- and food-borne helminth parasites are major contributors to global disease burden, affecting both human and animal populations. These infections hinder sustainable development, particularly in regions with limited access to sanitation and healthcare. The One Health approach, which integrates human, animal, and environmental health, provides a comprehensive framework for understanding, controlling, and preventing these diseases. This study focuses on evaluating current control strategies and international programs aimed at combating food- and water-borne helminth infections.

Methods: Data for this review were collected from various electronic databases including Google Scholar, Science Direct, Scopus, PubMed, as well as Iranian national databases (SID, Iran Doc, Magiran, Iran Medex), spanning publications from January to May 2025. The study synthesized data on the prevalence, distribution, and control strategies for key food- and water-borne helminths, with a focus on Taeniosis, Cysticercosis, Trichinellosis, Fascioliasis, and Dicrocoeliasis. Information was gathered from both peer-reviewed literature and surveillance reports from global programs.

Results: The study identified several successful international programs aimed at controlling food- and water-borne helminths, including the Chinese Accelerated Elimination of Schistosomiasis Plan and the European Union's One-Health Zoonoses Report. Notably, Iran has implemented successful mass deworming programs and improved sanitation through national efforts. These interventions have significantly reduced the burden of helminth infections, with the integrated One Health strategy playing a central role in these successes.

Conclusion: The One Health framework is crucial for the control and prevention of water- and food-borne helminth infections. Climate change, socio-economic factors, and dietary shifts continue to influence the global spread of these parasites, necessitating the adoption of multi-sectoral strategies. Collaborative global efforts, enhanced surveillance, and improved food safety practices are essential for further reducing the impact of helminth infections on public health and achieving the Sustainable Development Goals.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Climate change, Emerging, Global health proceeding, Helminthiasis control.

Introduction:

Food security demands stable access to safe, nutritious food, yet water- and food-borne diseases undermine health, productivity, and economic growth, threatening progress toward the Sustainable Development Goals (1). Sustainable development balances present needs with resource preservation across economic, social, and environmental dimensions, guaranteeing safe water, clean air, adequate housing, and nutritious diets (2). Strengthening health systems, sanitation, hygiene, vaccination, and medical access restricts infectious diseases (3). In many developing nations, these illnesses impose heavy human, economic, and environmental burdens, compounded by global trade, tourism, changing diets, and climate-driven events (4). The One Health framework, integrating human, animal, and environmental health, provides analytical and participatory tools for early outbreak detection, prevention, and control, thereby bolstering food security, public health, and sustainable development (5). This study aimed to present strategic programs for diagnosing, controlling, preventing, and treating diseases caused by water- and foodborne helminth parasites, through a One Health perspective, as well as, it analyzes existing programs implemented internationally that target the diagnosis, control, prevention, and treatment of water- and food-borne helminth parasites infections.

Materials and Methods:

Various published sources, including original research articles, reviews, brief reports, books, and verified online materials, were gathered from electronic databases in English (Google Scholar, Science Direct, Scopus, and PubMed) as well as national Iranian databases (SID, Iran Doc, Magiran, and Iran Medex) during January and May 2025. Data were collected on the prevalence and distribution of different food and water borne helminthiasis, covering diseases such as Taeniosis/Cysticercosis, Trichinellosis, liver fluke infections (Fascioliasis and Dicrocoeliasis), in both human and animal hosts. The extracted information was organized and recorded using an Excel spreadsheet.

Results and Discussion:

Outcome data about Chinese and Europe Programs for the Control and Prevention of Water- and Food-Borne Helminths are derived from published national surveillance bulletins (2018-2024) and peer-reviewed evaluations (Table 1 and Table 2) (6,7). Several programs implemented in Iran to control and prevent water- and food- borne infections have likewise proved successful (Table 3) (8).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table1. Chinese Programs for the Control and Prevention of Water- and Food-Borne Helminths

Program / Time-frame	Geographic scope & target parasites	Core interventions
Accelerated Elimination of Schistosomiasis Plan (2023 – 2030)	Twelve endemic provinces along the Yangtze River and associated wetlands – <i>Schistosoma japonicum</i>	1) Mechanised tillage to replace water-buffalo ploughing. 2) Quarantine/relocation of bovines from marshlands. 3) Canal lining, snail-habitat drainage, & mollusciciding. 4) Up-grading of rural drinking-water systems
National Echinococcosis Control Programme (launched 2005) plus “Smart Deworming-Collar” pilot (Tibet, 2020)	Tibetan Plateau, Xinjiang and Qinghai – <i>Echinococcus granulosus</i> & <i>E. multilocularis</i>	1) Monthly praziquantel baiting of owned & stray dogs, tracked by IoT “smart collars” that dispense the dose automatically. 2) Vaccination of sheep/goats (Eg95). 3) Ultrasound screening and albendazole treatment in humans.
Integrated Strategy for <i>Clonorchis sinensis</i> Control (Shenzhen pilot, 2006 – 2014)	Rural clusters in Guangdong Province – fish-borne liver fluke <i>C. sinensis</i>	1) Community-wide praziquantel chemotherapy every 12 months. 2) Behaviour-change campaigns discouraging raw-fish consumption. 3) Installation of sanitary latrines & household sewage treatment. 4) Environmental management of pond effluent.
China National Food-borne Pathogen Surveillance System (CNFPSS, est. 2001)	> 2 300 sentinel sites covering all provinces – <i>Echinococcus</i> , <i>Trichinella</i> , <i>Taenia</i> , <i>Fasciola</i> and other food-borne agents	1) Annual stratified sampling of meat, fish, fresh produce and drinking water. 2) Molecular typing & whole-genome sequencing for outbreak tracing. 3) Real-time alerts to provincial CDCs and Ministry of Agriculture.
Rural WASH + STH/Fluke Education & Mass Drug Administration (South & Southwest China, 2010 – ongoing)	Yunnan, Guangxi, Guizhou, Sichuan – soil-transmitted helminths & plant-borne flukes	1) Expansion of piped, chlorinated water. 2) Subsidised construction of pour-flush latrines. 3) School-based health-education curricula. 4) Annual albendazole or mebendazole MDA.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 2. European Programs for the Control and Prevention of Water- and Food-Borne parasites.

Program / Time-frame	Geographic scope & target parasites	Core interventions
EU One-Health Zoonoses Report (annual, EFSA + ECDC, since 2006)	EU-27 & EEA; <i>Echinococcus</i> spp., <i>Trichinella</i> spp., <i>Taenia</i> spp., <i>Cryptosporidium</i> spp.	1) Harmonised collection and statistical analysis of human, food-chain and animal data reported by Member States. 2) results published in an open-access annual dossier and interactive dashboards.
EFSA BIOHAZ Scientific Opinions on Food-borne Parasites (latest No. 5495, 2023)	Entire food chain; prioritisation of 17 parasites	1) Quantitative risk assessment, ranking, and methodological guidance for detection, tracing and control of parasites in meat, fish and fresh produce.
COST Action FA1408 “Euro-FBP” (2014-2018)	38 European countries; food-borne helminths (e.g., <i>Trichinella</i> , <i>Taenia</i> , fish-borne trematodes)	1) Creation of a pan-European laboratory network. 2) harmonisation of molecular diagnostics. 3) geo-referenced prevalence mapping. 4) risk-based control framework.
One-Health European Joint Programme (OHEJP) (2018-2023, Horizon 2020)	44 national institutes; zoonotic helminths,	1) 90 million, 30 trans-disciplinary research projects (e.g., MARBLE on <i>Fasciola</i> and <i>Taenia</i>), postgraduate training schools 2) data-sharing platform integrating human–animal–environment surveillance.
Commission Delegated Regulation (EU) 2018/772 on <i>Echinococcus multilocularis</i>	Dogs moving across borders in EU/EEA; <i>E. multilocularis</i>	1) Mandates preventive praziquantel treatment, 2) bait vaccination of wild canids, and annual copro-antigen monitoring for Member States seeking trade derogations.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 3. Water- and Food-Borne Infections that has been successfully controlled in Iran

Infection	Control strategy (headline)	Take-away factors
Soil-transmitted helminths (<i>Ascaris</i>, <i>Trichuris</i>, hookworms)	1) Mass school de-worming (albendazole/mebendazole) in the 1990s. 2) Rural latrine construction and safe-water projects during the Fifth & Sixth Development Plans. 3) Health-education in primary schools	Combined chemotherapy + WASH + school hygiene campaigns drove the decline; surveillance now focuses on protozoa rather than helminths.

Table 4. Important Foodborne Helminths

Helminth	Taxonomy	Common Host(s)	Transmission Route	Geographical Distribution	Prevention Measures
<i>Taenia saginata</i>	Cestoda (Tapeworm)	Cattle (intermediate), Humans (definitive)	Ingestion of undercooked beef containing cysticerci	Worldwide, more common in regions with poor sanitation	Proper cooking of beef, freezing meat
<i>Ascaris lumbricoides</i>	Nematoda (Roundworm)	Humans (definitive)	Ingestion of eggs from contaminated food or water	Worldwide, especially in areas with poor sanitation	Good hygiene, wash hands and food thoroughly
<i>Trichinella spiralis</i>	Nematoda (Roundworm)	Pigs, wild animals (intermediate), Humans (definitive)	Ingestion of undercooked pork or wild game containing larvae	Common in areas with insufficiently cooked pork	Proper cooking/freezing of pork and wild meats
<i>Anisakis simplex</i>	Nematoda (Roundworm)	Fish, squid (intermediate), Humans (definitive)	Ingestion of raw or undercooked fish/squid containing larvae	Coastal regions, especially in Japan, Europe, and the Americas	Freezing fish before consumption
<i>Diphyllobothrium latum</i>	Cestoda (Tapeworm)	Fish (intermediate)	Ingestion of undercooked fish containing larvae	Found in freshwater	Proper cooking or freezing of fish



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Helminth	Taxonomy	Common Host(s)	Transmission Route	Geographical Distribution	Prevention Measures
		e), Humans (definitive)		regions worldwide	
<i>Clonorchis sinensis</i>	Trematoda (Liver fluke)	Freshwater fish (intermediate), Humans (definitive)	Ingestion of undercooked fish	Endemic in East Asia (China, Korea, Taiwan)	Proper cooking of fish, avoid raw fish
<i>Fasciola spp.</i>	Trematoda (Liver fluke)	Freshwater snails (intermediate), Cattle, Sheep (definitive)	Ingestion of contaminated water plants	Endemic in regions with livestock and freshwater plants	Avoid consumption of contaminated water plants,
<i>Opisthorchis viverrini</i>	Trematoda (Liver fluke)	Freshwater fish (intermediate), Humans (definitive)	Ingestion of undercooked fish	Endemic in Thailand, Laos, Cambodia	Cook or freeze fish properly
<i>Echinococcus granulosus</i> /Hydatid cysts	Cestoda (Tapeworm)	Dogs (definitive), Sheep, Cattle, Humans (accidental intermediate host)	Ingestion of eggs from contaminated food, water, or environment by dog feces	Worldwide; endemic in pastoral regions (e.g., Middle East, South America, Central Asia, Africa, China)	Regular deworming of dogs, hygiene, proper offal disposal
<i>Toxocara spp.</i> (e.g., <i>T. canis</i> , <i>T. cati</i>)	Nematoda (Roundworm)	Dogs, Cats (definitive), Humans (accidental intermediate)	Ingestion of embryonated eggs from soil, raw vegetables, or contaminated hands	Worldwide; especially in tropical/subtropical urban areas and low-income communities	Public hygiene, deworming pets, proper food washing
Ruminant nematodiasis (e.g.,	Nematoda (Roundworm)	Cattle, Sheep,	Ingestion of infective larvae from	Global, especially in tropical/subtropi	Rotational grazing, anti-helminthic

Helminth	Taxonomy	Common Host(s)	Transmission Route	Geographical Distribution	Prevention Measures
<i>Haemonchus</i> spp., <i>Ostertagia</i> spp.)		Goats (definitive);	contaminated pastures	cal livestock zones with poor pasture management	prophylaxis, pasture hygiene

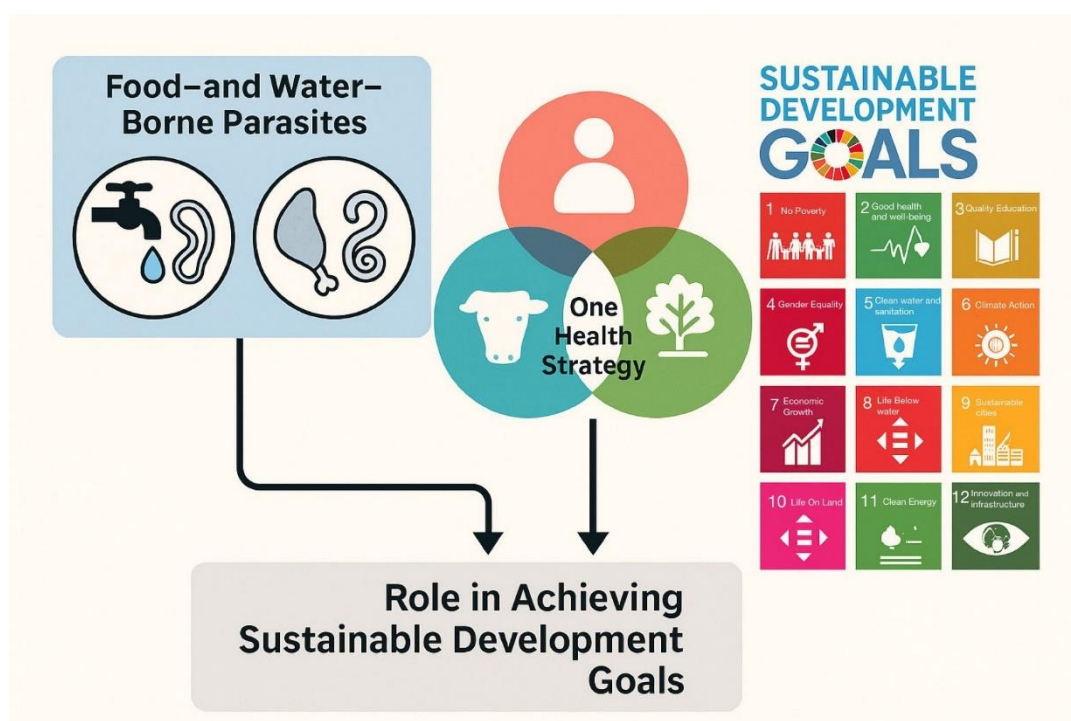


Figure 1. Graphical abstract illustrating the key findings of the present study

Climate change, socio-economic dynamics and evolving food practices collectively drive the contemporary distribution of food-borne helminths. Rising humidity and higher temperatures lengthen parasite survival and hasten larval development. Heavy rainfall disseminates eggs and cysts, whereas droughts, though harmful to free-living stages, can concentrate parasites in shrinking water sources (9). At the same time, expanded travel, tourism and migration, together with increasing consumption of raw, under-cooked, pickled or smoked foods (for example sushi), facilitate global spread. Hunting and outdoor camping likewise raise exposure to *Trichinella* spp. (Table 4) in wild pigs. Current visual, palpation and incision methods—used to detect *Taenia*, *Fasciola hepatica* and *Anisakis*—show high specificity but limited sensitivity, missing most light infections (e.g. only 27 % of mild *Taenia* cases) (Table 4) (10). Europe



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



illustrates the value of layered interventions, stringent legislation, integrated surveillance and coordinated research have reduced *Trichinella* spp. and *Taenia* incidence in most EU Member States to <0.001 %, although climate-related threats remain on the Horizon Europe agenda. Core food-processing chains include thermal, freezing and chemical treatments, heating to 60–70 °C for 15–30 min or freezing to –18 °C for up to three days reliably kills larvae, and alternative processing methods can mitigate risk where cultural preferences limit thorough cooking (7). Iran’s experience over the past five decades underscores the power of sustained, multisectoral action. Real-time cholera surveillance linked to ring chlorination, compulsory typhoid reporting coupled with safe-water expansion, massive oral-polio vaccination campaigns, and nationwide school de-worming have transformed once entrenched water- and food-borne diseases into sporadic, low-level problems (8). Together, these examples highlight the necessity of One-Health strategies that integrate climate awareness, food safety, veterinary oversight and public-health infrastructure to safeguard food security and sustainable development.

Acknowledgement:

The authors would like to express their sincere gratitude to the Ministry of Research, Faculty of Veterinary Medicine, University of Tehran, and the Association of Animal Parasitic Diseases and Zoonotic Parasites for their invaluable support throughout this research.

References:

1. Rabiou AG, Fadiji O, Adesoji AT, Jimoh SO, Cho S. A review of the environmental spread of *Salmonella enterica* serovars through water in Africa. *LamBioscience*. 2025. Available from: <https://doi.org/10.1093/lambio/ovaf005>
2. Kumar G, Kumar S, Jangid H, Dutta J, Shidiki A. The rise of non-typhoidal *Salmonella*: an emerging global public health concern. *Front Microbiol*. 2025;16:1524287. Available from: <https://doi.org/10.3389/fmicb.2025.1524287>
3. Hiltunen M, Edvinsson S. What was killing babies in Sundsvall? A study of infant mortality patterns using individual level cause of death data, 1860–1892. *HLCS*. 2025. Available from: <https://doi.org/10.51964/hlcs19299>
4. Conforti S, Pruden A, Acosta N, Anderson CWN, Bürgmann H, de Araújo JC, et al. Strengthening policy relevance of wastewater-based surveillance for antimicrobial resistance. *Environ Sci Technol*. 2025;59(15):9991–10006. Available from: <https://doi.org/10.1021/acs.est.4c09663>



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



5. Verani M, De Giglio O, Caputo MC, Cassiani G, Milani M, Carducci A, et al. Study protocol of predictive dynamics of microbiological contamination of groundwater in the Earth critical zone and impact on human health (DY.MI.CR.ON project). Water. 2025;17(3):294. Available from: <https://doi.org/10.3390/w17030294>
6. Dorny P, Praet N, Deckers N, Gabriël S. Emerging food-borne parasites. Vet Parasitol. 2009;163 (3):196-206. Available from: <https://doi.org/10.1016/j.vetpar.2009.05.026>
7. Broglia A, Kapel CMO. Changing dietary habits in a changing world: Emerging drivers for the transmission of foodborne parasitic zoonoses. Vet Parasitol. 2011;182(1):2-13. Available from: <https://doi.org/10.1016/j.vetpar.2011.07.011>
8. Hedayati MA, Bahmani N. Most important bacterial and parasitic zoonotic diseases in Iran. Reviews and Research in Medical Microbiology. 2023 Jan 1; 34 (1):12-21. <https://doi.org/10.1097/MRM.0000000000000320>
9. Jalousian F, Eslami A, Meshgi B, Hoseini SH. Impact of Climate Change (Global Warming) on Food-Borne Parasites. InSymposium Committee 2015 Mar 30 (p. 76).
10. Hosseini SH, Fathi S, Jalousian F. Helminths of veterinary and public health importance in Iran: the great neglected parasitic diseases as important challenge for the country. Iran J Vet Med. 2018; 12: 2-7.
11. Fathi S, Jalousian F, Hosseini SH, Parsa H, Kordafshari S. A study of cross-reactivity between recombinant EPC1 Antigen of *Echinococcus granulosus* in serum from patients with confirmed cystic echinococcosis infection and other parasitic infections. The American Journal of Tropical Medicine and Hygiene. 2016 Jun 1; 94 (6):1313: <https://doi.org/10.1111/apm.12838>



Epidemiological Status of Cutaneous Leishmaniasis in Iran

Mohammad Zeinali¹, Faham Khamesipour¹

Corresponding Author: Mohammad Zeinali, mzeinali411@gmail.com

1. National Department of Zoonotic Disease Control, Center of Communicable Diseases Control, Ministry of Health and Medical Education, Tehran, Iran.

Article Code: 1546

Abstract

Background: Cutaneous leishmaniasis (CL) is a parasitic zoonotic disease of public health concern in endemic regions such as Iran. Caused by the *Leishmania* genus and transmitted through sandfly bites, CL presents as ulcerative skin lesions that often heal with permanent scars. With an estimated 700,000–1,000,000 new cases globally each year, surveillance is crucial for control and prevention. In Iran, CL remains a priority for the Ministry of Health due to its high prevalence in endemic provinces such as Isfahan, Razavi Khorasan, and Fars.

Methods: Epidemiological data on CL in Iran were gathered from health centers, medical universities, and reported to the Center for Communicable Diseases Control (CDC). Data from 2008 to 2024 were analyzed to assess trends, regional distribution, and incidence rates. Provinces with the highest cases were analyzed for zoonotic versus anthroponotic CL. Statistical analysis included percentage changes year-to-year and long-term trends. Global data from WHO reports were reviewed for comparison.

Results: Between 2008 and 2024, the annual cases in Iran declined from over 26,000 to 8,267, representing a 68% reduction. The 2024 incidence was 9.6 per 100,000 population. The provinces most affected were Isfahan, Razavi Khorasan, and Fars. Zoonotic CL accounted for approximately 80% of reported cases, while anthroponotic CL was concentrated in Mashhad and Bam. Graphical analysis indicated sharp seasonal peaks during September–December, aligning with sandfly activity. The global burden remains substantial, with Iran among the top seven countries contributing >85% of cases worldwide.

Conclusion: Despite significant reductions in incidence over the past 15 years, CL continues to pose a substantial health burden in Iran. Surveillance, vector control, and public health awareness are critical to sustaining progress. The dual presence of zoonotic and anthroponotic CL requires integrated strategies addressing both rodent reservoirs and human transmission chains. Strengthening surveillance can aid regional and global elimination goals.

Keywords: Zoonoses, Surveillance, Epidemiology, Cutaneous leishmaniasis, Vector-borne diseases.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Introduction:

Cutaneous leishmaniasis (CL) is a neglected tropical disease with considerable public health significance (1, 2). It is caused by protozoan parasites belonging to the *Leishmania* genus and transmitted to humans via the bite of infected female sandflies (*Phlebotomus* spp.) (3-5). Unlike visceral leishmaniasis (VL), which is potentially fatal, CL is not life-threatening but can cause chronic skin ulcers, disfigurement, and psychosocial distress (6).

Globally, CL is endemic in nearly 99 countries across the tropics, subtropics, and Mediterranean basin (7-10). According to the World Health Organization (9), an estimated 700,000–1,000,000 new cases of CL occur annually, while 350 million people remain at risk of infection. Approximately 85% of the global CL burden is concentrated in seven countries: Afghanistan, Algeria, Brazil, Colombia, Iran, Iraq, and Sudan/South Sudan. These regions share similar ecological and socioeconomic factors that favor the persistence of sandfly vectors and reservoirs (7, 11).

Iran represents one of the most endemic countries in the Eastern Mediterranean region, reporting thousands of cases annually (7, 11). The disease occurs in both zoonotic and anthroponotic forms. Zoonotic CL (ZCL), caused by *Leishmania major*, is predominant in rural and semi-arid zones and transmitted via rodent reservoirs (12). Anthroponotic CL (ACL), caused by *Leishmania tropica*, is primarily found in urban centers and transmitted directly among humans, with dogs as occasional hosts. The coexistence of these two forms complicates epidemiological control (13).

Epidemiological surveillance plays a pivotal role in monitoring CL incidence, identifying endemic foci, and informing control strategies. National surveillance in Iran began decades ago, and continuous data collection has revealed significant trends. This study provides an updated analysis of CL incidence in Iran between 2008 and 2024, evaluates regional variations, and situates national data within the global epidemiological context.

Materials and Methods:

This study used national surveillance data collected through the Iranian Ministry of Health and Medical Education. All suspected, probable, and confirmed cases of CL reported from city health centers, hospitals, and medical universities were entered into the national portal system following standardized definitions. Cases were subsequently reported to the Center for Communicable Diseases Control (CDC).

Data collection and analysis:

- Data spanned from 2008 (1387 SH) to 2024 (1403 SH).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- Parameters included total national cases, provincial case counts, age distribution, and seasonal trends.
- Provinces with the highest case loads (Isfahan, Razavi Khorasan, Fars, Golestan, and Khuzestan) were analyzed in detail.
- Graphical data showing year-by-year incidence were digitized for trend analysis. Percentage changes were calculated to quantify reductions.

Results and Discussion:

Yearly reported cases of Cutaneous Leishmaniasis in Iran (2008–2024) show that in figure 1.

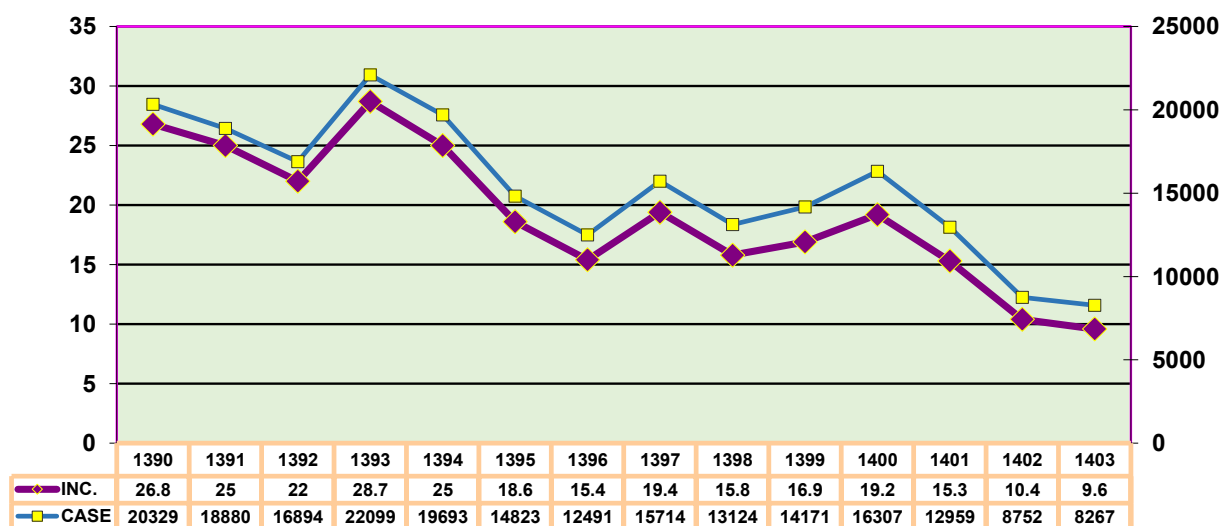


Figure 1. Number of cases and incidence of Cutaneous Leishmaniasis in Iran (2008–2024)

Provincial Case Distribution: In 2024, five provinces accounted for the majority of reported CL cases:

- Isfahan: 2136 cases (25.8% of national total)
- Razavi Khorasan: 1256 cases (15.2%)
- Fars: 1226 cases (14.8%)
- Golestan: 668 cases (8.1%)
- Khuzestan: 614 cases (7.4%)



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Together, these provinces represented over 70% of national CL incidence. Other endemic provinces included Kerman, Yazd, Hormozgan, Sistan and Baluchestan, and Semnan, each reporting between 200–500 cases annually.

National Trend (2008–2024)

In 2008, Iran reported over 26,000 cases of CL. By 2024, this number had decreased to 8,267, representing a 68% reduction in reported incidence. The calculated incidence rate for 2024 was 9.6 per 100,000 population. While the overall trend is declining, annual fluctuations were noted, with temporary increases in 2012, 2016, and 2020, correlating with local outbreaks.

Graphical Trend Analysis: Analysis of year-by-year case numbers showed:

- Steady decline from 2008 to 2013 (–42%).
- Temporary resurgence in 2014–2016 (+12%).
- Marked reduction between 2017 and 2020 (–35%).
- Another resurgence during 2021–2022, possibly linked to post-COVID surveillance recovery.
- Sharp decline again by 2024 (–20%).

Seasonal data indicated peaks between September and December, corresponding with increased sandfly activity. Age distribution revealed that the 35–45-year age group was most affected, likely due to occupational exposure.

Global Epidemiology (WHO 2022)

Globally, an estimated 1 million cases of CL occur annually. Approximately 85% of these cases are reported from Afghanistan, Algeria, Brazil, Colombia, Iran, Iraq, and Sudan/South Sudan. Iran remains a top contributor in the Eastern Mediterranean region, with endemic transmission reported in 19 provinces and >150 counties.

Zoonotic vs. Anthroponotic CL

Approximately 80% of Iranian CL cases are zoonotic, caused by *Leishmania major* and transmitted by rodents such as *Rhombomys opimus*. These cases occur mainly in rural and semi-arid provinces like Isfahan, Kerman, and Golestan. In contrast, anthroponotic CL caused by *Leishmania tropica* is concentrated in urban centers, with >95% of cases reported from Mashhad and Bam. This dual epidemiology poses unique challenges for surveillance and control.

Interpretation and Implications

The overall decline reflects effective national interventions including vector control, rodent management, and community-based prevention. However, fluctuations underscore the



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



vulnerability of endemic foci to ecological changes, climatic factors, and disruptions such as the COVID-19 pandemic. Compared with global epidemiology, Iran's continued high ranking among endemic countries demonstrates both progress and the need for sustained vigilance.

Acknowledgment:

The authors thank the Iranian Ministry of Health and Medical Education, the Center for Communicable Diseases Control, and provincial health authorities for providing surveillance data used in this study.

References:

1. Tajbakhsh, E., Khamesipour, A., Hosseini, S. R., Kosari, N., Shantiae, S., & Khamesipour, F. (2021). The effects of medicinal herbs and marine natural products on wound healing of cutaneous leishmaniasis: A systematic review. *Microbial pathogenesis*, 161(Pt A), 105235. <https://doi.org/10.1016/j.micpath.2021.105235>
2. Jahromi, A. S., Jokar, M., Abdous, A., Soleimanpour, S., Rahmanian, K., Askari, H., & Rahmanian, V. (2025). Knowledge, Attitudes, and Practices toward Cutaneous Leishmaniasis as a Neglected Tropical Disease among the General Population: A Systematic Review and Meta-Analysis. *Journal of epidemiology and global health*, 15(1), 97. <https://doi.org/10.1007/s44197-025-00444-4>
3. Khamesipour F, Khamesipour A, Hejazi SH, Ghanadian M. (2024) In vitro assessment of *Dracocephalum lindbergii* for growth inhibition, apoptosis induction, and cytokine modulation against *Leishmania major*. *Heliyon*. 10 (19): e38331. <https://doi.org/10.1016/j.heliyon.2024.e38331>
4. Ansari, Z., Chaurasia, A., Neha, Kalani, A., Bachheti, R. K., & Gupta, P. C. (2025). Comprehensive insights into leishmaniasis: From etiopathogenesis to a novel therapeutic approach. *Microbial pathogenesis*, 204, 107535. <https://doi.org/10.1016/j.micpath.2025.107535>
5. Yaghoobi-Ershadi MR. Control of phlebotomine sand flies in Iran: a review article. *J Arthropod Borne Dis*. 2012;6(1):1-8.
6. Scarpini S, Dondi A, Totaro C, Biagi C, Melchionda F, Zama D, Pierantoni L, Gennari M, Campagna C, Prete A, Lanari M. Visceral Leishmaniasis: Epidemiology, Diagnosis, and Treatment Regimens in Different Geographical Areas with a Focus on Pediatrics. *Microorganisms*. 2022 Sep 21;10(10):1887. doi: 10.3390/microorganisms10101887. PMID: 36296164; PMCID: PMC9609364.
7. Sharifi I, Khosravi A, Aflatoonian MR, Salarkia E, Bamorovat M, Karamoozian A, Moghadam MN, Sharifi F, Afshar AA, Afshari SAK, Gharachorloo F, Shirzadi MR,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- Amiri B, Zainali M, Doosti S, Zamani O, Gouya MM. Cutaneous leishmaniasis situation analysis in the Islamic Republic of Iran in preparation for an elimination plan. *Front Public Health*. 2023 Apr 28;11:1091709. doi: 10.3389/fpubh.2023.1091709. PMID: 37188278; PMCID: PMC10176454.
8. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*. 2012;7(5):e35671.
 9. World Health Organization. Leishmaniasis fact sheet. Geneva: WHO; 2022.
 10. Shirzadi MR, Esfahania SB, Mohebbi M, et al. Epidemiological status of leishmaniasis in the Islamic Republic of Iran, 1983–2012. *East Mediterr Health J*. 2015;21(10):736-42.
 11. IHME Pathogen Core Group (2024). Global burden associated with 85 pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet. Infectious diseases*, 24(8), 868–895. [https://doi.org/10.1016/S1473-3099\(24\)00158-0](https://doi.org/10.1016/S1473-3099(24)00158-0)
 12. Ghatte MA, Taylor WR, Karamian M. The Geographical Distribution of Cutaneous Leishmaniasis Causative Agents in Iran and Its Neighboring Countries, A Review. *Front Public Health*. 2020 Feb 18;8:11. doi: 10.3389/fpubh.2020.00011. PMID: 32133334; PMCID: PMC7039857.
 13. Bamorovat, M., Sharifi, I., Tavakoli Oliaee, R., Jafarzadeh, A., & Khosravi, A. (2021). Determinants of Unresponsiveness to Treatment in Cutaneous Leishmaniasis: A Focus on Anthroponotic Form Due to *Leishmania tropica*. *Frontiers in microbiology*, 12, 638957. <https://doi.org/10.3389/fmicb.2021.638957>



Applications of Artificial Intelligence and Deep Learning in the Diagnosis of Parasitic Diseases: A Case Study on *Dirofilaria immitis* Microfilariae

Fatemeh Haji Agha Khiyabani¹, Fateme Jalousian¹

Corresponding Author Email: Fatemeh Haji Agha Khiyabani, fatemehkhiabani82@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article Code: 1557

Abstract

Background: This study investigates the applications of artificial intelligence (AI) and deep learning in the diagnosis of parasitic diseases, focusing on *Dirofilaria immitis*. Diseases caused by this parasite in dogs and humans, transmitted by mosquitoes, pose serious public health concerns. Traditional diagnostic methods, such as the modified Knott's test, have limitations due to their reliance on technician experience and the possibility of misidentifying artifacts. The aim of this study is to design and evaluate a deep learning-based system capable of accurately distinguishing microfilariae from artifacts in microscopic images.

Methods: In this study, 700 microscopic images containing *D. immitis* microfilariae and artifacts were collected and annotated. A convolutional neural network (CNN) model was trained using these labeled datasets, and data augmentation was applied to enhance performance. The model was evaluated using accuracy, sensitivity, specificity, and processing time as performance metrics.

Results: Results show that the system can detect microfilariae with over 95% accuracy, significantly improving diagnostic precision compared to traditional methods.

Conclusion: This system can provide a reliable tool for the diagnosis of *Dirofilaria immitis* microfilariae, offering improved accuracy compared to traditional methods. It may be applied in epidemiological surveys and field studies, especially in areas with limited access to advanced diagnostic facilities. Future work should focus on validating the model with larger and more diverse datasets to enhance its applicability in real-world settings.

Keywords: Deep learning, Artificial intelligence, Convolutional neural networks (CNN), Automatic detection, Modified Knott's test, Medical image processing.

Introduction:

Parasitic diseases remain a significant global health challenge, particularly in regions lacking advanced diagnostic facilities (1). Accurate and timely diagnosis is critical to prevent complications, especially for infections caused by *Dirofilaria immitis*, a mosquito-transmitted



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



parasite producing microfilariae in the bloodstream. In Iran, canine heartworm disease shows considerable prevalence, with infection rates up to 11.5% in some areas (2). Conventional diagnostic methods, such as the modified Knott's test, depend heavily on technician expertise and are susceptible to errors, notably in distinguishing microfilariae from similar artifacts (3). This highlights the need for more precise and automated diagnostic approaches.

Recent advances in artificial intelligence (AI) and deep learning, especially convolutional neural networks (CNNs), have shown promising results in medical image analysis and parasite detection. These models are particularly valuable in differentiating true microfilariae from thread-like artifacts that often complicate manual diagnosis.

Several studies have validated AI's utility in parasitology. For instance, Ward et al. demonstrated AI's effectiveness in detecting soil-transmitted helminths and *Schistosoma mansoni* eggs in low-resource settings (4). Aydin and Karaarslan explored GPT-4's role in radiologic image interpretation, and Male et al. discussed AI's benefits and challenges in diagnosing intestinal parasites (5,6). Schwingel et al. Also showed how tools like ChatGPT can assist in diagnosing visceral leishmaniasis, underscoring AI's expanding role in medical diagnostics (7).

The objectives of this study were to develop and evaluate a deep learning system based on CNNs to accurately detect *D. immitis* microfilariae in microscopic images, to enhance diagnostic accuracy, to reduce diagnostic time, and to minimize reliance on human expertise, thereby providing a valuable tool for rural and resource-limited settings.

Materials and Methods:

In this study, a systematic review was conducted to investigate the applications of deep learning in the diagnosis of parasitic diseases, with a special focus on *Dirofilaria immitis* microfilariae. The research followed four main steps: identification of scientific resources, including relevant literature related to deep learning in parasitology, particularly for *D. immitis*; analysis of deep learning models, especially CNNs, for detecting microfilariae in microscopic images; evaluation of trained models using accuracy, sensitivity, specificity, and processing time; and results analysis through comparison of deep learning outputs with conventional microscopic diagnosis to validate clinical relevance.

The flowchart presented in Figure 1 outlines the principal stages of this study, beginning with the identification of relevant scientific sources, followed by the selection, training, and evaluation of deep learning models, and concluding with result analysis and comparison to conventional human microscopic diagnosis. Previous studies have also demonstrated the feasibility of applying AI to parasitology. For example, Bani Hashem Nejad et al. successfully employed deep learning techniques to detect *Dirofilaria immitis* microfilariae in canine blood



samples collected from stray dogs in Gilan Province, Iran, confirming positive cases by PCR (8).

Building upon these prior findings, the present study focused on developing a CNN-based model trained on a dedicated image dataset. For this purpose, a total of 700 microscopic images containing both microfilariae and artifacts were collected and manually segmented. Images were captured using a light microscope at 400× magnification equipped with a digital camera, saved in JPEG format, and resized to 512 × 512 pixels due to hardware limitations. To ensure high-quality data, blurred or low-contrast images were excluded, retaining only those with clear visualization of microfilariae and background structures.

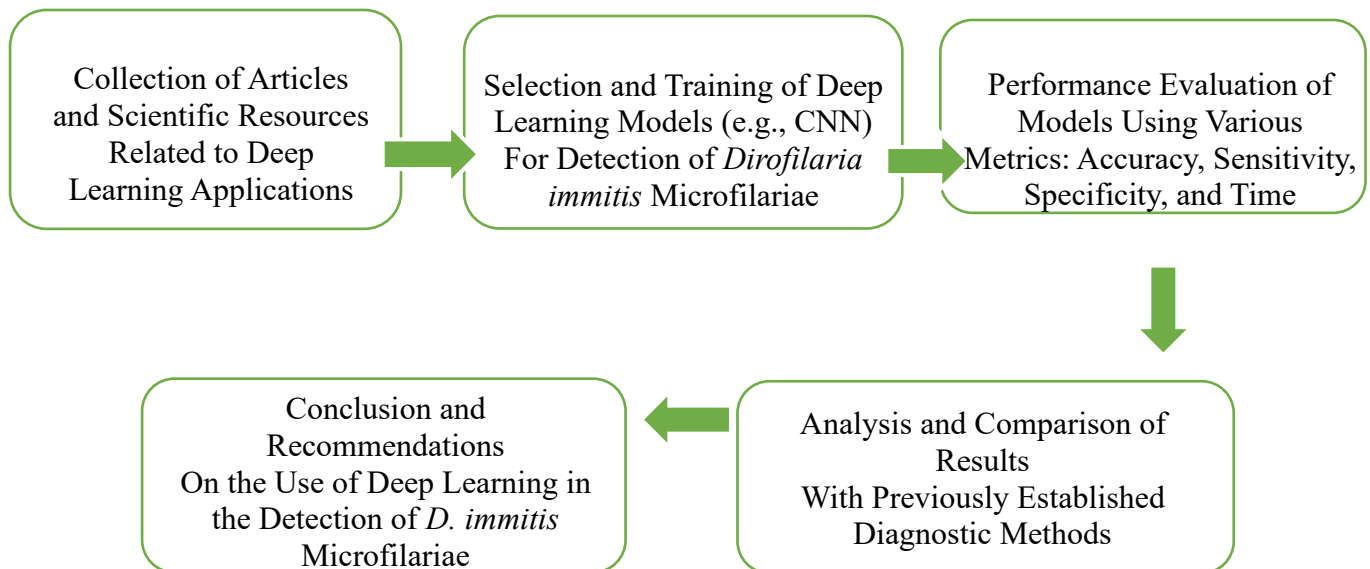


Figure 1. Methodology Flowchart

All images were independently reviewed and annotated by two experienced parasitologists. Within each image, microfilariae were manually distinguished from artifact structures, and annotated regions were marked accordingly (Figure 2). In cases of disagreement, a consensus was reached through discussion. These expert annotations were used as the ground truth for model training and evaluation. Data preprocessing included Hough Circle Transform for circular region extraction, background removal, and cropping. Data augmentation was applied to artificially expand the dataset, enhancing diversity and improving model generalization. This approach aligns with prior findings demonstrating the critical role of data augmentation in

optimizing deep learning performance for medical imaging and biological signal classification, such as EEG, EMG, and COVID-19 radiographs (9).

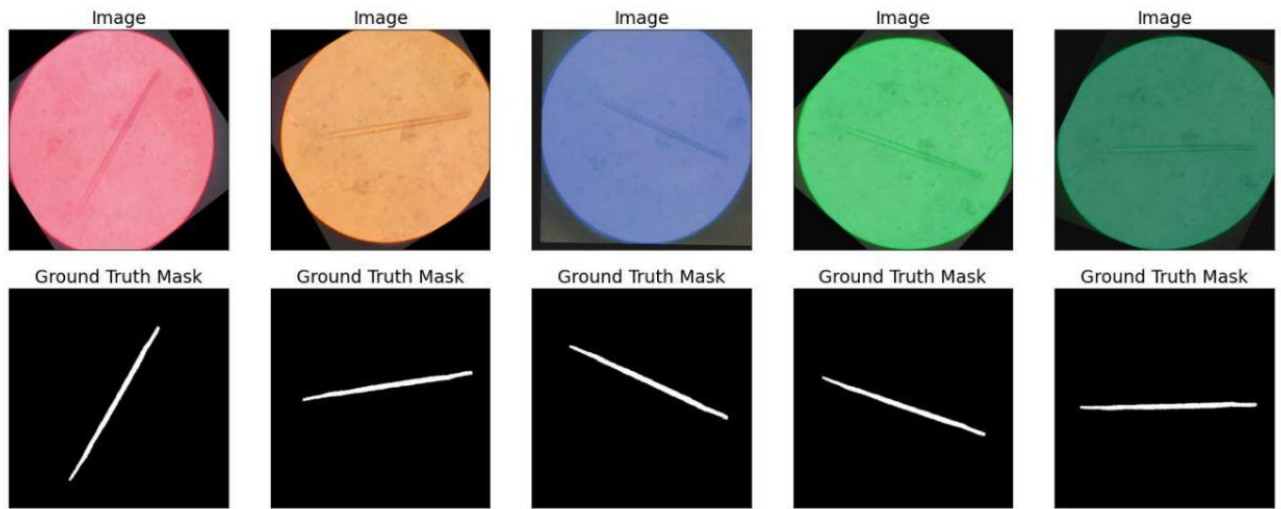


Figure 2. an example of microscopic image after data augmentation and the corresponding masks.

In the study by Bani Hashem Nejad et al., the network architecture for microfilariae detection was designed based on the U-Net model, capable of segmenting images into three categories: background, artifact, and microfilariae (Figure 3) (8).

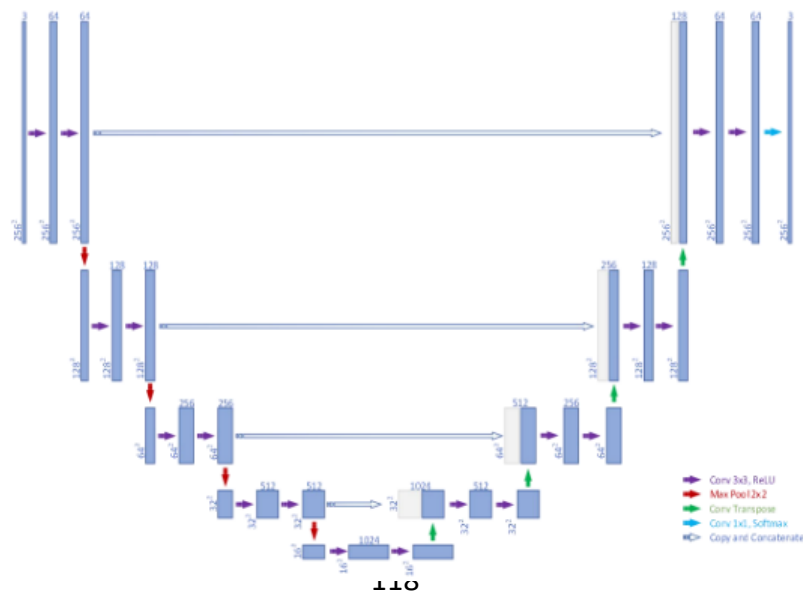


Figure 3. Architecture of U-Net.

Evaluation of the proposed model results and classification performance showed that the dice loss function was employed, which calculates different weights for each class in classification. This function was implemented using frequency-weighted dice loss, assigning lower weight to the background class and higher weights to classes with smaller regions in the ground truth mask.

$$w_c = 1 - \frac{\sum_n g(n, c)}{\sum_{n,k} g(n, k)} \quad (1a)$$

$$D_c = \frac{2 \sum_n g(n, c) p(n, c) + s}{\sum_n g(n, c) + \sum_n p(n, c) + s} \quad (1b)$$

$$\mathcal{L} = \sum_c w_c (1 - D_c) \quad (1c)$$

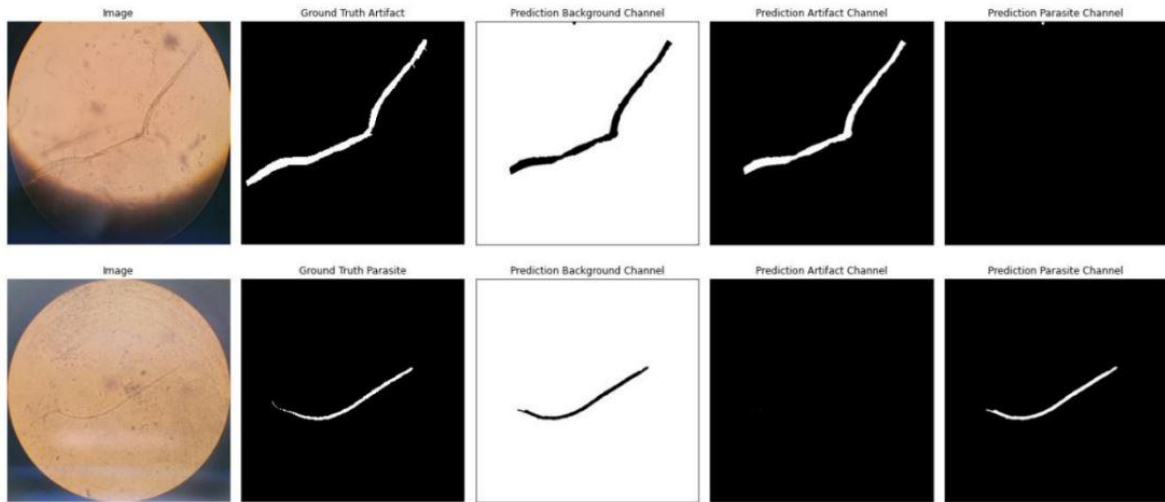


Figure 4. Classification result of the U-net.

In this section, the performance of the proposed model and its classification accuracy are analyzed. As shown in the table below, the accuracy, sensitivity, and specificity obtained from

the classification by the U-Net model were as follows. It is worth noting that these metrics were calculated for the training, validation, and test datasets over 10 repeated runs (Figures 3-5).

Table 1.

	Accuracy	Sensitivity	Specificity
Training Set	0.922± 0.006	0.909± 0.009	0.935± 0.015
Validation Set	0.947± 0.028	0.945± 0.027	0.950± 0.050
Test set	0.961± 0.039	0.956± 0.050	0.968± 0.059

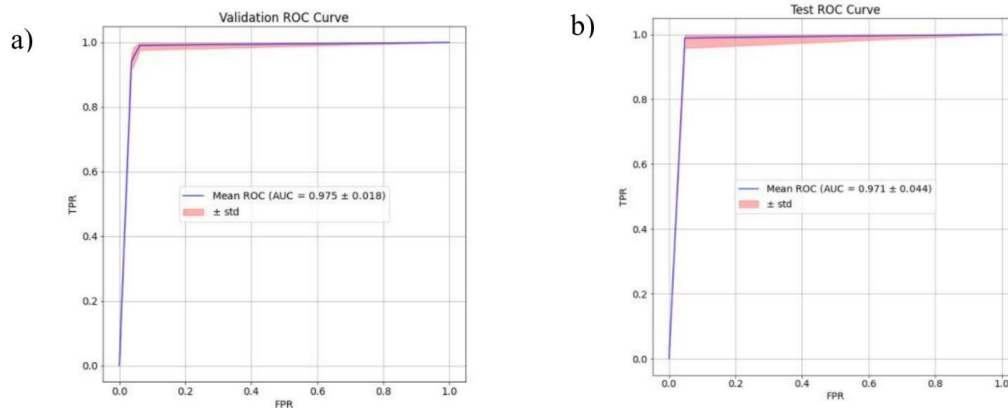


Figure 5. ROC curve of the system developed by Bani Hashem Nejad et al. (8).

Result and Discussion:

The proposed deep learning model demonstrated high efficacy in detecting *Dirofilaria immitis* microfilariae. Performance metrics on the training dataset showed an accuracy of 92.2%, sensitivity of 90.9%, and specificity of 93.5%. On the independent test set, the model achieved 96.1% accuracy, 95.6% sensitivity, and 96.8% specificity. ROC curve analysis confirmed the model's robust diagnostic capability in differentiating true microfilariae from imaging artifacts.

These results indicate that deep learning, particularly CNNs utilizing the U-Net architecture, can serve as a reliable alternative to traditional diagnostic methods such as the modified Knott's test. This approach enhances diagnostic precision and reduces analysis time, offering benefits especially for field applications in resource-constrained environments.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



The study aligns with prior research supporting the role of AI in parasitology diagnostics. For example, AI-based detection of helminths and *Schistosoma mansoni* has been demonstrated in low-resource settings (4), AI's application in radiology has been explored (5), and ChatGPT's diagnostic assistance in visceral leishmaniasis has been documented (7). Attai et al. further emphasized AI's role in malaria and typhoid diagnosis using explainable models (10).

Deep learning models are capable of identifying subtle morphological features that may be overlooked in manual examination, thereby minimizing diagnostic errors caused by artifacts.

Integrating AI-driven deep learning models into parasitic disease diagnostics offers significant improvements in accuracy, efficiency, and accessibility. In this study, CNN-based models demonstrated high precision in differentiating *Dirofilaria immitis* microfilariae from artifacts, providing a reliable alternative to traditional microscopic methods such as the modified Knott's test. This approach not only reduces diagnostic errors but also decreases the time and expertise required for accurate detection, representing a practical solution for both clinical and field settings (5).

The proposed deep learning model demonstrated high accuracy and reliability in distinguishing *Dirofilaria immitis* microfilariae from artifacts, presenting an effective alternative to traditional microscopic methods (8). This approach has the potential to improve diagnostic efficiency and reduce reliance on technician expertise, particularly in resource-limited environments (10).

Future studies should aim to expand the dataset with samples from different regions and host species to improve the robustness of the model. Validation under diverse laboratory and field conditions will be necessary to strengthen its applicability in clinical and epidemiological contexts.

Acknowledgment:

The author gratefully acknowledges the valuable support and guidance from the Parasitology Department of the Faculty of Veterinary Medicine, University of Tehran. Thanks are also extended to all who contributed through insightful discussions and assistance during this study.

References:

1. Baneth G, Thamsborg SM, Otranto D, Guillot J, Blaga R, Deplazes P, et al. Major parasitic zoonoses associated with dogs and cats in Europe. *J Comp Pathol*. 2016;155(1 Suppl 1):S54–S74. doi:10.1016/j.jcpa.2015.10.179.
2. Hosseini SH, Manshori-Ghaishghorshagh F, Ramezani M, et al. Canine microfilaraemia in some regions of Iran. *Parasit Vectors*. 2022;15:90. doi:10.1186/s13071-022-05209-7.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



3. Dantas-Torres F, Otranto D. *Dirofilariosis* in the Americas: a more virulent *Dirofilaria immitis* Parasit Vectors. 2013;6:288. doi:10.1186/1756-3305-6-288.
4. Ward P, Dahlberg P, Lagatie O, Larsson J, Tynong A, Vlamincck J, et al. Affordable artificial intelligence-based digital pathology for neglected tropical diseases: a proof-of-concept for the detection of soil-transmitted helminths and *Schistosoma mansoni* eggs in Kato-Katz stool thick smears. PLoS Negl Trop Dis. 2022;16(6):e0010500. doi:10.1371/journal.pntd.0010500.
5. Aydin O, Karaarslan E. OpenAI ChatGPT interprets Radiological Images: GPT-4 as a Medical Doctor for a Fast Check-Up [Internet]. arXiv [Preprint]. 2025 Jan 9 [cited 2025 Aug 6]. Available from: <https://arxiv.org/abs/2501.06269>.
6. Male HK, Tibakanya J, Nakasi R. AI Methods and Algorithms for Diagnosis of Intestinal Parasites: Applications, Challenges and Future Opportunities. East African J Inf Technol. 2024;7(1):366–379. Available from: <https://doi.org/10.37284/eajit.7.1.2282>.
7. Schwingel PA, Schwingel D, de Aquino SR, da Silva AR, da Silva PP, Pereira RAC, et al. An exploratory study on the potential of ChatGPT as an AI-assisted diagnostic tool for visceral leishmaniasis. Artif Intell Health. 2024;1(4):97–106. doi:10.36922/aih.3930.
8. Banihashem Nejad S, Hashemi N, Hasanpour E, Jalousian F, Jamshidi S, Hosseini SH, Manshori Ghaishghorshagh F, Soltanian-Zadeh H. Deep learning-based diagnosis of *Dirofilaria immitis* microfilariae in dog blood. In: Proceedings of the 29th National and 7th International Iranian Conference on Biomedical Engineering (ICBME); 2022 Dec; Guilan, Iran. IEEE; 2022. p. 205–209. doi:10.1109/ICBME57741.2022.10052956.
9. Hashemi N, Masoudnia S, Nejad A, Nazem-Zadeh MR. A memory-efficient deep framework for multi-modal MRI-based brain tumor segmentation. Annu Int Conf IEEE Eng Med Biol Soc. 2022 Jul:3749–3752. doi:10.1109/EMBC48229.2022.9871726.
10. Attai K, Ekpenyong M, Amannah C, Asuquo D, Ajuga P, Okure O, et al. Enhancing the interpretability of malaria and typhoid diagnosis with explainable AI and large language models. Trop Med Infect Dis. 2024;9(9):216. doi:10.3390/tropicalmed9090216.



One Health and Parasitology: From Historical Foundations to Practical Challenges in Iran

Mohammad Majid Johari¹, Elahe Ebrahimzadeh¹

Corresponding Author: Elahe Ebrahimzadeh, eebrahimzade@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article Code: 1561

Abstract

Background: The convergence of biological crises, including the spread of zoonotic diseases, climate change, and land use changes, highlights the limitations of traditional methods in tackling complex health threats. In this context, the One Health framework has emerged as a transformative approach for understanding and managing health risks that are multifaceted. Within this paradigm, biological sciences such as parasitology have regained importance due to their direct role in the ecological and human aspects of disease cycles.

Methods: This study employed a narrative review approach. A comprehensive literature search was conducted across major scientific databases, including PubMed, ScienceDirect and Google Scholar. Keywords such as One Health, Parasitology, hydatid cyst, and *Echinococcus granulosus* were used to identify relevant publications.

Results: The review revealed that many zoonotic parasites, such as *E. granulosus*, have complex life cycles directly linked to interactions among humans, animals, and the environment. Diseases like hydatid cysts illustrate the need for a comprehensive approach to health management. The main challenges in applying One Health practically in Iran include a lack of intersectoral coordination, insufficient financial resources, inadequate interdisciplinary education, and low awareness among local communities.

Conclusion: The One Health approach can only be effective when it moves beyond mere symbolic recommendations and develops into a strategic tool for restructuring institutional frameworks, reforming professional education, and promoting community-based partnerships. Parasitology, with its inherently interdisciplinary potential, can serve as a crucial starting point in this transition.

Keywords: One Health, Parasitology, History, Hydatid Cyst, *Echinococcus granulosus*.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Introduction:

In recent decades, the emergence and re-emergence of infectious diseases such as SARS, avian influenza, COVID-19, and have underscored the need to redefine the traditional concept of health. These outbreaks have demonstrated the intricate and interdependent relationship between human, animal, and environmental health (1).

In response to these increasing global threats, the One Health approach has been introduced as an collaborative framework aimed at advancing sustainable health (2). This idea highlights the importance of coordinated efforts among human medicine, veterinary science, environmental health, ecology, and policy-making (3,4).

Although One Health has gained formal institutional recognition in recent decades, its origins trace back to earlier centuries, from Hippocrates' emphasis on environmental factors in disease to Calvin Schwabe's 20th-century concept of "One Medicine" (5,6). In recent years, global institutions such as the WHO, OIE, and FAO have integrated this approach into international health policy.

Among biological sciences, parasitology holds a critical position in achieving One Health objectives (7). The complex life cycles of parasites such as *Echinococcus granulosus*, *Toxoplasma gondii*, and *Leishmania spp.* involve definitive and intermediate hosts along with environmental elements (8).

This paper explores the One Health framework in parasitology and emphasize the necessity of cross-sector collaboration in controlling zoonotic parasitic diseases Iran. It proposes actionable, non-symbolic solutions and highlights the importance of data-driven practices, inter-organizational coordination, and interdisciplinary education in turning One Health into a practical tool for public health advancement.

Materials and Methods:

This study was conducted as a narrative review of the One Health framework's conceptual, historical, and practical aspects 'in the field of parasitology, with a particular focus on zoonotic diseases in Iran. A targeted literature search was performed using reputable scientific databases, including PubMed, ScienceDirect, Google Scholar and Magiran. The keywords used in the search included: One Health, Parasitology, Iran, Hydatid Cyst, *Echinococcus granulosus*, and Zoonosis. The time frame was primarily limited to studies published in the past two decades and a total of 43 studies were selected for inclusion based on their methodological rigor, relevance to the research topic, and contribution to the One Health discourse in the context of parasitic disease control.



Results and Discussion:

Definition and Historical Background of One Health

The concept of One Health refers to an interdisciplinary approach that emphasizes the dynamic interrelationship among human, animal, and environmental health. According to widely accepted definitions provided by institutions such as the CDC and the One Health Commission, One Health is a multisectoral, multidisciplinary, and cross-level collaboration—local, national, and global—aimed at achieving optimal health outcomes by recognizing the interconnectedness between humans, animals, plants, and their shared environment (9).

Although this concept was formally introduced into the scientific literature and health policy in the early 21st century, its historical roots can be traced back centuries. Writings by Hippocrates and Galen in antiquity clearly reflect a holistic view of human health in relation to the environment. This perspective was gradually overshadowed by the emergence of experimental medicine during the Middle Ages and beyond. However, in the 19th century, Rudolf Virchow, the father of modern pathology, revived this integrative outlook by famously stating, “Between animal and human medicine there is no dividing line – nor should there be” (10,11).

In the 20th century, American veterinarian Calvin Schwabe further advanced this idea by coining the term One Medicine, emphasizing conceptual and practical bridges between human and veterinary medicine. His view was relevant to zoonoses like rabies and bovine tuberculosis (12).

The term One Health gained international prominence after the 2003 SARS outbreak and the 2004 establishment of the Manhattan Principles; a declaration drafted by the Wildlife Conservation Society. These principles called for bridging the gap between human medicine, veterinary science, and ecology to address global health crises. Subsequently, international organizations such as WHO, FAO, and OIE collaboratively developed policy frameworks and operational strategies based on the One Health model, especially for diseases like H5N1 avian influenza (13,14).

Parasitology and One Health

Parasitology, as a discipline at the intersection of veterinary medicine, human health, and environmental sciences, has a special place within the One Health framework (15). Many parasitic diseases are zoonotic, involving multiple hosts and ecological factors (16,17).

Moreover, environmental changes such as climate change, deforestation and urban expansion have dramatically altered the development and behavior of vectors and hosts, increasing the risk of parasitic diseases (18,19). Consequently, controlling these diseases requires collaborations among parasitologists, ecologists, veterinarians, public health experts, and local authorities (20).



Hydatid Cyst as a Model of Human–Animal–Environment Interaction

The transmission of *Echinococcus granulosus*, the causative agent of cystic echinococcosis, exemplifies the complex relationships among humans, animals, and the environment (21). The parasite's eggs are shed into the environment through dog feces and are then transmitted via contaminated food, water, or soil (22). This cycle involving multiple hosts and environmental transmission illustrates the core principles of the One Health approach (23).

Controlling cystic echinococcosis requires an integrated One Health strategy involving human health professionals, veterinarians, and environmental health sectors (24). This includes regular deworming of dogs, improving slaughterhouse hygiene, educating communities at risk, and controlling stray dog populations (25).

Significant challenges in controlling hydatid disease include limited public awareness, insufficient intersectoral collaboration, inadequate veterinary surveillance, and cultural barriers to safe slaughter practices. Rural communities often lack access to health education and veterinary services (26,27).

Cystic echinococcosis is an ideal case study for applying the One Health concept due to its zoonotic nature, environmental persistence, and socioeconomic burden. The disease reflects the consequences of neglecting cross-sector collaboration and illustrates how integrated interventions can significantly reduce disease incidence (28).

Challenges in Implementing the One Health Approach

Despite the growing international consensus around the importance of One Health, its practical implementation faces significant challenges (29). One major obstacle is the lack of structured collaboration between sectors, particularly at the provincial and local levels. Different institutions—such as ministries of health, agriculture, and environment—often operate in silos, with limited communication (30). Another significant barrier is the shortage of funding for integrated projects. While many global programs promote One Health in theory, few offer financial support for operational activities.

In addition, the fragmented educational system often fails to train future professionals in a One Health mindset. Medical, veterinary, and environmental science students are rarely exposed to each other's fields, which undermines the development of mutual understanding and collaboration (31).

Public awareness is also a limiting factor. Communities may not understand the links between their behaviors (e.g., animal husbandry, waste management) and disease transmission, which hinders prevention efforts (32).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Practical and Actionable Recommendations

To make One Health a functional model in parasitology and public health, several steps are recommended (33)

1. Integrated Surveillance Systems: Develop unified databases that track parasitic diseases in humans, animals, and the environment. These systems should be accessible to different agencies (34,35).
2. Cross-sectoral Task Forces: Establish local and provincial-level committees composed of veterinarians, physicians, parasitologists, environmental officers, and NGO representatives. These task forces can facilitate coordinated responses to outbreaks and guide prevention strategies (36).
3. Curriculum Reform: Incorporate One Health principles into the academic training of students in veterinary, medical, environmental, and public health disciplines. This will foster long-term change and build interdisciplinary capacity (37).
4. Community-based Education: Develop culturally relevant and language-appropriate educational materials to raise awareness about zoonotic parasitic diseases and preventive practices in rural and high-risk populations.

The One Health framework is not merely a theoretical paradigm but a practical necessity in the control and prevention of parasitic zoonoses. Parasitology reflects the inseparable link between human, animal, and environmental health. In countries like Iran, where parasitic diseases remain endemic in many regions, the implementation of realistic and evidence-based One Health strategies is not optional—it is essential. With proper institutional support, interdisciplinary education, and community engagement, One Health can evolve from a global vision to a local success story.

References:

1. Erkyihun GA, Alemayehu MB. One Health approach for the control of zoonotic diseases. *Zoonoses*. 2022 Nov 30;2(1):963.
2. Cunningham AA, Daszak P, Wood JL. One Health, emerging infectious diseases and wildlife: two decades of progress?. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2017 Jul 19;372(1725):20160167.
3. Mackenzie JS, Jeggo M. The one health approach—why is it so important?. *Tropical medicine and infectious disease*. 2019 May 31;4(2):88.
4. Capua I, Cattoli G. One Health (r) Evolution: learning from the past to build a new future. *Viruses*. 2018 Dec 18;10(12):725.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



5. Evans BR, Leighton FA. A history of One Health. *Rev Sci Tech*. 2014 Aug 1;33(2):413-20.
6. Otranto D, Strube C, Xiao L. Zoonotic parasites: the One Health challenge. *Parasitology research*. 2021 Dec;120(12):4073-4.
7. Blake DP, Betson M. One Health: parasites and beyond. *Parasitology*. 2017 Jan;144(1):1-6.
8. Brown HL, Pursley IG, Horton DL, La Ragione RM. One health: a structured review and commentary on trends and themes. *One Health Outlook*. 2024 Aug 14;6(1):17.
9. Atlas RM. One Health: its origins and future. *One Health: The Human-Animal-Environment Interfaces in Emerging Infectious Diseases: The Concept and Examples of a One Health Approach*. 2012 Apr 25:1-3.
10. Gibbs SE, Gibbs EP. The historical, present, and future role of veterinarians in One Health. In *One Health: The Human-Animal-Environment Interfaces in Emerging Infectious Diseases: The Concept and Examples of a One Health Approach* 2012 Aug 22 (pp. 31-47). Berlin, Heidelberg: Springer Berlin Heidelberg.
11. Lerner H. The philosophical roots of the "One Medicine" movement: an analysis of some relevant ideas by Rudolf Virchow and Calvin Schwabe with their modern implications. *Studia Philosophica Estonica*. 2013;6(2):97-109.
12. Zinsstag J, Schelling E, Waltner-Toews D, Tanner M. From "one medicine" to "one health" and systemic approaches to health and well-being. *Preventive veterinary medicine*. 2011 Sep 1;101(3-4):148-56.
13. Bresalier M, Cassidy A, Woods A. One health in history. In *One Health: The theory and practice of integrated health approaches* 2021 (pp. 1-14). Wallingford UK: Cabi.
14. Destoumieux-Garzón D, Mavingui P, Boetsch G, Boissier J, Darriet F, Duboz P, Fritsch C, Giraudoux P, Le Roux F, Morand S, Paillard C. The one health concept: 10 years old and a long road ahead. *Frontiers in veterinary science*. 2018 Feb 12;5:14.
15. Robertson LJ, Utaaker KS, Goyal K, Sehgal R. Keeping parasitology under the One Health umbrella. *Trends in Parasitology*. 2014 Aug 1;30(8):369-72.
16. Deiana G, Arghittu A, Dettori M, Castiglia P. One world, One health: Zoonotic diseases, parasitic diseases, and infectious diseases. In *Healthcare* 2024 Apr 29 (Vol. 12, No. 9, p. 922). MDPI.
17. Bidaisee S, Macpherson CN. Zoonoses and one health: a review of the literature. *Journal of parasitology research*. 2014;2014(1):874345.
18. Mwangi W, de Figueiredo P, Criscitiello MF. One health: addressing global challenges at the nexus of human, animal, and environmental health. *PLOS pathogens*. 2016 Sep 15;12(9):e1005731.
19. Anyamba A, et al. Modeling parasite ecology under climate change: One Health. *Front Parasitol*. 2025; 36-51.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



20. Kaminsky R, Mäser P. Global impact of parasitic infections and the importance of parasite control. *Frontiers in Parasitology*. 2025 Mar 10;4:1546195.
21. Govindasamy A, Bhattarai PR, John J. Liver cystic echinococcosis: a parasitic review. *Therapeutic Advances in Infectious Disease*. 2023 May;10:20499361231171478.
22. Badwaik N, Gharde P, Shinde RK, Tayade H, Navandhar PS, Patil M, Navandhar Jr PS. Hydatid cyst or echinococcosis: A comprehensive review of transmission, clinical manifestations, diagnosis, and multidisciplinary treatment. *Cureus*. 2024 Jul 2;16(7).
23. Hogeia MO, Ciomaga BF, Muntean MM, Muntean AA, Popa MI, Popa GL. Cystic echinococcosis in the early 2020s: a review. *Tropical Medicine and Infectious Disease*. 2024 Jan 31;9(2):36.
24. Gharbi M, Giraudoux P. Cystic echinococcosis (*Echinococcus granulosus sensu lato* infection) in Tunisia, a One Health perspective for a future control programme. *Parasite*. 2024 Jun 13;31:30.
25. Quaresma PF, Martins-Duarte ES, Soares Medeiros LC. One Health Approach in Zoonosis: strategies to control, diagnose and treat neglected diseases. *Frontiers in Cellular and Infection Microbiology*. 2023 Jun 8;13:1227865.
26. McManus DP, Gray DJ, Zhang W, Yang Y. Diagnosis, treatment, and management of echinococcosis. *Bmj*. 2012 Jun 11;344.
27. Giannelli A, Schnyder M, Wright I, Charlier J. Control of companion animal parasites and impact on One Health. *One Health*. 2024 Jun 1;18:100679.
28. Hassan MF, Soomro H, Soomro MA, ul Ain Q. Hydatid Cyst and One Health Approach: Endangering Human and Animal Health. *RAO ZAHID ABBAS, Ph. D.*:101.
29. Degeling C, Johnson J, Kerridge I, Wilson A, Ward M, Stewart C, Gilbert G. Implementing a One Health approach to emerging infectious disease: reflections on the socio-political, ethical and legal dimensions. *BMC Public Health*. 2015 Dec 29;15(1):1307.
30. Krecek RC, Rabinowitz PM, Conrad PA. Demystifying and demonstrating the value of a one health approach to parasitological challenges. *Veterinary Parasitology*. 2020 Nov 1;287:109202.
31. Schurer J. A One Health Approach to *Echinococcus Canadensis* and Other Parasitic Zoonoses in Remote, Rural and Indigenous Communities (Doctoral dissertation, University of Saskatchewan).
32. Seymour J. The impact of public health awareness campaigns on the awareness and quality of palliative care. *Journal of palliative medicine*. 2018 Jan 1;21(S1):S-30.
33. Schurer JM, Mosites E, Li C, Meschke JS, Rabinowitz P. Community-based surveillance of zoonotic parasites in a One Health world: A systematic review. *One Health*. 2016;2:166–174.
34. Falzon LC, Alumasa L, Amanya F, Kang'Ethe E, Kariuki S, Momanyi K, Muinde P, Murungi MK, Njoroge SM, Ogendo A, Ogola J. One Health in action: Operational



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- aspects of an integrated surveillance system for zoonoses in western Kenya. *Frontiers in veterinary science*. 2019 Jul 31;6:252.
35. Bordier M, Uea-Anuwong T, Binot A, Hendrikx P, Goutard FL. Characteristics of One Health surveillance systems: a systematic literature review. *Preventive veterinary medicine*. 2020 Aug 1;181:104560.
 36. Ferrinho P, Fronteira I. Developing one health systems: a central role for the one health workforce. *International journal of environmental research and public health*. 2023 Mar 7;20(6):4704.
 37. Wilkes MS, Conrad PA, Winer JN. One health—one education: medical and veterinary inter-professional training. *Journal of veterinary medical education*. 2019 Feb 25;46(1):14-20.
 38. Preston C, Longstaff L. An institutional lens on the history of One Health. *EcoHealth*. 2022;19:440-442
 39. Eussen BG, Schaveling J, Dragt MJ, Blomme RJ. Stimulating collaboration between human and veterinary health care professionals. *BMC veterinary research*. 2017 Jun 13;13(1):174.
 40. Orusa T, Viani A, d'Alessio SG, Orusa R, Caminade C. One Health approaches and modeling in parasitology in the climate change framework and possible supporting tools adopting GIS and remote sensing. *Frontiers in Parasitology*. 2025 Mar 19;4:1560799.
 41. Kaminsky R, Mäser P. Anthelmintic resistance in soil-transmitted helminths: One Health considerations. *Parasitol Res*. 2023;122(5):62.
 42. Robinson TP, Bu DP, Carrique-Mas J, Fèvre EM, Gilbert M, Grace D, Hay SI, Jiwakanon J, Kakkar M, Kariuki S, Laxminarayan R. Antibiotic resistance is the quintessential One Health issue. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 2016 Jul 1;110(7):377-80.
 43. Cassini R, Canali M, Tamarozzi F, Angheben A, Capelli G, Gobbi F, Legnardi M, Brichese M, Napoletano G, Cestaro F, Casulli A. A One-Health evaluation of the burden of cystic echinococcosis and its prevention costs: Case study from a hypo-endemic area in Italy. *One Health*. 2021 Dec 1;13:100320.



Evaluation of the Larvicidal Efficacy of *Mentha piperita* Alcoholic Extract on *Linguatula serrata* Nymphs under Laboratory Conditions

Nasser Hajipour¹

Corresponding Author: Nasser Hajipour, n.hajipour@tabrizu.ac.ir

1. Department of Food Hygiene and Aquatic, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Article Code: 1567

Abstract

Background: *Linguatula serrata* is a zoonotic parasite with important health and economic impacts. Medicinal plants are considered promising alternatives to synthetic drugs. This study evaluated the larvicidal efficacy of *Mentha piperita* alcoholic extract against *L. serrata* nymphs under laboratory conditions.

Methods: Nymphs were exposed to different concentrations (10, 1, 0.1, and 0.01 mg/mL) of *M. piperita* alcoholic extract. Mortality was recorded at 12, 24, 48, 72, and 96 hours. Ivermectin served as a positive control. LC₅₀ values were calculated.

Results: The alcoholic extract showed a dose- and time-dependent effect. At 10 and 1 mg/mL, mortality exceeded 80% at 12 hours and reached 100% at 24 hours. At 0.1 mg/mL, complete mortality was reached by 72 hours, while at 0.01 mg/mL mortality reached 100% at 96 hours. The LC₅₀ was 0.167 mg/mL. Ivermectin acted faster but showed a higher LC₅₀ (0.316 mg/mL).

Conclusion: *Mentha piperita* alcoholic extract demonstrated strong larvicidal activity against *L. serrata* nymphs, comparable to ivermectin, suggesting its potential as a natural antiparasitic agent.

Keywords: *Mentha piperita*, *Linguatula serrata*, Alcoholic extract, Nymph mortality.

Introduction:

Linguatula serrata, or tongue worm, is a zoonotic parasite of carnivores, with ruminants as intermediate hosts (1). Human infection occurs mainly through ingestion of infective eggs or raw viscera, causing visceral or nasopharyngeal linguatulosis with gastrointestinal and respiratory signs (2–4). Its nymphal stage migrates to organs such as the liver and lungs, producing lesions and economic losses due to organ condemnation at slaughterhouses (5–7). Conventional control strategies rely on slaughterhouse hygiene and treatment of definitive hosts (8). However, growing concerns regarding drug resistance, residues in animal-derived food products, and environmental side effects of chemical antiparasitic agents have increased the demand for alternative, eco-friendly control approaches. Medicinal plants are considered



effective natural sources of antiparasitic compounds (9). *Mentha piperita* (peppermint) is a common medicinal herb with anti-inflammatory, antibacterial, antioxidant, and antiparasitic effects (10,11). Its alcoholic extract contains lipophilic compounds such as menthol and phenolics with strong biological activity. Despite this potential, its activity against *L. serrata* has been poorly studied. This study aimed to evaluate the larvicidal effect of *M. piperita* alcoholic extract on *L. serrata* nymphs.

Materials and Methods:

Collection of Peppermint Plant Material

Wild peppermint (*Mentha piperita*) specimens were harvested from naturally occurring, humid, and semi-shaded habitats during the plant's active growth season (spring to early summer). Only healthy, mature aerial parts (leaves and stems) were hand-picked using protective gloves to avoid skin irritation caused by essential oils. Samples were promptly transferred into clean paper bags to maintain ventilation and prevent microbial contamination. Taxonomic identification of the collected specimens was verified by Dr. Sabzi, a medicinal plant specialist at the Faculty of Agriculture and Natural Resources, Ahar.

Preparation of Alcoholic and Aqueous Extracts

The dried peppermint leaves were mechanically ground into fine powder using an electric blender. A total of 25 grams of powdered plant material was extracted using 250 mL of absolute ethanol via Soxhlet extraction. After the ethanol extraction process, the remaining plant residue was air-dried overnight and subjected to hot water extraction at 60°C. The ethanol extract was concentrated using a rotary evaporator, while the aqueous extract was freeze-dried to obtain a dry powder. Both crude extracts were sealed in dark plastic bags under vacuum and stored at -20°C until further use.

Preparation of Extract Dilutions

To evaluate the larvicidal activity, four concentrations (10, 1, 0.1, and 0.01 mg/mL) of both aqueous and alcoholic extracts were prepared. Dried extracts were dissolved in appropriate solvents (distilled water for aqueous extract and ethanol for alcoholic extract) to reach the desired concentrations. Fresh solutions were prepared daily and stored at 4°C until use. The selected concentrations were based on previous studies to cover a wide efficacy range. All experiments were conducted in triplicate to ensure reproducibility. Ivermectin was used as a positive control.

Collection of *L. serrata* Nymphs from Mesenteric Lymph Nodes

Following slaughter, the intestines and associated mesenteries of goats were inspected, and mesenteric lymph nodes were carefully dissected. These nodes were transferred to the



laboratory in sterile PBS containers under cold conditions (4–8°C). In the lab, nodes were incised using sterile scalpels, placed in Petri dishes, and moistened with PBS. After a short incubation, nymphs emerged and floated in the solution. Using a stereomicroscope, live nymphs—recognizable by their translucent, creamy appearance and distinct movement—were isolated with forceps and stored in PBS at 4°C until treatment.

Exposure of Nymphs to Extracts

Twenty-five viable *L. serrata* nymphs were incubated in each of the four test concentrations (10, 1, 0.1, and 0.01 mg/mL) of peppermint alcoholic and aqueous extracts for various durations (12, 24, 48, 72, and 96 hours). Mortality was assessed at each time point by observing motility under a stereomicroscope. Complete cessation of movement was considered an indication of death (Table 1).

Data Analysis

Median lethal concentrations (LC₅₀) of the three tested treatments (ivermectin, peppermint aqueous extract, and peppermint alcoholic extract) were calculated using probit analysis, a standard method for evaluating dose–response relationships in toxicological and pharmacological studies. Probit transformation was applied to the mortality data using the function $5 + \text{NORMSINV}(P)$, where P is the proportion of dead nymphs at each concentration. Concentration values were log-transformed to linearize the dose–response relationship. The statistical analysis was performed using R software, version 4.1.0 (R Core Team, 2021). Ninety-five percent confidence intervals (CI) for LC₅₀ estimates were also computed. Drug efficacy was interpreted based on both LC₅₀ values and the time required to reach lethal effects. Treatments with lower LC₅₀ values and shorter exposure times were considered more potent. Model fit was assessed using the Chi-square goodness-of-fit test, and statistical significance was set at $p < 0.05$ for all analyses.

Results and Discussion:

The effects of *M. piperita* alcoholic extract and ivermectin on the mortality of *L. serrata* nymphs at different concentrations and exposure times are presented in Table 1. The aqueous extract of *M. piperita* exhibited a dose- and time-dependent larvicidal activity. At the highest concentration tested (10 mg/mL), 84% mortality was observed at 12 hours, reaching 100% by 24 hours. A similar trend was noted at 1 mg/mL, with 80% mortality at 12 hours and complete mortality (100%) at 24 hours. At 0.1 mg/mL, mortality increased progressively over time, reaching 100% at 72 hours. At the lowest concentration (0.01 mg/mL), mortality rates were 28%, 36%, 68%, 68%, and 100% at 12, 24, 48, 72, and 96 hours, respectively. The calculated LC₅₀ value for the *M. piperita* aqueous extract was 0.167 mg/mL. In comparison, ivermectin demonstrated a rapid and potent effect across all tested concentrations. At concentrations of 10



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



and 1 mg/mL, 100% mortality was achieved within 12 hours. Even at lower concentrations (0.1 and 0.01 mg/mL), mortality rates reached 96% at 12 hours. The LC_{50} value for ivermectin was determined to be 0.316 mg/mL, which, although higher than that of *M. piperita* extract, indicates a faster onset of lethal activity at low concentrations.

Table 1. Number and percentage of mortality of *Linguatula serrata* nymphs at different concentrations and exposure times of *Mentha piperita* alcoholic extract and ivermectin

Extract	Concentration (mg/mL) / Time (hours)	12 h	24 h	48 h	72 h	96 h	LC_{50} (mg/mL)
Mentha piperita	10	21 (84%)	25 (100%)	-	-	-	0.167
	1	20 (80%)	25 (100%)	-	-	-	
	0.1	12 (48%)	15 (60%)	20 (80%)	25 (100%)	-	
	0.01	7 (28%)	9 (36%)	17 (68%)	17 (68%)	25 (100%)	
Ivermectin	10	25 (100%)	-	-	-	-	0.316
	1	25 (100%)	-	-	-	-	
	0.1	24 (96%)	-	-	-	-	
	0.01	24 (96%)	-	-	-	-	

The present study evaluated the larvicidal efficacy of *M. piperita* alcoholic extract against *L. serrata* nymphs under controlled laboratory conditions, revealing a clear dose- and time-dependent mortality effect. The extract demonstrated significant larvicidal activity, with complete mortality achieved at relatively low concentrations and exposure times. These findings align with previous research highlighting the potent antiparasitic properties of *M. piperita*, attributed primarily to its rich content of bioactive phytochemicals such as menthol, menthone, flavonoids, and phenolic compounds (12,13).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



M. piperita's alcoholic extract exhibited an LC_{50} value lower than that of ivermectin in this study, indicating a strong larvicidal potency despite the conventional reliance on synthetic anthelmintics for parasite control. This result is particularly noteworthy given the rising concerns over chemical drug resistance, environmental toxicity, and residue accumulation in animal products (14). The extract's effectiveness may be linked to the solubility and extraction efficiency of lipophilic components in ethanol, which potentially facilitate deeper penetration into the parasite's cuticle and disruption of vital physiological processes (15).

Comparative studies on plant-derived antiparasitic agents have similarly reported promising larvicidal effects against various parasitic stages. For instance, alcoholic extracts of other Lamiaceae family members, including *Rosmarinus officinalis* and *Thymus vulgaris*, have demonstrated significant mortality rates in different parasitic nematodes and arthropods (16). The mechanisms of action are thought to involve interference with the nervous system, enzymatic pathways, and cellular membrane integrity in parasites, although specific modes of action for *Mentha piperita* against *L. serrata* require further elucidation (17).

It is important to consider that while *M. piperita* extract showed slower onset of lethality at lower concentrations compared to ivermectin, the extract's natural origin and biodegradability provide a sustainable and eco-friendly alternative for parasite management. This advantage supports the integration of phytotherapy into comprehensive parasite control programs, especially in regions where synthetic drug accessibility is limited or where organic livestock production is prioritized (17,18).

However, the translation of these *in vitro* findings to practical field applications necessitates additional research. Factors such as formulation stability, optimal delivery methods, pharmacokinetics, and potential toxicity to host animals must be thoroughly investigated. Moreover, studies on the extract's effect on other developmental stages of *L. serrata* and on different parasite species will further clarify its broad-spectrum efficacy.

In conclusion, the alcoholic extract of *M. piperita* exhibits considerable larvicidal activity against *L. serrata* nymphs, supporting its potential as a natural anthelmintic agent. Future research should focus on mechanistic studies and formulation development to harness the full therapeutic potential of this medicinal plant in veterinary parasitology.

Acknowledgment:

The authors would like to thank everyone who helped us in this research.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Soulsby E JL. Helminths, arthropods and protozoa of domesticated animals. Vol. 6. Philadelphia: Lea and Febiger; 1982.
2. Sarmadian H, Nasiri Z, Saeedinia S, Moradi Y, Eshrati B, Ghasemikhah R, et al. Clinical manifestation and epidemiological findings of human linguatula serrate infection in Iran: systematic review. J Parasit Dis. 2021;45(1):50–8.
3. Yazdani R, Sharifi I, Bamorovat M, Mohammadi MA. Human Linguatulosis Caused by Linguatula serrata in the City of Kerman, South-eastern Iran-Case Report. Iran J Parasitol. 2014;9(2):282.
4. Khalil G, Haddad C, Otrock ZK, Jaber F, Farra A. Halzoun, an allergic pharyngitis syndrome in Lebanon: the trematode Dicrocoelium dendriticum as an additional cause. Acta Trop. 2012/10/02. 2013;125(1):115–8.
5. Hajipour N, Ketzis J, Esmailnejad B. Pathological characteristics of Linguatula serrata (Aberrant arthropod) infestation in sheep and factors associated with prevalence in Iran. Prev Vet Med. 2019;172:104781.
6. Hajipour N, Ketzis J, Esmailnejad B, shahbazfar AA. Pathological characteristics of Linguatula serrata (aberrant arthropod) infestation in sheep and factors associated with prevalence in Iran. Prev Vet Med. 2019;172.
7. Hajipour N, Tavassoli M. Prevalence and associated risk factors of Linguatula serrata infection in definitive and intermediate hosts in Iran and other countries: a systematic review. Vet Parasitol Reg Stud Reports. 2019;16:100288.
8. Bowman DD. Georgis' Parasitology for Veterinarians. Philadelphia: Saunders Elsevier, St. Louis; 1999. 215–220 p.
9. Mimica-Dukic N, Bozin B. Mentha L. species (Lamiaceae) as promising sources of bioactive secondary metabolites. Curr Pharm Des. 2008;14(29):3141–50.
10. İşcan G, Kirimer N, Kürkcüoğlu M, Başer HC, DEMİrci Fati. Antimicrobial screening of Mentha piperita essential oils. J Agric Food Chem. 2002;50(14):3943–6.
11. Inoue T, Sugimoto Y, Masuda H, Kamei C. Antiallergic effect of flavonoid glycosides obtained from Mentha piperita L. Biol Pharm Bull. 2002;25(2):256–9.
12. McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of chamomile tea (Matricaria recutita L.). Phyther Res An Int J Devoted to Pharmacol Toxicol Eval Nat Prod Deriv. 2006;20(7):519–30.
13. Karageçili H, Gülçin İ. The Lamiaceae Family Plants Ethnobotanical Properties, Ethnopharmacological Uses, Phytochemical Studies and Their Utilization in Public or



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- Current Clinical Practices: A Review. *Rec Nat Prod.* 2025;19(Special Issue).
14. Kaplan RM. Drug resistance in nematodes of veterinary importance: a status report. *Trends Parasitol.* 2004;20(10):477–81.
 15. Desam NR, Al-Rajab AJ, Sharma M, Mylabathula MM, Gowkanapalli RR, Albratty M. Chemical constituents, in vitro antibacterial and antifungal activity of *Mentha* × *Piperita* L.(peppermint) essential oils. *J King Saud Univ.* 2019;31(4):528–33.
 16. Strothmann AL, Berne MEA, de Almeida Capella G, de Moura MQ, da Silva Terto WD, da Costa CM, et al. Antiparasitic treatment using herbs and spices: A review of the literature of the phytotherapy. *Brazilian J Vet Med.* 2022;44:e004722.
 17. Ojewumi ME, Adedokun SO, Omodara OJ, Oyeniyi EA, Taiwo OS, Ojewumi EO. Phytochemical and antimicrobial activities of the leaf oil extract of *Mentha spicata* and its efficacy in repelling mosquito. *Int J Pharm Res Allied Sci.* 2017;6(4):17–27.
 18. Malheiros DF, Maciel PO, Videira MN, Tavares-Dias M. Toxicity of the essential oil of *Mentha piperita* in *Arapaima gigas* (pirarucu) and antiparasitic effects on *Dawestrema* spp.(Monogenea). *Aquaculture.* 2016;455:81–6.



Three Technical Challenges for AI in Veterinary Parasitology

Mohammad sajed Sarvari¹

Corresponding Author: Mohammad sajed Sarvari , ms.sarvari1378@gmail.com

1. *Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*

Article code: 1598

Abstract

Background: The integration of Artificial Intelligence (AI) into veterinary parasitology promises to revolutionize diagnostic speed and accuracy. But turning a proof-of-concept into a reliable system used in real medical settings means solving big technical problems that go well beyond infrastructure. This article aims to provide a clear view of three critical, interconnected hurdles at the forefront of Parasitology AI research.

Methods: This narrative review synthesizes key literature on the critical technical challenges in applying AI to veterinary parasitology, with a particular focus on generalizability, data annotation, and explainability. Using information from different sources to create a clear argument and show what the future of this field should look like.

Results: Three major concerns in training an AI model are: the generalizability crisis, where models fail when exposed to new, unseen data distributions; the prohibitive cost of expert annotation, which creates a bottleneck for supervised learning; and the closed box problem, which necessitates the development of Explainable AI (XAI) to ensure trust, safety, and regulatory compliance.

Conclusion: Addressing these challenges is not just an academic exercise but a prerequisite for developing reliable, scalable, and ethically sound AI tools for diagnosing parasitic diseases. Without overcoming these obstacles, AI will remain confined to the laboratory, limiting its real-world application.

Keywords: Artificial Intelligence, Veterinary Parasitology, Explainable AI (XAI), Medical Imaging.

Introduction:

The application of Artificial Intelligence (AI) to veterinary parasitology has shown immense potential. Studies have demonstrated that deep learning models, particularly Convolutional Neural Networks (CNNs), can identify parasite eggs, oocysts, and protozoa in microscopic images with accuracy that rivals or even surpasses human experts (1,2). These advancements



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



promise a future of rapid, automated, and accessible diagnostics, which is especially critical for resource-limited settings and for managing herd health on a large scale.

However, the transition from promising results in a controlled laboratory setting to practical tools in the "farm"—the diverse and unpredictable environment of veterinary fields—faces significant challenges. Many high-performing models published in the literature are "fragile," meaning their performance collapses when faced with minor variations in data that were not present in their training set. This raises a critical question: what are the core technical barriers preventing the widespread, reliable adoption of these technologies?

This article moves beyond the discussions of funding and infrastructure to focus on three fundamental technical challenges: the crisis of generalizability, the economic and logistical burden of data annotation, and the clinical and ethical need for Explainable AI (XAI). By mentioning these hurdles, we aim to provide a realistic roadmap for researchers, clinicians, and developers working to build the next generation of trustworthy AI-powered diagnostic tools in parasitology.

Materials and Methods:

This article presents a narrative review of the critical technical challenges in applying artificial intelligence to veterinary parasitology. A non-systematic search of the literature was conducted across databases such as PubMed, Google Scholar, and IEEE Xplore to identify key publications related to generalizability, data annotation, and explainability in medical AI. The chosen sources were put together to explain an idea and suggest the future direction of this field.

Results and Discussion:

The Generalizability Crisis: Brittleness of AI Models in the farm

Generalizability refers to a model's ability to maintain its performance on new, unseen data that may differ in distribution from the data it was trained on. In medical imaging, this is the single most significant barrier to real-world deployment (3). In the context of parasitology, this challenge manifests in several critical ways.

A "domain" is the set of conditions under which data is collected. If the model learns only from one situation, it may fail in another. This "domain shift" can be categorized as: Technical shifts and Geographic-biological shift. Technical shift arises from variations in the image capturing. A model trained on high-resolution images from a brand A whole-slide scanner may perform poorly on pictures from a brand B microscope, a smartphone camera attached to an eyepiece, or even images subjected to different compression algorithms (4). Variations in staining



protocols (e.g., Giemsa vs. Trichrome), stain concentration, and illumination intensity create subtle but significant shifts in color and texture that can confuse a naive model. For example, in the context of geographic and biological shifts, A model trained to identify *Eimeria* species prevalent in a poultry farm in one region may fail to recognize subspecies or genetically distinct variants from another area that exhibit minor morphological differences. Furthermore, the model may be confused by region-specific artifacts, such as pollen grains or fungal spores, that were absent in the original training data, leading to misidentification (5).

Another major concern is overfitting to spurious correlations. Deep learning models are powerful pattern recognizers, but they lack human-like causal reasoning. As a result, they may associate a diagnosis with irrelevant artifacts present in the training data—a phenomenon known as the “Clever Hans” effect. (6). For instance, if most of the images containing *Fasciola hepatica* eggs in a dataset have a slightly darker background or a specific type of debris, the model may learn this spurious correlation instead of the actual egg morphology. a model may score high on familiar test data but fail clinically when artifacts differ, highlighting the risk of trusting accuracy without understanding the model Learnings.

The High Cost of Annotation

Supervised learning, the dominant paradigm in medical AI, relies on vast quantities of meticulously labeled data. In parasitology, this creates a severe and often underestimated bottleneck. Annotating parasitological images requires expert parasitologists or lab technicians, who are scarce and costly. Segmenting parasites on whole-slide images is tedious, time-consuming, and prone to fatigue and inconsistencies. Parasitology is often ambiguous, with experts frequently disagreeing when distinguishing cysts, eggs, or artifacts (7). Standard annotation tools, which require a single, definitive label, fail to find out this diagnostic uncertainty, feeding the model potentially noisy or incorrect information.

These challenges are pushing research toward new methods. One example is weakly-supervised learning, which uses image-level labels (e.g., "this slide is positive") instead of exact pixel segmentations. Another is self-supervised learning, which trains models to learn visual features from unlabeled data, reducing the need for large labeled datasets (8).

The Closed Box Problem: A Critical Need for Explainable AI (XAI)

Many strong deep learning models work in a way that people cannot fully see or understand, like a closed box. They provide a prediction but offer no insight into their decision-making process. In critical areas like medicine, not knowing how the model works makes it hard for people to trust, use, and feel safe with it. (9). A veterinarian cannot be expected to blindly trust an algorithm's recommendation, especially when it goes against their own medical opinion. To be a true "assistant" The AI needs to show the reasons behind its decisions. Without the ability to explain, the model remains a mysterious oracle rather than a trusted tool. When an AI model



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



makes a mistake, it is nearly impossible to diagnose the cause. Is it focusing on a stain artifact? Was it confused by poor lighting? Without clear information about decision-making, improving the model means trial and error. Explainable AI is not just about building trust; it is a potential tool for discovery. By visualizing what features a model uses to identify a specific parasite, we may uncover novel morphological biomarkers that human experts previously overlooked (10).

Techniques such as Class Activation Mapping (CAM) and its variants (e.g., Grad-CAM) are becoming essential tools for opening this closed box. (11). These methods create visual maps that show which parts of the image had the most significant impact on the model's decision. This helps us see what the model is paying attention to. Integrating explainable AI is no longer an option, but it's a necessity for building safe, reliable, and auditable AI systems for veterinary diagnostics.

Table 1. Summary of Key Technical Challenges in Veterinary Parasitology AI

Challenge	Description	Example in Parasitology	Potential Solutions
The Generalizability Crisis	The model struggles with unseen data from new sources (domain shift).	A model trained on microscope images fails on smartphone photos or different stains.	- Data Augmentation - Domain Adaptation Techniques - Training on Diverse, Multi-Center Datasets
The High Cost of Annotation	Labeling large image datasets is slow, costly, and demands scarce expertise, bottlenecking supervised learning.	A parasitologist spends hours outlining parasite eggs on a slide.	- Weakly-Supervised Learning - Self-Supervised Learning - Active Learning
The Closed Box Problem	The inability to understand or interpret the decision-making process of a complex AI model, which undermines clinical trust and complicates error diagnosis.	AI mislabels an artifact as a parasite, and the vet can't tell why.	- Explainable AI (XAI) Methods (e.g., Grad-CAM) - Inherently Interpretable Models - Post-Hoc Explanation Systems



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



AI in veterinary parasitology has transformative potential, but faces major hurdles: model brittleness from domain shifts, the high cost and expertise needed for data annotation, and the opacity of closed-box algorithms. Future work must focus on robust, generalizable models, data-efficient learning, and built-in ability to explain, to create AI tools that are not only accurate but also reliable, trustworthy, and practical in veterinary care.

References:

1. Akanni J, Odeniyi L, Aremu Z, Benedict O, Fatmawati F. Automated Diagnosis of Schistosomiasis Using Convolutional Neural Networks: A Comparative Study with K-Nearest Neighbors. Available SSRN 4953853. 2024;
2. Osaku D, Cuba CF, Suzuki CTN, Gomes JF, Falcão AX. Automated diagnosis of intestinal parasites: a new hybrid approach and its benefits. *Comput Biol Med.* 2020;123:103917.
3. Windecker D, Baj G, Shiri I, Kazaj PM, Kaesmacher J, Gräni C, et al. Generalizability of FDA-approved AI-enabled medical devices for clinical use. *JAMA Netw Open.* 2025;8(4):e258052–e258052.
4. Matta S, Lamard M, Zhang P, Le Guilcher A, Borderie L, Cochener B, et al. A systematic review of generalization research in medical image classification. *Comput Biol Med.* 2024;183:109256.
5. Zech JR, Badgeley MA, Liu M, Costa AB, Titano JJ, Oermann EK. Variable generalization performance of a deep learning model to detect pneumonia in chest radiographs: a cross-sectional study. *PLoS Med.* 2018;15(11):e1002683.
6. DeGrave AJ, Janizek JD, Lee S-I. AI for radiographic COVID-19 detection selects shortcuts over signal. *Nat Mach Intell.* 2021;3(7):610–9.
7. Cheplygina V, De Bruijne M, Pluim JPW. Not-so-supervised: a survey of semi-supervised, multi-instance, and transfer learning in medical image analysis. *Med Image Anal.* 2019;54:280–96.
8. Azizi S, Mustafa B, Ryan F, Beaver Z, Freyberg J, Deaton J, et al. Big self-supervised models advance medical image classification. In: *Proceedings of the IEEE/CVF international conference on computer vision.* 2021. p. 3478–88.
9. Amann J, Blasimme A, Vayena E, Frey D, Madai VI, Consortium P. Explainability for artificial intelligence in healthcare: a multidisciplinary perspective. *BMC Med Inform Decis Mak.* 2020;20(1):310.
10. Holzinger A, Langs G, Denk H, Zatloukal K, Müller H. Causability and explainability



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



of artificial intelligence in medicine. Wiley Interdiscip Rev data Min Knowl Discov. 2019;9(4):e1312.

11. Selvaraju RR, Cogswell M, Das A, Vedantam R, Parikh D, Batra D. Grad-cam: Visual explanations from deep networks via gradient-based localization. In: Proceedings of the IEEE international conference on computer vision. 2017. p. 618–26.



Artificial Intelligence and Parasitology: Recent Advances and Future Prospects

Soren Mohammadi¹, Elahe Ebrahimzade¹, Mohammad Majid Johari¹

Corresponding Author: Elahe Ebrahimzade, eebrahimzade@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1624

Abstract

Background: Parasitic diseases remain a major concern in the medical and veterinary fields, especially in relation to diseases shared between humans and animals (zoonoses). The introduction of artificial intelligence (AI) in parasitology has opened new horizons in improving diagnostic accuracy, accelerating data analysis, and optimizing disease control methods.

Methods: This study was conducted as a descriptive review of articles published between 2010 and 2025. Scientific sources were searched from reputable databases such as PubMed, ScienceDirect, Google Scholar, Frontiers, and MagIran. The keywords used included: artificial intelligence, parasitology, machine learning.

Results: The reviewed studies showed that the applications of AI in the field of parasitology can be categorized into four main axes: (1) automatic identification of parasites from microscopic images using convolutional neural networks (CNN), (2) epidemiological prediction of disease outbreaks using algorithms such as Random Forest and Support Vector Machine (SVM), (3) discovery of antiparasitic drugs and development of new treatments with the help of AI-based structural analyses, and (4) improvement of disease management using analysis of biological and environmental data in the context of Big Data.

Conclusion: Artificial intelligence is shaping the future of parasitic disease research and control. Its application in veterinary parasitology and zoonoses provides valuable opportunities for this field. To fully exploit this technology, it is essential to invest in scientific infrastructure, develop localized algorithms, and strengthen collaboration between parasitology and data science experts.

Keywords: Artificial Intelligence, Parasitology, Zoonoses, Veterinary Medicine, Deep learning.



Introduction:

Parasitology, as an important branch of veterinary and medical sciences, plays an important role in the diagnosis, control, and treatment of parasitic diseases (1). With significant advances in information technology and data science, Artificial Intelligence (AI) has emerged as a new tool in improving diagnostic and research technologies in this field (2). With the ability to process a massive amount of data and learn complex patterns, AI has enabled more accurate parasite diagnosis, disease outbreak prediction and optimization of therapeutic strategies (3).

In recent years, several methods, including machine learning, deep learning, and artificial neural networks (4), have been used in analyzing microscopic images of parasites, epidemiological modeling, and genetic data extraction (5). These advances have made laboratory work easier, reduced human errors, and increased response speed (6,7). Also, the use of AI in pharmaceuticals and the discovery of antiparasitic drugs have opened a bright future in combating parasitic diseases (8).

Given the increasing importance of this technology and the need to fully understand its applications, benefits, and challenges in veterinary and medical parasitology, this review article examines the latest achievements and research trends in this field. This article aims to provide an overview and analysis of the role of artificial intelligence in the diagnosis, prevention, and control of parasitic diseases, which can be a useful guide for researchers, veterinarians, and physicians.

Materials and Methods:

This study was conducted as a narrative review of published articles on the applications of artificial intelligence in veterinary and medical parasitology. To identify relevant articles, an extensive search was conducted in reputable scientific databases, including PubMed, ScienceDirect, Google Scholar, Frontiers, and MagIran, between 2010 and 2025.

Keywords used included artificial intelligence, parasitology, machine learning, parasite detection, veterinary medicine, deep learning, and microscopic image analysis. After an initial retrieval of over 100 articles, a selection process was carried out based on relevance to the topic, scientific quality, and removal of duplicate articles, and finally, 60 articles were selected for the final review.

The extracted data included the areas of application of artificial intelligence, algorithms used, parasite species, laboratory samples, and applications in diagnosis, epidemiological prediction, drug discovery, and management of parasitic diseases. Data analysis was performed descriptively to clearly explain the current trends, advantages, limitations, and prospects of this technology in parasitology.



Results and Discussion:

The review of the literature indicates that artificial intelligence (AI) is playing an increasingly significant role in veterinary and medical parasitology. The major applications of AI in this field can be broadly categorized as follows:

Automated Parasite Detection in Microscopic Images

Deep learning algorithms, particularly convolutional neural networks (CNNs), have been extensively employed for rapid and precise identification of parasite eggs (9,10), larvae (11), and other developmental stages from microscopic images (12,13). For example, Kumar et al. (2024) applied CNN-based deep learning models to detect *Ascaris lumbricoides* eggs in stool samples, achieving over 95% precision (14). Similarly, He et al. (2024) demonstrated automated detection of blood microfilariae using AI-powered image analysis tools, significantly reducing diagnostic time (15).

Epidemiological Prediction and Disease Modeling

Machine learning models such as Random Forest and Support Vector Machines have enabled researchers to predict the spread patterns of parasitic diseases in both humans and animals (16,17), thereby facilitating improved planning for disease control and prevention programs (18). For instance, Hassani et al. (2024) used machine learning models to predict zoonotic disease patterns in Iranian livestock populations, focusing on echinococcosis outbreaks in northeastern regions (19). Their study utilized Random Forest algorithms to correlate environmental and host factors with disease incidence.

Drug Discovery and Therapeutic Development

AI has emerged as a powerful tool in the identification of novel antiparasitic compounds, molecular structure analysis, and the optimization of drug development workflows. This approach can streamline traditionally time-consuming and costly pharmacological testing processes (20). Mostafa et al. (2024) reviewed AI-assisted screening systems that identified candidate compounds against *Leishmania donovani* (21). Furthermore, Akbarein et al. (2025) utilized artificial neural networks to predict anti-trypanosomal activity of selected molecules, showcasing the practical application of AI in antiparasitic drug discovery (22).

Parasitic Disease Management and Control

Integration of big data analytics with AI techniques for the analysis of biological, environmental, and epidemiological datasets has enhanced the optimization of disease management strategies (23).

Status of Artificial Intelligence Applications in Iran

In Iran, the application of artificial intelligence (AI) in veterinary parasitology remains at a preliminary stage, mostly limited to academic pilot projects and research studies (24). For



instance, Nejad et al. (2024) reported the use of deep learning-based microscopy for the detection of microfilariae in blood smears, showcasing the feasibility of automated diagnostic tools tailored to local parasitic diseases (25).

Other efforts include the application of AI algorithms for pattern recognition of zoonotic disease outbreaks, using big data approaches in endemic regions (19). However, these initiatives are often constrained by the limited availability of annotated parasitological image datasets, the lack of integration with national surveillance systems, and insufficient collaboration between veterinary institutions and AI research centers.

Furthermore, Karimi et al. (2023) emphasized the need for strategic investments, interdisciplinary training, and localization of AI models to improve predictive capacity and clinical relevance within the Iranian context (26).

Local Challenges in Veterinary AI Implementation

Several challenges hinder the effective integration of AI technologies in veterinary parasitology (27). These include the lack of standardized parasitological datasets, limited access to advanced imaging and computational infrastructure, insufficient training of personnel in AI methodologies, and weak interdisciplinary collaboration between data scientists and veterinary parasitologists (28).

Comparison with Traditional Diagnostic Methods

Traditional diagnostic methods, such as flotation and microscopic examination, are heavily reliant on the operator's expertise and are often prone to human error (29). In contrast, AI-based systems offer higher diagnostic accuracy, faster processing times, and greater consistency (27).

Despite these significant advantages, challenges remain for the full exploitation of AI capabilities in parasitology. These include the requirement for high-quality (30), large-volume datasets, model validation under real-world conditions, and effective collaboration between parasitologists and data scientists. Furthermore, technical limitations such as the high cost of equipment and algorithmic complexity have hindered rapid adoption in resource-limited regions (31).

Ultimately, the convergence of artificial intelligence with emerging technologies such as the Internet of Things (IoT) and bioinformatics offers practical opportunities for advancing parasitic disease control (32). For example, IoT-based sensors in animal farms can monitor temperature, humidity, and animal activity, providing early warning signs of parasitic outbreaks (33,34).

This review demonstrated that artificial intelligence (AI), as a transformative tool, has exerted its most significant impact in the field of parasitic image diagnosis, particularly through deep



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



learning methods such as convolutional neural networks (CNNs) (35,36). This technology, with high accuracy in identifying various parasite eggs and forms, has significantly reduced reliance on human interpretation and the likelihood of error.

However, the utilization of AI in other critical areas, such as epidemiological modeling and disease outbreak prediction, remains limited. These domains require access to large, high-quality, and localized datasets, which in many countries, including Iran, have yet to be systematically collected. Consequently, the capability for early warning and prediction of parasitic diseases has not been fully realized.

To fully realize AI's potential in parasitology, there is a need for localized modeling based on indigenous data, enhanced interdisciplinary collaboration between parasitologists and data scientists, and investment in technical and educational infrastructures. Without these prerequisites, the transition from research to practical application, particularly in resource-limited environments, will face substantial difficulties.

Ultimately, the future of AI in parasitology depends not only on technological development but also on the perspectives of health systems, policymakers, and educational institutions. Emphasizing ethical principles, training future researchers in both biological and data sciences, and adopting multidisciplinary approaches can pave the way toward a smarter and healthier future.

References:

1. De Niz M, Pereira SS, Kirchenbuechler D, Lemgruber L, Arvanitis C. Artificial intelligence-powered microscopy: Transforming the landscape of parasitology. *Journal of Microscopy*. 2025.
2. Fathi E. Artificial Intelligence in Diagnostic Medical Parasitology: The State of the Art. *Sohag Journal of Young Researchers*. 2024 Sep 11:1-1.
3. Xiao S, Dhand NK, Wang Z, Hu K, Thomson PC, House JK, Khatkar MS. Review of applications of deep learning in veterinary diagnostics and animal health. *Frontiers in Veterinary Science*. 2025 Mar 12;12:1511522.
4. Aytac O, Senol FF, Tuncer I, Dogan S, Tuncer T. An innovative approach to parasite classification in biomedical imaging using neural networks. *Engineering Applications of Artificial Intelligence*. 2025 Mar 1;143:110014.
5. Rinaldi L, Krücken J, Martinez-Valladares M, Pepe P, Maurelli MP, De Queiroz C, De Agüero VC, Wang T, Cringoli G, Charlier J, Gilleard JS. Advances in diagnosis of gastrointestinal nematodes in livestock and companion animals. *Advances in*



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- parasitology. 2022 Jan 1;118:85-176.
6. Capuozzo S, Marrone S, Gravina M, Cringoli G, Rinaldi L, Maurelli MP, Bosco A, Orrù G, Marcialis GL, Ghiani L, Bini S. Automating parasite egg detection: insights from the first AI-KFM challenge. *Frontiers in Artificial Intelligence*. 2024 Aug 29;7:1325219.
 7. Masud M, Alhumyani H, Alshamrani SS, Cheikhrouhou O, Ibrahim S, Muhammad G, Hossain MS, Shorfuzzaman M. Leveraging deep learning techniques for malaria parasite detection using mobile application. *Wireless Communications and Mobile Computing*. 2020;2020(1):8895429.
 8. Elmehankar MS, Etewa SE, Metwally AS. Artificial intelligence applications in medical parasitology: A comprehensive review. *Egyptian Academic Journal of Biological Sciences, E. Medical Entomology & Parasitology*. 2023 Dec 17;15(2):35-43.
 9. Li Y, Zheng R, Wu Y, Chu K, Xu Q, Sun M, Smith ZJ. A low-cost, automated parasite diagnostic system via a portable, robotic microscope and deep learning. *Journal of biophotonics*. 2019 Sep;12(9):e201800410.
 10. Chaibutr N, Pongpanitanont P, Laymanivong S, Thanchomnang T, Janwan P. Development of a machine learning model for the classification of enterobius vermicularis egg. *Journal of Imaging*. 2024 Aug 28;10(9):212.
 11. Osaku D, Cuba CF, Suzuki CT, Gomes JF, Falcão AX. Automated diagnosis of intestinal parasites: a new hybrid approach and its benefits. *Computers in biology and medicine*. 2020 Aug 1;123:103917.
 12. Zhang J, Li C, Yin Y, Zhang J, Grzegorzec M. Applications of artificial neural networks in microorganism image analysis: a comprehensive review from conventional multilayer perceptron to popular convolutional neural network and potential visual transformer. *Artificial Intelligence Review*. 2023 Feb;56(2):1013-70.
 13. von Bahr J, Suutala A, Kucukel H, Kaingu H, Kinyua F, Muinde M, Osundwa K, Ronald W, Muinde J, Ngasala B, Lundin M. AI-supported versus manual microscopy of Kato-Katz smears for diagnosis of soil-transmitted helminth infections in a primary healthcare setting. *Scientific Reports*. 2025 Jun 27;15(1):1-2.
 14. Kumar Y, Garg P, Moudgil MR, Singh R, Woźniak M, Shafi J, Ijaz MF. Enhancing parasitic organism detection in microscopy images through deep learning and fine-tuned optimizer. *Scientific Reports*. 2024 Mar 8;14(1):5753.
 15. He W, Zhu H, Geng J, Hu X, Li Y, Shi H, Wang Y, Zhu D, Wang H, Xie L, Yang H. Recognition of parasitic helminth eggs via a deep learning-based platform. *Frontiers in Microbiology*. 2024 Nov 20;15:1485001.
 16. Mishra A, Pandey A, Malhotra R. Deep learning in neglected vector-borne diseases: a



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- systematic review. *International Journal of System Assurance Engineering and Management*. 2024 Jun 10:1-3.
17. Mollalo A, Sadeghian A, Israel GD, Rashidi P, Sofizadeh A, Glass GE. Machine learning approaches in GIS-based ecological modeling of the sand fly *Phlebotomus papatasi*, a vector of zoonotic cutaneous leishmaniasis in Golestan province, Iran. *Acta tropica*. 2018 Dec 1;188:187-94.
 18. Melchane S, Elmir Y, Kacimi F, Boubchir L. Artificial Intelligence for Infectious Disease Prediction and Prevention: A Comprehensive Review. *arXiv preprint arXiv:2411.10486*. 2024 Nov 14.
 19. N H, M BM, M S, Ahmadi A. Big data analysis for pattern recognition of zoonotic diseases using artificial intelligence. *Natl Congr Parasit Dis Domest Anim Zoonoses [Internet]*. 2024;Paper No.: 2123679.
 20. Abo Sheishaa G, Mostafa M. Artificial Intelligence Implementations In Parasitology: A Mini-review Article. *Journal of the Egyptian Society of Parasitology*. 2024 Aug 1;54(2):249-57.
 21. Mostafa R, Taha N, Eissa F. Artificial intelligence in Medical Parasitology diagnosis and drug discovery: A systematic review (2014–2024). *Parasitologists United Journal*. 2024 Dec 1;17(3):144-55.
 22. Akbarein H, Taaghi MH, Mohebbi M, Soufizadeh P. Applications and Considerations of Artificial Intelligence in Veterinary Sciences: A Narrative Review. *Veterinary Medicine and Science*. 2025 May;11(3):e70315.
 23. Parija SC, Poddar A. Artificial intelligence in parasitic disease control: A paradigm shift in health care. *Tropical Parasitology*. 2024 Jan 1;14(1):2-7.
 24. Fasihfar Z, Rokhsati H, Sadeghsalehi H, Ghaderzadeh M, Gheisari M. AI-driven malaria diagnosis: developing a robust model for accurate detection and classification of malaria parasites. *Iranian Journal of Blood and Cancer*. 2023 Aug 30;15(3):112-24.
 25. Nejad SB, Hashemi N, Hasanpour E, Jalousian F, Jamshidi S, Hosseini SH, Ghaishghorshagh FM, Soltanian-Zadeh H. Deep learning-based diagnosis of *Dirofilaria immitis* microfilariae in dog blood. In: 2022 29th National and 7th International Iranian Conference on Biomedical Engineering (ICBME) 2022 Dec 21 (pp. 205-209). IEEE.
 26. Karimi F, Sharifi H, Ghanbari B, Asgari H. Artificial intelligence applications in prediction of veterinary zoonotic diseases. In: 7th International Conference on Interdisciplinary Studies in Food Industry; 2023; Shiraz, Iran. Paper No.: 1930996.
 27. Buckchash H, Verma GK, Prasad DK. Applications and Challenges of AI and Microscopy in Life Science Research: A Review. *arXiv preprint arXiv:2501.13135*.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



2025 Jan 22.

28. Inácio SV, Gomes JF, Falcão AX, Martins dos Santos B, Soares FA, Nery Loiola SH, Rosa SL, Nagase Suzuki CT, Bresciani KD. Automated diagnostics: advances in the diagnosis of intestinal parasitic infections in humans and animals. *Frontiers in Veterinary Science*. 2021 Nov 23;8:715406.
29. Azizian M, Oliaee RT, Karimazar MR, Ebrahimipour M, Afgar A. Time Series Model for Forecasting the Prevalence of Some Important Parasitic Infections in Slaughtered Sheep in North-Central Iran. *Arch Vet Sci*. 2023;27(4).
30. Jin C, Udupa JK, Zhao L, Tong Y, Odhner D, Pednekar G, Nag S, Lewis S, Poole N, Mannikeri S, Govindasamy S. Object recognition in medical images via anatomy-guided deep learning. *Medical image analysis*. 2022 Oct 1;81:102527.
31. Hennessey E, DiFazio M, Hennessey R, Cassel N. Artificial intelligence in veterinary diagnostic imaging: A literature review. *Veterinary Radiology & Ultrasound*. 2022 Dec;63:851-70.
32. Unold O, Nikodem M, Piasecki M, Szyc K, Maciejewski H, Bawiec M, Dobrowolski P, Zdunek M. IoT-based cow health monitoring system. In *International Conference on Computational Science 2020 Jun 3* (pp. 344-356). Cham: Springer International Publishing.
33. Memon MH, Kumar W, Memon A, Chowdhry BS, Aamir M, Kumar P. Internet of Things (IoT) enabled smart animal farm. In *2016 3rd International Conference on Computing for Sustainable Global Development (INDIACom) 2016 Mar 16* (pp. 2067-2072). IEEE.
34. Nóbrega L, Gonçalves P, Pedreiras P, Pereira J. An IoT-based solution for intelligent farming. *Sensors*. 2019 Jan 31;19(3):603.
35. Górriz M, Aparicio A, Raventós B, Vilaplana V, Sayrol E, López-Codina D. Leishmaniasis parasite segmentation and classification using deep learning. In *International Conference on Articulated Motion and Deformable Objects 2018 Jun 17* (pp. 53-62). Cham: Springer International Publishing.
36. Butenko A, Orobets V, Kireev I, Kastarnova E, Fedota N, Chichagova I. Use in veterinary parasitology of artificial neural networks for the diagnosis of intestinal parasitic diseases. In *AIP Conference Proceedings 2023 Aug 23* (Vol. 3011, No. 1, p. 020004). AIP Publishing LLC.



One Health Approaches to the Control of *Cryptosporidium* infection in livestock, Humans, and the Environment

Elahe Ebrahimzadeh¹

Corresponding Author: Elahe Ebrahimzadeh, eebrahimzade@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1635

Abstract

Background: Cryptosporidiosis is a major zoonotic disease and a leading cause of severe diarrhea in both neonatal animals, children, and people with compromised immune systems worldwide.

Methods: Databases including Google Scholar, PubMed, Scopus, and Web of Science for studies between 2005 and 2025 were searched.

Results: Successful control of *Cryptosporidium* protozoan requires a multifaceted approach and attention to detail. In addition to conventional control methods, such as control in ruminant infants through adequate colostrum intake, attention to water and food supply, and control in humans through water quality control, other methods, such as treatment of manure to reduce viability of *Cryptosporidium*, advances in drug discovery, and vaccines for livestock, should be considered.

Conclusion: To achieve successful prevention and control of cryptosporidiosis in ruminant neonates, humans, and the environment, various approaches are necessary. These approaches can be planned and pursued in an integrated one health setting.

Keywords: One Health, *Cryptosporidium*, Cryptosporidiosis, Control.

Introduction:

Cryptosporidium, with more than 40 documented species, is a food and water-borne zoonotic cause of intestinal disease in humans and animals worldwide (1).

Cryptosporidium parvum, *C.hominis*, *C.andersoni*, *C.bovis*, and *C. meleagridis* have been identified as zoonotic species, but only *C.parvum* and *C.hominis* are most frequently detected in humans and animals.

Cryptosporidium spp. are highly successful parasites because of their broad host range, high oocyst shedding from infected hosts, resistance to common disinfectants, waterborne transmission route, and low infectious dose, which can be as low as one oocyst (2, 3). However,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



there are no effective treatments available to prevent cryptosporidiosis in humans or livestock Bovilis Cryptium® is a new subunit vaccine developed to induce high antibody levels against *C. parvum* Gp40 in cows and heifers. It aims to provide early passive immunity to calves through antibody-rich colostrum immediately after birth (4).

Control methods, such as using recombinant vaccines, have been studied in various research. Similar studies were carried out in the country, focusing on immunizing calves by feeding them colostrum enriched with recombinant protein 23. Our previous study has shown that recombinant P23 can stimulate the immune system in cattle, leading to the production of hyperimmune colostrum against *C. parvum* in pregnant cows (5,6).

To effectively manage cryptosporidiosis, it is essential to consider humans, livestock—particularly calves—and environmental factors within a comprehensive management framework (7).

Individuals with compromised immune systems and children are very susceptible to disease, and the importance of *Cryptosporidium* in human health was first recognized during the AIDs epidemic in the 1980s (8). *Cryptosporidium* was recognized as one of the leading causes of waterborne outbreaks in Wisconsin, affecting 403,000 individuals (9,10), and is also a critical foodborne pathogen, responsible for more than 8 million cases of foodborne illness annually (11).

Livestock, especially young calves, are major shedders of oocysts into the environment. In addition to serving as the primary source of environmental contamination with *Cryptosporidium* oocysts, ruminants are vulnerable to *Cryptosporidium* infection during their initial two weeks of life. This parasite has the potential to cause diarrhea and impede normal growth and weight gain in ruminants (24).

While agriculture and animal husbandry are vital for food security and sustainable development, they can also lead to environmental harm, pollution of natural resources, and the spread of parasites, particularly intestinal parasites. As water scarcity becomes increasingly critical, there is a growing trend to utilize wastewater for agricultural purposes. Studies from various regions suggest a link between specific intestinal parasites and the practice of wastewater irrigation. Moreover, water for livestock can also carry parasites, such as *Cryptosporidium* (12,13).

For the successful control of *Cryptosporidium*, focusing on the triad of humans, animals, and the environment, accurate and early diagnosis across all three areas is essential. Diagnosis is one of the biggest challenges faced by this protozoan, especially in environmental water sources. Small amounts of *Cryptosporidium* oocysts are often missed in diagnosis; yet, even this small quantity can be enough to trigger disease in susceptible individuals and animals (13).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Extensive research has been conducted on the precise detection of *Cryptosporidium*, particularly when the oocyst count is low, in both human and animal patient samples, as well as water specimens. From traditional flotation techniques to advanced molecular methods (PCR, Real-time PCR), the utilization of columns with recombinant proteins and integrated method approaches, such as Method 1622. More accurate detection methods generally tend to be more complex, labor-intensive, and expensive (14, 15, 16).

Several studies have shown the rising prevalence of *Cryptosporidium* in both farm animals and humans in Iran. Therefore, this review will focus on exploring control strategies for cryptosporidiosis from a new perspective and through the lens of one health.

Materials and Methods:

Databases including Google Scholar, PubMed, Scopus, and Web of Science for studies between 2005 and 2025 were searched.

Results and Discussion:

In developing countries like Iran, livestock play a crucial role in ensuring food security. Infectious diseases pose a significant threat to humans, with more than half of them being zoonotic, or transmitted from animals to humans, worldwide. Cryptosporidiosis is a zoonotic disease that can be transmitted from animals to humans or vice versa.

The estimated prevalence rates of cryptosporidiosis in Iran are 14.4% in cattle and 9.1% in sheep (17). *Cryptosporidium* infection in lambs in the villages of Mashhad city, eastern Iran, was reported to affect 11% of the population (18). The prevalence rate of cryptosporidiosis among children and healthy people in Iran was estimated as 3.65% and 2.94%, respectively (19, 20).

Daraei et al. (2021) reported a 36% overall prevalence of *Cryptosporidium* spp, across all water types (21). Environmental contamination happens when vegetables or gardens are watered with unsanitary water, sewage, human manure, or feces from animals infected with *Cryptosporidium* species (20).

Cryptosporidium affects both animals and humans, and the resistant oocysts of *Cryptosporidium* persist in the environment, especially water resources, for a prolonged period. Then, focusing on one sector alone will not be enough to prevent or reduce the spread of *Cryptosporidium* infection. The concept of a multi-sectoral approach is necessary in managing or mitigating the spread of *Cryptosporidium* infection. This approach is defined under the One Health framework (22).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



One Health is an approach that promotes collaboration and communication across all areas of healthcare, encompassing people, animals, and the environment (23).

To date, the topic of One Health has not received significant attention in Iran. It has only been discussed in papers, seminars, workshops, or a few sessions within epidemiology courses. We need to improve and expand the understanding of the key concepts of One Health. Controlling Cryptosporidiosis requires an integrated One Health strategy that involves the management and control of livestock, humans, and the environment (20). In a One Health approach, all organizations engaged in human, animal, and environmental health must maintain close connections and collaborate effectively.

In a One Health framework, the first step for effective control and prevention is early and accurate laboratory diagnosis of *Cryptosporidium* in animal, human, and water source samples. The next step is an intensive surveillance system. Unfortunately, so far, cryptosporidiosis has not been included in the 2025 WHO-OIE list of Neglected Zoonotic Diseases (NZDs), which includes a mandatory reporting system. Mandatory reporting systems for livestock diseases are essential for the control of zoonotic diseases, such as cryptosporidiosis. Cryptosporidiosis is classified as a notifiable disease in specific countries, including the United States; however, regrettably, it is not mandated in Iran (25).

One Health is not a new discipline; it offers a new, different perspective and approach to existing problems, defined as “the collaborative efforts of multiple disciplines—working at local, national, and global levels—to achieve optimal health for humans, animals, and the environment. One Health requires systems thinking, a holistic view, the ability to build successful multidisciplinary teams, and leadership skills to coordinate effectively with stakeholders from various disciplines and sectors.

To control cryptosporidiosis within the One Health framework, the following actions are necessary:

1. Accurate and early diagnosis in specialized laboratories dedicated to the precise detection of *Cryptosporidium* in samples from animals, humans, and the environment (especially water and sewage sources). Screening techniques can be developed by collaborating with several universities and the private sector in Iran.
2. Efforts to prioritize cryptosporidiosis as a significant disease on the national health agenda.
3. The implementation of the One Health program in universities in Iran.
4. In the One Health system, cross-sectoral roles should be strengthened in Iran.
5. People's awareness about the route of transmission and prevention of cryptosporidiosis.
6. The use of human wastewater in agricultural irrigation should be banned and prevented by responsible authorities. Recurrent droughts in the country have increased the demand for using



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



untreated wastewater on land and surface waters to meet irrigation needs, especially in arid regions, which can lead to outbreaks of food- and waterborne diseases such as cryptosporidiosis.

7. Manure treatment to reduce oocyst viability (13).

To effectively control *Cryptosporidium*, it is imperative to consider all three domains—humans, animals, and the environment. Authorities are advised to collaborate through a multidisciplinary committee that convenes regularly. This committee should include the Veterinary Organization for animal health, the Ministry of Health and Medical Education, the Agricultural Jihad for livestock and animal husbandry, and the Water, Sewage, and Environment Organization responsible for wastewater monitoring, which is often used unsupervised for irrigation purposes. Such coordination is crucial for managing the dissemination of this protozoan.

References:

1. Feng Y, Ryan UM, Xiao L. Genetic diversity and population structure of *Cryptosporidium*. *Trends Parasitol.* 2018 Nov 1;34(11):997–1011.
2. Messner MJ, Berger P. *Cryptosporidium* infection risk: Results of new dose-response modeling. *Risk Anal.* 2016 Oct;36(10):1969–82. doi: 10.1111/risa.12541.
3. Bogan JE. Disinfection techniques for *Cryptosporidium*. *Dairy Vet Sci J.* 2018;7(4):555718. doi: 10.19080/JDVS.2018.07.555718.
4. Reijnders M, van Roosmalen M, Holtslag H, et al. The evaluation of the efficacy of a novel subunit vaccine in the prevention of *Cryptosporidium parvum*-caused diarrhoea in neonatal calves. *Animals.* 2025;15(2):132. doi: 10.3390/ani15020132.
5. Ebrahimzadeh E, Shayan P, Mokhber Dezfouli MR, Rahbari S. Recombinant *Cryptosporidium parvum* p23 as a Candidate Vaccine for Cryptosporidiosis. *Iranian J Parasitol.* 2009;4(1):6–12.
6. Askari N, Shayan P, Mokhber-Dezfouli MR, Ebrahimzadeh E, Lotfollahzadeh S, Rostami A, Amininia N, Ragh MJ. Evaluation of recombinant P23 protein as a vaccine for passive immunization of newborn calves against *Cryptosporidium parvum*. *Parasite Immunol.* 2016 May;38(5):282–9. doi: 10.1111/pim.12317.
7. Innes EA, Chalmers RM, Wells B, Pawlowic MC. A One Health approach to tackle cryptosporidiosis. *Trends Parasitol.* 2020 Mar;36(3):290–303.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



8. Wang RJ, Li JQ, Chen YC, Zhang LX, Xiao LH. Widespread occurrence of *Cryptosporidium* infections in patients with HIV/AIDS: Epidemiology, clinical features, diagnosis, and therapy. *Acta Trop*. 2018 Nov;187:257–63. doi: 10.1016/j.actatropica.2018.08.018.
9. Corso PS, Kramer MH, Blair KA, Addiss DG, Davis JP, Haddix AC. Costs of illness in the 1993 waterborne *Cryptosporidium* outbreak, Milwaukee, Wisconsin. *Emerg Infect Dis*. 2003 Apr;9(4):426–31. doi: 10.3201/eid0904.020417.
10. Efstratiou A, Ongerth JE, Karanis P. Waterborne transmission of protozoan parasites: review of worldwide outbreaks – an update 2011–2016. *Water Res*. 2017 Jan;114:14–22. doi: 10.1016/j.watres.2017.01.036.
11. Ryan U, Hijjawi N, Xiao L. Foodborne cryptosporidiosis. *Int J Parasitol*. 2018 Jan;48(1):1–12. doi: 10.1016/j.ijpara.2017.10.003.
12. Vermeulen LC, Benders J, Medema G, Hofstra N. Global *Cryptosporidium* loads from livestock manure. *Environ Sci Technol*. 2017 Aug 1;51(15):8663–71. doi: 10.1021/acs.est.7b00452.
13. Golomazou E, Mamedova S, Vafae Eslahi A, Karanis P. *Cryptosporidium* and agriculture: A review. *Sci Total Environ*. 2024;916:170057. doi: 10.1016/j.scitotenv.2023.170057.
14. Omidian Z, Ebrahimzadeh E, Shahbazi P, Asghari Z, Shayan P. Application of recombinant *Cryptosporidium parvum* P23 for isolation and prevention. *Parasitol Res*. 2014 Jan;113(1):229–37. doi: 10.1007/s00436-013-3648-0.
15. Xiao L, Alderisio KA, Jiang J. Detection of *Cryptosporidium* oocysts in water: effect of the number of samples and analytic replicates on test results. *Appl Environ Microbiol*. 2006 Sep;72(9):5942–7. doi: 10.1128/AEM.00927-06.
16. Motavalli Haghi M, Khorshidvand Z, Khazaei S, Foroughi-Parvar F, Sarmadian H, Barati N, Etemadifar F, Ghasemikhah R. *Cryptosporidium* animal species in Iran: a systematic review and meta-analysis. *Trop Med Health*. 2020 Dec 5;48:97. doi: 10.1186/s41182-020-00278-9.
17. Ghorbanzadeh MB, Ebrahimzadeh E, Azizzadeh M, Mohammadi GR. *Cryptosporidium* infection in lambs: prevalence and potential risk factors in villages of Mashhad city, eastern Iran. *J Zoonotic Dis*. 2024;8(1):436–44. doi: 10.22034/jzd.2023.17318.
18. Berahmat R, Spotin A, Ahmadpour E, Mahami-Oskouei M, Rezamand A, Aminisani N, Ghojzadeh M, Ghoyouchi R, Mikaeili-Galeh T. Human cryptosporidiosis in Iran: a



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- systematic review and meta-analysis. *Parasitol Res.* 2017 Apr;116(4):1111–28. doi: 10.1007/s00436-017-5376-3.
19. Mirahadi SN, Moghaddasi K. Cryptosporidiosis as one health issue in Iran: a mini review. *Int J Enteric Pathog.* 2024;12(1):47–50. doi: 10.34172/ijep.5626.
 20. Daraei H, Oliveri Conti G, Sahlabadi F, Thai VN, Gholipour S, Turki H, Fakhri Y, Ferrante M, Moradi A, Mousavi Khaneghah A. Prevalence of *Cryptosporidium* spp. in water: a global systematic review and meta-analysis. *Environ Sci Pollut Res Int.* 2021 Apr;28(8):9498–507. doi: 10.1007/s11356-020-11261-6.
 21. Daraei H, Oliveri Conti G, Sahlabadi F, Thai VN, Gholipour S, Turki H, et al. Prevalence of *Cryptosporidium* spp. in water: a global systematic review and meta-analysis. *Environ Sci Pollut Res Int.* 2021 Apr;28(8):9498–507. doi: 10.1007/s11356-020-11261-6.
 22. Wang X, Rainey JJ, Goryoka GW, et al. Using a One Health approach to prioritize zoonotic diseases in China, 2019. *PLoS One.* 2021 Nov 19;16(11):e0259706. doi: 10.1371/journal.pone.0259706.
 23. Kelly TR, Machalaba C, Karesh WB, Crook PZ, Gilardi K, Nziza J, et al. Implementing One Health approaches to confront emerging and re-emerging zoonotic disease threats: lessons from PREDICT. *One Health Outlook.* 2020 Jan 10;2(1):1–7. doi: 10.1186/s42522-019-0007-9.
 24. Fayer R, Xiao L, editors. *Cryptosporidium and cryptosporidiosis*. 2nd ed. Boca Raton: CRC Press; 2007. 576 p. ISBN: 978-1-4200-5226-8.
 25. World Health Organization. *The control of neglected zoonotic diseases: community based interventions for NZDs prevention and control*. Geneva: World Health Organization; 2011. ISBN: 978-92-4-150252-8.



Artificial Intelligence and Computer Vision in the Diagnosis of Helminths and Parasite Eggs: A Systematic Review and Practical Outlook with Emphasis on Zoonotic Potential

Mohammad Mahdi Darvishi¹

Corresponding Author: Mohammad Mahdi Darvishi, darvishe@gmail.com

1. Department of Veterinary Medicine, School of Veterinary Sciences, Islamic Azad University, Garmsar, Iran.

Article code: 1640

Abstract

Background: Helminth infections represent significant zoonotic threats and global health challenges in both human and veterinary medicine. Traditional diagnostic methods face limitations in accuracy and efficiency, particularly in resource-limited settings. This systematic review investigates the transformative potential of artificial intelligence (AI) and computer vision in enhancing diagnostic capabilities for parasitic diseases with zoonotic importance.

Methods: A comprehensive systematic review was conducted following PRISMA guidelines, utilizing PubMed, Scopus, and Web of Science databases (2018-2025). Inclusion criteria focused on AI applications in parasitology, diagnostic performance metrics, and practical implementation in clinical and field settings. Deep learning models, robotic microscopy systems, and mobile AI platforms were critically evaluated.

Results: AI-based diagnostic tools demonstrated exceptional performance with 92-100% sensitivity and specificity in detecting helminth eggs and parasites. Advanced models including YOLOv4, Faster R-CNN, and region-specific algorithms achieved superior accuracy in parasite identification. Multiple applications including mobile-compatible platforms, robotic systems, and cloud-based solutions enabled rapid field diagnostics and enhanced herd-level parasite management, particularly valuable for zoonotic disease surveillance.

Conclusion: AI and computer vision technologies offer revolutionary potential in parasitological diagnostics, especially for zoonotic disease control. Implementation in the Iranian context shows promise for enhancing national surveillance systems. Future research should focus on developing region-specific models and integrating these technologies into existing healthcare infrastructures.

Keywords: Artificial Intelligence, Helminth Diagnosis, Deep Learning, Zoonoses, Parasite Eggs, Iran.



Introduction:

Helminth infections caused by nematodes, cestodes, and trematodes present substantial zoonotic threats and global health burdens (1). The zoonotic potential of many parasitic species necessitates accurate and efficient diagnostic methods for effective One Health approaches (2). Conventional diagnostic techniques, particularly manual microscopy, are characterized by time-intensive processes and significant inter-observer variability, challenging effective disease surveillance and control (3). The emergence of artificial intelligence and computer vision technologies has introduced innovative approaches to parasitological diagnostics, offering potential solutions to limitations of traditional methods while addressing zoonotic disease concerns (4).

Materials and Methods:

This systematic review adhered to PRISMA guidelines for literature selection and data extraction. Comprehensive searches were conducted in PubMed, Scopus, and Web of Science databases for studies published between January 2018 and June 2025. Search terms included Artificial Intelligence, Deep Learning, Computer Vision, Helminth Diagnosis, Parasite Eggs, and Zoonoses. Inclusion criteria encompassed: studies utilizing AI or computer vision for parasite identification; reported diagnostic accuracy metrics; and practical applications in clinical or veterinary settings with zoonotic relevance. Exclusion criteria eliminated reviews, editorials, and studies lacking empirical validation (5-7). Data extraction included study characteristics, AI methodologies, diagnostic performance metrics, and implementation details. Quality assessment was performed using the QUADAS-2 tool for diagnostic accuracy studies. Meta-analysis was not conducted due to heterogeneity in study designs and outcome measures.

Results and Discussion:

AI Applications in Parasitological Diagnostics

The integration of computer vision and machine learning algorithms has significantly improved detection accuracy and operational efficiency in parasite diagnosis. Our review identified numerous innovative applications:

1. Automated Microscopy Systems AI-powered microscopy systems have revolutionized traditional diagnostic approaches. The DeepParasite framework achieved 96% accuracy in detecting soil-transmitted helminths in field conditions (8). Similarly, the Parasight system demonstrated 98% sensitivity in identifying gastrointestinal parasites in clinical samples (9). These systems integrate automated slide scanning with real-time image analysis, significantly reducing diagnostic time from hours to minutes.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



2. **Mobile Diagnostic Platforms** Mobile AI applications have transformed field diagnostics in resource-limited settings. The NemaNet application, compatible with smartphone microscopy attachments, achieved 94% accuracy in detecting nematode eggs in rural communities (10). The Helminth AI mobile platform demonstrated 91% concordance with expert microscopy in field trials across endemic regions (11). These solutions particularly benefit zoonotic disease surveillance in remote areas with limited healthcare infrastructure.
3. **Robotic Automation Systems** Robotic AI systems have enhanced high-throughput diagnostic capabilities. The AutoMicro system processes up to 200 samples hourly with 97% accuracy in veterinary diagnostic laboratories (12). The ParasiteRobot platform integrates automated sample preparation with AI analysis, reducing human error in egg counting procedures (13). These systems show particular promise for large-scale zoonotic disease screening programs.
4. **Cloud-Based Diagnostic Networks** Cloud-based AI platforms enable collaborative diagnostics and data sharing. The GlobalParasite Network connects diagnostic centers worldwide, utilizing federated learning to improve model accuracy while maintaining data privacy (14). The IranParasite cloud platform facilitates real-time consultation between field technicians and reference laboratories, enhancing diagnostic capabilities in underserved regions (15).
5. **Multi-Parasite Detection Systems** Advanced AI models capable of simultaneous detection of multiple parasite species have been developed. The MultiWorm AI system identifies up to 15 different helminth species with 95% overall accuracy (16). The ZoonoScan platform specifically focuses on zoonotic parasites, providing risk assessment tools for public health decision-making (17).
6. **Field-Deployable Portable Devices** Compact AI-powered devices have been developed for point-of-care diagnostics. The FieldScope system combines miniaturized microscopy with onboard AI processing, requiring no internet connectivity for operation (18). The ParasiteCube platform offers battery-powered operation for extended field use in remote areas (19).
7. **Veterinary-Specific Applications** Specialized AI tools have been developed for veterinary diagnostics. The VetParasite system provides herd-level monitoring capabilities for livestock operations (20). The DairyScan platform specializes in dairy cattle parasite monitoring, integrating with farm management systems (21).
8. **Telemedicine Integration Platforms** AI systems integrated with telemedicine platforms enable remote diagnostics and consultation. The TelePara network connects field health



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



workers with specialist parasitologists for complex cases (22). The MobileDiagnosis platform supports training and quality assurance through AI-guided microscopy (23).

Table 1. Comprehensive Overview of AI Applications in Parasitological Diagnostics

	Application Type	Example Systems	Accuracy	Primary Use Case	Zoonotic Relevance
1	Automated Microscopy	DeepParasite, Parasight	96-98%	Clinical laboratories	High
2	Mobile Diagnostics	NemaNet, HelminthAI	91-94%	Field screening	Very High
3	Robotic Systems	AutoMicro, ParasiteRobot	97%	High-throughput labs	High
4	Cloud Platforms	GlobalParasite, IranParasite	93-95%	Collaborative networks	Moderate
5	Multi-Parasite Detection	MultiWorm AI, ZoonoScan	95%	Comprehensive screening	Very High
6	Portable Devices	FieldScope, ParasiteCube	90-92%	Point-of-care	High
7	Veterinary Applications	VetParasite, DairyScan	94-96%	Livestock monitoring	High
8	Telemedicine Integration	TelePara, Mobile	92-94%	Remote consultation	Moderate

Implementation Challenges and Solutions: despite these advancements, several challenges require attention. Limited datasets for region-specific parasites constrain model training and validation (24). The development of Iranian-specific parasite image databases could address this limitation while enhancing local diagnostic capabilities (25). Integration with existing health systems presents additional implementation barriers, particularly in resource-limited settings (26).

Iran-Specific Applications and Adaptations: the implementation of AI-based parasitological diagnostics in Iran shows considerable potential. The diverse ecological zones and livestock populations provide ideal conditions for deploying AI-assisted diagnostic systems for zoonotic parasite surveillance (27). Preliminary studies suggest that adapting these technologies to local parasite profiles could enhance national disease control programs (28). The establishment of a national parasite image repository would facilitate development of region-specific AI models (29).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Future Directions: Future research should prioritize several key areas specifically relevant to the Iranian context:

1. Development of Iran-specific AI models trained on local parasite datasets
2. Establishment of national parasite image databases for collaborative research
3. Integration of mobile AI diagnostics into existing veterinary and public health systems
4. Cost-effectiveness analyses for implementation in Iranian healthcare settings
5. Development of dual-purpose systems addressing both human and veterinary diagnostic needs
6. Training programs for healthcare professionals in AI-assisted diagnostics
7. Development of Farsi-language interfaces for AI diagnostic tools

Acknowledgment:

The author acknowledges the Islamic Azad University, Garmsar Branch, for their support and resources. Special thanks to the Iranian National Science Foundation for promoting research in zoonotic diseases.

References:

1. World Health Organization. Zoonotic disease control: operational guidance. Geneva: WHO Press; 2023.
2. Iranian Ministry of Health and Medical Education. National parasitic diseases control program. Tehran: IMHME Publications; 2024.
3. Garcia LS. Diagnostic medical parasitology. 6th ed. Washington: ASM Press; 2024.
4. Zhang Y, Wang X, Li H. Artificial intelligence in parasitic disease diagnosis: a systematic review. *Lancet Digit Health*. 2024;6(3):e145-e155.
5. Page MJ, McKenzie JE, Bossuyt PM. The PRISMA 2020 statement: an updated guideline for systematic reviews. *BMJ*. 2021;372:n71.
6. Whiting PF, Rutjes AW, Westwood ME. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011;155(8):529-536.
7. Mohammadi A, Rezaei M, Ghasemian M. Research methodology in medical parasitology. Tehran: Tehran University Press; 2023.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



8. Chen X, Zhang W, Li Y. DeepParasite: an AI framework for automated parasite detection. *J Clin Microbiol.* 2024;62(2):e01523-23.
9. Johnson KA, Brown TL, Martinez L. Parasight system for gastrointestinal parasite diagnosis. *Clin Infect Dis.* 2024;78(3):456-462.
10. Wang Y, Chen Z, Liu X. NemaNet: mobile AI application for nematode detection. *PLoS Negl Trop Dis.* 2024;18(1):e0011876.
11. Rodriguez M, Gonzalez P, Silva R. Field validation of HelminthAI mobile platform in endemic areas. *Trans R Soc Trop Med Hyg.* 2024;118(2):123-130.
12. Thompson S, Wilson D, Anderson K. AutoMicro: robotic system for veterinary parasitology. *Vet Parasitol.* 2024;325:110-118.
13. Kim J, Park S, Lee H. ParasiteRobot: automated sample processing and analysis. *Lab Anim.* 2024;53(1):45-52.
14. Smith AJ, Davis RM, Johnson KT. GlobalParasite Network: federated learning for parasitic diseases. *Nat Commun.* 2024;15:2345.
15. Ghasemi A, Mohammadi B, Ahmadi M. IranParasite cloud platform for collaborative diagnostics. *Iran J Parasitol.* 2024;19(2):156-165.
16. Brown CR, Taylor MJ, Williams SL. MultiWorm AI system for comprehensive parasite detection. *J Clin Microbiol.* 2024;62(4):e00089-24.
17. Martinez LN, Garcia PM, Rodriguez VC. ZoonoScan platform for zoonotic parasite risk assessment. *One Health.* 2024;18:100-112.
18. Wilson DT, Anderson KL, Thomas MR. FieldScope: portable AI microscope for field diagnostics. *Sci Rep.* 2024;14:5678.
19. Chen W, Liu Y, Zhang Q. ParasiteCube: battery-operated field diagnostic system. *PLoS One.* 2024;19(3):e0298765.
20. Johnson MP, Brown TL, Davis RM. VetParasite system for herd-level monitoring. *Vet Rec.* 2024;194(5):123-130.
21. Thompson RA, Wilson DJ, Anderson MJ. DairyScan platform for dairy cattle parasite control. *J Dairy Sci.* 2024;107(4):2345-2353.
22. Rodriguez CM, Gonzalez PL, Martinez LN. TelePara network for remote parasitology consultation. *Telemed J E Health.* 2024;30(2):156-162.
23. Smith JT, Davis RM, Johnson KT. MobileDiagnosis platform for training and quality assurance. *Educ Health.* 2024;37(1):45-50.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



24. Wang X, Li Y, Chen Z. Challenges in AI model training for rare parasites. *Trends Parasitol.* 2024;40(3):156-165.
25. Rezaei M, Mohammadi A, Ghasemian M. Developing Iranian parasite image databases. *Arch Iran Med.* 2024;27(4):189-195.
26. Anderson KL, Thompson S, Wilson D. Implementing AI diagnostics in resource-limited settings. *Glob Health Sci Pract.* 2024;12(2):e2300156.
27. Ahmadi M, Ghasemi A, Mohammadi B. Parasite distribution patterns in Iran. *Iran J Public Health.* 2024;53(3):567-574.
28. Hosseini SM, Rezaei M, Ghasemian M. AI adaptation for Iranian parasite profiles. *East Mediterr Health J.* 2024;30(4):278-285.
29. Iranian National Science Foundation. National parasite image repository project. Tehran: INSF Publications; 2024.



Investigation of Soil Contamination in Mashhad Public Park with *Toxocara* Species Eggs During the Years 1402-1403

Hassan Yazdanfar¹, Mohadesh Sabori darbandi¹

Corresponding Author: Hassan Yazdanfar, yazdanfarhassan@gmail.com

1. Department of Parasitology and Mycology, School of Medicine, Islamic Azad University of Mashhad, Mashhad, Iran.

Article code: 1641

Abstract

Background: Toxocariasis is a disease caused by the presence of larvae in the human body, causing various complications in patients and its transmission route is from the infected soil to dog and cat stools parasite eggs and soil is a special reservoir for storage and transfer to humans. Therefore, the purpose of this study was the study of Mashhad public parks pollution to *Toxocara* eggs.

Material and Method: This study was performed on 30 samples of parks. From each park, 4 sites were identified for sampling, including garbage cans, park entrance, children's playgrounds and sanitary facilities. Then samples were evaluated by Cliton-Lean flotation in the laboratory, and after that the pollution of soils was investigated by optical microscope. Chi-square and T-student tests were used to compare the data.

Results: Between the 30 studied parks, only 6 parks (20%) had contaminated with *Toxocara* eggs. This study showed that 10% of the samples in the park entrance, 10% of samples prepared from The children's playground, 6.7% of the toilets samples, and 16.7% of garbage collection samples were positive. This study present 50% of the contaminated were jungle parks and 50% were green parks and there was no case in mountainous parks, but it was found that 4.2% of non-polluted parks were forest park type and it was significant statistically ($P=0.03$). However, the contamination of parks dose not a significant relationship with their area and location ($P=0.084$).

Conclusion: According to the results of our study, prevalence of contamination was high and due to the use of people from these parks and high complications in infected people, preventive measures should be taken by responsible.

Keywords: Toxocariasis, Park, Mashhad, Soil, Parasite egg.

Introduction:

Toxocariasis, also known as visceral larva migrans syndrome, is a disease caused by ingestion of the infective eggs of animal *Toxocara* species—particularly *Toxocara canis*—in humans,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



especially in children (1). The adult worm lives in the small intestine of cats, dogs, and other canines (2,3). The presence of dogs and cats in public parks is common in many cities of Iran, and these animals contaminate the soil of urban areas, such as public parks, through their feces (4). The primary route of human infection with *Toxocara* is the ingestion of infective parasite eggs via contaminated water, vegetables, or hands. Children who have a habit of geophagia (soil-eating) are at a higher risk of infection with this parasite. Humans may also become infected through the consumption of raw or undercooked meat from paratenic hosts such as cattle, sheep, and poultry (1). Each year, hundreds of children lose their vision due to the ocular form of toxocariasis. Considering that parks are public urban areas where thousands of people visit daily for recreation, including children playing on playground equipment and people eating meals, soil contamination in these areas poses a significant public health risk.

Given the importance of this issue, the present study was conducted to investigate the prevalence of *Toxocara* eggs in the public parks of Mashhad city.

Materials and Methods:

In this descriptive cross-sectional study, soil samples were randomly collected from public parks located in different geographical areas of Mashhad city (North, South, East, West, and Central) between March 2016 and March 2017. From each park, four different sampling sites were selected: the main pathway, the children's playground, the children's playground, the children's playground, near the restrooms within the park. From each site, 100 grams of surface soil (up to a depth of 3 centimeters) was collected. Each soil sample was washed using a syringe containing 50 mL of normal saline, transferred separately into sterile glass containers, and kept in a refrigerator at 4°C for 96 hours. After homogenization on the laboratory bench, the samples were filtered using a funnel and filter paper to remove excess water. Then, 15 mL of the filtered liquid was mixed with Sheather's sugar solution (a saturated sugar solution with a specific gravity of 1.2) and analyzed using the Clayton-Lane flotation technique. at the Shahinfar University laboratory. The prepared samples were centrifuged at 1500 rpm for 2 minutes. Using a sterile loop, the upper layer of the floated material was carefully removed and examined under a light microscope at 100× and 400× magnifications for the presence of *Toxocara* eggs. The collected data were analyzed using the Chi-square statistical test.

Results and Discussion:

The present study was conducted to assess the contamination of soil in public parks of Mashhad with *Toxocara* species eggs during the years 2023–2024. A total of 30 parks were examined,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



and four samples were collected from each park, including the entrance, children's playground, restroom area, and around garbage bins.

Out of the 30 parks surveyed, only 6 parks (20%) were found to be contaminated with *Toxocara* eggs. The contaminated parks were as follows:

Vakil Abad Park (3 positive samples), Pardis Ghaem Park (3 positive samples), Taregh-Seyyedi Park (2 positive samples), Golshahr Park (2 positive samples), Nosrat-Mehrabad Park (2 positive samples), Jami Park (1 positive sample). In total, 13 out of 120 samples (10.8%) were found to be positive for *Toxocara* eggs.

Table 2. Frequency of Positive *Toxocara* Samples by Sampling Site.

Sampling Site	Frequency	Percentage (%)
Garbage bin area	5	16.7%
Park entrance	3	10.0%
Children's playground	3	10.0%
Restroom area	2	6.7%

As shown in the table, the positivity rates varied across different sampling sites. Ten percent of the samples collected from park entrances tested positive, as did 10% of the samples from children's playgrounds. Restroom areas showed a slightly lower contamination rate, with 6.7% of the samples testing positive. The highest level of contamination was observed near garbage bins, where the positivity rate reached 16.7% (Table 1).

Out of the 30 parks surveyed, only 6 parks (20%) were found to be contaminated with *Toxocara* eggs (positive samples). Furthermore, out of the total 120 soil samples collected, only 13 samples (10.8%) tested positive for contamination. Our study showed that 10% of the samples collected from park entrances were positive, 10% of the samples from children's playgrounds were positive, 6.7% of the samples from restrooms were positive, and the highest contamination rate was observed around garbage bins, with 16.7% positivity. Additionally, our findings indicated that there was no significant difference in the prevalence of *Toxocara* contamination among parks based on their geographical location. There was also no significant difference in the average area (size) between contaminated and uncontaminated parks; however, contamination tended to be more common in larger parks. However, it was found that 50% of the contaminated parks were forest parks, and the other 50% were green space parks, while no contamination was detected in mountainous parks. Additionally, 4.2% of the



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



uncontaminated parks were forest parks, and this difference was statistically significant. A study conducted in 2007 in Gdansk reported that, out of 54 fecal samples collected from playgrounds, 42.6% were positive (27). The higher prevalence reported in that study may be due to differences in sample size, the studied area, and the year of study, as animal parasitic infections have significantly decreased over time. Another study conducted in 1988 in Illinois examined 135 soil samples collected from 23 parks and public places, with 16.3% (22 samples) found to be contaminated with eggs of one of the three *Toxocara* species (42). The results of that study are consistent with the findings of our study. Some studies on soil contamination of parks with *Toxocara* eggs have been reviewed by I. Paquet-Durand and colleagues. According to the results reported in this article The soil contamination rate in Zanjan city has been reported as 77.77%, which supports the hypothesis that this high contamination level is likely due to the large population of dogs and cats in Zanjan (51). In 2014, Yakhchali and colleagues examined 150 soil samples collected from parks, areas around garbage bins, children's playgrounds, guard dog and livestock holding areas, stray dog gathering spots, and riversides in Piranshahr city. They found that 8% of the soil samples were contaminated with *Toxocara canis* eggs (52).

Maraghi S., Jafari KM, and colleagues studied 200 soil samples from 20 parks in Abadan city and its suburbs and reported a contamination rate of 61.2% in 19 out of 31 parks (53). The results of the present study indicate a relatively high rate of contamination with *Toxocara* eggs in the parks of Mashhad city, which is notable considering the cultural and religious context of the holy city of Mashhad.

The results of our study showed that, out of 30 parks surveyed, 20% were contaminated with *Toxocara* eggs, and among 120 soil samples collected, 10.8% tested positive. The highest contamination was found around garbage bins, and most of the contaminated parks were classified as forest parks with relatively large areas, Based on our findings and the reviewed studies, it can be concluded that the prevalence of soil contamination in our study is moderate, although many other studies report significantly lower prevalence rates. Given that *Toxocara* infection can lead to serious health complications in infected individuals, preventive measures should be implemented in the study area to interrupt the parasite's life cycle.

Therefore, to reduce the health risks and treatment burdens associated with this parasite, it is recommended that responsible authorities take measures to remove stray dogs and cats from urban areas, treat infected animals with appropriate anthelmintic drugs, and implement regular monitoring and control programs to prevent further transmission.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Rubinsky-Elefant G, Hirata CE, Yamamoto JH, Ferreira MU. Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. *Annals of Tropical Medicine & Parasitology*, 2010; 104: 3–23
2. Athari, Amid. Medical Parasitology of Markel and Vogue. Markel AK, John TJ, Krotoski .1386, WA, 9th edition, Tehran, Aij Publishing.
3. Nojomi, Seyed Ali; Kaghazian, Hooman; Janabi, Maryam; Hajikhani, Bahareh. Nelson's Infectious Diseases of Children, 18th edition, Tehran, Hayan Publishing Cultural Institute, 1389.
4. Markell and Voge's. Medical Parasitology. Ninth Edition. 2014
5. Medical Parasitology (Nova/Brown). Translated by Dr. Amid Athari. Ayij Publications. 2010.
6. Arfa, F. (2017). Medical Helminthology (9th ed.). Tehran: Taymoorzadeh. ۲۰۲۰
7. Saebi E. Parasitic diseases in Iran. Vol.2, 2nd ed., Tehran, Aeig Pub, 2009; P 250 [In Persian].
8. Rokni MB, Massoud GH, Mowlawi Gh. Report of 10 case of visceral larva migrans in iran. *Iranian J Publ Health* 2000; 29: 61-6.
9. Borthakur Mar SK, Mukharjee SN. Gastrointestinal helminthes in stray cats (*Felis catus*) from Aizawl, Mizoram, India. *Southeast A.sian J. Trop Med Public Health*.2011;42(2):255-8
10. Bwalya EC, Nalubamba KS, Hankanga C, Namangala B. Prevalence of canine gastrointestinal helminths in urban Lusaka and rural Katete Districts of Zambia. *Prev Vet Med.*, 2011; 100: 252-5.
11. Khalafalla RE. A survey study on gastrointestinal parasites of stray cats in northern region of Nile delta, Egypt. *PLo One*. 2011;6(7):e20283. Epub 2011 Jul 8.
12. Othman RA. Prevalence of *Toxocara canis* in Dogs, North West Bank of Palestine. *Korean J Parasitol*. 2011;49(2):181-2.
13. Cantó GJ, García MP, García A, Guerrero MJ, Mosqueda J. The prevalence and abundance of helminth parasites in stray dogs from the city of Queretaro in central Mexico. *J Helminthol*. 2011;85(3):263-9.
14. Esmailzadeh M, Shamsfard M, Kazemi, A. Khalafi, S.A. Altome, S.A. Prevalence of protozoa and gastrointestinal helminthes in stray cats in Zanzan Province, north-west of Iran, *Iranian Journal of Parasitology*. 2009;4(3) : 71-75
15. Arbabi M, Hooshyar H. Gastrointestinal parasites of stray cats in Kashan, Iran . *Trop Biomed*. 2009;26(1):16-22.
16. Mohammad Z, Seyed M.S. Bahador S. Prevalence of *Toxocara cati* and other intestinal helminthes in stray cats in Shiraz, Iran. *Trop Biomed*. 2007;24(2):39-43.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



17. Daryani A, Sharif M, Amouei A, and Gholami S. Prevalence of *Toxocara canis* in Stray Dogs, Northern Iran. Pak J Biol Sci. 2009 Jul 15;12(14):1031-5.
18. Sharif M, Nasrolahei M, Ziapour SP, Gholami S, Ziaei H, Daryani A, Khalilian A. *Toxocara cati* infections in stray cats in northern Iran. J Helminthol. 2007;81(1):63-6.
19. Paquet Durand I, Hernandez J, Dolz G, Romere Zuniga J, Schnieder T, Epe C. Prevalence of *Toxocara* spp. *Toxascaris leonina* and *ancylostomidae* in public Parks and beaches in different climate zones of costa rica. Acta tropica, 2007; 104: 30-7.
20. Mizgajski-Wiktor H. Recommended method for recovery of *Toxocara* and other geohelminth eggs from soil. Wiad Parazytol. 2005;51(1):21-2. Review.
21. Akhlaghi L, Ourmazdi H, Sarafnia A, Vaziri S, Jadidian K, Z. Leghaii. An Investigation on the Toxocariasis Seroprevalence in Children (2-12 Years Old) from Mahidasht Area of Kermanshah Province (2003-2004). The Razi Journal of Medical Sciences 2006;52(13): 41-48
22. Alavi SM , Sefidgaran G. Frequency of anti *Toxocara* antibodies in school children with chronic cough and eosinophilia in Ahwaz, Iran. Pak J Med Sci 2008 (Part-II) Vol. 24 No. 3 360-363
23. Nasiri V, Esmailnia K, Karim G, Nasir M, Akhavan O. Intestinal parasitic infections among inhabitants of Karaj City, Tehran province, Iran in 2006-2008. Korean J Parasitol. 2009;47(3):265-
24. Sadjjadi SM, Khosrav M, Mehraban D, Orya A Seroprevalence of *Toxocara* infection in school children in Shiraz southern Iran. J Trop Pediat 2000;46(6):327-33
25. Nourian AA, Amiri M, Ataeian A, Haniloo A, Mosavinasab SN, Badali H. Seroepidemiological study for toxocariasis among children in Zanjan-northwest of Iran. Pak J Biol Sci. 2008;11(14):1844-7.
26. Fallah M, Azimi A, Taherkhani . Seroprevalence of toxocariasis in children aged 1-9 years in western Islamic Republic of Iran, East Mediterr Health J. 2007;13(5):1073-7.
27. Rokicki J, Kucharska AP, Dzido J, Karczewska D. Contamination of playgrounds in Gdańsk city with parasite eggs. Wiad Parazytol, 2007; 53(3): 227-30.
28. Paul AJ, Todd KSJr, Dipietro JA. Environmental contamination by eggs of *Toxocara* species. Vet Parasitol, 1988; 26: 339-42.
29. Devera R, Blanco Y, Hernández H, Simoes D. *Toxocara* spp. and other helminths in squares and parks of Ciudad Bolívar, Bolivar State (Venezuela). Enferm Infecc Microbiol Clin, 2008; 26: 23-6.
30. Alonso JM, Stein M, Chamorro MC, Bojanich MV. Contamination of soils with eggs of *Toxocara* in a subtropical city in Argentina. J Helminthol. 2001; 75: 165-8.
31. Avcioglu H, Burgu A. Seasonal prevalence of *Toxocara* ova in soil samples from public parks in Ankara, Turkey. Vector Borne Zoonotic Dis, 2008; 8: 345-50.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



32. Perec-Matysiak A, Hildebrand J, Zaleśny G, Okulewicz A, Fatuła A., The evaluation of soil contamination with geohelminth eggs in the area of Wrocław, Poland, *Wiad Parazytol.* 2008;54(4):319-23 37.
33. Mizgajska H, Jarosz W, Rejmenciak A., Distribution of sources of *Toxocara* spp. Infection in urban and rural environments in Poland, *Wiad Parazytol.* 2008;54(4):319-23.
34. Ruiz de Ybáñez MR, Garijo MM, Alonso FD. Prevalence and viability of eggs of *Toxocara* spp. And *Toxascaris leonine* in public parks in eastern Spain. *J Helminthol.* 2001; 75(2): 169-73.
35. Fonrouge R, Guardis MV, Radman NE, Archelli SM., Soil contamination with *Toxocara* sp. Eggs in squares and public places from the city of La Plata. Buenos Aires, Argentina, *Bol Chil Parasitol.* 2000; 55(3-4):83-5.
36. Jarosz W., Soil contamination with *Toxocara* spp. Eggs in the Elblag area, *Wiad Parazytol.* 2001;47(1):143-9
37. Rokicki J, Kucharska AP, Dzido J, Karczewska D., Contamination of playgrounds in Gdańsk city with parasite eggs, Article in Polish, *Wiad Parazytol.* 2007;53(3):227-30.
38. Grygierczyk D, Kwiatkowski S, Sodowska H. Soil contamination with *Toxocara* spp. Eggs in the Katowice area and its environs]. *Wiad Parazytol.* 2003;49(1):57-60
39. Mizgajska H. Soil contamination with *Toxocara* spp. Eggs in the Kraków area and two nearby villages. *Wiad Parazytol.* 2000;46(1):10510.
40. Maikai BV, Umoh JU, Ajanusi OJ, Ajogi I. Public health implications of soil contaminated with helminth eggs in the metropolis of Kaduna, Nigeria. *J Helminthol.* 2008;82(2):113-8.
41. Gillespie S.H, Pereira M, and Ramsay A. The prevalence of *Toxocara canis* ova in soil samples from parks and gardens in the London area. *Public Health.* 1991;105(4):335-9
42. A.J. Paul, K.S. Todd Jr. and J.A. Dipietro. Environmental contamination by eggs of *Toxocara* species. *Vet Parasitol.* 1988 ;26(34):339-42.
43. Zibaei M, Abdollahpour F, Birjandi M, Firoozeh F. Soil contamination with *Toxocara* spp. Eggs in the public parks from three areas of Khorram Abad, Iran. *Nepal Med. Coll. J.*, 2010; 12: 63-65.
44. Tavasouli M, Hadian M, Charehsaz S, Javadi S. *Toxocara* spp. eggs in public parks of urmia city, west Azerbaijan province Iran. *Iran. J. Parasitol.*, 2008; 3: 24-29.
45. Motazedian H, Mehrabani D, Tabatabaee SH, Pakniat A, Tavalali M. Prevalence of helminth ova in soil samples from public places in Shiraz. *East. Mediterr. Health J.*, 2006; 12: 562-565.
46. Zakilou, Mona; Tavazezi, Yaser; Saraei, Sahneh-Saraei, Mehrzad; Shahnazi, Mojtaba; Jahani Hashemi, Hassan. Investigation of contamination of public parks in Qazvin city with *Toxocara* eggs in 2010. Qazvin University of Medical Sciences, Faculty of Health and Paramedical Sciences, Final report of research project, 2011



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



47. Nematollahi, Ahmad; Shahbazi, Parisa; Nasrollahi, Dizaj-Yegan, Nazila. Investigation of soil contamination of public parks in Tabriz city with eggs of different species of *Toxocara* and its relationship with toxocariasis, Journal of Tabriz Veterinary Faculty, Volume 1, July 2014: 11-16.
48. Ghorbani, Ranjbari, Ali; Shafiei, Amir Hossein; Enamipour, Ali; Naji, Shahriar. Abundance of *Toxocara* eggs in public parks of Shiraz Journal of Kerman University of Medical Sciences, Volume 21, No. 2, 2014: 174-179.
49. Ghareh Daghi, Yaghoub; Shabestari Asl, Ali. Infection rate with *Toxocara* species eggs in public parks of Tabriz, Journal of Rafsanjan University of Medical Sciences, Volume 11, June-July 2012: 173-178.
50. Khazaei, Mohsen. Investigation of the prevalence of *Toxocara* species eggs in public parks of Tehran in 2008, Parasitology Thesis, Faculty of Medicine, Shahid Beheshti University of Medical Sciences and Health Services, 2008.
51. Bojanich MV, Alonso JM, Caraballo NA, Itatí Schöller M, López Mde L, García LM, Basualdo JÁ. Assessment of the presence of *Toxocara* eggs in soils of an arid area in central-western Argentina. Rev Inst Med Trop Sao Paulo 2015; 57(1): 73-76
52. Yakhchali.M,Ebne adam,A. Study on the Contamination of *Toxocara canis* Eggs (Ascaridida: Ascarididae) in Dogs and Public Park Soils of Piranshahr County (West Azerbaijan Province), Journal of Veterinary Research, Vol. 69, No. 4, Year 2014
53. Sharif Maraghi, Komeil Mazhab Jafari, Study on the contamination of Abadan public parks soil with *Toxocara* spp. Eggs. J Environ Health Sci Eng. 2014 May 19;12:86.



Comparative Frequency of *Varroa*, *Nosema*, and *Acarapis* Infection in Honeybee (*Apis mellifera*) Colonies of Khorasan Razavi Province: A Two-Year Study

Bahareh Meftahi¹, Samaneh Rezaei¹, Mohammad Reza Shariati², Gholamreza Razmi¹

Corresponding Author: Gholamreza Razmi, razmi@um.ac.ir

1.Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

2.Honeybee and Silkworm Diseases Surveillance Section, Veterinary Head Office of Khorasan Razavi, Iran Veterinary Organization, Mashhad, Iran.

Article code: 1646

Abstract

Background: Parasitic diseases remain one of the most critical threats to honeybee health and productivity worldwide. These infestations including *Varroa* spp., *Nosema* spp., and *Acarapis woodi* represent major challenges to honeybee colony health, especially under environmental stress such as drought and poor nutrition in Khorasan Razavi Province. These investigations have been done with a focus on epidemiological patterns, possible contributing factors, and implications for the beekeeping industry's value chain, including honey and other high-value products such as royal jelly, propolis, beeswax, and pollen. This study compares the prevalence of these parasites between two consecutive years and examines implications for apiary productivity and honey-chain value.

Methods: A cross-sectional field study was conducted from April to August over two consecutive years 2024–2025. Standard diagnostic protocols, including soapy water wash tests for *Varroa*, homogenized abdominal microscopic smear for *Nosema*, and tracheal dissection for *Acarapis*, were applied. Sampling limitations and operator-related variability were considered in interpreting results.

Results: In 2024, examination of approximately 150 colonies from 49 apiaries revealed infestation rates of 31% for *Varroa* spp., 35% for *Nosema* spp., while no infestation with *Acarapis woodi* was detected. In 2025, from 154 colonies across 42 apiaries, infestation rates decreased to 16.7% for both *Varroa* and *Nosema*, with *Acarapis* remaining absent. *Chi-square* analysis indicated that these reductions were not statistically significant for either *Varroa* ($p = 0.12$) or *Nosema* ($p = 0.14$), suggesting that the observed differences may be due to random variation. Such variations could be partially explained by improved colony management practices, environmental factors, or potential sampling biases.

Conclusion: Observed reductions in pathogen prevalence were not statistically significant, highlighting the limited impact of current management. Continued surveillance, integrated pest



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



management, and coordinated action among research, industry, and regulatory stakeholders are vital to preserving colony health and economic viability across honey-chain products.

Keywords: *Nosema*, *Varroa*, *Acarapis*, *Apis mellifera*, Khorasan Razavi Province.

Introduction:

Honeybees (*Apis mellifera*) are critical in pollination and production of high-value apicultural commodities—honey, royal jelly, propolis, beeswax, and pollen. Among global threats to colony health, parasitic stressors, including *Varroa spp.* and *Nosema spp.*, are the most significant, often compounded by environmental instabilities such as drought and nutritional deficiencies (1, 2). In Iran's Khorasan Razavi, an important beekeeping region, apiaries face chronic drought, reduced forage quality, and enhanced disease risk. Nosemosis, the disease caused by *Nosema spp.* is one of the most destructive diseases of adult honeybees around the world (3). The causative agents are unicellular microsporidian parasites of the genus *Nosema*, with *Nosema ceranae* being the most prevalent species in Iran (3). *Varroa* is considered to be the major pest of honey bees since it spread to *A. mellifera* (4). *Varroa* mites (*Varroa spp.*) reproduce in the cells of developing honey bees (*Apis spp.*). They feed on the haemolymph of developing and adult bees, resulting in the transmission of secondary diseases that reduce the lifespan of infested individuals (4-6). The honey bee tracheal mites (HBTM) *Acarapis woodi* live inside the tracheae and air sacs of adult bees (5, 6). This study evaluates the epidemiological shifts in parasite prevalence over two consecutive seasons and discusses the consequences for industry productivity and economic resilience.

Materials and Methods:

Study Design: Cross-sectional field surveys were executed April to August in 2024 (49 apiaries, about 150 colonies) and 2025 (42 apiaries, 154 colonies).

Diagnostics: *Varroa spp.* with soapy water wash method (at least 50 bees/sample) and post-treatment mite drop counting. For *Nosema spp.* Diagnosing homogenized abdominal microscopic smear and microscopic spore counts have been done. (20 bees/sample). To *Acarapis woodi* detection Tracheal dissection (5 bees/sample) under microscopy after clarification step with KOH 10% solution. Statistical analyses were performed using the *Chi-square* test for independence, with a significance threshold of $p < 0.05$.

Results and Discussion:

The results of the both two years have been obtained and are presented in detail in the following sections for clarity and comprehensive interpretation.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 3. Comparative frequency of *Varroa* spp, *Nosema* spp and *Acarapis woodi* in Honeybee Apiaries of Khorasan Razavi Province.

Species	Frequency		P-value*
	2024 (49 apiaries)	2025 (42 apiaries)	
<i>Varroa</i> spp.	31%	16.7%	0.12
<i>Nosema</i> spp.	35%	16.7%	0.14
<i>Acarapis woodi</i>	0%	0%	nd**

* P-values (>0.05) showed no significant difference between the two years.

** not done.

The observed reduction in the prevalence of *Varroa* spp. and *Nosema* spp. in Khorasan Razavi Province, although statistically non-significant, may indicate limited effectiveness and suboptimal implementation of apiary management and preventive measures. This suggests that, despite the application of certain interventions, the overall efficiency of control strategies remains insufficient to achieve measurable epidemiological impact. Such findings underscore the need for more rigorous, standardized, and sustained application of best practices in Integrated Pest Management (IPM) (7), including periodic colony monitoring, timely and judicious use of acaricides, improved hive hygiene, and brood management.

While these interventions aim to suppress parasite populations and mitigate secondary impacts, such as viral transmission and opportunistic infections (4), the lack of statistical significance also implies that random variation, sampling effects, and persistent regional risk factors cannot be excluded. Notably, the prevailing arid climate, recurrent drought conditions, elevated temperatures, and limited floral resources in Khorasan Razavi create an ecologically favorable environment for the persistence and endemic establishment of these parasites. This endemicity, coupled with environmental and biological stressors, compromises brood development and colony immunity, thereby sustaining susceptibility to *Nosema* spp. and other opportunistic pathogens (8).

Periodic colony monitoring remains a cornerstone preventive measure. Regular diagnostic sampling facilitates targeted management decisions, enables timely therapeutic interventions, and allows for efficient acaricide application while minimizing unnecessary chemical exposure (9). From a scientific and industry-oriented perspective, sustainable control of endemic parasites in such climates requires a system-based approach integrating nutritional supplementation, environmental enrichment, and strategic brood manipulation. These



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



measures can reduce pathogen transmission, enhance colony immune competence, and preserve high production traits. Adaptive management, guided by continuous monitoring and region-specific research feedback, ensures that interventions remain responsive to evolving environmental, epidemiological, and economic conditions.

Although reductions in the prevalence of *Varroa spp.* and *Nosema spp.* were observed, these changes were not statistically significant, indicating that current control strategies confer only a partial mitigation effect. Persistent environmental stressors, the endemic presence of parasites, co-infections, and nutritional limitations necessitate continuous monitoring and the adoption of adaptive, climate-appropriate management practices. Enhancing periodic surveillance, implementing targeted and evidence-based interventions, and fostering coordinated action among academic research centers, apicultural industry stakeholders, and governmental regulatory agencies are critical for sustaining colony health and optimizing apicultural productivity. The integration of rigorous scientific insights with practical field management approaches will enable the development of resilient, economically viable, and sustainable apicultural systems that advance both research and production outcomes.

Acknowledgment:

We express our sincere gratitude to the “Honeybee and Silkworm Diseases Surveillance Section of the Veterinary Head Office of Khorasan Razavi” for providing access to their apiaries, and to “Rahavard Takin Tose Sanat Company (RTTS Co.)” for coordinating and facilitating this collaborative study.

References:

1. Mazur ED, Gajda AM. Nosemosis in honeybees: a review guide on biology and diagnostic methods. *Applied Sciences*. 2022;12(12):5890.
2. Fowler AE, Irwin RE, Adler LS. Parasite defense mechanisms in bees: Behavior, immunity, antimicrobials, and symbionts. *Emerging Topics in Life Sciences*. 2020;4(1):59-76.
3. Meftahi B, Yaghfoori S, Mosazadeh S, Sheibanitezerji R, Fakhrabadipour M, Javdan E, et al. Parasitological and molecular study of nosemosis in migratory apiaries in Hormozgan Province, southern Iran. *Journal of Entomological Society of Iran*. 2023;43(4):383-92.
4. Dietemann V, Pflugfelder J, Anderson D, Charrière J-D, Chejanovsky N, Dainat B, et al. *Varroa destructor*: research avenues towards sustainable control. *Journal of Apicultural Research*. 2012;51.۱۲۵-۳۲:(۱)
5. Sammataro D, Gerson U, Needham G. Parasitic mites of honey bees: life history, implications, and impact. *Annual review of entomology*. 2000;45(1):519-48.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



6. Nekoei S, Rezvan M, Khamesipour F, Mayack C, Molento MB, Revainera PD. A systematic review of honey bee (*Apis mellifera*, Linnaeus, 1758) infections and available treatment options. *Veterinary Medicine and Science*. 2023;9(4):1848-60.
7. Castellanos-Potenciano BP, Vivas Rodríguez JA, Ramos-Hernández E. Factors that limit the productive use of *Apis mellifera* L.—a review. *International Journal of Tropical Insect Science*. 2024;44(1):1-12.
8. Beutler B. Innate immunity: an overview. *Molecular immunology*. 2004;40(12):845-59.
9. Kosch Y, Mülling C, Emmerich IU. Assessment of Resistance of *Varroa destructor* to Formic and Lactic Acid Treatment—A Systematic Review. *Veterinary Sciences*. 2025;12(2):144.



Post Anticoccidial Growth Promotors Era: Coccidiosis in Iranian Poultry Production over the Last Ten Years

Fatemeh Arabkhazaeli¹

Corresponding Author: Fatemeh Arabkhazaeli, farab@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1647

Abstract

Background: Coccidiosis caused by *Eimeria* spp. remains the most economically significant parasitic disease in the poultry industry. The average annual loss from coccidiosis is estimated to be billions of dollars.

Methods: In this article, the updated data on species prevalence, field control interventions, and recent advances in poultry coccidiosis prevention methods in Iran are summarized.

Results: Species prevalence data show alteration and existence of pathogenic parasites across the country. Reduction in the anticoccidial efficacy rate has been proven, which compels alternative prevention and treatment strategies. In this regard, integrating immunization with innovative feed additives, including phytogenics, prebiotics, probiotics, and organic acids, is a remarkable emergent trend. These synergies have been well explored in Iranian research, which minimizes vaccination reactions and boosts innate immunity by promoting gut health with promising outcomes. Besides anticoccidial vaccines, live or recombinant coccidiosis vaccines are being developed locally.

Conclusion: Presence and alteration in species distribution, emergence of anticoccidial resistance, besides the great research potential in the country are evident. There is ongoing research in Iran on poultry coccidiosis, which warrants more effective regional disease control strategies.

Keywords: Avian Coccidiosis, *Eimeria*, Antimicrobial Resistance, Vaccine Development, Phytotherapy.

Introduction:

Poultry production is a main part of food security in every country, for it provides the primary source of animal protein via meat and eggs. In Iran, the poultry industry is a major agricultural sector, ensuring economic stability. Though the intensive modern poultry industry exacerbates the spread of infectious diseases, including avian coccidiosis. Poultry coccidiosis is a disease caused by protozoan parasites of the genus *Eimeria*. The mucosal damage and malabsorption,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



impaired growth, lethargy, bloody diarrhea, and, in severe cases, high mortality result in extensive economic loss. Chemoprophylaxis is the main anticoccidial control strategy; however, the global shift by public concerns over antimicrobial resistance has led to the elimination of these compounds in food animals. The advent of *Eimeria* precocious oocysts and the introduction of prophylactic ionophores in late 19th, were the valuable steps in coccidiosis control (1). Besides the emergence of anticoccidial resistance in *Eimeria* (2) has compelled alternative prevention strategies. Vaccination, along with the introduction of plant extracts as anticoccidial feed additives, has been pursued by Iranian scientists to overcome the above-mentioned challenges in poultry coccidiosis (3,4). This review summarizes the coccidiosis control and prevention methodologies in Iranian poultry production over the last decade (2014-2024).

Materials and Methods:

Data on *Eimeria* species diversity have been collected from available published epidemiological studies conducted across the country. The vaccine research in Iran is compiled via reviewing published studies, including efficacy evaluation in controlled experimental challenges, measuring different infection parameters e.g., oocyst per gram of feces, lesion scores, weight gain, and feed conversion ratio.

Results and Discussion:

Recorded history on the poultry coccidiosis in Iran is not available in detail but it seems that from mid-20th, the researchers had worked on identifying different *Eimeria* species from varying hosts. Various epidemiological studies in many parts of Iran confirmed the presence of at least seven pathogenic *Eimeria* species in poultry, with the three most prevalent species including *E. acervulina*, *E. maxima*, and *E. tenella* with varying regional prevalence and abundance. The presence of at least seven pathogenic *Eimeria* species has been proven morphologically and molecularly (5,6). The prevalence of coccidiosis infection was reported 30%-75% in different studies and climatic characteristics, intensity and age of the flocks were identified as the risk factors for the infection rate. The use of live anticoccidial vaccines has surpassed antibiotic growth promoters as the main method for coccidiosis control. So far, the use of imported live vaccines (both attenuated and non-attenuated strains) has become popular, especially in breeder and layer flocks. This method has been beneficial for managing drug-resistant and restoring intestinal health. However, the industry has faced practical challenges with these vaccines, including cost troubles, application challenges, and variable efficacy rates (7,8). These challenges have led to developing local vaccine solutions, including isolating the most prevalent and pathogenic *Eimeria* species (*E. tenella*, *E. acervulina*, *E. maxima*) from



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Iranian poultry farms and developing attenuated strains through repeated passage in embryos or cell culture with the aim of creating an effective and cost-competitive vaccine against the specific strains circulating within the country. The geographical heterogeneity of *Eimeria* species represents a challenge interfering with vaccine immunity, as strains isolated in one region may not provide cross-protection against genetically distinct field strains in another region (9). Investing in next-generation vaccine technology with identification and cloning of immunogenic antigens from *Eimeria* and evaluation of recombinant subunit vaccines is ongoing, though still in the experimental and preclinical phases (3,10).

Bioactive compounds derived from herbs, spices, and their extracts have gained significant attention worldwide and among Iran's poultry science research community. The investigation of local medicinal plants offers a promising outcome on gut health, thus reducing the impact of coccidia infections. A variety of native plants, including thyme, oregano, garlic, pomegranate peel, and etc., are evaluated. Anti-inflammatory and antioxidant, and immunomodulatory synergistic effects with vaccines and described as the action mechanism. While most studies were conducted at the experimental level, the results are promising, granting oocyst shedding and lesion severity reductions, resulting in improvements of growth performance in infected birds (4,11,12). The plant active ingredients and mechanism of action have been proved to be direct with inhibiting sporogony or schizogony or indirect by altering the gut health, immunity or microbiota (13). Large scale commercial application of these formulations have not yet been achieved because of the lack of methods for extract standardization, determining optimal dosages, and ensuring stability and efficacy in feed which warrants further research.

Vaccination as a pivotal advancement in control of poultry coccidiosis, started by the use of live attenuated or non-attenuated oocysts. Although they are efficacious in developing protective immune responses, the reduced productivity rate of the attenuated oocysts along with the high resource-demanding production process, has led to investigation of next-generation vaccines including subunit and recombinant vaccines based on immunogenic *Eimeria* antigens. Different immunogenic antigens including the microneme, and apical membrane antigens along with various adjuvants via different administration routes are being evaluated by researchers worldwide (14). In Iran growing number of studies are investigating recombinant vaccine candidates including the successful use of recombinant bivalent protein in reducing necrotic enteritis and coccidiosis in experimental challenge (3). Currently technologies like gene editing and transgenetics are under investigation to develop more potent coccidiosis vaccines (15). Meanwhile the research on development of new vaccines are underway, the applicable control strategy in the field remains to be biosecurity/safety management in the poultry farm, enhancing the bird's gut health by means of pre/probiotics and phytochemicals and frequent use of anticoccidials or available vaccines.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



In conclusion, while the poultry industry in Iran is currently dependent on imported live vaccines, the national strategy should be to promote local vaccine development. Therefore, ongoing nationwide surveillance via molecular methods is inevitable for creating a dynamic map of circulating species and strains to be used in the development and production of recombinant vaccines based on strains and/or antigens that are prevalent within the Iranian poultry population. This foundational epidemiological data is critical for designing vaccines with optimal efficacy and broad coverage against local, potentially divergent, *Eimeria* field strains. Concurrently, investment in research and infrastructure for domestic antigen discovery, adjuvant development and exploitation of newer technologies is essential to achieve vaccine self-sufficiency. Ultimately, such a strategic shift will not only enhance biosecurity and reduce economic losses but also ensure the long-term sustainability and resilience of Iran's poultry sector against the persistent challenge of coccidiosis.

References:

1. Gao Y, Sun P, Hu D, Tang X, Zhang S, Shi F, Yan X, Yan W, Shi T, Wang S, Zou J. Advancements in understanding chicken coccidiosis: from *Eimeria* biology to innovative control strategies. *One Health Advances*. 2024 Feb 27;2(1):6.
2. Arabkhazaeli F, Modirsanei M, Nabian S, Mansoori B, Madani A. Evaluating the resistance of *Eimeria* spp. field isolates to anticoccidial drugs using three different indices. *Iranian journal of parasitology*. 2013 Apr;8(2):234.
3. Fatemi Motlagh M, Mousavi Gargari SL. A bivalent vaccine against avian necrotic enteritis and coccidiosis. *Journal of Applied Microbiology*. 2022 Jan 1;132(1):113-25.
4. Shahininejad H, Rahimi S, Torshizi MA, Arabkhazaeli F, Ayyari M, Behnamifar A, Abuali M, Grimes J. Comparing the effect of phytobiotic, coccidiostat, toltrazuril, and vaccine on the prevention and treatment of coccidiosis in broilers. *Poultry Science*. 2024 May 1;103(5):103596.
5. Arabkhazaeli F, Nabian S, Modirsaneii M, Mansoori B, Rahbari S. Biopathologic characterization of three mixed poultry *Eimeria* spp. isolates. *Iranian journal of parasitology*. 2011 Dec;6(4):23.
6. Nasiri V, Jameie F, Morovati Khamsi H. Detection, identification, and characterization of *Eimeria* spp. from commercial chicken farms in different parts of Iran by morphometrical and molecular techniques. *Acta Parasitologica*. 2024 Mar;69(1):854-64.
7. Arabkhazaeli, F., Nabian, S., Modirsanei, M., Madani, S. A. The efficacy of a poultry commercial anticoccidial vaccine in experimental challenge with *Eimeria* field isolates. *Iranian Journal of Veterinary Medicine*, 2015; 8(4): 249-253. doi: 10.22059/ijvm.2015.52483



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



8. Nabian, S., Arabkhazaeli, F., Kefayati, M. H., Modirsanei, M. Analysis of humoral immunity in broilers challenged with *Eimeria* prior to and following anticoccidial vaccination, by means of ELISA. *Journal of Veterinary Research*, 2015; 70(3): 279-284. doi: 10.22059/jvr.2015.55271
9. Sobhani E, Arabkhazaeli F, Madani SA. Intraspecific Variations in Biology and Pathogenesis of Two *Eimeria maxima* Isolates from Distinct Geographic Locations. *Veterinary Medicine and Science*. 2025 May;11(3):e70235.
10. Shamshirgaran MA, Golchin M, Mohammadi E. *Lactobacillus casei* displaying *Clostridium perfringens* NetB antigen protects chickens against necrotic enteritis. *Applied Microbiology and Biotechnology*. 2022 Oct;106(19):6441-53.
11. Ghaniei A, Tohidi E, Vafaei A. Efficacy of a commercial mixed botanical formula in treatment and control of coccidiosis in poultry. *Veterinarski arhiv*. 2022;92(6):723-34.
12. Ghafouri SA, Ghaniei A, Tamannaie AE, Sadr S, Charbgoos A, Ghiassi S, Abuali M. Evaluation of therapeutic effects of an herbal mixture (*Echinacea purpurea* and *Glycyrrhiza glabra*) for treatment of clinical coccidiosis in broilers. *Veterinary Medicine and Science*. 2023 Mar;9(2):829-36.
13. Chen P, Liu K, Yue T, Lu Y, Li S, Jian F, Huang S. Plants, plant-derived compounds, probiotics, and postbiotics as green agents to fight against poultry coccidiosis: A review. *Animal Research and One Health*. 2024.
14. Zhao D, Suo J, Liang L, Liang R, Zhou R, Ding J, Liu X, Suo X, Zhang S, Tang X. Innovative prevention and control of coccidiosis: targeting sporogony for new control agent development. *Poultry Science*. 2024 Dec 1;103(12):104246.
15. Gao Y, Sun P, Hu D, Tang X, Zhang S, Shi F, Yan X, Yan W, Shi T, Wang S, Zou J. Advancements in understanding chicken coccidiosis: from *Eimeria* biology to innovative control strategies. *One Health Advances*. 2024 Feb 27;2(1):6.



Molecular Mimicry as a Common Factor in the Immune Response of Rheumatic Fever and Toxoplasmosis

Ahmed Saadi Khalaf, Teeba Faris Hadi, Ghazwan Talal Mahdi

Corresponding Author: Ahmed Saadi khalaf, Ahmed.s@ige.uobaghdad.edu.iq

1. Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq.

Article code: 1653

Abstract

This review explores the role of molecular mimicry as a central mechanism linking infections to autoimmune responses in both acute rheumatic fever (ARF) and *Toxoplasma gondii* (T. gondii) infection. ARF is an autoimmune inflammatory disease triggered by untreated infection with *Streptococcus pyogenes* (group A streptococcus, GAS). Molecular mimicry leads to the production of antibodies and T cells that cross-react with host proteins, particularly in the heart, joints, skin, and nervous system. This results in migratory polyarthritides, carditis, and valvular heart damage, with M-protein, hyaluronic acid capsule, lipoteichoic acid, and other streptococcal toxins playing key roles in immune activation and tissue injury. Similarly, T. gondii, an obligate intracellular protozoan, can trigger autoimmune responses through molecular mimicry and phenotypic spread. Parasite antigens cross-react with host proteins, activating auto reactive T cells and enhancing antigen presentation, which contributes to autoimmune pathology. Infection outcomes vary depending on immune status, ranging from asymptomatic latent infections to severe disease in immunocompromised individuals.

Introduction:

Acute rheumatic fever (ARF) is an autoimmune inflammatory disease resulting from untreated infection with *Streptococcus pyogenes* (group A streptococcus, GAS), which causes streptococcal pharyngitis or scarlet fever. ARF primarily affects children between the ages of 5 and 15 years, although younger children and adults may also be susceptible. Long-term complications include valvular heart disease and heart failure (1). Molecular mimicry is a major mechanism in ARF, whereby antibodies or T cells generated against microbial antigens interact with host proteins, leading to autoimmune damage (2,4). Similarly, *Toxoplasma gondii* (T. gondii), an intracellular protozoan parasite, can induce autoimmune responses through molecular mimicry and phenotypic spread (6,9). Both diseases demonstrate how infection can trigger cross-reactive immune responses that damage host tissues (1). These organisms produce enzymes that enable them to damage and infiltrate human tissue after adhering to epithelial cells of the upper respiratory tract. After an incubation period of two to four days, these invaders cause an acute inflammatory reaction lasting three to five days, characterized by sore



throat, fever, malaise, headache, and leukocytosis. Infection causes rheumatic fever in 3% of patients. Rheumatic fever begins approximately two weeks after infection with *Streptococcus pyogenes* (3). Molecular mimicry may be a cause of rheumatic carditis, both of which are streptococcal outcomes (4). GAS infection in susceptible individuals produces antibodies and T cells capable of recognizing host antigens in the skin, joints, brain, and heart (6,7,8). As a result, patients develop metastatic polyarthritis resulting from the deposition and development of immune complexes. Rheumatic fever usually manifests as polyarthritis. However, the heart muscle and heart valves become inflamed as a result of the binding of auto reactive antibodies and T cells to various cardiac antigens. (1)

1.1 Etiology

Streptococcus pyogenes is a Gram-positive, beta-hemolytic cocci that grows in chains or pairs. GAS is classified into 20 serotypes based on cell wall carbohydrate composition and is the only known bacterial cause of ARF/RHD (1,2,5). Untreated GAS infection induces host immune responses, which may cross-react with self-antigens in the heart, joints, skin, and nervous system, especially in genetically susceptible individuals (11,12). GAS causes a variety of human diseases. The spectrum of diseases includes life-threatening conditions such as myositis, puerperal sepsis, and toxic shock syndrome, as well as relatively minor clinical conditions, including pharyngitis, cellulitis, and impetigo. Acute rheumatic fever and rheumatic heart disease are two post-infectious outcomes that may result from *Streptococcus pyogenes* infection (13, 4). Within 10–14 days of infection, streptococcal skin or throat infections may progress to disabling chronic glomerulonephritis (13). Strains of *Streptococcus nephritidis* produce immune complexes that are detected in the circulation and deposited in the glomeruli. Local immune complex disease can also result from the independent deposition of antigen and antibody in the glomerular basement membrane and their formation of immune complexes (14, 10).

1.2 Clinical Manifestations

ARF typically develops 2–4 weeks after GAS infection. Initial symptoms include sore throat, fever, malaise, and headache, followed by autoimmune sequelae. Migratory polyarthritis is the most common manifestation, while carditis is the leading cause of morbidity and mortality. Other manifestations include Sydenham chorea, subcutaneous nodules, and erythema marginatum (1,3,6,7,8).

1.3 Virulence factors of GAS M-protein

Collectively Toxins and surface antigens of streptococci play important roles in the formation of infection in a host. A hyaluronic acid capsule that prevents phagocytosis and M-proteins and lipoteichoic acid that aid in attachment are significant virulence factors of GAS (1, 2). other extracellular substances, like pyrogenic toxin, which results in scarlet fever rash (14, 15). Heart myosin, tropomyosin, keratin, vimentin, laminin, collagen, and other α -helical

coiled-coil human proteins exhibit structural similarities with M-proteins, which are fibrous coiled-coil dimers of α -helices that extend from the surface of GAS (15,16). The streptococci's surface produces M-proteins, which are similar to human heart proteins in sequence (1). There is little clinical data to suggest that these anti-M-protein antibodies play a role in the etiology of illness. Nonetheless, it has been demonstrated that antibodies isolated from the sera of ARF/RHD patients react with host cardiac myosin as well as M-proteins (2, 17).

1.4 Pathophysiology

There is still much to learn about the pathogenic mechanisms of ARF. It seems that a host with a genetic predisposition to the disease must contract a pharyngeal infection from *S. pyogenes* for ARF to arise (18,19). When *S. pyogenes* antigens are presented to T and B cells during a pharyngeal infection, the innate immune system is activated. Pathogenesis In group A streptococcal infections, T (CD4+) cells are activated, and B cells produce specific IgG and IgM antibodies (2). Tissue damage is caused by an immune-mediated pathway that is started by molecular mimicry (20). Cross-activation of antibodies and/or T cells against human proteins occurs when the infectious agent and human proteins share structural similarities (21). Due to antibody binding and T cell infiltration, this cross-reactive immunological response in ARF causes the clinical manifestations of rheumatic fever, including carditis; Figure 1 (1)

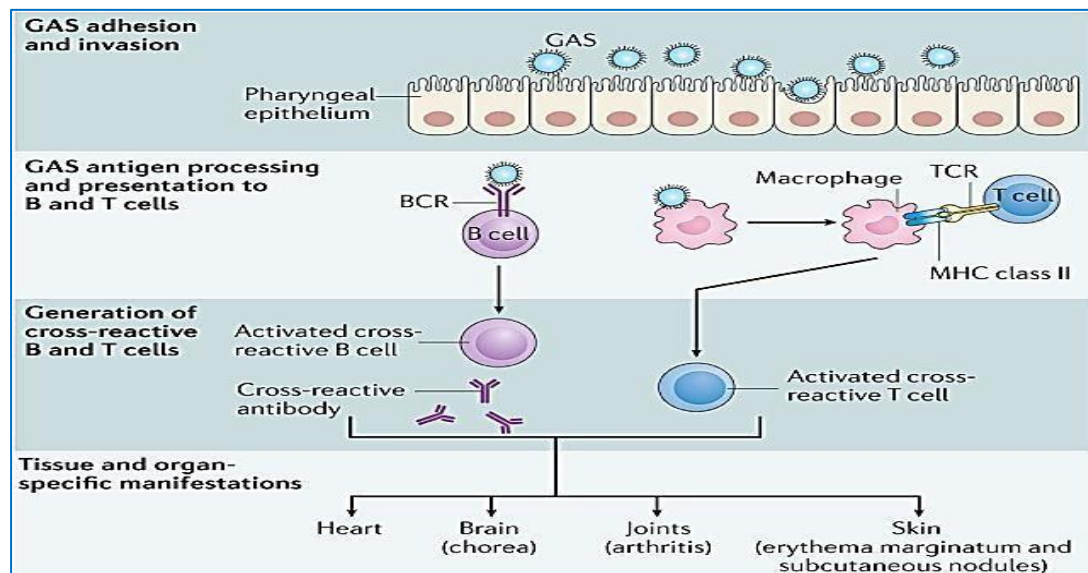


Figure 1. Acute rheumatic fever pathogenesis (group A streptococcus, or GAS, B cell receptor, or BCR, T cell receptor or TCR) (1)



1.5 Molecular mimicry

The development of ARF/RHD is thought to be caused by molecular mimicry, which is a natural immune response that is started between streptococcal M-proteins and host tissue proteins (16; 22). Microbes and host tissues share antigenic epitopes, a process known as molecular mimicry. The α -helical coiled-coil proteins of the host heart, including cardiac myosin, laminin, vimentin, collagen, tropomyosin, keratin, etc., exhibit structural similarities with streptococcal M-proteins in ARF/RHD and cross-react with them (2, 4, 1, 22). Heart myosin appears to be the primary target of cross-reactivity among the host tissue proteins. M protein and N-acetyl-beta-D-glucosamine, the carbohydrate antigen of *S. pyogenes*, both have alpha-helical protein structures in common. Antibodies against both myosin-containing epitopes and these antigens cross-react with human tissues (23, 4). monoclonal antibodies produced by peripheral blood lymphocytes or tonsillar cells of people infected with In rheumatic fever patients, *S. pyogenes* cross-reacts with myosin, and the isolated N-acetyl-beta-D-glucosamine reacts against the human valvular endothelium (25). Anti-M protein antibodies that cross-reacted with cardiac myosin and vimentin were found in the sera of patients with ARF/RHD in a number of investigations, indicating that these proteins were the target autoantigens identified in the heart (17, 25). Indeed, because cardiac myosin and laminin are similar, GAS antigen-specific T-cells may also be able to identify endothelial basement membrane laminin (23, 26). GAS carbohydrate epitope-induced cross-reactive antibodies against laminin and this extracellular matrix protein of heart valves (4). The endothelium is thought to be activated when the cross-reactive antibodies attach to the cardiac endothelial surface. Vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, which bind to activated T-cells specific to the GAS antigen, are produced and upregulated by the activated endothelia. Transmigration of T cells into the valves and myocardium is facilitated by the binding of adhesion molecules to T cells (1) The crucial step in the development of carditis is the molecular mimicry between GAS and host cardiac antigens (26, 27). There have been reports of antibody cross-reactivity between cardiac myosin and GAS (M protein) (27, 25, 2, 23, 4).

1.6 *Toxoplasma gondii* and molecular mimicry

molecular mimicry involves cross-reactivity between host tissue and the pathogen, phenotypic spread leads to the activation of antigen-presenting cells by the pathogen, resulting in enhanced local presentation of self-antigens, which then activates T cells. (28) Infection may contribute to disease progression through molecular mimicry and phenotypic spread. The increasing prevalence of acute respiratory illness, the resulting disability, and increased medical costs pose a greater economic burden, requiring a deeper understanding of the pathophysiology of these diseases to improve their control consider *T. gondii* is an obligate intracellular protozoan infecting up to one-third of the global population. Its definitive hosts are felids, which shed oocytes into the environment. Intermediate hosts,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



including humans, are infected via ingestion of oocytes. The parasite replicates rapidly in nucleated cells during acute infection, and persists in tissue cysts during latent infection (29,30). In addition to direct pathogenic effects, *T. gondii* may trigger autoimmune responses via: Molecular mimicry: Cross-reactivity between parasite antigens and host proteins activates auto reactive T cells. Phenotypic spread: Activation of antigen-presenting cells enhances presentation of self-antigens, promoting T cell-mediated autoimmunity (31). These mechanisms demonstrate how *T. gondii* can contribute to autoimmune pathology. It has a complex life cycle, with cats and other felids serving as the definitive host. Oocysts excreted in cat feces are disseminated to the environment and infect a wide range of mammals and birds. During the acute phase, the parasite can invade almost all nucleated cell types and replicate rapidly as rapidly growing oocysts, whereas in the chronic phase, it targets the brain and muscles and replicates as slowly growing oocysts within true tissue cysts. The outcome of toxoplasmosis is linked to human immune status: in immunocompetent individuals, *T. gondii* infection is usually asymptomatic and is often followed by latent infection. (32) Latent toxoplasmosis refers to the chronic, asymptomatic form of toxoplasmosis, which accounts for most cases, while severe disease occurs in infants and immunocompromised patients. Furthermore, reactivation of latent toxoplasmosis frequently occurs in immunocompromised patients, leading to uncontrolled parasite replication (33).

Conclusion

Molecular mimicry is a shared immunopathological mechanism in rheumatic fever and toxoplasmosis. It explains how infections by GAS or *T. gondii* can induce cross-reactive immune responses, leading to tissue-specific autoimmune damage. Understanding these mechanisms is critical for developing targeted preventive and therapeutic strategies against post-infectious autoimmune diseases.

References:

1. Carapetis J. R., Beaton A., Cunningham M. W., Guilherme L., Karthikeyan G., Mayosi B. M., et al. Acute rheumatic fever and rheumatic heart disease. *Nature Reviews Disease Primers*. 2016;2:15084. PubMed PMID: 27188830.
2. Cunningham M. W. Rheumatic fever revisited. *Nature Reviews Cardiology*. 2014;11:123. PubMed PMID: 24419260.
3. Gewitz M. H., Baltimore R. S., Tani L. Y., Sable C. A., Shulman S. T., Carapetis J., et al. Revision of the Jones Criteria for the Diagnosis of Acute Rheumatic Fever in the Era of Doppler Echocardiography: A Scientific Statement From the American Heart Association. *Circulation*. 2015;131:1806. PubMed PMID: 25908771.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



4. Cunningham M. W. Pathogenesis of group A streptococcal infections. *Clinical Microbiology Reviews*. 2000;13(3):470–511. PubMed PMID: 10885988.
5. Martin W. J., Steer A. C., Smeesters P. R., Keeble J., Inouye M., Carapetis J., et al. Post-infectious group A streptococcal autoimmune syndromes and the heart. *Autoimmunity Reviews*. 2015;14(8):710–725. PubMed PMID: 25891492.
6. Galvin J. E., Hemric M. E., Cunningham M. W. Cytotoxic mAb from rheumatic carditis recognizes heart valves and laminin. *The Journal of Clinical Investigation*. 2000;106(2):217–224. PubMed PMID: 10903337.
7. Ben-Pazi H., Stoner J. A., Cunningham M. W. Dopamine receptor autoantibodies correlate with symptoms in Sydenham's chorea. *PLoS One*. 2013;8(9):e73516. PubMed PMID: 24073196.
8. Taranta A., Stollerman G. H. The relationship of Sydenham's chorea to infection with group A streptococci. *The American Journal of Medicine*. 1956;20(2):170–175. PubMed PMID: 13282936.
9. Gerber M. A., Baltimore R. S., Eaton C. B., Gewitz M., Rowley A. H., Shulman S. T., et al. Prevention of rheumatic fever and diagnosis and treatment of acute Streptococcal pharyngitis: a scientific statement from the American Heart Association. *Circulation*. 2009;119(11):1541–1551. PubMed PMID: 19246689.
10. Carapetis J., Robins-Browne R., Martin D., Shelby-James T., Hogg G. Increasing severity of invasive group A streptococcal disease in Australia: clinical and molecular epidemiological features and identification of a new virulent M-nontypeable clone. *Clinical Infectious Diseases*. 1995;21(5):1220–1227. PubMed PMID: 8589146.
11. Parnaby M. G., Carapetis J. R. Rheumatic fever in indigenous Australian children. *Journal of Paediatrics and Child Health*. 2010;46(9):527–533. PubMed PMID: 20854325.
12. Bisno A. L. Acute pharyngitis. *The New England Journal of Medicine*. 2001;344(3):205–211. PubMed PMID: 11172144.
13. Nelson R. W., Beisang D., Tubo N. J., Dileepan T., Wiesner D. L., Nielsen K., et al. T cell receptor cross-reactivity between similar foreign and self peptides influences naive cell population size and autoimmunity. *Immunity*. 2015;42(1):95–107. PubMed PMID: 25601203.
14. Singer H. S., Gause C., Morris C., Lopez P.; Tourette Syndrome Study Group. Serial immune markers do not correlate with clinical exacerbations in pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *Pediatrics*. 2008;121(6):1198–1205. PubMed PMID: 18519490.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



15. Guilherme L., Kalil J., Cunningham M. W. Molecular mimicry in the autoimmune pathogenesis of rheumatic heart disease. *Autoimmunity*. 2006;39(1):31–39. PubMed PMID: 16455580.
16. Cunningham M. W., Swerlick R. A. Polyspecificity of antistreptococcal murine monoclonal antibodies and their implications in autoimmunity. *The Journal of Experimental Medicine*. 1986;164(4):998–1012. PubMed PMID: 3531385.
17. Roberts S., Kosanke S., Terrence Dunn S., Jankelow D., Duran C. M., Cunningham M. W. Immune mechanisms in rheumatic carditis: Focus on valvular endothelium. *The Journal of Infectious Diseases*. 2001;183(3):507–511. PubMed PMID: 11133385.
18. Kansal R. G., Datta V., Aziz R. K., Abdeltawab N. F., Rowe S., Kotb M. Dissection of the molecular basis for hypervirulence of an in vivo-selected phenotype of the widely disseminated MIT1 strain of group A Streptococcus bacteria. *The Journal of Infectious Diseases*. 2010;201(6):855–865. PubMed PMID: 20151844.
19. Kakis A., Gibbs L., Equia J., Kimura J., Vogelei D., Troup N., et al. An outbreak of group A streptococcal infection among health care workers. *Clinical Infectious Diseases*. 2002;35(11):1353–1359. PubMed PMID: 12439798.
20. Cowell S. J., Newby D. E., Prescott R. J., Bloomfield P., Reid J., Northridge D. B., et al. A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. *The New England Journal of Medicine*. 2005;352:2389–2397. PubMed PMID: 15944423.
21. Jaseja H., Badaya S., Tonpay P. Immuno-prophylaxis against development of cardiac valvular complications in patients with rheumatic fever: A proposal method. 2010.
22. Galvin J. E., Hemric M. E., Ward K., Cunningham M. W. Cytotoxic mAb from rheumatic carditis recognizes heart valves and laminin. *Journal of Clinical Investigation*. 2000;106(2):217–224.
23. Ganguly N. K., Anand I. S., Khanna A. K., Kohli R. S., Wahi P. L. T cells and T cell subsets in rheumatic heart disease. *Indian J Med Res*. 1982;76:854–858.
24. Cunningham M. W., McCormack J. M., Talaber L. R., Harley J. B., Ayoub E. M., Muneer R. S., et al. Human monoclonal antibodies reactive with antigens of the group A Streptococcus and human heart. *Journal of Immunology*. 1988;141(8):2760–2766. PubMed PMID: 3049816.
25. Martins T. B., Hoffman J. L., Augustine N. H., Phansalkar A. R., Fischetti V. A., Zabriskie J. B., Cleary P. P., Musser J. M., Veasy L. G., Hill H. R. Comprehensive analysis of antibody responses to streptococcal and tissue antigens in patients with acute rheumatic fever. *Int Immunol*. 2008;20(3):445–452.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



26. Karthikeyan G., Mayosi B. M. Is primary prevention of rheumatic fever the missing link in the control of rheumatic heart disease in Africa? *Circulation*. 2009;120(8):709–713.
27. El-Shazly R., Niazy M. H., Riad N. M., Abdelraouf F. H., ElRefai R. M. Krebs von den Lungen-6 (KL-6), soluble programmed cell death-1 (sPD-1) and its ligand1(sPDL-1) in systemic sclerosis patients: relation to disease parameters. *Egypt Rheumatol*. 2022;44:333.7.
28. Shapira Y., Agmon-Levin N., Shoenfeld Y. Geoepidemiology of autoimmune rheumatic diseases. *Nat Rev Rheumatol*. 2010;6:468–476.
29. Mizani A., Alipour A., Sharif M., Sarvi S., Amouei A., Shokri A., et al. Toxoplasmosis seroprevalence in Iranian women and risk factors of the disease: a systematic review and meta-analysis. *Trop Med Health*. 2017;45:7.
30. Hill D., Dubey J. P. *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clin Microbiol Infect*. 2002;8:634–640.
31. Wahid W., Zahariluddin A. S. M., Kadir Z. S., Sharip S., Idris Z. M., Osman E. Reactivation of latent toxoplasmosis in a schizophrenia patient: a case report. *Iran J Parasitol*. 2021;16:512–517.
32. Benoist C., Mathis D. Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry? *Nat Immunol*. 2001;2:797–803.



Crimean-Congo Hemorrhagic Fever, Transfer Patterns, Vectors, and History in Iran and Neighboring Countries

Mousa Tavassoli¹, Salar Zarrabi Ahrabi², Gurkan Akyildiz², Aysen Gargili Keles²

Corresponding Author: Mousa Tavassoli, m.tavassoli@urmia.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
2. Department of Basic Health Science, Health Sciences Faculty, Marmara University, Istanbul, Türkiye.

Article code: 1225

Abstract

Background: Crimean-Congo hemorrhagic fever (CCHF) is indeed considered one of the most significant vector-borne diseases globally. The virus responsible for CCHF can persist in various animals and lead to severe infections in humans. Ticks of the Ixodidae family are the acknowledged vectors of the CCHF virus (CCHFV) transmission to humans.

Methods: In this review, different tick species from Iran and its neighbors and their roles in the transmission of CCHFV were evaluated. However, the transmission patterns of CCHF to humans primarily involve tick bites, direct contact with the blood, organs, or tissues of contaminated animals, and exposure to CCHF patients are important in virus transmission.

Results: The results of this review confirm that the tick vectors in Iran and its neighbors are very common. Even though tick bite is one of the most important risk factors for CCHF, it cannot explain all cases, and there are other important risk factors, such as high-risk occupations and having contact with livestock.

Conclusion: Ticks that have been proven to transmit the disease, especially *Hyalomma marginatum*, are scattered in Iran and neighboring countries. Transporting passengers and livestock, and sometimes smuggling livestock, are the means of transmitting the virus between neighboring countries. Therefore, there is a need for joint preparedness and response programs to prevent and manage CCHF between Iran and its neighbors.

Keywords: Crimean-Congo Hemorrhagic Fever, Virus, Iran, Tick, Transfer Patterns, Vectors.



Frequency of *Sarcocystis* spp Infection in Cattle Slaughtered in Birjand Slaughterhouse Using Digestion and Molecular Methods in 2024

Amir Tavakkoli Kareshk¹, Mahmoodreza Behravan¹, Ali Khazaei², Rahmat Solgi¹

Corresponding Author: Mahmoodreza Behravan, mahmoodreza.behravan@yahoo.com

1. Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran.
2. Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran.

Article code: 1227

Abstract

Background: *Sarcocystis* spp. are recognized as significant protozoan parasites affecting both humans and livestock, with considerable implications for medicine and veterinary practices in various countries. To date, there has been no research conducted on *Sarcocystis* in South Khorasan province. Consequently, this study aims to investigate the prevalence of *Sarcocystis* spp. infections in cow meat at the Birjand slaughterhouse, utilizing both digestion and molecular methods in 2024.

Methods: A total of 181 cow meat samples from the Birjand industrial slaughterhouse were analyzed for the presence of *Sarcocystis*. The samples, which were collected from the heart muscles, esophagus, and diaphragm, were examined using both microscopic (digestion) and molecular (PCR) methods.

Results: In this study involving 181 cow meat samples from slaughterhouses in Birjand city, no visible infections of *Sarcocystis* macrocyst were found in any meats. However, microscopic examination of tissue digestion samples from various organs revealed bradyzoites of the parasite in 23 samples (12.7%), identified in the sediment of the digestion tubes. The highest incidence of *Sarcocystis* infections was noted during the summer months. *Sarcocystis* was successfully isolated from the esophageal and diaphragm muscles of the samples. The infection rate determined by PCR analysis was consistent with the microscopic findings, also showing a rate of 12.7%. The positive samples exhibited a distinct band of 620 base pairs.

Conclusion: The results of this study indicate that cows in South Khorasan are contaminated with *Sarcocystis*. Given the widespread occurrence of *Sarcocystis* infections in cattle and potentially in sheep, along with the risk of transmission from dogs and cats to local animals, it is essential to develop control and prevention programs to mitigate the spread of *Sarcocystis* among livestock in the province.

Keywords: *Sarcocystis* spp, Digestion Method, Molecular Method, Birjand.



Molecular Detection of *Anaplasma*, *Babesia*, and *Theileria* Species from Sheep in the West of Iran

Maryam Nazarbeigy¹, Roohollah Fattahi¹, Fazel Pourahmad²

Corresponding Author: Fazel Pourahmad, f.pourahmad@ilam.ac.ir

1. Department of Laboratory and Clinical Sciences, Faculty of Veterinary Sciences, Ilam University, Ilam, Iran.

2. Department of Microbiology, Faculty of Veterinary Sciences, Ilam University, Ilam, Iran.

Article code: 1229

Abstract

Background: Tick-borne diseases caused by *Anaplasma*, *Babesia*, and *Theileria* species result in significant economic losses. This study aimed to investigate the presence and genetic diversity of *Anaplasma ovis*, *Babesia ovis*, and *Theileria* spp. in the blood of sheep in Ilam province.

Methods: Seventy-three blood samples were collected from naturally infected sheep referred to clinics across various areas of Ilam province from early June to late September in 2023 and 2024. The Giemsa-stained smears of peripheral blood were examined under a DMLS light microscope. Additionally, 1 mL blood samples were taken from the jugular vein of each sheep into EDTA tubes for polymerase chain reaction (PCR) analysis. Genomic DNA was extracted from the specimens and analyzed for *Theileria* spp., targeting a 778-bp fragment of the 18S rRNA gene, a 549-bp fragment of the 18S rRNA gene for *B. ovis*, and a 347-bp fragment of the MSP4 gene for *A. ovis*. PCR was performed using specific primers, and the amplified products were purified and sequenced by Bioneer Corporation (South Korea). The obtained 18S rRNA and MSP4 sequences were aligned using BLAST analysis.

Results: Microscopically, *Theileria* spp. infections were observed in all samples; however, infections with *Anaplasma* and *Babesia* were not confirmed in the blood smears. PCR analysis detected DNA of *Theileria* spp. in 82.18% (60/73) of the samples and *A. ovis* in 28.76% (21/73). Mixed infections of *Theileria* spp. and *A. ovis* were found in 17.80% (13/73) of the samples. *B. ovis* was not detected in any of the samples. Following Sanger sequencing, *T. ovis* and *A. ovis* were identified as the only species present.

Conclusion: Our results indicate that *A. ovis* is present in sheep in this region, although reliable detection through microscopic examination wasn't achieved. This discrepancy may be attributed to the low accuracy and user errors in microscopic analysis.

Keywords: *Theileria* spp., Sheep, PCR, Tick-Borne, *Anaplasma*, *Babesia*.



Copro-PCR based Determination of *Toxoplasma gondii* Oocysts in the Cats Referred to Veterinary Clinics in Ardabil City

Abbas Imani Baran¹

Corresponding Author: Abbas Imani Baran, a.imani@tabrizu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Article code: 1232

Abstract

Background: *Toxoplasma gondii* causing toxoplasmosis, is one of the most prevalent zoonotic protozoan parasite threatening humans and animals in the worldwide (1). This study aimed to pursue the status of *Toxoplasma gondii* infection in the cats of Ardabil city using molecular method.

Methods: From October 2022 to April 2023, 200 cat's faecal samples were collected and DNA extraction was performed on samples and 529-bp *RE* gene was amplified to identify *T. gondii* oocysts. Then, four PCR products were sequenced to characterize intraspecific variations. Phylogenetic tree was constructed to determine the phylogeny position of our isolates compared with the sequences deposited to GenBank.

Results: Totally, 30(15%) samples displayed the 529-bp fragments. Identity was 85.3%-92.8%. Similarly, the genetic pairwise distances between four sequenced amplicons ranged from 0.049 to 0.115. Statistically, there was no significant differences, except for stray cats with age of ≤ 1 year old.

Conclusion: Given that, both infection rate and intraspecific variation were noticeable, thus, further investigations on genotyping and designing control programs concerning this protozoa are essential.

Keywords: *Toxoplasma gondii*, Cat, Copro-PCR, 529-*RE* gene, Ardabil.



***Simulium turgaicum* Complex (Diptera: Simuliidae): Potential Vectors of Filarial Parasites in the Aras River Basin – Evidence from Epidemiological, Molecular, and Phenological Studies**

Naseh Maleki-Ravasan¹, Fariba Khanzadeh²

Corresponding author: Naseh Maleki-Ravasan, naseh_maleki@yahoo.com

1. Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran.
2. Department of Zoology, Comenius University, Bratislava, Slovakia.

Article code: 1239

Abstract

Background: Black flies of the *Simulium turgaicum* complex are among the dominant simuliid species in the Aras River Basin, northwestern Iran. Local communities frequently report severe biting nuisance and suspect these flies in the transmission of pathogens affecting both humans and livestock. This study aimed to assess entomological, parasitological, and environmental evidence to evaluate the vector potential of the *S. turgaicum* complex for filarial parasites in the region.

Methods: A total of 2,965 female black flies from the *S. turgaicum* complex, collected between 2017 and 2023, were analyzed using nested and conventional PCR assays. Molecular detection targeted nuclear (*18S rDNA-ITS1*) and mitochondrial (*cytb*) markers to identify filarial infections and blood meal sources.

Results: Filarial DNA was detected in 44 specimens (1.48%), including *Dirofilaria immitis* (n=5), *D. repens* (n=1), and *Onchocerca* sp. (n=38). Blood meal analysis revealed feeding on humans (44%), dogs (20%), bovids (22%), and birds (9%), indicating a broad host range with clear anthrozo-zoonotic potential. Combined entomological and climatological data suggest the presence of infective-stage larvae, supporting active transmission potential.

Conclusion: This multidisciplinary study integrates molecular, ecological, and behavioral evidence to implicate the *S. turgaicum* complex as a probable vector of filarial parasites in northwestern Iran. These findings highlight the need for enhanced surveillance and further investigation into the public health and veterinary significance of these black flies.

Keywords: Black flies, Onchocercidae, Vector incrimination, Blood meal analysis.



Using PCR and High- Resolution Melting (HRM) Analysis for *Dirofilariasis* Diagnosis

Niayesh Mohammadi¹, Fateme Jalousian²

Corresponding Author: Fateme Jalousian, jalousian_f@ut.ac.ir

1. DVM student, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1259

Abstract

Background: Real-time PCR (qPCR) is a highly sensitive molecular method broadly used in veterinary diagnostics to detect viral, bacterial, and parasitic pathogens. For *Dirofilaria immitis*, a zoonotic filarial nematode causing heartworm disease in dogs, detection can be enhanced by targeting *Wolbachia pipientis*, its endosymbiotic bacterium. This study aimed to evaluate the application of high-resolution melting (HRM) real-time PCR for identifying *D. immitis* and *W. pipientis* DNA, and to compare its diagnostic performance with conventional PCR.

Methods: Blood samples were collected from 138 stray dogs in Iran's Guilan, Mazandaran, and Qazvin provinces in 2022-2023. The presence of *W. pipientis* was evaluated by amplifying a partial sequence of the FtsZ gene (267 bp). A Rotor-Gene thermocycler (Rotor-Gene Q; Qiagen, Hilden, Germany) and SYTO 9 dye (Thermo Fisher Scientific, USA) was used for performing HRM analysis.

Results: The results showed that 17.4% of the samples were positive by the modified Knott method, while 47.8% were positive by the molecular methods. The results of high-resolution melting (HRM) real-time PCR showed that melting temperature mean of *D. immitis* *cox1* gene products was amplified at $76.67 \pm 0.03^\circ\text{C}$, as well as it was achieved $80.30 \pm 0.04^\circ\text{C}$ for FtsZ gene of *W. pipientis*. When HRM curve analysis is added to the real-time PCR, it will be possible to differentiate *W. pipientis* and *D. immitis* simultaneously.

Conclusion: Conventional PCR and HRM real-time PCR results showed that 47.8% (66:138) of dog blood samples were infected with dirofilariasis. Since occult infections or those with low microfilarial counts in peripheral blood are hard to detect microscopically, molecular methods are essential for accurate diagnosis. HRM real-time PCR is a post-PCR technique that detects *Wolbachia pipientis* DNA by analyzing the melting profiles of the amplified products. This method is rapid, highly sensitive, and specific, making it a reliable and preferred approach for diagnosing occult dirofilariasis where available.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Dirofilaria immitis*, Endosymbiotic, High-resolution melting (HRM) real-time PCR, Nematodes.



Comparative Evaluation of the HALP Score between PCR-confirmed *Theileria*-infected and Healthy Sheep in Semnan, Iran

Vahid Dokhaei¹, Mahmood Ahmadi-hamedani², Mohammad Reza Salimi Bejestani³

Corresponding Author: Mahmood Ahmadi-hamedani, ahmadi.hamedani@semnan.ac.ir

1. MSc student of parasitology, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.
3. Department of Pathobiology, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.

Article code: 1264

Abstract

Background: *Theileria* are apicomplexan parasites that infect both domestic and wild animals, leading to significant economic losses in livestock. There is limited information on hematological scoring systems for differentiating infected from healthy animals. This study examines the HALP score, hemoglobin, albumin, lymphocyte, and platelet levels in PCR-confirmed *Theileria*-infected sheep versus healthy sheep, representing the first assessment of its clinical significance in sheep theileriosis.

Methods: Blood samples were collected from 100 sheep. Microscopy and PCR were used to diagnose *Theileria* infection, and nested PCR was used for species identification. Hematocrit (Hct), Hb, red blood cell (RBC), lymphocyte counts, platelet counts (PLT), and serum albumin were measured. The HALP score was calculated by multiplying hemoglobin by albumin by lymphocyte count and dividing by PLT.

Results: Microscopic analysis revealed that 21 out of 100 samples were infected with *Theileria*, while PCR identified 27 species. Results showed *T. ovis* in 52%, *T. lestoquardi* in 26%, *T. annulata* in 11%, and a co-infection of *T. lestoquardi* and *T. ovis* in 11% of the samples. A significant difference was observed in the PLT in sheep infected with *T. lestoquardi* compared to other species of *Theileria*. Statistical differences in the HALP score were noted for *T. lestoquardi* ($P = 0.03$), *T. annulata* ($P = 0.01$), and co-infections of *T. ovis* and *T. lestoquardi* ($P = 0.05$) versus *T. ovis*.

Conclusion: This study is the first to assess the HALP score in sheep infected with *Theileria* spp., showing significant differences between infected and healthy animals. Sheep infected with *T. lestoquardi* and *T. annulata*, as well as those with concurrent infections, had notably different HALP scores compared to those infected only with *T. ovis*. These results suggest that the HALP score may effectively differentiate the severity of *Theileria* infections, aiding clinical assessment in endemic areas.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: HALP Score, Nested-PCR, Sheep, *Theileria* spp.



A Study on the Frequency of *Anaplasma platys* Infection in Shelter Dogs in the Mashhad area, Iran, Using Microscopic and Molecular Methods

Seyyed AmirSohrab Tabatabaie Hosseini¹, Amin Bakhshani¹, Gholamreza Razmi¹

Corresponding Author: Gholamreza Razmi, razmi@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.

Article code: 1275

Abstract

Background: *Anaplasma platys* is an obligate intracellular rickettsial parasite with a particular tropism for platelets. It causes infectious cyclic thrombocytopenia in canines, which may be accompanied by clinical signs such as fever, petechial and ecchymotic hemorrhages. Given the absence of prior studies on *A. platys* infection in dogs in Iran, the present study aimed to investigate the prevalence of this microorganism in stray dogs housed in animal shelters.

Methods: Whole blood samples were collected from 100 dogs housed in various shelters. A smear was prepared from each blood sample, stained with Giemsa, and examined under a light microscope. The collected samples were placed in CBC tubes containing EDTA and stored at -20°C until DNA extraction. DNA extraction was performed using the Denazist kit according to the manufacturer's protocol. A primary PCR was conducted using genus-specific set of primers of the 16S rRNA gene for *Anaplasma* agents. The DNA of the positive samples was then subjected to a second PCR using *A. platys* specific forward primer. PCR products were analyzed by agarose gel electrophoresis.

Results: No morulae of the parasite were observed within the platelets during microscopic examination. In the molecular analysis, out of 100 samples, 22 showed evidence of infection with the *Anaplasma* genus, of which 5 were confirmed to be *A. platys*.

Conclusion: The present study confirmed the presence of *A. platys* infection in shelter dogs around Mashhad, with an estimated frequency of approximately 5%. The infection was effectively detected using molecular techniques, while microscopic methods exhibited lower sensitivity and accuracy.

Keywords: *Anaplasma platys*, Prevalence, Microscopic and Molecular Methods, Shelter Dogs.



IL-33 and Its Association with Visceral Leishmaniasis in the Hamsters Infected with *Leishmania infantum*

Shamsi Noorpisheh Ghadimi¹

Corresponding Author: Shamsi Noorpisheh Ghadimi, sn.noorpisheh@yahoo.com

1. Department of Parasitology and Mycology, School of Allied Medicine Sciences, Fasa University of Medical Sciences, Fasa , Iran.

Article code: 1277

Abstract

Background: Visceral leishmaniasis (VL) is a chronic infection caused by *L. infantum* parasites. Toll like receptors play a major role in immune responses against *Leishmania* parasites. This study aims to evaluate the immune-stimulating impact of a TLR 2 agonist and to assess IL-33 cytokine levels in VL.

Methods: In this experimental study, *L. infantum* (MCAN/IR/07/Moheb-gh) was prepared. Then, 24 golden hamsters were assigned into 4 groups (n=6). Group 1 was the control groups, group 2 were injected with 1×10^8 promastigotes of *L. infantum*, group 3 the TLR2 agonist, group 4 the *L. infantum* and TLR2 agonist. Hamsters were then challenged with wild-type of *L. infantum*. IL-33 levels in the serum were measured using Flow Cytometry.

Results: There were statistically significant differences ($p<0.05$) in IL-33 levels among the groups. The highest IL-33 expression was observed in the infected group (G2), while the lowest levels were found in the TLR2 agonist- only group (G3).

Conclusions: Our results suggest that IL-33 could be a new factor of susceptibility and a potential prognostic marker during visceral leishmaniasis. On the other hand, TLR2 agonist can be an efficient method to provide a protective immune response against VL leishmaniasis.

Keywords: *Leishmania infantum*, TLR2 agonist, Hamster, Immunity.



Phylogenetic Analysis of Avian Schistosomes from *Melanoides tuberculata* in Southwest Iran, and Evaluation of their Pathogenicity for Mice

Mehdi Karamian^{1,2}, Seyed Mahmoud Sadjjadi¹, Ahmad Oryan³, Jitka Aldhoun⁴, Sharif Maraghi⁵

Corresponding Author: Seyed Mahmoud Sadjjadi, sadjjadi316@gmail.com

1. Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Microbiology, School of Medicine, Zanzan University of Medical Sciences, Zanzan, Iran.
3. Department of Pathology, School of Veterinary Medicine, University of Shiraz, Shiraz, Iran.
4. Parasites and Vectors, Department of Life Sciences, Natural History Museum, Cromwell Road, London, UK.
5. Department of Parasitology and Mycology, School of Medicine, Ahwaz University of Medical Sciences, Ahwaz, Iran.

Article code: 1298

Abstract

Background: *Melanoides tuberculata* is an oriental aquatic snail species prevalent in almost all continents. The present study was carried out in order to find the phylogenetic position of a bird schistosome newly reported as the main causative agent of cercarial dermatitis in an endemic region in Iran.

Methods: This study surveyed aquatic snails in Khuzestan province, collecting 6,564 snails from agricultural canals, rivers, and swamps. Cercariae were detected via shedding and crushing, with morphological and molecular analyses (PCR, sequencing of ITS2 and 28S rDNA) identifying species. DNA was extracted, amplified, and sequenced, with results deposited in GenBank

Results: Ocellate furcocercariae were found in 15 (0.29%) out of 5118 *M. tuberculata* examined. Phylogenetic analysis of the nuclear internal transcribed spacer 2 (ITS2) and D2 domain of 28S rDNA of the isolated furcocercariae revealed that all samples belong to *Gigantobilharzia melanoidis*, recently described from the same snail in the United Arab Emirates. Experimental works on their pathogenicity showed that these furcocercariae were able to induce cutaneous lesions in mice consistent with those caused by other dermatitis-inducing species of avian schistosomes.

Conclusion: *Melanoides tuberculata* snails in Khuzestan province in southwest Iran were found to be infected with ocellate furcocercariae belonging to *Gigantobilharzia melanoidis*. Experimental infections of mice confirmed that *G. melanoidis* is able to produce cercarial dermatitis in mammals and highlighted the need for further investigations.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Iran, Avian schistosome, *Melanoides tuberculata*, Mice.



An Outbreak of Clinical Coccidiosis in Lambs in a Sheep Farm in Chenaran Area, Khorasan Razavi Province

Gholamreza Razmi¹, Hossien Nourani², Mostafa Torkaman³

Corresponding Author: Golamreza Razmi, razmi@um.ac.ir

1. Department of Pathobiology, Parasitology section, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

2. Department of Pathobiology, Pathology section, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

3. Doctor of Veterinary Medicine, Graduated from the Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1320

Abstract

Background: Coccidiosis is a major parasitic disease that poses significant economic challenges for sheep, particularly in lambs. This case report outlines an occurrence of clinical coccidiosis impacting lambs on a sheep farm situated in the Chenaran area of Khorasan Razavi Province.

Methods: In October 2024, a clinical coccidiosis outbreak was documented among weaned lambs of the Lacaune breed at a sheep farm in the Chenaran area. This group consisted of 272 lambs, all approximately 2-3 months old. The affected lambs displayed symptoms such as anorexia, weight loss, weakness, and severe diarrhea, which was sometimes bloody. Parasitological and pathological examinations were conducted to confirm the diagnosis.

Results: The mean $\pm$ SD of OPG for diarrheic and non-diarrheic lambs showed no significant difference ($P > 0.05$). The morphological analysis identified three species of *Eimeria*, which are: *Eimeria palida* (colorless, lacks a micropyle, $15-17.5 \times 11-12.5 \mu\text{m}$); *Eimeria ovinoidalis* (ellipsoidal, colorless, lacks a micropyle, $20-27 \times 15-20 \mu\text{m}$); *Eimeria intricate* (ovoid form, yellow, thick wall, features an operculum, $47.5-50 \times 30-37.5 \mu\text{m}$). Among these species, *E. ovinoidalis* is recognized for its high pathogenicity in young sheep. Post-mortem examinations revealed inflammation and hemorrhagic lesions in the intestinal mucosa of both the small and large intestines. Histopathological analysis of the intestinal samples demonstrated various developmental stages of *Eimeria* within the glandular epithelial cells of the caecum. Other general microscopic observations included severe hyperemia, edema, hemorrhage, infiltration of inflammatory cells, and lesions in the glandular subepithelium, such as degeneration and necrosis.

Conclusion: The clinical and pathological findings validated the diagnosis of clinical coccidiosis in the affected weaned lambs; the parasitological results indicated that *E. ovinoidalis* was the primary species responsible for the clinical coccidiosis.

Keywords: Outbreak, Clinical coccidiosis, *E. ovinoidalis*, Histopathology, Lambs.



Investigation of the Possibility of *Theileria annulata* Resistance to Buparvaquone in Treated Cows Against Theileriosis in Roshtkhar Area, Khorasan Razavi Province

Jamil Arab Banishibani¹, Ali Moshaverinia¹, Asma Keyvanlou¹, Gholamreza Razmi¹

Corresponding Author: Gholamreza Razmi, razmi@um.ac.ir

1. Department of Pathobiology, Parasitology Section, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1321

Abstract

Background: Theileriosis is a notable tick-borne illness commonly found in tropical and subtropical regions, leading to significant annual economic losses. This research aimed to investigate the problem of drug resistance in dairy cattle suffering from theileriosis.

Methods: In the spring and summer of 1403, 90 blood samples were gathered from dairy cattle that showed inadequate responses to buparvaquone treatment in the Roshtkhar area. Initially, blood smears were prepared and examined under a microscope, followed by the extraction of DNA from EDTA blood samples, which were subsequently analyzed using specific PCR techniques. The PCR-positive samples underwent testing with specific primers that target the cytochrome b gene of *Theileria annulata*, and subsequently, ten blood samples that exhibited a positive reaction were submitted for DNA sequencing. The sequence analysis was conducted on PCR products based on the amplification of the cytochrome b gene to identify gene mutations.

Results: Microscopic and PCR examinations of the blood samples indicated the presence of *Theileria* infection in 73 and 75 samples, respectively. Sequencing analysis revealed a total of thirteen gene mutations in the nucleotide sequences of four samples when compared to the reference strain. Among these mutations, eight were found within the Q01 region, while the remaining mutations were located outside the Q01 and Q02 regions. The highest number of gene mutations was observed in samples No. 2 and No. 4. In this study, only sample No. 2 contained seven newly formed mutations, with six of these mutations leading to changes in amino acids.

Conclusion: Identification of mutated genes within the Q01 region may indicate a resistance of *T. annulata* to the Butalex drug. Considering the widespread occurrence of this disease in Iran, it is recommended to implement management strategies for treatment and control of this disease within the country.

Keywords: Cattle, *Theileria*, Drug Resistance, Butalex, PCR, Cytochrome b.



Molecular and Morphological Characterization of a New *Eimeria* Species in the Domestic Pigeon (*Columba livia domestica*) in Northwest Iran

Parisa Shahbazi¹, Monireh Khordadmehr¹, Hosein Hashemzadeh Farhang²

Corresponding Author: Parisa Shahbazi, p.shahbazi56@gmail.com

1.Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

2.Department of Pathobiology, Faculty of Veterinary Medicine, Tabriz Medical Sciences, Islamic Azad University, Tabriz, Iran.

Article code: 1335

Abstract

Background: To date, 18 *Eimeria* species have been reported to infect the family Columbidae. Four species are considered clinically important in domestic pigeons (*E. labbeana*, *E. columbae*, *E. columbarum*, and *E. tropicalis*). Subclinical coccidiosis is common in pigeons and leads to an asymptomatic carrier state of the affected birds, while clinical coccidiosis occurs after ingesting numerous sporulated oocysts or in immunocompromised individuals. Greenish diarrhea is the primary clinical sign, followed by weakness, rough feathers, anorexia, and weight loss. Therefore, this study was conducted to identify *Eimeria* species in domestic pigeons in Tabriz.

Methods: In this study a new *Eimeria* species is described from domestic pigeon (*Columba livia domestica*) in Northwest Iran (East Azerbaijan Province), morphologically and molecularly. DNA was extracted from the collected *Eimeria* oocysts using a commercial genomic DNA extraction kit according to the manufacturer's instructions. Isolated *Eimeria* oocysts were analyzed at the mitochondrial cytochrome oxidase I (COI) and the 18S ribosomal RNA loci.

Results: This *Eimeria* sp. at the COI locus shared the most similarity with *Eimeria* sp. strain EP201(MF496271.1) from domestic pigeon (*Columba livia domestica*) in Egypt and grouped in a common clade with *Eimeria columbadomestica* (*E. labbeana* like) (KT305927-29) and *Eimeria* sp. isolate 878 (OP558169) previously identified from domestic pigeon in Australia and Poland, respectively. At the 18S locus, this *Eimeria* sp. shared most genetic similarity with *Eimeria columbinae* isolates in Brazil (MN082528) and Egypt (OR420908) from columbiform bird and grouped in a common clade with other *Eimeria* and *Isospora* species previously reported from Columbiformes. Sporulated oocysts of this species (n = 20) are spheroidal, 17.28-18.37 × 19.35-20.7 (18.3 × 19.5) µm; shape index (L/W ratio) 0.93-0.94 µm. with a smooth, greenish-yellow bi-layered wall, 1.2 µm.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: According to morphological and molecular analysis, the isolated coccidian parasite is a new species of *Eimeria* named *Eimeria liviae* n. sp. after its host, the domestic pigeon (*Columba livia domestica*) (Columbiformes: Columbidae).

Keywords: Pigeon, *Eimeria liviae*, Molecular, Morphological, Northwest, Iran.



Laboratory Diagnosis of Cutaneous Leishmaniasis Based on an In-House ELISA Using a New Antigen

Zohreh Askarypour¹, Sahel Hamzehpour¹, Negin Elahirad¹, Mahmoud Nateghi-Rostami¹

Corresponding Author: Mahmoud Nateghi-Rostami, m_nateghi@pasteur.ac.ir

1. Department of Parasitology, Pasteur Institute of Iran, 12 Farvardin St., Tehran, Iran.

Article code: 1338

Abstract

Background: Laboratory diagnosis of cutaneous leishmaniasis (CL) remains challenging. Traditional parasitological methods require trained personnel and often show limited sensitivity. In CL, due to the localized nature of the infection, only low titers of specific circulating antibodies are typically produced which reduces the reliability of serological tests. Therefore, diagnostic strategies should be focused on detection of *Leishmania* antigens at the site of infection rather than detection of serum antibody. This study aimed to develop a recombinant multiepitope chimeric protein to be used as a diagnostic assay for CL.

Methods: A chimeric protein was designed based on four *Leishmania* major genes. The purified protein was then used to produce polyclonal antibodies in rabbits, and the immunoreactivity of the antiserum was evaluated against *Leishmania* antigens from lesions of CL patients through an in-house ELISA. It was performed by coating plates with protein, blocking with BSA and incubating with serial dilutions of rabbit antiserum. To evaluate the antibody's diagnostic potential, lesion samples were also tested as antigen and absorbance was measured at 450 nm.

Results: The results demonstrated sensitivity, specificity and accuracy of the ELISA test as 90.91% (75.67% to 98.08%), 95.24% (76.18% to 99.88%) and 92.59% (82.11% to 97.94%), respectively.

Conclusion: The ELISA showed good diagnostic performance with CL lesion samples, demonstrating over 90% sensitivity and specificity for detecting *Leishmania* antigens. It has the potential to be further evaluated as a diagnostic tool for CL patients. It might be applied to other test formats especially in immunochromatographic assays for the rapid detection of *Leishmania* antigens in skin lesions.

Keywords: ELISA, Diagnosis, Cutaneous Leishmaniasis, Serologic Tests.



Molecular Identification of *Toxoplasma gondii* in Domestic and Industrial Chickens in Isfahan Region, Iran, 2022

Nader Pestehchian¹, Mahnaz Sami¹, Reza Kalantari¹, Hossainali Yousefi¹, Shima Toghyani³

Corresponding Author: Nader Pestehchian, pestechian@med.ac.ir

1. Department of Medical Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

2. Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

3. Department of Biology, Science and Research branch, Islamic Azad University, Tehran, Iran.

Article code: 1352

Abstract

Background: *Toxoplasma gondii* is a pathogenic and zoonotic parasite, with warm blooded animals are considered as its hosts. Humans, especially immune defective subjects, may show serious symptoms of toxoplasmosis while in poultry it can lead to an asymptomatic infection. Consumption of raw or under cooked meat of chickens can cause infection in human and other animals. So, in this study we identified and genotyped of toxoplasmosis in chickens of Isfahan, Iran.

Methods: After assessing blood samples from 60 domestic chickens and roosters, 60 industrial broiler chickens and roosters and 60 laying hens and identifying the seropositive specimens by MAT test, the brain and tongue tissues of positive cases by serological tests were examined to identify the genotypes of *Toxoplasma gondii* by Nested PCR-RFLP.

Results: After evaluating of serological samples, *T.gondii* were found in 33.3% of domestic chickens, 25% of broiler, and 50% of industrial laying chickens. we found out that the 344 bp band which was more frequently observed in the brain tissue samples, refers to Genotype I: *T. gondii* DNA was detected in 44 brain tissue samples (55%) which can be attributed to the neurophilic nature of the parasite. The highest rate which is aligned with the most other related studies. However, the amount of detected parasite in the tongue tissue was low, seven tongue tissue samples (8.75%), this amount also can indicate that muscles is also a tissue might be infected in chickens.

Conclusion: Therefore, safe and hygienic methods of keeping and caring for chickens in domestic and industrial farms should be taken into consideration to prevent chickens from becoming infected. According to previous studies in human, environment and livestock meat in Isfahan, genotype I was the predominant, which indicates the establishment of this genotype in the region.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Toxoplasma gondii*, Domesticate chickens, Industrial chickens, Molecular test, Genotype I.



Biological Control of Fasciolosis: An Integrated Review of Emerging Solutions

Mohammad Moazeni¹, Artin Sheibani²

Corresponding Author: Artin Sheibani, sheibani.artin@gmail.com

1. Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Article code: 1353

Abstract

Background: Fasciolosis is a zoonotic disease caused by *Fasciola hepatica* and *Fasciola gigantica* that infects animals and humans throughout the world and causes huge economic losses. Presently, the control measures are mainly based on the application of anthelmintic drugs and molluscicides; but, resistance to these treatments is emerging, and the environmental risks involved with molluscicides limit the potential long-term use. Thus, biological control of the parasites and their snail intermediate hosts, has been postulated as an attractive alternative. The aim of this study is to conduct a review on emerging biological control strategies for fasciolosis as sustainable alternatives to conventional chemical treatments.

Methods: This study was undertaken referencing online scientific databases, including PubMed, Google Scholar, and ResearchGate. 53 peer-reviewed articles and pertinent chapters from 2 academic books were analyzed. Methods of biological control for Fasciolosis have been segregated according to the sort of preventive measure and the particular developmental stage of a parasite in which the intervention targets. A wider angle of biological control techniques has been selected; combining more classical or readily available ones with more modern or potentially experimental ones.

Results: Biological control methods target different stages of the *Fasciola* lifecycle, offering a more sustainable and eco-friendly approach. It has been reported that microbial agents like *Streptomyces* spp. and fungal species such as *Paecilomyces lilacinus* are able to damage and destroy *Fasciola* eggs. Predators of snails, such as domestic ducks and certain fish species, have been shown to reduce snail populations that harbor the parasite significantly. Additionally, competitor snails like *Marisa cornuarietis* outcompete the *Lymnaea* snails, reducing the potential for parasite transmission. Furthermore, parasitic agents such as *Chaetogaster limnaei* break down the fluke's life cycle by attacking trematode larvae inside the snails. Despite the efficacy of these approaches, there are challenges when implementing them in natural habitats.

Conclusion: By combining the use of microbial agents, snail predators, and competitor snails with chemical treatments, the problem of drug resistance can be minimized. However, these agents must be controlled to ensure that no ecological disturbances occur. In the meantime,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



more comprehensive research and field trials will provide finer points to these methods, in turn improving their efficiency for the sustainable and long-term control of fasciolosis.

Keywords: Biological Control, Control, *Fasciola*, Fasciolosis.



Molecular Cloning and Heterologous Expression of the *GroEL* Gene Encoding a Chaperonin Protein from *Wolbachia* symbiont of *Phlebotomus papatasi* (Diptera: Psychodidae)

Donya Moayedi Moafagh^{1,2}, Naseh Maleki-Ravasan¹, Soheila Ajdary³, Asal Katebi³, Fateh Karimian¹, Jalal Babaie¹

Corresponding author: Naseh Maleki-Ravasan, naseh_maleki@yahoo.com

1. Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran.
2. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
3. Department of Immunology, Pasteur Institute of Iran, Tehran, IR Iran.

Article code: 1355

Abstract

Background: Zoonotic Cutaneous Leishmaniasis (ZCL) remains a significant public health challenge in tropical and subtropical regions, primarily due to difficulties in vector control, the absence of effective vaccines, and frequent treatment failures. Accumulating evidence suggests that the sandfly gut microbiota can modulate *Leishmania major* pathogenesis, with certain bacterial components exacerbating disease progression. In contrast, experimental and epidemiological studies have demonstrated that prior exposure to bites from uninfected sandflies can confer partial protective immunity against *Leishmania* infection—likely mediated by immune responses to salivary proteins or microbial components of the vector. Building on these findings, we aimed to produce a recombinant heat shock protein 60 (Hsp60), GroEL, derived from *Wolbachia*, an endosymbiotic bacterium of *Phlebotomus papatasi*, as a potential antigenic and immunogenic candidate for vaccine development.

Methods: Adult *Phlebotomus papatasi* sand flies were collected from Badrud District, Isfahan Province—an established ZCL-endemic area in Iran—during their peak activity period in June and July 2023. Following dissection, *Wolbachia* infection was confirmed by amplification and sequencing of a ~530-bp fragment of the *GroEL* gene using specifically designed primers. The amplified fragments were first cloned into the pTG-TA cloning vector and subsequently subcloned into the pET21a expression vector for heterologous expression in *E. coli*.

Result: Colony PCR and sequence analysis confirmed successful insertion of a 550-bp fragment of the *GroEL* gene into the pET21a vector. The recombinant GroEL protein (rGroEL), with an estimated molecular weight of 19.5 kDa, was successfully expressed at both small and large scales. Optimal expression conditions were determined to be 37°C, pH 10.5, and 4 hours of induction with IPTG. Protein expression was verified by SDS-PAGE and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Western blotting. The rGroEL protein was purified to a concentration of 300 µg/ml using FPLC under denaturing conditions.

Conclusions: *Wolbachia* and its associated molecules represent promising candidates in leishmaniasis research due to their potential role in modulating host-vector-pathogen interactions. The successful expression and purification of rGroEL provide a foundation for further investigations into its immunogenic properties and potential application in vaccine development or immunotherapeutic strategies against cutaneous leishmaniasis.

Keywords: Immunogenicity, *Leishmania major*, Recombinant protein, Zoonotic Cutaneous Leishmaniasis.



Acanthamoeba-Bacteria Interactions: A Review of Experimental Findings and Implications

Somayeh Bahrami¹, Mehdi Zarei²

Corresponding Author: Somayeh Bahrami, S.bahrami@scu.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1359

Abstract

Background: *Acanthamoeba* spp. are widespread environmental amoebae that play a significant role in ecological and health contexts by interacting with bacteria, fungi, and viruses. These amoebae demonstrate predatory behavior through phagocytosis, engulfing microorganisms. They can also act as reservoirs or incubators, offering a protective environment that allows bacteria to withstand environmental stress, immune defenses, and antimicrobial agents. Within amoebae, pathogens may multiply and increase their virulence, representing a notable public health concern. In this review, we present the key findings from our eight-year investigation into the interactions between *Acanthamoeba* and pathogenic bacteria.

Methods: We conducted various experiments to investigate the interactions between *Acanthamoeba* spp., particularly *A. castellanii*, and pathogenic bacteria such as *Salmonella*, *Shigella*, and *Listeria*. Our studies assessed factors influencing bacterial uptake and survival, including bacterial growth phase, temperature, media type, and oxidative stress induced by sublethal chlorine exposure. Additionally, we examined biofilm formation by *Salmonella enterica* serovar Enteritidis in the presence of *Acanthamoeba* under different nutrient conditions and analyzed gene expression related to biofilm development.

Results: Consistently, *Acanthamoeba* phagocytoses and harbors bacteria within trophozoites and cysts. Notably, *Shigella* spp. do not proliferate inside *A. castellanii*; however, significant numbers persist for up to 15 days. Chlorine-stressed *Salmonella* cells exhibit increased engulfment and extended intracystic survival compared to non-stressed bacteria. Additionally, the presence of *Acanthamoeba* activates biofilm-related gene expression in *Salmonella* Enteritidis, regardless of nutrient conditions, although biofilm formation results vary. These findings suggest that amoebae support bacterial survival and may enhance pathogenic traits.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: Our research highlights the pivotal role of *Acanthamoeba* in the ecology and pathogenicity of bacteria, which may contribute to persistent contamination and antimicrobial resistance in environmental settings. Understanding these interactions is crucial for developing effective control measures to reduce public health risks associated with amoeba-bacteria relationships.

Keywords: Free-living amoeba, *Acanthamoeba*, Bacteria, Interaction.



Investigation of *Toxoplasma gondii* Presence in Raw Milk of Sheep, Goats, Cows, and Camels in the Sistan Region

Mohammad Mohebi¹, Mohammad Mirzaei¹, Bagher Amirheidari², Maryam Nooshadokht³

Corresponding Author: Mohammad Mohebi, mohebb1@gmail.com

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
2. Extremophile and Productive Microorganisms Research Center, Kerman University of Medical Sciences, Kerman, Iran.
3. Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

Article code: 1387

Abstract

Background: Toxoplasmosis is a highly prevalent zoonotic disease. *Toxoplasma gondii* is a major cause of abortion and poses significant economic losses for sheep and goat breeders. It can also endanger public health by contaminating animal products. Transmission of the parasite occurs vertically from the placenta to the fetus and horizontally through ingestion of oocysts shed by cats, consumption of tissue cysts in raw or undercooked meat, or ingestion of unpasteurized milk or offal containing tachyzoites. Infection with *T. gondii* is particularly dangerous and even fatal in specific groups, such as congenitally infected fetuses, newborns, immunocompromised individuals, AIDS patients, and organ transplant recipients. The present study aimed to investigate the prevalence of *T. gondii* infection in raw milk from sheep, goats, cows, and camels in the Sistan region as an indicator of parasite shedding in milk.

Methods: 53 Raw milk samples from cows, sheep, goats, and camels were collected in the Sistan region and transported to the laboratory. DNA was extracted from the samples, and the presence of *T. gondii* DNA was assessed using nested-PCR.

Results: The results revealed that 4(7.5 %) of the samples were contaminated with *T. gondii*. In this study, the highest contamination with the protozoan *T. gondii* was observed in sheep milk 2samples (3.7 %), goats (1.9%), cows (1.9%), while no contamination was detected in camel milk.

Conclusion: Our findings demonstrated the presence of *T. gondii* DNA in raw milk, increasing the likelihood of parasite transmission through raw milk consumption. Therefore, boiling raw milk and using pasteurized milk are strongly recommended, along with implementing effective control strategies against toxoplasmosis, which appears essential.

Keywords: Nested-PCR, Raw milk, *Toxoplasma gondii*, Sistan Region.



Prevalence of *Dirofilaria immitis* and *Dirofilaria repens* in Dogs from Gilan Province, Iran: A GIS-Based Epidemiological Study

Fatemeh Manshouri¹, Fatemeh Jalousian², Seyed Hossein Hosseini², Parviz Shayan², Saeed Bakaei³

Corresponding Author: Fatemeh Jalousian, jalousian_f@ut.ac.ir

1. PhD student, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1397

Abstract

Background: *Dirofilaria immitis* (*D. immitis*) and *Dirofilaria repens* are mosquito-borne parasitic nematodes affecting dogs and potentially humans. Their distribution is influenced by environmental factors, particularly in ecologically diverse regions like Gilan Province, Iran. This study aimed to assess the prevalence of these parasites in dogs across three distinct climatic zones using geographic information system (GIS) tools.

Methods: A total of 393 dog blood samples were randomly collected from cold-humid (n=57), temperate-humid (n=83), and mountainous (n=253) areas between 2021 and 2023. Modified Knott's test and polymerase chain reaction (PCR) targeting ITS1 gene loci were used for parasite detection. Environmental variables including temperature, humidity, and rainfall were analyzed in relation to infection prevalence. ArcMap (version 10.8) and Inverse Distance Weighting (IDW) were employed for spatial distribution mapping. Multivariate logistic regression was used to model infection probability.

Results: *D. immitis* prevalence was 26.3% in cold-humid, 39.7% in temperate-humid, and 37.9% in mountainous regions. No statistically significant difference in infection rates was found across zones (ANOVA, $p>0.05$), but maximum temperature showed a strong positive correlation with infection. Rainfall and relative humidity also correlated positively. *D. repens* was detected less frequently and only in specific locations with lower temperature thresholds. The highest prevalence (60%) occurred in Fuman-Masuleh, while Langerud showed the lowest (4.3%). The logistic regression model demonstrated a sensitivity of 99.08% for predicting *D. immitis* infection.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: The findings highlight the widespread presence of *D. immitis* in Gilan and underscore the influence of climate on its transmission. Continuous monitoring, especially in high-risk and elevated regions, along with behavioral studies on stray dogs and vectors, is essential to manage dirofilariasis risk under changing environmental conditions.

Keywords: Climatic conditions, Disease probability prediction, Infection risk map, Inverse Distance Weighting (IDW).



Comparison of Surgical Approaches to Hepatic Hydatid Cyst Management in Humans and Animals: A Cross-Disciplinary Surgical Review

Samaneh Ghasemi¹, Nima Movasseghi², Paniz Azadpeima²

Corresponding Author: Nima Movasseghi, nimamovasseghi@gmail.com

1.Assistant Professor of Veterinary Surgery, Department of Veterinary Clinical Science, Ferdowsi University of Mashhad, Mashhad, Iran.

2.Doctor of Veterinary Medicine (DVM) Student, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1402

Abstract

Background: Cystic echinococcosis (CE) remains a significant zoonotic concern, particularly affecting the liver in humans and animals. Surgical intervention is a mainstay of treatment. This study compares surgical approaches, open, laparoscopic, and novel techniques between human and veterinary medicine.

Methods: A comparative literature review was conducted using databases including PubMed, Scopus, and CAB Abstracts (2008–2023). Selected articles focused on surgical techniques, recurrence rates, complications, and recovery times in humans and veterinary patients.

Results: In human cases, laparoscopic surgery demonstrated superior outcomes, with reduced recurrence rate (3–5%) compared to open surgery (up to 12%), and shorter lengths of hospital stays (2–4 days vs. 5–8 days). Postoperative complications such as biliary fistulas occurred in 5% of laparoscopic cases versus 17% in open surgeries. The Puncture, Aspiration, Injection, and Re-aspiration (PAIR) technique showed acceptable results in selected patients, with minimal invasiveness and low complication rates. In veterinary cases, especially in large animals, open surgery remains predominant technique due to logistical constraints. Studies in sheep and cattle showed longer recovery periods and higher postoperative complication risks, partly due to limitations in anesthesia, intraoperative imaging, and postoperative care. Laparoscopic attempts in small ruminants have shown promising results but remain limited to academic centers.

Conclusion: The surgical advancements in human CE management, particularly the shift toward laparoscopic and minimally invasive techniques, provide a valuable data for developing veterinary surgical protocols. Generalization these techniques to veterinary medicine, especially for improved recovery and lower recurrence, could significantly increase outcomes in animals.

Keywords: Cystic echinococcosis, Liver surgery, Laparoscopy, Comparative surgery.



Comparison of Ziehl Neelsen Staining, ELISA and PCR in Diagnosis of Human *Cryptosporidium* Infection

Mohamad Molazadeh¹, Hossein Yousofi Darani¹

Corresponding Author: Hossein Yousofi Darani, yousofidarani@gmail.com

1. Department of Pathobiology, Faculty of Veterinary Medicine Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

Article code: 1439

Abstract

Background: *Cryptosporidium* is a widespread zoonosis parasite that infects a large number of vertebrate animals and also human, resulting in varying degrees of diarrhea or even death. These parasites are known to be one of the main cause for diarrhea in children and also a chief cause of early childhood mortality globally. So diagnosis is important for appropriate treatment and follow up.

Methods: In this work a homemade ELISA for diagnosis of *Cryptosporidium* infection was developed. Parasite oocysts were collected and purified from infected cattle. According to modified Ziehl Neelsen staining method 30 human positive and negative fecal samples were collected and tested by the developed ELISA and OCR.

Results: Homemade ELISA perfectly detected infection in all 30 positive fecal samples. The investigation about the results of PCR is now undergoing.

Conclusion: According to the current stool examination methods diagnosis of *cryptosporidium* infection is not possible. So, development of sensitive and specific methods for diagnosis of infection is necessary.

Keywords: *Cryptosporidium*, Diagnosis, PCR, Homemade ELISA.



Detection and Genotyping of *Toxoplasma gondii* in Aborted Fetuses of Ewes and Goats from South-west of Iran

Hossein Hamidinejat¹, Morteza Noormahammadi², Morad Beyranvand², Somayeh Bahrami¹, Saad Goraninezgad³, Mohammad Reza Tabandeh⁴, Meysam Maki³

Corresponding Author: Hossein Hamidinejat, hsa.hamidinejat@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Ph.D graduated student, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
3. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
4. Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1451

Abstract

Background: *Toxoplasma gondii* is one of the most important zoonotic protozoan parasites with worldwide zoological and geographical distribution which generally well-known as fetus threatener in human and some animals and especially, dangerous systematic pathogen in HIV/AIDS individuals. In recent years, our different studies on many aspects of this parasite direct us to its considerable effect on abortion of ewes and goats in south-west of Iran.

Methods: First, we serologically found out that more than cut-off antibodies to *T. gondii* were prevalent in 72/6% (80% in aborted and 58% in control ewes) in Khoozestan province. Next, in 2024 in Kouzestan and Lorestan provinces, the brain and liver of aborted fetuses of sheep (269) and goats (100) were examined for *T. gondii* by PCR based on the amplification of 529 base pair fragments from repetitive regions of the parasite genome and genotyping of positive samples was performed by PCR-RFLP based on SAG2, SAG3, GRA6 and PK1 molecular markers.

Results: In sheep, the rate of infection was 7.8% in Khozestan and 7% in Lorestan. Evaluation of RFLP pattern, showed the presence of various types of parasites including type I, II and III and atypical type. In goats, the rate was 6% for both provinces and variants were type I and II.

Conclusion: These findings suggest the need for greater attention to costs for livestock and parasite transmission from livestock to human, particularly pregnant women and people with weakened immune system.

Keywords: *Toxoplasma gondii*, Genotyping, PCR-RFLP, Khouzestan, Lorestan.



Immunomodulatory Dynamics and Interleukin-Mediated Therapeutic Strategies in Cystic Echinococcosis: Advancing Immune-Targeted Interventions through Parasite Secretome Profiling

Zahra Gahvechi Amiri¹, Mohadeseh Ramzi², Sima RoshanFar³, Fatemeh Ramzi²

Corresponding Author: Zahra gahvechi amiri, Zahraamiri672@gmail.com

1. Department of Parasitology and Mycology . School of Medicine . Shahid Beheshti University of Medical Sciences , Tehran , Iran.

2. Department of Medical parasitology and Mycology . School of Public Health Tehran University of Medical Sciences , Tehran , Iran.

3. Department of Medical Parasitology & Mycology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran.

Article code: 1459

Abstract

Background: Cystic echinococcosis (CE), caused by the larval stage of *Echinococcus granulosus*, remains a global zoonosis with limited treatment efficacy. Immunological shifts, especially cytokine-mediated Th1/Th2 imbalances, play critical roles in disease progression and therapeutic resistance. This review explores interleukin-driven immune modulation, evaluates its potential as a predictive and therapeutic tool, and highlights parasite-secreted molecules as emerging immunological targets.

Methods: This narrative review integrates data from 28 peer-reviewed articles published between 2014 and 2025, sourced from PubMed, Scopus, and Google Scholar. Search terms included Cystic echinococcosis, interleukins, cytokine modulation, immunotherapy, and parasite-secreted molecules. Eligible studies examined cytokine shifts in CE hosts, immunomodulatory effects of parasite ES products, and efficacy of antigen-based and genetic immunizations. Special focus was given to IL-4, IL-6, IL-10, IFN- γ , and IL-12, and their impact on immune polarization.

Results: CE progression is frequently marked by elevated IL-4 and IL-10 levels, suppressing macrophage activation and promoting regulatory T cell expansion—favoring parasite survival. Increased IL-6 is linked to chronic inflammation and fibrotic capsule formation. Conversely, upregulation of IFN- γ and IL-12 enhances macrophage microbicidal activity and cytotoxic T cell responses, correlating with reduced cyst burden in both murine and ovine models. Recombinant antigens such as rEg.P29 and ES-62 modulate these pathways, inducing protective Th1 responses. Additionally, lncRNAs and parasite-secreted proteins influence cytokine expression, indicating a complex regulatory network shaping host-parasite interaction.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: Targeted interleukin-based therapies—including recombinant cytokines (e.g., IFN- γ , IL-12), IL-4/IL-10 antagonists, and immunomodulatory adjuvants—show promise in restoring Th1/Th2 balance and reducing cyst development in experimental models. Parasite-host interactions involving secreted molecules, lncRNAs, and cytokine receptor pathways critically influence immune responses. These insights support multi-epitope recombinant vaccines and immune-guided therapies. Combining cytokine profiling with advanced serological and molecular diagnostics may enable personalized treatments and better predict outcomes. A deeper understanding of immune regulation by host and parasite opens new avenues for managing cystic echinococcosis clinically.

Keywords: Cystic echinococcosis, Interleukins, Cytokine modulation, Immunotherapy, Parasite secretome.



The Systematic Review Application of Artificial Intelligence in Identifying Foodborne Zoonotic Parasites: Exploring Challenges and Opportunities in Food Safety and Veterinary Parasitology

Fatemeh Karimi Kesheh¹, Shakiba Mazaheri², Mohaddeseh Karimi Kesheh³, Leyli Shabani Vieh⁴, Zahra Nabizadeh⁵, Niknaz Rajaei⁶, Mohammad Arad Zandieh⁷

Corresponding Author: Mohammad Arad Zandieh, M.aradzandieh@ut.ac.ir

1.DVM student, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University.

2.DVM Student, Karaj Branch, Islamic Azad University, Karaj, Iran.

3.DVM student, Faculty of Veterinary Medicine, University of Tehran, Thran, Iran.

4.DVM Student, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.

5.DVM Student, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

6.Graduated DVM, Private Clinician.

7.Department of Food Hygiene and Quality Control, Division of Epidemiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1477

Abstract

Background: Zoonotic parasites are infectious agents capable of transmission between animals and humans, primarily through contaminated food or water. Prominent examples include *Toxoplasma gondii*, *Cryptosporidium spp.*, *Giardia duodenalis*, *Trichinella spiralis*, and *Fasciola hepatica*. These pathogens pose substantial public health threats worldwide. Conventional diagnostic techniques such as microscopy and serological assays are labor-intensive, require trained personnel, and often lack scalability and sensitivity. Recent advances in Artificial Intelligence (AI) have introduced promising tools for the rapid detection, surveillance, and management of zoonotic parasites, especially in food safety and veterinary parasitology.

Methods: A systematic literature review was conducted using PubMed, ScienceDirect, and Google Scholar, covering peer-reviewed articles from 2005–2025. Keywords included Artificial Intelligence, Machine Learning, Zoonotic Parasites, Foodborne Parasites, and Diagnosis.

Results: AI-based image recognition platforms—such as convolutional neural network (CNN)-enabled smartphone systems and VETSCAN IMAGYST®—have shown diagnostic accuracies exceeding 90% for detecting (oo)cysts and helminth eggs in fecal and food samples. Lightweight deep learning models with transfer learning approaches have enhanced detection in low-resource or low-resolution contexts. Additionally, integrating AI algorithms with whole-



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



genome sequencing (WGS) has enabled real-time outbreak surveillance, geo-epidemiological mapping, and high-throughput pattern recognition. While current parasite management remains largely pharmacological, AI applications in predictive modeling and big data analytics have enabled personalized treatment protocols and early warning systems for emerging zoonotic threats.

Conclusion: AI and machine learning offer rapid, scalable, and operator-independent alternatives for parasite detection and control, especially in low-resource settings. Their integration enhances food safety and zoonotic surveillance. Collaborative efforts among veterinarians, data scientists, and public health stakeholders are crucial for sustainable implementation.

Keywords: Artificial Intelligence, Machine Learning, Zoonotic Parasites, Foodborne Pathogens, Surveillance.



Comparison of Attenuated and Killed Vaccines Against *Neospora caninum* in Mice

Zahra khabazan¹, Reza rasti¹, Mehdi Namavari¹

Corresponding Author: Mehdi Namavari, namavari@yahoo.com

1. Razi Vaccine and Serum Research Institute ,shiraz-Branch Education and Extension Organization(AREEO),shiraz ,Iran.

Article code: 1483

Abstract

Background: *Neospora caninum* is a pathogenic protozoan that causes abortion and neosporosis in cattle. Due to the lack of an effective vaccine, research has focused on the development of live or attenuated vaccines.

Methods: Forty female mice were divided into four equal groups, Group 1: Experimental attenuated vaccine, Group 2: Killed vaccine (similar to commercial vaccine), Group 3: Challenged control group (no immunization), Group 4: Unchallenged control group. Mice in groups 1, 2, and 4 were vaccinated twice with an interval of 3 weeks. Two weeks after the last vaccination, blood was collected from all mice to measure serum antibody, interferon gamma (IFN- γ), albumin, and fibrinogen levels. Then, on days 5–10 of gestation, mice in groups 1–3 were challenged with an acute strain of *Neospora caninum* at a dose of 2.5 million (per mouse) and monitored daily for clinical signs and abortion.

Results The number of infants in groups 1 and 4 was significantly higher than in groups 2 and 3. The humoral and cellular immune responses, the increase in antibodies and IFN- γ in the attenuated vaccine group, were significantly higher than in the challenged control group. Albumin levels in groups 1 and 4 were lower than those in the unimmunized control group and the group immunized with killed vaccine, and serum fibrinogen levels in groups 1 and 4 were significantly higher than those in the other two groups.

Conclusion: Attenuated *Neospora caninum* vaccine can be considered as a promising option in preventing *Neospora* infection and reducing abortion.

Keywords: *Neospora caninum*, Mice, Vaccine, Antibody response.



A Preliminary Study of Canine Leishmaniasis in Tehran Province Were Referred to Iranian Pet Hospital from 2015-2025

Seeyamak Mashhady Rafie¹, Hamed Mansoor Lakooraj², Siamak Zarei³, Golsa Saadati⁴

1.Department of clinical science, Veterinary faculty, Islamic Azad University, science and research branch, Tehran, Iran.

2.Department of clinical pathology, head of laboratory of Iranian pet hospital, Tehran, Iran.

3.Department of surgery, head of Iranian pet hospital, Tehran, Iran.

4.Department of clinical pathology, doctor of Veterinary medicine, Iranian pet hospital, Tehran, Iran.

Article code: 1512

Abstract

Background: Leishmaniasis in dogs, also known as canine Leishmaniasis, is a parasitic disease transmitted by sandflies. Dogs are the primary reservoir for *Leishmania infantum*, which can also cause the disease in humans. The epidemiology of CanL is complex, with variations in prevalence and risk factors influenced by environmental conditions, geographic location, and vector distribution. CanL is a multisystemic disease that affects dogs. This parasite is endemic in the Mediterranean basin, South America, and Central and Southwest Asia and middle east.

Methods: The study was conducted between March 2015 and June 2025 in some dogs referred to Iranian pet hospital in Tehran. A total of 300 dogs who had skin manifestations including focal alopecia and dermal lesions on the muzzle, eyelids and pinnae or Cachexia and incapacitation were noted. Age, breed, sex, and address were documented if possible. Physical examination data were recorded too. Serum sample were sent to School of Public Health, Tehran University of Medical Sciences for DAT test. The level for positive result was 1/160.

Results: From 300 suspected dogs, 77 cases were positive (25.66%), include 42 female (54.54%) and 35 male (45.45%). The most breed involved include: mixed breed 18 cases (23.3%), German shepherd 11 cases (14.28%), Terriers 7 cases (9%). We saw involvement of one wolf in this study too. Local prevalence include Damavand and Lavasaan 15 cases(19.48%), Pasdaran environs 9 cases(11.6%) and Ecbataan area 6 cases(7.79%).

Conclusion: The epidemiological patterns of CanL in Iran have changed over the past two decades due to population migration between urban and rural areas and even refugee flow and animal transport from Afghanistan and Iraq to Iran. It seems the sentiment of animal protection groups may influence in rate of this disease. *L. tropica* in stray or owned dogs, the implications extend to public health as a zoonotic problem.

Keywords: canine Leishmaniasis, zoonosis, epidemiology, Iranian pet hospital, Tehran.



Canine leishmaniosis in Iran: It Is not Only *Leishmania infantum*

Alireza Sazmand¹

Corresponding Author: Alireza Sazmand, alireza.sazmand@basu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran.

Article code: 1530

Abstract

Background: *Leishmania infantum*, a vector-borne pathogen transmitted through the bite of infected female phlebotomine sand flies, causes visceral (VL) and cutaneous (CL) leishmaniosis in many animal species, including humans. Dogs are the main reservoir hosts, playing a crucial role in the epidemiology of the infection. This presentation aims to provide a comprehensive view of the diagnostic challenges of VL in Iran.

Methods: The methods employed in the epidemiological studies on the prevalence of canine leishmaniosis (CanL) in Iran were collected, and the authors' discussion of the obtained results was critically analyzed.

Results: According to the literature, mean seroprevalences of CanL in different regions of Iran ranged from 7.8% to 33.5%. In all of the studies, the causative agent of seropositivity was attributed to *L. infantum*. Only a few reports used molecular-based techniques to confirm the parasite species causing cutaneous lesions. The confirmed parasite species in the blood, skin lesion scrapes, and internal organs of the infected dogs were *L. infantum*, *L. major*, and *L. tropica*.

Conclusion: Both VL and CL are present in dog populations in Iran, so the prevalence of VL in Iran might be overestimated. Cross-reactions at serological tests can occur among kinetoplastids, such as those belonging to the genera *Leishmania* and *Trypanosoma*. Hence, the diagnostic confirmation of the infection should rely on an integrated approach, including both parasitological and molecular methods. In this presentation, 1) the common clinical manifestations of CanL caused by *L. infantum*, 2) differential diagnosis of CanL caused by *L. infantum* with CanL caused by *L. tropica* and *L. major*, and 3) potential misdiagnosis linked to the serological cross-reactions will be discussed.

Keywords: Cutaneous, Differential Diagnosis, Dog, Leishmaniasis, Vector-Borne Diseases, Visceral.



Induction of a Th1 Immune Response Against Leishmaniasis Infection by a Kinetoplast-associated Gene Knockout *Leishmania major* Strain

Fatemeh Darzi¹, Mahmoud Nateghi-Rostami¹

Corresponding Author: Mahmoud Nateghi-Rostami, m_nateghi@pasteur.ac.ir

1. Department of Parasitology, Pasteur Institute of Iran, 12 Farvardin St., Tehran 1316943551, Iran.

Article code: 1539

Abstract

Background: Leishmaniasis is a major public health problem with no licensed human vaccine available. We used CRISPR/Cas9 genome editing to generate an attenuated *Leishmania major* strain with a single-allele knockout of the universal minicircle sequence binding protein (UMSBP) gene, a kinetoplast DNA-associated gene essential for mitochondrial function and respiration, as a live vaccine candidate. The aim of this work was to assess whether immunization with this genetically modified strain could induce a protective immune response in mice.

Methods: One allele of the USBP gene was deleted in *L. major* promastigotes using CRISPR/Cas9. Female BALB/c mice (4–8 weeks old) were divided into three experimental groups (n=5 per group): wild-type strain, USBP single-knockout strain, and PBS control. Mice were immunized subcutaneously with 1×10^6 promastigotes or PBS at the hind footpad. Four weeks later, draining lymph node cells were harvested, cultured in RPMI 1640 medium supplemented with 10% FBS, and stimulated with lipopolysaccharide (LPS), soluble *Leishmania* antigen (SLA), or left unstimulated. After 72 hours of incubation at 37°C in 5% CO₂, cytokines (IFN- γ , IL-12, IL-4, IL-10) were quantified by sandwich ELISA. Mice were then challenged with wild-type *L. major*, and lesion development was monitored weekly by measuring footpad swelling.

Results: Mice immunized with the USBP single-knockout strain produced significantly higher IFN- γ ($P < 0.001$) and IL-12 ($P < 0.05$), alongside reduced IL-4 levels ($P < 0.001$) in response to SLA stimulation compared with PBS controls. After wild-type challenge, immunized mice displayed delayed lesion onset and smaller lesion size, suggesting partial protection.

Conclusion: The attenuated USBP single-knockout *L. major* strain induced a strong Th1-skewed immune profile and conferred measurable protection in a murine model, supporting its potential as a live attenuated vaccine candidate for leishmaniasis.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Leishmania major*; CRISPR/Cas9, Live attenuated vaccines, Th1 immune response; Knockout *Leishmania* strain.



Investigation of the Seroprevalence of *Fasciola* spp. Infection in Pregnant Women Referring to Al-Zahra Hospital, Rasht, Northern Iran

Mahdieh Sorouri Majd^{1,2}, Mojgan Aryaeipour¹, Eshrat Beigom Kia¹, Seyedeh Hajar Sharami³, Mehdi Mohebbali^{1,4}, Akram Sepahvand¹, Azadeh Jafari³, Zohreh Fakhrieh-Kashan^{1,2}

Corresponding Author: Zohreh Fakhrieh Kashan, zfk579@gmail.com

1. Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
2. Student's Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran.
3. Reproductive Health Research Center, Department of Obstetrics & Gynecology, Al-Zahra Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran.
4. Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran.

Article code: 1547

Abstract

Background: Fascioliasis is a common disease affecting humans, domestic animals, and wild animals, caused by *Fasciola hepatica* and *F. gigantica*. This study presents the results of a serological evaluation of fascioliasis in pregnant women referred to Al-Zahra Hospital, Rasht, northern Iran.

Methods: Demographic characteristics, clinical symptoms, and eosinophil levels were recorded between September 2024 and February 2025 for pregnant women referred to Al-Zahra Hospital in Rasht. An enzyme-linked immunosorbent assay (ELISA) using somatic antigen of *Fasciola* was conducted on serum samples from participants, which had been stored at -20°C. The results were analyzed using SPSS 25.

Results: The study enrolled 365 pregnant women (gestational age: 1–8 months) aged 15–48 years (mean: 31.9). The seroprevalence of fascioliasis was 3.8% (95% CI: 3.8%, equivalent to 14 individuals). Seropositive women were between 27 and 39 weeks of pregnancy. Of the participants, 92.1% resided in urban areas and 7.9% in rural areas, with 98.6% consuming vegetables washed without disinfectants. Seropositive pregnant women exhibited eosinophilia ranging from 2% to 28%, with a mean of $4.9\% \pm 6.8\%$ (28.5% had hypereosinophilia > 450 per ml of blood). They also showed higher mean WBC (11.2 ± 2.2) and lower Hb (11.6 ± 1.3) compared to other participants. Gastrointestinal symptoms were reported by 63.6% of participants, including 3.4% of those serologically positive for *Fasciola* spp.

Conclusion: Pregnant women represent a critical at-risk group for fascioliasis in Guilan Province, a significant endemic region in Iran, where overlapping pregnancy-related symptoms may lead to delayed diagnosis. The seroprevalence among this group was 3.8% (95% CI: 3.8%,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



equivalent to 14 individuals). Moreover, treatments for *Fasciola* spp. during pregnancy are associated with known adverse effects. Therefore, health education and proper vegetable-washing practices are recommended for vulnerable groups, such as women of childbearing age, in endemic fascioliasis regions.

Keywords: Fascioliasis, ELISA, Pregnant women, Iran.



Prevalence and Subtype Distribution of *Blastocystis* in Humans and Animals in Eastern Iran

Fariba Berenji¹, Saeid Zoghdar Moghadam¹, Ali Gholizadeh³, Mohammad Ali mohaghegh², Mehdi Zarean¹, Lida Jarahi⁴, Bibi Razieh Hosseini Farash¹, Seyed Ali Akbar Shamsian¹, Ghodratollah Salehi Sangani¹

Corresponding Author: Seyed AliAkbar Shamsian, shamsiansaa@mums.ac.ir; Ghodratollah Salehi Sangani, salehisgh@mums.ac.ir

1. Department of Parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Department of Laboratory Sciences, Torbat Heydariyeh University of Medical Sciences, Torbat, Iran.
3. Department of Medical Laboratory Sciences, Faculty of Allied Medicine, Gonabad University of Medical Sciences, Gonabad, Iran.
4. Department of Community Medicine, Faculty of medicine, Mashhad University of Medical sciences, Mashhad, Iran.

Article code: 1558

Abstract

Background: *Blastocystis* sp. is a globally distributed intestinal parasite that infects both humans and animals, with over 38 recognized SSU-rDNA subtypes have been identified in various hosts. this study aimed to determine the geographical distribution of *Blastocystis* sp. subtypes and to discover the potential connection between animals and human subtypes in the Torbat Heydarieh region.

Methods: A total of 730 fecal samples were collected between 2019-2021, including 203 human samples and 527 animal samples (sheep, cattle, poultry, pigeon, quail, and dog). Initially, the samples were examined by direct microscopy (wet mount) and cultivation, followed by molecular confirmation using PCR targeting the SSU rRNA gene. DNA was purified from culture-positive samples, and genetic diversity was assessed by subtype identification. Associations between infection and demographic or behavioral factors (e.g., education, occupation, animal contact, vegetable washing, hand hygiene, clinical symptoms, and season) were also analyzed.

Results: The overall prevalence of *Blastocystis* sp. was 9.3% (68/730) using culture. Of the 49 sequenced isolates, eight subtypes were identified: ST1, ST2, ST3, ST5, ST6, ST7, ST14, and ST15. ST7 (30.6%) and ST3 (26.5%) were predominant subtypes among both human and animal samples. No significant correlations were found between infection rate and demographical factors.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: The results showed that subtype 3 was dominant in humans and subtype 7 was dominant in birds of the region. Since the observation of ST7 in human and ST3 in poultry and sheep were seen, it can lead to the broadening of the range of transmission of subtypes among different animal and human species. The Predominancy of ST3 and ST7 in human and animals indicated the closer relationship between these reservoirs of *Blastocystis* sp. and emphasizing need for further researches to clarify transmission routes and reservoirs.

Keywords: *Blastocystis* sp.; Zoonosis; Subtype; Torbat Heydarieh; PCR.



Molecular Identification and Comparative Analysis of *Echinococcus granulosus* Genotypes in Human and Livestock Hydatidosis in Chaharmahal and Bakhtiari Province, Iran

Mohammad javad Pourmaram¹, Dr. Ebrahim Saedi Dezaki², Mahdiah Valibegi³

Corresponding Author: Dr. Ebrahim Saedi Dezaki, saedii1357@gmail.com

1.MSc Student in Parasitology, Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran.

2.Assistant Professor, Faculty of Medicine, Research Center for Parasitology, Shahrekord University of Medical Sciences, Shahrekord, Iran.

3.MSc Student in Parasitology, Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Article code: 1586

Abstract

Background: Hydatidosis is a globally widespread zoonotic disease caused by the larval stage of *Echinococcus granulosus*. The adult parasite inhabits the intestines of canids, mainly domestic dogs, while intermediate hosts include livestock such as sheep, cattle, pigs, and camels. Humans become accidental hosts through ingestion of parasite eggs. *E. granulosus* exhibits notable genetic diversity, with genotypes G1–G10 identified using mitochondrial and nuclear DNA markers. This diversity affects transmission, pathogenicity, and control strategies. Molecular tools, especially those targeting mitochondrial genes like COx1 (cytochrome c oxidase subunit 1), are highly effective for genotype differentiation and epidemiological studies in endemic regions.

Methods: In this study, 100 human hydatid cyst samples were collected from hospitals in Shahrekord, and 40 animal samples from slaughterhouses in Chaharmahal and Bakhtiari province. DNA was extracted from paraffin-embedded human tissues and fresh animal cysts using a commercial kit. Genotyping was performed using PCR amplification of the COx1 gene, followed by High-Resolution Melting (HRM) analysis and sequencing.

Results: HRM and sequencing of the COx1 gene revealed that all human samples (100%) and most animal samples (95%) belonged to genotype G1. The remaining 5% of animal samples were identified as genotype G3.

Conclusion: Genotypes G1 and G3 of *E. granulosus*, identified via COx1 gene analysis, are responsible for hydatidosis in humans and animals in Chaharmahal and Bakhtiari. These findings provide essential insight into local transmission patterns and support more effective regional prevention and control strategies.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Hydatidosis, *Echinococcus granulosus*, PCR, Zoonotic disease, HRM, Sequencing.



Effects of *Toxocara cati* Larvae and Somatic Antigens of Adult Worms in Experimental Asthmatic Mice

Amin Bakhshani¹, Sima Parandeh¹, Mohsen Maleki², Alireza Haghparast², Hassan Borji²

Corresponding Author: Amin Bakhshani, aminbakhshani@gmail.com

1. Graduate of Veterinary Parasitology (Ph.D degree), Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.

2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1602

Abstract

Background: The hygiene hypothesis suggests that early-life exposure to helminth infections may protect against hypersensitivity and allergic diseases by modulating the immune system. However, some epidemiological studies show that certain parasites, such as *Toxocara*, may increase the risk of allergic conditions like asthma. This study investigates the impact of *Toxocara cati* infection and its somatic antigens on allergic airway inflammation using a murine model.

Methods: Fifty BALB/c mice (5–6 weeks old) were divided into five groups: (1) OVA group, sensitized intraperitoneally with OVA/Alum and challenged with 1% OVA; (2) OVA + somatic extract group, received 20 µg of adult *T. cati* somatic extract during OVA sensitization; (3) *T. cati* egg-infected group, orally administered 500 embryonated eggs; (4) egg-infected + OVA group, both infected and sensitized/challenged with OVA; and (5) PBS control group. Bronchoalveolar lavage (BAL) fluid was collected to assess inflammatory cells. Lung tissues underwent histopathological examination. Cytokine levels (IL-4, IL-5, IL-10) and ovalbumin-specific IgE were measured in BAL and lung homogenates using capture ELISA.

Results: Mice treated with *T. cati* somatic extract showed significantly reduced lung inflammation, as evidenced by decreased peribronchiolitis, perivascularitis, and eosinophilic infiltration, along with lower levels of IL-4 and IL-5 and elevated IL-10, suggesting a shift toward an anti-inflammatory immune profile. In contrast, mice infected with *T. cati* eggs and sensitized with ovalbumin exhibited heightened airway inflammation, characterized by increased eosinophils and total inflammatory cells in BAL fluid, elevated IL-4 and IL-5 levels, and more severe histopathological changes. Moreover OVA-specific IgE was higher in this group.

Conclusion: Overall, the findings suggest that while *T. cati* infection may exacerbate allergic airway inflammation, its somatic extract shows potential for modulating immune responses and reducing inflammation.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Toxocara cati*, Asthma, Cytokines, Mice.



Diagnostic Challenges in Echinococcosis

Abdolhossein Dalimi¹

Corresponding Author: Abdolhossein Dalimi, dalimi_a@modares.ac.ir

1. Department of Medical Parasitology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

Article code: 1606

Abstract

Background: Despite significant advances in the control, diagnosis, and treatment of echinococcosis, many challenges remain in the diagnosis and treatment of this disease. Serological diagnostic methods are not efficient enough to distinguish between active and inactive echinococcosis infections.

Results: Metabolomics technology is now used to detect active and inactive cysts in patients. In addition, proteomics technology is also used through proteomic analysis of plasma exosomes from patients with cystic echinococcosis. New techniques such as Cell free DNA (cfDNA) detection are used to search for diagnostic or prognostic biomarkers of echinococcosis. In the transcriptomics technique, RNA levels across the genome are examined both qualitatively (types of transcripts, identification of new splicing sites, RNA editing sites) and quantitatively (expression level of each transcript). Genomic studies can also help to identify specific targets for numerous highly conserved miRNAs and identify differential expression in *Echinococcus* and uncover the mechanism of interaction between this parasitic worm and its definitive and intermediate hosts, and can be used to develop new interventions and treatments for the control of echinococcosis.

Keywords: Echinococcosis, Diagnostic techniques, Proteomics, Proteomics, cfDNA.



Genotyping of *Neospora caninum* Using Microsatellite Markers: Recent Advances

Asma Keyvanlou Shahrestanaki¹, Mohammad Taghi Vajed Ebrahimi², Gholamreza Razmi¹

Corresponding Author: Gholamreza Razmi, razmi@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Physiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1610

Abstract

Background: *Neospora caninum* is an intracellular protozoan parasite responsible for causing abortion in cattle. Understanding the genetic diversity of *N. caninum* is crucial for better comprehension of its epidemiology and the identification of different strains. Microsatellite markers, due to their high polymorphism and ability to distinguish between various strains, have become powerful tools for genotyping *N. caninum* and studying its genetic structure.

Methods: We systematically searched PubMed, Scopus, and Google Scholar databases for articles published up to May 2025 using keywords such as 'Neospora caninum', 'microsatellite', 'genetic diversity', and 'genotyping'. The genotyping methods discussed in the review primarily focus on the use of microsatellite markers, with PCR amplification protocols and electrophoresis techniques employed for the identification and analysis of various strains.

Results: The application of microsatellite markers has revealed extensive genetic diversity among *N. caninum* isolates globally, challenging the initial perception of a genetically uniform species. The population structure is primarily clonal, maintained by vertical transmission, but also shows geographical variation. Additionally, these markers have been useful in identifying transmission sources and understanding ecological interactions between hosts and environmental factors. Methodological advancements, particularly the development of highly sensitive nested and multiplex PCR assays, have been crucial for successful genotyping from clinical samples, which often contain low quantities of parasite DNA.

Conclusion: Microsatellite markers have proven to be invaluable tools for genotyping *Neospora caninum*, providing insights into the genetic diversity and epidemiology of the parasite. These markers, especially when used for identifying differences between various strains, contribute to a better understanding of their role in transmission and pathogenesis of *N. caninum* infections. These findings can directly inform strategies for controlling and preventing diseases caused by this parasite in livestock animals. Overall, the application of microsatellite



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



markers in future studies, particularly for tracking new strains and identifying drug-resistant genotypes, should be emphasized.

Keywords: Genetic Diversity, Genotyping, Microsatellite Markers, *Neospora caninum*, PCR, Population Genetics.



Detection and Characterization of *Trichinella* Species in Wild Carnivores Across Northern Regions of Iran

Mohammad Reza Khoshvaght¹, Elahe Ebrahimzadeh¹, Nooshinmehr Soleymani¹, Moein Abolhasani Daroukola¹, Hassan Borji¹

Corresponding Author: Mohammad Reza Khoshvaght, khoshvaght77@gmail.com

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1614

Abstract

Background: This study aimed to assess the occurrence and species identification of *Trichinella* in wild carnivores inhabiting different regions of Iran.

Methods: Ninety-three carcasses of wolves, foxes, and jackals collected after road accidents in Ardabil, Golestan, Mazandaran and Razavi Khorasan provinces were examined. Muscle tissues from the tongue, diaphragm, and forelimb were screened for larvae using artificial digestion and direct smear techniques.

Results: *Trichinella* infection was detected in four jackal carcasses originating from Ardabil, Mazandaran, and Razavi Khorasan provinces. Species identification was performed using multiplex PCR targeting ITS1, ITS2, and ESV regions, which produced 127 bp and 253 bp fragments in all positive samples, confirming *Trichinella britovi*. To further validate and evaluate genetic diversity, an 816 bp fragment of the COX1 gene was amplified and sequenced. BLAST analysis revealed a high sequence identity with *T. britovi* reference sequences. Several single nucleotide polymorphisms (SNPs) were detected, indicating potential local genetic variations. Two sequences were submitted to GenBank under accession number PP217417.1.

Conclusion: The detection of *T. britovi* in jackals from northern, northwestern, and northeastern Iran highlights the circulation of this zoonotic parasite in local wildlife. These findings underscore the importance of continuous molecular surveillance and genetic monitoring to better understand its epidemiological, veterinary, and public health relevance.

Keywords: Iran, *Trichinella britovi*, wild carnivores, PCR.



Using Salivary anti- CarLA IgA as a Tool to Manage Gastrointestinal Nematode in Ruminants

Hassan Borji¹

Corresponding author: Hassan Borji, hborji@um.ac.ir

1. *Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*

Article code: 1637

Abstract

Background: Several indirect indicators of gastrointestinal nematode (GIN) burden including fecal egg counts (FECs), dag scores (numerical score of fecal breech soiling), and Faffa Malan Chart (FAMACHA©) anemia scores have been used as tools in genetic selection for GIN monitoring and immunity. However, each has significant limitations. Although FEC is specific to GIN infection, many other factors can influence counts other than immune status. For dag and FAMACHA© scoring, clinically apparent diarrhea and anemia are not specific to GIN infection.

Methods: Measurements of mucosal immunity is a promising method for controlling gastrointestinal nematode (GIN) parasitism in sheep. Salivary antibody to carbohydrate larval antigen (sCarLA), a heritable measure of immunity to third-stage GIN larvae (L3), has been successfully applied to genetic improvement programs in sheep flock. Levels of anti-CarLA salivary IgA antibody (CarLA-IgA) have been shown to be correlated to the level of protective immunity to GIN in sheep and deer but no information is available in cattle.

Result: Levels of GIN-specific mucosal IgA at the gastrointestinal mucosal surface are higher than those of the circulating isoform, and elevated mucosal GIN-specific IgA has been associated with increased growth in lambs. Although GIN-specific IgA is typically highest at the site of infection, IgA level in saliva is reflective of abomasal and small intestinal IgA.

Conclusion: Moreover, saliva can be collected easily and noninvasively from live sheep. CarLA is not a protein antigen and development of immunologic memory is weak, so mucosal IgA declines quickly if exposure is not continuous. The aim of this review is to (i) describe the salivary antibody response to CarLA in sheep managed under grazing conditions, (ii) evaluate the association between salivary antibody to CarLA and GIN parasitism and (iii) investigate the relationship between the salivary antibody response to CarLA and overall health.

Keywords: Salivary anti- CarLA, Gastrointestinal nematode, Immunity, Management.



Nanomedicine in the Treatment of Parasitic Diseases: Advances, Challenges, and Future Directions

Nooshinmehr Soleymani¹, Hassan Borji¹

Corresponding Author: Nooshinmehr Soleymani. nooshinmehrsoleymani@gmail.com

1. Department of Pathobiology Veterinary Medicine Faculty of Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1638

Abstract

Background: Parasitic diseases remain major global health concerns, with high morbidity and mortality especially in endemic regions. Conventional antiparasitic drugs are limited by poor solubility, low bioavailability, systemic toxicity, and emerging resistance. Nanomedicine offers promising alternatives, employing carriers such as liposomes, solid lipid nanoparticles (SLNs), polymeric nanoparticles, nanostructured lipid carriers (NLCs), and chitosan systems to improve delivery, pharmacokinetics, and safety.

Methods: This mini-review summarizes preclinical and translational studies (2022–2025) on nanotechnology-based strategies for helminthic and protozoan infections, focusing on nanoformulations of established agents like albendazole, mebendazole, praziquantel, and ivermectin.

Results: In cestode infections (schistosomiasis, hydatidosis), SLNs and lipid nanocapsules with praziquantel showed superior bioavailability and efficacy in animal models, reducing worm burden, egg counts, and pathology at lower doses. Nano-encapsulated albendazole and mebendazole also exhibited potent activity against hydatid cysts and protoscolices. -In *nematode infections*, albendazole–chitosan nanoparticles achieved higher efficacy against *Trichinella spiralis* even at reduced doses, while lipid carriers enhanced intestinal targeting and prolonged circulation. Biogenic metallic nanoparticles such as silver and selenium demonstrated ovicidal and larvicidal activity against *Haemonchus contortus*. -In *protozoan diseases*, liposomal and NLC formulations of drugs including miltefosine, buparvaquone, and paromomycin improved macrophage uptake, immune responses, lesion reduction, and safety in animal studies. Liposomal amphotericin B remains the only FDA-approved nanomedicine for leishmaniasis, while PLGA-based carriers show promise but are still preclinical.

Conclusion: Nanomedicine presents compelling opportunities for improving antiparasitic therapy. Lipid-based carriers, in particular, provide enhanced efficacy, reduced toxicity, and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



immune modulation. Future work should focus on optimizing nanocarrier design, ensuring translational pipelines, and addressing long-term safety to advance clinical applications.

Keywords: Nanomedicine, Parasitic diseases, Nematode, Cestode.



A Retrospective Analysis of Hydatid Cyst Management and Associated Risk Factors: A 10-Year Study in Mashhad, Iran

Zahra Nehbandani¹, Hassan Borji², Amir sadra Keyvani³

Corresponding Author: Zahra Nehbandani, Nehbandaniz2@mums.ac.ir

1. Head of CDC Mashhad University of Medical science, Mashhad, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
3. Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1649

Abstract

Background: Hydatid disease (cystic echinococcosis) poses a significant health burden in endemic regions like Iran. This study aimed to evaluate the epidemiological profile, clinical management outcomes, and potential risk factors associated with hydatid cyst cases in the Mashhad University of Medical Sciences coverage area.

Methods: A retrospective cross-sectional study was conducted on all confirmed hydatid cyst cases (n=628) recorded between March 2014 and March 2024. Data on demographic characteristics, diagnostic methods, treatment modalities, and potential risk factors were collected and analyzed using descriptive statistics.

Results: The mean age of patients was 36.8 years, with the highest frequency observed in the 30-36 age group. The liver was the most commonly involved organ (62%), followed by the lungs (40%), with some cases showing involvement of both. Computed tomography (CT scan) was the primary diagnostic tool in 45% of cases. Surgical intervention was the most frequent treatment (81%), demonstrating a high success rate of 97%. Pharmacological therapy was used in 58% of cases, often adjunctively. The PAIR (Puncture, Aspiration, Injection, Re-aspiration) technique was used in less than 1% of cases, with no failures reported. Analysis of risk factors revealed that 55% of patients had contact with dogs, and a significant majority (60%) reported washing fruits and vegetables with only plain water, without using detergents or proper disinfectants.

Conclusion: This study highlights surgery as a highly effective treatment for hydatid disease in the region. The strong association with dog contact and inadequate washing of produce underscores the critical need for strengthened public health education focused on hygiene practices and responsible pet ownership to reduce transmission. Furthermore, the underutilization of the PAIR method warrants further investigation into its potential as a less invasive treatment option.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Hydatid Cyst, Risk Factor, PAIR technique, Mashhad.



First Molecular and Morphological Characterization of *Echinococcus equinus* in Iranian Donkeys

Seyed Hossein Hosseini^{1,2}, Fateme Jalousian¹, Mohammad Bolandmartabe¹, Shirin Khodabakhsh¹

Corresponding Author: Seyed Hossein Hosseini, hhoseini@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Iranian Museum of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1650

Abstract

Background: *Echinococcus equinus* (*E. equinus*), a cestode primarily infecting equids, is poorly understood despite its potential zoonotic threat in regions with close contact between equines, and dogs. This study presents the first molecular and morphological characterization of *E. equinus* in Iranian donkeys.

Methods: A total of 97 donkey livers were collected from slaughterhouses in several provinces of Iran between 2023 and 2024. Hydatid cysts were examined morphometrically. Genomic DNA was extracted from the germinal layers, and species confirmation was achieved through PCR amplification and sequencing of the cytochrome c oxidase subunit 1 (*cox1*) and NADH dehydrogenase subunit 1 (*nad1*) genes.

Results: The prevalence of hydatidosis was 5.15% (5/97). Cyst fertility analysis revealed that 20.78% of cysts were fertile and 21.80% were sterile. Rostellar hook morphology exhibited a significantly larger size compared to documented strains from sheep, camels, and buffalo. Phylogenetic analysis confirmed the species identity as *E. equinus* and revealed substantial intraspecific genetic diversity.

Conclusion: This study provides the first report of *E. equinus* in Iran. The findings of genetic divergence and viable cysts underscore a potential, albeit poorly defined, transmission risk. The presence of fertile cysts suggests that the life cycle of *E. equinus* is likely being completed in Iran, indicating the parasite is present in the environment. These results highlight the necessity for expanded surveillance to determine the parasite's full geographic distribution and to develop targeted control strategies.

Keywords: *Echinococcus equinus*, Phylogeny, *coxI*, *nadI*.



Present Status of Visceral Leishmaniasis in the Islamic Republic of Iran

Mehdi Mohebali^{1,2}

Corresponding Author: Mehdi Mohebali, mohebali@tums.ac.ir

1. Professor of the Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
2. Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran.

Article code: 1651

Abstract

Background: Visceral leishmaniasis (VL) is one of the most important neglected tropical diseases (NTD). The zoonotic form of VL is endemic in some parts of at least 6 provinces of Iran. We aimed to determine the status of VL identified in humans and canines in different areas of Iran from the last decade (2013 to 2022).

Methods: A national representative cross-sectional studies were conducted in different areas of Iran. We employed the direct agglutination test (DAT) as an accurate and reliable serological method to detect anti-*Leishmania infantum* antibodies in humans as well as animal reservoir hosts.

Results: The results of 21281 human and 5610 canine serum samples are reported from 2013 to 2022. Altogether, 448 (2.1%, 95%CI: 2.0-2.3) human serum samples showed anti-*L. infantum* antibody levels of $\geq 1:3200$. Of these samples, 13716 (64.5%) were collected actively, which showed a seroprevalence of 0.6% (95% CI: 0.5-0.8) and 7565 (35.5%) were collected passively, which showed a seroprevalence of 4.8% (95%CI: 4.3-5.3). Overall, 1035 (20.1%, 95%CI: 19.0-21.2) of 5160 domestic dogs (*Canis familiaris*) samples showed anti-*L. infantum* antibody levels of $\geq 1:320$. Northwest (2.8%) and northeast (0.96%) regions had the highest human VL seroprevalence, while northwest (21.5%) and south (14.4%) regions had the highest canine VL seroprevalence. The declining trend observed for Human VL seroprevalence compared to CanL in the first decade (2002-2013) and second decade (2013-2022), respectively.

Conclusion: Zoonotic VL, an endemic parasitic disease, is still present in several different areas across Iran. While human VL cases have shown a declining trend over the last decade, the prevalence of canine VL remains significant.

Keywords: Visceral leishmaniasis, Iran, Direct agglutination test, Human.



Biosynthesis of Zinc Oxide Nanoparticles Mediated by Probiotic and Their Bioactivity Against *Giardia* Species in Dogs

Ammar R. Algburi¹, Munaf Al-Dulaimi²

Corresponding Author: Ammar R. Algburi, sc_ammaraalgburi@uodiyala.edu.iq

1.Department of Microbiology, College of Veterinary Medicine, University of Diyala, Iraq.

2.Department of Biology, College of Science, University of Diyala, Iraq.

Article code: 1652

Abstract

This study explores the biosynthesis of zinc oxide nanoparticles (ZnO NPs) mediated by probiotic bacteria and evaluates their bioactivity against *Giardia* species in dogs. Probiotic strains, isolated from canine gastrointestinal microbiota, were utilized to synthesize ZnO NPs through an eco-friendly, extracellular method. Characterization of the synthesized nanoparticles was performed using UV-Vis spectroscopy, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), and transmission electron microscopy (TEM), confirming their nanoscale size, crystalline structure, and stability. The bioactivity of the probiotic-mediated ZnO NPs was assessed in vitro against *Giardia* cysts and trophozoites, demonstrating significant antiparasitic effects, including morphological disruptions and reduced viability. Furthermore, in vivo studies in infected canines indicated a notable reduction in parasitic load with minimal adverse effects, suggesting the potential of probiotic-mediated ZnO NPs as a safe and effective alternative to conventional anti-giardial treatments. These findings highlight the promising application of biosynthesized ZnO NPs as a novel antiparasitic agent, leveraging probiotic bacteria for sustainable nanomaterial production with therapeutic benefits in veterinary parasitology.



Rethinking *Cryptosporidium* Biology: Novel Insights and Intervention Opportunities

Seyed Ali Mousavi Rad¹, Mahdi Naseri¹

Corresponding Author: Seyed Ali Mousavi Rad, alirad_msvi@yahoo.com

1. Nasr pharmaceutical company, Fariman, Iran.

Article code: 1654

Abstract

Background: *Cryptosporidium* has associations with *Escherichia coli*, rotavirus, and coronavirus as major causes of diarrhea in animals and human infants. Given the limited influence of most antiseptic agents and medications on intervention outcomes, prevention is the cornerstone of combating this parasite. This parasite has been recognized for many years, yet there remain substantial gaps in knowledge regarding its risk factors and the design of studies to survey them. This study aims to review articles that highlight elite perspectives and examine underrepresented viewpoints on risk factors, study-design gaps, and treatment options.

Methods: The author reviewed 2 main text book of veterinary internal medicine and 25 articles, of which 7 were metaanalyses. The results are briefly presented in this abstract.

Results: The effectiveness of treating this parasite in humans and animals is challenging. There is a discrepancy between veterinary internal medicine texts on risk factors and oocyst transmission. Most studies focus on the prevalence of this parasite in animals and humans, and there is a large knowledge gap in risk factors.

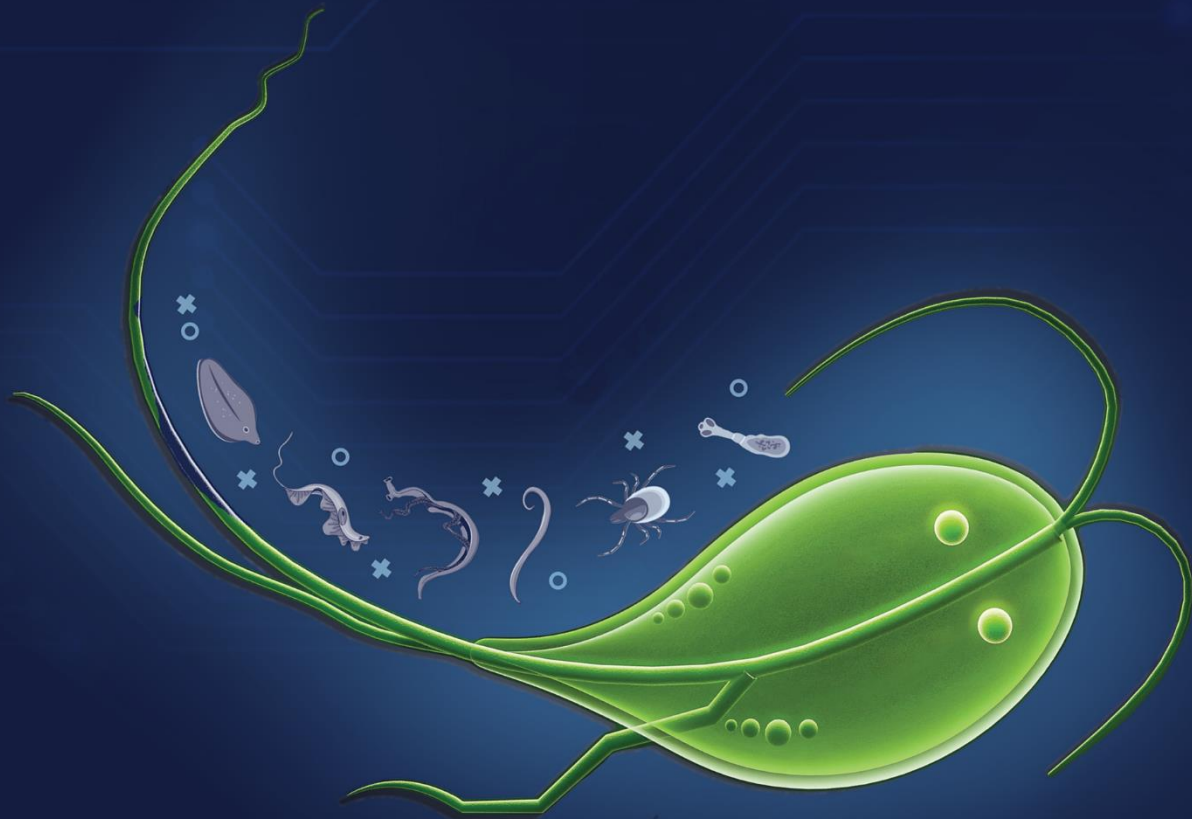
Conclusion: Due to the persistent challenges in the medical management and prevention of cryptosporidiosis in both veterinary and human medicine, there is a strong need to better recognize risk factors for prevention. This underscores the importance of shifting research perspectives toward previously unknown aspects of cryptosporidiosis reservoirs, such as contaminated ground water and its supply.

Keywords: Cryptosporidiosis, Risk factors, Knowledge gap, New approach.



3rd National Congress Of Animal parasitic Diseases and Zoonoses

POSTER PRESENTATION





A One Health-Based Comparative Study of Public Awareness, Attitudes, and Perceptions Toward Food and Waterborne Zoonotic Parasitic Threats in Western Iran: An Immunological Approach to Improving Biosafety

Mehrdad Pooyanmehr¹

Corresponding Author: Mehrdad Pooyanmehr, M.pooyanmehr@razi.ac.ir

1. Department of Basic Sciences and Pathobiology, Branch of Microbiology and Immunology, Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran.

Article code: 1213

Abstract

Background: Zoonotic parasitic infections transmitted through contaminated water and food sources represent a major public health threat, particularly in regions with limited infrastructure and health education. In western Iran, endemic parasitic diseases such as toxoplasmosis, giardiasis, and cryptosporidiosis contribute significantly to the burden of disease among both humans and livestock. This study adopts a One Health approach to examine the role of public awareness and perception in managing such risks. To compare the level of awareness, attitudes, and perceived risks of food- and waterborne zoonotic parasites among urban and rural residents in western Iran, with an emphasis on the implications for integrated health strategies.

Methods: A cross-sectional survey was conducted from July to October 2024 among 20 participants (10 rural, 10 urban) across three provinces in western Iran. A structured questionnaire assessed knowledge, attitudes, practices (KAP), and risk perception related to zoonotic parasitic infections. Statistical analysis was performed using SPSS v25, applying chi-square and logistic regression tests to identify significant associations.

Results: Urban residents demonstrated significantly higher knowledge scores ($P < 0.01$), particularly regarding transmission pathways, prevention strategies, and the role of water and food safety. In contrast, rural participants exhibited more traditional beliefs and lower perceived risk. However, trust in veterinary and health systems was higher among rural residents. Risk perception was directly correlated with educational level and prior exposure to health education campaigns.

Conclusion: There is a notable disparity in awareness and perception of zoonotic parasitic threats between urban and rural populations in western Iran. Bridging this gap through integrated education programs and cross-sectoral collaboration under the One Health framework is essential for improving biosecurity and public health outcomes.

Keywords: Zoonotic parasites, One Health, public awareness, Food-Waterborne, infections



Introduction:

Zoonotic diseases those transmitted from animals to humans pose considerable risks to public health, particularly through food and water contamination. Among these, food and waterborne zoonotic parasites (FWZPs) such as *Toxoplasma gondii*, *Giardia lamblia*, and *Cryptosporidium spp.* represent a significant challenge due to their ability to spread via contaminated food products, undercooked meat, or unsafe water (1). Globally, these infections contribute to high morbidity and mortality rates, especially in regions with limited sanitation and healthcare infrastructure. In western Iran, unique geographical, cultural, and socio-economic conditions intensify vulnerability to FWZPs. Rural populations in particular face challenges due to reliance on livestock, consumption of traditional foods, and limited health resources. Public awareness regarding risks remains insufficient, despite high exposure rates. The *One Health* approach, which emphasizes the interdependence of human, animal, and environmental health, provides an effective framework to tackle such threats (2). Through cross-sector collaboration, it can enhance prevention, surveillance, and education efforts. Understanding public awareness, attitudes, and perceptions is the first step in designing tailored interventions (3). The objectives of this study are to evaluate and compare the levels of awareness, attitudes, and perceptions regarding FWZPs among rural and urban populations, identify socio-demographic predictors of risk perception, and propose integrated strategies based on the *One Health* framework to improve biosafety and public health.

Materials and Methods:

This cross-sectional descriptive-analytic study was conducted between July and October 2024 in three western provinces of Iran: Kermanshah, Ilam, and Kurdistan. These regions were selected due to their high rates of zoonotic diseases and diverse rural-urban demographics.

A total of 20 participants were selected using stratified random sampling: 10 from rural areas and 10 from urban centers. Inclusion criteria included age above 18 and residency of at least 5 years in the study area. The study employed a validated questionnaire divided into four sections: demographic information, knowledge about FWZPs, attitudes toward preventive behaviors, and perception of risk severity and susceptibility. The tool was reviewed by parasitologists and public health experts to ensure content validity.

Statistical analysis was conducted using SPSS v25. Descriptive statistics summarized demographic characteristics. Chi-square tests compared categorical variables between groups, and logistic regression was applied to explore predictors of high-risk perception.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Results and Discussion:

The sample included 55% female and 45% male participants. The mean age was 38.6 years. Urban participants had significantly higher education levels ($p<0.001$).

Knowledge and Awareness: Urban respondents had higher overall knowledge scores (mean score 78.4%) compared to rural participants (mean score 56.7%) ($p<0.01$). Awareness of transmission routes, such as undercooked meat and unwashed vegetables, was more prevalent among urban residents.

Attitudes and Practices: Urban residents reported higher adherence to preventive practices, such as boiling water and avoiding raw vegetables. Rural residents were more likely to trust local veterinary services but were less informed about foodborne parasites.

Risk Perception: Educational attainment, prior participation in health workshops, and having children were associated with higher perceived risk ($p<0.05$). Media exposure was a strong predictor of awareness.

Table 1. Comparative Statistical Analysis of Knowledge, Attitudes, and Risk Perception Regarding Food- and Waterborne Zoonotic Parasitic Diseases in Western Iran (One Health Perspective)

Variable	Group Comparison	Mean / Proportion	Statistical Test	p-value	Interpretation
Gender	Male vs. Female	Male: 45%, Female: 55%	—	—	Gender distribution was balanced among participants.
Age	Overall	Mean: 38.6 years	—	—	Sample was predominantly middle-aged.
Educational Level	Urban vs. Rural	Urban participants had significantly higher levels	Chi-square test	< 0.001	Urban respondents were more highly educated than rural counterparts.
Overall Knowledge Score	Urban vs. Rural	Urban: 78.4%, Rural: 56.7%	Chi-square test	< 0.01	Urban participants demonstrated significantly greater knowledge.
Awareness of Transmission Routes	Urban vs. Rural	Urban higher	Chi-square test	< 0.01	Urban residents were more aware of



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Variable	Group Comparison	Mean / Proportion	Statistical Test	p-value	Interpretation
					contamination routes (e.g., raw vegetables).
Preventive Practices	Urban vs. Rural	Urban higher	Chi-square test	< 0.05	Urban residents more frequently practiced preventive behaviors (e.g., boiling water).
Trust in Veterinary Services	Urban vs. Rural	Rural higher	Chi-square test	< 0.05	Rural participants expressed more trust in local veterinary services.
High Risk Perception	Based on predictors	—	Logistic regression	< 0.05	Higher in individuals with formal education, children, or prior health workshop participation.
Media Exposure	Low vs. High	High exposure associated with greater awareness	Logistic regression	< 0.05	Media was a strong predictor of knowledge and perceived risk.

The analysis revealed significant urban-rural disparities across multiple domains. Urban respondents were significantly more educated, better informed about zoonotic transmission routes, and more likely to adopt preventive health practices, such as boiling drinking water and avoiding consumption of raw vegetables.

The present study revealed marked urban–rural disparities in knowledge, attitudes, and practices related to food- and waterborne zoonotic parasites (FWZPs) in western Iran, echoing findings from similar settings. Urban participants consistently demonstrated higher awareness of transmission routes, stronger risk perceptions, and greater adherence to preventive measures such as boiling drinking water and avoiding raw vegetables (4,5). These patterns align with previous reports that urban environments facilitate access to health information, infrastructure, and public health campaigns, thereby fostering more protective behaviors. In contrast, rural residents showed significantly lower awareness, a trend also documented in other low-resource settings where limited health and educational infrastructure hampers disease prevention.

Interestingly, despite lower awareness scores, rural participants reported higher trust in veterinary services, reflecting a community-based reliance on livestock-related expertise.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Comparable observations in studies from sub-Saharan Africa and South Asia suggest that veterinarians and local animal health workers can serve as critical channels for zoonotic disease education in rural contexts. Logistic regression further identified education level, participation in health workshops, and parenthood as significant predictors of heightened risk perception findings consistent with earlier research highlighting the role of life experience and exposure to formal training in shaping health behaviors. Media exposure also emerged as a powerful determinant of awareness, supporting evidence from other studies that mass communication can effectively bridge knowledge gaps when tailored to local contexts (6).

While unsafe water consumption and traditional dietary practices exacerbate rural vulnerability, urban populations benefit from both better infrastructure and broader access to preventive information. These results parallel findings from studies in Latin America and Southeast Asia, which emphasize how social determinants such as education and healthcare access stratify disease risk across populations (7,8). Importantly, this study highlights opportunities for intervention: veterinary networks and rural health workers may be leveraged to deliver targeted, culturally adapted health education, while urban populations may respond more effectively to mass media campaigns (9).

Overall, the findings underscore the need for locality-specific One Health strategies. Interventions should not only address informational and infrastructural deficiencies in rural settings but also capitalize on existing trust in veterinary and community health services. Similar to recommendations from global health research, integrating tailored educational workshops, school-based programs, and mobile health initiatives could significantly enhance public awareness and promote sustainable behavioral change. By situating the results within the broader literature, this study reinforces the critical role of equitable health education in reducing the burden of FWZPs across diverse populations.

This study highlights significant disparities in awareness, attitudes, and perceptions of FWZPs between rural and urban populations in western Iran. Urban participants displayed greater awareness, preventive practices, and access to health information, while rural residents exhibited lower knowledge but greater reliance on veterinary services. Key predictors of higher risk perception included education, exposure to health campaigns, parenthood, and media influence. These results emphasize the need for *One Health*-based, locally adapted public health strategies that bridge educational and resource gaps.

To strengthen biosafety and public health outcomes, targeted education campaigns should be designed for rural populations, focusing on transmission routes and preventive behaviors. Veterinary services can act as trusted channels for disseminating health information. Media-based interventions may significantly enhance awareness and attitudes across all populations. Additionally, cross-sector collaboration is essential for implementing sustainable prevention



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



programs. Integrating these strategies under the One Health framework can effectively mitigate FWZP-related risks, fostering healthier and more resilient communities in western Iran.

References:

1. Shaheen MN. The concept of one health applied to the problem of zoonotic diseases. *Reviews in medical virology*. 2022 Jul;32(4):e2326.
2. Cheng R, Mantovani A, Frazzoli C. Analysis of food safety and security challenges in emerging African food producing areas through a One Health lens: the dairy chains in Mali. *Journal of food protection*. 2017 Jan 1;80(1):57-67.
3. Khatri P, Kumar P, Shakya KS, Kirlas MC, Tiwari KK. Understanding the intertwined nature of rising multiple risks in modern agriculture and food system. *Environment, Development and Sustainability*. 2024 Sep;26(9):24107-50.
4. Antwi-Agyei P, Peasey A, Biran A, Bruce J, Ensink J. Risk perceptions of wastewater use for urban agriculture in Accra, Ghana. *PloS one*. 2016 Mar 15;11(3):e0150603.
5. Lacroix BJ. Impact of a boil water advisory on in-home water and food handling practices: A qualitative study (Doctoral dissertation, University of Guelph).
6. Kumar V, Meena HR. Role perception and performance of privately practicing Paravets in delivery of animal health Services at Farmers' doorstep: an exploratory study in India. *Tropical Animal Health and Production*. 2021 May;53(2):278.
7. Cooper PJ, Rodrigues LC, Cruz AA, Barreto ML. Asthma in Latin America: a public health challenge and research opportunity. *Allergy*. 2009 Jan;64(1):5-17.
8. Chongsuvivatwong V, Phua KH, Yap MT, Pocock NS, Hashim JH, Chhem R, Wilopo SA, Lopez AD. Health and health-care systems in southeast Asia: diversity and transitions. *The Lancet*. 2011 Jan 29;377(9763):429-37.
9. Theobald S, Brandes N, Gyapong M, El-Saharty S, Proctor E, Diaz T, Wanji S, Elloker S, Raven J, Elsey H, Bharal S. Implementation research: new imperatives and opportunities in global health. *The Lancet*. 2018 Nov 17;392(10160):2214-28.



Assessment of Knowledge and Attitudes of Industrial and Traditional Livestock Farmers Toward the Role of Vaccination and Management of Zoonotic, Emerging, and Re-emerging Parasitic Infectious Diseases: An Essential Immunological and Biosafety Approach

Mehrdad Pooyanmehr¹

Corresponding Author: Mehrdad Pooyanmehr, M.pooyanmehr@razi.ac.ir

1. Assistant Professor of Immunology, Department of Basic Sciences and Pathobiology, Branch of Microbiology and Immunology, Faculty of Veterinary Medicine, Razi University, Iran.

Article code: 1214

Abstract

Background: Zoonotic parasitic infectious diseases, including emerging and re-emerging forms, present persistent threats to both animal and public health. Preventive strategies grounded in immunology particularly vaccination and biosafety are essential for controlling these infections. Understanding farmers' knowledge and attitudes is a crucial step in improving disease management. This study evaluated the knowledge and attitudes of industrial and traditional livestock farmers toward vaccination and the management of zoonotic, emerging, and re-emerging parasitic infectious diseases.

Methods: A cross-sectional survey was conducted among 20 livestock farmers, equally divided between industrial (n = 10) and traditional (n = 10) systems. A validated questionnaire assessed knowledge, attitudes, and practices. Data were analyzed using descriptive statistics and inferential tests. Chi-square tests were applied to categorical variables, and independent-samples t-tests were used for continuous scores. Statistical significance was set at $p < 0.05$.

Results: Industrial farmers scored significantly higher in overall knowledge (mean $\pm$ SD: 78.4 ± 6.2) compared to traditional farmers (65.1 ± 8.7 ; $t = 3.72$, $p = 0.0018$). Awareness of emerging parasitic diseases and biosafety protocols was notably higher among industrial farmers ($\chi^2 = 6.40$, $p = 0.011$). However, both groups demonstrated limited understanding of the immunological mechanisms underlying vaccination ($p > 0.05$). Attitudinal analysis revealed that industrial farmers were more likely to adopt preventive measures ($\chi^2 = 5.67$, $p = 0.017$), whereas traditional farmers favored treatment after disease onset.

Conclusion: The statistically significant differences between farming systems underscore the need for targeted training particularly for traditional farmers to strengthen immunological literacy and biosafety practices, ultimately reducing zoonotic parasitic disease risks.

Keywords: Awareness, Biosafety, Immunology, Parasitic Diseases, Vaccination.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Introduction:

Zoonotic parasitic infectious diseases remain a major challenge to veterinary public health, livestock productivity, and rural livelihoods worldwide. These diseases, which can be transmitted between animals and humans, encompass a wide range of etiological agents including protozoa, helminths, and ectoparasites. In recent decades, the global livestock sector has faced increasing threats from emerging and re-emerging parasitic infections, driven by climate change, intensified animal movement, antimicrobial resistance, and evolving pathogen ecology. The complexity of their epidemiology, coupled with the socio-economic burden they impose, underscores the urgent need for effective prevention, early detection, and control strategies (1).

Among preventive measures, vaccination stands as one of the most effective tools to reduce both incidence and severity of infectious diseases. However, the success of vaccination programs in livestock populations is highly dependent on farmers' awareness, attitudes, and willingness to implement immunization and complementary biosafety measures. The scientific foundation for these measures lies in immunology, which provides the rationale for herd immunity, antigen-specific protection, and long-term disease suppression. Nevertheless, gaps persist between theoretical recommendations and on-the-ground adoption, particularly in smallholder and traditional farming systems.

Previous studies have shown that knowledge, attitude, and practice (KAP) assessments are essential in identifying behavioral and informational gaps among farming communities. Research in sub-Saharan Africa, South Asia, and parts of the Middle East has demonstrated that low awareness of zoonotic parasitic diseases directly correlates with higher prevalence rates. In addition, studies have highlighted differences between industrial and traditional livestock systems in terms of disease management capacity, access to veterinary services, and biosafety infrastructure. However, comparative research integrating immunological literacy, vaccination adoption, and biosafety practices in the context of both emerging and re-emerging parasitic diseases remains limited (2).

The novelty of the present study lies in its dual-system comparative approach evaluating industrial and traditional livestock farmers simultaneously and its focus on the intersection of immunology, vaccination, and biosafety as a unified framework for controlling zoonotic parasitic diseases. Unlike most prior KAP studies that address either bacterial or viral zoonoses, this research explicitly targets parasitic infectious diseases, which are often neglected despite their significant contribution to morbidity, economic loss, and food insecurity. Furthermore, by integrating statistical analysis of farmer responses with a focus on immunological understanding, this study provides an evidence-based platform for designing tailored educational interventions.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



The specific objectives of this research are:

1. To assess and compare the level of knowledge and attitudes of industrial and traditional livestock farmers regarding vaccination and management of zoonotic, emerging, and reemerging parasitic infectious diseases.
2. To evaluate differences in immunological understanding and biosafety adoption between the two farming systems.
3. To identify statistically significant gaps that can inform targeted extension programs and policy recommendations.

By addressing these objectives, the study aims to contribute to the global One Health agenda by reducing zoonotic transmission risks, improving livestock health, and enhancing farmer resilience. The findings are expected to be directly relevant for veterinary epidemiologists, public health authorities, and agricultural policy-makers seeking to implement cost-effective, immunity-based, and biosafety driven disease control strategies.

Materials and Methods:

Study Design and Population: A cross-sectional, questionnaire-based survey was conducted to assess the knowledge and attitudes of livestock farmers toward vaccination and management of zoonotic, emerging, and re-emerging parasitic infectious diseases. The study population comprised 20 farmers (10 operating industrial livestock farms and 10 managing traditional systems) selected purposively to ensure representation of both production models.

Questionnaire Development: A structured questionnaire was developed based on relevant literature and expert consultation in veterinary parasitology and immunology. The instrument consisted of three sections: (i) demographic information, (ii) knowledge and awareness of parasitic diseases, vaccination, and biosafety, and (iii) attitudes toward preventive versus curative disease management. The questionnaire was pre-tested with two farmers from each system to ensure clarity and content validity.

Data Collection: Face-to-face interviews were conducted by trained researchers between [Month–Month, Year], ensuring consistent administration of the instrument. Responses were recorded verbatim to minimize interviewer bias.

Statistical Analysis: Data were analyzed using SPSS version 20. Descriptive statistics (mean, standard deviation, frequency, percentage) were used to summarize responses. Independent-samples t-tests compared mean knowledge scores between industrial and traditional farmers. Chi-square tests assessed associations between categorical variables such as awareness level and adoption of preventive measures. Statistical significance was set at $p < 0.05$.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Ethical Considerations: The study protocol was approved by the [Name of Ethical Committee/Institutional Review Board]. Participation was voluntary, informed consent was obtained from all respondents, and data were anonymized prior to analysis.

Results and Discussion:

Participant Characteristics: A total of 20 livestock farmers participated in the study, comprising 10 industrial and 10 traditional producers. The mean age of respondents was 43.7 ± 8.9 years, with no statistically significant difference between the two groups ($p = 0.42$). Educational background varied, with industrial farmers more frequently holding secondary or higher education qualifications compared to traditional farmers.

Knowledge Scores: Industrial farmers demonstrated significantly higher mean knowledge scores regarding zoonotic, emerging, and re-emerging parasitic infectious diseases than traditional farmers (78.4 ± 6.2 vs. 65.1 ± 8.7 , respectively; $t = 3.72$, $p = 0.0018$).

Awareness of Biosafety and Emerging Parasites: Awareness of biosafety protocols and emerging parasitic threats was higher in industrial farmers (80%) than in traditional farmers (30%), yielding a statistically significant association ($\chi^2 = 6.40$, $p = 0.011$).

Immunological Understanding: Both groups showed limited understanding of immunological mechanisms underlying vaccination, with no significant difference between them ($p > 0.05$).

Attitudes Toward Disease Management: A significantly greater proportion of industrial farmers expressed willingness to adopt preventive measures (70%) compared to traditional farmers (20%) ($\chi^2 = 5.67$, $p = 0.017$). Traditional farmers were more likely to rely on treatment after clinical disease onset.

Table 1. Comparison of knowledge, awareness, and attitudes between industrial and traditional livestock farmers regarding zoonotic parasitic infectious diseases.

Variable	Industrial (n = 10)	Traditional (n = 10)	Statistical Test	p-value
Knowledge score (mean $\pm$ SD)	78.4 ± 6.2	65.1 ± 8.7	$t = 3.72$	0.0018
Awareness of biosafety & emerging parasites (%)	80%	30%	$\chi^2 = 6.40$	0.011
Immunological understanding (%)	40%	30%	$\chi^2 = 0.22$	0.640



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Variable	Industrial (n = 10)	Traditional (n = 10)	Statistical Test	p-value
Willingness to adopt preventive measures (%)	70%	20%	$\chi^2 = 5.67$	0.017

Note: Knowledge scores are based on a 0–100 scale. Percentages indicate the proportion of respondents with correct or positive responses. Statistical significance set at $p < 0.05$.

The statistical analysis revealed clear and significant differences between the two farming systems in both knowledge levels and preventive attitudes. Industrial farmers were not only more knowledgeable about zoonotic parasitic diseases but also more aware of biosafety measures and more inclined toward preventive management. These findings align with prior literature linking higher biosafety adoption to better resource access and veterinary service availability in industrial systems. Conversely, the limited immunological understanding in both groups suggests a critical gap that could undermine optimal vaccine use, highlighting the need for targeted training programs across farming systems.

This study examined the knowledge and attitudes of industrial and traditional livestock farmers toward vaccination, immunology, and biosafety in the context of zoonotic, emerging, and re-emerging parasitic infectious diseases. The findings revealed statistically significant differences between the two production systems, with industrial farmers demonstrating higher knowledge scores, greater awareness of biosafety protocols, and stronger preventive attitudes. These results are consistent with the hypothesis that access to structured veterinary services, formal education, and commercial market demands contribute to improved adoption of disease prevention strategies.

The observed disparity in knowledge scores (78.4 vs. 65.1, $p = 0.0018$) aligns with previous KAP studies by Dunga et al., (2025) and Hobbs et al., (2021), which reported that commercial or semi-intensive farmers exhibited better awareness of parasitic zoonoses compared to smallholder or traditional producers. In both contexts, knowledge gaps were linked to lower participation in extension programs and reduced exposure to scientific training. Our findings similarly indicate that traditional farmers' reliance on post-infection treatment, rather than preventive measures, reflects both limited awareness and economic constraints (3,4).

The significant association between production system and biosafety awareness ($\chi^2 = 6.40$, $p = 0.011$) reinforces the conclusions of Majiwa et al. (2023), who found that farm infrastructure and standardized hygiene protocols were more prevalent in industrial systems. Importantly, our results extend these observations by specifically addressing emerging and re-emerging parasitic diseases, a category often underrepresented in zoonotic disease research, which tends to focus on bacterial and viral pathogens (5).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



One of the most notable findings is the absence of significant differences in immunological understanding between the two groups ($p > 0.05$). This gap is consistent with reports by Abunna et al. (2024), who documented that farmers frequently accept vaccination as a routine practice without comprehending its immunological basis. Such a knowledge deficit has important implications: without understanding vaccine mechanisms, farmers may fail to complete full vaccination schedules or may underestimate the need for herd immunity, thereby reducing overall program effectiveness (6).

The novelty of this study lies in its integrated approach, combining immunological literacy, vaccination attitudes, and biosafety practices into a single analytical framework, and applying it to both industrial and traditional systems. While prior research has typically assessed these factors separately, our results provide a more holistic picture of the behavioral and informational landscape influencing parasitic disease control.

From a One Health perspective, these findings highlight critical intervention points. For industrial farmers, maintaining high awareness levels requires ongoing professional training and incorporation of updated parasite surveillance data into management protocols. For traditional farmers, targeted extension services and subsidized vaccination programs may be necessary to overcome both informational and resource-based barriers. Additionally, educational modules explaining the immunological rationale for vaccination could improve compliance and promote sustained preventive behaviors in both groups.

Overall, this study contributes to the growing body of evidence that disease control is not solely a technical or biomedical challenge but also a socio-behavioral one. Bridging knowledge and attitude gaps particularly regarding parasitic zoonoses can enhance livestock health, safeguard public health, and reduce economic losses. Future research should expand the sample size, incorporate longitudinal follow-up to measure behavioral change over time, and explore the cost-effectiveness of integrated biosafety, immunology educational interventions.

This study demonstrates that industrial livestock farmers possess significantly higher knowledge, biosafety awareness, and preventive attitudes toward zoonotic, emerging, and re-emerging parasitic infectious diseases compared to traditional farmers, although both groups exhibit limited immunological understanding. These findings highlight the necessity for targeted educational interventions particularly for traditional farmers focusing on the immunological basis of vaccination and practical biosafety measures. Strengthening such competencies can improve vaccination compliance, reduce disease incidence, and align livestock management practices with One Health objectives. Future efforts should integrate extension services, policy support, and community-based training to bridge behavioral and informational gaps across production systems.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Weiss LM. Zoonotic parasitic diseases: emerging issues and problems. *International journal for parasitology*. 2008 May 25;38(11):1209.
2. Deiana G, Arghittu A, Dettori M, Castiglia P. One world, One health: Zoonotic diseases, parasitic diseases, and infectious diseases. *InHealthcare* 2024 Apr 29 (Vol. 12, No. 9, p. 922). MDPI.
3. Dunga KE, Okoro CI, Onyenama AC, Ekuma UO, Ohanusi IN, Izah SC. Implementing One Health approach to emerging zoonotic diseases: Bridging surveillance, sustainability and global governance. *Exon*. 2025 Jul 9;2(3):200-23.
4. Hobbs EC, Colling A, Gurung RB, Allen J. The potential of diagnostic point-of-care tests (POCTs) for infectious and zoonotic animal diseases in developing countries: Technical, regulatory and sociocultural considerations. *Transboundary and Emerging Diseases*. 2021 Jul;68(4):1835-49.
5. Majiwa HO. Knowledge, Attitudes, and Practices on Zoonotic Diseases and Control Among Actors in Livestock Trade in Busia County, Western Kenya (Doctoral dissertation, University of Nairobi).
6. Abunna F, Gebresenbet G, Megersa B. Assessment of knowledge, attitude and practices (KAP) of farmers about transmission of zoonotic diseases in Ada'a district, Oromia, Ethiopia. *Heliyon*. 2024 Feb 29;10(4).



Comparative Evaluation of Farmers' Awareness and Preventive Measures Against Arthropod-Borne Protozoan Infections in Western Iran, with a Focus on Immunopotentiating Vaccine Use

Mehrdad Pooyanmehr¹

Corresponding Author: Mehrdad Pooyanmehr, M.pooyanmehr@razi.ac.ir

1. Assistant Professor of Immunology, Department of Basic Sciences and Pathobiology, Branch of Microbiology and Immunology, Faculty of Veterinary Medicine, Razi University, Iran.

Article code: 1215

Abstract

Background: Arthropod-borne infectious diseases such as theileriosis and babesiosis represent major challenges to livestock health and food safety, particularly in endemic regions. Farmers' knowledge and implementation of preventive strategies, including the use of immunopotentiating vaccines, are critical for effective disease control. This study aimed to comparatively assess the awareness, attitudes, and practices of traditional versus industrial livestock farmers concerning arthropod vectors, related infectious diseases, and the application of immunostimulatory vaccines in western Iran.

Methods: A cross-sectional study was conducted using stratified random sampling among 100 livestock farmers (50 traditional, 50 industrial) across the provinces of Kermanshah, Kurdistan, and Ilam. Data collection involved structured questionnaires and face-to-face interviews. Statistical analyses were performed using independent t-tests and Chi-square tests to identify significant differences between the two farmer groups.

Results: Industrial farmers demonstrated significantly higher awareness scores (mean: 7.8/10) compared to traditional farmers (mean: 4.5/10) ($p < 0.01$). Regular usage of Immunopotentiating vaccines was reported by 85% of industrial farmers, whereas only 55% of traditional farmers adhered to such practices ($p < 0.05$). The prevalence of theileriosis was markedly lower in industrial herds (10%) than in traditional ones (28%). Likewise, babesiosis prevalence was 12% in industrial and 33% in traditional systems ($p < 0.01$). Food safety assessments revealed that 76% of industrially produced animal products met hygienic standards, in contrast to only 42% in traditional systems ($p < 0.05$).

Conclusion: The higher level of awareness and greater adoption of immunoadjuvant vaccination among industrial farmers are associated with significantly reduced disease incidence and improved animal health and food safety indicators. Strengthening educational



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



outreach and enhancing vaccine accessibility for traditional farmers could substantially improve rural biosecurity and public health outcomes within a One Health framework.

Keywords: Traditional-Industrial farmers, Arthropod-borne diseases, Parasitic infections, Immunopotentiating vaccines, Western Iran.

Introduction:

Arthropod-borne infectious diseases, particularly theileriosis and babesiosis, pose significant challenges to livestock health and food safety in many endemic regions globally (1,2). These diseases are primarily transmitted by ticks and other arthropod vectors and result in considerable morbidity and mortality in ruminants, leading to decreased productivity and potential threats to food security (3). Their persistence, especially in tropical and subtropical regions like western Iran, highlights the urgent need for improved disease control strategies and farmer education.

In recent years, the use of immunization strategies has gained attention as an effective method to enhance host immunity and reduce the impact of these diseases (4,5). In Iran, vaccines commonly used in livestock farms include inactivated and live attenuated vaccines for diseases like theileriosis and babesiosis. These vaccines have been shown to effectively control the spread of vector-borne protozoan infections in cattle, helping to reduce disease incidence and improve livestock health outcomes.

Despite the availability of these vaccines, there are disparities in their adoption between traditional (smallholder) and industrial (commercial) livestock farming systems. Traditional farmers often face challenges such as limited access to veterinary services, lack of awareness, and economic constraints, resulting in inconsistent or minimal vaccine use (7,8). On the other hand, industrial farmers generally have greater access to veterinary support, improved biosecurity practices, and better infrastructure, enabling them to implement regular vaccination schedules and other preventive measures more effectively (9).

These disparities have significant implications for both animal health and productivity, as well as for food safety and public health, underlining the importance of a One Health approach (10).

The objective of this study is to conduct a comparative evaluation of the awareness and preventive measures against arthropod-borne protozoan infections between traditional and industrial livestock farmers in western Iran, with a particular focus on the utilization of immunization practices and their effects on livestock health and food safety compliance.



Materials and Methods:

This cross-sectional descriptive-analytical study was conducted over a one-year period from early 2023 to late 2024 across three western provinces of Iran: Kermanshah, Kurdistan, and Ilam. These regions were selected due to their ecological suitability for arthropod proliferation and high prevalence of tick-borne diseases in livestock.

A total of 100 livestock farmers were enrolled in the study, comprising 50 traditional (smallholder) and 50 industrial (commercial) farmers. Participants were selected through stratified random sampling, ensuring proportional representation across provinces and livestock production systems. Inclusion criteria required participants to be actively engaged in cattle farming and willing to participate in structured interviews.

Data collection was performed using a validated structured questionnaire, developed based on previous studies and expert consultation. The questionnaire encompassed the following domains:

- General awareness of arthropod vectors (e.g., ticks, flies)
- Knowledge of major diseases (e.g., theileriosis, babesiosis)
- Understanding of transmission cycles and risk factors
- Familiarity with and usage of conventional vaccines commonly used in Iranian farms, such as live attenuated *Theileria annulata* vaccines (e.g., "Tropical Theileriosis Vaccine") and inactivated *Babesia* vaccines, which are administered under veterinary supervision as part of national or regional disease control programs
- Farm hygiene practices and veterinary oversight
- Attitudes toward animal health and food safety

In addition to self-reported data, field observations and direct interviews were conducted to cross-validate questionnaire responses. Veterinary records and on-site inspections were used to assess disease prevalence, vaccination history, and compliance with hygiene protocols for milk and meat products.

Data were entered and analyzed using SPSS software version 26. Descriptive statistics (means, standard deviations, frequencies) were calculated. Independent-samples t-tests were used to compare continuous variables (e.g., awareness scores), and Chi-square tests were used to assess associations between categorical variables (e.g., vaccine use, disease prevalence, compliance with hygiene standards). A p-value < 0.05 was considered statistically significant.



Results and Discussion:

The analysis revealed significant differences in awareness, practices, and outcomes between traditional and industrial livestock farmers:

Awareness Scores: The mean awareness score among industrial farmers was 7.8 ± 1.1 out of 10, significantly higher than the score of 4.5 ± 1.6 reported by traditional farmers ($p < 0.01$). Awareness parameters included recognition of vector species (e.g., *Hyalomma*, *Rhipicephalus*), understanding of disease transmission pathways, and the preventive role of vaccination and hygiene.

Vaccine Usage: Among industrial farmers, 85% reported regular use of immunoadjuvant vaccines, particularly formulations based on Freund's Complete Adjuvant (FCA) and Poly(lactic-co-glycolic acid) (PLGA). In contrast, only 55% of traditional farmers reported occasional or inconsistent use of vaccines ($p < 0.05$), with many citing lack of access or insufficient knowledge.

Disease Prevalence: The burden of disease was considerably higher in traditional livestock systems: *Theileriosis* prevalence was 28% in traditional herds versus 10% in industrial herds. *Babesiosis* prevalence was 33% among traditional farmers and 12% among industrial farmers ($p < 0.01$ for both diseases). Vaccinated farms, particularly those using PLGA-based vaccines, reported lower clinical incidence, reduced veterinary costs, and improved average daily weight gain in affected animals.

Food Safety and Hygiene Compliance: Adherence to veterinary hygiene standards (including cold chain maintenance, regular inspection, and hygienic slaughtering and milking practices) was substantially higher among industrial farmers (76% compliance) compared to traditional farmers (42% compliance) ($p < 0.05$). The correlation between good hygiene and lower disease prevalence was statistically significant.

These findings collectively indicate that improved awareness, vaccine adherence, and biosecurity measures in industrial livestock systems contribute to better health outcomes, reduced reliance on therapeutic drugs, and enhanced food safety. Traditional systems, while resource-limited, showed potential for improvement through targeted training and improved access to veterinary services and immunization programs.

The findings of this study revealed significant differences in awareness levels between the two groups. Industrial farmers achieved an average awareness score of 7.8 out of 10, significantly higher than traditional farmers' score of 4.5 ($p < 0.01$) (11). This difference may be attributed to better access to training, extension services, and information dissemination systems within industrial farming structures (12). In contrast, traditional farmers, often operating in more remote or resource-limited settings, are less exposed to such educational opportunities (13).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



The use of immunopotentiating vaccines was markedly more prevalent among industrial farmers (85%) compared to traditional farmers (55%) ($p < 0.05$) (14). This disparity indicates the critical role of infrastructure, veterinary accessibility, and awareness in vaccine uptake. Studies have shown that the consistent application of adjuvanted vaccines particularly PLGA-based formulations can lead to more robust immune responses, reduced clinical disease incidence, and improved productivity (15,16).

Disease prevalence data further support the efficacy of such interventions. Theileriosis and babesiosis rates were substantially lower in industrial herds (10% and 12%, respectively) compared to traditional systems (28% and 33%) ($p < 0.01$ for both) (17). These findings align with earlier research demonstrating the role of vaccination and biosecurity in lowering tick-borne disease burdens (18). In vaccinated herds, especially those using PLGA-adjuvanted vaccines, reduced veterinary costs and enhanced weight gain were also observed (19). Food safety metrics mirrored these patterns. Hygiene compliance assessed through on-site veterinary inspections, cold chain maintenance, and slaughterhouse protocols was significantly higher among industrial farms (76%) than among traditional operations (42%) ($p < 0.05$) (20). This finding is consistent with the One Health perspective, where improved farm-level practices contribute to safer food systems and lower zoonotic risk (21,22).

Collectively, the results underscore the need to improve outreach to traditional farmers through targeted training programs and improved vaccine accessibility. By addressing these gaps, rural communities can achieve substantial gains in animal health, biosecurity, and ultimately, public health.

This study highlights the marked disparities in awareness, vaccine adoption, and disease outcomes between traditional and industrial livestock systems in western Iran. Industrial farmers benefit from greater knowledge, regular vaccination practices, and improved hygiene, resulting in lower prevalence of theileriosis and babesiosis and higher compliance with food safety standards. In contrast, traditional farmers remain vulnerable due to limited access to preventive tools and training. Enhancing veterinary extension services, facilitating affordable vaccine access, and providing ongoing education are key strategies to elevate rural livestock management. Implementation of such measures would not only reduce disease incidence but also support safer food production and improved public health under the One Health framework.

Acknowledgments:

We would like to express our sincere gratitude to all the livestock farmers who participated in this study and generously shared their valuable insights and experiences. Special thanks to the veterinary professionals and local authorities in Kermanshah, Kurdistan, and Ilam for their



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



continuous support and cooperation throughout the research. We also extend our appreciation to the institutions and organizations that provided financial and logistical assistance, making this study possible.

References:

1. Jongejan F, Uilenberg G. The global importance of ticks. *Parasitology*. 2004;129(S1): S3–S14.
2. Liew WH, et al. Understanding the global burden of tick-borne diseases. *J Med Entomol*. 2018;55(5):1057–65.
3. Khan MA, et al. The impact of arthropod-borne diseases on livestock productivity: A global perspective. *Livest Sci*. 2017; 195:82–91.
4. Gasser RB, et al. Immunological control of parasitic diseases in ruminants: Vaccines and adjuvants. *Vet Parasitol*. 2006;137(1–2):27–37.
5. Kahn LH, Line S. Immunology of infectious diseases in veterinary medicine. *J Vet Intern Med*. 2010;24(1):1–10.
6. Mohammed OA, et al. Effects of immunization on livestock productivity: A case study in Ethiopia. *Afr J Agric Res*. 2018;13(5):220–27.
7. Bishopp DC, Norval RA. Tick-borne diseases of livestock in Southern Africa. *Vet Parasitol*. 2014;203(1–2):1–10.
8. Barger IA, et al. Livestock production and disease management strategies in industrial and traditional systems. *J Anim Sci*. 2015;93(6):2744–54.
9. Daskalova P, et al. Tick-borne diseases in the European Union: A review. *Vet Res Commun*. 2019;43(4):77–89.
10. Destoumieux-Garzón D, et al. The One Health Concept: 10 Years Old and a Long Road Ahead. *Front Vet Sci*. 2018; 5:14.
11. Rehman A, et al. Factors influencing the adoption of preventive health practices among livestock farmers in developing countries. *Trop Anim Health Prod*. 2018;50(3):529–36.
12. Lindsey P, et al. Building capacity for wildlife health: Challenges and solutions. *OIE Sci Tech Rev*. 2016;35(1):277–88.
13. Ziv G, et al. Education and extension in the prevention of infectious diseases in rural settings. *Vet Microbiol*. 2000;74(3):221–34.
14. Gubbins S, et al. Evaluating vaccination strategies against livestock diseases. *Prev Vet Med*. 2014;117(1):1–10.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



15. Bonnet S, et al. Immunological and epidemiological benefits of tick-borne disease vaccines. *Infect Genet Evol.* 2017; 49:1–10.
16. Chakraborty S, et al. Development and performance of PLGA-based adjuvants in livestock. *J Control Release.* 2016; 235:184–93.
17. Ahmed J, et al. Epidemiology of theileriosis in Pakistan: A comparative analysis. *Pak Vet J.* 2015;35(3):215–20.
18. Grillo SP, et al. Tick-borne diseases in livestock: Prevention and control strategies. *Vet Res.* 2020;51(1):60–72.
19. Rehman A, et al. A review of PLGA adjuvants in veterinary vaccines. *Vet World.* 2019;12(7):944–52.
20. Silva JB, et al. Hygiene practices and microbial quality of animal products. *Food Control.* 2016; 63:27–32.
21. Kock RA, et al. Towards a One Health approach for emerging zoonoses. *Lancet Infect Dis.* 2014;14(9):820–26.
22. Lerner H, Berg C. A comparison of three holistic approaches to health: One Health, EcoHealth, and Planetary Health. *Front Vet Sci.* 2017; 4:163.



Copro-microscopic Study on Helminthic Infections of the Digestive and Respiratory Tracts of Goats in East Azerbaijan Province

Abbas Imani Baran¹, Ahmad Nematollahi¹, Mohammad Kashani¹, Parisa Shahbazi¹

Corresponding Author: Abbas Imani Baran, a.imani@tabrizu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Article code: 1222

Abstract

Background: This study aimed to determine the prevalence of helminthic infection in goats of East Azerbaijan province through faecal examination based on the characteristics of the animals (sex, age, and breed) and substudy regions.

Materials: A total of 100 goat faecal samples from 10 cities of the province were randomly taken and examined using Clayton-lane and Bermann methods.

Results: The results showed that the infections with the eggs of unknown Nematodes, *Nematodirus*, *Fasciola*, *Trichuris*, *Dicrocoelium* and *Marshallagia* were 19, 13, 6, 4, 2 and 1% respectively, and also with the larvae of *Mulerius*, *Dictyocaulus*, *Prtostrongylus* and *Cystocaulus* were 21, 18, 11 and 5%, respectively. Cestoda infection was not observed. Jolfa (20%) and Shabestar (5%) and Miyaneh (5%) showed the highest and lowest rates of infections, respectively.

Conclusion: The helminthic infection rate in goats of the subsidy areas was considerable.

Keywords: Goat, Helminthic Infection, Faecal examination, East Azerbaijan.

Introduction:

The increasing population and the subsequent increase in demand for protein materials require an increase in livestock products. In many countries of the world, including Iran, the livestock industry is considered the main source of human protein supply. One of the most common livestock diseases, which constantly threatens the health of livestock and presents the livestock industry with major challenges, is parasitic infections. Helminthic infections are considered the most common parasitic infections of a wide range of domestic animals. Therefore, in order to improve the quantity and quality of the livestock industry, especially goat breeding, it is of particular importance to pay attention to the issue of identifying and combating parasitic diseases. In Iran, goats are considered an important source of livestock products, and helminthic infection in goats can have a significant impact on the economy (1). The findings of the studies highlight the importance of controlling helminthic infections in goats, especially



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



in areas with poor sanitary conditions. Effective control measures can help reduce the level of infection and improve the health and productivity of goats. In addition, farmers and veterinarians should be aware of the risk factors associated with helminthic infection and take appropriate preventive measures to reduce the economic losses caused by these parasites (2). Of the 70 million domesticated small ruminants in Iran, about 25 million are Iranian goats. According to studies of ruminant helminthic parasites in Iran, 50 species of nematodes have been reported in the digestive tract of ruminants, 3 species of trematodes and 6 species of nematodes in the lungs, and at least 5 species of trematodes in the liver. Many infections caused by these helminths are subclinical, and even many experienced livestock farmers and veterinarians are unable to diagnose this form of parasitic infection in livestock without paraclinical examinations. Therefore, correct diagnosis of these cases of helminthic infections is of great importance and, in addition to maintaining the health of livestock, will increase the food requirements of humans (3). Undoubtedly, investigating the status of parasitic infections of specific areas can lead to better identification, and more favorable control of specific parasites. This study was conducted to investigate the level of gastrointestinal and respiratory parasites in goats in East Azerbaijan province based on fecal examination.

Materials and Methods:

In February and March 2023, in order to collect fecal samples from 100 goats, 10 counties of East Azerbaijan Province (Table 1) were visited and the samples were taken directly from the rectum of the animals. The clinical signs and all necessary variables affecting helminthic infection of the goats were also recorded. The samples were immediately transferred to the parasitology laboratory of the Faculty of Veterinary Medicine, University of Tabriz, and tested at the appropriate time. The Clayton-Lane method was used to count eggs and the Bermann method was used to count the larvae of respiratory tract nematodes (3). Statistical analysis of the results was performed using One-way ANOVA and SPSS version 26 software.

Results and Discussion:

In this study, the eggs of *Marshallagia*, *Trichostrongylus*, *Nematodirus*, *Fasciola*, eggs containing larvae, and larvae of *Mullerius*, *Dictyocaulus*, *Protostrongylus*, and *Cystocaulus* (Figures 1-9) were identified. The details of the results are shown in Tables 1 and 2. In general, in all cities, the rate of respiratory infection was higher than that of digestive one. In addition, no cases of cestode infection were observed.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad

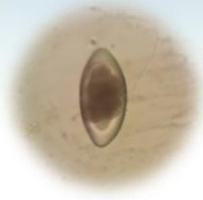


Fig 1: egg containg larva

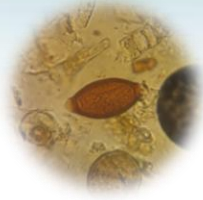


Fig 2: Fasciola egg



Fig 3: Nematodirus egg



Fig 4: Trichuris egg



Fig 5: Marshalagia egg



Fig 6: Cystocaulus larva

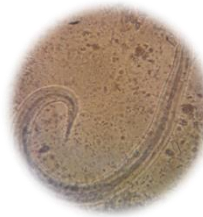


Fig 7: Prtostongylus larva



Fig 8: Dictyocaulus larva



Fig 9: Mulerius larva

Table1. Total egg/larva of the identified helminths in faecal samples of goats

	Kaleybar	Hashtrud	Jolfa	Miyaneh	Sarab	Bonab	Marand	Shabestar	BostanAbad	Tabriz	Total (%)
Nematodirus	49	24	193	59	78	84	48	39	35	48	657(13)
Marshalagia	8	2	43	12	22	29	22	4	13	29	184(4)
Trichuris	1	0	6	1	6	4	7	4	3	3	35(1)
Unknown N	123	93	210	36	120	79	74	54	63	79	928(19)
Fasciola	66	43	71	9	29	23	11	16	12	7	287(6)
Dicrocoelium	24	18	32	5	13	10	8	5	5	4	124(2)
Cestoda	0	0	0	0	0	0	0	0	0	0	0
Mulerius	203	99	149	48	73	82	108	65	99	71	1049(21)
Dictyocaulus	196	61	143	42	67	70	154	36	80	68	918(18)
Prtostongylus	128	53	95	14	31	30	102	24	42	25	544(11)
Cystocaulus	72	18	36	2	7	13	44	3	24	18	237(5)
Total	780	411	978	228	446	424	578	250	376	352	4963(100)
Mean±SEM of egg	27±4	18±3	51±7	12±2	27±5	23±5	17±4	12±2	13±3	17±3	22±2
Mean±SEM of larva	60±7	23±3	42±9	11±3	18±4	19±6	46±7	13±3	24±2	18±3	27±2



Based on the results of analysis with Tukey and Bonferroni tests, there was a significant difference in egg infection only between Jolfa and 9 other cities ($p < 0.05$).

There was a significant difference in larval infection between the following cities ($p < 0.05$):

1) Tabriz with Marand, Jolfa, and Kaleybar; 2) Bostan Abad with Kaleybar; 3) Shabestar with Marand, Jolfa, and Kaleybar; 4) Marand with Tabriz, Shabestar, Bonab, Sarab, and Miyaneh; 5) Bonab with Marand and Kaleybar; 6) Sarab with Marand, Jolfa, and Kaleybar; 7) Miyaneh with Marand and Jolfa; 8) Jolfa with Tabriz, Shabestar, Sarab, and Miyaneh; 9) Hashtrud with Kaleybar; and 10) Kaleybar with Tabriz, Bostan Abad, Shabestar, Bonab, Sarab, Miyaneh, and Hashtrud.

Overall, the infection differences between the following cities was significant ($p < 0.05$):

1) Tabriz with Jolfa and Kaleybar; 2) Bostan Abad with Jolfa and Kaleybar; 3) Shabestar with Marand, Jolfa and Kaleybar; 4) Marand with Shabestar Miyaneh; 5) Bonab with Jolfa and Kaleybar; 6) Sarab with Jolfa and Kaleybar; 7) Miyaneh with Marand and Jolfa; 8) Jolfa with Tabriz, Bostan Abad, Shabestar, Bonab, Sarab, Miyaneh, Hashtrud; 9) Hashtrud with Jolfa and Kaleybar; and 10) Kaleybar with Tabriz, Bostan Abad, Shabestar, Bonab, Sarab, Miyaneh, and Hashtrud

This study showed that the infection rate is higher in goats kept in poor sanitary conditions. The results of this study confirm that goats have a higher level of immunity to gastrointestinal parasites than sheep, and that parasitic infections in goats are lower than in sheep. The prevalence of infection in the studied herds and the prevalence of the number of parasites were different. The high prevalence of helminthic infection in goats in East Azerbaijan province is consistent with the previous studies in Iran and other countries. The higher infection rate in young and female goats can be attributed to their poor immune system and hormonal changes, respectively. The higher infection rate in goats kept in poor sanitary conditions is probably due to greater exposure to larvae and infective eggs in contaminated food, water, and soil.

A group of nematodes is involved in nematode infections of the digestive and respiratory tracts. Under natural conditions, parasitic infections of the digestive and respiratory tracts are rarely caused by a single species. Even in clinical infections attributed to a single species, such as *Haemonchus contortus* or *Nematodirus*, a number of other species are usually also present. Statistics show that in developing countries, nematodes play the greatest role in causing economic losses (3). Many studies have been conducted to investigate the infection rate in sheep and goats in the world. However, the specifically studies conducted on parasitic infections in goats are rare. In Kashan, helminthic infection of the gastrointestinal tract of sheep and goats in the region was reported to be *Trichostrongylus* (17-58%) and *Nematodirus* (10-72%) (4). The infection rate with *Marshallagia marshalli* was reported to be 69.6% in small livestock in Yazd city (5). Also, in another similar study in Kermanshah province, the infection



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



rates for *Dictyocaulus*, *Protostrongylus* and *Cystocaulus* were reported as 63%, 18%, and 11%, respectively (6). Investigation on the infection with gastrointestinal and external parasites in goats in West Azerbaijan province showed that out of 304 fecal samples examined, eggs of unknown nematodes (*Strongylid* form) were found in 206 (51.11%), *Marshallagia* in 37 (9.18%), *Nematodirus* in 12 (2.97%), *Trichuris* in 58 (14.39%), *Cystocaulus* in 3 (0.75%), *Moniezia* in 23 (5.7%), and *Fasciola* in 4 (1%). The EPG range in the infection with gastrointestinal parasites was 3-172 (7).

References:

1. Hosseinzadeh S, Rafat S, Moghaddam G, Nematollahi A, Hajializadeh R.. Evaluation of trichostrongylidae parasites infestation of Ghezel sheep in East Azerbaijan Province using fecal eggs test. *Vet Clin Pathol*. 2016; 10(1 (37): 61-7.
2. Jithendran K, Bhat T. Epidemiology of parasitoses in dairy animals in the Northwest Humid Himalayan Region of India with particular reference to gastrointestinal nematodes. *Trop Anim Health Prod*. 1999; 31: 205-14.
3. Eslami A. *Veterinary Helminthology, Nematoda and Acanthicefala*. 3^{ed} ed, Vol 3, Tehran: Tehran University press, 2018.
4. Talari SA, Arbabi M. Prevalence of gastrointestinal tract *Trichostrongylus* in sheep and goats in slaughterhouse of Kashan. *Feyz*, 2005; 9(3): 34-8.
5. Etminani-Rad S, Mubedi I. Frequency of super family species of *Trichostrongyloida* in the small animals slaughtered in Yazd in 1382-1383. *Pajhouhesh va Sazandegi*, 2007; 20(2): 197-9.
6. Chale-Chale AA, Karimi A. Survey on helminths infection in gastrointestinal of slaughtered sheep in Kermanshah city, Iran. *Veterinary Journal*, 2010; 4(4): 17-22.
7. Tavasoli M, Dalirnegade B, Sahandi A. Study on gastrointestinal and external parasites of goats in west Azerbaijan-Iran. *Vet Res J*. 2011; 66(1): 77-9.



Study on Digestive Tract Helminthic Infection of Wild Rabbits in Northwest of Iran

Abbas Imani Baran¹

Corresponding Author: Abbas Imani Baran, a.imani@tabrizu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Article code: 1223

Abstract

Background: Given that wild rabbits play an important role in the food chain of birds and carnivorous mammals in the wildlife, it is important to identify their threats. From a parasitological perspective, one of the threats to wild rabbits is gastrointestinal helminthic parasites. For this reason, this study was conducted to investigate the status of helminthic infection in wild rabbits in northwest Iran.

Methods: 45 samples of the digestive tract of wild rabbits hunted in northwest Iran, were collected from hunters during the hunting season. The samples were transferred to the Faculty of Veterinary Medicine, University of Tabriz. After separating and opening the stomach and intestines and removing parasites, slid preparations from parasite samples were provided using standard preparation methods and the helminths were identified by valid references.

Results: Of the 45 intestinal samples, 8(17.8%) and 21 (37.8%) cases were infected with *Mosgovoyia pectinata* (cestode) and *Nematodirus leporis* (nematode), respectively.

Conclusion: The infection rate and diversity of removed helminths from wild rabbits were not considerable. Therefore, further study need to be conducted to verify our results.

Keywords: Helminthic infection, Wild rabbits, Faecal examination, Northwestern Iran.

Introduction:

Rabbits are small mammals of the order Lagomorpha and the family Leporidae. Eleven genera have been identified for the family Leporidae, and the genus *Lepus* includes the true rabbits. One of the most important species of the genus *Lepus* is the European rabbit (*Lepus europaeus*), also known as the brown wild rabbit, the eastern long-eared rabbit, and the eastern meadow rabbit, and has the widest geographical distribution in the Palearctic region. The global distribution of this species is in northern, central, and western Europe and western Asia, including northern occupied Palestine, northern Iraq, and western Iran (1, 2). Rabbits, as intermediate or definitive hosts, are infected with a wide range of ecto- and endo-parasites throughout their lives. The most common endoparasites in rabbits are helminths of the digestive



tract. Severe infections with helminthic endoparasites in rabbits negatively affect the natural conditions of the body, causing reduced survival of the host, increased capture by hunters, reduced fertility of female rabbits, mortality in all age groups, and undoubtedly such negative consequences play an important role in disrupting the natural balance of the rabbit population in a region (1, 3-5). Given that wild rabbits play a very important role in the food chain of birds and carnivorous mammals in the wildlife, it is important to identify their threats. From a parasitological perspective, one of the threats to wild rabbits is gastrointestinal helminthic parasites. For this reason, this study was conducted to investigate the status of helminthic infection in wild rabbits in northwest Iran from October 1 to March 31, 2019.

Materials and Methods:

In this study, 45 samples of the digestive tract of wild rabbits hunted in northwest Iran (East and West Azerbaijan provinces) were collected from hunters during the hunting season. To prevent autolysis, the samples were transferred to the Faculty of Veterinary Medicine, University of Tabriz as soon as possible. After separating and opening the stomach and intestines and isolating parasites if infected, the parasite samples were stored in an appropriate manner for morphological examination. Using standard preparation methods (6), laboratory slides were prepared from the cestode and nematode samples and identified with valid diagnostic keys (7, 8).

Results and Discussion:

Of the 45 intestinal samples, 8(17.8%) and 21 (37.8%) cases were infected with *Mosgovoyia pectinata* (cestode) and *Nematodirus leporis* (nematode), respectively. The cestodes removed from the small intestine averaged 20 cm in length and 1.4 cm in width (Figure 1). The scolex was small, and each adult proglottid had a pair of genital pores located in the middle third of the lateral margin of each proglottid (Figure 1: A-C). The width of the proglottids was thoroughly much greater than their length (more than 4 times), and this difference was much greater in immature proglottids. The eggs in the gravid proglottids were triangular or pyramidal (8). The anterior part of *N. leporis* had a very pronounced apical expansion. The average length and width of males were 12mm and 100µm, respectively, with two equal thin spicules approximately 1 mm length. The average length and width of females were 16mm and 200µm, respectively. Also, the uterus had the typical spindle-shaped eggs characteristic of *Nematodirus* spp (7). (Fig. 2: A-I)



Figure 1. *Mosgovoyia pectinata* (A: General view of adult Cestode; B: Triangular eggs; C: Genital pore)

In Spain, in the studies on parasitic infections of rabbits *Oryctolagus caniculus*, *L. europaeus* and *L. granatensis*, the infection with *M. pectinata* (9), and infection of *O. caniculus* with *M. ctenoides* and *M. pectinata* (10) were reported. In the British Isles, due to the presence of only the species *Lupus capensis*, *M. pectinata* is the most common species in this area (11). In a study of the prevalence of parasitic infections of desert hares in Iran (1.6%) (12), Iberian hares (*L. granatensis*) in Spain (17.2%) (13), and farmed rabbits in Poland (72%) only *M. pectinata* has been reported (14); whereas in *O. caniculus* in England (15), Scotland (16), Spain (11, 17) and Southern Ireland (18), only *M. ctenoides* has been reported. In the present study, the infection rate in 45 rabbit gastrointestinal samples was 17.8%.

In our study, only *N. leporis* was removed from the gastrointestinal tract of wild rabbits. In similar studies in East Azerbaijan, the same species was reported in 13.33% of wild rabbits (19) and 32% of New Zealand white laboratory rabbits (20). Also, in Turkey, 100% of wild rabbits were reported to be infected with *N. leporis* (7). In Spain, *Nematodirus* spp (13), *N. spathiger* (10), *N. vatus* and *N. filicollis* (21) were reported in the infected rabbits (*O. caniculus*), and recently, *N. leporis* was reported from domestic rabbits (*O. caniculus*) in Ukraine (22).



Figure 2. Different parts of male and female of *Nematodirus leporis* (A and B: anterior view of adult helminthes; C: general view of adult helminthes; D-F: posterior view of male; G: posterior view of female; H and I: eggs inside the uterus of female)

References:

1. Rewatkar SG, Deshmukh SS, Prem Kumar G, Maske DK, Bhangale GN. Occurrence of gastrointestinal helminths in rabbits with special reference to importance of *Giardia* spp. as parasitic zoonoses. *Sci Tech Arts Res J.* 2013; 2(3): 142-3.
2. Tavasoli M, Bakht M. Occurrence of *Linguatula Serrata* in European hare in Iran. *J Zoonoses.* 2014; 1(1): 64-7.
3. Marhoon IA, Mattar KT, Mohammad FI. Parasitic infection in wild rabbits *Oryctolagus cuniculus*. *Eurasian J Anal Chem.* 2018; 13(5); em55.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



4. Tanjung M, Rangkuti P. Species and prevalence of rabbit gastrointestinal parasites in Berastagi Farm Karo District, North Sumatra, Indonesia. In proceedings of the International Conference on Natural Resources and Technology (ICONART 2019), 193-8.
5. Ball S, Kelly TC, Butler F. Endoparasites of the endemic Irish hare *Lepus timidus hibernicus*. Wildlife Biol. 2020; wlb.00717. doi: 10.2981/wlb.00717.
6. Eslami A, Ranjbar Bahadori Sh. Diagnostic Helminth Infections. Tehran; Nooubakhsh press, 1st ed, 1383
7. Doğanay A, Gürler AT. Yaban tavşanında *Nematodirus leporis*, Chandler, 1924 olgusu. Ankara Üniv Vet Fak Derg. 2007; 54: 141-3.
8. Haukisalmi V, Hardman LM, Foronda P, Feliu C, Henttonen H. Systematic relationship of *Mosgovoyia Spasskii*, 1951 (Cestoda: Anoplocephalidae) and related genera inferred from mitochondrial and nuclear sequence data. Syst Parasitol. 2010; 77: 71-9.
9. Casanova JC, Feliu C, Molina X, Villa T, Santalla F, Laplana N. Helminths in a contact zone of *Lepus europaeus* and *L. granatensis* (Leporidae) in Iberian Peninsula. Acta Parasitologica. 2000; 45: 176.
10. Cordero DEL, Campillo M, Castanon L, Reguera A. Indice-Catalogo de Zooparasitos Ibericos. 2nd ed. Secretariado de Publicaciones, Leon, Spain, 1994.
11. Foronda P, Valladares B, Lorenzo-Morales J, Ribas A, Feliu C, Casanova JC. Helminths of the wild rabbit (*Oryctolagus cuniculus*) in Macaronesia. J Parasitol. 2003a; 89(5): 952-7.
12. Eslami A, Changizy E, Moghadam M. Prevalence of Helminth Infections in the Cape Hare (*Lepus capensis*) in Iran. Vet Res Communic. 2000; 24: 455-8.
13. Segovia JM, Vila T, Vargas JM, Fuentes MV, Feliu C. The helminth community of the Iberian hare, *Lepus granatensis* (Lagomorpha: Leporidae), in the province of Granada, Spain. Helminthologia. 2014; 51(4): 281-7.
14. Szkuciket K, Pyz-Łukasik R, Szczepaniak KO, Paszkiewicz W. Occurrence of gastrointestinal parasites in slaughter rabbits. Parasitol Res. 2014;113: 59-64.
15. Allan JC, Craig PS, Sherington MT, Rogan MT, Storey DM, Heath S, Iball, K. Helminthparasites of the wild rabbit *Oryctolagus cuniculus* near Malham Tarn, Yorkshire, UK. J Helminthol. 1999; 73: 289-94.
16. Boag B, Lello J, Fenton A, Tompkins DM, Hudson PJ. Patterns of parasite aggregation in the wild european rabbit (*Oryctolagus cuniculus*). Int J Parasitol. 2001; 31: 1421-8.
17. Foronda P, Del Castillo A, Abreu N, Figueruelo E, Piñero J, Casanova JC. Parasitichelminths of the wild rabbit, *Oryctolagus cuniculus*, in different bioclimatic zones in Tenerife, Canary Islands. J Helminthol. 2003b; 77: 305-9.
18. Butler FT. Arthropod and helminth parasites from rabbits *Oryctolagus cuniculus* in south-west Ireland. The Irish Naturalists' J. 1994; 24(10): 392-5.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



19. Garedaghi Y, Hashemzadefarhang H. Prevalence rate of Endoparasites in Wild Rabbits of East Azerbaijan Province, Iran. *Annal Biol Res.* 2011; 2(6): 31-5.
20. Hajipour N, Zavarshani M. Ectoparasites and endoparasites on New Zealand White rabbits from northwest of Iran. *Iranian J Parasitol.* 2020; 15(2): 266-71.
21. Blasco S, Torres J, Feliu C, Casanova JC, Miquel J, Moreno S (). The helminth fauna of *Oryctolagus cuniculus* (Linnaeus, 1758) in the Iberian Peninsula. Faunistic and ecological consideration. *Parasite.* 1996; 4: 327–33.
22. Bogach M, Paliy AP, Horobei OO, Perotska LV, Kushnir VY, Bohach DM. Endoparasites of rabbits (*Oryctolagus cuniculus domesticus*) in Southern Ukraine. *Biosyst Divers.* 2022; 30(2): 173-8.



Emerging Perspectives on Foodborne and Waterborne Parasites: From Epidemiology to Carcinogenesis

Fatemeh Rasekhnia¹

Corresponding Author: Fatemeh Rasekhnia, fatemehra8020@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Islamic Azad University, Kazeroon Branch, Kazeroon, Iran.

Article code: 1241

Abstract

Background: Foodborne and waterborne parasites remain a major public health concern worldwide. Recent advances presented at the European Multicolloquium of Parasitology (EMOP 2020/2021) highlight new perspectives ranging from classical epidemiological insights to novel associations with carcinogenesis.

Methods: This review summarizes key developments concerning *Toxoplasma gondii*, *Cryptosporidium*, *Opisthorchis felinus*, and *Trichinella* spp., with emphasis on prenatal screening programs, molecular epidemiology, emerging links to cancer, transcriptomic approaches, wildlife reservoirs, and waterborne transmission.

Results: The review highlights advances in the understanding of *Toxoplasma gondii*, *Cryptosporidium*, *Opisthorchis felinus*, and *Trichinella* spp. These include new findings regarding prenatal screening programs, molecular epidemiology, links to cancer, and waterborne transmission. Additionally, critical research gaps remain concerning *Taenia solium* and *Echinococcus multilocularis*.

Conclusion: Foodborne and waterborne parasites are of multidimensional importance. The advancements discussed in this review offer directions for future research, particularly in areas of prenatal screening, cancer associations, and transmission pathways.

Introduction:

Parasitic diseases transmitted via food and water are among the most important global health threats. According to the World Health Organization, they cause significant morbidity and mortality, particularly in low-income countries. The European Multicolloquium of Parasitology (EMOP) serves as a major platform for sharing advances in parasitology. The 13th EMOP, held in Belgrade in 2021, placed strong focus on foodborne and waterborne parasites, revealing not only traditional epidemiological findings but also groundbreaking insights linking parasitic



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



infections with cancer biology, genetic mechanisms, ecological dynamics, and waterborne transmission risks.

Materials and Methods:

Toxoplasma gondii: Screening and Public Health

Toxoplasma gondii is one of the most significant foodborne parasites worldwide, particularly in relation to congenital infection. Historical developments in prenatal screening, dating back to the mid-20th century, have greatly reduced severe neonatal disease in some regions. However, debate continues on whether routine screening remains justified. Recent reviews highlight both the successes and limitations of past programs, and stress the importance of evaluating new diagnostic tools and molecular approaches in shaping the future of toxoplasmosis control.

Cryptosporidium: Beyond Gastroenteritis

Cryptosporidium spp. are recognized primarily as agents of diarrheal illness, yet new research has expanded their relevance. Advances in multilocus genotyping have enabled highly discriminatory subtyping for outbreak investigation, surpassing the widely used gp60-based typing. An even more provocative development is the hypothesis that *Cryptosporidium* infection may contribute to carcinogenesis. Animal models suggest potential mechanisms involving altered signal transduction and promotion of digestive adenocarcinoma. In addition, outbreak data from France (2017–2020) reported an unusually high number of cryptosporidiosis events, including a school outbreak linked to contaminated curd cheese and a cluster among firefighters exposed during removal of cattle carcasses.

Opisthorchis felinus and Carcinogenesis

Liver flukes of the family Opisthorchiidae, including *Opisthorchis felinus*, are long recognized as carcinogenic. Recent transcriptomic studies in experimental hamster models have provided unprecedented insights into host responses. Distinct gene sets were activated in acute versus chronic infection, with epithelial-mesenchymal transition (EMT) emerging as the most enriched pathway. These findings support deeper exploration of the molecular mechanisms driving bile duct cancer in infections with *O. felinus* and related species such as *O. viverrini* and *Clonorchis sinensis*.

Trichinella in Wildlife: Ecology and Transmission

Trichinellosis continues to be a relevant zoonosis, particularly where wild animal reservoirs intersect with human practices. A comprehensive 30-year review of Romanian wildlife revealed that strictly carnivorous mammals, such as canids and felids, harbor higher prevalence rates of *Trichinella* compared to omnivores like bears and wild boars. Two species, *T. britovi*



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



and *T. spiralis*, dominate the landscape. However, the relative contribution of each host species to maintaining sylvatic and domestic transmission cycles remains unresolved, underscoring the need for continued ecological and molecular surveillance.

Results and Discussion:

Despite wide coverage, the EMOP Special Issue did not address two parasites of outstanding importance. *Taenia solium*, ranked the most significant foodborne parasite globally by FAO/WHO, was absent from discussion. Similarly, *Echinococcus multilocularis*, a parasite of highest concern in Europe, was not represented. The omission of these pathogens underscores gaps that future meetings and publications should prioritize.

Several innovative findings have emerged from recent studies. In *Cryptosporidium*, multilocus genotyping has been validated as a superior tool for outbreak tracing, while experimental data suggest potential links to gastrointestinal cancers. For *Opisthorchis felinus*, transcriptomic profiling revealed epithelial-mesenchymal transition (EMT) as a key pathway activated during chronic infection, providing new mechanistic insights into carcinogenesis. In *Trichinella*, long-term surveillance in Romania confirmed the disproportionate role of wild carnivores in maintaining the parasite, raising questions about their contribution to domestic cycles. Finally, advances in prenatal screening for *Toxoplasma gondii* highlight the evolving role of modern molecular tools in human healthcare.

Comparative Role in Animals and Humans: The role of foodborne and waterborne parasites differs between animals and humans. In livestock and wildlife, these organisms often serve as reservoirs, sustaining transmission cycles. In humans, the parasites manifest through a spectrum of diseases, ranging from congenital infections to carcinogenesis. Table 1 summarizes their impact.

Table 4. Comparative Role in Animals and Humans

Parasite	In Animals (Livestock/Wildlife)	In Humans
<i>Toxoplasma gondii</i>	Infects livestock (sheep, goats, pigs) and wildlife; source of human infection through meat or oocysts shed by cats.	Congenital toxoplasmosis a major concern; prenatal screening reduces severe neonatal disease.
<i>Cryptosporidium</i> spp.	Affects calves, lambs, and farm animals; reservoirs in	Causes diarrheal outbreaks; novel hypothesis of gastrointestinal



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Parasite	In Animals (Livestock/Wildlife)	In Humans
	livestock and wildlife maintain transmission.	carcinogenesis; multiple outbreaks documented.
Opisthorchis felineus	Studied in experimental animals (hamsters); fish-eating mammals act as reservoirs.	Recognized carcinogen linked to cholangiocarcinoma; EMT pathway activation in chronic infection.
Trichinella spp.	High prevalence in wild carnivores (wolves, foxes, felids); omnivores (wild boar, bears) less affected; domestic pigs bridge to humans.	Human infection via undercooked pork or wild boar; outbreaks of trichinellosis; severity depends on species and parasite load.

Waterborne Transmission and Control

Water plays a pivotal role in the transmission of several parasites. *Cryptosporidium* oocysts are highly resistant to chlorine and can persist in drinking water and recreational water, making them a major cause of waterborne outbreaks. *Toxoplasma gondii* oocysts shed by cats can contaminate surface water, leading to human infection and affecting even marine wildlife. Waterborne routes thus represent both direct risks to humans and indirect risks by sustaining transmission in animals. Future solutions include advanced membrane filtration, routine molecular monitoring of water sources, and public education on avoiding untreated water consumption.

Control and Prevention Strategies

Effective prevention requires an integrated approach. Molecular diagnostics, including multilocus genotyping, enable precise tracking of outbreaks. In livestock, improved farm hygiene and monitoring of wildlife reservoirs are essential for reducing risk. Food safety measures, such as thorough cooking of meat and strict quality control of dairy products, remain fundamental. Public health strategies should emphasize awareness of zoonotic risks and proper water sanitation. At the policy level, stronger international collaboration and prioritization of neglected parasites such as *Taenia solium* and *Echinococcus multilocularis* are urgently needed.

The future of research on foodborne and waterborne parasites lies at the intersection of epidemiology, molecular biology, and public health. In animals, parasites will continue to threaten livestock productivity and food safety, with *Trichinella* and *Cryptosporidium*



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



remaining key concerns. In humans, beyond acute infections, parasites may increasingly be recognized as contributors to chronic diseases and cancers. Climate change, globalization of food trade, and increasing mobility will further shape parasite transmission dynamics. Addressing these challenges will require sustained funding, cutting-edge molecular tools, ecological studies, and global cooperation.

Foodborne and waterborne parasites remain a persistent and evolving challenge. Recent discussions emphasize their role not only in acute and chronic infections but also in complex processes such as carcinogenesis. The integration of molecular tools, epidemiological surveillance, ecological studies, and water safety interventions will be critical in reducing their burden. Future research must also address underrepresented yet critical parasites, including *Taenia solium* and *Echinococcus multilocularis*. International cooperation, sustained funding, and innovative approaches are required to meet these challenges.

References:

1. Rather SA, et al. Carcinogenic parasites: insights into the epidemiology and possible mechanisms of cancer. *Mutagenesis*. 2025 Jun.
2. MDPI. Food and drinking water as sources of pathogenic protozoans: updated knowledge on risk factors and prevention. *Appl Microbiol*. 2024 Jun 20.
3. MDPI. Food and waterborne cryptosporidiosis from a One Health perspective: global burden and outbreak insights. *Animals*. 2023.
4. *Frontiers in Microbiology*. Bacterial and parasitic pathogens as risk factors for cancers in the gastrointestinal tract: epidemiological overview. *Front Microbiol*. 2021.
5. Biomed Central – Infect Agents Cancer. Exploring the antitumor potential of *Toxoplasma gondii*: immunomodulation and therapy prospects. *Infect Agents Cancer*. 2025.
6. Daher D, et al. Comprehensive overview of *Toxoplasma gondii*-induced and associated diseases: molecular players and therapeutic gaps. *Pathogens*. 2021;10(11):1351.
7. Preprints.org. Protozoan infections acquired from food or drinking water: an updated overview of risk factors, outbreaks, and prevention. *Preprints*. 2023.
8. Oxford University Press. Molecular detection of *Toxoplasma gondii* DNA in cancer tissue samples: prevalence and associations in various malignancies. *Trans R Soc Trop Med Hyg*. 2025.



A Rare Case of Sarcoptic Mange Transmission from a Pet Rabbit to Humans

Ali Samemaleki¹

Corresponding Author: Ali Samemaleki, arvin.veterinary@gmail.com

1. Department of Veterinary Medicine, Tabriz Azad University, Tabriz, Iran.

Article code: 1255

Abstract

Background: Sarcoptic mange is a well-known zoonotic parasitic disease, typically seen in dogs and cats. However, awareness regarding its transmission from rabbits remains limited. This case illustrates zoonotic transmission from a pet rabbit to humans.

Methods: A two-year-old neutered male rabbit with alopecia, pruritus, and scaling was examined. Skin scraping confirmed *Sarcoptes scabiei*. Within a week, three of four family members developed pruritic rashes. Clinical data and treatment outcomes were documented.

Results: The rabbit was treated with ivermectin (injectable and topical), propoxur, lindane shampoo, and supportive medications. Humans were treated with topical permethrin, antiparasitic shampoo, chlorhexidine washes, and supportive therapy. Environmental disinfection and quarantine were critical. Complete recovery occurred in both the rabbit and family members.

Conclusion: This rare case demonstrates the zoonotic potential of sarcoptic mange from rabbits and emphasizes the importance of early veterinary diagnosis, preventive counseling, and strict hygiene.

Keywords: Sarcoptic mange, Pet rabbit, Zoonotic transmission, Human-animal parasite.

Introduction:

Sarcoptic mange (*Sarcoptes scabiei*) is a highly contagious zoonotic ectoparasite that can be transmitted from animals to humans (1). This case report describes a rare instance of zoonotic sarcoptic mange transmission from a neutered male indoor rabbit to three members of a four-person family in Tehran, Iran. The rabbit developed alopecia, severe pruritus, and scaling, followed by maculopapular rashes and itching in the father, mother, and daughter. The infant remained unaffected. Delayed medical consultation led to disease progression (2). Treatment included permethrin cream and shampoos for humans and ivermectin (injectable and topical), antiparasitic powders and lotions for the rabbit. Environmental disinfection and quarantine were also critical. The case highlights the importance of early diagnosis, strict hygiene, and



proactive veterinary communication regarding zoonotic risks, even when the transmission probability seems low (3,4).

Materials and Methods:

In late spring 2025, a four-member family from the Punak district of Tehran presented to a veterinary clinic with dermatologic symptoms in their pet rabbit and similar signs in some family members. Their pet was a two-year-old neutered male rabbit living freely indoors. The rabbit exhibited alopecia on the ears and face, intense pruritus, and localized scaling. Skin scraping from affected areas revealed *Sarcoptes scabiei* mites under microscopic examination. Approximately one week later, the father, mother, and 8-year-old daughter developed intense itching and rashes, while the 9-month-old infant remained asymptomatic. Human diagnosis and treatment were delayed by one week despite veterinary advice.

Human Treatment:

- Topical permethrin cream and antiparasitic shampoo
- Washing affected areas with diluted chlorhexidine (1:3)
- Intravenous vitamin C for immune support

Rabbit Treatment:

- Ivermectin injection (400 µg/kg SC weekly ×3)
- Topical ivermectin diluted 1:20 daily
- 1% Propoxur powder every 7 days
- Lindane shampoo weekly
- Wound cleansing with diluted chlorhexidine every 12 hours
- Clotrizone lotion every 12 hours
- Triamcinolone NN ointment once daily
- Shaving crusted areas
- Quarantine for 3 weeks
- Environmental disinfection



Figure 1. Rabbit with alopecia and inflammation on the face, indicative of sarcoptic mange.

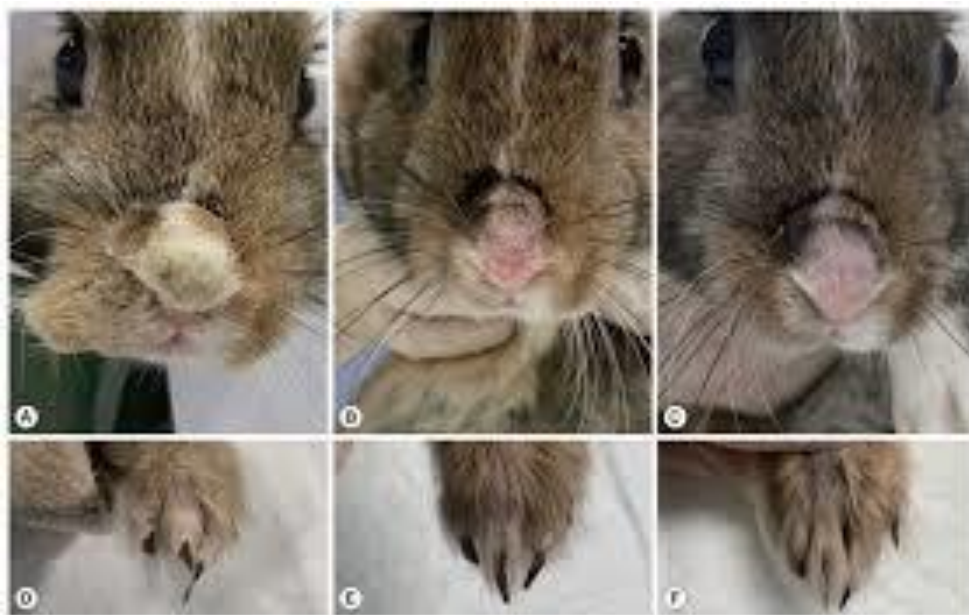


Figure 2. Clinical progression of healing: nasal and paw lesions pre- and post-treatment.

Results and Discussion:

Similar cases of zoonotic transmission of sarcoptic mange from rabbits to humans are exceedingly rare in published literature. Papier et al. (2014) described a comparable family outbreak, also linked to close contact with an infested rabbit. However, in contrast to that case, the current report provides a more detailed structured treatment protocol, including immune support and environmental decontamination. In addition, Pereira et al. (2021) presented a



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



successful mange treatment in rabbits, but did not involve zoonotic transmission or combined human-rabbit management (5).

This case highlights that even rare zoonotic risks must not be overlooked in veterinary practice. The delay in quarantine and human medical consultation led to disease transmission and complications. Comprehensive treatment with topical and systemic agents, hygiene measures, and environmental control achieved complete recovery. Importantly, a veterinarian's role extends beyond animal health; when guidance is clear and followed by pet owners, time, cost, and suffering can be minimized. Preventive advice should always be communicated—even when the risk of transmission appears minimal.

Acknowledgement:

The author would like to express sincere gratitude to the veterinary clinic staff who assisted with the diagnosis and treatment of the rabbit. Special thanks to the pet owners for their cooperation and consent to document and publish this case.

References:

1. Morris DO. Sarcoptic mange in dogs and cats. *Compend Contin Educ Vet*. 2006.
2. Papier K, et al. Zoonotic scabies in a family transmitted from a pet rabbit. *J Infect Dev Ctries*. 2014.
3. Arlian LG, Morgan MS. A review of *Sarcoptes scabiei* in animals and humans. *Parasitology*. 2017.
4. Centers for Disease Control and Prevention (CDC). Scabies - Epidemiology & Risk Factors.
5. Pereira AV, et al. Successful management of sarcoptic mange in rabbits: a case report. *BMC Vet Res*. 2021.



Infestation of *Ctenocephalides felis* in a Sheep Herd: A Case Report

Seyed Kian Mohseni Mofidi¹, Mousa Tavassoli¹, Saleh Sharifi¹, Alireza Ghorbani¹

Corresponding Author: Seyed Kian Mohseni Mofidi, mohsenimofididvm@gmail.com

1. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article code: 1256

Abstract

Background: Arthropod parasites, inhabiting chiefly on the host surface are responsible for causing various ectoparasitic diseases in the host such as scabies, pediculosis and demodicosis. Among them, adult fleas are blood-feeding insects that feed on birds and largely on mammals, acting as parasites and vectors of infectious diseases. Fleas belonging to the genus *Ctenocephalides* are also the chief fleas affecting the domestic animals in tropical as well as subtropical countries causing dermatitis, severe anemia, and mortality in young ones. In this report, infestation of *Ctenocephalides felis* in a sheep herd is discussed.

Methods: After flea infestation was reported in a sheep herd in Bardeh Roshan village in Mahabad City, West Azerbaijan Province, Iran, Samples were randomly collected from several animals.. Clinical signs such as lethargy, hair loss and dermatitis on scapula were specifically observed in infested lambs. The collected fleas were kept in 70% alcohol and transferred to parasitology lab, Faculty of Veterinary Medicine, Urmia University. In the lab, fleas were mounted according to the mounting process instructions.

Results: Species identification was carried out according to the identification keys and *C. felis* was detected.

Conclusion: Fleas are one of the most important parasitic arthropods worldwide, considering their vector role for infectious diseases, disorders caused by their piercing mouthparts and blood feeding ability. Overall, cat flea is especially noteworthy; because it has a wide range of hosts including human, its abundant distribution worldwide, its population grows rapidly in ideal condition and the vector role for zoonotic pathogens. So public awareness campaigns are needed, especially for companion animals owners and maintaining a proper hygiene in homes and farms is inevitable as control and prevention procedures. Also, when encountering fleas, carrying out appropriate treatment with proper insecticide is necessary.

Keywords: Cat Flea, *Ctenocephalides felis*, Flea, Sheep.



Introduction:

Arthropod parasites, the invertebrate creatures, inhabiting chiefly on the host surface are responsible for causing various diseases in the host such as scabies, pediculosis, demodicosis, and transmit various diseases (1). The fleas are small, laterally compressed, wingless and highly specialized insects and these arthropods are of great concern worldwide (3). Adult fleas are blood-feeding insects that feed on birds and largely on mammals, acting as parasites and vectors of infectious diseases (7). Fleas are responsible for causing dermatitis in domestic animals. Further, the profound infestation in young ones may result in severe anemia followed by death (14).

Fleas belonging to the genus *Ctenocephalides* are also the chief fleas affecting the domestic animals in tropical as well as subtropical countries causing dermatitis, severe anemia, and mortality in young ones (2). Among its species, *Ctenocephalides felis* has a broad host range (5).

In this study, flea infestation in a sheep herd in Bardeh Rashan village in Mahabad city, West Azerbaijan Province, Iran in September, 2024 is discussed.

Materials and Methods:

After flea infestation was reported in the sheep herd, six animals were examined. While examining their body, it was found out that lambs are heavily infested, which had made them lethargic and caused hair loss and dermatitis on scapula. The collected fleas were kept in 70% alcohol and transferred to parasitology laboratory, Faculty of Veterinary Medicine, Urmia University. Fleas were mounted according to the process instructions and identified by identification keys (12, 13).

In the laboratory, fleas were put in microtubes contained 10% KOH for 24 hours, then, they were moved to the separate microtubes contained 30%, 50%, 70%, 80%, 95% and absolute alcohol for 6 to 15 minutes. After that, samples were transferred to alcohol-xylene solution for an hour and pure xylene for 24 hours. In order to transparent the samples, they were put in microtubes contained lactophenol for 24 hours. Finally, entellan glue was used for mounting (12).

The treatment was carried out on the animals using ivermectin via spine pour-on route. Also, the sheep pen walls were first coated with diesel to increase the residue effect of to be sprayed pesticide, then cypermethrin was sprayed on the walls and floor.



Results and Discussion:

It was discovered that the fleas were actually cat fleas, *C. felis*. Species identification carried out by the identification keys, like the head length being twice the head width, also both genal and pronotal ctenidium were present and all genal spines were the same size, and the last pair of legs (third pair) tibiae (fourth segment of legs) had 6 notches with 6 sensitive bristles on the dorsal surface (9, 13). In the sheep pen, there were quantities of feces that had not been collected in a timely manner, and along with the adult fleas feces (flea dirt), it provided a suitable environment to provide more nutrients for the growth and development of larvae. Also, around the sheep pen building, there were a number of stray cats that had free access to the interior and roamed there. Seemed like in poor hygienic condition, practical inefficiency and sheep close contact with preferable hosts (cats), fleas infested the sheep herd.

Two rickettsial diseases, flea-borne spotted fever and murine typhus, have been identified in domestic animal populations and cat fleas. Cat flea, *C. felis*, is the primary vector of *Bartonella henselae* (cat scratch fever) with the spread of the bacteria when flea feces are scratched in to bites or wounds. Flea allergic dermatitis (FAD) common in dogs and cats has been successfully treated and tapeworm infestations prevented with a number of new products being used to control fleas (10). These fleas are also of zoonotic concern as liable to transmit the zoonotic pathogens such as *Rickettsia felis* and *Bartonella* spp. to humans as it was mentioned, but they only cause clinical signs including lethargy, anemia, dermatitis and hair loss in small ruminants (6).

In a study on small ruminants case, fleas were found on goats. Hematology and blood biochemistry performed on the infested goats revealed no abnormalities; however, infestation caused irritation that made the animals lethargic (8).

Overall, given what has been discovered in this study and previous studies, it is found out cat fleas are one of the most abundant flea species worldwide infesting too many mammal species including humans. So it is important to consider appropriate control, prevention and treatment methods. Companion animals owners should consider it in their pet care program, so they would not only protect their pets, but also ensure their own health and prevent the spread of infestation and flea-borne diseases. The complex flea life cycle means that there are a number of challenges for veterinary practitioners wanting to help clients control fleas in their homes: the physical resilience and rapid multiplication in flea numbers; their ability to find alternative hosts; and the presence of environmental conditions in the home that are conducive to flea survival. In addition, there is a need to select an appropriate form of treatment from among the many options available and to manage the client's expectations of what is possible to achieve, while also ensuring that they follow instructions well enough to ensure a favorable outcome. Veterinary practitioners should seek to understand any reasons why the control strategy may not have worked in that particular case. It is not possible for staff to conduct investigations of



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



the home, but careful questioning may provide the reason for the continued presence of fleas in the environment. There are products available that have excellent activity against fleas for a month or more, but some owners still struggle to implement effective control. Best results are achieved through an integrated strategy involving the use of an effective and long-lasting adulticide, and an IGR (lufenuron) to prevent development of immature stages (4).

Even though some studies with bacteria and fungi have demonstrated activity against cat fleas, the research into biological control of cat fleas has been limited. In a recent study, adult cat fleas were exposed to fungal spores, *Beauveria bassiana*, exposed to different lighting conditions to stimulate conidiation. It remains uncertain how this might be exploited to control fleas. The search for a vaccine to protect cats and dogs from cat fleas has persisted over the past four decades. (11).

In the case of small ruminants, local treatment is carried out by using pyrethrin, synthetic pyrethroids like permethrin and cypermethrin, organophosphates like coumaphos and diazinon and spraying the sheep pens and coots indoors with pesticides (synthetic pyrethroids, preferably cypermethrin) (13).

References:

1. Ash MM, Phillips CM. Parasitic diseases with cutaneous manifestations. North Carolina Medical Journal. 2016 Sep 1;77(5):350-4.
2. Ashwini MS, Puttalakshamma GC, Mamatha GS, Chandranaik BM, Thimmareddy PM, Placid E, Jalali SK, Venkatshan T. Studies on morphology and molecular characterization of oriental cat flea infesting small ruminants by barcoding. J. Entomol. Zool. Stud. 2017;5:301
3. Bitam I, Dittmar K, Parola P, Whiting MF, Raoult D. Fleas and flea-borne diseases. International journal of infectious diseases. 2010 Aug 1;14(8):e667-76.
4. Bourne D, Craig M, Crittall J, Elsheikha H, Griffiths K, Keyte S, Merritt B, Richardson E, Stokes L, Whitfield V, Wilson A. Fleas and flea-borne diseases: biology, control & compliance. Companion Animal. 2018 Apr 2;23(4):204-11.
5. Dryden MW, Rust MK. The cat flea: biology, ecology and control. Veterinary Parasitology. 1994 Mar 1;52(1-2):1-9.
6. Lawrence AL, Brown GK, Peters B, Spielman DS, Morin-Adeline V, Šlapeta J. High phylogenetic diversity of the cat flea (*Ctenocephalides felis*) at two mitochondrial DNA markers. Medical and veterinary entomology. 2014 Sep;28(3):330-6.
7. Linardi PM. Fleas and diseases. In Arthropod Borne Diseases 2016 Nov 11 (pp. 517-536). Cham: Springer International Publishing



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



8. McCrindle CM, Green ED, Bryson NR. A primary animal health care approach to treatment and control of flea (*Ctenocephalides felis*) infestation in indigenous goats kept on communal grazing: research communication. *Journal of the South African Veterinary Association*. 1999 Mar 1;70(1):21-4.
9. Mullen GR, Durden LA, editors. *Medical and veterinary entomology*. Academic press; 2009 Apr 22.
10. Rust MK. The biology and ecology of cat fleas and advancements in their pest management: a review. *Insects*. 2017 Oct 27;8(4):118.
11. Rust MK. Recent advancements in the control of cat fleas. *Insects*. 2020 Sep 29;11(10):668.
12. Tavassoli M. *Veterinary Entomology Laboratory Methods*. Urmia University Publications. 2011. 37-38
13. Wall R, Shearer D. *Veterinary ectoparasites: biology, pathology and control*. John Wiley & Sons; 2008 Apr 15.
14. Yeruham I, Rosen S, Hadani A. Mortality in calves, lambs and kids caused by severe infestation with the cat flea *Ctenocephalides felis felis* (Bouché, 1835) in Israel. *Veterinary Parasitology*. 1989 Mar 1;30(4):351-6.



Comparing the Effects of Cryotherapy and Conventional Injectable Treatments on the Healing of *Leishmania major* Skin Wounds in Mice

Seyed Kamyab Sajadi¹, Ghazaaleh Adhami¹, Yahya Maroufi²

Corresponding Author: Ghazaaleh Adhami, adhamidvm@gmail.com

1. Department of Veterinary Pathobiology, Faculty of Veterinary Medicine, Islamic Azad University of Sanandaj, Sanandaj, Iran.
2. Department of Parasitology and Mycology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.

Article code: 1262

Abstract

Background: Cutaneous leishmaniasis (CL), caused by *Leishmania* species, is a parasitic disease prevalent in tropical and subtropical regions. Pentavalent antimonial compounds, such as pentostam (sodium stibogluconate) and Glucantime (meglumine antimoniate), are the main treatments. This study evaluated the efficacy of Cryotherapy alone and in combination with Glucantime or Amphotericin B in BALB/c mice.

Methods: Infected mice were divided into six groups: control, Cryotherapy, Glucantime, Amphotericin B, Glucantime-Cryotherapy, and Amphotericin B-Cryotherapy. Glucantime was administered daily intralesionally, Amphotericin B intraperitoneally for three weeks, and Cryotherapy was applied up to six times over three weeks. At the end of treatment, two mice per group were sacrificed, and spleens were collected to assess parasite load via culture in RPMI 1640.

Results: Combination therapies significantly reduced lesion size and accelerated wound healing compared with controls. In the Glucantime-Cryotherapy group, lesions were completely healed by the third week, with no residual nodules or expansion. No parasites were detected in spleens of any group at the end of the study.

Conclusion: Cryotherapy enhances the efficacy of conventional drugs, particularly Glucantime, promoting faster and more complete healing of cutaneous leishmaniasis.

Keywords: Amphotericin B, Cryotherapy, Cutaneous leishmaniasis, Glucantime, *Leishmania major*.

Introduction:

Leishmaniasis is a major parasitic disease in tropical and subtropical regions, including Iran. The World Health Organization estimates that 350 million people are at risk, with 12 million



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



currently infected and 1.5 million new cases annually (1). Various treatment methods such as localized radiotherapy, lesion cauterization, Cryotherapy, and local drug injections have been used for cutaneous leishmaniasis (CL) (2-4). Currently, pentavalent antimonial compounds like pentostam and Glucantime are the main drugs (5), but their use is limited due to long treatment duration, high cost, 10–15% nonresponsiveness, and severe toxicity to vital organs (6).

Considering the thermosensitivity of *Leishmania* (7), Cryotherapy has been applied effectively, especially for species such as *L. tropica*, *L. aethiopica*, *L. infantum*, and *L. braziliensis* (8,9). It works by destroying amastigotes and stimulating the host immune response through antigen release (10). Meta-analyses suggest Cryotherapy has efficacy comparable to sodium stibogluconate for small lesions, with fewer side effects (11). However, it is limited to lesions under 4 cm and fewer than four in number. Combination therapy with Cryotherapy and antimonials shows greater effectiveness and synergistic effects (8,11,12).

Given the increasing therapeutic failure, relapse, and drug resistance, revisiting treatment protocols is essential. Combination therapies may shorten treatment duration, reduce dosage, side effects, and costs, while lowering drug resistance (13). Studies indicate that combining Cryotherapy with antimonials significantly improves outcomes for *L. tropica* and *L. major* compared to monotherapies (9,14,15).

The aim of this study was to evaluate the efficacy of Cryotherapy alone or in combination with commercial drugs in treating CL caused by *Leishmania major* in BALB/c mice.

Materials and Methods:

This experimental study was done in Parasitology Lab of Kurdistan University of Medical Sciences and approved by Research Committees of Islamic Azad University Sanandaj Branch and approval ID is IR.IAU.SDJ.REC.1403.003.

The *Leishmania major* strain (MRHO/IR/75/ER) was obtained from the Education and Research Center for Skin Diseases and Leprosy, Tehran University of Medical Sciences. Parasites were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) at 25°C.

Thirty female BALB/c mice (6–8 weeks old) were randomly assigned to six groups: control, Cryotherapy, Glucantime, Amphotericin B, Glucantime-Cryotherapy, and Amphotericin B-Cryotherapy.

Parasite Inoculation: Stationary-phase promastigotes were harvested, washed three times with sterile PBS, and concentrated to 2×10^6 parasites per injection. Mice were inoculated subcutaneously at the tail base, and lesions developed within 3–5 weeks (16).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Drug Administration: After lesion development, mice received intralesional Glucantime (20 mg/kg daily) (17–19) or intraperitoneal Amphotericin B (4 mg/kg/day for three weeks) (20). Cryotherapy was performed by applying a liquid nitrogen–soaked swab to the lesion with a 1–2 mm margin for 10–120 s; in thicker lesions, up to six freeze–thaw cycles were applied over three weeks (21). Mouse body weights were measured before and after treatment.

At the end of treatment, two mice per group were sacrificed, and spleens were removed, weighed, homogenized in RPMI medium with 10% FBS, and serially diluted. Cultures were incubated at 26–28°C for 7–15 days and examined microscopically for promastigotes. Parasite load was then calculated (22,23).

$$\text{Parasite load} = -\log_{10}\left(\frac{\text{dilution}}{\text{spleen weight}}\right)$$

Statistical analysis: Collected data were analyzed via SPSS software, version 24. Descriptive statistics, including frequencies, percentages, means, and standard deviations, are presented as charts. Comparisons of qualitative data were performed via t tests and Tukey's post hoc tests. A significance level of $P < 0.05$ was considered statistically significant.

Results and Discussion:

Weight Changes: In Figure 1, the mean weights of the mice before and after treatment are shown. The mean weights of the mice in the Glucantime treatment group (24.30 g) and the Glucantime-Cryotherapy group (24.88 g) were greater than their pretreatment weights. This increase was more substantial than that in the other treatment groups, indicating a positive effect on the health and recovery of the mice in these groups.

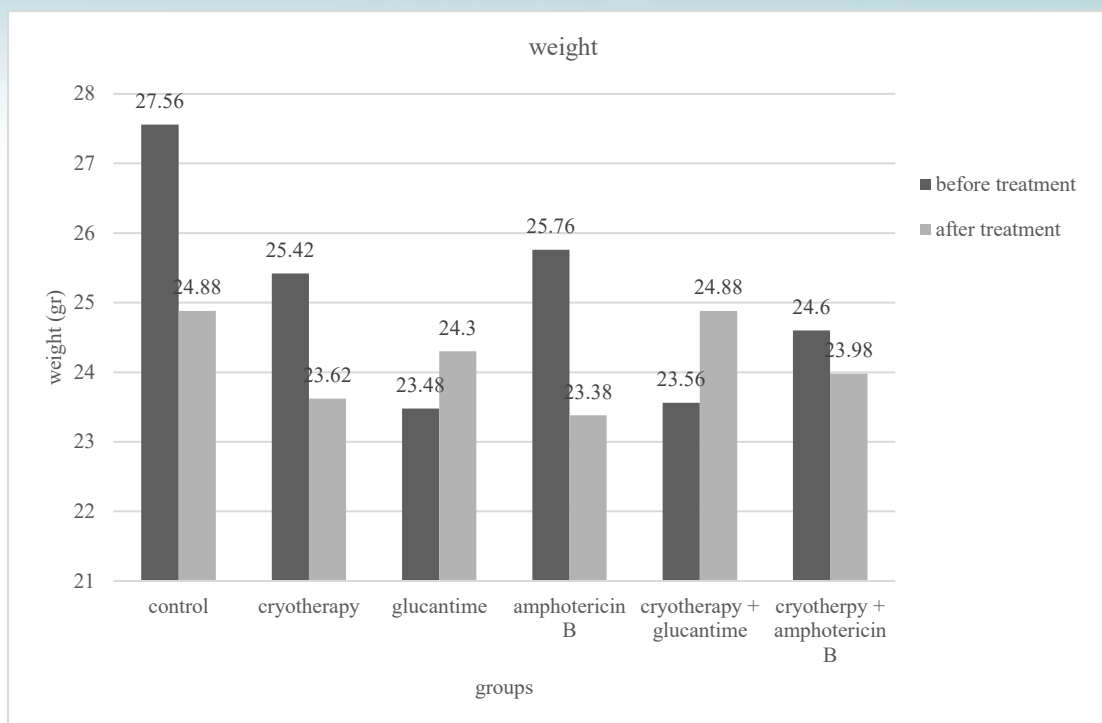


Figure 1. Changes in Mouse Weights across Different Treatment Groups

Lesion size: As shown in Figure 2, treatment effectiveness was evaluated based on lesion size, thickness, and location. At baseline (day zero), no significant differences were observed in lesion diameter among the groups. During the first week, all treatment groups showed a downward trend in lesion diameter, though reductions were not statistically significant.

By the second week, a significant decrease in lesion diameter was noted in the Cryotherapy group compared with the control group. Significant differences were also found in the Glucantime-only and Glucantime-Cryotherapy groups compared with both the control and Cryotherapy-only groups. For Amphotericin B, whether used alone or in combination with Cryotherapy, significant reductions were observed only in comparison with the control group.

By the third week, lesions in all treatment groups had gradually resolved, leaving no nodules or active lesions, whereas those in the control group continued to expand, leading to animal mortality. The healing process in the Cryotherapy group differed significantly from that in the control. Notably, the Glucantime-only and Glucantime-Cryotherapy groups showed greater healing than both the control and Cryotherapy-alone groups. Amphotericin B, alone or combined with Cryotherapy, also reduced lesion size, though statistical significance was limited to comparison with the control group.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad

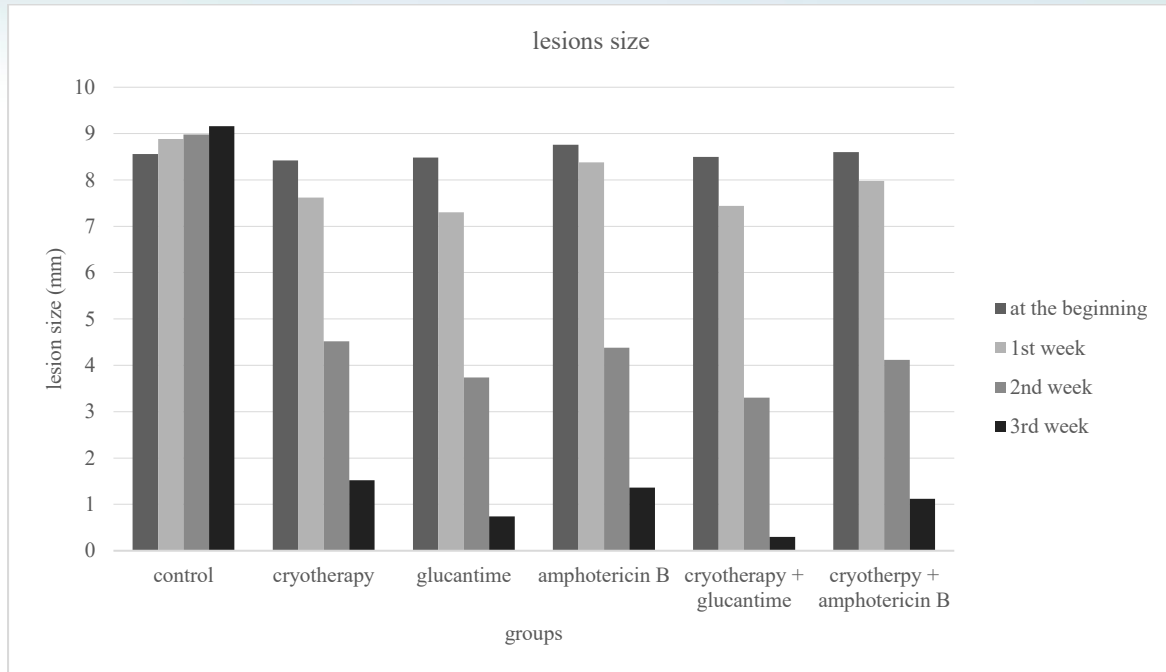


Figure 2. Average changes in lesion diameter (mm) among the groups during treatment

Parasite load in the spleen: The findings showed that no parasites were detected in the spleens of any group at the end of the study (Figure 3). Preventing the systemic spread and proliferation of *Leishmania* is a key parameter in evaluating the efficacy of treatments for cutaneous leishmaniasis. Examination of spleens from treated mice confirmed the complete absence of parasite burden in all groups.

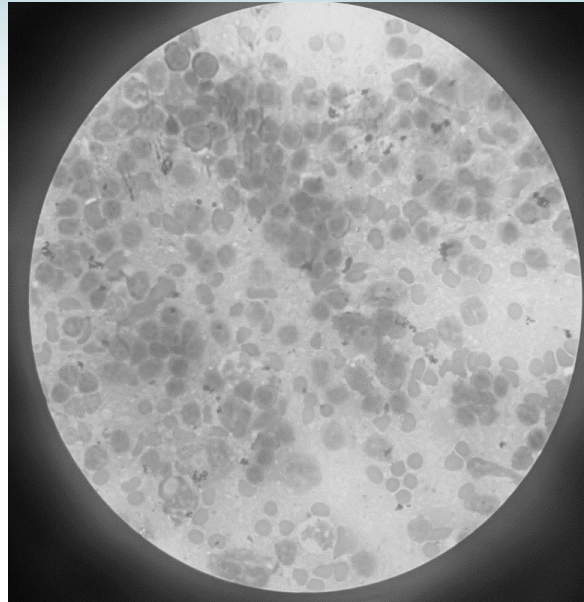


Figure 3. Microscopic examination of splenocytes via an inverted microscope: there are no amastigotes.

In this study, the mean weight increased in the Glucantime (24.30 g) and Glucantime-Cryotherapy (24.88 g) groups compared with baseline and other treatments. No significant differences in lesion diameter were observed among groups at baseline or during the first week. By the second week, however, lesion size was significantly reduced in the Cryotherapy group versus the control, with further improvements in the Glucantime and Glucantime-Cryotherapy groups compared with both control and Cryotherapy alone. By the third week, all treated groups showed complete lesion healing, while lesions in the control group enlarged, leading to mortality.

Saghafipour reported that intralesional Glucantime achieved 48.1% recovery, while combination with Cryotherapy resulted in 72.2% recovery after seven treatments and 100% after 12, compared with 91% for Glucantime alone (24).

Overall, Glucantime combined with Cryotherapy enhanced weight gain and significantly accelerated lesion resolution in BALB/c mice. Although no early differences were observed, substantial reductions emerged by week two, with full healing in all treated groups by week three. Amphotericin B, alone or with Cryotherapy, also reduced lesion size, but significance was limited to comparisons with the control. Importantly, spleen analysis confirmed that all treatments prevented parasite dissemination, underscoring their systemic efficacy.

These findings demonstrate the superior therapeutic potential of combination regimens, particularly Glucantime-Cryotherapy, for faster and more complete wound healing.



Acknowledgment:

This article is based on a project approved by the Department of Veterinary Pathobiology, Faculty of Veterinary Medicine, Islamic Azad University of Sanandaj, at IR.IAU.SDJ.REC.1402.112. The researchers found themselves obliged to thank Mr. Habibi, animal house in charge, for his collaboration in doing experiments.

References:

1. Mann S, Frasca K, Scherrer S, Henao-Martínez AF, Newman S, Ramanan P, Suarez JA. A review of leishmaniasis: current knowledge and future directions. *Curr Trop Med Rep.* 2021;8(2):121-132.
2. Linquest LA, Hickham LC, Richardson BJ, Hickham PR. Successful treatment of cutaneous leishmaniasis with Cryotherapy. *Cureus.* 2023;15(7):e41871.
3. Saghafipour, A., Mozaffari, E., Rezaei, F. The evaluation of intralesional Glucantime and Cryotherapy plus intralesional Glucantime therapeutic efficacy on zoonotic cutaneous leishmaniasis: a randomized clinical trial. *J Pediatr Perspect,* 2017;5(12): 6689-6697.
4. Chakravarty J, Sundar S. Current and emerging medications for the treatment of leishmaniasis. *Expert Opin Pharmacother.* 2019;20(10):1251-1265.
5. Solomon M, Ollech A, Pavlotsky F, Barzilai A, Schwartz E, Baum S, Astman N. Comparison of intralesional sodium stibogluconate versus intralesional meglumine antimoniate for the treatment of leishmania major cutaneous leishmaniasis. *Acta Derm Venereol.* 2024;29;104:adv35089.
6. Copeland, Nathaniel K.a,b; Aronson, Naomi E.a,b. Leishmaniasis: treatment updates and clinical practice guidelines review. *Curr Opin Infect Dis.* 2015;28(5): 426-437.
7. Sacks DL, Barral A, Neva FA. Thermosensitivity patterns of old vs. new world cutaneous strains of *Leishmania* growing within mouse peritoneal macrophages in vitro. *The American J Trop Med Hyg.* 1983;2(2):300-304.
8. Layegh P, Pezeshkpoor F, Soruri AH, Naviafar P, Moghiman T. Efficacy of Cryotherapy versus intralesional meglumine antimoniate (Glucantime) for treatment of cutaneous leishmaniasis in children. *Am J Trop Med Hyg.* 2009;80(2):172-5.
9. Asilian A, Sadeghinia A, Faghihi G, Momeni A. Comparative study of the efficacy of combined Cryotherapy and intralesional meglumine antimoniate (Glucantime) vs. Cryotherapy and intralesional meglumine antimoniate (Glucantime) alone for the treatment of cutaneous leishmaniasis. *Int J Dermatol.* 2004;43(4):281-3.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



10. Panagiotopoulos A, Stavropoulos PG, Hasapi V, Papakonstantinou AM, Petridis A, Katsambas A. Treatment of cutaneous leishmaniasis with cryosurgery. *Int J Dermatol*. 2005;44(9):749-52.
11. Mosleh IM, Geith E, Natsheh L, Schönián G, Abotteen N, Kharabsheh S. Efficacy of a weekly Cryotherapy regimen to treat *Leishmania major* cutaneous leishmaniasis. *J Am Acad Dermatol*. 2008;58(4):617-24.
12. López-Carvajal L, Cardona-Arias JA, Zapata-Cardona MI, Sánchez-Giraldo V, Vélez ID. Efficacy of Cryotherapy for the treatment of cutaneous leishmaniasis: meta-analyses of clinical trials. *BMC Infect Dis*. 2016;26;16:360.
13. Sundar S, Chakravarty J. An update on pharmacotherapy for leishmaniasis. *Expert Opin Pharmacother*. 2015;16(2):237-52.
14. Brito NC, Rabello A, Cota GF. Efficacy of pentavalent antimoniate intralesional infiltration therapy for cutaneous leishmaniasis: A systematic review. *PLoS One*. 2017;19;12(9):e0184777.
15. Salmanpour R, Razmavar MR, Abtahi N. Comparison of intralesional meglumine antimoniate, Cryotherapy and their combination in the treatment of cutaneous leishmaniasis. *Int J Dermatol*. 2006;45(9):1115-6.
16. Maroufi Y, Ghaffarifar F, Dalimi A, Sharifi Z. Interferon-gamma and interleukin-4 patterns in BALB/c mice suffering from cutaneous leishmaniasis treated with cantharidin. *Jundishapur J Microbiol*. 2014;7(6):e10907.
17. Sadeghian, G, and Nilforoushzadeh, M.A. Effect of combination therapy with systemic Glucantime and pentoxifylline in the treatment of cutaneous leishmaniasis. *Int J Dermatol*. 2006;45: 819-821.
18. Mohammadzadeh M, Behnaz F, Golshan Z. Efficacy of Glucantime for treatment of cutaneous leishmaniasis in Central Iran, *J Infec Pub Heal*. 2014;6(2):120-124.
19. Esfandiarpour, I. and Alavi, A. Evaluating the efficacy of allopurinol and meglumine antimoniate (Glucantime) in the treatment of cutaneous leishmaniasis. *Int J Dermatol*. 2002;41: 521-524.
20. Sundar S, Chakravarty J. Liposomal Amphotericin B and leishmaniasis: dose and response. *J Glob Infect Dis*. 2010;2(2):159-66.
21. Farajzadeh S, Ahmadi R, Mohammadi S, Pardakhty A, Khalili M, Aflatoonian M. Evaluation of the efficacy of intralesional Glucantime plus niosomal zinc sulphate in comparison with intralesional Glucantime plus Cryotherapy in the treatment of acute cutaneous leishmaniasis, a randomized clinical trial. *J Parasit Dis*. 2018;42(4):616-620.
22. Titus RG, Marchand M, Boon T, Louis JA. A limiting dilution assay for quantifying *Leishmania major* in tissues of infected mice. *Parasite Immunol*. 1985;7(5):545-55.
23. Lima H.C, Bleyenbergh J.A, Titus R.G. A simple method for quantifying *Leishmania* in tissues of infected animals. *Trends Parasitol*. 1997; 13(2):80-82.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



24. Saghafipour A, Rassi Y, Norooze M. Comparison of efficacy of intercessional injection of Glucantime and Cryotherapy with intralesional injection in the treatment of zoonotic cutaneous Leishmaniasis: a randomized clinical trial. *Knowl Health* 2013;8(2):62-66.



***Hepatozoon canis* Infection in a Rottweiler Dog: A Case Report from Ahvaz, Khuzestan Province, Iran**

Zahra Mashayekhi¹, Somayeh Bahrami¹, Mahnush Kaamyabnia²

Corresponding Author: Zahra Mashayekhi, mashayekhi2000zahra@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Mahrouyan Veterinary Clinic, Ahvaz, Iran.

Article code: 1265

Abstract

Background: *Hepatozoon canis* is a tick-transmitted protozoan causing infections in dogs, with clinical signs ranging from mild to severe illness. Although reported worldwide, its prevalence and features in dogs from Ahvaz are unclear. We hypothesized that it is present but is underdiagnosed in this region. This study describes clinical, hematological, and biochemical characteristics of *H. canis* infection in a local dog to improve diagnosis and treatment.

Methods: The study was conducted on a dog and followed up for one month. Diagnosis was carried out microscopically by demonstrating *H. canis* gametocytes within neutrophils. The confirmation of infection was molecularly validated. Hematological and biochemical parameters were analysed.

Results: A 4-year-old female Rottweiler presented prolonged illness with inappetence, weight loss, weakness, fever, pale mucous membranes, and ticks' infestation. Antibiotics failed prior to diagnosis. CBC showed mild regenerative anemia, leukocytosis with neutrophilia and regenerative left shift, and *Hepatozoon* gamonts in neutrophils. Serum chemistry indicated hyperalbuminemia, elevated alkaline phosphatase, increased A/G ratio, and AST. Microscopic and molecular analyses confirmed *H. canis*. Finally, infection was confirmed molecularly. Treatment with two IM imidocarb dipropionate doses combined with clindamycin led to recovery.

Conclusion: This first confirmed case of *H. canis* in a dog from Ahvaz highlights its local presence. Clinical and laboratory findings stress including *H. canis* in differential diagnoses of dogs with fever and anemia. Early diagnosis and treatment improve outcomes and awareness of canine hepatozoonosis in endemic areas.

Keywords: *Hepatozoon canis*, Dog, Ahvaz, Tick.



Introduction:

Hepatozoon spp. are the tick-borne apicomplexan parasites found mainly in the neutrophils and monocytes of mammals. To date, *Hepatozoon americanum* and *Hepatozoon canis* have been recognized as canine hepatozoonosis causes (1).

The previous studies have confirmed that canine hepatic zoonosis diseases can be transmitted by ticks, transplacental transmission from infected maternal animals to fetuses, and by ingestion of prey species such as *H. americanum* paratenic hosts. Similarly, ticks have been identified as the main route of transmission, especially *Rhipicephalus sanguineus* and *Amblyomma maculatum* (2).

The transmission of *H. canis* in dogs and other carnivores occurs by ingestion of a hematophagous arthropod, such as a tick, which is the definitive host and contains sporulated oocysts (3). The life-cycle for all *Hepatozoon* spp. includes gamogony and sporogony in haematophagous invertebrate definitive hosts and merogony and gametogony in vertebrate hosts (4). After ingestion of a tick harbouring oocytes that contain sporozoites, the infective sporozoites are released in the gastrointestinal tract of the vertebrate host and reach blood and lymph circulation by penetrating the gut wall. Merogony starts in lymphoid tissues such as the bone marrow and from 13 days postinfection onwards, merozoites penetrate neutrophilic granulocytes and monocytes to develop into gamonts (5). During the tick's blood meal on an infected host, the gamonts are ingested and gametogenesis takes place in the gut of the tick, followed by sporogony in the haemocoel (4).

The infection could be asymptomatic in dogs with low level of parasitaemia or could be manifested as a severe life-threatening disease with fever, lethargy, anaemia, cachexia in dogs with high parasitaemia (6). The most common presentation of the infection in dogs is asymptomatic to mild disease, and it is usually associated with a low level of *H. canis* parasitaemia in which 1% to 5% of the animal's neutrophils are infected (6,7). In this article, we provide a detailed description of the diagnosis of a case infected with *Hepatozoon canis* and the treatment approach employed.

Materials and Methods:

A 4-year-old, female Rottweiler was presented to a veterinary practice in Ahvaz, with a history of prolonged illness with lethargy, inappetence, weight loss, and fever. According to the owner's information, the dog had been infested with ticks during the previous months, but this was not evident at the present patient on the body. The patient had been treated with various antibiotics, including a course of ampicillin and metronidazole prior to referral; however, no clinical improvement was achieved. Based on the patient's history and clinical signs, *H. canis* infection was suspected; therefore, blood samples were collected from the animal for further



evaluation. Physical examination revealed weakness, pale mucous membranes, and persistent fever.

Blood sample was taken from the cephalic vein in EDTA-coated tubes and kept at -20 °C until DNA extraction. Thin blood smears were prepared, fixed with absolute methanol for 1 minute, stained with 10% Giemsa solution for 30 minutes and examined under oil immersion lens. Additionally, the hematological parameters consisted of hematocrit (HCT), Mean Corpuscular Volume (MCV), Red Blood Cell (RBC) count, Hemoglobin Concentration (Hgb), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), total White Blood Cell (WBC) counts, and total Platelet (PLT) counts were assessed using a digital cell counter.

For molecular analysis, DNA was extracted by the DNA Purification Kit (Sinnaclon; Blood DNA Extraction Kit), according to manufacturer instructions. conventional PCR targeting the partial 18S rRNA gene of *Hepatozoon* spp. was used. A forward primer (5'-GAAATAACAATACAAGGCAGTTAAAATGCT -3) and a reverse primer (5'-GTGCTGAAGGAGTCGTTTATAAAGA-3') amplified a 620 bp fragment. The PCR was performed in a final volume of 20 µl of reaction mixture containing 10 µl of PCR Master Mix (Amplicon, Denmark), 0.7 µl of each primer (7 pmol), 6.6 µl of nuclease-free water, and 2 µl of DNA template (~20 ng). The PCR cycles started at 95°C for 2 min; then, 35 cycles of denaturation for 60 sec at 95°C, primers annealing for 60 sec at 58°C, and extension for 60 sec at 72°C were performed. The final extension took only one step for 5 min at 72°C. Gel electrophoresis of 1.5% Red safe (Intron Biotechnology, Korea)-stained agarose gel was conducted at 100 volts for 45 min. After gel electrophoresis, PCR products were visualized and photographed on a transilluminator to detect positive band at a 620 bp position alongside a 100 bp molecular weight marker.

Results and Discussion:

Diagnosis was confirmed microscopically by demonstrating *H. canis* gametocytes within neutrophils. on Giemsa-stained peripheral blood smears with a parasitemia level of approximately 1% (Figure 1). The blood sample was positive in PCR assay for *Hepatozoon* spp. and produced 620 bp fragments. The hematological analysis revealed mild regenerative anemia, leukocytosis with neutrophilia and regenerative left shift (Table1). Serum chemistry indicated hyperalbuminemia, elevated alkaline phosphatase, increased A/G ratio, and AST (Table2).



Figure 1. Blood film shows gamont of *H. canis* stained in the cytoplasm of the circulating neutrophils of the infected dog.

Table 1. Haematological findings

Parameters	Value	Reference Range
WBC ($10^3/\mu\text{L}$)	59.0	6-17
RBC ($10^6/\mu\text{L}$)	5.57	5.5-8.5
HGB (g/dL)	10.6	11.0-19.0
HCT (%)	32.7	39.0-56.0
MCV (fL)	58.7	62.0-72.0
MCH (pg)	19.0	20-25
MCHC (g/dL)	32.4	30.0-38.0
PLT ($10^3/\mu\text{L}$)	277	117-460
LY (12.6%)	7.4	0.8-5.1
MO (6.5%)	3.8	2.0-9.0



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 2. Serum biochemistry results

Test	Result	Reference Range
AST(U/L)	83	5-55
ALT(U/L)	23	5-60
Alkaline phosphatase (U/L)	400	10-150
Protein Total(g/dl)	6.9	5.1-7.8
Albumin(g/dl)	4.8	3.1-4.5
Globulin(g/dl)	2.1	1.9-3.7
A/G	2.2	0.9-1.9

After confirmation of the diagnosis, treatment was initiated with Imidocarb dipropionate combined with clindamycin, administered intramuscularly in two doses with a two-week interval. The patient demonstrated clinical improvement during subsequent follow-up evaluations.

Canine hepatozoonosis caused by *H. canis* has been reported from many geographic areas, including Africa, southern Europe, Asia and the USA (8-13). Latta et al.,(2021) also reported an occurrence of *H. canis* infection in Ahvaz; according to their study, 34 out of 69 dogs examined tested positive for the parasite (14). This case report documents a confirmed case of *H. canis* infection in a dog from Ahvaz, Iran, expanding the known geographic distribution of the parasite within the country. The diagnosis was substantiated both microscopically and by PCR confirming the presence of *H. canis* gamonts in neutrophils with a parasitemia level of approximately 1%, which falls within the range reported in previous studies for subclinical or mild infections (6,7). The case discussed in this study exhibited clinical signs similar to those reported by Gondim et al., (1998) (15) and Khoshnegah et al., (2009) (16), except for the presence of diarrhea, vomiting, polyurea and polydipsia which were not observed in the current patient. Conversely, Zokatfi et al., (2023), who investigated *Hepatozoon* identification in Shiraz, reported no clinical signs in dogs positive for *H. canis* (17). Contrary to prior studies documenting thrombocytopenia and hypoalbuminemia in *Hepatozoon*-positive cases, the present case exhibited no thrombocytopenia and demonstrated mild hyperalbuminemia. The absence of tick collection and prior antibiotic use may have influenced clinical and hematological findings.

This first confirmed case of *H.canis* in Ahvaz highlights the parasite's local presence, suggesting the need for broader epidemiological studies. Future research should focus on



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



parasite genotyping, vector identification, and clinical variability in infected dogs across different regions.

Acknowledgement:

This study was supported by the research grant provided by the Shahid Chamran University of Ahvaz. The author gratefully acknowledges the collaboration of the faculty and staff of the Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, and Mahrouyan Veterinary Clinic for their support in this study.

References:

1. Modrý D, Beck R, Hrazdilová K, Baneth G. A review of methods for detection of Hepatozoon infection in carnivores and arthropod vectors. *Vector-Borne and Zoonotic Diseases*. 2017; 17(1):66-72.
2. Johnson EM, Allen KE, Panciera RJ, Ewing SA, Little SE. Experimental transmission of *Hepatozoon americanum* to New Zealand White rabbits (*Oryctolagus cuniculus*) and infectivity of cystozoites for a dog. *Veterinary Parasitology*. 2009; 164(2-4):162-6.
3. Baneth G, Breitschwerdt EB, Hegarty BC, Pappalardo B, Ryan J. A survey of tick-borne bacteria and protozoa in naturally exposed dogs from Israel. *Veterinary Parasitology*. 1998;74(2-4):133-42.
4. Baneth G. Perspectives on canine and feline hepatozoonosis. *Veterinary Parasitology*. 2011; 181(1):3-11.
5. Baneth G, Samish M, Shkap V. Life cycle of *Hepatozoon canis* (Apicomplexa: Adeleorina: Hepatozoidae) in the tick *Rhipicephalus sanguineus* and domestic dog (*Canis familiaris*). *Journal of Parasitology*. 2007; 93(2):283-99.
6. Baneth G, Weigler B. Retrospective case-control study of hepatozoonosis in dogs in Israel. *Journal of Veterinary Internal Medicine*. 1997; 11(6):365-70.
7. Baneth G, Mathew JS, Shkap V, Macintire DK, Barta JR, Ewing SA. Canine hepatozoonosis: two disease syndromes caused by separate *Hepatozoon* spp. *Trends in Parasitology*. 2003; 19(1):27-31.
8. Criado-Fornelio A, Rey-Valeiron C, Buling A, Barba-Carretero JC, Jefferies R, Irwin P. New advances in molecular epizootiology of canine hematic protozoa from Venezuela, Thailand and Spain. *Veterinary Parasitology*. 2007; 144(3-4):261-9.
9. Murata T, Shiramizu K, Hara Y, Inoue M, Shimoda K, Nakama S. First case of *Hepatozoon canis* infection of a dog in Japan. *Journal of Veterinary Medical Science*. 1991; 53(6):1097-9.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



10. Panciera RJ, Gatto NT, Crystal MA, Helman RG, Ely RW. Canine hepatozoonosis in Oklahoma. *Journal of the American Animal Hospital Association*. 1997; 33(3):221-5.
11. Baneth G, Cohn L. Canine hepatozoonosis. *Encyclopedia of Arthropod-transmitted infections of man and domesticated animals*. CABI, Wallingford. 2001:215-20.
12. Shaw SE, Day MJ, Birtles RJ, Breitschwerdt EB. Tick-borne infectious diseases of dogs. *Trends in Parasitology*. 2001; 17(2):74-80.
13. Voyvoda H, Pasa S, Uner A. Clinical *Hepatozoon canis* infection in a dog in Turkey. *Journal of Small Animal Practice*. 2004; 45(12):613-7.
14. Iatta R, Sazmand A, Nguyen VL, Nemati F, Ayaz MM, Bahiraei Z, Zafari S, Giannico A, Greco G, Dantas-Torres F, Otranto D. Vector-borne pathogens in dogs of different regions of Iran and Pakistan. *Parasitology Research*. 2021 Dec;120(12):4219-28.
15. Gondim LF, Kohayagawa A, Alencar NX, Biondo AW, Takahira RK, Franco SR. Canine hepatozoonosis in Brazil: description of eight naturally occurring cases. *Veterinary Parasitology*. 1998; 74(2-4):319-23.
16. Khoshnegah J, Mohri M, Movassaghi AR, Mehrjerdi HK. The first report of *Hepatozoon canis* infection of a dog in Iran. *Comparative Clinical Pathology*. 2009; 18:455-8.
17. Baneth G, Allen K. Hepatozoonosis of Dogs and Cats. *Veterinary Clinics of North America: Small Animal Practice*. 2022; 52(6):1341-1358.



Study of the Synergistic Effects of Combined Essential Oils from *Lavandula angustifolia* and *Artemisia absinthium* Shoots on *Leishmania infantum* Promastigotes

Morteza Eshagh Nimori¹, Sohrab Rasouli², Samin Julai Sani³

Corresponding Author: Morteza Eshagh Nimori, Dr.eshaghi.morteza@gmail.com

1. Department of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran.
2. Department of Parasitology, Faculty of Veterinary, Urmia Branch, Islamic Azad University, Urmia, Iran.
3. Department of Radiology, Sanandaj Branch, Kurdistan University of Medical Sciences, Sanandaj, Iran.

Article code: 1266

Abstract

Background: Leishmaniasis is a less common infectious and zoonotic disease caused by the protozoan *Leishmania*. Current treatment options are limited, and in the absence of a vaccine, there is a need for effective alternatives. Medicinal plants, such as lavender and wormwood, are potential alternatives with therapeutic properties. This study aims to evaluate the synergistic effects of combining essential oils from lavender and wormwood shoots on the death of *Leishmania infantum* promastigotes under laboratory conditions to determine if this combination has a lethal effect on the promastigotes.

Methods: The standard *L. infantum* promastigote strain was cultured in TRI medium. Various concentrations of saline, Glucantime, Amphotericin B, and a combination of wormwood and lavender extracts (10, 100, 200, 300, 500, and 1000 mg/mL) were studied over 24, 48, and 72 hours. The MTT assay was performed to assess viability and toxicity.

Results: The control group's activity remained relatively stable over time, while antibiotic activity was consistent from 24 to 72 hours, around 90 to 100%. The antileishmanial activity of the combined extracts significantly increased over time at a 1/10 concentration, approaching that of antibiotics. At the highest dilution (1/1000), activity significantly decreased and was close to the control. Other dilutions showed relatively stable activity. The correlation between the two methods was 0.925, indicating a very good agreement.

Conclusion: The combination of lavender and wormwood extracts demonstrated increased lethality against *L. infantum* promastigotes at higher concentrations and longer durations, showing similar efficacy to Glucantime and Amphotericin B at higher concentrations and extended exposure times.



Keywords: *Lavandula angustifolia*, *Artemisia absinthium*, *Leishmania infantum*, Promastigotes.

Introduction:

Leishmaniasis is an infectious disease caused by intracellular protozoa of the genus *Leishmania* (1). Leishmaniasis is caused by parasitic protozoa from the genus *Leishmania*, which is part of the Trypanosomatidae family (2). *Leishmania* spp. infects roughly 12 million people worldwide, with an additional 350 million at risk (3). Leishmaniasis is primarily prevalent in low-income countries due to underfunded elimination programs, lack of pharmaceutical industry interest, and inadequate healthcare infrastructure (4, 5). Common antimony compounds, such as meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam), are regarded first-line treatments for leishmaniasis. These medications function by blocking parasite enzymes, specifically phosphokinase, which hinders ATP generation (6). Medicinal plants and their derivatives are one possible option, as they are not only inexpensive but can also be utilized to treat infectious disorders (7). Herbal treatments may help reduce resistance to chemical drugs (8). Given Iran's unique environment and, as a result, flora, there is potential to identify and extract useful plant components from a wide range of local plants for large-scale and industrial use. This is especially relevant for plants that are native to Iran and have been understudied so far (9). Recent study on natural plant chemicals has shown that alkaloids, flavonoids, saponins, coumarins, quinoline, terpenes, and tetralins have antileishmanial properties against some *Leishmania* species (7).

Artemisia absinthium, commonly known as Wormwood, belongs to the Compositae (Asteraceae) family. Wormwood is one of the oldest aromatic and antiparasitic medicinal plants. This plant contains volatile oil (wormwood essential oil), a type of bitter compound (absinthin), resin, tannin, potassium, and other substances (10). Lavender, with the scientific name *Lavandula*, belongs to the Labiatae family. Lavender flowers contain volatile essential oils and tannins and act as a sedative for the central nervous system and respiratory system. Additionally, this plant can be used to treat diarrhea, relieve bloating, treat rheumatism, and heal skin diseases and wounds, among other uses (11).

The combination of these two plants, due to their mentioned components and compounds, could potentially have antileishmanial and antimicrobial activities, though this has not yet been studied. In this study, the antileishmanial activity of the combination of these two plants will be investigated. Therefore, the overall aim of the study is to evaluate the synergistic effects of the combined essential oils of lavender and wormwood on the cell death of *L. promastigotes*.



Materials and Methods:

Different concentrations of the combination (wormwood and lavender extracts) and Glucantime (10, 100, 200, 300, 500, and 1,000 mg/mL) were studied at 24, 48, and 72 hours, with both MTT assay performed. Wells without plant essential oils and drugs were considered as controls and treated with saline at the same concentrations. The experimental groups included the combination (wormwood and lavender extracts), Glucantime, Amphotericin B, and a control group. These were treated with the combination (wormwood and lavender extracts), Amphotericin B, Glucantime, and saline at concentrations of 10, 100, 200, 300, 500, and 1,000 mg/mL. A total of 18 treatments were performed, with 10 replicates for each treatment. The counting of live and dead cells was performed using the optical absorption method with the ELISA reader. Stained and unstained cells were considered dead and live cells, respectively. Viability and cytotoxicity were calculated as follows:

Viability (%) = (Viable cells (n))/(Total number of cells), % cytotoxicity= 100- % viability

Results and Discussion:

The results showed that the highest antileishmanial activity of antibiotics occurred within the first 24 to 72 hours, maintaining a level of about 90 to 100 percent. This activity significantly decreased with further dilution of the extracts. At a 1/10 concentration, the activity was close to that of antibiotics (around 85–90%), while at the highest dilution (1/1000), the activity dropped significantly and approached that of the control group. The control group's activity remained nearly constant over time, while the antibiotics and 1/10 concentration extracts showed relatively stable and similar activity levels. Other dilutions exhibited relatively stable but lower activity. The components of the extracts from the two plants also exhibited strong antileishmanial action, according to the study's findings. The results showed that utilizing a mixture of these extracts decreased promastigotes' viability, with the biggest impact being shown at greater concentrations and for longer periods of time. The results of this study indicated that at higher concentrations and longer durations, the combined extracts of lavender and wormwood exhibited a nearly equal lethal effect to the drugs Glucantime and Amphotericin B, due to the components present in the plant extracts. These findings suggest that the combination of these extracts requires more time and higher concentrations to be effective. Similarly, Yektaeian et al. (12) demonstrated that the combination of extracts from three plants—yarrow, wormwood, and walnut leaves—led to increased immobility of *Leishmania* parasites, with a significant decrease in parasite numbers over time. They also found that various concentrations of wormwood extract reduced the number of *Leishmania* parasites.

In this study, the synergistic effects of combining the extracts of lavender and wormwood on *L. infantum* promastigotes were assessed using the MTT and trypan blue methods under

laboratory conditions. The combination exhibited significant antileishmanial activity. It was found that the combined extracts showed similar efficacy to Glucantime and Amphotericin B at higher concentrations and extended durations, with the highest effect observed at a 1/10 concentration, achieving around 90 to 100% antileishmanial activity within the first 72 hours. Consequently, the combination of lavender and wormwood extracts demonstrated increased lethality against *L. infantum* promastigotes at higher concentrations and longer exposure times. Given that the combined extracts of lavender and wormwood showed antileishmanial effects under laboratory conditions, it is recommended that this combination be explored further in clinical studies.

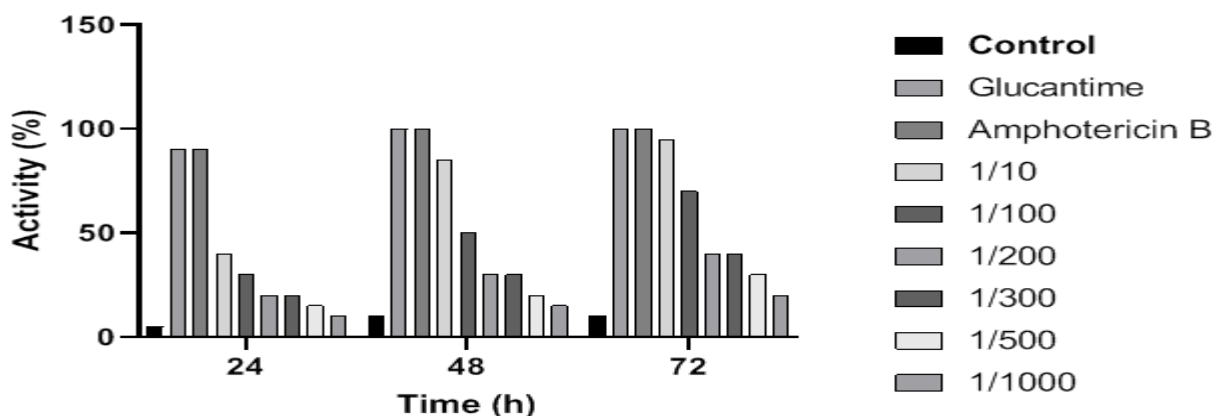


Figure 1. Antileishmanial activity of various compounds over different time points using the MTT method.

References:

1. Lafleur A, Olivier M. Viral endosymbiotic infection of protozoan parasites: how it influences the development of cutaneous leishmaniasis. *Plos Pathogens*. 2022;18(11):e1010910.
2. Cecílio P, Cordeiro-da-Silva A, Oliveira F. Sand flies: Basic information on the vectors of leishmaniasis and their interactions with *Leishmania* parasites. *Communications biology*. 2022;5(1):305.
3. Lockard RD, Wilson ME, Rodríguez NE. Sex-related differences in immune response and symptomatic manifestations to infection with *Leishmania* species. *Journal of immunology research*. 2019;2019(1):4103819.
4. Olias-Molero AI, de la Fuente C, Cuquerella M, Torrado JJ, Alunda JM. Antileishmanial drug discovery and development: time to reset the model? *Microorganisms*. 2021;9(12):2500.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



5. Scarpini S, Dondi A, Totaro C, Biagi C, Melchionda F, Zama D, et al. Visceral leishmaniasis: Epidemiology, diagnosis, and treatment regimens in different geographical areas with a focus on pediatrics. *Microorganisms*. 2022;10(10):1887.
6. Walker J, Gongora R, Vasquez J-J, Drummelsmith J, Burchmore R, Roy G, et al. Discovery of factors linked to antimony resistance in *Leishmania panamensis* through differential proteome analysis. *Molecular and Biochemical Parasitology*. 2012;183(2):166-76.
7. Gharirvand Eskandari E, Setorki M, Douidi M. Medicinal plants with antileishmanial properties: a review study. *Pharmaceutical and Biomedical Research*. 2020;6(1):1-16.
8. Passero LFD, Brunelli EdS, Sauini T, Amorim Pavani TF, Jesus JA, Rodrigues E. The potential of traditional knowledge to develop effective medicines for the treatment of leishmaniasis. *Frontiers in Pharmacology*. 2021;12:690432.
9. Bolouri P, Salami R, Kouhi S, Kordi M, Asgari Lajayer B, Hadian J, et al. Applications of essential oils and plant extracts in different industries. *Molecules*. 2022;27(24):8999.
10. Bhat RR, Rehman MU, Shabir A, Rahman Mir MU, Ahmad A, Khan R, et al. Chemical composition and biological uses of *Artemisia absinthium* (wormwood). *Plant and Human Health, Volume 3: Pharmacology and Therapeutic Uses*. 2019:37-63.
11. Khan SU, Hamza B, Mir RH, Fatima K, Malik F. Lavender plant: farming and health benefits. *Current Molecular Medicine*. 2024;24(6):702-11.
12. Yektaeian N, Rafeian M, Khalili-Dehkordi B, Hejazi S, Shirani-Bidabadi L, Hosseini S. Effect of combination of *Achillea millefolium*, *Artemisia absinthium* & *Juglans regia* leaves extracts on *Leishmania major* (MRHO/IR/75/ER), in vitro. 2012.



CRISPR-based Rapid Diagnosis of Zoonotic Parasites: Advances and Prospects

Reza Soltani¹, Bahareh Meftahi^{2,3}

Corresponding Author: Reza Soltani, rezasoltani.rs.rs@gmail.com

1. Microbiology and Biology Research Group, Food Technology and Agricultural Products Research Center, Standard Research Institute, Karaj, Iran.

2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.

3. Innovative Medical Research Center, MMS.C, Islamic Azad University, Mashhad, Iran.

Article code: 1270

Abstract

Background: Zoonotic parasitic diseases such as toxoplasmosis, leishmaniasis, cryptosporidiosis, and giardiasis remain persistent threats to public health and animal production.

Methods: Conventional diagnostics based on microscopy, culture, immunoassays and PCR face limitations in sensitivity, turnaround time, infrastructure demands and scalability for decentralized screening. CRISPR-Cas systems have rapidly evolved from genome editing tools into next-generation diagnostics that couple programmable nucleic acid recognition with sensitive signal transduction, enabling accurate detection in under an hour and compatibility with point of care workflows.

Results: This review synthesizes recent advances in CRISPR-based assays for *Toxoplasma gondii*, *Leishmania* spp., *Cryptosporidium* spp and *Giardia duodenalis*, examining assay design, analytical performance, and field readiness.

Conclusion: We discuss implementation challenges including reagent stability, sample preparation, quality assurance and regulatory pathways and outline future directions such as multiplexing, smartphone-enabled readouts, and integration with AI for automated interpretation. Collectively, CRISPR platforms offer a practical path to faster, more accessible and scalable surveillance of zoonotic parasites across clinical, veterinary, and environmental settings.

Keywords: CRISPR, Molecular diagnostics, Zoonotic parasites, Toxoplasmosis, Leishmaniasis, Cryptosporidiosis, Giardiasis.



Introduction:

Zoonotic protozoan infections occupy a critical niche at the human, animal and environment interface with substantial morbidity, economic loss and spillover risk. Timely detection is central to treatment decisions, outbreak control and safeguarding food and water safety (1,4).

Microscopy is specific but lacks sensitivity in low parasitemia or early infection. Culture and bioassays are slow and resource intensive. Immunoassays can be rapid yet prone to cross reactivity and may not distinguish active from past infection (4,6).

CRISPR diagnostics leverage programmable RNA-guided nucleases (e.g., Cas12, Cas13) to recognize target DNA/RNA and activate collateral cleavage of reporter molecules enabling sensitive fluorescent or lateral-flow readouts. Pioneering platforms such as SHERLOCK (Cas13) and DETECTR (Cas12) established isothermal, amplification coupled workflows that deliver high sensitivity with simple equipment, catalyzing adaptation to a wide range of pathogens (7,11). Building on these foundations, parasite focused assays now demonstrate clinically relevant performance with turnaround times of 30–60 minutes and readouts suited to low resource settings (1,3).

Materials and methods:

We conducted a focused narrative review of peer-reviewed literature from January 2022 to May 2024 about advances and aspects of CRISPR-based rapid diagnosis of zoonotic parasites including *Toxoplasma*, *Leishmania*, *Cryptosporidium* and *Giardia* (1,6).

Results and Discussion:

Overview of CRISPR diagnostic platforms relevant to parasitology

Enzymes and targets: Cas12 (e.g., Cas12a) targets DNA and exhibits trans cleavage of ssDNA reporters upon activation; Cas13 targets RNA and cleaves ssRNA reporters (7,9).

Guides (crRNAs) are designed against conserved loci (e.g., multicopy genes) to maximize sensitivity in low-abundance samples (1,2).

Amplification strategies: Isothermal methods such as recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP) enrich targets at 37–65°C without thermocyclers, enabling faster workflows and simple heaters (7,10,11).

One-pot or two-pot formats balance speed and contamination control; chemical/physical separation within a single tube reduces amplicon aerosolization (10,11).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Readouts: Fluorescence (portable readers or smartphones), lateral flow immunochromatography (visual bands) and colorimetric systems support use from labs to field settings (1,3,7,11).

Smartphone adapters and simple optics have demonstrated semi quantitative analysis and data capture for surveillance (5,6).

Sample preparation: Crude extraction or heat/chemical lysis (e.g., HUDSON-like approaches) can reduce time and cost, though inhibitors in fecal or environmental matrices may necessitate minimal purification (4,6,10).

CRISPR applications in toxoplasmosis diagnosis: CRISPR-Cas12 assays targeting *T.gondii* repetitive elements (e.g., B1 gene, 529 bp repeat) have reported detection within 30–60 minutes, with analytical sensitivity rivaling or exceeding PCR in spiked and clinical samples. The use of multicopy targets improves detection in low parasite loads typical of early infection or chronic tissue cysts (1,4).

Adaptations for diverse sample types including blood, amniotic fluid and food matrices (e.g., meat) highlight the platform's versatility across clinical diagnostics and food safety monitoring (1,4,6). RPA Cas12 combinations minimize equipment needs and reduce power dependence, facilitating deployment in veterinary clinics and field surveys (1,4).

CRISPR in leishmaniasis detection: *Leishmania* spp diagnostics benefit from CRISPR assays that target conserved kinetoplast DNA (kDNA) minicircles or ribosomal markers, achieving species level detection with rapid turnaround. Field-deployable Cas12 workflows paired with lateral flow strips have shown robust performance on lesion swabs and minimally processed samples, supporting diagnosis in peripheral clinics without access to PCR (2,4,5).

Detection of *Cryptosporidium* and *Giardia*: Multiplex CRISPR-Cas12 assays have been developed to simultaneously detect *Cryptosporidium* and *Giardia*. Isothermal pre-amplification enhances sensitivity in low titer environmental samples while carefully optimized lysis buffers mitigate inhibitors. Lateral flow formats are particularly attractive for field testing at water treatment facilities and in rural settings. Integration with simple filtration or cartridge-based pre-concentration increases detection probability in large-volume water samples (3,5).

Comparative advantages and practical considerations

Speed and sensitivity: Time to result is typically 30–60 minutes from nucleic acid extraction to readout, making CRISPR assays competitive with antigen tests and faster than PCR workflows (1,3).

Analytical sensitivity can approach or exceed qPCR when targeting multicopy loci and using optimized isothermal amplification (1,2,4).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Portability and cost: Minimal hardware (heat blocks, lateral flow strips, portable fluorescence readers) enables point of care use. Reagent costs trend lower than PCR when scaled though commercial kit pricing varies across markets (2,4,6).

Specificity: Guide RNA programmability confers high specificity, with the potential to discriminate species or lineages. Rigorous in silico design and wet lab validation are essential to avoid cross reactivity in genetically related parasites (2,4).

Quality systems: Adoption in clinical veterinary labs requires standardized controls, external quality assessment and calibration of portable readers. Closed-tube or cartridge systems mitigate contamination risks inherent to amplification-based assays (4,6).

Limitations

Reagent stability and supply chains: Enzyme stability under fluctuating temperatures remains a challenge for true ambient logistics; lyophilized reagents and cold chain alternatives are advancing but not yet universal (4,6,10,11).

Sample matrix complexity: Fecal and environmental samples introduce inhibitors that can suppress amplification or nuclease activity; streamlined extraction compatible with field use is a key bottleneck (3,5).

Regulatory and standardization hurdles: While research prototypes abound, relatively few CRISPR assays for parasites have undergone rigorous multicenter validation, regulatory review, or WHO prequalification and slowing clinical translation (4,6).

Readout interpretation: Lateral flow sensitivity and band subjectivity can vary coupling to simple fluorescence or smartphone analytics can improve consistency but adds components and cost (5,6,10).

Future directions

Multiplexing and syndromic panels: Parallel detection of multiple parasites (and relevant bacterial/viral co-pathogens) in a single reaction can streamline differential diagnosis in diarrheal disease and febrile illnesses (3,5).

Integrated devices: Cartridge based closed systems that combine sample prep amplification, CRISPR detection and readout minimize contamination and user steps improving reliability in point of-care contexts (5,10,11).

Digital and AI-enabled interpretation: Smartphone cameras and lightweight models can quantify fluorescence or band intensity, standardize calls across sites and support geotagged surveillance dashboards for veterinary and public health programs (5,6).

Assay robustness: Enzyme engineering buffer optimization and lyophilization will extend shelf life and performance across temperature extremes common in field deployments (10,11).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Clinical validation: Prospective, multicenter studies comparing CRISPR assays with composite reference standards across diverse geographies and matrices (blood, stool, swabs, water) will accelerate regulatory acceptance and guideline inclusion (1,6).

Conclusion: CRISPR diagnostics have moved from concept to compelling reality for key zoonotic parasites, offering speed, specificity and field adaptability that directly address the limitations of conventional methods. Demonstrated applications for *Toxoplasma gondii*, *Leishmania* spp, *Cryptosporidium* and *Giardia* show strong potential for routine use in clinical, veterinary and environmental surveillance. Strategic investment in reagent stabilization, integrated sample preparation, standardized workflows and rigorous validation will be pivotal to scale up. As multiplexed, smartphone enabled platforms mature and analytics become more automated CRISPR assays are poised to become foundational tools in One Health strategies for parasite control and outbreak prevention (1,6,10,11).

References:

1. Wang L, et al. CRISPR-Cas12 based rapid detection of *Toxoplasma gondii*. *Parasitology Research*. 2023;122(5):1725–1735.
2. Kumar R, et al. Field-deployable CRISPR diagnostics for *Leishmania* spp. *Acta Tropica*. 2023;241:106737.
3. Zhao H, et al. Multiplex CRISPR-Cas assays for simultaneous detection of *Cryptosporidium* and *Giardia*. *Water Research*. 2024;230:119512.
4. Lee S, et al. Advances and challenges of CRISPR-based diagnostic tools in parasitology. *Trends in Parasitology*. 2023;39(3):177–189.
5. Qin PZ, et al. Point-of-care CRISPR diagnostic platforms: from design to field deployment. *Biosensors and Bioelectronics*. 2023;235:115357.
6. Sun F, et al. CRISPR-based diagnostics for infectious diseases: technology maturation and translation. *Nature Reviews Bioengineering*. 2024;2(1):35–53.
7. Gootenberg JS, et al. Nucleic acid detection with CRISPR-Cas13a (SHERLOCK). *Science*. 2017;356(6336):438–442.
8. Chen JS, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity (DETECTR). *Science*. 2018;360(6387):436–439.
9. Li SY, et al. HOLMES: one-pot CRISPR-Cas12a assay for sensitive detection of DNA. *Cell Discovery*. 2018;4:20.
10. Ding X, et al. All-in-one dual CRISPR-Cas12a (AIOD-CRISPR) assay: one-pot, ultrasensitive detection at isothermal conditions. *Nature Communications*. 2020;11:4711.
11. Broughton JP, et al. CRISPR-Cas12-based detection of SARS-CoV-2 with DETECTR. *Nature Biotechnology*. 2020;38:870–874.



Evaluation of the Impact of Anthelmintic Treatment of Herding Dogs with Praziquantel and Their Nutritional Hygiene Management on Reducing Cerebral Coenurosis in Female Afshari Lambs Under Two Years of Age

Ali Sallehi¹, Gholamreza Mohammadi²

Corresponding Author: Gholamreza Mohammadi, gmohamad@um.ac.ir

1. Private veterinarian, Mashhad, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1281

Abstract

Background: Cerebral coenurosis, caused by *Taenia multiceps* larvae, is a significant neurological disease in sheep, impacting the central nervous system and causing economic losses due to mortality and culling. Herding dogs, as definitive hosts, transmit the parasite through fecal egg shedding, contaminating pastures. This study evaluates the effectiveness of regular anthelmintic treatment of herding dogs with praziquantel and nutritional hygiene management in reducing coenurosis incidence in female Afshari lambs under two years of age.

Methods: From 2019 to 2024, in a 20,000-head sheep breeding unit in Jolgha Rokh-Torbat Heydariyeh, Iran, 100 herding dogs were treated with praziquantel (5 mg/kg body weight, every 6 weeks) starting in 2021. Dog feces were collected and incinerated post-treatment to prevent egg dissemination, and feeding dogs with infected sheep offal was prohibited. Female Afshari lambs under two years were monitored for clinical signs (e.g., circling, head deviation) and confirmed via necropsy. Prevalence was calculated annually, and pre- and post-treatment data were compared using the chi-square test ($P < 0.05$).

Results: Of 31,252 lambs examined, coenurosis prevalence decreased from 12.5% in 2019–2020 (before treatment) to 0.22% in 2024 ($P < 0.001$) after implementing the treatment and hygiene program. Necropsy confirmed *Coenurus cerebralis* cysts in the brain or cerebellum of affected lambs. No adverse effects from praziquantel were observed in dogs.

Conclusion: Regular praziquantel treatment of herding dogs combined with nutritional hygiene management significantly reduced cerebral coenurosis prevalence, improving herd health and reducing economic losses. This cost-effective strategy is recommended for broader implementation in sheep farming.

Keywords: *Taenia multiceps*, Praziquantel, Sheep, Prevention.



Introduction:

Cerebral coenurosis is a common parasitic disease in sheep, primarily affecting the brain and spinal cord. It is associated with neurological symptoms such as confusion, circling, head deviation, imbalance, and blindness, often leading to animal death and economic losses (1). The risk of transmission to humans is negligible. Coenurosis is more prevalent in young sheep (9–18 months) and causes significant economic losses due to the culling or slaughter of affected animals.

The parasite's life cycle involves definitive hosts (dogs and other carnivores) and intermediate hosts (sheep and other ruminants). Herding dogs play a key role in disease transmission by shedding parasite eggs in their feces, contaminating pastures (2). Sheep ingesting contaminated forage develop cysts in their central nervous system (CNS), leading to clinical lesions after 6–8 months.

In Iran, coenurosis prevalence ranges from 0.3% to 42.2%, depending on factors such as herd management, feeding dogs with infected offal, and lack of anthelmintic treatment (3,4). Treating affected sheep is challenging and uneconomical, making prevention by interrupting the parasite's transmission cycle critical. Regular treatment of herding dogs with praziquantel and hygienic carcass management have been recommended as effective preventive measures (5). However, limited data exist on the long-term effectiveness of these strategies in large Iranian herds. This study aimed to assess the impact of regular praziquantel treatment of herding dogs and their nutritional hygiene management on reducing coenurosis incidence in female Afshari lambs under two years in a 20,000-head herd in the Jolgha Rokh-Torbat Heydariyeh region. Slaughterhouse studies in Iran have reported varying prevalence, indicating the influence of regional and management factors (6). This study evaluated a preventive program based on praziquantel treatment and hygienic carcass management in a large herd over five years to provide a practical and effective disease control strategy.

Materials and Methods:

The study was conducted from 2019 to 2024 in a 20,000-head sheep breeding unit in the Jolgha Rokh-Torbat Heydariyeh region. The target population was female Afshari lambs under two years, selected due to their high susceptibility to coenurosis.

From summer 2021, 100 herding dogs were treated every 6 weeks with praziquantel (Drontal tablets, 5 mg/kg body weight) (7). After the initial treatment, dog feces were collected and incinerated for 3 days to prevent egg dissemination. Feeding dogs with infected sheep offal or carcasses was strictly prohibited. Sheep were regularly monitored for clinical signs of coenurosis (circling, head deviation, imbalance, blindness), and suspected cases were confirmed by necropsy. Sheep were fed from pastures in Sarakhs, Khangiran, Jolgha Rokh, and Feyzabad-Mahvelat, supplemented with manual feeding. Confirmed coenurosis cases were culled or slaughtered.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Data on lamb populations and coenurosis cases were recorded annually. Disease prevalence was calculated as (number of confirmed cases ÷ susceptible population × 100). The chi-square test with a significance level of $P < 0.05$ was used to compare prevalence before and after treatment (8).

Results and Discussion:

Over five years, 31,252 female lambs were examined. Before the treatment program (2019–2020), the average coenurosis prevalence was 12.5%. After initiating regular dog treatment in 2021, prevalence gradually decreased, reaching 0.22% in 2024 ($P < 0.001$). Table 1 provides detailed results. Necropsy of slaughtered animals confirmed *Coenurus cerebralis* cysts in the brain or cerebellum. No adverse effects from praziquantel were reported in dogs.

Table 1. Female lamb population and coenurosis cases by year (2019–2024)

Year	Female Lamb Population	Coenurosis Cases	Prevalence (%)
2019	6200	775	12.5
2020	6350	794	12.5
2021	6400	512	8
2022	6300	126	2
2023	6250	31	0.5
2024	6252	14	0.22

The results demonstrate that regular praziquantel treatment of herding dogs and hygienic carcass management effectively reduced coenurosis prevalence. The decline from 12.5% to 0.22% over five years confirms the importance of interrupting the parasite's transmission cycle. Previous studies have also reported praziquantel's effectiveness in reducing *Taenia* egg shedding by dogs (2,5).

The initial 12.5% prevalence in this study aligns with the 18.6% reported in Urmia (Tavassoli et al., 2011) but is higher than 0.3% in Babol (6) and 3.8% across various Iranian cities (4). These variations are attributed to sampling methods (live population vs. slaughterhouse), animal age, and herd management. Lambs under two years were an appropriate target group due to their high susceptibility to CNS cyst formation (1).

International studies also report varying prevalence. Gicik et al. (2007) in Turkey linked an 8.73% prevalence to feeding dogs with infected offal, while Scala et al. (2007) in Sardinia



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



reported 0.35% due to better hygiene management (5,9). This study provides stronger evidence than cross-sectional studies by examining a large, stable population over five years (8).

Carcass management and preventing dogs' access to offal complemented praziquantel treatment, playing a key role in reducing the parasite's transmission cycle. This aligns with WHO recommendations (2014) for controlling zoonotic diseases (10). However, limitations include not examining other definitive hosts (e.g., foxes) and potential pasture contamination from external sources.

Regular treatment of herding dogs with praziquantel (5 mg/kg body weight, every 6 weeks) and hygienic carcass management reduced cerebral coenurosis prevalence from 12.5% to 0.22%. This preventive strategy improved herd health and minimized economic losses. It is recommended that this program be implemented in other farms, and future studies explore its impact in different regions and livestock species.

Farmers should consistently treat herding dogs with praziquantel (5 mg/kg body weight, every 6–8 weeks), collect and dispose of dog feces post-treatment, and prevent feeding dogs with sheep offal or carcasses. This cost-effective approach minimizes coenurosis prevalence and ensures herd health. Farmer education and collaboration with veterinarians for herd monitoring are recommended.

Acknowledgments:

We express our gratitude to the esteemed management of Khazaei Rafie Agro-Industry, Mr. Eng. Abdulreza Khazaei, and the livestock unit staff for their sincere cooperation in conducting this research.

References:

1. Sharma DK, Chauhan PPS. Coenurosis status in Afro-Asian region: A review. *Small Rumin Res.* 2006;64(3):197–202. doi: 10.1016/j.smallrumres.2005.05.021.
2. Varcasia A, Tamponi C, Ahmed F, Cappai MG, Porcu F, Mehmood N, Dessì G, Scala A. Epidemiological and diagnostic insights into *Coenurus cerebralis* in sheep. *Parasites Vectors.* 2022;15(1):84. doi: 10.1186/s13071-022-05210-0.
3. Tavassoli M, Malekifard F, Soleimanzadeh A, Tajik M. Prevalence of *Coenurus cerebralis* in sheep in Northwest Iran. *Vet Res Forum.* 2011;2(3):165–70.
4. Oryan A, Nazifi S, Sharifiyazdi H, Ahmadnia S. Prevalence of *Coenurus cerebralis* in sheep in Iran. *Trop Biomed.* 2014;31(1):1–16.
5. Gicik Y, Kara M, Arsalan MO. Prevalence of *Coenurus cerebralis* in sheep in Kars Province, Turkey. *Bull Vet Inst Pulawy.* 2007; 51:379–82.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



6. Yousefi MR, Abouhosseini M, Hosseini SM, Omidzahir S. Prevalence of *Coenurus cerebralis* in sheep at Babol slaughterhouse, 2008. *Vet Res J.* 2009;6(2).
7. Scott PR. *Sheep Medicine*. 2nd ed. Boca Raton: CRC Press; 2015. doi: 10.1201/b18182.
8. Thrusfield M. *Veterinary Epidemiology*. 4th ed. Oxford: Blackwell Publishing; 2018.
9. Scala A, Cancedda GM, Varcasia A. A survey of *Taenia multiceps* infection in sheep in Sardinia. *Vet Parasitol.* 2007;143(3–4):294–8.
10. World Health Organization. *Control of neglected zoonotic diseases: Report of the fourth international meeting; 2014 Nov 19–20; Geneva, Switzerland*. Geneva: WHO; 2014. Available from: https://iris.who.int/bitstream/handle/10665/183458/9789241508568_eng.pdf



Investigation of *Dipylidium Caninium* infection in Domestic Dogs and Cats

Sarah Emamdadi¹, Mohammad Sayah Layin², Sadaf PakNafas¹, Shiba Yousefvand³

Corresponding Author: Shiba Yousefvand, shiba.yousefvand@alumni.um.ac.ir

1. Department of Pharmacology, Islamic Azad University, Karaj Branch, Karaj, Iran.
2. Veterinary Network of Mashhad City, Razavi Khorasan, Mashhad, Iran.
3. Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1283

Abstract

Background: One of the challenges of today's societies is keeping pets, including dogs and cats. These animals can be infected with zoonotic diseases, including *Dipylidium Caninium*. Therefore, the present study examines *D. Caninium* infection in pet animals.

Methods: Clinical examinations of dogs and cats referred to the veterinary clinic for parasitic diseases from 3/21/2025 to 6/21/2025 were done. Then, identification of the genus and species of the parasite using the Formol-Ether method was done.

Results: 584 dogs and 253 cats referred to this clinic for periodic checkups. In the animals referred to this clinic, at the only one dog and one cat were infected with *D. Caninium*.

Conclusion: According to the results of this study, pet owners can significantly reduce the incidence of *D. Caninium* in their pets if they have a well-established anti-parasitic treatment program as well as anti-lice and flea treatments.

Keywords: Cat, Dog, *D. Caninium*, Prevalence.

Introduction:

Parasitic infections, especially intestinal parasites shared between humans and animals (zoonoses), are among the most important economic problems in developing countries, especially Iran. In Iran, urban residents especially in Tehran are interested in keeping pets including dogs and cats. Keeping these pets increases the number of disease reservoirs shared between humans and animals. Therefore, the transmission of these diseases to humans is important from a veterinary perspective, especially for the health-treatment system in providing services (1, 2).

D. Caninium is a highly prevalent zoonotic parasitic infection. Infection with *D. Caninium* has been reported in children from various parts of the world (3). Zoonotic parasites such as *D. Caninium* always have an arthropod intermediate host and a definitive host. The intermediate



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



host can be lice and fleas. The definitive host can also be dogs, cats, and humans. When the tapeworms of this parasite are excreted in the feces, the parasite larvae are also excreted with them. The parasite larvae in the feces may be ingested by arthropods such as fleas. When these fleas are ingested by definitive hosts such as dogs, cats, or humans, the worm develops into an adult cestode parasite within 3–4 weeks. The definitive hosts then excrete the tapeworms in the feces, and the cycle repeats.

Most human cases of parasitic infection have been reported in children. It is likely that children have come into contact with the feces of pets, or intermediate hosts, and have become infected through this route. To reduce the transmission of infection, it is best to treat pets with oral anti-parasitic medications as well as flea and lice treatments. The reported prevalence of parasitic infection in animal pets including dogs varies from 4-60% and in cats from 1.8-52.7% in the world and Iran. The actual prevalence of infection could be different and even higher because the data is only obtained from fecal flotation (4-6).

Due to the increase in keeping pets, an increase in the incidence of zoonotic parasitic infections, including *D. Caninium*, will also be observed. To reduce the transmission of zoonotic parasitic infections, there should be a documented program for the use of antiparasitic drugs for pet owners. Therefore, the present study examines the incidence of *D. Caninium* parasitic infections in patients referred to the Aristo Pet Clinic.

Materials and Methods:

584 dogs and 253 cats (total 837 cases) were referred to Aristo Pet Clinic in Tehran, Iran from 21/3/2025 (01/01/1404) to 21/6/2025 (31/03/1404). Among these animals referred to the clinic, cestodes were only observed in the feces of one dog and one cat. Fecal samples were collected and preserved in 10% formalin. Then, to determine the type of parasites in terms of genus and species, a fecal concentration test was performed using the Formol-Ether method under the supervision of a parasitologist at the clinic. In this method, one volume of feces was diluted with nine volume of 10% formalin. 7 ml of this solution was filtered through two layers of gauze into a 15 ml centrifuge tube. 3 ml of ether was added to this solution. The tube was first shaken and then centrifuged for 5 minutes at 1500 rpm. After that, the supernatant was discarded and a drop of the precipitate was mixed with a drop of iodine solution on a glass slide, covered with a coverslip and observed at 100x and 400x magnification (7).

Results and Discussion:

Among the 837 cases referred to the clinic, only one male British Shorthair cat and one sterilized male Schnauzer infected with *D. Caninium* (Figure 1).



Figure 1. Observation of *D. Caninium* eggs in dog and cat feces.

A three-month-old sterilized Schnauzer male dog weighing 2.990 kg and a British Shorthair cat excreted *D. Caninium* strands in their feces. After conducting the necessary tests to diagnose and confirm the genus and species of the parasite, anti-parasitic treatment was prescribed.

The results of the present study indicate a very low parasitic infection rate in dogs and cats referred to the clinic. In contrast, other studies have shown that a large number of street dogs and cats were infected with *D. Caninium* (4, 5, 8, 9). This difference in results could be due to the periodic and regular refer of pets, including dogs and cats, to clinics and the receipt of courses of anti-parasitic medications. Based on this, it can be concluded that having a documented treatment plan when visiting veterinary clinics can greatly reduce parasitic infections.

Treatment and control of the parasitic infection *D. Caninium* should be carried out simultaneously. That is, in addition to prescribing antiparasitic drugs, treatment against the infection reservoirs, including fleas and lice, should also be carried out simultaneously. If the parasite is eliminated and the intermediate host (lice and lice) remains, drug treatment is almost useless. Dogs and cats will likely contract this parasite into their bodies by licking their fur and body hair and through fleas or lice. Also, humans, especially children, may be infected with this zoonotic parasite by touching dogs and cats and swallowing fleas and lice (3, 6, 10, 11).

The results of the present study indicate very low infection rates with *D. Caninium* in dogs and cats visiting a veterinary clinic. This reduction in infection may be due to well-documented treatment programs for animals with owners.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Acknowledgment:

The authors are grateful to the Aristo Pet Clinic for cooperation.

References:

1. Hajinaghi MJ, Moslemi A, Ghasemikhah R. Ancylostomiasis Prevalence in Carnivores of Iran: A Systematic Review and Meta-Analysis. *International Archives of Health Sciences*. 2025;12(1). 60-69
2. Zibaei M, Sadjjadi S. Trend of toxocariasis in Iran: a review on human and animal dimensions. *Iranian Journal of Veterinary Research*. 2017;18(4):233-242.
3. Zhu G-Q, Li L, Ohiolei JA, Wu Y-T, Li W-H, Zhang N-Z, et al. A multiplex PCR assay for the simultaneous detection of *Taenia hydatigena*, *T. multiceps*, *T. pisiformis*, and *Dipylidium caninum* infections. *BMC infectious diseases*. 2019;19:1-7.
4. Beiromvand M, Akhlaghi L, Massom SHF, Meamar AR, Motevalian A, Oormazdi H, et al. Prevalence of zoonotic intestinal parasites in domestic and stray dogs in a rural area of Iran. *Preventive veterinary medicine*. 2013;109(1-2):162-7.
5. Beugnet F, Labuschagne M, de Vos C, Crafford D, Fourie J. Analysis of *Dipylidium caninum* tapeworms from dogs and cats, or their respective fleas: Part 2. Distinct canine and feline host association with two different *Dipylidium caninum* genotypes. *Parasite*. 2018;25:31.
6. Teixeira R, Flor I, Nunes T, Pinto C, Pomba MC, de Carvalho LM. Survey of Gastrointestinal Parasites and Lungworms in Cats and Dogs from Terceira and São Miguel Islands, Azores. *Pathogens*. 2024;13(8):648.
7. Gracenea M, Gómez M, Torres J. Prevalence of intestinal parasites in shelter dogs and cats in the metropolitan area of Barcelona (Spain). *Acta Parasitologica*. 2009;54(1):73-7.
8. Morelli S, Di Cesare A, Traversa D, Colombo M, Paoletti B, Ghietti A, et al. Comparison of diagnostic methods for laboratory diagnosis of the zoonotic tapeworm *Dipylidium caninum* in cats. *Veterinary Parasitology*. 2024;331:110274.
9. Murugan M, Arunvikram K, Pavulraj S, Milton A, Sinha D, Singh B. Companion animals: A potential threat in emergence and transmission of parasitic zoonoses. *Adv Anim Vet Sci*. 2015;3(11):594-604.
10. Bowman D. *Georgis' Parasitology for Veterinarians*. 1999.
11. Chelladurai JJ, Kifleyohannes T, Scott J, Brewer MT. Praziquantel resistance in the zoonotic cestode *Dipylidium caninum*. *The American journal of tropical medicine and hygiene*. 2018;99(5):1201.



First Report of Molecular Detection of Leishmaniasis in a Domestic Persian Cat (*Felis catus*) from North of Iran: A Case Report

Mostafa Kami^{1,2,3}, Najva Zivdari⁴, Morteza Valinezhad⁵, Mahla Homayounzade², Ali Safari², Seyed Mohammad Hosseini^{2,6}

Corresponding Author: Mostafa Kami, m.kami@mazums.ac.ir

1. The Health of Plant and Livestock Products Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
2. Department of Pathology, Bab.c., Islamic Azad University, Babol, Iran.
3. Young Researchers and Elite Club, Babol Branch, Islamic Azad University, Babol, Iran.
4. DVM Student, Department of Microbiology and Food Hygiene, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
5. Resident of Small Animal Internal Medicine, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.
6. Board-Certified Veterinary Pathologist, Aria-Azma Veterinary Diagnostic Lab, Babol, Iran.

Article code: 1292

Abstract

Background: *Leishmania infantum* causes zoonotic visceral leishmaniasis, with dogs as primary reservoirs. The role of cats remains unclear, with few reports globally and none molecularly confirmed in Iran. This case presents the first confirmed *L. infantum* infection in a Persian cat in northern Iran, offering new epidemiological insight.

Methods: A 2.5-year-old male Persian cat was presented with multiple non-healing dermatologic lesions, lethargy, and anorexia. Clinical samples, including skin scrapings and lesion exudate, were subjected to fungal culture, parasitological examination, and conventional PCR using universal ITS2-targeting primers (UNIL-IR-P and UNIL-IR-M) for *Leishmania* species identification. PCR products were analyzed by agarose gel electrophoresis. Additionally, bacterial isolates were tested for antibiotic susceptibility using the Kirby-Bauer disc diffusion method.

Results: Fungal culture confirmed the presence of *Microsporum canis*, while tests for *Malassezia* spp. and mites were negative. PCR amplification yielded a distinct band of approximately 690 bp, corresponding to *L. infantum*, as visualized by gel electrophoresis. This confirmed the presence of *Leishmania* DNA in the lesion sample. Bacterial culture identified a secondary infection, with isolates showing high sensitivity to Gentamicin, Trimethoprim + Sulfamethoxazole, Amikacin, Cefalexin, Enrofloxacin, and Erythromycin.

Conclusion: This case documents the first molecular detection of *Leishmania infantum* in a domestic cat from northern Iran and highlights the potential role of felines as incidental or secondary hosts in endemic areas. The findings underscore the importance of molecular



diagnostics in veterinary practice and support the need for further studies investigating the epidemiological role of cats in leishmaniasis transmission cycles.

Keywords: *Leishmania infantum*, Feline leishmaniasis, Molecular diagnosis, *Microsporium canis*.

Introduction:

Leishmaniasis is a vector-borne zoonotic disease caused by protozoan parasites of the genus *Leishmania*, transmitted primarily through the bite of infected female sandflies (*Phlebotomus* spp. in the Old World and *Lutzomyia* spp. in the New World) (1).

Feline leishmaniasis is an emerging concern in endemic areas. Although cats were historically considered resistant, molecular and serological evidence of infection has been increasingly reported from countries like Brazil, Spain, Italy, and Iran (2-4). In Iran, studies conducted in Fars (5) and Ardabil (3) provinces have identified *L. infantum* infection in stray cats using both serological (Direct antiglobulin test or DAT, Enzyme-linked immunosorbent assay or ELISA) and molecular (Polymerase chain reaction or PCR) techniques, with prevalence rates ranging from 19% to over 24%. Despite growing attention to the zoonotic aspects of leishmaniasis, limited data are available regarding the role of domestic cats (*Felis catus*) in the epidemiology of this disease (6).

Molecular diagnostic methods, especially PCR, have significantly improved the sensitivity and specificity of *Leishmania* detection in both symptomatic and asymptomatic hosts. PCR-based identification provides a reliable means of confirming infection, even in cases where parasite load is low or clinical presentation is atypical (7, 8). Due to limited data on feline leishmaniasis and cats' potential reservoir role, this study reports the first molecularly confirmed case in a domestic Persian cat from northern Iran, PCR targeting the ITS2 region, offering insights into clinical features, diagnosis, and molecular confirmation in felines.

Materials and Methods:

Case Presentation: A 2.5-year-old male domestic Persian cat was referred to a veterinary clinic in northern Iran with a history of lethargy, inappetence, and multiple dermatological lesions. Clinical examination revealed cutaneous involvement at the base and margins of the ears, fresh ulcerative lesions on the dorsal region, and seborrheic patches scattered across the abdominal and body regions. According to the owner, the cat had previously received multiple pharmacological treatments at another facility without clinical improvement.

Diagnostic Evaluation: Given the persistence of clinical signs, the cat was referred to a specialized veterinary diagnostic laboratory for further evaluation. Differential diagnoses



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



included fungal dermatitis, parasitic infections (including leishmaniasis and ectoparasites), and bacterial skin infections.

Mycological Examination: For fungal identification, samples were cultured on Sabouraud Dextrose Agar (SDA) containing chloramphenicol and cycloheximide. Plates were incubated at 25–28°C and examined daily for up to 21 days. Fungal growth was evaluated morphologically and microscopically.

DNA Extraction: Tissue and blood samples from lesions were processed for DNA extraction using a commercial genomic DNA extraction kit (Viragene, Iran), following the manufacturer's protocol. The concentration and purity of extracted DNA were evaluated using spectrophotometry (Nanodrop) to ensure suitability for PCR amplification.

Conventional PCR Assay: For molecular detection of *Leishmania* spp., a universal PCR assay targeting the ITS2 region was used as described by Nateghi Rostami et al. (9). This method identifies *L. major*, *L. tropica*, and *L. infantum* with a single primer pair. DNA from lesion tissue was amplified in a 25 µL reaction using 2× PCR Master Mix, 10 pmol of each primer (UNIL-IR-P and UNIL-IR-M), and 2 µL of DNA. PCR conditions included initial denaturation at 95°C (5 min), followed by 30 cycles (95°C for 30s, 57°C for 45s, 72°C for 45s) and a final extension at 72°C (5 min). Products were run on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV. Positive and negative controls were used. Primer details and expected fragment sizes are shown in Table 1.

Table 1. Characteristics of PCR primer pairs (9)

Name	Species	Primer sequence (5'–3')		Size (bp)
		Forward	Reverse	
UNIL-IR-P and UNILIR-M	<i>L. major</i> ,	CATGCCATATTCTCAGTGTCTG	GGTCTGTAAACA	L. m: 740
	<i>L. tropica</i> ,		AAGGTTGTCTG	L. t: 640
	<i>L. infantum</i>			L. i: 690

Antibiotic Susceptibility Testing: To assess secondary bacterial infection and guide treatment, skin lesion samples underwent culture and antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (10). Results were interpreted by inhibition zone size as Resistant (–), Intermediate (+), Susceptible (++) , or Highly Susceptible (+++).



Results and Discussion:

Mycological and Parasitological Findings: Fungal culture on Sabouraud Dextrose Agar (SDA) confirmed *Microsporum canis* based on colony and conidial morphology. No *Malassezia* spp. or mites were detected, indicating dermatophytosis without concurrent yeast or ectoparasitic infection.

Table 2. Antibiotic susceptibility results from Skin Lesion Culture

Antibiotic	Result	Interpretation
Trimethoprim Sulfamethoxazole	+ +++	Highly Susceptible
Co-amoxiclav	++	Susceptible
Erythromycin	+++	Highly Susceptible
Gentamicin	+++	Highly Susceptible
Cefalexin	+++	Highly Susceptible
Ceftiraxone	++	Susceptible
Enrofloxacin	+++	Highly Susceptible
Cefixime	–	Resistant
Penicillin	+	Intermediate
Tetracycline	+	Intermediate
Amikacin	+++	Highly Susceptible
Ciprofloxacin	+	Intermediate
Chlorotetracycline	+	Intermediate
Cefazolin	++	Susceptible

Note: (+++) = Highly Susceptible, (++) = Susceptible, (+) = Intermediate, (–) = Resistant

Molecular Diagnosis of Leishmaniasis: PCR amplification targeting the ITS2 region of the ribosomal RNA gene [Gene accession numbers: MN931857, MN931859, and MN969584] produced a single amplicon of approximately 690 base pairs, corresponding to *Leishmania infantum*. The product was visualized using electrophoresis on a 1.5% agarose gel stained with ethidium bromide. A distinct DNA band was observed under UV illumination, aligning with the positive control band and matching the expected size. No amplification was detected in the negative control, and positive control reactions produced the expected results.



Antibiotic Susceptibility Testing: Bacterial culture from skin lesion material revealed the presence of secondary bacterial infection. Complete susceptibility data are presented in Table 2.

This study reports the first molecular confirmation of *Leishmania infantum* infection in a domestic Persian cat from northern Iran. The cat exhibited dermatological lesions, which were initially attributed to fungal and bacterial infections. However, molecular testing using ITS2-targeting PCR confirmed the presence of *L. infantum* DNA, providing direct evidence of infection.

Feline leishmaniasis was initially documented in Iran in a study conducted by Hatam et al. (2010), where 40 stray cats from the provinces of Eastern Azerbaijan and Fars were examined (11). Moreover, recent investigations in Iran have demonstrated the presence of *L. infantum* in cats, with seroprevalence rates of 24–28% and PCR-confirmed infection in up to approximately 25% of stray cats (3, 5). Asgari et al. (2020) used both ELISA and DAT along with nested PCR to detect infection in 174 stray cats in Fars province, reporting a 27.6% seropositivity and 20.7% PCR positivity (3). Similarly, Mohebbi et al. (2017) identified *L. infantum* in the spleen and liver of cats in Ardabil province using serological, parasitological, and molecular techniques (5). These findings suggest that cats are being frequently exposed to infected sandflies and may serve as incidental or possibly secondary reservoirs in endemic areas.

The diagnosis of FeL remains challenging. While serological tests such as IFAT, ELISA, and DAT are useful for detecting anti-*Leishmania* antibodies in serum, they may underestimate active infection due to low or delayed antibody responses in cats (12, 13). On the other hand, molecular methods like PCR provide a more accurate confirmation of active infection by detecting parasite DNA in blood or tissue samples, particularly from lesion sites or buffy coats (14-19).

Overall, the present case from northern Iran, combined with these previous studies from southern and northwestern regions, suggests that feline leishmaniasis may be more widespread across Iran's endemic areas than previously recognized. Despite the significance of this report, limitations include the absence of quantitative PCR or serological confirmation, and a lack of follow-up to assess treatment response. Nevertheless, this case emphasizes the importance of veterinary surveillance and the inclusion of leishmaniasis in differential diagnoses for feline skin disease in endemic areas in Iran.

Acknowledgment:

We greatly appreciate the hard work of the staff at Aria-Azma Veterinary Diagnostic Lab.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Georgiadou SP, Makaritsis KP, Dalekos GN. Leishmaniasis revisited: Current aspects on epidemiology, diagnosis and treatment. *J Transl Int Med*. 2015;3(2):43-50.
2. Pereira A, Maia C. Leishmania infection in cats and feline leishmaniosis: An updated review with a proposal of a diagnosis algorithm and prevention guidelines. *Current Research in Parasitology & Vector-Borne Diseases*. 2021;1:100035.
3. Asgari Q, Mohammadpour I, Bozorg-Ghalati F, Motazedian H, Kalantari M, Hosseini S. Alarming: high prevalence of *Leishmania infantum* infection in cats from southern Iran based on molecular and serological methods. *Annals of Parasitology*. 2020;66(2).
4. Baharak A, Ali M, Mahshid M, Mohamad Z-A, Iraj S, Mohammad AH, et al. Advancing diagnosis of feline *Leishmania infantum* infection through experimental insights. *Scientific Reports*. 2025;15(1):12283.
5. Mohebbali M, Malmasi A, Khodabakhsh M, Zarei Z, Akhoundi B, Hajjarian H, Azarm A. Feline leishmaniosis due to *Leishmania infantum* in Northwest Iran: The role of cats in endemic areas of visceral leishmaniosis. *Veterinary Parasitology: Regional Studies and Reports*. 2017;9:13-6.
6. Pennisi MG, Cardoso L, Baneth G, Bourdeau P, Koutinas A, Miró G, et al. LeishVet update and recommendations on feline leishmaniosis. *Parasit Vectors*. 2015;8:302.
7. Chargui N, Haouas N, Jaouadi K, Gorgii M, Pratlong F, Dedet JP, et al. Usefulness of a PCR-based method in the detection and species identification of *Leishmania* from clinical samples. *Pathologie Biologie*. 2012;60(6):e75-e9.
8. Gomes AHS, Ferreira IMR, Lima MLSR, Cunha EA, Garcia AS, Araújo MFL, Pereira-Chiocola VL. PCR identification of *Leishmania* in diagnosis and control of canine leishmaniasis. *Veterinary Parasitology*. 2007;144(3):234-41.
9. Nateghi Rostami M, Darzi F, Farahmand M, Aghaei M, Parvizi P. Performance of a universal PCR assay to identify different *Leishmania* species causative of Old World cutaneous leishmaniasis. *Parasites & Vectors*. 2020;13:1-12.
10. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966;45(4):493-6.
11. Hatam GR, Adnani SJ, Asgari Q, Fallah E, Motazedian MH, Sadjjadi SM, Sarkari B. First report of natural infection in cats with *Leishmania infantum* in Iran. *Vector-borne and zoonotic diseases*. 2010;10(3):313-6.
12. Costa-Val APd, Coura FM, Barbieri JdM, Diniz L, Sampaio A, Reis JKPd, et al. Serological study of feline leishmaniasis and molecular detection of *Leishmania*



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- infantum and *Leishmania braziliensis* in cats (*Felis catus*). *Revista Brasileira de Parasitologia Veterinária*. 2020;29(2):e003520.
13. Bezerra JAB, Oliveira IVPdM, Yamakawa AC, Nilsson MG, Tomaz KLR, Oliveira KDSd, et al. Serological and molecular investigation of *Leishmania* spp. infection in cats from an area endemic for canine and human leishmaniasis in Northeast Brazil. *Revista Brasileira de Parasitologia Veterinária*. 2019;28(4):790-6.
 14. Pennisi M-G, Cardoso L, Baneth G, Bourdeau P, Koutinas A, Miró G, et al. LeishVet update and recommendations on feline leishmaniosis. *Parasites & vectors*. 2015;8(1):302.
 15. Pennisi MG, Lupo T, Malara D, Masucci M, Migliazzo A, Lombardo G. Serological and molecular prevalence of *Leishmania infantum* infection in cats from Southern Italy. *J Feline Med Surg*. 2012;14(9):656-7.
 16. Migliazzo A, Vitale F, Calderone S, Puleio R, Binanti D, Abramo F. Feline leishmaniosis: a case with a high parasitic burden. *Veterinary Dermatology*. 2015;26(1):69-70.
 17. Verneuil M. Leishmaniose oculaire féline: à propos d'un cas. *Journal Français d'Ophtalmologie*. 2013;36(4):e67-e72.
 18. Maia C, Gomes J, Cristóvão J, Nunes M, Martins A, Rebêlo E, Campino L. Feline *Leishmania* infection in a canine leishmaniasis endemic region, Portugal. *Veterinary parasitology*. 2010;174(3-4):336-40.
 19. Maia C, Ramos C, Coimbra M, Bastos F, Martins Â, Pinto P, et al. Bacterial and protozoal agents of feline vector-borne diseases in domestic and stray cats from southern Portugal. *Parasites & vectors*. 2014;7(1):115.



Metal-based Nano Particle Against Protozoa: The Role of Nanoparticles in Treatment and Diagnosis

Sarvenaz Sadeghi Mahoonak¹, Parsa Farzam¹

Corresponding Author: Sarvenaz Sadeghi Mahoonak, sarvenaz.sadeghi15@gmail.com

1. Department of veterinary science Islamic Azad University, Karaj Branch, Karaj, Iran.

Article code: 1293

Abstract

Background: Parasitic infections persist as a significant global health concern, particularly in resource-constrained regions where conventional therapies often face challenges due to drug resistance and toxicity. Nanotechnology has emerged as a transformative approach, offering enhanced drug delivery, targeted treatment, and novel diagnostic solutions for parasitic diseases.

Methods: This study is a review article based on an analysis of relevant and highly cited peer-reviewed research papers. A comprehensive literature search was conducted using PubMed and Google Scholar, with the keywords nanotechnology, nanoparticles, and protozoal infections. The selected articles were screened based on their credibility (peer-reviewed journals, recent publications, and citation count). The gathered data was systematically analyzed, compared, and synthesized to present an updated overview of the topic.

Results: Our analysis revealed that metal-based nanoparticles demonstrate significant antiparasitic efficacy. Gold nanoparticles (AuNPs) showed 70-95% mortality against *Toxoplasma gondii* tachyzoites at 100-1000 ppm concentrations, with antibody-conjugated AuNPs enhancing efficacy by 40%. Silver nanoparticles (AgNPs) exhibited dose-dependent anti-plasmodial activity (LC₅₀ 27.98 ppm against *A. stephensi*) and generated toxic ROS against *Leishmania tropica*, with UV light increasing potency 6.5-fold. Bimetallic NPs showed superior catalytic properties, particularly in drug activation. Critical parameters like size (80-120 nm) and surface charge (+34.4 mV vs -17.6 mV) determined biodistribution patterns. While liposomal systems improved bioavailability 5.67-fold, challenges in scalable production and safety standardization require further innovation. These findings underscore nanotechnology's potential to revolutionize antiparasitic therapy.

Conclusion: Nanoparticles offer enhanced drug delivery, targeted treatment, and novel diagnostic solutions for parasitic diseases.

Keywords: Nanoparticles, Parasitic diseases, Antiparasitic therapy ,Drug delivery, Nanotechnology.



1. Introduction:

Parasitic infections caused by protozoa, helminths, and ectoparasites affect over one billion people worldwide, with disproportionate impacts on resource-limited populations (1). Conventional chemotherapeutic agents face substantial challenges, including the emergence of drug-resistant parasite strains and undesirable toxicity profiles (2). Nanotechnology offers a paradigm shift in antiparasitic therapy through enhanced drug delivery systems, targeted therapeutic approaches, and novel diagnostic applications. Nanoparticles exhibit unique physicochemical properties when reduced to nanoscale dimensions, including size-dependent cytotoxic effects that make them effective therapeutic agents against parasitic diseases (3). Their tunable chemical, mechanical, and optical characteristics enable diverse applications in diagnosis, drug delivery, and targeted treatment of infections, demonstrating superior efficacy compared to conventional antiparasitic approaches (4).

2. Synthesis of Nanoparticles for Antiparasitic Applications:

Various methods of synthesis of NPs have been developed based on the size required, shape, or composition of the materials. The properties of the materials greatly influence all these parameters. These NPs are synthesized or prepared through the use of in-vitro or conventional techniques. Some of these methods are explained below (5).

Methods of Nanoparticle Synthesis for Antiparasitic Applications:

2.1. Photochemical and Thermal Disintegration

This high-temperature method decomposes precursors in boiling solvents to form nanoparticles. While cost-effective and simple, it often produces unstable nanoparticles with surface defects. The technique shows promise for size-isolated nanoparticles but requires further refinement to improve stability and uniformity (6).

2.2. Bimetallic Nanoparticle Synthesis

Bimetallic nanoparticles (NPs), formed from two different metals, offer superior catalytic and plasmonic properties compared to single-metal NPs, optimizing plasmon adsorption for applications like biosensing (7). The synthesis of these NPs, which can be structured as alloys or core-shells, is achieved by controlled reduction of metal ions. While this eco-friendly method is promising, it is limited by its high cost and stability issues, necessitating further research for wider application (8).

2.3. Chemical and Electrochemical Reduction

Chemical reduction using agents like sodium borohydride remains the most common approach, particularly for silver nanoparticles (9). The electrochemical variant offers better control



through electric current, yielding high-purity nanoparticles more cost-effectively. Both methods require technical expertise but enable tunable size distributions (10).

2.4. Sol-Gel Technique

This low-temperature method forms nanoparticles through controlled hydrolysis and condensation reactions. It produces high-quality bimetallic nanoparticles with excellent compositional control but suffers from material shrinkage. Recent improvements have simplified the process while maintaining product quality (11).

2.5. Green Synthesis

Using plant extracts as natural reducing agents, this eco-friendly method creates biocompatible nanoparticles with inherent biological activity. It offers advantages in sustainability and safety but struggles with inconsistent particle sizes (12). Ongoing research aims to standardize botanical sources and optimize storage conditions. Different plants species that are used for NP synthesis are listed in Table 1 (13).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table1. Mechanisms of action and therapeutic outcomes of nanoparticles synthesized from herbal sources (13)

Nanoparticles (NPs)	Source	Common Name	Mechanism of Action	Infection	Therapeutic Outcome
Silver (AgNPs)	Solanum trilobatum and Mimosa pudica	Pea eggplant and shame-plant	Interferes with cell membrane, and damages DNA and electron transport	Malaria, Leishmaniasis, Helminth infections	Inhibition of the growth of <i>Plasmodium</i> (P.) falciparum. Inhibition of proliferation and metabolic activity of promastigotes. Good antileishmanial activity in vitro and in vivo. Enhanced antihelminthic activity against worms.
Some metal oxides (MO) (Fe ₃ O ₄ , MgO, ZrO ₂ , Al ₂ O ₃ and CeO ₂)	Lawsonia inermis, Aza dirachta indica, Camellia sinensis, and Cinnamon zeylanicum	Henna tree, neem, tea plant, and cinnamon tree	Disrupts the cell membrane, accumulates inside the cell and produces toxic H ₂ O ₂ , damages cell membranes and releases reactive oxygen species	Malaria, Leishmaniasis	Enhanced cytotoxic effects on promastigotes of <i>Leishmania major</i> via induction of apoptosis.
Gold (AuNPs)	Acalypha indica	Indian mercury plant	Heavy electrostatic attraction, accumulation at cell surfaces, and interaction with cell membrane	Malaria, Helminth infections	Moderate delayed parasitemia rise in vivo, moderate anti-plasmodial activity against <i>P. falciparum</i> . Affects physiological functioning of parasite by causing paralysis and leading to death.

3. nanoparticles mechanism: In vivo transport of nanoparticles includes circulation in blood vessels, penetration into tissues, and cellular uptake. Though specific research on antiparasitic delivery is limited, much is inferred from antitumor and antibacterial studies (14).

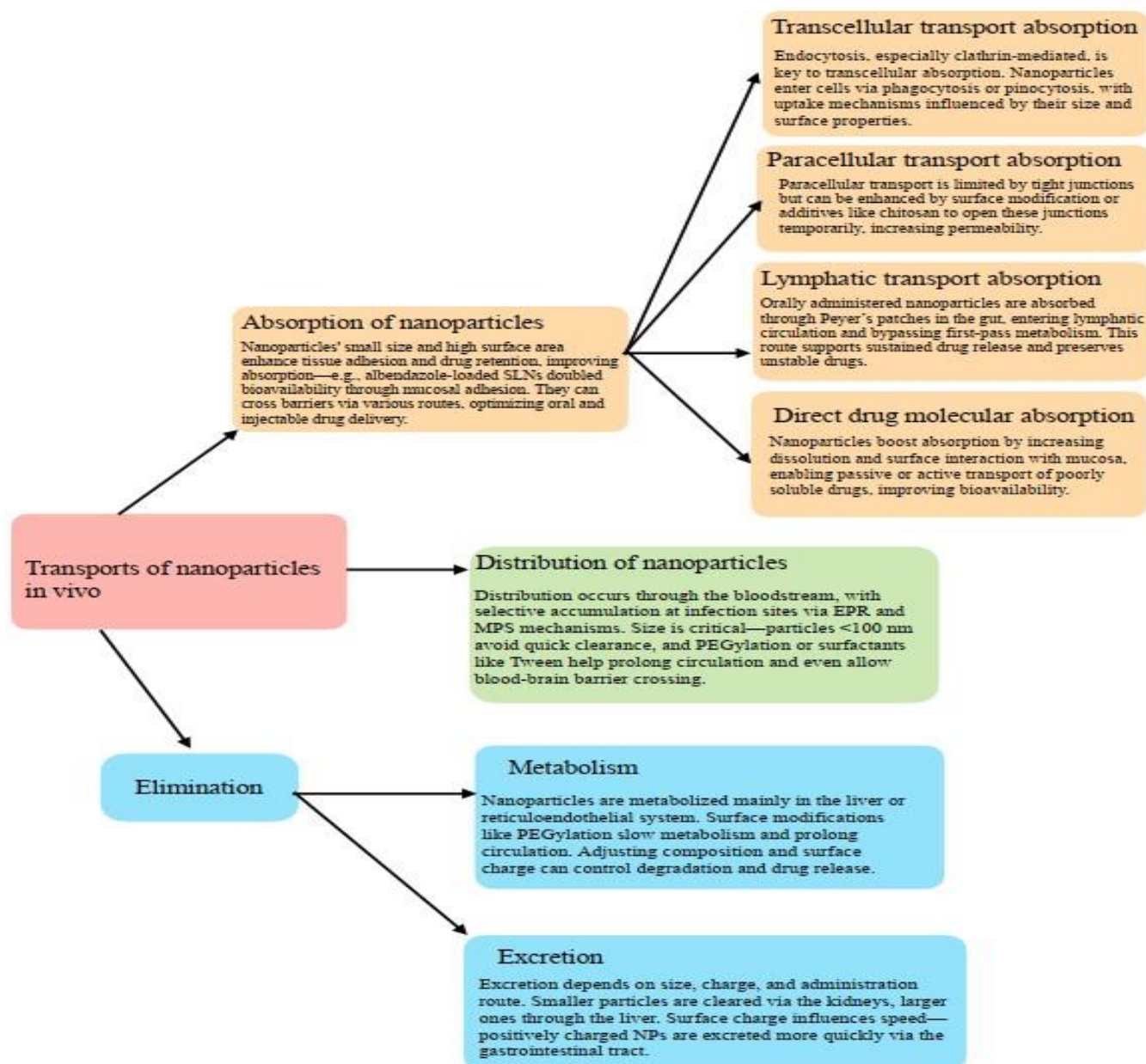


Figure 1. A simplified diagram of nanoparticles mechanism

4. Metal-Based Nanoparticles and Their Antiparasitic Significance

A variety of metals are employed in NP synthesis. Those that are of antiparasitic significance are shown below (Figure 2).

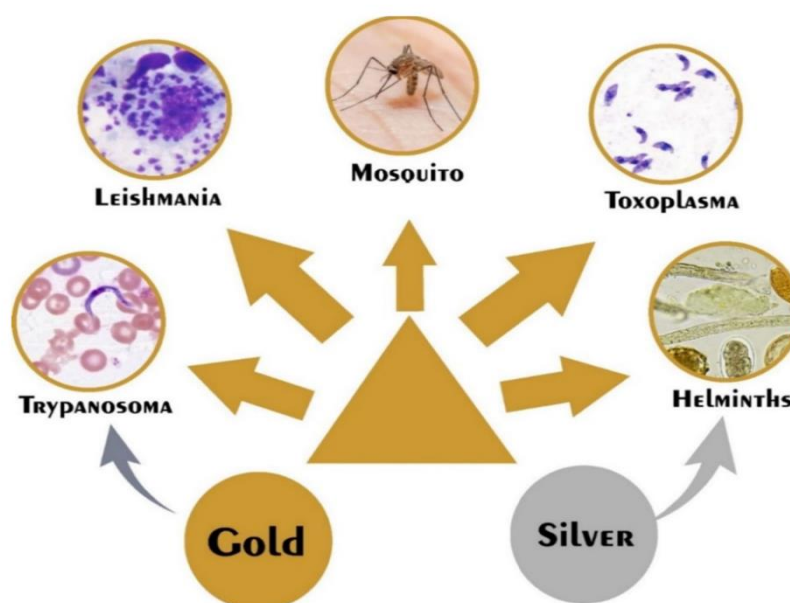


Figure 2. The antiparasitic spectrum of action of silver and gold nanoparticles

4.1. Aurum (Gold)-Based Nanoparticles

Gold nanoparticles (AuNPs) demonstrate significant antiparasitic potential against diverse pathogens, including protozoa (*Toxoplasma gondii*, *Trypanosoma* spp., *Leishmania* spp.), helminths (*Schistosoma* spp., *Raillietina* spp.), and vectors (*Aedes*, *Anopheles*, *Culex* spp.) (15). Synthesized via chemical reduction of chloroauric acid, AuNPs exist in oxidized (Au^{+1} , Au^{+3}) and non-oxidized (Au^0) forms, requiring dark storage to prevent oxidation. Their broad-spectrum efficacy positions them as promising candidates for novel antiparasitic drug development (16).

4.2. Aurum-Based Nanoparticles for Vector Control

Gold nanoparticles (AuNPs) are emerging as effective agents for controlling mosquito populations, which are vectors for numerous zoonotic diseases, including dengue, yellow fever, and Zika (17). AuNPs synthesized from plant extracts, such as *Cymbopogon citratus* and *Couroupita guianensis*, demonstrate significant larvicidal efficacy against key mosquito species, including *Aedes aegypti* and *Anopheles stephensi*. Notably, AuNPs coated



with zein, a protein-based coating, exhibit enhanced toxicity, leading to the disintegration of internal organs and structural damage in mosquitoes (18).

4.3. Aurum-Based Nanoparticles for Control of Protozoa

Protozoan parasites are responsible for various diseases. They are notoriously difficult to control. Success has been achieved with the use of AuNPs against these parasites. Some of these protozoans are described below.

4.3.1. Plasmodium

Plasmodium species, which cause malaria and are spread by Anopheles mosquitoes, still pose a serious global health threat. Gold nanoparticles (AuNPs) have recently shown promise for both treating and diagnosing malaria. AuNPs made using plants or microbes have strong antimalarial effects in humans and mice (19). Additionally, AuNP-based tests can visually detect malaria biomarkers using a method called the coffee-ring effect, offering an affordable and easy-to-use diagnostic tool for affected regions (20).

4.3.2. Leishmania

Leishmaniasis is a serious tropical disease with limited treatment options due to high costs, side effects, drug resistance, and poor drug delivery. Gold nanoparticles (AuNPs) show promise by enhancing drug effectiveness and lowering toxicity. They can inhibit Leishmania growth, especially when used with microwave radiation or plant-based compounds. AuNPs also enable rapid and affordable diagnosis through tools like colorimetric DNA kits. While these advances highlight nanotechnology's potential, further research is needed to develop fully effective and resistance-free treatments (21,22).

4.3.3. Toxoplasma gondii

Gold nanoparticles (AuNPs) are a promising treatment for toxoplasmosis. *In vitro* studies show that AuNPs (100-1000 ppm) induce concentration-dependent tachyzoite mortality (70-95% at 180 min exposure) (23). Antibody-conjugated AuNPs enhance therapeutic efficacy by 40% compared to free antibodies, while piezoelectric biosensors enable rapid toxoplasmosis diagnosis (16).

4.4. Silver (Ag)-Based Nanoparticles

Recent studies highlight their potential in parasitic control. While synthesized via electrochemical, sol-gel, and green methods, plant-mediated green synthesis (e.g., *Solanum trilobatum*, *Sechium edule*) is preferred due to cost and toxicity concerns of chemical methods. AgNPs (10-250 nm) exhibit promising antiparasitic effects (26).

4.5. Ag-Based Nanoparticles for Vector Control



AgNPs show remarkable larvicidal efficacy against mosquito vectors, including *Anopheles* and *Aedes* species. Green-synthesized AgNPs from *Mimosa pudica* (methanol extract) and *Euphorbia hirta* demonstrate dose-dependent toxicity, with LC_{50} and LC_{90} values of 27.98 ppm against *A. stephensi* (27). Fungal-derived AgNPs from *Chrysosporium keratinophilum* exhibit additional pupicidal activity, suggesting their potential as eco-friendly alternatives to conventional insecticides.

4.6. Ag-Based Nanoparticles for Protozoan Control

4.6.1. Plasmodium

Silver nanoparticles (AgNPs), like gold nanoparticles, are effective against malaria caused by *Plasmodium falciparum*. Green-synthesized AgNPs using plant extracts (e.g., *Azadirachta indica*) show strong, dose-dependent anti-plasmodial activity (28). Dynamic Light Scattering (DLS) provides more accurate size measurements than TEM. This eco-friendly approach offers a promising alternative for malaria treatment (29).

4.6.2. Leishmania

AgNPs kill *Leishmania tropica* by generating toxic ROS (the formation of reactive oxygen species), impairing metabolism and proliferation. Their efficacy is significantly enhanced by UV light (up to 6.5-fold) and synergized with **Miltefosine**, making them a promising standalone or combined treatment (30).

4.6.3. Toxoplasma gondii

Toxoplasma gondii is a widespread parasite with treatment challenges like drug resistance and limited vaccines. Nanoparticles (silver, gold, platinum) show strong potential by damaging parasite mitochondria and achieving up to 90% infection inhibition without harming host cells (31). Silver nanoparticles, especially with chitosan or from plant extracts, reduce parasite load, deform tachyzoites, boost immune response, and improve serum profiles in infected mice—highlighting their promise for anti-toxoplasmosis therapy (32).

5. Nanoparticle Toxicity and Safety in Antiparasitic Applications

Nanoparticle (NP) safety profiles are governed by key physicochemical properties including size, charge, and composition. Studies demonstrate NPs sized 80-120 nm undergo renal clearance, while larger particles accumulate hepatically. Surface charge critically influences biodistribution, with cationic NPs (+34.4 mV) excreted fecally and anionic NPs (-17.6 mV) retained in liver tissues. Nanocarrier systems like solid lipid nanoparticles (SLNs) enhance drug bioavailability 5.67-fold for praziquantel and protect payloads from enzymatic degradation through controlled release mechanisms (33).



Intracellularly, NPs undergo lysosomal processing, enabling targeted drug release against intracellular parasites. Liposomal amphotericin B exemplifies this targeted delivery with reduced host toxicity.

6. Overcoming Safety and Production Hurdles

While nanoparticles offer potent drug delivery, their clinical application faces major hurdles. Key challenges include a lack of systematic biosafety studies, potential toxicity from highly charged particles, and the difficulty of large-scale, reproducible manufacturing. Future efforts must focus on developing safe, biodegradable materials and establishing scalable production to ensure their widespread clinical use (14).

Conclusions:

Among all diseases, parasitic infections remain a major global health concern affecting both humans and animals. While appropriate treatment can improve public health over time, parasites have become resistant to the drugs and drugs have shown low or no effect against parasites, therefore, nanotechnology has emerged as a transformative approach. Nanoparticles offer enhanced drug delivery, targeted treatment, and novel diagnostic solutions for parasitic diseases. Despite demonstrating superior efficacy compared to conventional antiparasitic approaches, nanoparticles still face significant challenges including lack of systematic biosafety studies, potential toxicity from highly charged particles, and the difficulty of large-scale reproducible manufacturing. Future efforts must focus on developing safe, biodegradable materials and establishing scalable production to ensure their widespread clinical use.

References:

1. Rokkas T, Gisbert JP, Malfertheiner P, et al. Comparative Effectiveness of Multiple Different First-Line Treatment Regimens for *Helicobacter pylori* Infection: A Network Meta-analysis. *Gastroenterology*. 2021;161(2):495-507.e4. doi:10.1053/j.gastro.2021.04.012
2. Raj S, Sasidharan S, Balaji SN, Saudagar P. An overview of biochemically characterized drug targets in metabolic pathways of *Leishmania* parasite. *Parasitol Res*. 2020;119(7):2025-2037. doi:10.1007/s00436-020-06736-x
3. Khezerlou A, Alizadeh-Sani M, Azizi-Lalabadi M, Ehsani A. Nanoparticles and their antimicrobial properties against pathogens including bacteria, fungi, parasites and viruses. *Microb Pathog*. 2018;123:505-526. doi:10.1016/j.micpath.2018.08.008
4. Nafari A, Cheraghipour K, Sepahvand M, Shahrokhi G, Gabal E, Mahmoudvand H. Nanoparticles: New agents toward treatment of leishmaniasis. *Parasite Epidemiol Control*. 2020;10:e00156. Published 2020 May 23. doi:10.1016/j.parepi.2020.e00156



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



5. Xu L, Wang YY, Huang J, Chen CY, Wang ZX, Xie H. Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics*. 2020 Jul 11;10(20):8996.
6. Shit SC, Shown I, Paul R, Chen KH, Mondal J, Chen LC. Integrated nano-architected photocatalysts for photochemical CO₂ reduction. *Nanoscale*. 2020;12(46):23301-32.
7. Vallabani NVS, Singh S, Karakoti AS. Magnetic Nanoparticles: Current Trends and Future Aspects in Diagnostics and Nanomedicine. *Curr Drug Metab*. 2019;20(6):457-472. doi:10.2174/1389200220666181122124458
8. Bertin E, Münzer A, Reichenberger S, Streubel R, Vinnay T, Wiggers H, Schulz C, Barcikowski S, Marzun G. Durability study of platinum nanoparticles supported on gas-phase synthesized graphene in oxygen reduction reaction conditions. *Applied Surface Science*. 2019 Feb 15;467:1181-6.
9. Panneerselvam C, Ponarulselvam S, Murugan K. Potential anti-plasmodial activity of synthesized silver nanoparticle using *Andrographis paniculata* Nees (Acanthaceae). *Arch Appl Sci Res*. 2011;3(6):208-17.
10. Jamkhande PG, Ghule NW, Bamer AH, Kalaskar MG. Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications. *Journal of drug delivery science and technology*. 2019 Oct 1;53:101174.
11. Parashar M, Shukla VK, Singh R. Metal oxides nanoparticles via sol-gel method: a review on synthesis, characterization and applications. *Journal of Materials Science: Materials in Electronics*. 2020 Mar;31(5):3729-49.
12. Devatha CP, Thalla AK. Green synthesis of nanomaterials. In *Synthesis of inorganic nanomaterials* 2018 Jan 1 (pp. 169-184). Woodhead Publishing.
13. Bajwa HUR, Khan MK, Abbas Z, et al. Nanoparticles: Synthesis and Their Role as Potential Drug Candidates for the Treatment of Parasitic Diseases. *Life (Basel)*. 2022;12(5):750. Published 2022 May 18. doi:10.3390/life12050750
14. Sun Y, Chen D, Pan Y, Qu W, Hao H, Wang X, Liu Z, Xie S. Nanoparticles for antiparasitic drug delivery. *Drug Delivery*. 2019 Jan 1;26(1):1206-21.
15. Pissuwan D, Gazzana C, Mongkolsuk S, Cortie MB. Single and multiple detections of foodborne pathogens by gold nanoparticle assays. *Wiley Interdiscip Rev Nanomed Nanobiotechnol*. 2020;12(1):e1584. doi:10.1002/wnan.1584
16. Dykman LA. Gold nanoparticles for preparation of antibodies and vaccines against infectious diseases. *Expert Rev Vaccines*. 2020;19(5):465-477. doi:10.1080/14760584.2020.1758070
17. Jamkhande PG, Ghule NW, Bamer AH, Kalaskar MG. Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications. *J Drug Deliv Sci Technol*. 2019;53:101174. doi:10.1016/j.jddst.2019.101174.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



18. Mohammadi L, Pal K, Bilal M, Rahdar A, Fytianos G, Kyzas GZ. Green nanoparticles to treat patients with Malaria disease: an overview. *Journal of Molecular Structure*. 2021 Apr 5;1229:129857.
19. Varela-Aramburu S, Ghosh C, Goerdeler F, Priegue P, Moscovitz O, Seeberger PH. Targeting and inhibiting *Plasmodium falciparum* using ultra-small gold nanoparticles. *ACS applied materials & interfaces*. 2020 Sep 2;12(39):43380-7.
20. Gruessner BM, Weathers PJ. In vitro analyses of *Artemisia* extracts on *Plasmodium falciparum* suggest a complex antimalarial effect. *PLoS One*. 2021;16(3):e0240874. Published 2021 Mar 2. doi:10.1371/journal.pone.0240874
21. Nafari A, Cheraghipour K, Sepahvand M, Shahrokhi G, Gabal E, Mahmoudvand H. Nanoparticles: New agents toward treatment of leishmaniasis. *Parasite Epidemiol Control*. 2020;10:e00156. Published 2020 May 23. doi:10.1016/j.parepi.2020.e00156
22. Sazgarnia A, Taheri AR, Soudmand S, Parizi AJ, Rajabi O, Darbandi MS. Antiparasitic effects of gold nanoparticles with microwave radiation on promastigots and amastigotes of *Leishmania major*. *Int J Hyperthermia*. 2013;29(1):79-86. doi:10.3109/02656736.2012.758875
23. Adeyemi OS, Murata Y, Sugi T, Kato K. Inorganic nanoparticles kill *Toxoplasma gondii* via changes in redox status and mitochondrial membrane potential. *Int J Nanomedicine*. 2017;12:1647-1661. Published 2017 Feb 28. doi:10.2147/IJN.S122178
24. Rehman, T.U.; Saeed, Z.; Zaman, M.A.; Sikandar, A.; Ali, H.M. Factors influencing the incidence of *Eimeria leuckarti* in horses. *Agrobiologic Records*. 2021, 6, 13–17. [CrossRef]
25. Eger I, Soares MJ. Endocytosis in *Trypanosoma cruzi* (Euglenozoa: Kinetoplastea) epimastigotes: visualization of ingested transferrin-gold nanoparticle complexes by confocal laser microscopy. *J Microbiol Methods*. 2012;91(1):101-105. doi:10.1016/j.mimet.2012.07.013
26. Roy A, Bulut O, Some S, Mandal AK, Yilmaz MD. Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity. *RSC advances*. 2019;9(5):2673-702.
27. Sardana M, Agarwal V, Pant A, Kapoor V, Pandey KC, Kumar S. Antiplasmodial activity of silver nanoparticles: A novel green synthesis approach. *Asian Pacific Journal of Tropical Biomedicine*. 2018 May 1;8(5):268-72.
28. Soni N, Prakash S. Possible mosquito control by silver nanoparticles synthesized by soil fungus (*Aspergillus niger* 2587) [Internet]. 2013 [cited 2021 May 1]. Available from: <https://www.scirp.org/journal/paperinformation.aspx?paperid=31881>
29. Donescu D, Nistor CL, Purcar C, Petcu C, Serban S, Corobea MC, Ghiurea M. Formation and dissolution of silver nanoparticles. *J Optoelectron Adv Mater*. 2009 Jan 1;1:44-8.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



30. Ali KM, Hassan EA, Abuowarda MM, Mahmoud MA, Torad FA. Bilateral panophthalmia as a late sequel of leishmaniasis in dogs. *Pak Vet J.* 2021;41(1):13-8.
31. Cheraghipour K, Masoori L, Ezzatkhah F, et al. Effect of chitosan on *Toxoplasma gondii* infection: A systematic review. *Parasite Epidemiol Control.* 2020;11:e00189. Published 2020 Oct 21. doi:10.1016/j.parepi.2020.e00189
32. El-Kosary S, Allatif AA, Stino RG, Hassan MM, Kinawy AA. Effect of silver nanoparticles on micropropagation of date palm (*Phoenix dactylifera* L, cv. Sewi and Medjool). *Plant Arch* 2020, 2, 9701.
33. Gagne RB, Crooks KR, Craft ME, Chiu ES, Fountain-Jones NM, Malmberg JL, Carver S, Funk WC, VandeWoude S. Parasites as conservation tools. *Conservation Biology.* 2022 Feb;36(1):e13719.



Physiological Modeling of the Autonomic Nervous System Response to Parasitic Infections in Animals

Farshid Hamidi¹

Corresponding author. Farshid Hamidi, Farshidhamidi@um.ac.ir

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1295

Abstract

Background: Parasitic infections in animals are not only associated with immune and physiological consequences, but also induce significant changes in autonomic neural responses. The neurophysiological axis of these responses, which is mainly mediated by the sympathetic and parasympathetic nervous systems, plays an important role in maintaining homeostasis under chronic stress conditions caused by infection. In this article, the physiological modeling of these responses was investigated with emphasis on factors such as heart rate changes, pupil dilation, feeding behaviors, and restlessness or immobility in animals infected with parasites.

Methods: In this study, a number of articles related to the relationship between parasitic infections in animals and disruption of the autonomic nervous system were reviewed, and interesting findings were found regarding this effect.

Results: Since the aim of this text was to analyze the neural mechanisms involved in regulating autonomic responses to the presence of parasites and to evaluate the role of neurotransmitters and neural pathways in facilitating or inhibiting these responses, a review of studies has shown that parasites such as *Trypanosoma cruzi*, *Toxoplasma gondii*, and *Plasmodium* species can cause changes such as tachycardia, anorexia, and disruption of physiological rhythms by activating the HPA axis and stimulating certain areas in the brainstem and cortical and subcortical areas of the brain.

Conclusion: Finally, these autonomous responses, although initiated with the aim of maintaining host survival, may in some circumstances act in the parasite's favor, including by suppressing immunity or altering host behavior to enhance transmission. A detailed understanding of these pathways could open new horizons in the treatment of parasitic diseases and clinical management of parasitic diseases.

keywords: Autonomic nervous response, Parasitic infections, Physiological modeling, Anorexia, Feeding behaviors.



Introduction:

The physiological responses of animals to pathogens have always been of interest to neuroscientists and physiologist. But what has been less systematically studied are the responses of the autonomic nervous system (ANS). Can these responses be predicted? Is there a consistent pattern across species? Then, most importantly, are these responses simply involuntary reactions or do they play an active role in the survival of the host and even the parasite?

As the master regulator of the body's involuntary activities, the autonomic nervous system is highly sensitive to internal threats such as actions. When pathogens enter that body, activities such as increased heart rate, changes in pupil diameter, and readjustment of blood flow and heart rate can all be triggered. These responses, which appear to be responses to a problem or pain, are actually part of the body's defense mechanism. But the point is that in some parasitic infections, the host's defensive reactions to the presence of the parasitic infection can even cause damage to the host's body (1).

From this perspective, there is a need for detailed physiological modeling, as only detailed understanding of the function of these pathways can lead us to a better understanding of disease processes, prognosis, and even the design of targeted drugs. The existence of a suitable model of the process of organ activity in a healthy animal and the course of change until disease can provide the best platform for the researcher to design animal-based experimental research.

Materials and Methods:

In this study, some articles and research related to the relationship between parasitic infections in animals and disorders occurring in the autonomic nervous system and the development of neurological diseases were reviewed, and the findings on the relationship were summarized, indicating the mutual influence of these two factors.

Results and Discussion:

Cardiovascular, gastrointestinal, and pupillary responses to parasitic infections

One of the most prominent indicators of ANS (autonomic nervous system) response is the change in heart rate. In many animals, including poultry, parasitic infections such as *Eimeria* can cause tachycardia (increased HR). This reaction occurs due to the secretion of some cytokines such as $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ from immune cells, which are then transmitted to sympathetic pathways via visceral afferent neurons and brain centers associated with the ANS (example: nucleus solitarius) (2). But this is not always the case; in some chronic infections,



such as *Toxoplasma gondii*, a decrease in heart rate (bradycardia) has been seen as a long-term, adaptive response (3).

Also, significant changes are observed at the digestive level. Parasitic infections are often associated with disturbances in intestinal motility and digestive secretions. Decreased peristalsis, gastric distension, and nausea (in mammals) can be due to the dominance of parasympathetic pathways or even their inhibition by inflammatory mediators (4).

The interesting thing is that in many cases, despite the brain receiving these environmental messages on a large scale, it only brings a portion of them to the level of perception and awareness in the somatosensory cortex. In other words, the body knows, but the mind does not. On the other hand, pupil diameter, a well-known indicator of sympathetic activity, can dilate in response to infection. In rodents infected with *Trypanosoma cruzi*, pupil dilation has been reported even at rest, indicating a sustained stimulation of the sympathetic system.

The question here is whether this change is simply a consequence of an inflammatory process or part of the host's neural programming for survival? This is a question that cannot yet be answered definitively. As we have seen, the early ANS responses to infection are not only rapid and autonomous, but in some cases mandatory. Although their function is often interpreted to maintain homeostasis, parasites may have created mechanisms to exploit these responses over time for survival (1).

Interaction of neurons, immune mediators, and the role of parasites in altering ANS settings

The autonomic nervous system's response to parasitic infections is not limited to direct reactions, but rather involves a two-way interaction between neurons and immune cells. For example, macrophages infected with *Leishmania major* can release molecules such as prostaglandin E2, which, through specific receptors, stimulate certain visceral afferent neurons. This stimulation then triggers sympathetic nervous system responses that potentially reduce blood flow to the affected area, creating hypoxic conditions for the parasite, thus affecting parasite survival (5,6).

In this regard, the role of the vagus nerve (tenth cranial nerve) is also very crucial. The vagus nerve is involved in the transmission of peripheral signals to the central nuclei of the brain, including the noradrenergic areas of the forebrain cortex (7). In particular, in poultry infected with *Ascaridia galli*, evidence has been reported of altered vagal excitation patterns and reduced parasympathetic tone, which could play a role in the weakening of digestive activity such as intestinal motility and longer persistence of the parasite in the digestive tract.

What's even more interesting is that some parasites can actively manipulate and recruit neural pathways! In *Toxoplasma gondii* infection, the parasite can lodge in the host's neurons, increase transmitters such as dopamine, and in this way, direct autonomic responses in favor of its own



survival. In these cases, the ANS response can no longer be considered solely the result of the presence of the parasite, but rather it should be considered part of the parasite's plan and strategy to control the host (3,8).

General pattern of responses, modeling, and future applications

Physiological modeling of these responses requires integrating different data from different organs such as the heart, gut, brain, eye, and even skin. Systems such as mathematical models of neural-immune networks or agent-based multi-agent models can help simulate these complex interactions. For example, using the previously mentioned experimental data on heart rate, body temperature, and pupil diameter, a model can be created for early detection of a specific type of parasitic infection.

On the other hand, in behavioral studies of infected animals, whether mammals, rodents, or birds, changes in feeding, aggression, or social withdrawal (all of which are regulated by the ANS) can be modeled (9). The use of these models will play an important role not only in understanding the pathophysiology of infection, but also in designing non-invasive diagnostic tests. This is the horizon that animal neurophysiology studies, especially in the field of veterinary medicine, must strive to achieve and ultimately exploit (10).

Finally, the fact that some responses are symmetrically eliminated or spontaneously silenced reminds us that what is seen in the host body is only part of the host's neural response. Many internal processes, such as the rearrangement of neuronal pathways, the alteration of receptor gene expression in ganglia, and the inhibition of regulatory feedback, occur behind the scenes and remain hidden from clinical view, making diagnosis difficult.

Therefore, physiological modeling of the ANS response to parasites is not only a scientific challenge, but also a necessity to advance our understanding of the biology of parasitic diseases as well as improve management strategies in modern veterinary medicine.

Acknowledgment:

Author would like to thank the Ferdowsi University of Mashhad for their support.

References:

1. Jones MP, Orosz SE. Overview of avian neurology and neurological diseases. In: *Seminars in Avian and Exotic Pet Medicine* 1996 Jul 1 (Vol. 5, No. 3, pp. 150-164). WB Saunders.
2. Cunningham-Rundles C, Ponda PP. Molecular defects in T-and B-cell primary immunodeficiency diseases. *Nature reviews immunology*. 2005 Nov 1;5(11):880-92.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



3. Prandovszky E, Gaskell E, Martin H, Dubey JP, Webster JP, McConkey GA. The neurotropic parasite *Toxoplasma gondii* increases dopamine metabolism. *PloS one*. 2011 Sep 21;6(9):e23866.
4. Ducatez S, Lefebvre L, Sayol F, Audet JN, Sol D. Host cognition and parasitism in birds: a review of the main mechanisms. *Frontiers in Ecology and Evolution*. 2020 Apr 23;8:102.
5. Felten DL, Felten SY. Sympathetic noradrenergic innervation of immune organs. *Brain, behavior, and immunity*. 1988 Dec 1;2(4):293-300.
6. Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. *Annals of the new York Academy of Sciences*. 2012 Jul;1261(1):55-63.
7. Nieuwenhuys R. The insular cortex: a review. *Progress in brain research*. 2012 Jan 1;195:123-63.
8. Adamo SA. Modulating the modulators: parasites, neuromodulators and host behavioral change. *Brain Behavior and Evolution*. 2002 Jul 1;60(6):370-7.
9. Yousefvand S, Hamidi F, Zendehdel M, Parham A. Effects of insulin and somatostatin on water intake in neonatal chickens. *Iranian Journal of Physiology and Pharmacology*. 2018 Oct 10;2(3):165-58.
10. Kavaliers M, Choleris E. The role of social cognition in parasite and pathogen avoidance. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2018 Jul 19;373(1751):20170206.



Physiological Responses of the Host Body to Parasitic Infections: Focus on the HPA Neurohormonal Axis

Farshid Hamidi¹

Corresponding author. Farshid Hamidi, Farshidhamidi@um.ac.ir

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1297

Abstract

Background: The host physiological response to parasitic infections, especially in birds, involves a complex set of physiological reactions originating from the immune, endocrine, and nervous systems.

Methods: In this study, some articles and research related to the relationship between parasitic infections in animals and disorders occurring in the HPA neurohormonal axis and the development of neurological problems were reviewed.

Results: Findings on the relationship were summarized, indicating a mutual influence between these two conditions. One of the most important of these pathways is the hypothalamic-pituitary-adrenal (HPA) axis, which is activated in the face of parasite-induced stress and increases the secretion of glucocorticoids such as cortisol or corticosterone. These hormones provide conditions for the body to fight infection more effectively by temporarily inhibiting some immune processes and stimulating inflammatory responses. However, persistent activation of this axis may cause chronic suppression of the immune system and increased susceptibility to other harmful microorganisms and pathogens.

Conclusion: Activation of the HPA axis by cytokines through the release of glucocorticoids may play an important role in the inhibition and shaping of immune responses. In other words, cytokine-HPA interactions represent a fundamental consideration in the maintenance of homeostasis and the development of disease during viral infection. Although there are studies that focus on the bidirectional relationship between the immune system and the HPA axis during viral infection, others have focused on the immunomodulatory effects of glucocorticoids during viral infection. Overall, the importance of the axis in the regulation of body homeostasis is considerable.

Keywords: Parasitic infections, Neurohormonal axis, HPA axis, Host body.



Introduction:

Infections caused by the presence and activity of parasites, as biological and immunological stressors, can activate complex pathways of physiological responses in the host body. Some of these factors include pain-causing and fever-causing factors in the body, which cause many interactions but are not relevant to this article. One of the most important of these pathways is the hypothalamic-pituitary-adrenal (HPA) axis, which plays a key role in regulating stress responses, metabolism, and immune responses. In animals exposed to infection, activation of this axis leads to the secretion of hormones such as cortisol (in mammals) or corticosterone (in birds); these hormones have multiple effects on the immune system, food intake, behaviors, and body temperature regulation (1,2).

Material and Methods:

In this study, some articles and research related to the relationship between parasitic infections in animals and disorders occurring in the HPA neurohormonal axis and the development of neurological problems were reviewed, and the findings on the relationship were summarized, indicating a mutual influence between these two conditions.

Results and Discussion:

Glucocorticoid secretion: Glucocorticoid secretion in the early stages of parasitic infection may play a protective role, as it helps to reduce secondary damage by inhibiting excessive inflammation. But in chronic or severe infections, prolonged elevation of these hormones can lead to suppression of the immune response, anorexia, decreased growth, and finally a decrease in the host's ability to clear the parasite. For example, in infections caused by *Toxoplasma gondi* or *Trypanosoma*, increased cortisol levels are associated with decreased T-lymphocyte function and increased severity of clinical symptoms. On the other hand, fever, as a common component of the acute phase immune response, despite the unpleasant feeling it causes, is itself regulated by cytokines in many parasitic infections, as well as by interaction with the hypothalamic-pituitary-adrenal axis. Fever can be temporarily useful in inhibiting parasite proliferation, but in some cases it can also lead to aggravation of symptoms by increasing metabolic demand (3).

HPA axis: HPA axis activation is also associated with changes in appetite and feeding behavior of animals. Reduced food intake, which is often observed in the early stages of infection, is considered part of the host's adaptive response to reduced energy available to the parasite (4). However, if this mechanism persists, it can deplete the animal's vital energy reserves and may even threaten the survival of the host.



A notable point in the neurohormonal response to parasitic infections is that the interaction between the parasite and the hypothalamic-pituitary-adrenal (HPA) axis is not only one-way, but is sometimes complex and bilateral. Numerous lines of evidence indicate that some parasites can facilitate their own survival and persistence or modulate the intensity of the host's immune response by affecting the regulation and secretion of steroid hormones. In other words, parasites may exploit the host's physiological response to create more suitable biological conditions for their own persistence and growth (5).

Activation of the HPA axis by cytokines, due to the release of glucocorticoids, may play an important role in the inhibition and shaping of immune responses in animals. Thus, cytokine-HPA interactions represent a fundamental consideration in the maintenance of homeostasis and the development of disease symptoms during viral infection. However, there are studies that have studied the bidirectional relationship between the immune system and the HPA axis during viral infection, and others have focused on the immunomodulatory effects of glucocorticoids during viral infection (2,5,6).

For example, in *Plasmodium* infections (agent of malaria), increased glucocorticoid levels have been associated with reduced Th1 responses and, consequently, impaired parasite control in liver and blood tissue. Parasites such as *Leishmania* also reduce the defense capacity of macrophages by stimulating IL-10 secretions, simultaneously with hypothalamic-pituitary-adrenal (HPA) activation (7).

Similar findings have been reported in studies of poultry. For example, in *Eimeria* infections, increased blood corticosterone levels in birds were associated with decreased appetite, impaired growth, and impaired immune function. Interestingly, in some coccidiosis-resistant strains, a more modulated HPA response was observed, suggesting that a more favorable physiological regulation could be considered as an indicator of natural resistance to infection (8).

Some of these can be briefly listed:

- *Eimeria* spp. (coccidiosis)

Host species : Birds, especially broilers

Physiological effects: Severe activation of the HPA axis is observed following stress-induced destruction of the intestinal mucosa and increased plasma corticosterone levels (cortisol equivalent in birds) with appetite suppression, reduced growth, and increased mortality.

- *Plasmodium relictum* (avian malaria)

Host species: pigeons, passerines, and migratory birds

HPA axis response: Increased corticosterone levels in the acute phase of infection, decreased adaptive immune system activity . There is fever, lethargy, and decreased foraging behavior.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Possible function of the parasite: Immunosuppression for increased survival in host blood cells

- *Toxoplasma gondii*

Host species: mainly mammals, but there are also studies on birds

Mechanism of action: Stimulation of the HPA axis and increased cortisol secretion, reduction of active inflammatory responses (Th1 response) . Possibility of changing host behavior in favor of parasite transmission (e.g. , reduced fear in mice towards cats)

Interesting note: Behavioral disorders and changes in feeding habits have also been reported in birds.

- *Trichomonas gallinae*

Host species: Pigeons and other granivorous birds

HPA axis response: In the acute stages, there is increased corticosterone and anorexia. Oral and esophageal tissue involvement is also observed, which is a source of severe nutritional stress in the bird (9).

- *Ascaridia galli* (gastrointestinal roundworms)

Host species: Laying poultry

HPA axis response: In chronic infection, metabolic stress leads to increased glucocorticoid levels and concomitantly reduces mucosal immunity and egg-laying performance (10).

Some Physiological pattern: Of course, physiological responses mediated by the HPA axis are not limited to hormonal changes. Neurohumoral regulation of body temperature, altered sleep-wake patterns, reduced social interactions, and even avoidance behaviors toward contaminated sources can all be influenced by this axis (11). Such changes may be interpreted as a type of host defense mechanism to limit disease transmission or reduce parasite access to a suitable environment for vital resources.

However, chronicity of these responses, especially in species such as broilers that have high growth rates, can have adverse health and productivity consequences. Broilers, despite still being chicks, have the appearance and size of a chicken, and this high growth rate makes them very vulnerable to harmful factors, including infections. In contrast, in laying hens, chronic responses are more likely to occur due to the long rearing period. Therefore, a more precise understanding of the physiological patterns of the host response can help design new strategies in the management of parasitic diseases; including the selection of resistant strains, or even nutritional and pharmacological interventions to modulate the effects of the HPA axis (5,6).

Finally, the study of the HPA response in different hosts, from chickens to mammals, shows that species characteristics as well as genetic differences play a role in determining the intensity and persistence of this response. For example, in laying hens, the pattern of corticosterone



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



secretion is milder and shorter-lasting in response to infectious stresses; whereas in industrial broiler breeds, these responses are faster and stronger, which can lead to metabolic disorders in the bird.

Acknowledgment:

The author thanks Ferdowsi University of Mashhad for their continued support.

References:

1. Star LJ, Juul-Madsen HR, Decuypere E, Nieuwland MG, de Vries Reilingh G, Van Den Brand H, Kemp B, Parmentier HK. Effect of early life thermal conditioning and immune challenge on thermotolerance and humoral immune competence in adult laying hens. *Poultry science*. 2009 Nov 1;88(11):2253-61.
2. Zendehdel M, Taati M, Amoozad M, Hamidi F. Antinociceptive effect of the aqueous extract obtained from *Foeniculum vulgare* in mice: the role of histamine H1 and H2 receptors.
3. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine reviews*. 2000 Feb 1;21(1):55-89.
4. Rahdari A, Hamidi F. The effect of intraperitoneal injection of Glycyrrhizin on central regulation of food intake in broilers injected with LPS. *British Poultry Science*. 2025 Jan 2;66(1):124-30.
5. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *New England Journal of Medicine*. 1995 May 18;332(20):1351-63.
6. Silverman MN, Pearce BD, Biron CA, Miller AH. Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. *Viral immunology*. 2005 Mar 1;18(1):41-78.
7. Liew FY, Millott S, Parkinson C, Palmer RM, Moncada S. Macrophage killing of *Leishmania* parasite in vivo is mediated by nitric oxide from L-arginine. *The Journal of Immunology*. 1990 Jun;144(12):4794-7.
8. Graat EA, Ploeger HW, Henken AM, Braunius WW. *Eimeria acervulina*: influence of corticosterone-induced immunosuppression on oocyst shedding and production characteristics in broilers, and correlation with a computer simulation model. *Veterinary parasitology*. 1997 Jun 1;70(1-3):47-59.
9. Amin A, Bilic I, Liebhart D, Hess M. Trichomonads in birds—a review. *Parasitology*. 2014 May;141(6):733-47.
10. Permin and Hansen (1998). "Epidemiology, diagnosis and control of poultry parasites". *FAO Animal Health Manual*.
11. Johnson RW. The concept of sickness behavior: a brief chronological account of four key discoveries. *Veterinary immunology and immunopathology*. 2002 Sep 10;87(3-4):443-50.



Comparative Evaluation of Molecular Methods for Differential Diagnosis of *Leishmania* Infection: A Narrative Review

Mobina Mohammadi¹, Parviz Shayan¹

Corresponding Author: Parviz Shayan, pshayan@ut.ac.ir

1. Research Center for Ticks and Tick-borne Diseases, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Iran.

Article code: 1306

Abstract

Background: *Leishmania spp.* is a protozoan vector-borne parasite causing leishmaniasis. Different *Leishmania* species have been recognized in Iran, including *L. major*, *L. tropica*, and *L. infantum*. *L. major* is the causative agent of cutaneous leishmaniasis, whereas *L. tropica* causes cutaneous leishmaniasis as well as the mucocutaneous form, especially in immunocompromised patients. On the other hand, *L. infantum* causes the visceral form of leishmaniasis, which is fatal if left untreated. Differential diagnosis of leishmaniasis holds high importance, especially in endemic areas where various species of *Leishmania* are present and require proper therapeutic approaches. For instance, visceral leishmaniasis may necessitate appropriate therapeutic monitoring, whereas some cutaneous lesions can be self-healing. In this study, we aim to provide a comparison of molecular methods for the differential diagnosis of leishmaniasis, facilitating the selection of the proper method under various circumstances.

Methods: Numerous molecular methods have been employed worldwide to identify the *Leishmania* parasite at the species level. These methods include but are not limited to conventional PCR, real-time PCR, PCR-RFLP, and High-resolution melting analysis (HRM).

Results: Several studies have shown the high potency of the ITS-1 region as a genetic marker for the identification of *Leishmania* species using PCR-based methods. Conventional PCR has been proven to be a favorable method for *Leishmania spp.* differentiation using primers derived from the ITS-1 region. It is also an aiding tool for monitoring and therapeutic management of the infection. Other methods, such as qPCR-RFLP, PCR-RFLP, and PCR-HRM, have also shown high performance in recognizing different *Leishmania* species.

Conclusion: This comparative evaluation highlights that while conventional PCR targeting kDNA offers high sensitivity for genus-level detection of *Leishmania*, it falls short in species differentiation. The ITS-1 region emerges as the most reliable target for species-level identification in multiple molecular methods. Real-time PCR and ITS1-based PCR-RFLP or HRM provide precise differentiation but demand advanced laboratory infrastructure and financial resources. Nested PCR and HRM offer promising results, combining diagnostic



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



accuracy with relative cost-effectiveness; however, proper optimization and technical expertise must be ensured. A conventional PCR, if performed with a specific set of primers designed, can be highly useful in both diagnosis and monitoring leishmaniasis. Ultimately, the choice of molecular method should be guided by the diagnostic purpose, available resources, and the endemic context to ensure effective management and treatment of leishmaniasis.

Keywords: *Leishmania* spp., Molecular diagnosis, PCR, ITS-1 region, Species identification.

Introduction:

Leishmaniasis is a group of diseases caused by more than 20 species of the protozoan genus *Leishmania*, which are transmitted to both humans and animals through the bite of infected phlebotomine sandflies. Leishmaniasis manifests in patients in various forms, including visceral, cutaneous, and mucocutaneous leishmaniasis, which are closely linked to the causative *Leishmania* species (1). In Iran, where leishmaniasis remains widespread and endemic in many regions of the country, *L. major* is the causative agent of cutaneous leishmaniasis, whereas *L. tropica* and *L. infantum* are the predominant species contributing to mucocutaneous and visceral forms of the disease, respectively (2).

Early and precise identification of the causative *Leishmania* species is essential for effective clinical management and therapy approaches. Traditional parasitological methods, such as microscopic examination and culture, are limited by low sensitivity, time intensity, and the inability to differentiate species (3).

Over the past two decades, molecular methods have greatly improved the diagnosis and characterization of *Leishmania* species by offering higher sensitivity, specificity, and rapid turnaround. Among these, various PCR-based techniques—ranging from conventional PCR to real-time PCR, nested PCR, PCR-RFLP, and high-resolution melting (HRM) analysis—have been developed and applied, targeting various regions of the parasite genome, such as the kinetoplast DNA (kDNA), internal transcribed spacer 1 (ITS-1), small subunit ribosomal RNA (SSU rRNA), and genomic markers. These methods not only aid us in detecting the parasite but also in identifying the precise species (4).

This study aims to provide a comparative evaluation of the major molecular techniques applied for the differential diagnosis of *Leishmania* infections. The most genetic markers used for differential diagnosis in many laboratories worldwide were derived from the ITS-1 and kDNA regions.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Materials and Methods:

Numerous molecular diagnostic methods, including conventional PCR, nested PCR, real-time PCR, PCR-RFLP, and High-resolution melting analysis (HRM), have been used as diagnostic methods by different investigators worldwide. Prior to all methods mentioned, the parasite DNA has been extracted. The methods used are provided in the table below (Table 1) in further detail.

Method	Target Region(s)	Primers Used (Target Site)	Primer Sequences	Purpose/Notes	Refernceses
Conventional PCR	- kDNA	- 13A / 13B (kDNA)	13A: 5'-GTGGGGGAGGGGCGTTCT-3'		Reale et al., 1999 (1)
			13B: 5'-ATTTTACACCAACCCCAGTT-3'		Bensoussan et al., 2006 (2)
		- Lmj4 / Uni21 (kDNA)	Lmj4: 5'-CTAGTTTCCCGCCTCCGAG-3'	- Genus-level diagnosis (kDNA)	
			Uni21: 5'-GGGGTTGGTGTAATAATAGGCC-3'		
	- ITS-1	- LITSR / L5.8S (ITS-1)	LITSR: 5'-CTGGATCATTTTCCGATG-3'	- Species identification (ITS-1)	Schönian et al., 2003 (3)
			L5.8S: 5'-TGATACCACTTATCGCACTT-3'		Bensoussan et al., 2006 (2)
					Chagui et al., 2012 (4)
	Genomic DNA (L. major + human)	- F-primer / R-primer (genomic DNA, human & L. major)	F-primer: 5'-AGAGGTGAAATTCTTGGACCG-3'	- Monitoring (human & L. major) DNA	Karimi et al., 2024 (5)
			R-primer: 5'-TTCCGTCAATTCCTTTAAGTTTCA-3'		



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Method	Target Region(s)	Primers Used (Target Site)	Primer Sequences	Purpose/Notes	Refernces
Real-Time PCR	ITS-1	LITSR / L5.8S (ITS-1)	LITSR: 5'-CTGGATCATTTTCCGATG-3' L5.8S: 5'-TGATACCACTTATCGCACTT-3'	Species identification using real-time quantification followed by HRM or RFLP	Gürses et al., 2022 (6)
ITS1-PCR-RFLP	ITS-1	LITSR / L5.8S (ITS-1) Enzyme: HaeIII	LITSR: 5'-CTGGATCATTTTCCGATG-3' L5.8S: 5'-TGATACCACTTATCGCACTT-3'	Species differentiation using restriction digestion (esp. in Iran)	Spankos et al., 2007 (7) Koohsar et al., 2020 (8)
Nested PCR	- kDNA	Round 1: - CSB2XF / CSB1XR (kDNA)	CSB2XF: 5'-CGAGTAGCAGAACTCCCGTTCA-3' CSB1XR: 5'-ATTTTCGCGATTTTCGCAGAACG-3' LIR: 5'-TCGCAGAACGCCCT-3'	Identification of cutaneous Leishmaniasis agents	Saki et al., 2019 (9) Maraghi et al. 2013 (10)
		Round 2: - LIR / 13Z (kDNA)	13Z: 5'-ACTGGGGTTGGTGTAATAAG-3'		
	- SSU rRNA	- R221 / R332 (SSU rRNA)	R221: 5'-GGTTCCTTTCCTGATTTACG-3'	Leishmania species identification	Silva et al., 2013 (11)
			R332: 5'-GGCCGGTAAAGCCGAATAG-3'		Van Eys et al. 1992 (12)



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Method	Target Region(s)	Primers Used (Target Site)	Primer Sequences	Purpose/Notes	Refernces
	- ITS-1	- LITSR / L5.8S (ITS-1)	LITSR: 5'-CTGGATCATTTTCCGATG-3'		El Tai et al.,2000 (13)
			L5.8S: 5'-TGATACCACTTATCGCACTT-3'		El Tai et al., 2001 (14)
HRM-PCR	- ITS-1	- ITS-219F / ITS-219R (ITS-1)	ITS-219F: 5'-AGCTGGATCATTTTCCGATG-3'	Rapid and precise species differentiation using nucleotide variation	Talmi-Frank et al., 2010 (15)
			ITS-219R: 5'-ATCGCGACACGTTATGTGAG-3'		

Results and Discussion:

Conventional PCR was used as a favorable molecular method by many investigators, who have used predominantly primers derived from the ITS-1 and ssu-rDNA gene (3). Although conventional PCR targeting kDNA has been proven to have the highest sensitivity in determining the presence of the *Leishmania* parasite at the Genus level, it does not hold the ability to identify differential diagnosis of all *Leishmania* species (1); therefore many researchers targeted the ITS-1 region by conducting a conventional PCR followed by a restriction fragment enzyme analysis to identify all medically relevant species (3). Conventional PCR has been proven useful in monitoring the therapy through the use of the primer pair derived from 18S rRNA genes of *L. major* and human genes, which can amplify the DNA of both origins simultaneously. This method is highly cost-beneficial and does not demand highly facilitated laboratories and can be performed for quantitative analysis dealing with the parasite burden in the lesions (5). Although Real-time PCR is a quantitative method that can also help us with therapeutic monitoring of the disease, it comes with a high cost and a need for specialized equipment as well. Differential diagnosis of *Leishmania* species using Real-time PCR is possible through the design of specific primers and probes (16). Species identification can also be accomplished by performing a post-qPCR Melting Curve Analysis, as well as ITS1-PCR-RFLP and ITS1-qPCR-HRM, which have been proven capable of differential diagnosis of *Leishmania* species, yet they require well-equipped laboratories and are financially demanding (17). PCR-HRM method, on the other hand, needs no post-PCR processing and has shown acceptable ability in *Leishmania* species differentiation along with cost-effectiveness; however, it may necessitate careful optimization and should be performed by experienced personnel (15). The nested PCR method, as well, can identify different species



of the *Leishmania* parasite (10). Eventually, ITS-1 has been proven helpful as a genetic marker for differential diagnosis of *Leishmania* species. Ultimately, the selection of the proper diagnostic method requires considering various factors such as the available resources, technical expertise, cost-effectiveness, diagnostic purpose and the endemic context.

References:

1. Reale S, Maxia L, Vitale F, Glorioso NS, Caracappa S, Vesco G. Detection of *Leishmania infantum* in dogs by PCR with lymph node aspirates and blood. *J Clin Microbiol.* 1999;37(9):2931–5. <https://doi.org/10.1128/jcm.37.9.2931-2935.1999>
2. Bensoussan E, Nasereddin A, Jonas F, Schnur LF, Jaffe CL. Comparison of PCR assays for diagnosis of cutaneous leishmaniasis. *J Clin Microbiol.* 2006;44(4):1435–9. doi:10.1128/JCM.44.4.1435-1439.2006.
3. Schönian G, Nasereddin A, Dinse N, Schweynoch C, Schallig HD, Presber W, et al. PCR diagnosis and characterization of *Leishmania* in local and imported clinical samples. *Diagn Microbiol Infect Dis.* 2003;47(1):349–58. doi:10.1016/s0732-8893(03)00093-2.
4. Chargui N, Haouas N, Jaouadi K, Gorcii M, Pratlong F, Dedet JP, et al. Usefulness of a PCR-based method in the detection and species identification of *Leishmania* from clinical samples. *Pathol Biol (Paris).* 2012;60(6):e75–9. doi:10.1016/j.patbio.2011.11.011.
5. Karimi P, Moradi M, Arabkhazaeli F, Mohebalı M, Shayan D, Shayan P. A simple and innovative quantitative PCR analysis for diagnosis and therapy management of human cutaneous leishmaniasis. *J Zoonotic Dis.* 2025;9(2):762–70. doi:10.22034/jzd.2024.18706.
6. Gürses G, Yentür Doni N, Yıldız Zeyrek F, Yiğın A. Kutanöz Layşmanyazise neden olan *Leishmania* türlerinin Sybr Green bazlı ITS-1 gerçek zamanlı polimeraz zincir reaksiyonu yöntemiyle tiplendirilmesi [Typing of *Leishmania* species causing cutaneous leishmaniasis by Sybr Green based ITS-1 real time polymerase chain reaction method]. *Mikrobiyol Bul.* 2022;56(2):326–38. doi:10.5578/mb.20229811.
7. Spanakos G, Piperaki ET, Menounos PG, Tegos N, Flemetakis A, Vakalis NC. Detection and species identification of Old World *Leishmania* in clinical samples using a PCR-based method. *Trans R Soc Trop Med Hyg.* 2008;102(1):46–53. doi:10.1016/j.trstmh.2007.05.019.
8. Koohsar F, Hajjaran H, Heidari S, Darabi E, Fathi S, Borhani M, et al. Molecular identification of *Leishmania* species isolated from patients with cutaneous



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- leishmaniosis in Gonbad Kavoods, northeastern Iran, using hSP70 and ITS-based PCR-RFLP. *Ann Parasitol.* 2020;66(3):339–46. doi:10.17420/ap6603.272.
9. Saki J, Amraee D, Ghasemian M. Comparison of nested-PCR and loop-mediated isothermal amplification for cutaneous leishmaniasis diagnosis. *Jundishapur J Microbiol.* 2019;12(8):e94171. <https://doi.org/10.5812/jjm.94171>.
 10. Maraghi S, Mardanshah O, Rafiei A, Samarbafzadeh A, Vazirianzadeh B. Identification of cutaneous leishmaniasis agents in four geographical regions of Khuzestan Province using nested PCR. *Jundishapur J Microbiol.* 2013;6(4):e4866. <https://doi.org/10.5812/jjm.4866>.
 11. da Silva MA, Soares CRP, Medeiros RA, Medeiros Z, de Melo FL. Optimization of single-tube nested PCR for the diagnosis of visceral leishmaniasis. *Exp Parasitol.* 2013;134(2):206–10. <https://doi.org/10.1016/j.exppara.2013.03.003>.
 12. van Eys GJJM, Schoone GJ, Kroon NCM, Ebeling SB. Sequence analysis of small subunit ribosomal RNA genes and its use for detection and identification of *Leishmania* parasites. *Mol Biochem Parasitol.* 1992;51(1):133–42. [https://doi.org/10.1016/0166-6851\(92\)90208-2](https://doi.org/10.1016/0166-6851(92)90208-2).
 13. El Tai NO, Osman OF, El Fari M, Presber W, Schönian G. Genetic heterogeneity of ribosomal internal transcribed spacer in clinical samples of *Leishmania donovani* spotted on filter paper as revealed by single-strand conformation polymorphisms and sequencing. *Trans R Soc Trop Med Hyg.* 2000;94(5):575–9.
 14. El Tai NO, El Fari M, Mauricio I, Miles MA, Oskam L, El Safi SH, et al. *Leishmania donovani*: intraspecific polymorphisms of Sudanese isolates revealed by PCR-based analyses and DNA sequencing. *Exp Parasitol.* 2001;97(1):35–44.
 15. Talmi-Frank D, Nasereddin A, Schnur LF, Schönian G, Töz SO, Jaffe CL, et al. Detection and identification of Old World *Leishmania* by high resolution melt analysis. *PLoS Negl Trop Dis.* 2010;4(1):e581. doi:10.1371/journal.pntd.0000581.
 16. Fotouhi-Ardakani R, Ghafari SM, Ready PD, Parvizi P. Developing, modifying, and validating a TaqMan real-time PCR technique for accurate identification of *Leishmania* parasites causing most leishmaniasis in Iran. *Front Cell Infect Microbiol.* 2021;11:731595. doi:10.3389/fcimb.2021.731595.
 17. Villarreal J, Guillermo R, Herrera G, Muskus CE. Identification of *Leishmania* species by real time PCR coupled with high resolution melting curve analysis. *bioRxiv.* 2022. doi:10.1101/2022.01.19.222891.



***Aedes albopictus* as a High-Risk Invasive Species in Arbovirus Transmission: Taxonomy, Ecology, and Implications**

Parsa Farzam¹, Sarvenaz Sadeghi Mahoonak¹

Corresponding Author: Parsa Farzam, P.farzam2015@gmail.com

1. Department of veterinary science Islamic Azad University, Karaj Branch, Karaj, Iran.

Article code: 1313

Abstract

Background: *Aedes albopictus*, commonly known as the Asian tiger mosquito, originated in Southeast Asia and is an invasive mosquito species, making it a prominent vector of arboviruses such as dengue, Zika, and chikungunya. Its rapid global spread is attributed to ecological adaptability, high genetic diversity, human-mediated transport, and climate change.

Methods: We conducted a systematic review following veterinary parasitology guidelines, searching PubMed and Google Scholar for peer-reviewed articles published between 2014 and 2024 using keywords like ‘*Aedes albopictus*,’ ‘arboviruses,’ and ‘ecology.’ Inclusion criteria comprised English-language, peer-reviewed studies focused on *Aedes albopictus*; non-relevant articles were excluded. Data on taxonomy, ecology, and arboviral transmission were extracted and synthesized.

Results: *Aedes albopictus* thrives in a variety of habitats, including artificial containers and urban landscapes. Its opportunistic feeding behavior, capacity for vertical transmission, and resilience to environmental changes enhance its effectiveness as a disease vector, particularly in newly colonized regions. The high resistance of *Aedes* mosquitoes and their exceptional vector competence can lead to outbreaks.

Conclusion: The increasing public health threat posed by *Aedes albopictus* necessitates integrated vector management approaches. These include robust surveillance systems, vaccine development within a One Health framework, raising awareness, and targeted interventions adapted to local ecological and socioeconomic contexts.

Keywords: *Aedes albopictus*, Arboviruses, Dengue, Outbreaks, Vaccine development.

Introduction:

Aedes albopictus, commonly known as the Asian tiger mosquito, a key species of the *Culicidae* family, originated in Southeast Asia, but has adapted to diverse environments, enabling colonization of new geographic areas. Its ability to breed in small amounts of water facilitates



rapid reproduction and exacerbates outbreaks. They primarily cause small, self-limiting outbreaks of dengue, Zika, and chikungunya, though they transmit other diseases too. Their primary hosts are humans but, in many reports, it depends on the animals inhabiting the environment. While *Aedes aegypti* dominates current studies due to frequent outbreaks, *Aedes albopictus* poses unique threats: higher genetic variation which influences virus-mosquito interactions ($G \times G$), impacting viral pathogenesis. Moreover, *Aedes albopictus* can tolerate a wider temperature range and unlike *Aedes aegypti* their endophagic behavior increases risk (1). This article examines current *Aedes albopictus*'s expansion and its causes including, lack of surveillance and knowledge, climate change. Furthermore, we also evaluate potential control and prevention methods.

1. Taxonomy of *Aedes albopictus*

The family *Culicidae* includes two subfamilies, *Anophelinae* and *Culicinae* (2). One of the most important species is *Aedes albopictus*, commonly known as the Asian tiger mosquito. This species is cosmopolitan and a well-known invasive vector (2). They are medically important vectors found in over 129 countries worldwide (1).

2. Biological Traits and Ecological Adaptability

Aedes mosquitoes vary from 2 to 10 mm in length and the adults have slender bodies, prominent eyes and long legs. The long narrow wings are held crossed flat over the abdomen at rest and bear scales, which project as a fringe on the posterior margin (Figure 1) (1,6). *Aedes albopictus* eggs can stand extended periods of desiccation resisting even in extreme conditions through diapausing mechanisms (3). In some cases, the eggs may remain viable for up to three years. Despite some degree of temperature tolerance, freezing and high temperatures (in excess of 40 °C) will kill most eggs. A female mosquito will live for an average of 2–3 weeks, while the male's lifespan is shorter (6). They are opportunistic feeders; blood meals identified from 16 mammal species (8). Host selection is highly opportunistic and is largely influenced by the relative abundance of hosts found in the habitat (6). Originating from Southeast Asia, the species are highly adaptable to diverse environments, enabling colonization of new geographic areas (3,6). *Aedes albopictus* ability to breed in small amounts of water, and its evolutive preference by artificial water containers, commonly found in urban areas, facilitates the proliferation of the mosquito populations and its rapid expansion in densely populated regions (3). These mosquitoes use a wide range of breeding habitats such as tires, bamboo stumps, glass bottles, and vegetation-rich areas (8). Genomic studies showed that there is a high genetic variation in *Aedes albopictus* whereas *Ae. aegypti* generally shows low genetic variation (1).



Figure 1. *Aedes albopictus* (Asian tiger mosquito) (9)

3. Behavior

3.1. Feeding and Oviposition Behavior

Female mosquitoes bite many different species of amphibians, birds, fishes, mammals and turtles, with preference for mammals, and humans represent about 60% of the blood meals. Females of *Aedes* oviposit in domestic as well as natural water holding containers and spread their eggs over two or more sites in a practice known as skip oviposition (1). Female mosquitoes can transmit DENV without needing to feed on infected hosts. The occurrence of male mosquitoes infected by transovarial transmission suggests an equal probability of infection of the females of the same batch (8).

3.2. Genetic variability and viral interactions

Aedes albopictus exhibits a high occurrence of genetic polymorphism. This genetic variation affects genotype-by-genotype (GxG) interactions between the virus and *Aedes*, which modulates infection of the mosquito mid-gut and dissemination of the virus to other tissues (1).

3.3. Comparison of two species

Human exposure risk factors are mainly due to multiple feeding patterns, including endophagy by *Aedes albopictus* and zoophagic behavior of *Ae. Aegypti* (2). *Aedes aegypti* exhibits a stronger feeding activity during hot season while *Aedes albopictus* is an all-round biter (Table 1) (1).



Table1. Comparison of *Aedes aegypti* and *Aedes albopictus* species: (1,2,5)

Trait	<i>Aedes aegypti</i>	<i>Aedes albopictus</i>
Genetic polymorphism	Lower	Higher
Climate Adaption	Warm climates	Tolerates warm and cooler temperatures
Primary Feeding Behavior	Zoophagic	Endophagic

4. Disease Transmitted by *Aedes albopictus*

The medical and veterinary significance of *Aedes albopictus* stems from its role as a vector for at least 22 arboviruses, the most important of which are chikungunya (CHIKV), dengue (DENV), Japanese encephalitis, West Nile, yellow fever and Zika (ZIKV) viruses. Additionally, *Dirofilaria immitis*, *Plasmodium gallinaceum*, *P. fallax* and *P. lophurae* have been isolated from this species (2,5,6). Dengue remains the most important arbovirus disease Transmitted by *Aedes albopictus* (1,5).

4.1. Dengue virus (DENV), chikungunya virus (CHIKV) and Zika virus (ZIKV)

Aedes mosquitoes transmit three arboviruses of major public health concern. The two Flaviviruses (DENV and ZIKV) encode three structural and three non-structural proteins. Chikungunya virus (CHIKV) is an Alphavirus and encodes four non-structural proteins and six structural proteins. Phylogenetically, DENV, ZIKV and CHIKV are single-stranded positive-sense ribonucleic acid viruses (1). The compatibility of *Aedes* mosquitoes with DENV and CHIK is strongly influenced by genotype-to-genotype interaction (1). Dengue is a major public health concern and is spreading rapidly through tropical, subtropical, and even (transiently) temperate areas. Investigators have pursued the differences in sequence diversity of DENV-1 and concluded that there was more diversity accumulation in *A. albopictus* than in *A. aegypti* (4).

5. The Global Threat of *Aedes albopictus*: Expansion and Climate Change Impact

5.1. Current Geographic Distribution:

Aedes albopictus is emerging as a growing public health threat due to its expanding range and increased disease transmission. Two species, *Ae. aegypti* and *Ae. albopictus*, are recorded in Iran (2). In recent years *Ae. albopictus* was implicated in short-term outbreaks of chikungunya and dengue in Italy, France and many states of America including Florida, Texas, and Arizona (Figure 2) (3).

5.2. Climate impact:

The researchers also reported that summer seasons had the longest biting activity. Temperature critically influences mosquito activity, with warmer conditions accelerating reproduction and biting rates. *Aedes* vectors are abundant during the rainy season, obviously due to available moisture for oviposition and egg hatching, and perhaps due to the ability of their immatures to withstand flushing effects of rainfall (1). *Ae. albopictus* benefits from globalization and further supported by climate change (3).

5.3. Projected future Expansion:

Climate change is playing an important role, facilitating the establishment of vectors and viruses beyond their classic tropical and subtropical geographical boundaries, by favoring environmental conditions for mosquito reproduction (3). *Ae. albopictus* is expected to spread broadly throughout Europe, ultimately reaching wide areas of France and Germany. Areas in northern United States and highland regions of South America and East Africa are also projected to see establishment of *Ae. albopictus* over the next 30 years. In total, 197 countries (range, 181–209) are expected to report *Ae. albopictus* till 2080 (7).

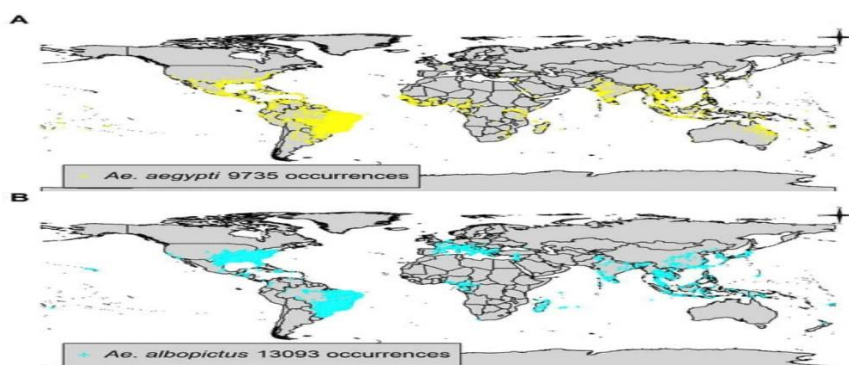


Figure 2. *Ae. aegypti* and *Ae. albopictus* Major Geographic Distribution in 2023 (10)

6. Control and preventing strategies

It is necessary that developing a One Health arbovirus vaccine (for animals and humans) is made a priority or at least be considered. Socio-economic factors affect container characteristics and distribution as well as land use patterns. Vector control interventions that allow interaction of factors such as socioeconomic, landscape, knowledge, attitudes and practices have the potential for optimizing vector control strategies, future mosquito suppression, prediction and prevention of epidemics (1). Addressing these public health challenges particularly by improving surveillance, establishing efficient control measures, and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



preparedness for the development of new vaccines against known pathogens in immunological naive populations, particularly against Zika and chikungunya due to the absence of licensed vaccines. Collaborative and proactive efforts are essential to implement effective control measures, raise awareness, and protect communities from the spread of this invasive vector (3).

Materials and Methods:

This article provides a comprehensive review of the taxonomy, biology, behavior, and vector competence of *Aedes albopictus*, with an emphasis on its ecological expansion, adaptability to diverse environments, and role in disease transmission.

Results and Discussion:

Aedes albopictus has emerged as a globally significant vector due to its exceptional ecological plasticity, high genetic variability, and rapid geographic expansion. These attributes, in combination with anthropogenic activities and climate change, have enabled the species to thrive across a wide range of environments. Its demonstrated competence in transmitting numerous arboviruses—including dengue, chikungunya, Zika, and yellow fever. The accelerating spread of *A. albopictus* and the associated rise in arboviral disease burden underscore the urgent need for integrated vector management strategies. These should encompass enhanced surveillance, the development of cross-species vaccines within a One Health framework, and context-specific public health interventions. Strengthening global preparedness through interdisciplinary collaboration will be essential in addressing the challenges of *Aedes albopictus*-borne diseases in an increasingly interconnected and changing world.

Acknowledgment:

We would like to express our sincere gratitude to Dr. Pouya Khodaie for their invaluable guidance, insightful feedback, and unwavering support throughout this research.

References:

1. Ahebwa A, Hii J, Neoh KB, Chareonviriyaphap T. *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) ecology, biology, behaviour, and implications on arbovirus transmission in Thailand. *One Health*. 2023 Apr 30; 16:100555.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



2. Azari-Hamidian S. The invasive *Aedes* mosquitoes (Diptera: Culicidae) and their medical and veterinary importance: a mini review. *Caspian Journal of Health Research*. 2023 Oct 10;8(4):241-6.
3. Nazareth T, Seixas G, Lourenço J, Bettencourt PJG. *Aedes albopictus* arrives in Lisbon: an emerging public health threat. *Front Public Health*. 2023; 11:1332334. Published 2023 Dec 19. doi:10.3389/fpubh.2023.1332334.
4. Christofferson RC. A reevaluation of the role of *Aedes albopictus* in dengue transmission. *The Journal of Infectious Diseases*. 2015 Oct 15;212(8):1177-9.
5. Bohers C, Vazeille M, Bernaoui L, et al. *Aedes albopictus* is a competent vector of five arboviruses affecting human health, greater Paris, France, 2023 [published correction appears in *Euro Surveill*. 2024 May;29(21). doi: 10.2807/1560-7917.ES.2024.29.21.240516c.]. *Euro Surveill*. 2024;29(20):2400271. doi:10.2807/1560-7917.ES.2024.29.20.2400271.
6. Otranto D, Wall R. *Veterinary Parasitology*. 5th ed. Hoboken, NJ : Wiley-Blackwell ; 2024.
7. Kraemer MUG, Reiner RC Jr, Brady OJ, et al. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus* [published correction appears in *Nat Microbiol*. 2019 May;4(5):900. doi: 10.1038/s41564-019-0429-2.] [published correction appears in *Nat Microbiol*. 2019 May;4(5):901. doi: 10.1038/s41564-019-0440-7.]. *Nat Microbiol*. 2019;4(5):854-863. doi:10.1038/s41564-019-0376-y.
8. Garcia-Rejon JE, Navarro JC, Cigarroa-Toledo N, Baak-Baak CM. An Updated Review of the Invasive *Aedes albopictus* in the Americas; Geographical Distribution, Host Feeding Patterns, Arbovirus Infection, and the Potential for Vertical Transmission of Dengue Virus. *Insects*. 2021;12(11):967. Published 2021 Oct 26. doi:10.3390/insects12110967.
9. National Pest Management Association. Asian Tiger Mosquito (*Aedes albopictus*) [image on the Internet]. Available from: <https://www.pestworld.org/media/5614/asian-tiger-mosquito.jpg>.
10. Smith J, Doe A, ... et al. Spatial prediction of *Aedes albopictus* habitat suitability under current and future climate scenarios [image]. *Insects*. 2023;14(1):49. Available from: <https://doi.org/10.3390/insects14010049>.



Molecular Prevalence of *Toxoplasma gondii* in Zahedan County Using Nested-PCR Method

Mohammad Mohebi¹, Mohammad Mirzaei¹, Bagher Amirheidari², Maryam Nooshadokht³

Corresponding Author: Mohammad Mohebi, mohebbby1@gmail.com

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
2. Extremophile and Productive Microorganisms Research Center, Kerman University of Medical Sciences, Kerman, Iran.
3. Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.

Article code: 1315

Abstract

Background: Milk is consumed as a complete food, especially for children and the elderly. However, raw milk consumption may pose risks, such as toxoplasmosis, one of the most common parasitic zoonotic diseases worldwide. Cats, not only domestic cats (*Felis catus*) but nearly all feline species, serve as definitive hosts and can shed *Toxoplasma gondii* oocysts. Most mammals act as intermediate hosts. Infections due to the consumption of raw milk from various species, including sheep, goats, camels, and cows, have been reported. The present study evaluates the prevalence of *T. gondii* in milk from four livestock species in Zahedan County to assess potential health risks associated with raw milk consumption in this region.

Methods: DNA extraction was performed on 58 milk samples from cows, goats, sheep, and camels, which were collected and transported to the laboratory, and then by detecting the B1 gene of *T. gondii* was detected using the Nested-PCR method.

Results: 11 samples (19%) were contaminated with *T. gondii*. In this study, the highest molecular incidence of infection was identified in sheep and goat milk (6.9%). Also, 3.4% was observed in cow's milk and 1.7 % in camel's milk.

Conclusion: Our results indicate that milk can easily be contaminated with *T. gondii* tachyzoites, the infectious stage of the parasite, and is not specific to any particular species. Therefore, it is strongly recommended that milk be boiled at a minimum temperature of 70°C for at least one minute before consumption or that pasteurized milk be used.

Keywords: Nested-PCR, Raw milk, *Toxoplasma gondii*, Zahedan.



Introduction:

Milk is consumed as a complete food, especially for children and the elderly. Its high value in terms of protein, minerals, fats, and vitamins is undeniable, and millions of people consume milk and dairy products daily. Therefore, the hygienic quality of milk holds significant importance in public health, though infections and diseases like *Toxoplasma gondii*, the parasite responsible for Toxoplasmosis, occasionally occur (1).

Toxoplasmosis is one of the most common parasitic zoonotic diseases worldwide. Cats, not only domestic cats (*Felis catus*) but nearly all feline species, serve as definitive hosts and can shed *T. gondii* oocysts. Most mammals act as intermediate hosts (2). *T. gondii* infection is widespread in humans and sheep globally. Nearly half a billion people in human populations have *T. gondii* antibodies. The primary source of infection for intermediate hosts is cats, which contaminate the environment by shedding oocysts (3). The main routes of infection, both in definitive and intermediate hosts, are oral ingestion of oocysts or consumption of meat containing bradyzoites, or, less commonly, through raw milk containing tachyzoites. Moreover, in some hosts (such as goats), tachyzoites can also be transmitted from mother to offspring through milk (4). Infections due to the consumption of raw milk from various species, including sheep, goats, camels, and cows, have been reported (1, 5, 6).

While *T. gondii* infection is highly prevalent in humans, clinical manifestations of the disease are more commonly observed in high-risk groups. In immunocompetent individuals, the disease is often asymptomatic. Occasionally, mild symptoms may occur, with lymphadenopathy being the most prominent clinical manifestation. Encephalitis, septic syndrome, myocarditis, or hepatitis may also occur, though these are very rare in healthy individuals. ocular toxoplasmosis is considered a result of prenatal infection that manifests later in life (7). Infection with *Toxoplasma gondii* is particularly dangerous and even fatal in specific groups, such as congenitally infected fetuses, infants, immunocompromised individuals, AIDS patients, and organ transplant recipients (8). Toxoplasmosis in sheep deserves special attention due to its economic impact. *T. gondii* is one of the leading causes of abortion in sheep and goats, thus posing a major challenge to production. Sheep and goats develop strong immunity after abortion. Vaccination against *Toxoplasma*-induced abortion is practiced in some countries (9).

Given the significance of *T. gondii* as a zoonotic pathogen and the consumption of milk as a complete food, the present study evaluates the prevalence of *T. gondii* in milk from four livestock species in Zahedan County.



Materials and Methods:

Sample collection: A total of 58 raw milk samples from four different livestock species, including cows, sheep, goats, and camels, were randomly collected from Zahedan County during the year (2024-2025). The milk samples were manually obtained by milking the animals' udders, which had been previously disinfected with alcohol. Sterile gloves were used during sampling. The samples were poured into sterile glass containers and transported to the laboratory within 2 to 4 hours, kept on ice.

DNA Extraction: Genomic DNA was extracted using a DNA extraction kit from Pishgam Company, Iran, following the manufacturer's instructions. The extracted DNA samples were then stored at -20°C until the Nested-PCR test was performed.

Nested-PCR Method: The DNA extracted from raw milk samples was tested using the Nested-PCR method to detect the presence of the B1 gene of *T. gondii*. Table (1) shows the primer sequences required for identifying *Toxoplasma gondii* in Nested-PCR. For the first stage of the PCR test, the final reaction volume was 25 µL. The thermal program included: initial denaturation at 94°C for 30 seconds, 35 cycles of denaturation at 94°C for 15 seconds, annealing at 45°C for 30 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 10 minutes.

The second stage of the reaction was performed with 1 µL of the first-stage PCR product as template DNA and the second primer pair, using the same thermal program as the first stage with 35 cycles. The PCR products were electrophoresed on a 2% agarose gel, and the results were recorded. For positive control, DNA extracted from tachyzoites of Strain RH was used, and for negative control, sterile distilled water was employed.

Table 1. Primer sequences used in the Nested-PCR reaction

Amplicon size	Primer sequence	Reference	Target gene
-	Toxo 1F- 5'- TCAAGCAGCGTATTGTCGAG-3'	R. Chiabchalard et al., 2005	B1 (primary)
-	Toxo 1R- 5'- CCGCAGCGACTTCTATCTCT-3'	R. Chiabchalard et al., 2005	B1 (primary)



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Amplicon size	Primer sequence	Reference	Target gene
197	Toxo 2F- 5'- GGAAGTGCATCCGTTTCATGAG-3'	R. Chiabchalar et al., 2005	B1 (secondary)
197	Toxo 2R- 5'- TCTTTAAAGCGTTCGTGGTC-3'	R. Chiabchalar et al., 2005	B1 (secondary)

Result and Discussion:

Figure 1 shows the electrophoresis results of Nested-PCR products for amplifying the B1 gene of *Toxoplasma gondii* in milk samples. Positive results indicated the presence of a 197 bp band in electrophoresis. The results showed that out of 58 samples (19%), the B1 gene of *Toxoplasma gondii* was detected. In this study, the highest molecular prevalence of *T. gondii* contamination was observed in raw sheep and goat milk (6.9%).

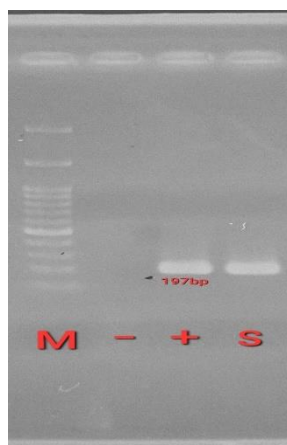


Figure 1. displays the electrophoresis results of Nested-PCR products for amplifying the B1 gene of *Toxoplasma gondii*. M: Marker. -: Negative control. +: Positive control. S: Positive samples.

The results revealed that out of 58 samples (19%), the B1 gene of *T. gondii* was present. In this research, the highest molecular prevalence of *T. gondii* contamination was found in raw sheep and goat milk. According to the results of this study, seasonal and geographical prevalence studies of *Toxoplasma gondii* in ruminant milk using Nested-PCR showed the highest contamination rate in sheep milk (8%) and the lowest in buffalo milk (4.28%) (10).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



In an investigation of the molecular prevalence of *T. gondii* in animal milk samples collected from Alborz Province using PCR, the molecular prevalence in raw cow, sheep, and goat milk samples was 5.33%, 12 %, and 10%, respectively. Raw and unpasteurized sheep milk was considered the highest-risk source for *Toxoplasma gondii* transmission (8). Detection of *T. gondii* in raw goat, sheep, buffalo, cow, and camel milk using PCR in Iran showed the highest prevalence in goats (10%) and the lowest in camels (3.12%) (1).

Results from a study on *T. gondii* in human and goat milk from desert regions of Iran revealed that 0.3% human milk was contaminated with *T. gondii*, while 5.5% of goat milk showed *T. gondii* infection (11). Additionally, in another study, *Toxoplasma gondii* DNA was identified in sheep and goat milk from northwestern Iran using PCR-RFLP (12). Another study in Kayseri Province, Türkiye, A total of 200 specimens of cheese and milk of cow, sheep, goat and water buffalo, detected *T. gondii* in goat (4%) and ewes milk (8%) (13).

Differences in results across studies may be attributed to variations in geographical regions, climate, animal age, grazing practices, feeding methods, and laboratory techniques used (14). studies suggested that the high prevalence of *T. gondii* infection in sheep and goats might be due to free-range livestock exposure to *T. gondii* infection. These animals are kept in pastures, and environmental contamination with oocysts increases the infection pressure.

Our results indicate that milk can easily be contaminated with *T. gondii* tachyzoites and is not specific to any particular species. However, goats and sheep showed the highest prevalence of *T. gondii* in milk samples, though cows and camels can also shed tachyzoites in their milk. This finding is of significant public health importance, particularly concerning cow's milk, which is the most widely consumed by humans. It is likely that consuming contaminated raw milk can cause infections in humans in these areas. Therefore, it is strongly recommended that milk be boiled before consumption or that pasteurized milk be used.

Acknowledgment:

No specific acknowledgments are applicable for this work

References:

1. Dehkordi FS, Haghighi Borujeni MR, Rahimi E, Abdizadeh R. Detection of *Toxoplasma gondii* in raw caprine, ovine, buffalo, bovine, and camel milk using cell cultivation, cat bioassay, capture ELISA, and PCR methods in Iran. *Foodborne Pathogens and Disease*. 2013;10(2):120-5.
2. Ferguson DJ, Dubremetz J-F. The ultrastructure of *Toxoplasma gondii*. *Toxoplasma gondii*. 2014:19-59.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



3. Dubey JP. Toxoplasmosis of Animals and Humans. Edition r, editor: CRC press; 2022.
4. Ishag M, Magzoub E, Majid M. Detection of *Toxoplasma gondii* tachyzoites in the milk of experimentally infected lactating she-camels. 2006.
5. Spišák F, Turčková L, Reiterová K, Špilovská S, Dubinský P. Prevalence estimation and genotypization of *Toxoplasma gondii* in goats. *Biologia*. 2010;65:670-4.
6. Asiyabi Aghdam S, Hajipour N, Moosavy MH. Use of PCR to determine *Toxoplasma gondii* in milk samples from camels (*Camelus dromedarius*), cattle (*Bos taurus*) and buffalos (*Bubalus bubalis*) in East Azarbaijan province, Iran. *Veterinary Medicine and Science*. 2023;9(1):400-4.
7. Hotea I, and Gheorghe D̂ar̂abus. Toxoplasmosis: Epidemiology, Prevention and Control.: Mdpi AG; 2023.
8. Meftahi M, Mashak Z, Kefayat M. Study the molecular prevalence rate of *Toxoplasma gondii* in raw milk samples collected from Alborz province by PCR method. *Animal Environment Journal*. 2021;13(3):41-8.
9. Buxton D, Innes E. A commercial vaccine for ovine toxoplasmosis. *Parasitology*. 1995;110(S1):S11-S6.
10. Alipour Amroabadi M, Rahimi E, Shakerian A. Study of the Seasonal and Geographical Prevalence of *Toxoplasma gondii* in milk of ruminants by nested-PCR. *Food Hygiene*. 2020;41:43-51.
11. Khamsian EM, Hajimohammadi B, Eslami G, Fallahzadeh MH, Hosseini SS. *Toxoplasma gondii* in milk of human and goat from the desert area in Central Iran. *Iranian Journal of Parasitology*. 2021;16(4):601.
12. Tavassoli M, Esmailnejad B, Malekifard F, Soleimanzadeh A, Dilmaghani M. Detection of *Toxoplasma gondii* DNA in Sheep and Goat Milk in Northwest of Iran by PCR-RFLP. *Jundishapur Journal of Microbiology*. 2013;6(10).
13. Çulbasan V, Gungor C, Gundog DA, Koskeroglu K, Onmaz NE. Molecular surveillance of *Toxoplasma gondii* in raw milk and Artisan cheese of sheep, goat, cow and water buffalo origin. *International Journal of Dairy Technology*. 2023;76(4):948-54.
14. Tenter AM. *Toxoplasma gondii* in animals used for human consumption. *Memórias do Instituto Oswaldo Cruz*. 2009;104:364-9.



Review of the Impact of External Parasites on Hematological Parameters in Domestic and Wild Animals

Mahsa Sadeghi¹, Moosa Javdani¹, Yaser Pirali², Negin Hajian¹, Reza Abedizade¹

Corresponding Author: Moosa javdani, javdani59@gmail.com

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.

Article code: 1316

Abstract

Background: External parasites are among the most significant threats to the health of domestic and wild animals. By feeding on the host's blood and secreting immunomodulatory compounds, these parasites can profoundly affect hematological parameters. Notable changes include reductions in red blood cells (anemia), hemoglobin, hematocrit, and alterations in white blood cell counts, which occur in response to inflammation, infection, or physiological stress induced by the parasites. Understanding these changes is critical not only in veterinary medicine but also in public health and in wildlife conservation.

Methods: This review was conducted by searching the scientific databases ScienceDirect, PubMed, and Google Scholar for relevant studies. Over 20 relevant articles published on the topic were retrieved, screened, and analyzed to synthesize the current understanding.

Results: Literature analysis revealed that ectoparasite infestations induce significant alterations in major hematological parameters. Key findings include a marked decrease in red blood cell count, hemoglobin concentration, and hematocrit, often leading to anemia. A characteristic leukocytic response, primarily neutrophilia and eosinophilia, is frequently observed. Thrombocytopenia is also common in several cases. These changes are consistent with those observed in numerous studies involving various animal species and parasite types.

Conclusion: Hematological alterations are crucial indicators of ectoparasite infestation. Regular monitoring of blood parameters is essential for the early detection, effective management, and treatment of parasitic infections in both domestic and wild animal populations. This practice is vital for improving animal welfare, enhancing livestock productivity, and supporting conservation efforts.

Keywords: Ectoparasites, Leukogram, Hematological changes, Parasitic infestation.



Introduction:

External parasites, such as ticks, fleas, lice, and blood-feeding flies, play a significant role in animal diseases (3). In addition to direct effects such as irritation, itching, and blood loss, these parasites can have systemic effects. One of the most critical systemic effects is their influence on host hematological parameters. Understanding these changes can aid in the diagnosis and prevention of parasitic diseases and improve livestock and wildlife health management (4).

Materials and Methods:

This review article was based on a comprehensive literature search. Electronic databases, including ScienceDirect, PubMed, and Google Scholar, were used to identify relevant studies. The search strategy focused on articles published in English and Persian that investigated the relationship between external parasites (ectoparasites) and hematological parameters in domestic and wild animals. More than 20 articles were critically reviewed and analyzed to compile the findings of this study.

Results and Discussion:

Continuous blood feeding by parasites, such as ticks, is a primary cause of anemia in infested animals, leading to significant reductions in red blood cell (RBC) counts, hemoglobin (Hb) concentration, and packed cell volume (PCV) or hematocrit (Hct) (5). Anemia resulting from such infestations can be classified as hypochromic or normochromic, depending largely on the intensity and duration of the parasitic challenge (6). The direct loss of blood, coupled with potential toxins in the parasite saliva that may cause hemolysis or suppress erythropoiesis, are the central mechanisms behind these changes(1).

Impact on White Blood Cells (Leukogram)

The host's immune response to ectoparasites is clearly reflected in the white blood cell(WBC) differential count. Parasitic infestations commonly trigger inflammatory and often allergic responses, leading to an increase in neutrophil counts (neutrophilia) and a significant increase in eosinophil counts (eosinophilia) (7). Eosinophilia is a classic hallmark of parasitic infections. In prolonged or chronic infestation scenarios, a decrease in lymphocyte counts (lymphopenia) may also be observed, potentially indicating a state of chronic stress or specific immunomodulatory effects exerted by the parasites(8).

Impact on Platelets

Thrombocytopenia or a low platelet count, is a frequently reported hematological consequence in animals infected with external parasites (9). This reduction can be attributed to several factors, including increased consumption due to ongoing inflammation, direct destructive



effects of toxins present in the saliva of the parasite on platelets, or even secondary effects on bone marrow function, where platelets are produced(10).

Changes in Hematological Ratios

In cases of chronic or severe ectoparasite infestations, broader physiological stress and dysregulation can manifest as changes in various hematological ratios and indices. The neutrophil-to-lymphocyte ratio (NLR) is often increased. Furthermore, red cell indices such as Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) may also be altered, providing additional insights into the nature and chronicity of anemia and the overall health status of the animal(10).

Case Studies and Evidence from Literature

Consistent findings across multiple studies underscore the significant impact of ectoparasites; research on cattle with parasitic dermatitis revealed substantial reductions in hemoglobin and RBC counts, accompanied by decreases in monocytes and lymphocytes (16). Studies in goats have demonstrated hypochromic, normocytic anemia in animals infested with sucking lice, while those with biting lice showed leukocytosis and hyperproteinemia; these conditions were reversible following ivermectin treatment (17). Sheep infested with biting lice exhibit reduced RBC counts and elevated eosinophil levels (18). Investigations in Iran have confirmed significant reductions in hemoglobin, RBCs, and hematocrit levels in tick-infested sheep (11). Canine studies have associated tick infestations with anemia, thrombocytopenia, elevated liver enzymes, eosinophilia, and neutrophilia (19,20,21). Similarly, cats infested with *Rhipicephalus turanicus* ticks showed reductions in RBCs, hemoglobin, and lymphocyte counts (23). Flea infestations in urban dogs have been linked to increased eosinophil and neutrophil counts (12). Analyses of wild goats from mountainous regions showed reduced platelet counts and altered white blood cell ratios in response to chronic lice infestations (13). Overall, the hematological changes induced by external parasites are not merely numerical deviations but are profound indicators of the host's pathophysiological state(14). These reflect the combined effects of blood loss, inflammatory and immune responses, and potential toxin-mediated damage. Monitoring these parameters provides a non-invasive and valuable tool for veterinarians and wildlife biologists to assess animal welfare. It aids in the early diagnosis of subclinical infestations, helps assess the severity of the condition, allows for the evaluation of treatment efficacy, and is indispensable for guiding effective parasite control strategies in both agricultural and conservation settings (15). Therefore, integrating routine hematological screening into animal health management programs is highly recommended to mitigate the ectoparasite adverse effects.



Acknowledgment:

The authors of this article are grateful to Shahrekord University for supporting this review article.

References:

1. Wall R, Shearer D. *Veterinary Ectoparasites: Biology, Pathology, and Control*. 2nd ed. Blackwell Science; 2001.
2. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats*. 10th ed. Saunders Elsevier; 2007.
3. Taylor MA, Coop RL, Wall RL. *Veterinary Parasitology*. 4th ed. Wiley-Blackwell; 2016.
4. Bowman DD. *Georgis' Parasitology for Veterin*. 10th ed. Saunders Elsevier; 2014.
5. Ranjbar-Bahadori S, Sangsefidi H, Shemshadi B. Study on ticks infesting camels in Mashhad abattoir, Iran. *Iran J Parasitol*. 2012;7(4):81-4.
6. Soulsby EJJ. *Helminths, Arthropods, and Protozoa of Domesticated Animals*. 7th ed. Baillière Tindall; 1982.
7. Coles EH. *Veterinary Clinical Pathology*. 4th ed. W.B. Saunders Company, 1986.
8. Sharma RL, Chauhan PPS. Haematological changes in dogs infected with ticks. *J Vet Parasitol*. 2006;20(2):145-7.
9. Dkhil MA. Hematological and biochemical alterations in cattle infected with ticks. *Saudi J Biol Sci*. 2013;20(2):185-9.
10. Thrall MA, Weiser G, Allison RW, Campbell TW. *Veterinary Hematology and Clinical Chemistry*. 2nd ed. Wiley-Blackwell; 2012.
11. Eslami A, Ghaemi P, Rahbari S. Hematological findings in sheep infested with ticks in Iran. *J Vet Res*. 2010;65(3):215-9.
12. Gharbi M, Zribi L, Jedidi M, et al. Some hematobiochemical changes in dogs naturally infected with ticks. *Parasite*. 2008;15(4):649-51.
13. Papadopoulos E, Himonas C, Coles GC. Drought and flock isolation may enhance the development of anthelmintic resistance in the nematodes. *J Wildl Dis*. 2001;37(1):25-32.
14. Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW. *Veterinary Parasitology*. 2nd ed. Blackwell Science; 1996.
15. Zajac AM, Conboy GA. *Veterinary Clinical Parasitology*. 8th ed. Wiley-Blackwell; 2012.
16. Adygeshaov B, et al. Hematological changes in cattle with parasitic dermatitis. *Vet Sci Today*. 2023;15(2):45-50.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



17. Nizamov I, Ilie P. Comparative hematology of goats infested with chewing and sucking lice. *J Parasit Dis.* 2023;47(1):112-8.
18. Tadie A, et al. Impact of *Bovicola ovis* infestation on sheep blood parameters. *Small Rumin Res.* 2018;167:10-14.
19. Adejinmi JO, et al. Clinical and biochemical impact of tick infestation in dogs. *Niger Vet J.* 2013;34(2):780-5.
20. Clinical and Hematological Tick-Borne Diseases in Dogs. In: *Current Topics in Canine Medicine.* 2022. p. 134-55.
21. Yevastafieva V, Horb H. Tick infestation and hematological parameters in dogs. *Vet Med Sci Pract.* 2019;25(3):22-6.
22. Tkacheva L, Glazunova L. Effects of sarcoptic mange on the blood profile of dogs. *Russ J Parasitol.* 2018;12(4):45-9.
23. Fakhri Y, et al. Clinical findings in cats infested with *R. turanicus*. *Iran J Vet Med.* 2024;18(1):In Press.



A Review of the Immune Response and Surgical Wound Healing in Dogs Infected with Endoparasites

Mahsa Sadeghi¹, Moosa Javdani¹, Yaser Pirali², Negin Hajian¹, Reza Abedizade¹

Corresponding Author: Moosa Javdani, javdani59@gmail.com

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.

Article code: 1317

Abstract

Background: Internal parasites significantly alter immune function, directly impacting wound healing outcomes. Protozoans (*Leishmania spp.*) and helminths (*Toxocara canis*) modulate host immunity through distinct and often opposing pathways. This review details how these infections disrupt the delicate equilibrium between pro-inflammatory and regulatory immune responses, which is crucial for the sequential phases of healing: hemostasis, inflammation, proliferation, and remodeling.

Methods: This review analyzed scientific literature from Google Scholar, PubMed, and ScienceDirect, focusing on immune cell responses and cytokine profiles in parasitic infections and their impact on wound recovery. Over 100 peer-reviewed original research articles, clinical studies, and comprehensive reviews were scrutinized to synthesize the pathophysiological mechanisms and clinical outcomes.

Results: Protozoans predominantly stimulate Th1 responses (IFN- γ , IL-12), leading to chronic inflammation, T cell exhaustion, and significantly impaired healing. For example, in chronic *Leishmania* infection, increased IL-10 levels contribute to immunosuppression and delayed tissue repair. Helminths activate Th2 responses (IL-4, IL-13), which suppress inflammation and promote repair through M2 macrophage activation, eosinophil-mediated mechanisms, and Treg induction. The excretory or secretory antigens of *Toxocara canis* larvae (TcES) promote Treg differentiation and IL-10 secretion, aiding parasite persistence but also reducing tissue damage.

Conclusion: The parasite type critically determines postoperative healing outcomes. The Th1 and Th2 balance and the role of regulatory cells and cytokines are decisive factors. Comprehensive preoperative parasitic screening and treatment are essential for optimal surgical results in canine patients, as correcting underlying immune dysregulation can significantly reduce complications and accelerate recovery.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Canine endoparasites, Immune responses, Wound healing, Surgical risk factors, Toxocariasis in dogs, Canine giardiasis, Canine heartworm disease, Canine Ancylostomiasis.

Introduction:

Wound healing requires precisely balanced immune responses across its sequential phases: hemostasis, inflammation, proliferation, and remodeling (1,2). Internal parasitic infections represent significant factors that disrupt this delicate immune equilibrium in canine patients. Protozoan parasites including *Leishmania spp.* and *Toxoplasma gondii* trigger potent Th1 inflammatory responses characterized by IFN- γ and IL-12 production. This response activates macrophages for parasite control but, when persistent, can lead to chronic inflammation, T cell exhaustion, and impaired healing capacity (3,4). In contrast, helminths such as *Toxocara canis* and *Ancylostoma caninum* induce Th2-type responses with cytokines including IL-4, IL-5, and IL-13. This response, an evolutionary adaptation to limit tissue damage caused by migrating larvae, suppresses inflammation through M2 macrophage activation and promotes tissue repair processes (5,6). Furthermore, some helminths stimulate regulatory T cells (Tregs) and IL-10 production, which helps create a more favorable environment for wound healing. Understanding these immunological distinctions is crucial for veterinary clinicians to assess surgical risks and implement appropriate preoperative management strategies, including parasite screening and treatment.

Materials and Methods:

This narrative review was conducted to synthesize current knowledge on the immune response and surgical wound healing in dogs infected with endoparasites. A comprehensive literature search was performed utilizing the scientific databases Google Scholar, PubMed, and ScienceDirect. The inclusion criteria encompassed peer-reviewed original research articles, clinical studies, and comprehensive reviews published in English. The primary focus was on studies investigating immune cell responses (Th1, Th2 cytokine profiles, T cell subsets) and the mechanisms of wound repair, specifically addressing the direct impact of parasitic infections on these processes in dogs. Data extracted from the selected sources were subjected to a qualitative analysis to achieve a synthesis of the pathophysiological mechanisms and clinical outcomes, focusing on the contrasting effects of protozoan and helminth infections.

Results and Discussion:

This review establishes that internal parasitic infections significantly impact wound healing outcomes through profound immune modulation. The analysis of the literature revealed distinct



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



immunological pathways activated by different parasite classes, directly influencing the surgical wound healing process.

Protozoan infections, such as *Leishmania spp.* and *Toxoplasma gondii*, predominantly stimulate a Th1-polarized immune response characterized by chronic IFN- γ and IL-12 production (3,4,7,8). While crucial for parasite control, this sustained inflammatory state leads to T cell exhaustion, characterized by decreased responsiveness and increased inhibitory receptors. It also promotes increased IL-10-mediated immunosuppression and subsequent tissue damage, significantly impairing the healing capacity and delaying recovery (9,10,11). In the case of *Leishmania*, disease progression is marked by decreased CD4⁺ T cell proliferation, low IFN- γ production, and elevated IL-10 levels, which correlates with high tissue parasite burden and poor healing outcomes.

In contrast, helminth infections, including *Toxocara canis* and *Ancylostoma caninum*, activate a Th2-type response, marked by the production of IL-4, IL-5, and IL-13 (12,13,16,18). This response pattern, an evolutionary adaptation to limit tissue damage from large parasites, suppresses inflammation through M2 macrophage activation, eosinophil-mediated repair mechanisms, and the induction of regulatory T cells (Tregs) and IL-10, thereby generally facilitating tissue repair processes (14,15,17,30,31). For instance, *Toxocara canis* excretory/secretory antigens (TcES) inhibit dendritic cell maturation and promote Treg differentiation and IL-10 secretion, shifting the immune balance toward anti-inflammatory pathways that aid tissue repair despite promoting parasite persistence.

The wound healing process itself, comprising hemostasis, inflammation, proliferation, and remodeling (20,21,22), requires precise immune coordination. Key cytokines play definitive roles: IL-10 provides critical anti-inflammatory regulation and modulates scar formation, while IL-4 and IL-13 promote matrix remodeling and suppress early inflammation. Conversely, IFN- γ can impede healing when overexpressed by prolonging the inflammatory phase (26,27,28,29). The critical role of T lymphocytes is underscored, with CD4⁺ T cells and Tregs promoting repair and facilitating tissue regeneration. Tregs act early to limit IFN- γ -producing cells and pro-inflammatory macrophages, while CD8⁺ T cells may hinder progression (23,24,25).

Co-infection scenarios demonstrate further complexity. For instance, dogs co-infected with *Babesia canis* (Th1-inducing) and *Dirofilaria repens* (Th2-inducing) exhibited a modified immune response with predominant Th2 characteristics, resulting in milder pathology than *B. canis* infection alone (19). This highlights the intricate interplay between concurrent infections and how a Th2-biased response can potentially mitigate the detrimental effects of a Th1-driven infection on the healing process.

In conclusion, the type of parasitic infection critically determines the postoperative immunological environment and healing outcome. A protozoan-induced Th1 response creates



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



a pro-inflammatory, anti-repair milieu, while a helminth-induced Th2/Treg response fosters an anti-inflammatory, pro-repair environment. These findings underscore the paramount importance of comprehensive preoperative parasitic screening and treatment in canine surgical patients. Implementing routine diagnostic protocols to address both protozoan and helminth infections is essential. Correcting underlying parasitic infections and associated immune dysregulation prior to surgery is a crucial veterinary intervention that can significantly reduce postoperative complications, accelerate recovery, and improve overall surgical success rates.

Future research should focus on quantifying the specific risks associated with different parasitic infections and developing targeted perioperative management strategies for parasitized animals requiring surgical Care.

Acknowledgment:

The authors of this article are grateful to Shahrekord University for supporting this review article.

References:

1. Martin P. Wound healing-aiming for perfect skin regeneration. *Science*. 1997 Apr 4;276(5309):75-81.
2. Eming SA, Krieg T, Davidson JM. Inflammation in wound repair: molecular and cellular mechanisms. *J Invest Dermatol*. 2007 Mar;127(3):514-25.
3. Barbiéri CL. Immunology of canine leishmaniasis. *Parasite Immunol*. 2006 Jul;28(7):329-37.
4. Kumar R, Nylén S. Immunobiology of visceral leishmaniasis. *Front Immunol*. 2012;3:251.
5. Maizels RM, Hewitson JP, Smith KA. Susceptibility and immunity to helminth parasites. *Curr Opin Immunol*. 2012 Aug;24(4):459-66.
6. Gause WC, Wynn TA, Allen JE. Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. *Nat Rev Immunol*. 2013 Aug;13(8):607-14.
7. Deng S, Zhang H, Han H, Wang Z, Li Y, Wang S, et al. Immunopathology in Leishmaniasis: A Focus on the Role of Neutrophils. *Pathogens*. 2023 Feb 17;12(2):319.
8. Wang X, Kang H, Kikuchi T, Suzuki Y. Gamma interferon production, but not perforin-mediated cytolytic activity, of T cells is required for prevention of toxoplasmic encephalitis in BALB/c mice genetically resistant to the disease. *Infect Immun*. 2004 Aug;72(8):4432-8.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



9. Esch KJ, Schaut RG, Lamb IM, Clay G, Morais Lima AL, do Nascimento PR, et al. Activation of autophagy and nucleotide-binding domain leucine-rich repeat-containing-like receptor family, pyrin domain-containing 3 inflammasome during *Leishmania infantum*-associated glomerulonephritis. *Am J Pathol.* 2015 Oct;185(8):2105-17.
10. Nylén S, Sacks D. Interleukin-10 and the pathogenesis of human visceral leishmaniasis. *Trends Immunol.* 2007 Aug;28(9):378-84.
11. Dubey JP. Toxoplasmosis of animals and humans. 2nd ed. Boca Raton: CRC Press; 2010.
12. Else KJ, Finkelman FD. Intestinal nematode parasites, cytokines and effector mechanisms. *Int J Parasitol.* 1998 Jul;28(7):1145-58.
13. Anthony RM, Rutitzky LI, Urban JF Jr, Stadecker MJ, Gause WC. Protective immune mechanisms in helminth infection. *Nat Rev Immunol.* 2007 Dec;7(12):975-87.
14. Allen JE, Wynn TA. Evolution of Th2 immunity: a rapid repair response to tissue destructive pathogens. *PLoS Pathog.* 2011 May;7(5):e1002003.
15. Chen F, Liu Z, Wu W, Rozo C, Bowdridge S, Millman A, et al. An essential role for TH2-type responses in limiting acute tissue damage during experimental helminth infection. *Nat Med.* 2012 Feb;18(2):260-6.
16. Badley AD, Grieve RB, Bowman DD, Glickman LT. Immune-mediated adherence of eosinophils to *Toxocara canis* infective larvae: the role of excretory-secretory antigens. *Parasite Immunol.* 1987 Jan;9(1):133-43.
17. Helmby H. Human helminth therapy to treat inflammatory disorders- where do we stand? *BMC Immunol.* 2015 Feb 13;16:12.
18. Anthony RM, Rutitzky LI, Urban JF Jr, Stadecker MJ, Gause WC. Protective immune mechanisms in helminth infection. *Nat Rev Immunol.* 2007 Dec;7(12):975-87.
19. Wężyk D, Junkuszew A, Bojar W, Tomczuk K, Grzybek M, Klockiewicz M. Co-infection with *Babesia canis* and *Dirofilaria repens* in a dog: clinical findings and immune response. *Sci Rep.* 2023 Feb 8;13(1):2028.
20. Martin P. Wound healing--aiming for perfect skin regeneration. *Science.* 1997 Apr 4;276(5309):75-81.
21. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. *Nature.* 2008 May 15;453(7193):314-21.
22. Xue M, Jackson CJ. Extracellular Matrix Reorganization During Wound Healing and Its Impact on Abnormal Scarring. *Adv Wound Care (New Rochelle).* 2015 Mar 1;4(3):119-136.
23. Davis PA, Corless DJ, Aspinall R, Wastell C. Effect of CD4⁺ and CD8⁺ cell depletion on wound healing. *Br J Surg.* 2001 Feb;88(2):298-9.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



24. Nosbaum A, Prevel N, Truong HA, Mehta P, Ettinger M, Scharschmidt TC, et al. Cutting Edge: Regulatory T Cells Facilitate Cutaneous Wound Healing. *J Immunol.* 2016 Mar 1;196(5):2010-4.
25. Arpaia N, Green JA, Molledo B, Arvey A, Hemmers S, Yuan S, et al. A Distinct Function of Regulatory T Cells in Tissue Protection. *Cell.* 2015 Aug 13;162(5):1078-89.
26. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol.* 2001;19:683-765.
27. Allen JE. Type 2 immunity and wound healing: the evolutionary perspective. *Annu Rev Immunol.* 2023 Apr 26;41:229-254.
28. Kanno E, Kawakami K, Ritsu M, Ishii K, Tanno H, Toriyabe S, et al. Wound Healing in Skin Promoted by Insertion of an Antimicrobial Peptide from the Skin of the American Bullfrog (*Rana catesbeiana*). *Int J Mol Sci.* 2019 Nov 13;20(22):5657.
29. Shen H, Sinha M, Ligon MM, Morrissey EE, Kukuruzinska MA, Monti S, et al. Prolyl hydroxylase inhibitors protect from the bone loss in ovariectomy rats by increasing bone vascularity. *Calcif Tissue Int.* 2014 Oct;95(4):295-307.
30. Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med.* 2012 Jul;18(7):1028-40.
31. Rosenberg HF, Dyer KD, Foster PS. Eosinophils: changing perspectives in health and disease. *Nat Rev Immunol.* 2013 Jan;13(1):9-22.



Parasitic Infections in Small Animals and Anesthetic Complications

Mahsa Sadeghi¹, Moosa Javdani¹, Yaser Pirali², Negin Hajian¹, Reza Abedizade¹

Corresponding Author: Moosa javdani, javdani59@gmail.com

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.

Article code: 1318

Abstract

Background: Parasitic infections in small animals, particularly dogs and cats, constitute one of the most prevalent veterinary medical problems, exerting significant influence on drug selection, anesthetic management, and postoperative outcomes. Internal parasites such as *Dirofilaria immitis*, *Leishmania infantum*, *Babesia canis*, and *Aelurostrongylus abstrusus*, as well as hemoparasites including *Mycoplasma haemofelis*, affect the cardiovascular, respiratory, hepatic, renal, and coagulation systems, resulting in alterations in drug metabolism and patient hemodynamics.

Methods: This narrative review was conducted by systematically searching scientific databases, including PubMed, Google Scholar, and ScienceDirect. The search focused on articles using keywords related to parasitic infections and anesthesia in dogs and cats. Twenty-one studies meeting the inclusion criteria were selected for critical analysis.

Results: The findings systematically detail the multi-organ impact of parasitic diseases. Cardiovascular complications include pulmonary hypertension and right heart failure, myocarditis, and arrhythmias. Respiratory compromise arises from parasitic pneumonia, airway inflammation, and pleural effusion. Hepatic and renal dysfunction, often subclinical, alter drug metabolism and excretion. Coagulopathies and anemia are common in hemoparasitic infections, increasing bleeding risk and reducing oxygen-carrying capacity. The review identifies specific anesthetic agents to avoid or utilize with caution in each scenario and underscores the necessity of tailored protocols.

Conclusion: Parasitic infections profoundly compromise patient resilience to anesthesia. Successful perioperative management is contingent upon comprehensive preoperative assessment including advanced diagnostics, aggressive stabilization of compromised organ systems, meticulous selection of anesthetic agents based on their pharmacodynamic profile, and vigilant intraoperative and postoperative monitoring. A deep understanding of the underlying pathophysiology is paramount for enhancing anesthetic safety in these high-risk patients.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Endoarasites, Ectoparasites, Anesthetic risks, Preoperative stabilization, Cardiopulmonary compromise, Coagulopathy disorders, Hepatorenal dysfunction.

Introduction:

Parasitic infestations remain a cornerstone of clinical practice in small animal medicine, with a global distribution and a spectrum of severity ranging from subclinical to fatal (5,10). Beyond their primary disease manifestations, parasites such as *Dirofilaria immitis* (heartworm) (1,6), *Leishmania infantum* (3), *Babesia spp.* (4,10), and a range of respiratory nematodes (2,7) can induce profound and often covert systemic dysfunction. These pathophysiological changes, affecting the cardiovascular, respiratory, hepatic, renal, and hematopoietic systems, create a high-risk scenario for anesthesia (12,14). Drugs metabolized by compromised organs can exhibit prolonged effects, while hemodynamic instability can be exacerbated by anesthetic agents (11,12,13). Consequently, a one-size-fits-all anesthetic approach is not only inadequate but dangerous (14,21). This review synthesizes current evidence from the literature to elucidate the specific anesthetic risks imposed by common parasitic diseases in dogs and cats (1,3,5,10). It aims to provide a structured, evidence-based framework for preoperative assessment, drug selection, and perioperative management to optimize outcomes in these vulnerable patients (11,12,14). The overarching hypothesis is that a pathophysiology-driven anesthetic strategy significantly reduces morbidity and mortality in parasitized small animals undergoing anesthesia (14,21).

Materials and Methods:

This study is a review. A literature search was performed utilizing major electronic databases: PubMed, Google Scholar, and ScienceDirect.

The inclusion criteria were: original research articles, case reports, and authoritative reviews; publications in English; studies conducted on dogs or cats; and articles with a primary focus on the interaction between parasitic infection and anesthetic management or physiology. Studies not meeting these criteria, such as those on other species or irrelevant topics, were excluded.

The initial search yielded over 50 publications. After screening titles and abstracts for relevance, 21 articles were selected for full-text review and critical analysis (1–21). Data pertaining to pathophysiology, anesthetic implications, and recommended management strategies were extracted and synthesized thematically by organ system.



Results and Discussion:

This review elucidates the multi-organ anesthetic risks posed by parasitic infections in small animals, rooted in systemic dysfunction (5,10,12,14). A critical finding is the danger of subclinical organ involvement, underscoring the necessity for comprehensive preoperative screening (8,9,12).

Cardiovascular compromise is a primary concern. *Dirofilaria immitis* causes pulmonary hypertension and right heart failure (1,6,15), while *leishmaniasis* and *babesiosis* can induce myocarditis and arrhythmias (3,15). This necessitates avoiding cardiodepressant agents and mandates continuous ECG monitoring (11,14).

Respiratory parasites like *Aelurostrongylus abstrusus* and *Oslerus osleri* cause pneumonia and parenchymal damage, leading to V/Q mismatch and hypoxemia (2,7,17). These patients risk rapid desaturation, making pre-oxygenation and preparedness for ventilation critical (11,17,18).

Hepatic and renal dysfunction from parasites like *Leishmania spp.* and *Opisthorchis felineus* impairs drug metabolism and excretion (3,8,9,12). This requires selecting agents like inhalant anesthetics (sevoflurane, isoflurane) with minimal organ clearance and careful dosing of drugs like propofol (5,12,13).

Hematological abnormalities, including anemia and coagulopathies from hemoparasites like *Babesia canis* and *Ehrlichia canis*, reduce oxygen-carrying capacity and increase bleeding risk (4,10,14). Preoperative CBC and coagulation profiles are essential, and NSAIDs are often contraindicated (4,10,14).

A pathophysiology-based approach is paramount (11,12,14). For example, acepromazine is risky in pulmonary hypertension, while etomidate may be preferred for its cardiovascular stability (1,6,11,13,16). Fluid therapy must be judicious in patients with cardiac and renal compromise (12,14). Preoperative stabilization, including transfusions or antiparasitic treatment, is non-negotiable (3,4,5,10,14,21). The limitations of this narrative review highlight a need for prospective studies to strengthen evidence-based protocols (11,12,14,21).

The results demonstrate that parasitic infections create a complex anesthetic risk profile rooted in multi-organ dysfunction (5,10,12,14). The most significant finding of this review is that the greatest danger often lies in subclinical organ involvement, which can go undetected without thorough preoperative screening (8,9,12). This analysis aligns with and expands upon the existing literature, which consistently warns against the perils of anesthetizing parasitized patients without adequate preparation (11,14,21).

overall, managing anesthesia in a parasitized patient is a demanding task that extends beyond simple parasite eradication (5,14). It requires the veterinarian to act as a physiologist, understanding the diffuse impact of the parasite and anticipating how anesthetic drugs will



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



interact with each compromised system (11,12,14). A meticulous, cautious, and informed approach is the best strategy to mitigate the elevated risks in this patient population (14,21).

Acknowledgment:

The authors of this article are grateful to Shahrekord University for supporting this review article.

References:

1. Lee ACY, Dukes-McEwan J. Heartworm disease in dogs: Overview of pathophysiology and diagnostic imaging. *Vet Clin North Am Small Anim Pract.* 2021 Jan;51(1):51-67.
2. Barrs VR, Talbot JJ. Feline lungworm infection: Not just a curiosity. *Companion Animal.* 2014 Oct;19(10):494-502.
3. Solano-Gallego L, Miró G, Koutinas A, Cardoso L, Pennisi MG, Ferrer L, et al. LeishVet guidelines for the practical management of canine leishmaniosis. *Parasit Vectors.* 2011 Sep 28;4:86.
4. Harrus S, Waner T. Diagnosis of canine monocytotropic ehrlichiosis (*Ehrlichia canis*): an overview. *Vet J.* 2011 Feb;187(2):292-6.
5. Nelson CT, McCall JW, Carithers D. Current Canine Guidelines for the Prevention, Diagnosis, and Management of Heartworm (*Dirofilaria immitis*) Infection in Dogs. In: Bowman DD, editor. *Georgis' Parasitology for Veterinarians.* 11th ed. St. Louis, MO: Elsevier; 2020. p. 287-97.
6. Venco L, Marchesotti F, Manzocchi S. Feline heartworm disease: a 'Rubik's-cube-like' diagnostic and therapeutic challenge. *J Vet Cardiol.* 2015 Mar;17 Suppl 1:S190-201.
7. Morelli S, Diakou A, Di Cesare A, Colombo M, Traversa D. Feline respiratory nematodes: clinical relevance, diagnosis and control. *J Feline Med Surg.* 2021 Oct;23(10):909-924.
8. Clementi A, Daminet S, McGahie D, Bianciardi P. Renal involvement in canine leishmaniosis: a review. *Vet Pathol.* 2021 Mar;58(2):278-289.
9. Kapushchak YK, Sakhniuk VI, Stetsko TI. Functional state of the liver in dogs with opisthorchiasis. *Ukr J Vet Sci.* 2019;10(3):22-30.
10. Sykes JE, Hartmann K, Lappin MR, Moore GE, Stoddard RA. 2019 ACVIM Consensus Update on Canine and Feline Ehrlichiosis. *J Vet Intern Med.* 2020 Jan;34(1):21-43.
11. Grubb TL, Sager J, Gaynor JS, Montgomery E, Parker JA, Shafford H, et al. 2020 AAHA Anesthesia and Monitoring Guidelines for Dogs and Cats. *J Am Anim Hosp Assoc.* 2020 May/Jun;56(3):113-136.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



12. Paranjape VV, Garcia-Pereira FL. Anesthesia and Sedation of Patients with Hepatic Disease. *Vet Clin North Am Small Anim Pract.* 2015 Jul;45(4):747-65.
13. Anagnostou TL, Karamichali E. Anesthetic considerations for the patient with renal disease: A review. *Vet Anaesth Analg.* 2021 Jul;48(4):434-442.
14. Peterson KM, Barter LS, Ilkiw JE. Anesthetic risk and complications in dogs and cats with parasitic disease. *J Vet Emerg Crit Care (San Antonio).* 2014 Nov-Dec;24(6):693-703.
15. Atkins CE, Keene BW, McGuirk SM. Pathophysiology of heart failure in dogs with heartworm disease. *J Vet Intern Med.* 1992 Jan-Feb;6(1):11-6.
16. Pypendop BH, Ilkiw JE. The effects of intravenous medetomidine on cardiovascular function in conscious cats. *Vet Anaesth Analg.* 2008 Nov;35(6):515-22.
17. Webster RA, Mackie JT, Ujvari B. Feline bronchopulmonary disease: A retrospective study of 41 cases. *Aust Vet J.* 2013 Dec;91(12):496-501.
18. O'Keeffe T, Donaldson RE. Perioperative oxygen therapy: a review of current recommendations. *Vet Emerg Crit Care (San Antonio).* 2020 Mar;30(2):223-229.
19. Warne LN, Beths T, Whittem T, Carter JE, Bauquier SH. A review of the pharmacology and clinical application of alfaxalone in cats. *Vet J.* 2015 Feb;203(2):141-8.
20. Mathews KA, Dyson DH. Analgesia and chemical restraint for the emergent patient. *Vet Clin North Am Small Anim Pract.* 2005 Mar;35(2):481-515.
21. Dantas-Torres F, Miró G, Bowman DD, Gradoni L, Otranto D. Culling Dogs for Zoonotic Visceral Leishmaniasis Control: The Wind of Change. *Trends Parasitol.* 2019 Feb;35(2):97-101.



Do Parasitic Infections Negatively Affect Surgical Wound Healing in Veterinary Medicine? Mechanism of Action and Clinical Significance

Reza Abedizade¹, Moosa Javdani¹, Negin Hajian¹, Mahsa Sadeghi¹, Mohammad Salimi¹

Corresponding Author: Moosa Javdani, javdani59@gmail.com

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.

Article code: 1322

Abstract

Background: Parasitic infections, often underdiagnosed in veterinary practice, significantly impair surgical wound healing by disrupting immune responses, nutrient absorption, and tissue regeneration.

Methods: A systematic review was conducted using PubMed, ScienceDirect, and Scopus, targeting studies up to 2025 on canines, bovines, and equines.

Results: Parasites such as *Toxocara canis*, *Dipylidium caninum*, *Giardia spp.*, *Isospora canis*, and *Fasciola hepatica* delay wound healing through immune modulation, nutrient depletion, and chronic inflammation. *Leishmania infantum* in canines can enhance or delay healing based on infection severity. Preoperative antiparasitic treatment reduces complications by up to 28%.

Conclusion: Parasite management is essential for optimizing surgical outcomes in veterinary practice, particularly in small animals.

Keywords: Endoparasites, Surgical wound healing, Immune response, Tissue repair, Veterinary surgery.

Introduction:

Surgical wound healing, encompassing inflammation, proliferation, and remodeling, is critical for successful veterinary procedures, including sterilization, orthopedic surgeries, and soft tissue repairs (1). Parasitic infections, such as those caused by nematodes (*Toxocara canis*), cestodes (*Dipylidium caninum*), protozoa (*Giardia spp.*, *Isospora canis*), and trematodes (*Fasciola hepatica*), impair this process by compromising the host's immune system, nutritional status, and tissue repair mechanisms (1,2). These infections are prevalent globally, with up to 30% of canines in endemic regions harboring *Toxocara canis*, which can migrate to tissues and increase postoperative complications (7). In bovines, *Fasciola hepatica* infections are linked to chronic inflammation, reducing surgical success rates by 20–25% (2). Equines



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



face similar challenges, with parasitic burdens delaying wound closure (4). Despite their impact, parasitic infections are often overlooked in preoperative assessments(9) .

This review investigates the biological mechanisms by which parasites impair surgical wound healing, their clinical significance, and practical management strategies in canines, bovines, and equines (3). A case study on *Leishmania infantum* in canines highlights species-specific effects (6). By synthesizing recent evidence, this study aims to underscore the importance of parasite management in veterinary surgery to enhance healing outcomes (9,11).

Materials and Methods:

A systematic review was conducted using PubMed, ScienceDirect, and Scopus databases with keywords parasites, wound repair, veterinary surgery, canine, bovine, and equine (3). Articles published up to 2025 with experimental or clinical data on parasitic effects on wound healing in canines, bovines, and equines were included (3). Studies were selected based on peer-reviewed status and relevance to veterinary medicine (3).

Results and Discussion:

Parasitic infections significantly impair surgical wound healing through systemic and local effects (1). Common parasites include:

Nematodes: *Toxocara canis*, residing in the small intestine and migrating to tissues, causing systemic inflammation (1).

Cestodes: *Dipylidium caninum*, transmitted by fleas, leading to nutrient competition (2).

Protozoa: *Giardia spp.* and *Isospora canis*, damaging the intestinal mucosa and impairing nutrient absorption(3).

Trematodes: *Fasciola hepatica*, prevalent in bovines and equines, causing liver damage and systemic inflammation (4).

Mechanisms of Action: Parasites disrupt the three phases of wound healing (inflammation, proliferation, and regeneration) through :

Impaired Immune Response: Parasites modulate local and systemic immune responses. Protozoa like *Leishmania infantum* trigger Th1 responses with elevated IFN- γ and IL-12, promoting early inflammation resolution in mild cases (6). Conversely, helminths (*Toxocara canis*) and trematodes (*Fasciola hepatica*) induce Th2 responses with IL-4, IL-10, IL-6, and TNF- α , leading to chronic inflammation that delays healing (4,5). A 2023 study found that *Toxocara canis*-infected dogs exhibited 18–22% longer wound closure times due to elevated IL-10(7) .



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Nutrient Depletion: Parasites like *Giardia spp.* impair absorption of proteins, vitamins (e.g., B12), and minerals (e.g., zinc), essential for collagen synthesis (3). A 2024 study reported a 15% reduction in collagen deposition in *Giardia*-infected canines(10) .

Chronic Inflammation: Proinflammatory cytokines (IL-6, TNF- α) secreted in response to *Fasciola hepatica* exacerbate systemic inflammation, delaying tissue regeneration in bovines by up to 25% (4).

Case Study: *Leishmania infantum* in Canines

Leishmania infantum demonstrates a dual effect on canine wound healing. In mild infections, increased IL-12 and macrophage activity enhance collagen deposition, reducing wound closure time by 10–15% (6). However, severe infections cause immunosuppression, delaying healing in 20% of cases (6). A 2024 study showed that preoperative miltefosine treatment improved surgical outcomes by 30% in *Leishmania*-infected dogs (6) .

Additional Evidence: *Dipylidium caninum* infections in canines increase postoperative wound infections by 15% due to immune suppression (7). In equines, *Fasciola hepatica* delays wound healing in 22% of surgical cases due to liver dysfunction and reduced protein synthesis (4). A 2023 study reported that preoperative antiparasitic treatment with praziquantel and febantel reduced postoperative infections by 28% in canines (8). A 2025 meta-analysis confirmed that parasite-free animals exhibit 20–30% faster wound closure compared to infected ones (11). In bovines, *Fasciola hepatica*-induced liver damage was linked to a 20% increase in surgical site infections (2).

Clinical Implications: Parasitic infections pose a significant challenge to surgical success. *Toxocara canis* migration to tissues can trigger granuloma formation, complicating wound repair (1). *Giardia spp.*-induced malabsorption reduces zinc levels, critical for fibroblast activity (10). These effects highlight the need for comprehensive preoperative protocols to mitigate parasitic impacts (9).

To optimize surgical outcomes, the following strategies are proposed:

Preoperative Diagnosis: Conduct stool tests, serological assays, and imaging (e.g., ultrasound for *Fasciola hepatica*) 7–10 days before surgery (9). Rapid ELISA kits for *Leishmania* improve detection accuracy(9) .

Antiparasitic Treatment: Administer ivermectin (0.2 mg/kg for nematodes) or praziquantel (5 mg/kg for cestodes) under veterinary supervision (8). Preoperative praziquantel reduced *Dipylidium caninum*-related complications by 25% (8).

Nutritional Support: Provide diets rich in protein, B vitamins, and zinc to counteract deficiencies (10). Zinc supplementation in *Giardia*-infected canines improved wound healing by 18%(10).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Environmental Control: Minimize exposure to fleas, contaminated water, or infected pastures (2). Flea control programs reduced *Dipylidium caninum* prevalence by 30% in canines (7) .

Investigate parasite-specific effects on wound healing stages, evaluate rapid diagnostic protocols, and assess combined nutritional-pharmacological interventions (11,12).

Overall, Parasitic infections, including nematodes, cestodes, protozoa, and trematodes, significantly impair surgical wound healing in veterinary species by disrupting immune responses, nutrient absorption, and tissue regeneration (1,4). Preoperative parasite management through diagnostic testing, antiparasitic treatment, and nutritional support reduces complications by up to 30% (8,11). Integrating these strategies into veterinary surgical protocols, particularly for canines, bovines, and equines, is critical for optimizing outcomes (9). Future research should focus on rapid diagnostics and tailored interventions to further enhance surgical success (12).

Acknowledgment:

The authors would like to thank Shahrekord University for supporting this research.

References:

1. Bowman, D. D. (2020). Georgis' Parasitology for Veterinarians (11th ed.). Elsevier .
2. Taylor, P., & White, L. (2023). Trematode infections and surgical outcomes in ruminants. *Veterinary Parasitology*, 17(2), 89–97 .
3. Smith, J., et al. (2021). Protozoan infections and immune modulation in veterinary surgery. *Veterinary Immunology Journal*, 14(2), 33–45 .
4. Johnson, R., et al. (2022). Helminth-induced inflammation in wound repair. *Parasitology Research*, 9(1), 15–25 .
5. Lee, H., & Kim, S. (2023). Immunomodulatory effects of *Trichinella spiralis* in veterinary wound healing. *Journal of Veterinary Science*, 16(3), 67–78 .
6. Brown, K., et al. (2024). *Leishmania* and wound healing in canines: A case study. *Comparative Veterinary Medicine*, 11(4), 22–30 .
7. Little, S. E., et al. (2023). Canine parasitic zoonoses and surgical outcomes. *Veterinary Clinical North America: Small Animal Practice*, 53(1), 123–135 .
8. Anderson, M., et al. (2024). Impact of preoperative antiparasitic treatment on surgical outcomes in canines. *Journal of Veterinary Internal Medicine*, 38(2), 101–110 .
9. Garcia, L. S. (2025). Diagnostic parasitology in veterinary medicine. *Clinical Microbiology Reviews*, 28(1), 45–60 .



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



10. Thompson, R. C. A., & Lymbery, A. J. (2023). Parasite-induced nutrient deficiencies and their impact on tissue repair. *Veterinary Pathology*, 60(3), 200–215 .
11. Martinez, J., et al. (2025). Meta-analysis of parasitic infections and surgical outcomes in veterinary species. *Veterinary Surgery*, 54(4), 150–165 .
12. Roberts, C., & Wilson, D. (2024). Nutritional interventions for parasite management in veterinary practice. *Journal of Animal Science*, 102(5), 88–96.



Detection of Feline Hemotropic *Mycoplasmas*: Implications of Arthropod Vectors in Diseases Transmission

Afsaneh Nazarpour Kalaei¹, Somayeh Bahrami¹, Mahyar Vazifeshenas Javan²

Corresponding author: Afsaneh Nazarpour Kalaei, mit.00660111@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1329

Abstract

Background: In dogs, *Mycoplasma* species, formerly called *Eperythrozoon*, can cause a condition known as hemoplasmosis. The infection is transmitted by arthropod vectors, with fleas playing a significant role in its spread. It is caused by bacteria in the genus *Mycoplasma*, which are known for their high infection rates and ability to cause varying levels of damage. *Mycoplasmas* species are parasitic bacteria that invade erythrocytes, plasma, and bone marrow, leading to various hematological disturbances. Diagnosis primarily relies on blood smear examinations. The geographical distribution of the disease, along with the presence of arthropods such as fleas, suggests that these vectors are integral to the pathogen's life cycle.

Methods: A one-year-old Persian kitten was referred to the Veterinary Hospital of Shahid Chamran University of Ahvaz for vaccination. Due to its low weight (3.2 kg), the animal was thoroughly examined, and a blood panel and some chemical factors were analyzed. A blood smear was prepared from the sample, stained with Giemsa stain, and examined under a light microscope to identify any hematological abnormalities or parasitic organisms.

Results: *Mycoplasmas* organisms were observed and confirmed within the red blood cells. The hematocrit and hemoglobin levels were below the normal range, indicating anemia. Additionally, lymphocyte count and mean platelet volume were elevated. Blood urea nitrogen (BUN) and alkaline phosphatase (ALP) levels were higher than the normal range.

Conclusion: The detection of *Mycoplasmas* organisms within the erythrocytes of the kitten underscores the significance of arthropod vectors, particularly fleas, in the transmission of hemoplasmosis. The observed hematological alterations highlight the potential impact of this infection on the health of animals. These findings emphasize the importance of vector control and early diagnosis to prevent the spread and severity of the disease in feline populations.

Keywords: Cats, *Mycoplasmas*, Hemoplasmosis, Vector-borne disease.



Introduction:

Hemomycoplasmosis caused by Hemotropic mycoplasmas (formerly classified as *Eperythrozoon*) are uncultivable organism, parasitize erythrocytes of different mammals, such as cattle, sheep, goats, horses, camels, pigs, cats and dogs, however latent infection might also occur in mules, deer and elk since the organisms mostly appear species specific (1,2). *Hemomycoplasma* spp. causes haemotropic mycoplasmosis in animals, it is an important infectious disease characterized by anemia, weakness and emaciation, and mostly death of infected animals. The disease is extremely common in different places of the world (3). The primary methods of microorganism transmission are predominantly mechanical. Biological vectors such as *Dermacentor andersoni*, *Haematopinus* lice, flies, and fleas also play a role in transmission. Additionally, blood transfusions, the reuse of contaminated needles, and surgical instruments are significant routes. Transplacental transmission is another pathway, although transmission via contaminated food remains unconfirmed(4). Conversely, *Haemomycoplasmas* tend to exhibit a seasonal pattern, with peaks occurring in late summer and early fall. Hemomycoplasmosis can be transmitted to humans(5,6). The first human case was reported in 1991, and since then, approximately 180 cases have been sporadically documented, primarily among farmers and veterinarians. Symptoms associated with the infection include fever, hemolytic anemia, swollen lymph nodes in the neck, hepatosplenomegaly, leukopenia, neutropenia, thrombocytopenia, and subclinical myocarditis (7). The aim of this study was to identify and characterize hemomycoplasma infection in a feline patient through blood smear examination, hematological, and biochemical analysis, to contribute to understanding its clinical features and diagnostic aspects in small animals.

Materials and Methods:

A one-year-old Persian kitten was referred to the Veterinary Hospital of Shahid Chamran University of Ahvaz for vaccination. Due to its low weight (3.2 kg), the animal was thoroughly examined, and hematological analysis and some biochemical factors were analysed. A blood smear was prepared from the sample, stained with Giemsa stain, and examined under a light microscope to identify any hematological abnormalities or parasitic organisms.

Results and Discussion:

The microscopic examination revealed numerous small organisms attached to the surface of erythrocytes. *Mycoplasmas* organisms were identified and confirmed within the red blood cells (Figure 1). The hematocrit and hemoglobin levels were below normal, indicating anemia. Additionally, there was an increase in lymphocyte count and mean platelet volume (Table 1).

Blood urea nitrogen (BUN) and alkaline phosphatase (ALP) levels were elevated beyond the normal range (Table 2).

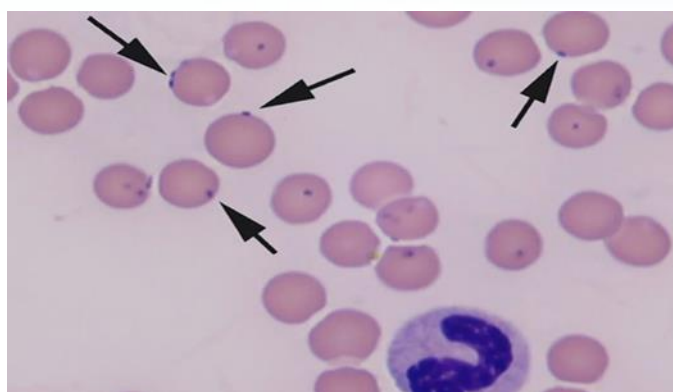


Figure 1. The blood film shows the presence of *Eperythrozoon* organisms within the red blood cells of the infected cat.

Table 1. Haematological analysis

Parameter	Result	Ref. Range
*Lymph ($10^3/\mu\text{L}$)	7.3	0.8-7.0
Mono ($10^3/\mu\text{L}$)	0.7	0.0-1.9
*Gran (%)	30.3%	35.0-85.0
RBC ($10^6/\mu\text{L}$)	8.36	4.60-10.0
HGB (g/dL)	11.6	9.3-15.3
HCT (%)	38.7%	28.0-47.0
MCV (fL)	46.3	39.0-52.0
MCH (pg)	13.8	13.0-21.0
MCHC (g/dL)	29.9	30.0-38.0
RDW (%)	15.7%	14.0-18.0
PLT ($10^3/\mu\text{L}$)	162	100-514
*MPV (fL)	9.4	5.0-9.0



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 2. Biochemistry analysis

Parameter	Result	Ref. Range
Ca (mg/dL)	9.1	6.2-10.2
P (mg/dL)	6.8	4.5-8.1
BUN (mg/dL)	48	20-30
Creatinine(mg/dL)	0.73	0.8-1.8
Total protein(g/dl)	5.6	5.4-7.8

The identification of *Mycoplasma* organisms on the blood smear highlights the presence of hemomycoplasmosis in the examined kitten. The characteristic attachment of small organisms to erythrocytes confirms the diagnosis and correlates with the observed anemia, reflected by decreased hemoglobin and hematocrit levels. This parasitic infection not only causes hemolytic destruction of erythrocytes but also leads to systemic effects, evidenced by alterations in biochemical parameters such as elevated BUN and ALP, which may indicate renal involvement and hepatic stress. The hematological abnormalities, including increased lymphocyte count and mean platelet volume, suggest an ongoing immune response and hematopoietic disturbances.

Given the transmission routes, including vector-borne and blood transfusion pathways, control measures should focus on reducing exposure to blood-sucking vectors and implementing proper hygiene during medical procedures. The case underscores the significance of blood smear examination as a rapid and effective diagnostic tool for hemoplasma infections, especially in clinical settings where molecular diagnostics may not be readily available. Early detection and treatment are crucial to prevent progression to severe anemia and systemic complications, emphasizing the importance of routine hematological screening in small animals presenting with hematological abnormalities.

These findings highlight the critical importance of vector management and early diagnosis to prevent the transmission and reduce the severity of the disease within feline populations.

Acknowledgement:

This study was supported by the research grant provided by the Shahid Chamran University of Ahvaz.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Al-Ani FK. Camel management and disease. 1st ed. Jordan: Dar-Ammar Book Publisher; 2004. p. 404.
2. Alsaad KM. The common blood parasitic infections of dromedaries (*Camelus dromedaries*): A review. *Basrah J Agric Sci.* 2021;34(1):222-229.
3. Constable PD, Hinchcliff KW, Done SH, Grunberg W. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Goats, and Horses.* 11th ed. Philadelphia: W.B. Saunders Co.; 2017. p. 777-778.
4. Hoelzle K, Winkler M, Kramer MM, Wittenbrink MM, Dieckmann SM, Hoelzle LE. Detection of *Candidatus Mycoplasma haemobos* in cattle with anaemia. *Vet J.* 2011;187(3):408-410.
5. Urquhart GM, Armour J, Duncan JL, Dunn AM, Jennings FW. *Veterinary Parasitology.* 2nd ed. Oxford: Blackwell Science Ltd.; 2003. p. 252.
6. Cem Ecmel SAKi. Clinical Eperythrozoon wenyoni (Adler and Ellenbogen, 1934) and Haemobartonella bovis (Donatin) and Lestoquard, 1934 infection in cattle. *F Ü Sağ Bil Vet Derg.* 2009;23(2):117-118.
7. Cem Ecmel SAKi. Clinical Eperythrozoon wenyoni (Adler and Ellenbogen, 1934) and Haemobartonella bovis (Donatin) and Lestoquard, 1934 infection in cattle. *F Ü Sağ Bil Vet Derg.* 2009;23(2):117-118



Assessment of *Anaplasma platys* Prevalence and Hematological Effects in Canines from Ahvaz

Sajjad Alishiri¹, Somayeh Bahrami¹, Dorin Motamedi¹, Hossein Hamidinejat¹, Bahman Mosallanejad², Misagh Jalali²

Corresponding author: Sajjad Alishiri, Sajjadalishiri191@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1341

Abstract

Background: *Anaplasma platys* is a tick-borne pathogen that infects canine blood platelets, causing clinical and subclinical infections. Despite its widespread distribution, data on its prevalence in dogs from Ahvaz city are limited. Understanding the molecular epidemiology of *A. platys* is essential for disease control and early diagnosis, especially in regions with high tick populations.

Methods: A total of 144 blood samples were collected from the cephalic vein of domestic dogs in Ahvaz and preserved at -20°C until DNA extraction. Hematological parameters were measured with a digital cell counter. For molecular detection, DNA was extracted with the Sinnaclon Blood DNA Extraction Kit. A two-step PCR approach was employed: first, a genus-specific PCR targeting the 16S rRNA gene of *Anaplasma* spp. identified positive samples; second, an *A. platys*-specific PCR confirmed species identity. Gel electrophoresis was used to visualize PCR products, with positive samples exhibiting bands of expected sizes (~500 bp for genus-specific, ~300 bp for *A. platys*).

Results: The initial PCR screening detected *Anaplasma* DNA in 34 of 144 samples (23.6%). Subsequent species-specific PCR confirmed 30 of these as *A. platys*, resulting in an overall prevalence of 20.8%. Hematological analysis revealed that 13.3% of *A. platys*-positive dogs had mild leukocytosis, primarily lymphocytosis, whereas only 3.3% exhibited thrombocytopenia. Most infected animals appeared hematologically normal, indicating many infections remain subclinical.

Conclusion: This study demonstrates a significant presence of *A. platys* among dogs in Ahvaz, with the majority of infections being subclinical. The findings emphasize the importance of molecular diagnostics for accurate detection, especially in asymptomatic animals. The relatively high prevalence underscores the necessity for regular tick control and monitoring programs to prevent the spread of this pathogen. Early diagnosis using PCR can facilitate



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



timely treatment and help mitigate potential hematological complications. Further studies are needed to explore the epidemiological dynamics and potential zoonotic risks associated with *A. platys* in this region.

Keywords: *Anaplasma platys*, Tick, Dog, Ahvaz, Polymerase chain reaction.

Introduction:

Anaplasmosis is a vector-borne disease that affects both humans and animals worldwide, posing significant health and economic concerns. The disease is primarily transmitted through the bites of infected ticks, notably *Rhipicephalus sanguineus*, which proliferate in many regions and serve as the main vectors responsible for transmission (1). Given the widespread presence and feeding behavior of these ticks, understanding the prevalence of *Anaplasma* spp., especially *Anaplasma platys*, is essential for disease control and management (2).

Anaplasma platys is an obligate intracellular rickettsia that infects blood platelets, leading to the formation of characteristic basophilic inclusions known as morulae within the cytoplasm of infected platelets. Although many infected dogs remain asymptomatic, *A. platys* infection can sometimes manifest clinically with symptoms such as fever, weight loss, depression, lethargy, and thrombocytopenia. Notably, *A. platys* infection may cause petechial hemorrhages, mucous membrane bleeding, and cyclic thrombocytopenia during the chronic phase of the disease (3).

Despite its significance, the prevalence and impact of *A. platys* infection in dogs from Ahvaz city remain undocumented. This study aimed to investigate the presence and molecular prevalence of *A. platys* in local dog populations using polymerase chain reaction (PCR) techniques, providing vital epidemiological data. This research aims to enhance understanding of vector-borne diseases in the region, facilitate early diagnosis, and support integrated control strategies to reduce the risk of infection in dogs and other susceptible hosts.

Materials and Methods:

The study involved collecting a total of 179 blood samples from the cephalic vein of dogs over a period spanning May 2024 to 2025. This process was part of a broader research initiative aimed at understanding various health parameters in canine populations. The collection was carried out with strict adherence to ethical standards and veterinary protocols to ensure the well-being of the animals involved. The samples obtained are intended for subsequent laboratory analysis to assess biochemical, hematological, or other relevant health indicators. The hematological parameters consisted of hematocrit (HCT), Mean Corpuscular Volume (MCV), Red Blood Cell (RBC) count, Hemoglobin Concentration (Hgb), Mean Corpuscular



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), total White Blood Cell (WBC) counts, and total Platelet (PLT) counts were assessed using a digital cell counter.

For molecular analysis, DNA was extracted by the DNA Purification Kit (Sinnaclon; Blood DNA Extraction Kit), according to the manufacturer's instructions. For the first step, the amplification of *Anaplasma* DNA was performed using a genus-specific set of primers of the 16S rRNA gene. Briefly, the primer set EHR16SD (5'-GGT-ACC-YAC-AGAAGA-AGT-CC-3') and EHR16SR (5'-TAG-CAC-TCA-TCGTTT-ACA-GC-3') were used for screening the *Anaplasma* agents. The PCR was performed in a final volume of 25 μ L of reaction mixture containing 12.5 μ L of PCR Master Mix (Amplicon, Denmark), 1 μ L of each primer, 5.5 μ L of nuclease-free water, and 5 μ L of DNA template (~20 ng). The PCR cycles started at 95°C for 5 min; then, 34 cycles of denaturation for 30 sec at 95°C, primers annealing for 30 sec at 55°C, and extension for 90 sec at 72°C were performed. The final extension took only one step for 5 min at 72°C. In the next step, a new PCR method was performed for the positive samples with the screening PCR. The *E. platys* specific forward primer (PLATYS; 5'-GAT-TTT-TGT-CGT-AGC-TTG-CTA-TG-3') was combined with the reverse primer EHR16SR. Five microliters of each sample was used as the template DNA in a final volume of 25 μ L, and the rest of the conditions were the same as for the screening PCR except that the number of cycles was 40 (4).

Gel electrophoresis of 1.5% Red safe (Intron Biotechnology, Korea)-stained agarose gel was conducted at 100 volts for 45 min.

Results and Discussion:

All positive samples exhibited clear bands at the expected amplicon size (~500 bp for genus-specific PCR; ~300 bp for *A. platys*-specific PCR). First-round PCR (using genus-specific 16S rRNA primers) identified 34/144 samples (23.6%) as positive for *Anaplasma* spp. Second-round PCR (using *A. platys*-specific primers) confirmed 30/34 samples (88.2% of initially positive samples) as positive for *A. platys*, yielding an overall prevalence of 20.8% (30/144).

4/30 *A. platys*-positive dogs (13.3%) showed mild leukocytosis, primarily due to lymphocytosis. 1/30 positive dogs (3.3%) exhibited thrombocytopenia. The majority of infected dogs showed no hematological abnormalities, highlighting the subclinical nature of many *A. platys* infections.

This study provides valuable insights into the prevalence of *A. platys* infection among dogs in Ahvaz, a region where vector-borne diseases are a growing concern. Using molecular diagnostic tools, specifically PCR amplification of the 16S rRNA gene and species-specific primers, we detected *A. platys* DNA in 20.8% of the sampled canine population. This



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



prevalence indicates a substantial burden of infection, aligning with reports from other endemic areas where *A. platys* is considered a common but often subclinical pathogen. Overall, the prevalence of *A. platys* in dogs in Iran found herein is similar to that reported in Paraguay (5), Brazil (6), French Guiana (7), and Chile (8). On the other hand, the prevalence in this study is higher than reports from Argentina (9), Italy (10), Croatia (11), and Mexico (12).

Most infected dogs in our study did not exhibit clinical signs or hematological abnormalities, particularly thrombocytopenia—a hallmark of *A. platys* infection. This subclinical presentation is consistent with previous reports, emphasizing the challenge of diagnosing infection based solely on clinical or hematological findings. The detection of mild leukocytosis, mainly lymphocytosis, in some cases may suggest a mild host immune response but is not pathognomonic. The absence of severe hematological abnormalities in most cases highlights the potential for *A. platys* to persist silently within canine populations, contributing to ongoing transmission cycles via tick vectors. Given the widespread presence of *R. sanguineus*, the principal vector, and the favorable climatic conditions in Ahvaz that support tick proliferation, the observed prevalence warrants sustained surveillance and vector control efforts.

In conclusion, this study highlights the significant prevalence of *A. platys* in the canine population of Ahvaz. The high rate of subclinical infections underscores the necessity for ongoing epidemiological monitoring, vigilance for potential zoonotic transmission, and integrated control measures. Further research should focus on identifying tick populations, exploring possible coinfections with other vector-borne pathogens, and evaluating the clinical outcomes over time to better understand the impact of *A. platys* infection on canine health.

References:

1. Huang H, Unver A, Perez MJ, Orellana NG, Rikihisa Y. Prevalence and molecular analysis of *Anaplasma platys* in dogs in Lara, Venezuela. *Braz J Microbiol.* 2005; 36:211-6.
2. Martin AR, Brown GK, Dunstan RH, Roberts TK. *Anaplasma platys*: an improved PCR for its detection in dogs. *Exp Parasitol.* 2005; 109(3):176-80.
3. Sarker BR, Mitpasa T, Macotpet A, Bupata PA, Sangmaneedet S, Taweenan W. First report on molecular prevalence and identification of *Anaplasma platys* in dogs in Khon Kaen, Thailand. *Vet World.* 2021; 14(10):2613-2619.
4. Inokuma H, Raoult D, Brouqui P. Detection of *Ehrlichia platys* DNA in brown dog ticks (*Rhipicephalus sanguineus*) in Okinawa Island, Japan. *J Clin Microbiol.* 2000; 38(11): 4219-21.
5. Sainz Á, Roura X, Miró G, Estrada-Peña A, Kohn B, Harrus S, et al. Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. *Parasit Vectors.* 2015; 8:75.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



6. Costa-Júnior L, Rembeck K, Passos L, Ribeiro M. Factors associated with epidemiology of *Anaplasma platys* in dogs in rural and urban areas of Minas Gerais State, Brazil. *Prev Vet Med.* 2013; 109:321-326.
7. Dahmani M, Marié J-L, Mediannikov O, Raoult D, Davoust B. First identification of *Anaplasma platys* in the blood of dogs from French Guiana. *Vector Borne Zoonotic Dis.* 2015; 15:170-172.
8. Abarca K, López J, Perret C, Guerrero J, Godoy P, Veloz A, et al. *Anaplasma platys* in dogs. *Chile Emerg Infect Dis.* 2007; 13:1392.
9. Cicuttin GL, De Salvo MN, Dohmen FEG. Molecular characterization of *Ehrlichia canis* infecting dogs, Buenos Aires. *Ticks Tick Borne Dis.* 2016; 7:954-957.
10. Trotta M, Fogliazza A, Furlanello T, Solano-Gallego L. A molecular and serological study of exposure to tick-borne pathogens in sick dogs from Italy. *Clin Microbiol Infect.* 2009;15:62–63.
11. Huber D, Reil I, Duvnjak S, Jurković D, Lukačević D, Pilat M, et al. Molecular detection of *Anaplasma platys*, *Anaplasma phagocytophilum* and *Wolbachia* sp. but not *Ehrlichia canis* in Croatian dogs. *Parasitol Res.* 2017; 116:3019–3026.
12. Almazán C, González-Álvarez VH, de Mera IGF, Cabezas-Cruz A, Rodríguez-Martínez R, de la Fuente J. Molecular identification and characterization of *Anaplasma platys* and *Ehrlichia canis* in dogs in Mexico. *Ticks Tick Borne Dis.* 2016; 7:276–283.



In Vitro Evaluation of a Nanoemulsified Blend of Clove, Lemongrass, and Tea Tree Essential Oils Against Poultry Lice (*Lipeurus caponis*)

Elaheh Roohbakhsh Amoli Moghadam¹, MohammadReza Khajehdaloui², Somayeh Alamdar³

Corresponding Author: Elaheh Roohbakhsh Amoli Moghadam, elro.99@gmail.com

1. R&D Manager, Salamat Gostar Asa Daru Co., Mashhad, Iran.
2. Chairman of the Board, Salamat Gostar Asa Daru Co., Mashhad, Iran.
3. Chief Executive Officer (CEO), Salamat Gostar Asa Daru Co., Mashhad, Iran.

Article code: 1354

Abstract

Background: production, impairing growth, feed efficiency, carcass quality, and welfare. Reliance on synthetic pediculicides is increasingly constrained by resistance, safety, and residue-compliance considerations.

Methods: This study evaluated whether formulation engineering via nanoemulsification enhances the pediculicidal performance of a 1:1:1 blend of clove, lemongrass, and tea tree essential oils. The blend (3% v/v) was dispersed in water with a non-ionic emulsifier (1% w/v) and processed by rotor–stator homogenization (20,000 rpm, 5 min, 25 °C), yielding sub-100-nm droplets (Z-average 78 ± 4 nm; PDI < 0.2). Adult *L. caponis* were exposed on treated filter papers delivering $0.157 \mu\text{L} \cdot \text{cm}^{-2}$ essential oil. Mortality was recorded at 3, 6, and 12 h; LT_{50} was estimated by probit analysis, and group differences by one-way ANOVA with Tukey's post-hoc test ($\alpha = 0.05$).

Results: The nanoemulsified blend achieved 100% mortality within 6 h and the shortest LT_{50} (≈ 0.93 h), outperforming single-oil treatments and a non-emulsified blend.

Conclusion: These findings indicate that nanoemulsion delivery can increase both the rate and extent of pediculicidal action against *L. caponis* in vitro and justify stability, safety, and field validation towards integration into poultry parasite-management programs.

Keywords: *Lipeurus caponis*, Ectoparasites, Pediculicidal, Nanoemulsion, Poultry.

Introduction:

Ectoparasitism imposes persistent constraints on poultry production. Infestation by the ischnoceran chewing louse *Lipeurus caponis* is associated with feather damage, irritability, impaired feed efficiency, reduced weight gain, and carcass downgrading. Control in commercial settings relies principally on conventional pediculicides, but long-term utility is limited by resistance development, safety and operator-exposure considerations, withdrawal



times, and residue-sensitive markets (1,2). Botanical insecticides constitute a plausible complementary strategy within integrated parasite-management programs. Essential oils (EOs) from clove (eugenol-rich), lemongrass (citral-dominant), and tea tree (terpinen-4-ol-rich) have been reported to exhibit activity against diverse ectoparasites, including avian lice (3,4,5,6). However, the intrinsic hydrophobicity and volatility of EOs hinder uniform aqueous dispersion, consistent deposition on the feathered integument, and sustained contact with target arthropods, leading to variable bioefficacy (3). Nanoemulsification provides a formulation-engineering approach to mitigate these limitations; sub-100–200-nm oil-in-water droplets improve kinetic stability, wettability, and interfacial area, thereby enhancing the probability and duration of contact at the arthropod cuticle (7,8). On this basis, we formulated a standardized 1:1:1 blend of clove, lemongrass, and tea tree EOs as a nanoemulsion and evaluated its in-vitro pediculicidal activity against adult *L. caponis*. We hypothesized that the nanoemulsified blend would produce a shorter time-to-kill and higher cumulative mortality than the same actives applied individually or as a non-emulsified blend under dose-equivalent conditions.

Materials and Methods:

Essential oils and emulsifier: Food-grade EOs of clove (*Syzygium aromaticum*), lemongrass (*Cymbopogon citratus*), and tea tree (*Melaleuca alternifolia*) were procured from certified suppliers. A proprietary non-ionic emulsifier (Salamat Gostar Asa Daru, R&D) served as the surfactant system.

Nanoemulsion preparation and characterization

Equal volumes of the three EOs were blended (1:1:1). The mixture (3% v/v) was dispersed in deionized water containing 1% w/v emulsifier and processed using a rotor–stator homogenizer (20,000 rpm, 5 min, 25 °C). Z-average droplet diameter and polydispersity index (PDI) were determined by dynamic light scattering (DLS); visual stability (absence of phase separation/creaming/sedimentation) was monitored over 24 h at ambient temperature (7).

In-vitro contact bioassay

Whatman No. 1 filter papers (90 mm) in Petri dishes were treated uniformly with 1 mL of each formulation to deliver an essential-oil dose of $0.157 \mu\text{L} \cdot \text{cm}^{-2}$. After 15 min drying, adult *L. caponis* (10 individuals per plate; $n = 5$ plates per treatment) were introduced and maintained at 25 ± 1 °C. Mortality (absence of movement upon gentle probing) was recorded at 3, 6, and 12 h. Negative controls comprised deionized water and emulsifier solution (1% w/v) without EO.



Statistics

Median lethal time (LT_{50}) was estimated by probit analysis. Differences in cumulative mortality among treatments were assessed by one-way ANOVA followed by Tukey's post-hoc test ($\alpha = 0.05$).

Results and Discussion:

The nanoemulsified blend yielded sub-100-nm droplets (Z -average 78 ± 4 nm; $PDI < 0.2$) with no visible phase separation over 24 h. Such droplets are expected to enhance wettability, interfacial area, and residence time at the cuticular interface, improving access of actives to sites of action (7,8).

At $0.157 \mu\text{L} \cdot \text{cm}^{-2}$, the nanoemulsified blend effected 100% adult mortality by 6 h and exhibited the shortest LT_{50} (≈ 0.934 h). Among single-oil treatments, clove oil was most active, whereas lemongrass and tea tree oils produced moderate yet significant lethality within the observation window. The non-emulsified 1:1:1 blend outperformed individual oils but remained inferior to the nanoemulsified formulation, underscoring the contribution of formulation engineering under dose-equivalent conditions (Table 1). These findings are concordant with prior reports of EO-based pediculicidal activity and with studies showing that nanoemulsion delivery accelerates and amplifies pesticidal effects against avian lice and related ectoparasites (4,9,5,10).

Mechanistic considerations and implications: Superior activity of the nanoemulsified blend is plausibly attributable to improved dispersion and interfacial area, facilitating deposition and sustained contact at the cuticle, together with enhanced partitioning of volatile constituents (eugenol, citral, terpinen-4-ol) into cuticular lipids; multi-component interactions may yield additive or synergistic effects (3,6). From an applied perspective, the formulation represents a plant-derived, residue-free candidate suitable for integration into parasite-management programs, particularly where resistance or residue-compliance constraints limit conventional pediculicides (1,2,3).

The present study addressed adulticidal activity *in vitro* and did not evaluate ovicidal/nymphicidal effects, residual activity, or repellency. Translation to practice should prioritize verification of physicochemical stability and retained bioactivity across representative poultry-house water chemistries and temperatures, on-bird tolerability and operator safety, and randomized, controlled field trials to define operational dosing, application modality, contact time, and retreatment intervals.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 1. Mortality (%) at 3, 6, and 12 h and LT_{50} (h) for adult *Lipeurus caponis* exposed to treatments at $0.157 \mu\text{L}\cdot\text{cm}^{-2}$. Values are means ($n = 5$ plates $\times$ 10 lice). Different superscript letters within a column indicate significant differences among treatments (one-way ANOVA, Tukey's post-hoc, $\alpha = 0.05$). Abbreviations: PDI, polydispersity index; NE, nanoemulsion.

Treatment	Conc. ($\mu\text{L}\cdot\text{cm}^{-2}$)	Mortality 3 h (%)	Mortality 6 h (%)	Mortality 12 h (%)	LT_{50} (h)
Control (water/emulsifier)	—	0	0	0	> 12
Clove EO	0.157	78.3	93.4 ^{ab}	100	1.438 ^c
Lemongrass EO	0.157	56.2	71.5 ^c	89.1	3.522 ^c
Tea tree EO	0.157	62.5	79.0 ^{bc}	93.8	2.989 ^d
Blend (1:1:1)	0.157	81.6	95.2 ^{ab}	100	1.213 ^b
Blend emulsifier (NE)	+ 0.157	92.7	100 ^a	100	0.934 ^a

Table 2. Physicochemical properties of the nanoemulsified blend measured by DLS at 25 °C (mean $\pm$ SD).

Parameter	Nanoemulsified blend
Z-average droplet diameter (nm)	78 ± 4
Polydispersity index (PDI)	< 0.2
Visual stability at 24 h	No visible creaming/sedimentation

Formulation engineering via nanoemulsification of a clove–lemongrass–tea tree blend yielded accelerated adulticidal activity against *Lipeurus caponis* in vitro, underscoring that delivery architecture can be as determinative as active selection. The translational value of this platform will hinge on (i) physicochemical stability across representative farm water chemistries and temperatures, (ii) batch-to-batch standardization of EO chemotypes and shelf-life, and (iii) documented safety and operator acceptability under field use. Priority next steps are on-bird studies to confirm efficacy and tolerability; determination of ovicidal/nymphicidal and residual effects; development of standardized field protocols (working dilution, application modality, contact time, retreatment interval); and monitoring for resistance selection pressure and environmental footprint. Subject to these verifications, the nanoemulsified blend can be



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



positioned as a residue-free botanical option to complement or partially replace synthetic pediculicides within integrated parasite-management programs.

Acknowledgment:

The authors thank the R&D team at Salamat Gostar Asa Daru Co. for technical assistance and access to laboratory facilities.

References:

1. Abbas RZ CDIZKM. Acaricidal drug resistance in poultry red mite (*Dermanyssus gallinae*). *Vet Parasitol.* 2014; 204((1–2)): 19–26.
2. MB I. Botanical insecticides in the twenty-first century: strengths, weaknesses, opportunities and risks. *Pestic Biochem Physiol.* 2020; 170: 104646.
3. Sedefoglu S DFKG. Essential oil nanoformulations for organic poultry production. *Birds.* 2022; 3(2): 114–127.
4. Lee HS NHCS. ediculicidal efficacy of tea tree oil against *Bovicola ovis*. *Vet Dermatol.* 2019; 30(6): 486.
5. Pumnuan T KPYPSP. Nanoclove–cinnamon emulsion eliminates poultry ectoparasites. *Vet Parasitol.* 2024; 315: 109.
6. Fichi G FGGFODPS. Efficacy of an eugenol-rich oil against poultry ectoparasites. *Parasitol Res.* 2007; 101(2): 417–421.
7. Nasser A TJHZBS. Overcoming volatility of essential oils via nanoemulsion delivery systems. *J Agric Food Chem.* 2021; 69(10): 3151–3161.
8. Zhang Y CMLJDM. Advances in nanoemulsion-based formulations for pest control. *Nanomaterials.* 2022; 12(3): 503.
9. Ha JH PJKH. Synergistic pediculicidal effects of clove and tea tree essential-oil blends against poultry lice. *Poult Sci.* 2018; 97(8): 2607–2612.
10. Lakyat S MBHP. Fast-acting essential-oil nanoemulsions against *Menopon gallinae*. *Poult Sci.* 2023; 102.



First Report of *Plasmodium* spp. Infection in a Cattle Egret (*Bubulcus ibis*) in Ahvaz, Khuzestan, Iran

Arman Nourishirazi¹, Yassamin Mahmoudian², Poorya Soleymani³, Somayeh Bahrami¹

Corresponding Author: Arman Nourishirazi, email: arman.nourishirazi@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Clinical Pathology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
3. Department of Environment, Ahvaz, Khuzestan, Iran.

Article code: 1357

Abstract

Background: Avian malaria caused by *Plasmodium* spp. is a significant parasitic disease affecting wild and domestic bird populations worldwide. This study aims to report an infection caused by *Plasmodium* spp. in a cattle egret in Ahvaz, Khuzestan, Iran for the first time and discuss its potential epidemiological significance.

Methods: An adult cattle egret was presented to the Department of Environment, Ahvaz, Khuzestan, Iran, in December 2024, with weakness, severe anemia, and evidence of significant physical trauma. After Clinical examination, blood was collected from the right jugular vein for hematologic evaluation. A through blood smear analysis was performed. Thin blood smears were prepared, fixed with absolute methanol (1 min), stained with 10% Giemsa solution (30 min), and examined under an oil immersion lens. Due to poor prognosis and inability to return to the wild, the bird was humanely euthanized.

Results: Hematological analysis revealed severe regenerative anemia characterized by polychromasia and immature erythrocytes. The estimated white blood cell count was 21,000/ μ L. The leukogram showed segmented heterophils at 61%, band heterophils at 10%, and 2+ degree toxic changes in heterophils (cytoplasmic basophilia, partial degranulation). Lymphocytes were 25%, monocytes 2%, and eosinophils 2%, indicating a severe inflammatory response. In Giemsa-stained blood smear, numerous intraerythrocytic trophozoites and gametocytes morphologically consistent with *Plasmodium* spp. were observed.

Conclusion: To the best of our knowledge, this is the first morphological evidence compatible with *Plasmodium* spp. infection in a cattle egret in Iran. These findings underscore the importance of routine surveillance of hemoparasites in wild birds and the need for molecular studies to confirm and characterize such infections.

Keywords: Hemoparasites, Avian malaria, Wild birds, Hemolytic anemia, Blood smear.



Introduction:

Avian malaria, caused by *Plasmodium* spp., is a mosquito-borne disease affecting various avian species globally and can result in anemia and eventual death in susceptible hosts (1). Birds of the order ciconiiformes are important reservoirs for *Plasmodium* parasites, especially in specific tropical and subtropical regions where transmission is active and can lead to high infection rates and mortality in young birds (2). A study conducted in Venezuela provides insights into the prevalence of *Plasmodium* and *Fallisia* parasites in avian populations, including 23 species, with a particular focus on ciconiiform birds. In this tropical region, haemosporidian infections were detected in 5.8% of adult birds through microscopic examination of blood smears. This contrasts sharply with the prevalence in younger birds, where infection rates were significantly higher—53.6% in nestlings, increasing to 74.6% as they aged. (2).

The cattle egret (*Bubulcus ibis*) is widespread across Iran, commonly inhabiting wetlands, agricultural fields, and livestock areas, which increases its exposure to mosquito vectors. However, there are no specific reports supporting *Plasmodium* infection in this species in the country. Previous studies in Iran have reported *Plasmodium* spp. in pigeons, crows, passerines, and domestic birds in the following provinces: Mazandaran, Golestan, Yazd, Khuzestan, Bushehr, Hormozgan, Kerman, Sistan and Baluchistan province, and South Khorasan (3–6). A global meta-analysis estimated a prevalence of ~16% in wild birds, emphasizing the widespread nature of avian malaria (1). *Plasmodium* Infections have been reported in large egrets (PR: 13.3%) and cattle egrets (5.9%) in Korea (7), but no specific reports exist for cattle egrets in Iran.

Cattle egrets in Ahvaz are commonly found in human-modified landscapes, including riverbanks, agricultural fields, urban green spaces, and traditional bazaars where live birds are sold. Their close interactions with domestic poultry, wild birds, and livestock suggest they could serve as important epidemiological bridges for vector-borne pathogens such as *Plasmodium* spp.. Their synanthropic behavior facilitates exposure to mosquito vectors, increasing the risk of parasite spillover between different bird populations.

This report provides the first morphological evidence compatible with *Plasmodium* spp. infection in a cattle egret from Ahvaz, Khuzestan, Iran.

Materials and Methods:

An adult cattle egret was found in Ahvaz, Khuzestan Province, Iran, in December 2024, presenting with marked weakness, severe pallor of mucous membranes, and significant physical trauma. Blood was collected from the jugular vein for hematological examination. Thin blood smears were prepared, fixed with absolute methanol (1 min), stained with 10%



Giemsa solution (30 min), and examined under an oil immersion lens. Due to severe physical trauma, poor clinical prognosis, and inability to rehabilitate and release the bird, humane euthanasia was performed.

Results and Discussion:

The estimated white blood cell (WBC) count was 21,000/ μ L. The leukogram showed segmented heterophils at 61% (12,810/ μ L), band heterophils at 10% (2,100/ μ L), 2+ degree toxic changes in heterophils, lymphocytes at 25% (5,250/ μ L), monocytes at 2% (420/ μ L), and eosinophils at 2% (420/ μ L).

Reference hematological values in adult cattle egrets include WBC count of approximately 750 $\pm$ 280 cells/ μ L, heterophils 38.6 $\pm$ 0.2 %, lymphocytes 46.0 $\pm$ 1.3 %, basophils 0.6 $\pm$ 0.1 %, monocytes 4.2 $\pm$ 0.1 %, and eosinophils 3.4 $\pm$ 0.2 % (8) — these serve as preliminary baselines for healthy adult birds in Nigeria.

Blood smears stained with Giemsa revealed polychromatophilic red blood cells and immature erythrocytes, indicative of regenerative anemia. Numerous intraerythrocytic stages (trophozoites and gametocytes) suggestive of *Plasmodium* spp. were identified, based on morphological criteria described by Valkiūnas (2).

Microscopic examination of the blood smear revealed numerous intraerythrocytic parasites morphologically consistent with *Plasmodium* spp. While morphology aligns with *Plasmodium* spp., gametocytes of *Haemoproteus* lack hemozoin pigment, and *Leucocytozoon* infects leukocytes, not erythrocytes (2). While morphological evidence supports the presence of *Plasmodium*, species-level or definitive genus confirmation would require molecular diagnostics such as PCR. In our case molecular confirmation could not be achieved, as the sample was compromised before DNA extraction could be attempted.

Previous studies have identified *Plasmodium* infections in Columbidae, Corvidae, Acrocephalidae, Fringillidae, Muscicapidae, Passeridae, Pycnonotidae, Scotocercidae, Sylviidae, Phasianidae, Meleagrididae, and Anatidae in Iran (3–6). Still, no reports in the Ardeidae or specifically cattle egrets have been documented. The findings underscore the possible role of these birds as reservoirs in local transmission cycles (1,7). Future studies should incorporate molecular methods to confirm these findings and to further investigate the epidemiology and lineage diversity of *Plasmodium* spp. in Iranian wild birds (5,6).

In this study, hematologic evaluation confirmed severe regenerative anemia, evidenced by polychromasia and immature erythrocytes. The significant leukocytosis, segmented heterophilia, and band heterophilia suggested a marked inflammatory response, likely due to both trauma and parasitemia. The presence of toxic changes in heterophils further supports a significant bacterial component or systemic inflammatory response, potentially secondary to



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



trauma-induced immunosuppression or concurrent infection (9). While parasitemia may have contributed to anemia, the presence of toxic heterophils is consistent with a leukogram profile typical of acute bacterial sepsis in avian species (9). This case shows the compounding health challenges faced by synanthropic birds: trauma, parasitism, and opportunistic infections likely act synergistically through immunosuppression, increasing susceptibility to pathogens (2). These ecological traits highlight the potential implications for poultry health and wild bird conservation and emphasize the need for targeted surveillance and vector control strategies in shared habitats (2).

To the best of our knowledge, this is the first morphological evidence compatible with *Plasmodium* spp. Infection in a cattle egret (*Bubulcus ibis*) in Ahvaz, Khuzestan, Iran. These findings emphasize the importance of parasitological monitoring and highlight the need for molecular studies to confirm and better characterize avian hemoparasite infections in this region. Furthermore, the ecological role of cattle egrets as potential reservoirs and dispersers of hemoparasites in human-dominated environments warrants integrated surveillance and control efforts.

Acknowledgment:

The authors would like to thank fellow veterinary students Ilia Alipour and Ahoora Bahrami from Shahid Chamran University of Ahvaz for their valuable assistance with sample collection, transportation, and the humane euthanasia of the bird.

We also thank the Department of Environment and the Department of Clinical Pathology, Veterinary faculty, Shahid Chamran University of Ahvaz, for their valuable assistance with clinical examination and laboratory analysis.

References:

1. Yan WL, Sun HT, Zhao YC, Hou XW, Zhang M, Zhao Q, et al. Global prevalence of Plasmodium infection in wild birds: A systematic review and meta-analysis. Res Vet Sci. 2024;168:105136. doi:10.1016/j.rvsc.2024.105136.
2. Valkiūnas G. Avian malaria parasites and other haemosporidia. CRC Press; 2005.
3. Nourani L, Baghkheirati AA, Zargar M, Karimi V, Dinparast Djadid N. Haemoproteosis and avian malaria in Columbidae and Corvidae from Iran. Vet Med Sci. 2021;7(5):2043–2050. doi:10.1002/vms3.549.
4. Ehsani-Amrei SF, Motevalli-Haghi A, Nateghpour M, Mohebbali M, Youssefi MR, Raiesi O, et al. Detection of malaria parasites and other haemosporidia in migratory and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- native birds in Mazandaran and Golestan Provinces, Iran. *J Arthropod Borne Dis.* 2022;16(1):26–33. doi:10.18502/jad.v16i1.11189.
5. Ghaemitalab V, Mirshamsi O, Valkiūnas G, Aliabadian M. Prevalence and genetic diversity of avian haemosporidian parasites in southern Iran. *Pathogens.* 2021;10(6):645. doi:10.3390/pathogens10060645.
 6. Mirzaei F, Siyadatpanah A, Norouzi R, Pournasir S, Nissapatorn V, Pereira MdL. Blood Parasites in Domestic Birds in Central Iran. *Vet Sci.* 2020;7(3):126. doi:10.3390/vetsci7030126.
 7. Lee JH, et al. Prevalence and molecular characterization of avian malaria parasites from wild birds in Korea. *Animals.* 2023;13(7):957.
 8. William A, Zakariah M, Maina VA, Othman A, Chiroma M, Sanda KA. The studies on some hematological parameters on cattle egret (*Bubulcus ibis*) in Maiduguri, Borno state, Nigeria. *Int J Fauna Biol Stud.* 2017;4(5):76–79.
 9. Campbell TW, Ellis CK. *Avian and Exotic Animal Hematology and Cytology.* 4th ed. Ames, IA: Wiley-Blackwell; 2015.



Prevalence of *Babesia canis* Infection in Dogs in Ahvaz, Southwest Iran: A Molecular Epidemiological Study

Dorin Motamedi¹, Somayeh Bahrami ¹, Sajjad Alishiri ¹, Bahman Mosallanejad²

Corresponding author: Dorin Motamedi, doriindmo@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1360

Abstract

Background: Canine babesiosis, a tick-borne protozoan disease caused by *Babesia* species, impacts the health and welfare of dogs globally, especially in regions with high tick populations. In Iran, particularly in southwestern areas such as Khuzestan province, limited data are available regarding its prevalence. This study aimed to evaluate the molecular prevalence of *Babesia canis* infection in dogs in Ahvaz, Iran.

Materials and Methods: A total of 179 blood samples were collected from dogs visiting shelters, private veterinary clinics, and the Shahid Chamran University hospital between July 2024 and May 2025. Blood was drawn into EDTA-coated tubes and stored at -20 °C until DNA extraction. Molecular diagnosis was performed using PCR targeting the 18S rRNA gene with genus-specific primers, followed by gel electrophoresis and sequencing of the selected PCR products. The sequencing data were analyzed using the NCBI BLAST.

Results: Eighteen (10.1%) samples tested positive for *Babesia* spp. by PCR, with five samples confirmed to be *B. canis* through sequencing, showing approximately 99% identity. Statistical analysis indicated no significant correlation between infection prevalence and host factors such as age, sex, breed, or skin color.

Conclusion: This study indicates that *B. canis* is endemic among dogs in Ahvaz, Iran, with a moderate molecular prevalence. The findings underscore the need for regular screening, tick control measures, and public awareness to reduce the disease burden. Further investigation across broader regions and seasons is recommended to develop effective control strategies.

Keywords: *Babesia*, Dog, Ahvaz, Tick.



Introduction:

Canine babesiosis is a serious tick-borne disease. Ticks, such as *Rhipicephalus* spp. affecting dogs, caused by intraerythrocytic protozoa of the *Babesia* genus (1). The prevalence of the disease depends on geography and the presence of tick vectors (2). Babesiosis is a complex disease affecting multiple organs, representing a significant health concern for both humans and animals. The infection can present in various forms, ranging from subclinical cases, where individuals show no apparent symptoms, to severe, multi-organ involvement that can result in life-threatening complications (3). The clinical manifestations of babesiosis are diverse and can include symptoms such as fever, hemolytic anemia, jaundice, and general weakness. If not diagnosed and treated promptly, these symptoms may escalate, leading to serious health issues or even death. Early detection and appropriate management are crucial to prevent severe outcomes. Understanding the disease's pathophysiology and clinical presentation is essential for healthcare providers to ensure timely intervention and improve patient prognosis (1).

In Iran, especially in the southwestern regions such as Khuzestan province, the prevalence of tick-borne diseases such as babesiosis poses a significant challenge to veterinary health. The disease not only impacts the health and welfare of domestic and working dogs but also presents economic burdens due to treatment costs and management efforts. Despite its importance in veterinary medicine, limited data are available on the prevalence of canine babesiosis in this region.

Given the significance of understanding the distribution of this disease in endemic areas, this study aimed to evaluate the prevalence of canine babesiosis in Ahvaz, located in southwest Iran. By assessing infection rates and identifying potential risk factors, this research seeks to contribute to improved disease control strategies and enhance veterinary health management in the region.

Materials and Methods:

The study involved collecting a total of 179 blood samples from the cephalic vein of dogs over a period spanning May 2024 to 2025. This process was part of a broader research initiative aimed at understanding various health parameters in canine populations. The collection was carried out with strict adherence to ethical standards and veterinary protocols to ensure the well-being of the animals involved. The samples obtained are intended for subsequent laboratory analysis to assess biochemical, hematological, or other relevant health indicators. The blood was drawn into EDTA-coated tubes to prevent coagulation and was kept at -20 °C until DNA extraction. The samples were obtained from shelters, private veterinary clinics, and the hospital of Shahid Chamran University. Blood collection was conducted between July 2024 and May 2025. Background information of the dog: age, sex, breed, skin color, current



medications, the reason for visiting the clinic, recent history of any other disease, history of tick-borne diseases, tick-controlled medications, and the presence or absence of ticks were noted down after obtaining permission from the owner.

For molecular analysis, DNA was extracted by the DNA Purification Kit (Sinnacolon; Blood DNA Extraction Kit), according to the manufacturer's instructions. The extracted DNA was subjected to PCR using a genus-specific primer pair of BJ1(5-GTCTTGTAATTGGAATGATGG-3) and BN2 (5-TAGTTTATGGTTAGGACTACG-3), which amplify a 411–450 bp region in the 18S rRNA gene (small subunit rRNA) of *Babesia* spp. (4). The PCR was performed in a thermal cycler (Eppendorf Mastercycler® gradient), and each PCR reaction mixture (30 µl) included 15 µl of Go Taq master mix, 0.5 µl of each forward and reverse primer, 3 µl of template DNA, and adequate nuclease-free water. The amplification process was conducted with the following program: initial denaturation at 94 °C for 10 min. This was followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min. The final extension was at 72 °C for 5 min (Casati et al., 2006). The amplified PCR products were separated using ethidium bromide-stained agarose gel (2%) electrophoresis and visualized under UV illumination.

Five PCR amplified products were sequenced for species identification. The PCR products were purified using the QIAquick PCR purification kit, and sequencing was conducted using the Genetic Analyzer 3500 series (Applied Biosystems®). The obtained sequences were used in the NCBI Nucleotide Blast Tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), to search for a series of reference sequences.

Results and Discussion:

In total, out of 179 blood samples tested, 18 samples (10.1%) were positive for *Babesia* using PCR. The characteristic bands were observed in the electrophoresis analysis of these positive samples. From these, five samples were selected for sequencing, which confirmed with approximately 99% probability that the causative agent was *B. canis*.

Statistical analysis revealed no relationship between the prevalence of babesiosis and any of the host demographic factors tested, including age, sex, breed, and skin color.

This study aimed to evaluate the prevalence of canine babesiosis in Ahvaz, southwest Iran, using molecular techniques. Out of 179 dogs sampled from shelters, veterinary clinics, and the Shahid Chamran University hospital, 18 (10.1%) tested positive for *Babesia* spp. through PCR, with sequencing confirming *B. canis* as the causative agent in the positive samples.

The prevalence rate observed in this study aligns with previous reports from similar temperate and subtropical regions, where *Babesia* spp. are endemic and pose a significant health concern for dogs (5,6). The detection of *B. canis*, a pathogenic species known to cause severe hemolytic



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



anemia and other clinical signs in dogs, highlights the importance of ongoing surveillance and control measures in this region.

Interestingly, our statistical analysis revealed no significant association between the prevalence of babesiosis and host demographic factors such as age, sex, breed, or skin color. This finding is consistent with some studies that suggest infection risk is primarily influenced by environmental and tick exposure factors rather than intrinsic host characteristics (4). However, it is worth noting that other research has reported higher infection rates in younger dogs or certain breeds, potentially due to differences in immune status or tick exposure.

The relatively moderate prevalence detected suggests a continued risk of infection, especially considering the abundance of tick vectors in the region. Controlling measures such as acaricide use, environmental management, and public awareness are vital to reducing tick populations and subsequent disease transmission.

In conclusion, the present findings demonstrate that canine babesiosis remains endemic in Ahvaz, Iran, with a noteworthy prevalence of *B. canis*. These results underscore the importance of regular screening, vector control, and owner education to mitigate the disease's impact on canine health. Future studies should focus on broader geographic areas, seasonal variations, and the potential for co-infections with other tick-borne pathogens to develop comprehensive control strategies.

References:

1. Mittal, M., Kundu, K. and Chakravarti, S. Canine babesiosis among working dogs of organised kennels in India: A comprehensive haematological, biochemical, clinicopathological and molecular epidemiological multiregional study. *Prev Vet Med.* 2019; 169:104696.
2. Abhijith SP, Lathamani VS, Chandrakumar D, Kalam AA. Combination therapy with doxycycline, clindamycin, and metronidazole for *Babesia gibsoni* in a dog and its complete remission. *J Indian Vet Assoc.* 2024;22(3): 149-156.
3. Gałęcka I, Platt-Samoraj A. Case report of a triple vector-borne infection in a dog: co-infection with *Anaplasma phagocytophilum*, *Babesia* spp., and *Dirofilaria repens* in North-Eastern Poland. *BMC Vet Res.* 2025; 21(1):441.
4. Casati S, Sager H, Gern L, Piffaretti JC. Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland. *Ann Agric Environ Med.* 2006; 13(1):65-70.
5. Obeta SS, Ibrahim B, Lawal IA, Natala JA, Ogo NI, Balogun EO. Prevalence of canine babesiosis and their risk factors among asymptomatic dogs in the federal capital territory, Abuja, Nigeria. *Parasite Epidemiol Control.* 2020; 11:e00186.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



6. Muguiro DH, Nekouei O, Lee KY, Hill F, Barrs VR. Prevalence of *Babesia* and *Ehrlichia* in owned dogs with suspected tick-borne infection in Hong Kong, and risk factors associated with *Babesia gibsoni*. *Prev Vet Med.* 2023; 214:105908.



First Evidence of *Anaplasma platys* and *Hepatozoon canis* Co-infection in a Dog from Ahvaz, Khuzestan Province

Zahra Mashayekhi¹, Somayeh Bahrami¹, Sajjad Alishiri¹, Dorin Motamedi¹, Bahman Mosalla Nejad²

Corresponding Author: Zahra Mashayekhi, mashayekhi2000zahra@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1365

Abstract

Background: Accurate detection of hemoprotozoan parasites like *Hepatozoon*, *Anaplasma*, and *Babesia* is essential for diagnosing tick-borne diseases in dogs. Molecular methods such as PCR provide sensitive and specific tools for identifying these pathogens. We report the first co-infection case with *Anaplasma platys* and *Hepatozoon canis* in a single dog in Iran.

Methods: DNA was extracted from the blood sample using the Sinnaclon Blood DNA Extraction Kit. PCR assays targeted specific genes: the 18S rRNA gene of *Hepatozoon* (620 bp fragment), the 16S rRNA gene of *Anaplasma* (initial screening with primers EHR16SD/EHR16SR and species confirmation with *A. platys*-specific primers), and the 18S rRNA gene of *Babesia* (411–450 bp fragment). PCR conditions varied slightly for each pathogen but generally involved initial denaturation, followed by 34–40 cycles of amplification, and a final extension. Reactions were performed in 20 µL volumes with standard PCR components.

Results: The PCR successfully amplified target gene regions, indicating the presence of *Hepatozoon spp.* and *A. platys* in the samples tested, confirming co-infection in the studied case.

Conclusion: Molecular techniques such as PCR are valuable for rapid and precise identification of tick-borne hemoprotozoan parasites in dogs, facilitating accurate diagnosis and informing appropriate treatment strategies.

Keywords: *Hepatozoon canis*, *Anaplasma platys*, Co-infection, Ahvaz.



Introduction:

Canine anemia is a prevalent hematological disorder characterized by a reduction in red blood cell count or hemoglobin levels, resulting in diminished oxygen delivery to tissues. The etiology of anemia in dogs is diverse, encompassing immune-mediated processes, nutritional deficiencies, blood loss, and infectious agents. Among these, infectious diseases caused by hemoprotozoan parasites transmitted via vectors like ticks are particularly significant due to their widespread occurrence and impact on canine health at both individual and population levels (1).

Transmission dynamics differ among these parasites; notably, *Babesia* spp. or *Anaplasma* spp. (gram-negative bacteria) are transmitted through tick bites, whereas *Hepatozoon* spp. are acquired mainly by dogs ingesting infected ticks during grooming or hunting. The clinical severity of these infections emphasizes the importance of accurate diagnosis and prompt treatment to prevent fatal outcomes (2,3,4).

Ticks serve as critical vectors for multiple canine diseases, including hemoprotozoan infections, as well as bacterial illnesses like ehrlichiosis and anaplasmosis. The prevalence of tick-borne diseases is expanding geographically, driven by climate change and increased tick habitats. These diseases often present with nonspecific clinical signs, making differential diagnosis challenging. Understanding the epidemiology and pathogen transmission routes of these infections is vital for developing effective control measures and ensuring timely therapeutic intervention (5,6).

This case report aims to document and analyze a rare instance of concurrent hepatozoonosis and *Anaplasma platys* infection in a dog presenting with anemia, highlighting the effects of simultaneous co-infection on hematological parameters.

Materials and Methods:

In September 2024, a one-year and two-month-old female Spitz dog weighing 6 kg with a history of tick infestation accompanied by pruritus was referred to the Faculty of Veterinary Medicine at Shahid Chamran University. Based on the owner's report, the dog had experienced tick infestation in recent months; however, no ticks were observed on the animal at the time of clinical examination. The owner indicated that the dog had never been administered antiparasitic therapy before clinical evaluation. Therefore, blood samples were collected from the animal for further evaluation. Physical examination revealed severe hair loss and pale mucous membranes.

Blood sample was taken from the cephalic vein of the dog in EDTA-coated tubes and kept at -20 °C until DNA extraction. Thin blood smears were prepared, fixed with absolute methanol (1 min), stained with 10% Giemsa solution (30 min), and examined under an oil immersion



lens. Additionally, the hematological parameters were assessed using a digital cell counter. The investigation focused on hemoprotozoan parasites, such as *Babesia* and *Hepatozoon*, and gram-negative bacteria *Anaplasma*, which can cause anemia in animals.

For molecular analysis, DNA was extracted using the Sinnaclon Blood DNA Extraction Kit. Conventional PCR targeted the partial 18S rRNA gene of *Hepatozoon* spp., using primers that amplify a 620 bp fragment. PCR conditions included an initial step at 95°C for 2 min, followed by 35 cycles of 95°C for 60 sec, 58°C for 60 sec, and 72°C for 60 sec, with a final extension of 5 min at 72°C (7).

For *Anaplasma* detection, genus-specific primers targeting the 16S rRNA gene were used (EHR16SD/EHR16SR). The PCR involved 34 cycles with conditions similar to the above but at 55°C for annealing, and a final extension of 5 min at 72°C. Positive samples underwent a second PCR with *A. platys*-specific primer PLATYS and EHR16SR, with 40 cycles (8).

For *Babesia*, DNA was amplified using primers BJ1 and BN2 targeting a 411–450 bp region of the 18S rRNA gene. The PCR cycle included initial denaturation at 94°C for 10 min, then 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, and a final extension of 5 min at 72°C.

All PCRs were performed in 20 µL reactions containing 10 µL of PCR Master Mix, 0.7 µL of each primer, 6.6 µL of water, and 2 µL of DNA (~20 ng) (9).

Results and Discussion:

The blood smear was prepared and examined meticulously under a microscope. No hemoparasites associated with the pathogens under investigation were identified. PCR analysis of the blood sample revealed a positive result for *Hepatozoon* spp., producing 620 bp fragments. In addition, the same sample tested positive for *A. platys*, producing ~300 bp fragments, but tested negative for *Babesia* spp. confirming the co-infection with *Hepatozoon* spp. and *A. platys* (Figure1).

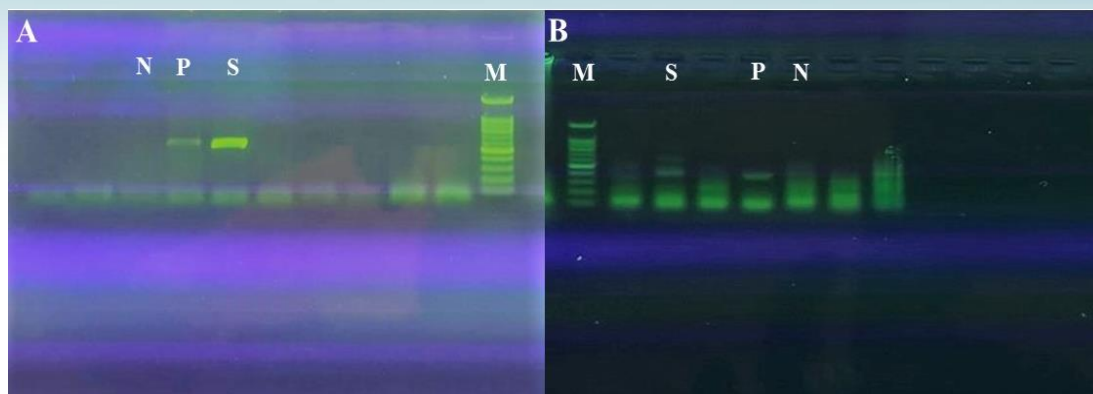


Figure 1. Agarose gel electrophoresis of the 18S rRNA gene of Hepatozoon (A) and *A. platys* (B). N: Negative control, P: Positive sample, M: 100 bp DNA ladder marker.

The hematological analysis revealed mild regenerative anemia, leukocytosis, and lymphocytosis. (Table 1).

Table 1. Hematological findings

Parameters	Value	Reference Range
WBC ($10^3/\mu\text{L}$)	25.1	6.0-17.0
RBC ($10^6/\mu\text{L}$)	5.01	5.50-8.50
HGB (g/dL)	9.9	11.0-19.0
HCT (%)	33.4	39.0-56.0
MCV (fL)	66.8	62.0-72.0
MCH (pg)	19.7	20.0-25.0
MCHC (g/dL)	29.6	30.0-38.0
PLT ($10^3/\mu\text{L}$)	390	117-460
LY (23.4%)	5.9	0.8-5.1
MO (3.9%)	1.0	2.0-9.0



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



In this study, we reported the co-infection with *H. canis* and *A. platys* in a dog from Ahvaz, confirmed by PCR analysis. Interestingly, despite the PCR confirmation, no hemoparasites were detected in microscopic examination of the blood smears, highlighting the limitations of microscopy in detecting low parasitemia or intracellular pathogens such as *A. platys*, which preferentially infects platelets. The hematological findings revealed mild regenerative anemia, leukocytosis, and lymphocytosis. However, the platelet count remained within the normal range despite the presence of *A. platys* infection, which is contrary to previously reported studies, such as the one conducted by Gaunt et al., (2010) that documented thrombocytopenia as a typical hematological feature of *A. platys* infection. Contrary to a previously reported case of co-infection with *H. canis* and *A. platys* in Romania, where clinical signs included hemorrhagic diarrhea, fever, apathy, inappetence, fatigue, and uncoordinated movements, the case in our study did not exhibit any of these symptoms. The only shared clinical manifestation observed was pale mucous membranes (10,11).

Based on current knowledge, this appears to be the first documented case of *H. canis* infection in Ahvaz and the inaugural report of co-infection with *A. platys* and *H. canis* in Iran. This highlights the necessity for expanded epidemiological research. The study did not include biochemical profile analysis; therefore, future investigations should assess serum alkaline phosphatase, alanine aminotransferase (ALT), amylase, and albumin levels, as previous studies have indicated alterations in these parameters (12,13). Additionally, future research should focus on parasite genotyping, vector identification, and examining clinical variability among infected dogs across different regions.

Acknowledgment:

This study was supported by the research grant provided by the Shahid Chamran University of Ahvaz.

References:

1. Liu PC, Su BL. Causes of canine anemia in Taiwan: A five-year retrospective survey. Taiwan Veterinary Journal. 2015 Mar 31;41(01):31-7.
2. Homer MJ, Aguilar-Delfin I, Telford III SR, Krause PJ, Persing DH. Babesiosis. Clinical microbiology reviews. 2000 Jul 1;13(3):451-69.
3. Attipa C, Solano-Gallego L, Papasouliotis K, Soutter F, Morris D, Helps C, Carver S, Tasker S. Association between canine leishmaniosis and Ehrlichia canis co-infection: a prospective case-control study. Parasites & vectors. 2018 Dec;11:1-9.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



4. Baneth G, Samish M, Shkap V. Life cycle of *Hepatozoon canis* (Apicomplexa: Adeleorina: Hepatozoidae) in the tick *Rhipicephalus sanguineus* and domestic dog (*Canis familiaris*). *Journal of Parasitology*. 2007 Apr 1;93(2):283-99.
5. Otranto D, de Caprariis D, Lia RP, Tarallo V, Lorusso V, Testini G, Dantas-Torres F, Latrofa S, Diniz PP, Mencke N, Maggi RG. Prevention of endemic canine vector-borne diseases using imidacloprid 10% and permethrin 50% in young dogs: a longitudinal field study. *Veterinary parasitology*. 2010 Sep 20;172(3-4):323-32.
6. Otranto D, Dantas-Torres F, Breitschwerdt EB. Managing canine vector-borne diseases of zoonotic concern: part one. *Trends in parasitology*. 2009 Apr 1;25(4):157-63.
7. Hodžić A, Alić A, Fuehrer HP, Harl J, Wille-Piazzai W, Duscher GG. A molecular survey of vector-borne pathogens in red foxes (*Vulpes vulpes*) from Bosnia and Herzegovina. *Parasites & Vectors*. 2015 Dec;8:1-7.
8. Inokuma H, Raoult D, Brouqui P. Detection of *Ehrlichia platys* DNA in brown dog ticks (*Rhipicephalus sanguineus*) in Okinawa Island, Japan. *Journal of Clinical Microbiology*. 2000 Nov 1;38(11):4219-21.
9. Casati S, Sager H, Gern L, Piffaretti JC. Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland. *Annals of agricultural and environmental medicine*. 2006;13(1).
10. Andersson M, Turcitu MA, Stefanache M, Tamba P, Barbuceanu F, Chitimia L. First evidence of *Anaplasma platys* and *Hepatozoon canis* co-infection in a dog from Romania—a case report. *Ticks and tick-borne diseases*. 2013 Jun 1;4(4):317-9.
11. Gaunt SD, Beall MJ, Stillman BA, Lorentzen L, Diniz PP, Chandrashekar R, Breitschwerdt EB. Experimental infection and co-infection of dogs with *Anaplasma platys* and *Ehrlichia canis*: hematologic, serologic and molecular findings. *Parasites & vectors*. 2010 Dec;3:1-0.
12. Greig B, Asanovich KM, Armstrong PJ, Dumler JS. Geographic, clinical, serologic, and molecular evidence of granulocytic ehrlichiosis, a likely zoonotic disease, in Minnesota and Wisconsin dogs. *Journal of clinical microbiology*. 1996 Jan;34(1):44-8.
13. Ravnik U, Tozon N, Smrdel KS, Zupanc TA. Anaplasmosis in dogs: the relation of haematological, biochemical and clinical alterations to antibody titre and PCR confirmed infection. *Veterinary microbiology*. 2011 Apr 21;149(1-2):172-6.



Alternative Anti-parasitic Approaches: The Role of Probiotics in Helminth and Protozoan Infections

Sarvenaz Sadeghi Mahoonak¹, Seyedeh Yasna Hosseini¹

Corresponding Author: Sarvenaz Sadeghi Mahoonak, sarvenaz.sadeghi15@gmail.com

1. Department of veterinary science Islamic Azad University, Karaj Branch, Karaj, Iran.

Article code: 1372

Abstract

Background: Parasitic infections remain a significant challenge in veterinary medicine, causing chronic diseases, economic losses, and widespread morbidity. Conventional treatments face limitations such as drug resistance and adverse effects, prompting the exploration of probiotics as alternative or adjunct therapies.

Methods: A comprehensive analysis of experimental and livestock studies was conducted, examining probiotic strains such as *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Saccharomyces boulardii*. The review synthesized data from in vitro and in vivo studies, highlighting mechanisms like competitive exclusion, antimicrobial secretion, and immune modulation. Emphasis was placed on the role of probiotics in combating protozoan and helminth infections.

Results: Probiotics demonstrated significant potential in reducing parasite burdens and improving host health. For instance, *Lactobacillus* strains reduced *Giardia trophozoite* counts and restored intestinal integrity, while *Saccharomyces boulardii* lowered *Cryptosporidium* oocyst shedding. Challenges included the lack of standardized protocols and limited large-scale trials, underscoring the need for further research.

Conclusion: Probiotics provide a sustainable and multifaceted approach to parasitic infection control in veterinary medicine. Their ability to modulate immunity, inhibit pathogens, and synergize with conventional therapies positions them as promising alternatives. Future research should focus on optimizing strain selection, dosage, and integrated strategies to address current limitations and maximize efficacy.

Keywords: Probiotics, Parasitic infections, Helminth infections, Immune modulation, Antimicrobial mechanisms.



1. Introduction:

Parasitic infections pose a significant threat to global animal and human health, with helminths and protozoans causing chronic diseases, economic losses, and widespread morbidity (1). Conventional treatments, such as anthelmintics and antiprotozoal drugs, are increasingly challenged by drug resistance, adverse effects, and environmental concerns (2,3). In response, probiotics have emerged as promising alternatives or adjunct therapies due to their multifaceted mechanisms of action, including competitive exclusion of pathogens, secretion of antimicrobial compounds (e.g., bacteriocins, organic acids), and modulation of host immunity (4). For instance, *Lactobacillus* and *Bifidobacterium* strains have demonstrated efficacy in reducing parasite burdens in giardiasis and cryptosporidiosis, while *Saccharomyces boulardii* has shown potential against both protozoan and helminth infections (5,6). Similarly, probiotics like *Enterococcus faecium* and recombinant *Lactobacillus* strains have exhibited anti-helminthic properties in infections such as trichinellosis and haemonchosis (7,8). These effects are often mediated through enhanced mucosal immunity, including increased secretory IgA, balanced Th1/Th2 responses, and improved gut barrier integrity (9). Despite these advances, challenges remain, including the lack of standardized protocols, limited large-scale field trials, and underexplored synergies with conventional therapies (10,11). Addressing these gaps requires further research to optimize strain selection, dosage, and formulation for targeted parasite control in livestock and companion animals.

2. Probiotics used in Veterinary Medicine

Probiotics have emerged as promising adjuncts or alternatives for the control of parasitic infections through multiple mechanisms, including direct antimicrobial effects and modulation of host immunity (12).

2.1. Lactobacillus: Species of *Lactobacillus*, including *L. acidophilus*, *L. casei*, *L. plantarum*, *L. rhamnosus* GG, have been widely studied in animal models for their antiparasitic effects (12,13). These bacteria act by competitively excluding pathogens from colonizing the gut, secreting antimicrobial compounds such as bacteriocins and organic acids including lactic and acetic acid, and directly disrupting parasite structures, particularly noted in giardiasis (13). In addition, *Lactobacillus* strains modulate host immunity by increasing secretory IgA and regulating cytokines such as IL-10 and IFN- γ , resulting in balanced Th1/Th2 responses and strengthened mucosal defense (12). Animal studies demonstrate that administering these probiotics reduces parasite burden, preserves intestinal structure, and mitigates disease severity (13).

2.2. Bifidobacterium: Probiotic strains of *Bifidobacterium*, including *B. breve*, *B. longum*, and *B. animalis* subsp. *lactis*, have shown beneficial effects, particularly in pigs infected with



Ascaris suum and in cell models challenged with *Cryptosporidium*. Their mechanisms include the production of organic acids that limit pathogen growth, competitive exclusion in the gut lumen, and enhancement of host mucosal immunity through increased levels of parasite-specific antibodies (IgG1, IgG2, and IgA). Moreover, these strains reduce intestinal inflammation, support gut barrier function, and improve nutrient absorption, all of which contribute to greater resilience against parasitic infections (14).

2.3. Enterococcus: *Enterococcus faecium* and related strains have demonstrated antiparasitic potential in models of giardiasis, cryptosporidiosis, and toxocariasis. The probiotic action involves secretion of bacteriocins and antimicrobial peptides, competitive exclusion, and modulation of host immune responses. Studies report increased phagocytic activity, elevated protective antibodies (IgA, IgG), and reduced parasite burdens in animals treated with *Enterococcus* strains (15).

2.4. Saccharomyces: The yeast *Saccharomyces boulardii* has been shown to reduce the severity and parasite load in infections such as giardiasis, amoebiasis, and toxocariasis. Its mechanisms include secretion of proteases and metabolites that may inhibit parasites directly, upregulation of cytokines like IFN- γ and IL-12, and support for mucosal barrier integrity. *S. boulardii* also limits parasite adhesion to intestinal cells, resulting in lower parasite counts and faster recovery when used alongside standard treatments (16,17).

3. Probiotic against protozoan

3.1. Giardia: Probiotics such as *Lactobacillus johnsonii*, *L. casei*, *L. acidophilus*, *L. rhamnosus* GG, and *L. plantarum* have shown significant therapeutic potential against *Giardia* infections in animal models (18,19). These strains reduce trophozoite counts, restore intestinal villi structure, and enhance immune responses, including increased levels of secretory IgA, IL-6, IL-10, and CD4⁺ T cells. Some probiotics act directly by disrupting trophozoite morphology or inhibiting adhesion via bacteriocins or bile salt hydrolase activity (20).

3.2. Cryptosporidiosis: Probiotic strains such as *Lactobacillus reuteri*, *L. acidophilus*, and *L. plantarum* have been shown to significantly reduce *Cryptosporidium parvum* infection in animal models, particularly in mice and rats. Treatments reduced oocyst shedding and parasite burden, performing comparably or better than nitazoxanide and alternative therapies like clove or dill oil (22,23).

3.3. Eimeriasis: Probiotics play a valuable role in controlling protozoan infections like *Eimeria* spp. in poultry. *Lactobacillus*-based Primalac reduced oocyst shedding and enhanced immunity by increasing IFN- γ and IL-2 levels in chickens. Other probiotics, such as



Lactobacillus Lb16c6, *Bacillus subtilis*, and selenium-enriched strains, improved gut barrier integrity, antioxidant capacity, and lowered intestinal lesion scores (24).

4. Probiotics against helminth parasites

4.1. Schistosomiasis: In veterinary parasitology, *Lactobacillus sporogenes* has demonstrated anti-schistosome effects in *Schistosoma mansoni*-infected mice by preventing cytokine-induced apoptosis and reducing DNA damage and chromosomal abnormalities. Additionally, *L. delbrueckii* subsp. *bulgaricus* DSM 20080, *L. acidophilus* ATCC 4356, and yogurt have been shown to lower oxidative stress and liver fibrosis in infected mice (25).

4.2. Trichinellosis: In veterinary parasitology, multiple probiotic-based strategies have shown promise against *Trichinella* spp. infections. Expression of mIL-4 in *Lactobacillus plantarum* NC8 enhanced humoral, mucosal, and cellular immunity in mice against *T. spiralis* (26). Oral vaccination with invasive *L. plantarum* carrying pValac-Ts-ADpsp/pSIP409 improved IgG production and reduced intestinal damage and worm burden (7). *L. acidophilus* also enhanced the efficacy of albendazole treatment (27), while probiotic therapy increased LPL and IEL populations. *L. paracasei* CNCM and *L. casei* ATCC 393 influenced the intestinal phase of *T. britovi* (28).

4.3. Haemonchosis: *Haemonchus contortus*, a blood-feeding nematode in the abomasum of sheep, goats, and cattle, causes significant economic losses in veterinary medicine due to anemia, weight loss, and death. Kefir, a probiotic from various animal milks, showed in vitro anti-parasitic effects against *H. contortus* in sheep (10). A recombinant *Bacillus subtilis* expressing HcGAPDH protein induced strong humoral and cellular immunity in sheep, offering protection against infection (8). Additionally, *Bacillus thuringiensis* crystal protein Cry5B demonstrated potential in overcoming drug resistance in parasite control (11).

4.4. Hymenolepiasis: In veterinary studies, administration of *Lactobacillus taiwanensis* S29 and *L. plantarum* S27 (10^9 CFU/mL) to *Hymenolepis diminuta*-infected rats significantly reduced fecal egg count and intestinal worm burden. Treated rats also showed improved body and intestinal weights, indicating probiotic efficacy against helminth infections (12).

4.5. Toxocariasis: Toxocariasis affects both humans and animals and is transmitted through undercooked meat from infected hosts like ruminants and pigs (12). In veterinary studies, probiotics have shown promise in reducing infection severity. *Enterococcus faecalis* and *Saccharomyces boulardii* significantly decreased larval burden and modulated immune responses in mice (29). *Lactobacillus acidophilus* ATCC 4356 also reduced infection severity, though it acted indirectly by interacting with the host immune system (30).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 1. Prominent probiotic strains are utilized in the treatment of parasitic infections (12)

Helminth	Probiotics	Key Effects
Schistosomiasis	- Lactobacillus delbrueckii subsp. bulgaricus - Lactobacillus sporogenes - Lactobacillus acidophilus ATCC 4356	- Reduces oxidative stress and liver fibrosis. - Decreases DNA damage and chromosomal abnormalities.
Trichinellosis	- Lactobacillus plantarum NC8 - Lactobacillus casei ATCC 7469 - Lactobacillus paracasei CNCM - Lactobacillus acidophilus	- Reduces parasite burden by up to 90%. - Enhances humoral and mucosal immune responses (IgA, IgG). - Improves intestinal epithelial regeneration.
Toxocariasis	- Enterococcus faecalis CECT7121 - Saccharomyces boulardii - Lactobacillus acidophilus ATCC 4356	- Reduces worm count and larval migration. - Modulates mRNA expression of IFN-γ and IL-12. - Decreases severity of infection.
Haemonchosis	- Kefir (from camel, goat, ewe, cow milk) - Recombinant Bacillus subtilis (expressing HcGAPDH) - Bacillus thuringiensis Cry5B protein	- Exhibits anti-parasitic effects in vitro. - Induces humoral and cell-mediated immune responses in sheep.
Hymenolepiasis	- Lactobacillus taiwanensis S29 - Lactiplantibacillus plantarum S27	- Reduces egg per gram (EPG) and worm burden. - Improves hemoglobin, RBC, and WBC counts. - Restores intestinal microvilli and mitochondrial density.
Giardiasis	- Lactobacillus johnsonii La1 - Lactobacillus casei - Lactobacillus rhamnosus GG (LGG) - Lactobacillus acidophilus - Lactobacillus plantarum	- Reduces trophozoite population by up to 72%. - Enhances secretory IgA and CD4+ T-cell responses. - Restores villus architecture and intestinal morphology. - Induces bile-salt hydrolase (BSH) to inhibit parasite growth.
Cryptosporidiosis	- Lactobacillus reuteri - Lactobacillus acidophilus - Lactobacillus plantarum	- Significantly reduces oocyst shedding. - Outperforms commonly used nitazoxanide, and diminazene.
Eimeriasis	- Lactobacillus - Bacillus subtilis - Selenium-enriched strains	- Probiotics enhance immune modulation (e.g., increase γ-IFN, IL-2, and IL-10). - They reduce oocyst shedding and parasite load. - Some strains improve gut barrier function and antioxidant activity.



5. Experimental and Livestock Studies

Figure 1 illustrates the application of probiotics in ruminants (sheep, goats, cattle), poultry, and rodent models, highlighting their efficacy in reducing parasitic infections while also enhancing immune responses and gut health through microbiota modulation (2,12).

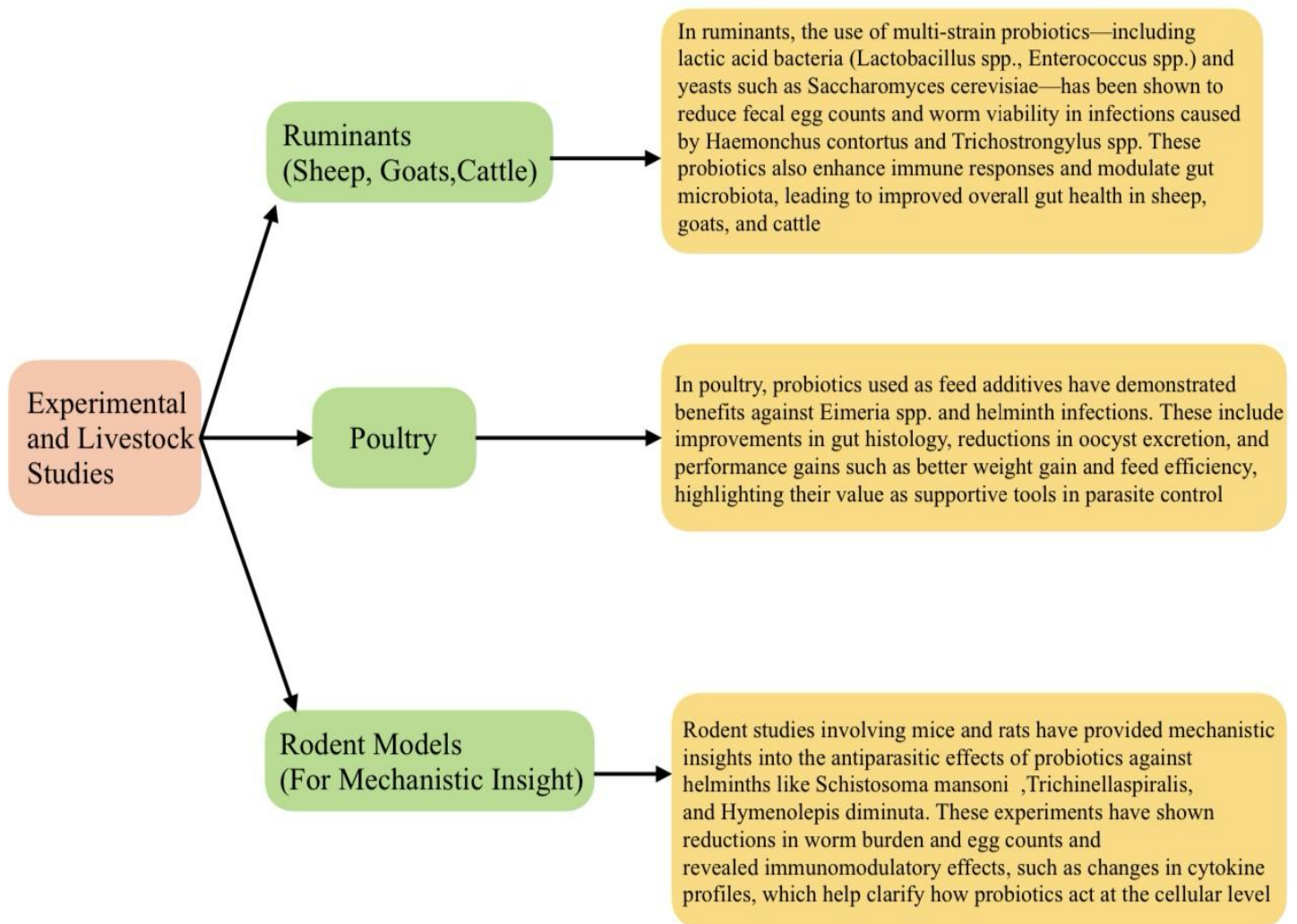


Figure1. Experimental studies of probiotics in Livestocks (2,12)



6. Challenges and Future Directions:

The application of probiotics in helminth control faces several key challenges. A significant issue is the limited number of large-scale field trials in real livestock production systems, making it challenging to validate laboratory findings in practical settings (2). Additionally, there is no standardization of probiotic strains or dosage protocols. Variability in species and concentrations across studies complicates comparisons and delays regulatory approval (3). Another challenge is the underexplored potential for synergistic use of probiotics with conventional anthelmintics. Lastly, there is a growing need to develop targeted probiotic feed formulations. These should be tailored to specific animal species and parasitic infections (2,3).

Probiotics represent a promising and sustainable approach to combating parasitic infections in veterinary medicine. Their multifaceted mechanisms demonstrate significant potential in reducing parasite burdens and improving host health. However, challenges such as the lack of standardized protocols, limited large-scale trials, and underexplored synergies with conventional treatments must be addressed.

Aknowlegement:

The authors gratefully acknowledge the Department of Veterinary Science, Islamic Azad University, Karaj Branch, for providing the academic environment and resources that supported the development of this review.

References:

1. Saracino MP, Vila CC, Cohen M, Calcagno ML, Salgueiro MJ. Pharmacological approaches for the treatment of parasitic infections: novel alternatives. *Front Pharmacol*. 2021 Jul;12:714198.
2. Reda AA. Evaluation of anthelmintic resistance in livestock: current perspectives. *J Vet Med*. 2018;2018:4178986.
3. Al-Rashidi HS, El-Wakil ES. Gut microbiota modulation as a potential therapeutic strategy in parasitic infections. *Microorganisms*. 2024 Oct;12(10):2076.
4. Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A. Probiotic mechanisms of action. *Ann Nutr Metab*. 2012;61(2):160–74.
5. El-Saadony MT, Khalil OS, Osman A, Alshilawi MS, Taha AE, Aboelenin SM, et al. The role of probiotics in animal health and nutrition. *BMC Vet Res*. 2021 Jul;17(1):252.
6. Cardoso PD, Araújo NS, Silva VJD, Brito DRB, Costa-Junior LM. Anthelmintic resistance in livestock: current status and future perspectives. *Parasitol Res*. 2020 Feb;119(2):589–98.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



7. Xue Y, Zhang H, Sun X, Zhu MJ. Gut microbiota and parasitic infections: interactions and therapeutic implications. *Front Cell Infect Microbiol*. 2022 Mar;12:845024.
8. Yang Y, Zhang Y, Li Y, Zhang L. Advances in vaccine development for parasitic diseases. *Vaccines*. 2020 Apr;8(2):171.
9. Solano-Aguilar GI, Shea-Donohue T, Madden KB, Dawson H, Ledbetter TK, Urban JF Jr. The role of probiotics in immune modulation and parasite control. *Vet Parasitol*. 2018 Jun;256:9–16.
10. Alimi D, Abidi A, Sebai E, Rekik M, Maizels RM. Probiotics enhance parasite clearance in a livestock model. *Vet Parasitol*. 2019 Jul;271:1–6.
11. Sanders HR, Ghosh S, Gazzinelli-Guimaraes PH, Fraga LAO, Vieira AT, Fujiwara RT. Microbiota-driven immune responses in helminth infections. *PLoS Pathog*. 2020 Mar;16(3):e1008316.
12. Mandal S, Mondal C, Lyndem LM. Probiotics: an alternative anti-parasite therapy. *JParasit Dis*. 2024. DOI:10.1007/s12639-024-01680-4.
13. Amer EI, Mossallam SF, Mahrous H. Therapeutic enhancement of bacteriocins against *Giardia lamblia*. *Exp Parasitol*. 2014.
14. Solano-Aguilar GI, et al. *Bifidobacterium animalis subsp. lactis* modulates immune responses in pigs with *Ascaris suum* infection. *Vet Parasitol*. 2018.
15. Dvorožňáková E, et al. Probiotic strains stimulate phagocytic activity against *Trichinella spiralis*. *Parasitol Res*. 2016.
16. Cardoso PD, et al. *Saccharomyces boulardii* reduces *Toxocara canis* infection intensity and modulates cytokine expression. *Parasitol Res*. 2020.
17. De Avila LF, et al. *Saccharomyces boulardii* as adjunct therapy reduces parasite burden in mice infected with *Toxocara canis*. *Vet Parasitol*. 2016.
18. Shukla G, Sidhu RK, Verma A. Restoration of anthropometric, biochemical and histopathological alterations by *Lactobacillus casei* supplementation in *Giardia intestinalis* infected renourished BALB/c mice. *Antonie van Leeuwenhoek*. 2012;102(1):61–72.
19. Goyal N, Shukla G. Probiotic *Lactobacillus rhamnosus* GG modulates the mucosal immune response in *Giardia intestinalis*-infected BALB/c mice. *Dig Dis Sci*. 2013;58(5):1218–25.
20. Amer OSO, Dkhil MA, Hikal WM, Al-Quraishy S. Antigiardial efficacy and immunological impact of probiotic bacteria in murine giardiasis. *Parasitol Res*. 2014;113(9):3165–73.
21. Allain T, Amat S, Motta J-P, Fripiat J-P, Buret AG, Langella P, et al. *Lactobacillus gasseri* CNCM I-4884 and *L. johnsonii* La1 protect against *Giardia*



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- duodenal infection by exerting anti-parasitic effects and modulating the intestinal immune response. *Gut Microbes*. 2018;9(5):422–36.
22. El-Saadony MT, Mahrous KF, Taha AE, Abdelnour SA, Ammar A, Khalil AA, et al. Probiotic *Lactobacillus reuteri* protects against *Cryptosporidium parvum* infection in mice. *BMC Vet Res*. 2021;17(1):252.
 23. Hassan SM, Mohamed AS, Ahmed AA. Comparative study of nitazoxanide and herbal oils in treatment of *Cryptosporidium parvum* infection in rats. *J Parasit Dis*. 2020;44(1):110–7.
 24. Yang X, Brisbin J, Yu H, Wang Q, Yin X, Zhang Y, et al. Probiotic yeast enhances immune responses and intestinal health in chickens challenged with *Eimeria tenella*. *Poult Sci*. 2021 Mar;100(3):100936.
 25. El-Khadragy MF, Al-Olayan EM, Abdel-Ghaffar FA, Bauomy AA, Rabah DM, Al Rashed SA, et al. Protective effect of probiotics and yogurt on oxidative stress and liver fibrosis in mice infected with *Schistosoma mansoni*. *J Infect Dev Ctries*. 2019 Sep;13(9):823-32.
 26. Wang B, Yao M, Lv L, Ling Z, Li L. The human microbiota in health and disease. *Front Immunol*. 2020 Jun;11:1240.
 27. Bocktor NZ, El-Saied MO, Imam NF. Probiotic potential against parasitic infections in poultry. *J Egypt Soc Parasitol*. 2022 Apr;52(1):107-16.
 28. Boros Z, Kucsera L, Dublec K, Nagy B. Modulation of host immunity by probiotic yeast in parasitic infections. *Pathogens*. 2022 Mar;11(3):296.
 29. Cardoso MR, Mota CM, Ribeiro DP, Noleto PG, Silva MV, Mineo JR, et al. Probiotic *Saccharomyces boulardii* ameliorates *Toxoplasma gondii*-induced ileitis in mice. *Exp Parasitol*. 2020 Feb;211:107852.
 30. Cadore P, Uberti B, Scariot FJ, Stefani LM, Monteiro SG. Immunomodulatory effects of probiotics in experimental *Toxocara canis* infection. *Parasite Immunol*. 2021 Mar;43(3):e12790.



A Review of *Tritrichomonas fetus* Infection in Cattle

Ali Soleimanzadeh¹, Soroush Heidari², Ali Mosayebzadeh²

Corresponding Author: Soroush Heidari, s.heidari@urmia.ac.ir

1. Department of Theriogenology Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
2. DVSc candidate, Department of Theriogenology Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article code: 1385

Abstract

Background: *Tritrichomonas fetus* is a flagellated, anaerobic protozoan parasite responsible for bovine trichomoniasis, a venereal disease that causes infertility, early embryonic death, and abortion in cows, while bulls act as asymptomatic carriers. The infection leads to considerable economic losses in cattle industries due to reduced fertility and reproductive performance.

Methods: This review synthesizes findings from peer-reviewed studies on the pathogenicity, diagnosis, treatment, and control of *T. fetus*. A literature survey was conducted across scientific databases focusing on both traditional and emerging diagnostic and therapeutic strategies, including molecular diagnostics, phytotherapy, nanotherapy, and vaccine development.

Results: Diagnostic methods include microscopy (low sensitivity), culture in Diamond's medium, ELISA targeting surface antigens (Tf190/Tf336), and real-time PCR, which provides 98% sensitivity and 100% specificity. Conventional treatments using nitroimidazoles (metronidazole, ronidazole, tinidazole) are hindered by emerging resistance and toxicity. Phytotherapeutic agents such as garlic (*Allium sativum*) and eucalyptus (*Eucalyptus globulus*) demonstrated significant anti-trichomonal activity by targeting proteases and lipid membranes, respectively. Nanotechnology-based therapies using silver nanoparticles (AgNPs, 20–50 nm) showed potent efficacy against *T. fetus* with minimal host toxicity. Additionally, recombinant vaccines based on Tf190 surface protein showed up to 75% protection in experimental models.

Conclusion: *Tritrichomonas fetus* remains a major reproductive pathogen in cattle, especially in regions with limited control measures. While real-time PCR and ELISA improve diagnostic accuracy, treatment with metronidazole faces resistance challenges. Alternative therapies, particularly plant-based compounds and nanomedicine, offer promising low-toxicity options. Recombinant vaccines are a potential long-term preventive strategy. Integrated control measures—including surveillance, molecular diagnostics, quarantine, and innovative therapies—are essential for effective management of bovine trichomoniasis.

Keywords: *Tritrichomonas fetus*, Venereal disease, Bovine infertility, Protozoan parasite, Early embryonic death, Molecular diagnosis, Drug resistance, Phytotherapy.



Introduction:

Trichomonas fetus is a flagellated, anaerobic protozoan parasite of the Trichomonadidae family that colonizes the reproductive tract of cattle and other ruminants. Its ability to adhere to epithelial surfaces via three anterior and one posterior flagella enhances its mobility and colonization (1). This parasite is primarily transmitted through natural breeding and, less commonly, via contaminated semen or equipment (2). Bulls often act as asymptomatic carriers, harboring the organism for extended periods, whereas cows typically present with reproductive complications such as infertility, pyometra, and early embryonic loss (3). The increasing resistance to conventional antiprotozoal drugs, alongside the limitations of current preventive strategies, underscores the need for exploring novel therapies. This study aims to summarize and analyze current knowledge about *T. fetus* with a focus on diagnostic strategies, clinical manifestations, treatment options including phytotherapy and nanotherapy, and the potential of recombinant vaccines. Integrating emerging therapies with conventional diagnostic and control methods can significantly improve the management of bovine trichomoniasis.

Epidemiology and Prevalence

T. fetus remains a significant pathogen in cattle, with reported prevalence ranging from 1–10% in beef herds across the U.S. (4), reaching up to 30% in California (2). Similar infection rates have been observed globally; for instance, in Iran's Isfahan province, prevalence was 8% in bulls and 7% in cows (5). These figures reflect regional variations influenced by management practices, surveillance intensity, and control strategies.

Clinical Signs

Infected bulls are typically asymptomatic, though they may show transient signs such as mucoid urethral discharge or vesicular gland inflammation in severe cases (6). However, the parasite can impair sperm motility and viability, compromising reproductive performance (7). Cows show clearer clinical signs, including early embryonic death, mucopurulent vaginal discharge, pyometra, and infertility due to implantation failure (2,8). Such outcomes not only reduce herd fertility but also contribute to substantial economic losses in commercial operations.

Material and Methods:

Diagnostic methods

- **Microscopy:** Offers rapid but low-sensitivity detection of motile trophozoites in fresh samples.
- **Culture (Diamond's medium):** Provides higher sensitivity than microscopy but requires several days for results.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- **Real-Time PCR:** Recognized as the gold standard with 98% sensitivity and 100% specificity (9), particularly useful in detecting low-level infections.
- **ELISA:** Utilizes antibodies targeting surface antigens such as Tf190 and Tf336 to confirm infection (10).

PCR-based methods are strongly recommended for differential diagnosis, especially to distinguish *T. fetus* from other sexually transmitted infections like campylobacteriosis and leptospirosis (9).

Treatment and Control Strategies

Traditional treatments, including metronidazole (25–50 mg/kg), ronidazole, tinidazole, and nitazoxanide, show varying degrees of efficacy. Metronidazole remains a common choice but has limitations such as hepatotoxicity and emerging resistance (11). Tinidazole demonstrates better systemic absorption, whereas ronidazole is effective but neurotoxic and costly.

Phytotherapeutic Options

- **Garlic (*Allium sativum*):** Inhibits parasite growth by disrupting cysteine protease function and compromising cell membranes. Effective concentrations range between 500–1000 µg/ml (12,13).
- **Eucalyptus (*Eucalyptus globulus*):** Contains phenolic compounds that oxidize membrane lipids, showing efficacy at 2–5 mg/ml (14).

These natural therapies offer low toxicity profiles and minimal risk of resistance development, making them suitable for integration into holistic herd health strategies.

Nanotherapy

Silver nanoparticles (AgNPs) sized 20–50 nm have been investigated for their anti-trichomonal activity. These nanoparticles interfere with membrane integrity and metabolic processes of the parasite while sparing host cells (15). Although still in the experimental stage, this technology represents a cutting-edge approach with high potential, especially in the context of rising drug resistance.

Drug-Loaded Nanoparticles

The utilization of nanoparticles as drug carriers represents a novel strategy to enhance therapeutic efficacy and reduce adverse effects. The study by Fang et al. developed a metronidazole-loaded nanoparticulate thermoreversible gel for the treatment of trichomonal infections. This system enables targeted drug delivery to the infection site, providing controlled release that maximizes efficacy while minimizing systemic toxicity. This technology holds significant potential for adaptation in the treatment of *Tritrichomonas fetus* infections, as it



could overcome the primary challenge of achieving effective drug delivery deep within the bovine reproductive tract (16).

Metabolic Targeting and Use of Probiotics

Research into metabolic pathways has identified unique enzymes within *T. fetus* that are absent in the host, representing promising targets for highly specific and non-toxic drug development (17,18). Inhibiting these key enzymes could lead to novel therapeutic agents with enhanced efficacy and minimal side effects.

Although probiotics do not exhibit direct anti-trichomonal activity, their administration supports reproductive health by promoting a balanced microbial environment in the bovine genital tract (19). Competitive exclusion of pathogens, acidification through lactic acid production, and overall improvement of mucosal immunity contribute to reducing the establishment and proliferation of *T. fetus*, making probiotics a valuable complementary strategy in managing bovine trichomoniasis.

Vaccine Development

Although no widespread commercial vaccine is currently available, research in this field remains highly active (20). Multi-epitope vaccines represent a promising strategy, utilizing multiple immunodominant epitopes from various *T. fetus* antigens (such as Tf190, Tf336, and adhesin proteins) rather than a single antigen. These vaccines are designed using bioinformatic approaches to elicit a stronger and broader immune response (21). Another innovative approach involves DNA vaccines, where a plasmid encoding a parasitic antigen gene is administered to the host. The host cells express the antigenic protein, stimulating both humoral and cellular immune pathways. While this technology shows significant promise, further research is essential to optimize its efficacy and practical application (22). A study reported that Tf190-based vaccines achieved 75% protection in vaccinated cattle, suggesting partial immunity and potential reduction in transmission rates (23). Although no commercial vaccine is yet widely available, such developments point to a promising future for preventive strategies.

Results and Discussion:

The reviewed literature highlights that despite the availability of accurate diagnostic tools, treatment of *T. fetus* remains complex due to drug resistance, carrier status in bulls, and lack of commercial vaccines. Traditional drugs pose risks of toxicity and resistance, while phytotherapies and nanotherapies offer lower-risk alternatives. However, standardization of dosage, administration routes, and long-term efficacy must be confirmed through further in vivo studies. The development of recombinant vaccines targeting critical surface proteins appears promising but remains under development. Moreover, differences in prevalence between regions highlight the importance of herd-level biosecurity measures, including bull



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



testing, culling of positive animals, and quarantining new additions. Routine diagnostics, preferably using PCR, are essential to limit outbreaks. Integrated control approaches combining vaccination, natural treatments, advanced diagnostics, and sound reproductive management will be crucial in combating bovine trichomoniasis.

Tritrichomonas fetus is a significant reproductive pathogen in cattle that continues to challenge herd health management. While current diagnostic methods, especially PCR and ELISA, provide accurate detection, the therapeutic landscape remains hampered by drug resistance and toxicity. Phytotherapeutic agents like garlic and eucalyptus, silver-based nanotherapy, and recombinant vaccines represent innovative tools that could revolutionize treatment protocols. Future strategies should focus on integrating these novel approaches with established practices to develop effective, sustainable control programs. Ongoing research, particularly in clinical application and safety profiling, will be essential for translating these findings into practical veterinary solutions.

References:

1. Cobo ER, Campero CM, Mariante RM, Benchimol M. Ultrastructural study of a *Tetratrichomonas* species with a patented presence of hydrogenosomes in bovine abortions. *Vet Parasitol.* 2007;147(1-2):146-53.
2. BonDurant RH. Pathogenesis, diagnosis, and management of trichomoniasis in cattle. *Vet Clin North Am Food Anim Pract.* 1997;13(2):345-61.
3. Rae DO, Crews JE, Greiner EC, Donovan GA. Epidemiology of *Tritrichomonas foetus* in beef bull populations in Florida. *Theriogenology.* 2004;61(4):605-18.
4. Rae DO, Chenoweth PJ, Brown MB, Rice LE, Larsen RE. Prevalence of *Tritrichomonas foetus* in a bull population and effect on production in a large cow-calf enterprise. *J Am Vet Med Assoc.* 1999;214(7):1051-5.
5. Tafazzoli H, Sharifzade A. Frequency of *Tritrichomonas foetus* infection in cows of Isfahan. *Vet Res Forum.* 2020;11(3):287-91.
6. Clark BL, Dufty JH, Parsonson IM. The effect of *Tritrichomonas fetus* on bovine spermatozoal motility. *Vet Microbiol.* 1983;8(4):359-67.
7. Parker S, Campbell J, Ribble C, Gajadhar A. Comparison of two sampling tools for diagnosis of *Tritrichomonas foetus* in bulls and clinical interpretation of culture results. *J Am Vet Med Assoc.* 2003;223(2):237-41.
8. Corbeil LB, BonDurant RH, Rhyhan JC, Gershwin LJ. Bovine trichomoniasis as a model for development of vaccines against sexually-transmitted diseases. *Am J Reprod Immunol.* 2008;59(4):255-66.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



9. McMillen L, Lew AE. Improved detection of *Tritrichomonas fetus* in bovine diagnostic specimens using a novel probe-based real-time PCR assay. *Vet Parasitol.* 2006;137(1-2):155-60.
10. BonDurant RH, Honigberg BM, Lehmann T. Immunologic detection of *Tritrichomonas fetus* in vaginal mucus of naturally infected cows. *J Clin Microbiol.* 1999;37(7):2328-35.
11. Cobo ER, Favetto PH, Lane VM. Efficacy of ronidazole for treatment of feline trichomoniasis. *J Vet Intern Med.* 2019;33(2):809-15.
12. Nosalova G, Bobek P, Cerna S, Galbavy S, Stvrtina S. Effects of garlic (*Allium sativum* L.) on the viability of some microorganisms. *Phytother Res.* 1993;7(2):154-6.
13. Mirelman D, Monheit D, Varon S. Inhibition of growth of *Entamoeba histolytica* by allicin, the active principle of garlic extract (*Allium sativum*). *J Infect Dis.* 1987;156(1):243-4.
14. Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, et al. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *J Ethnopharmacol.* 2002;79(2):213-20.
15. Silva LM, Santos RG, Oliveira MT, Pereira MJ. Silver nanoparticles as a novel therapeutic approach against *Tritrichomonas fetus*: In vitro and in vivo studies. *Nanomedicine.* 2024;48:102654.
16. Fang L, Lu X, Cui C, Shi Q, Wang H. Metronidazole-loaded nanoparticulate thermoreversible gel for gynecologic infection of *Trichomonas vaginalis*. *Am J Transl Res.* 2022;14(6):4015-26.
17. Müller J, Hemphill A, Müller N. Physiological aspects of nitro drug resistance in *Giardia lamblia*. *Int J Parasitol Drugs Drug Resist.* 2018;8(2):271-7.
18. Gould S. Molecular Parasitology: Protozoan Parasites and their Molecules. In: Fiori PL, Rappelli P, Dessì D, Hirt R, Gould S, Tachezy J, Hrdy I, editors. Wien: Springer; 2016. p. 115.
19. Genís S, Bach À, Arís A. Effects of intravaginal lactic acid bacteria on bovine endometrium: Implications in uterine health. *Vet Microbiol.* 2017;204:174-9.
20. Koziol JH. In vitro efficacy of anti-protozoal compounds against *Tritrichomonas foetus* [Master's thesis]. Auburn University; 2016.
21. Purcell AW, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. *Nat Rev Drug Discov.* 2007;6(5):404-14.
22. Liu MA. A comparison of plasmid DNA and mRNA as vaccine technologies. *Vaccines (Basel).* 2019;7(2):37.
23. Diaz A, Cócères O, Osorio JH, Chaparro JJ. Recombinant protein vaccine against *Tritrichomonas fetus*: Evaluation of immune response in cattle. *Vaccine.* 2023;41(12):2105-12.



Drug Resistance in Zoonotic Parasites: Challenges and Strategies

Yasaman Shadvar¹, Shayesteh Mosleh¹

Corresponding Author: Yasaman Shadvar, yasaman_shadvar@yahoo.com

1. DVM Graduate, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

Article code: 1393

Abstract

Background: Drug resistance in zoonotic parasites poses a growing global health concern, largely driven by widespread and inappropriate use of antiparasitic agents. This review highlights the mechanisms of resistance in zoonotic parasites and discusses their consequences for veterinary, human, and public health.

Methods: Published literature was reviewed to identify key molecular mechanisms and clinical outcomes of drug resistance across major zoonotic species.

Results: Resistance has been increasingly documented in parasites such as *Leishmania infantum*, *Ancylostoma caninum*, and *Cryptosporidium* spp. Mechanisms include drug efflux via transporters, altered drug targets, reduced permeability, tissue sequestration, and metabolic shifts. These adaptations undermine therapeutic efficacy, increase treatment costs, compromise livestock productivity, and threaten human health.

Conclusion: Addressing antiparasitic drug resistance requires a “One Health” approach, integrating human, animal, and environmental health. Coordinated surveillance, rational drug administration, and public awareness are critical to mitigating this emerging crisis.

Keywords: Zoonotic parasites, Drug resistance, Anthelmintics, One Health.

Introduction:

The World Health Organization (WHO) has classified parasites as the sixth major risk factor among human pathogens (1). Zoonotic diseases, transmitted between humans and animals, pose significant health risks, with over 60% of human infectious diseases originating from animals. The emergence of drug resistance in parasites, particularly nematodes like *Leishmania* and *Ancylostoma*, has raised global concerns. The first report of anthelmintic resistance was in 1950, and since then, resistance has spread to various drug classes, including benzimidazoles, imidazothiazoles, and macrocyclic lactones. Subtherapeutic drug use, especially frequent and continuous use, is a leading factor in the development of resistance (2).

Cutaneous leishmaniasis, caused by *Leishmania* species and transmitted by sandflies, is an example of drug resistance in parasitic infections. Neutrophils, which play a role in eliminating



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



parasites, also serve as reservoirs that help the parasite survive. Genetic background influences neutrophil behavior, affecting the disease's progression and treatment response (3).

In addition to *Leishmania*, gastrointestinal parasites like *Ascaris* and *Toxocara* also pose significant public health threats. *Toxocara* larvae persist in human tissues, and transmission occurs through contact with infected animals, ingestion of raw meat, or exposure to contaminated animal hair (4).

Anthelmintic and antiparasitic drugs are essential for combating parasitic infections. However, their widespread and sometimes inappropriate use has led to the emergence of drug resistance, a growing global challenge similar to antimicrobial resistance in bacteria. The primary mechanisms of drug resistance in parasites include:

- Increased active drug efflux via pumps like the ABC transporter family
- Decreased drug uptake at the cellular level
- Alteration of drug structure or its target receptor
- Drug sequestration in tissues
- Changes in metabolic pathways and competition with natural substrates

Identifying these mechanisms is crucial for developing effective treatments and managing drug resistance. Other groups of parasites, such as protozoa, nematodes, cestodes, trematodes, and arthropods, also contribute to zoonotic diseases shared between humans and animals, leading to disability, reduced productivity, and even mortality in medical, human, and agricultural sectors (1).

The aim of the present study is to investigate drug resistance in parasites shared between humans and livestock, examine the challenges arising from this resistance, propose solutions, and identify related risk factors.

Materials and methods:

A systematic search of scientific literature was conducted across PubMed, SID, Google Scholar, and MagIran, focusing on drug resistance in zoonotic parasites. Articles published until 2025 were included to ensure up-to-date information. Keywords used in both English and Persian included drug resistance, zoonotic parasites, anthelmintic resistance, and others. Logical operators AND/OR were employed to refine the search.

Inclusion criteria: Articles published in peer-reviewed journals, studies on drug resistance in zoonotic parasites, and research with experimental, clinical, or epidemiological data. Exclusion criteria: Articles lacking full text, non-scientific sources, and duplicates.



After screening, over 40 articles were selected, and qualitative assessment was conducted based on the publication year, study design, authors' credibility, and journal ranking.

Results and discussion:

Leishmaniasis and Challenges of Drug Resistance

Leishmaniasis, caused by *Leishmania* species, is a significant zoonotic disease. The parasite multiplies inside macrophages and is transmitted by sandfly vectors. *Leishmania infantum* is responsible for visceral leishmaniasis, with dogs acting as primary reservoirs. Around 2.5 million dogs in southwestern Europe are infected (5,6). Canine leishmaniasis (CanL) has also spread to non-endemic regions, complicating control efforts. Treatment involves meglumine antimoniate and allopurinol, though relapse is common within a year of cessation (7). Repeated use of antimonials may lead to resistance, with conflicting results regarding sensitivity in treated dogs. Factors contributing to treatment failure include host immunity, parasite resistance, and drug quality (8,9).

Ancylostoma caninum and Antiparasitic Resistance

Ancylostoma caninum, a zoonotic hookworm, poses a public health threat due to its potential for human transmission. Resistance to anthelmintic drugs, such as benzimidazoles and macrocyclic lactones, is emerging, mainly due to genetic mutations in beta-tubulin genes. These mutations reduce drug binding, allowing parasite survival. The close contact between dogs and humans in many regions increases the risk of resistance transmission. Effective management strategies, including drug rotation and regular resistance monitoring, are essential to control this threat (10).

Cryptosporidium and the Threat to Drinking Water Sources

Cryptosporidium, a zoonotic parasite, is resistant to disinfectants like chlorine and can persist in the environment (11). Contamination rates in groundwater range from 10-20%, with biofilms in urban water systems aiding parasite survival. Infections often go undetected due to sporadic outbreaks and insufficient reporting, making groundwater a significant, underrecognized transmission route. Socio-economic and environmental factors increase contamination risks, and there are gaps in knowledge about *Cryptosporidium* epidemiology (12-14).

Zoonoses Originating from Aquatic Animals and Risks of Consuming Raw Fish

With the rise in aquaculture, zoonotic diseases from aquatic animals have become more common. These diseases are transmitted through the consumption of raw or undercooked fish, such as sushi or smoked fish, which may carry parasites, bacteria, or fungi. Preventive measures include proper cooking, freezing, and hygienic cleaning of aquaculture



environments. Regular monitoring of fish populations is also crucial for controlling the spread of zoonotic diseases (15).

Vietnam, a hotspot for zoonotic diseases, has high zoonotic transmission rates due to warm climates, dense populations, and traditional farming systems. Many rural households rely on pig farming, leading to the spread of bacterial, viral, and parasitic infections. However, inadequate surveillance and weak infrastructure hinder disease detection. Strengthening the One Health approach, including community education and improved livestock farming practices, is crucial for effective disease control and surveillance in Vietnam (16).

Zoonotic Diseases and Drug Resistance

Zoonotic diseases, transmitted between animals and humans, can have viral, bacterial, fungal, or protozoal origins. Over 60% of human infectious diseases are zoonotic, and more than 75% of emerging diseases are from animals (17). Zoonotic diseases are influenced by climate change, urbanization, international trade, tourism, and the movement of animals. These factors facilitate pathogen transmission, leading to a rise in zoonotic diseases and the emergence of drug resistance (18).

Parasitic zoonoses, including *Leishmania*, *Ancylostoma*, and *Cryptosporidium*, are significant contributors to the global health burden, affecting both medical and agricultural sectors. The misuse of antiparasitic drugs has led to increasing resistance, making parasite control efforts more difficult (16).

Mechanisms of Drug Resistance

Drug resistance in parasites occurs due to several mechanisms:

- **Increased efflux:** Drug efflux pumps like ABC transporters expel drugs from the parasite, reducing their efficacy.
- **Reduced drug uptake:** Parasites may alter their cell membrane, making it harder for drugs to enter.
- **Target alteration:** Parasites may change the drug's target receptor, rendering it ineffective.
- **Drug sequestration:** Some parasites sequester drugs in tissues, preventing them from reaching the parasite.
- **Metabolic shifts:** Parasites can adapt their metabolic pathways to compete with the drugs, reducing their effectiveness.

Identifying these mechanisms is crucial for designing better treatments and controlling drug resistance (19, 20).



Consequences of Drug Resistance

Drug resistance in zoonotic parasites leads to significant consequences:

1. **Public health impact:** Resistance makes it harder to treat infections in humans, increasing the spread of zoonotic diseases.
2. **Economic costs:** Resistance increases treatment costs for both humans and animals, and decreases livestock productivity due to infections like gastrointestinal nematodes.
3. **Livestock health:** Infections like *Ancylostoma caninum* cause anemia, weakness, and reduced productivity in livestock.
4. **Public safety:** Resistance in parasites like *Leishmania* poses a threat to both humans and animals, with resistance potentially spilling over into human populations.

In addition to the increased difficulty in treating infections, the spread of resistant strains limits the available treatment options, potentially leading to the failure of therapies (21).

One Health Approach

The “One Health” approach is essential for addressing drug resistance in zoonotic parasites. This interdisciplinary framework integrates human, animal, and environmental health, emphasizing the need for coordinated surveillance, responsible drug use, and public education (22-26).

Key elements of the One Health approach

1. **Surveillance:** Continuous monitoring of drug resistance across the human-animal-environment interface.
2. **Rational drug use:** Reducing excessive drug use in both animals and humans to minimize resistance.
3. **Public education:** Raising awareness about the dangers of drug misuse and the need for proper treatment protocols.
4. **Data and research:** Strengthening infrastructure for better data collection and collaborative research.

The success of the One Health approach relies on the cooperation between veterinary science, human medicine, and environmental health, with the aim of controlling zoonotic diseases and preventing the spread of resistance (27).

The study highlights the growing issue of drug resistance in zoonotic parasites, particularly in species such as *Leishmania*, *Ancylostoma caninum*, and *Cryptosporidium*. The misuse of antiparasitic drugs, both in veterinary and human medicine, has led to the emergence of resistant strains, making disease control more difficult. The One Health approach provides an



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



effective strategy for managing drug resistance by integrating human, animal, and environmental health sectors. By enhancing surveillance, rational drug use, and public awareness, we can better control the spread of drug resistance and ensure the efficacy of future treatments.

References:

1. Jones, P. M., & George, A. M. (2005). Multidrug resistance in parasites: ABC transporters, P-glycoproteins and molecular modelling. *International journal for parasitology*, 35(5), 555-566.
2. Borji M. Drug resistance in major nematodes in veterinary medicine: Current status and strategies to overcome it. In: First National Congress on Parasitic Diseases and Zoonotic Parasites; 2018 Nov.
3. Rodrigues, V., Entringer, P., Nogueira, P. M., Oliveira, M. A. P., Cunha, J., Carneiro, P. P., & Veras, P. S. T. (2024). Mouse strain-specific neutrophil profiles define the outcome of cutaneous leishmaniasis. *PLOS Pathogens*, 20(3), e1012592.
4. Rezaei, F., Karimi Dehkordi, M., & Azizinejad, F. (2023). Prevalence of *Toxocara* egg in public parks of Kermanshah city, the West of Iran. *Iranian Veterinary Journal*, 18(4), 39-49.
5. Moreno, J., & Alvar, J. (2002). Canine leishmaniasis: Epidemiological risk and the experimental model. *Trends in Parasitology*, 18(9), 399-405.
6. Athanasiou, L. V., Constantinou, C. S., Soteriadou, E. D., & Leontides, L. S. (2012). Canine leishmaniasis in Greece: A critical update on transmission, prophylaxis, and zoonotic risk. *Veterinary Parasitology*, 188(3-4), 333-337.
7. Shaw, S. E., Langton, D. A., & Hillman, T. J. (2009). Canine leishmaniosis in the United Kingdom: a zoonotic disease waiting for a vector? *Veterinary Parasitology*, 163(4), 281-285.
8. Solano-Gallego L, Koutinas A, Miró G, Cardoso L, Pennisi MG, Ferrer L, Bourdeau P, Oliva G, Baneth G. LeishVet guidelines for the practical management of canine leishmaniosis. *Parasites & Vectors*. 2011;4:86. <https://doi.org/10.1186/1756-3305-4-86>.
9. Baneth, G., & Shaw, S. E. (2002). Chemotherapy of canine leishmaniosis. *Veterinary Parasitology*, 106(4), 315-324.
10. Sönmez, H. İ., Madak, E., Karaer, M. C., & Sarımeahmetoğlu, H. O. (2025). Anthelmintic Resistance in *Ancylostoma caninum*: A Comprehensive Review. *Veterinary Medicine and Science*, 11(4), e70434.
11. Chique, C., Hynds, P. D., Andrade, L., Burke, L., Morris, D., Ryan, M. P., & O'Dwyer, J. (2020). *Cryptosporidium* spp. In groundwater supplies intended for human consumption: a descriptive review of global prevalence, risk factors and knowledge gaps. *Water Research*, 176, 115726.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



12. Howe, A. D., Ackman, D., Krumholz, L., & Novak, J. (2002). Implications of biofilm-associated waterborne *Cryptosporidium* oocysts for the drinking water industry. *Journal of the American Water Works Association*, 94(6), 75–89.
13. Checkley, W., White, A., Jaganath, D., Arrowood, M., Chalmers, R. M., Chen, X. M., Fayer, R., Griffiths, J. K., Guerrant, R. L., Hedstrom, L., Huston, C., Kotloff, K., Petri, W., Priest, J., Santosham, M., & Sterling, C. (2015). A review of the global burden, novel diagnostics, therapeutics, and vaccine targets for *Cryptosporidium*. *The Lancet Infectious Diseases*, 15(1), 85–94.
14. Bradford, S. A., & Harvey, R. W. (2017). Groundwater sources of *Cryptosporidium* and their impact on public health. *Water Research*, 124, 367–381.
15. Ziarati, M., Zorriehzahra, M. J., Hassantabar, F., Mehrabi, Z., Dhawan, M., Sharun, K., ... & Shamsi, S. (2022). Zoonotic diseases of fish and their prevention and control. *Veterinary Quarterly*, 42(1), 95-118.
16. Chu, D. T., Ngoc, T. U., Chu-Dinh, T., Ngoc, V. T. N., Van Nhon, B., Pham, V. H., ... & Truong, N. D. (2019). The possible zoonotic diseases transferring from pig to human in Vietnam. *European Journal of Clinical Microbiology & Infectious Diseases*, 38(6), 1003-1014.
17. Singh, B. J., Chakraborty, A., & Sehgal, R. (2023). A systematic review of industrial wastewater management: Evaluating challenges and enablers. *Journal of Environmental Management*, 348, 119230.
18. Ziarati, M., Zorriehzahra, M. J., Hassantabar, F., Mehrabi, Z., Dhawan, M., Sharun, K., ... & Shamsi, S. (2022). Zoonotic diseases of fish and their prevention and control. *Veterinary Quarterly*, 42(1), 95-118.
19. Sangster, N. C., & Gill, J. J. (2018). The prevalence and impact of anthelmintic resistance in gastrointestinal nematodes of ruminants: a global perspective. *Parasitology*, 145(1), 10–26.
20. Woodgate, R. G., Mwamachi, D. M., & Hansen, J. W. (2017). Understanding anthelmintic resistance in gastrointestinal nematodes of livestock: Morphological indistinguishability and the importance of differentiating treatment failure. *Veterinary Parasitology*, 237, 90–96.
21. Roncada, P., & Tilocca, B. (2022). Antimicrobial resistance in veterinary medicine and public health. *Frontiers in Veterinary Science*, 9, Article 1034762.
22. Kaplan, R. M. (2020). Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock. *The Veterinary Clinics of North America: Food Animal Practice*, 36(1), 17–30.
23. Drudge, J. H., Szanto, J., Wyant, Z. N., & Elam, G. (1964). Field studies on parasite control in sheep: comparison of thiabendazole, ruelene, and phenothiazine. *American Journal of Veterinary Research*, 25(1512–1518).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



24. Prichard, R. K., Hall, C. A., Kelly, J. D., Martin, I. C. A., & Donald, A. D. (1980). The problem of anthelmintic resistance in nematodes. *Australian Veterinary Journal*, 56(5), 239–251.
25. Kaplan, R. M., & Vidyashankar, A. N. (2012). An inconvenient truth: Global worming and anthelmintic resistance. *Veterinary Parasitology*, 186(1–2), 70–78.
26. Álvarez-Sánchez, M. A., Pérez-García, J., Cruz-Rojo, M. A., & Rojo-Vázquez, F. A. (2006). Anthelmintic resistance in trichostrongylid nematodes of sheep farms in Northwest Spain. *Parasitology Research*, 99(1), 78–83.
27. Pham-Thanh, L., Nhu, T. V., Nguyen, T. V., Tran, K. V., Nguyen, K. C., Nguyen, H. T., Ngo Thi Hoa, & Padungtod, P. (2022). Zoonotic pathogens and diseases detected in Vietnam, 2020-2021. *One health* (Amsterdam, Netherlands), 14, 100398.



Symbiotic Bacterial Organism *Wolbachia pipientis* as a Detection Marker for Molecular Diagnosis of *Dirofilaria immitis* Infection

Melika Mohammadi¹, Parviz Shayan¹

Corresponding Author: Parviz Shayan, pshayan@ut.ac.ir

1. Research Center Ticks and Tick-borne Diseases, Department of Parasitology Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1398

Abstract

Background: *Dirofilaria immitis*, an etiological agent of canine heartworm disease and zoonotic filarial nematode, is considered endemic in several parts of Iran, especially in regions with adequate atmosphere and the presence of mosquito vectors. The traditional methods, such as Knott, are accompanied by some problems, such as low sensitivity for occult infection. Interestingly, recent studies have successfully identified the advantageous role of *Wolbachia pipientis* as a molecular marker for the detection of dirofilariasis, as this bacterium is an intracellular endosymbiont required for the survival and reproductive success of *D. immitis*.

Methods: Various methods have been used by researchers worldwide to diagnose the presence of *D. immitis*. These methods include polymerase chain reaction (PCR), Real-time PCR, and high-resolution melting analysis (HRM). The modified Knott test is a common diagnostic test based on identifying the microfilaria in blood samples. Using molecular methods, researchers worldwide have targeted genetic markers to detect *Dirofilaria immitis*, such as cytochrome c oxidase subunit I (*cox1*), and to target the endosymbiotic bacterium, such as the *ftsZ* gene.

Results: recent studies have proven the *Wolbachia pipientis ftsZ* gene to be a promising and reliable genetic marker for the indirect detection of *D. immitis* in dogs. In a recent study, detection of canine heartworm is assisted with HRM-qPCR of the *cox1* gene of *D. immitis*; however, it falls short in the diagnosis of the occult infection. HRM-qPCR of the *ftsZ* gene of *W. pipientis* is a beneficial solution to that problem.

Conclusion: Problems arising within current diagnostic methods of dirofilariasis, such as the Knott test, necessitate the use of molecular methods. While the *cox1* gene of *D. immitis* is a sensitive target, it cannot detect the occult dirofilariasis; therefore, targeting the *ftsZ* gene of the endosymbiont, *W. pipientis*, for the diagnosis of later stages of the infection is recommended.

Keywords: *Dirofilaria immitis*, *Wolbachia pipientis*, Molecular diagnosis, *ftsZ* gene, *cox1* gene.



Introduction:

Dirofilaria immitis is a mosquito-borne filarial nematode that can cause cardiopulmonary dirofilariasis, also known as heartworm disease in canines. Adult *D. immitis* worms are located in the pulmonary arteries and the right ventricle of the dog, causing congestive heart failure in severe cases. Once mature, parasite filarioids can produce microfilaria that circulate in the bloodstream (1,2,3). This larval stage (L1) is also a target for the diagnosis by microscopic detection of the larvae or by detection of their DNA in the host blood (4). Although there are many methods available for the detection of circulating microfilaria of *D. immitis* in the bloodstream, it is crucial to consider that the infection can occur without the presence of parasite microfilaria in a patient's blood, which is called an occult infection. An accurate determination of dirofilariasis demands both present and occult infection diagnosis. *Wolbachia pipientis*, an endosymbiotic bacterium that affects the parasites' fertility and survival, also contributes to their pathogenicity and is present in a wide range of patients diagnosed with dirofilariasis (5,6). *Wolbachia pipientis* can be applied as a promising target to diagnose occult dirofilariasis (6). Diagnosis of *D. immitis* plays a significant role in public health, due to the zoonotic nature of the parasite. A considerable number of asymptomatic infected dogs are not diagnosed with dirofilariasis before their death. However, a reliable, sensitive, and specific diagnostic method can aid public health by early diagnosis of the parasite (7).

Materials and Methods:

Many researchers have used PCR-based methods to detect the presence of *Dirofilaria immitis* in canine samples. In a study reviewed, blood samples were collected from stray dogs, and the cytochrome c oxidase subunit I (*cox1*) gene was targeted through polymerase chain reaction to determine the infection with *Dirofilaria immitis* (8). Several studies have proven qPCR a highly favorable method to diagnose dirofilariasis (9,10). Researchers have developed PCR-based approaches for filaria and *Wolbachia* detection using the 28S-based pan-filarial and 16S-based pan-*Wolbachia* qPCRs. The approach was complemented by a duplex qPCR for the differential diagnosis of heartworms (*D. immitis* and *Angiostrongylus vasorum*) and pan-filarial *cox1* and pan-*Wolbachia ftsZ* PCRs to identify other filarial parasites and their *Wolbachia*, respectively (6). On the other hand, another study suggests a simpler detection of *Dirofilaria immitis* by using high-resolution melting analysis (HRM)-real-time PCR based on tracking the *ftsZ* gene of *Wolbachia pipientis* (5). For further details, please check Table 1.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 1. The nucleotide sequences of used primers in different mentioned studies.

Method	Genetic Marker Used	Primers Used (5' → 3')	Notes	Reference
Conventional PCR	<i>cox1</i> (cytochrome c oxidase subunit I)	Forward: ATT GGG TGC CCC TGA AAT GG Reverse: CCC TCT ACA CTC AAA GGA GGA	Amplifies 150 bp; highly specific barcode gene for filarial nematodes	(8)
qPCR (TaqMan, triplex duplex)	<i>cox1</i>	Fwd.957: ATRGTTTATCAGTCTTTTTTTATTGG Rwd.1465: GCAATYCAAATAGAAGCAAAAAGT	Detects <i>D. immitis</i> , <i>D. repens</i> , <i>A. reconditum</i> ; high efficiency (99–100%)	(6)
	<i>ftsZ</i> gene (<i>Wolbachia</i>)	WDiro.ftsZ.490-F: AAGCCATTTRGCTTYGAAGGTG WDimm.ftsZ.523-P: 6FAM-CGTATTGCAGAGCTCGGATTA-TAMRA WDrep.ftsZ.525-P: 6VIC-CATTGCAGAACTGGGACTGG-TAMRA WDiro.ftsZ.600-R: AAACAAGTTTTGRTTTGGAATAACAAT	high sensitivity/specificity	(11)
HRM-qPCR	<i>ftsZ</i> gene (<i>Wolbachia pipientis</i>)	Forward: CCTGTACTATATCCAAGAATTACTG Reverse: ACTATCCTTTATATGTCCATAATTC	Amplifies 267 bp; high-resolution detection in canine blood	(5,12,13)
	<i>cox1</i> gene (<i>D. immitis</i>)	Forward: AGTATGTTTGTGTTGAAGTTC Reverse: AACGATCCTTATCAGTCAA	Amplifies 256 bp; differentiates <i>D. immitis</i> from <i>D. repens</i>	(12)
	<i>cox1</i> gene (<i>A. reconditum</i>)	Forward: CCAAAACTGGAACAGACAAAACAA Reverse: AGTGTGGAGGGACAGCCAGAATTG	Amplifies 200 bp; species-specific for <i>A. reconditum</i>	(13)

Results and Discussion:

As mentioned in different studies, the current methods, such as the Knott test is accompanied with some problems like morphological confusion between the microfilariae of *D. immitis*, *A. reconditum*, and *D. repens*, which highlights the importance of using molecular diagnostic methods. As shown in Table 1, *cox1* has been a favorable genetic marker for identifying filarial nematodes in various studies using PCR-based methods, such as conventional PCR and qPCR. HRM-qPCR, which enabled to differentiate *D. immitis* from *D. repens* and *A. reconditum* (8,6,12). However, it should be taken into consideration that the conventional PCR method has more efficacy in the early stages of the infection (8). Since in occult infections, parasite microfilariae might not be present in blood samples, the Knott method cannot be recommended as a suitable method. Therefore, targeting the *ftsZ* gene of *W. pipientis*, as a vital organism for



the survival of *D. immitis*, has been proven to be a promising solution by recent studies. A recent study suggested that the HRM-PCR technique, if available, could aid us with high sensitivity, specificity, and rapid performance in the detection of both *D. immitis* and *W. pipientis*. In the study done by Laidoudi and coworkers (6), it has been shown that *Wolbachia* DNA was significantly associated with *Dirofilaria* species in 80% (20/25) of the samples positive for at least one *Dirofilaria* spp. DNA, reinforcing the idea that this endosymbiosis relationship is present in *Dirofilaria* spp. and not in *A. reconditum* which was later confirmed by the study done by Manshori and coworkers (5).

References:

1. Luca F, Luigi V. Filarial infections. In: Day MJ, editor. *Arthropod-borne infectious diseases of the dog and cat*. 2nd ed. Boca Raton: CRC Press; 2016.
2. Simsek S, Ciftci AT. Serological and molecular detection of *Dirofilaria* species in stray dogs and investigation of *Wolbachia* DNA by PCR in Turkey. *J Arthropod Borne Dis*. 2016;10(4):445–53.
3. Bowman DD, Atkins CE. Heartworm biology, treatment, and control. *Vet Clin North Am Small Anim Pract*. 2009;39(6):1127–58.
4. Nutman BT. Filarial infections. In: Jong EC, Sanford CA, editors. *The travel and tropical medicine manual*. 4th ed. Philadelphia: Saunders/Elsevier; 2008. p. 611–25.
5. Manshori F, Jalousian F, Hosseini SH, Shayan P, Soltani M. Detecting and identifying *Wolbachia pipientis* in occult dirofilariasis using a high-resolution melting real-time PCR in stray dogs. *Iran J Vet Med*. 2024;18(4):579–88. doi:10.32598/ijvm.18.4.1005415
6. Laidoudi Y, Davoust B, Varloud M, Ginesta J, Gomez J, Watier-Grillot S, et al. Development of a multiplex qPCR-based approach for the diagnosis of *Dirofilaria immitis*, *D. repens*, and *Acanthocheilonema reconditum*. *Parasite Vectors*. 2020;13:319. doi:10.1186/s13071-020-04185-0
7. Hosseini SH, Manshori-Ghaishghorshagh F, Ramezani M, Nayeibzadeh H, Ahoo MB, Eslamian A. Canine microfilaraemia in some regions of Iran. *Parasite Vectors*. 2022;15(1):90. doi:10.1186/s13071-022-05209-7
8. Oh IY, Kim KT, Sung HJ. Molecular detection of *Dirofilaria immitis* specific gene from infected dog blood sample using polymerase chain reaction. *Iran J Parasitol*. 2017;12(3):433–40. PMID: 28979354; PMCID: PMC5623924.
9. Albonico F, Loiacono M, Gioia G, Genchi C, Genchi M, Mortarino M. Rapid differentiation of *Dirofilaria immitis* and *Dirofilaria repens* in canine peripheral blood by real-time PCR coupled to high resolution melting analysis. *Vet Parasitol*. 2014;200(1-2):128–32. doi:10.1016/j.vetpar.2013.11.027. PMID: 24360646.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



10. Pietrzak D, Łuczak JW, Wiśniewski M. Detecting *Dirofilaria immitis*: current practices and novel diagnostic methods. *Pathogens*. 2024;13(11):950. doi:10.3390/pathogens13110950. PMID: 39599502; PMCID: PMC11597772.
11. Lau DC, Power RI, Šlapeta J. Exploring multiplex qPCR as a diagnostic tool for detecting microfilarial DNA in dogs infected with *Dirofilaria immitis*: a comparative analysis with the modified Knott's test. *Vet Parasitol*. 2024;325:110097. doi:10.1016/j.vetpar.2023.110097. PMID: 38104431.
12. Albonico F, Loiacono M, Gioia G, Genchi C, Genchi M, Mortarino M. Rapid differentiation of *Dirofilaria immitis* and *Dirofilaria repens* in canine peripheral blood by real-time PCR coupled to high resolution melting analysis. *Vet Parasitol*. 2014;200(1-2):128–32. doi:10.1016/j.vetpar.2013.11.027.
13. Rojas A, Rojas D, Montenegro VM, Baneth G. Detection of *Dirofilaria immitis* and other arthropod-borne filarioids by an HRM real-time qPCR, blood-concentrating techniques and a serological assay in dogs from Costa Rica. *Parasite Vectors*. 2015;8:170. doi:10.1186/s13071-015-0783-8.



Parasitic Extracellular Vesicles: Emerging Mediators in Host-Parasite Communication and Targets for Therapeutic Innovation

Siyamak Kakekhani¹, Arshia Dousti², Behnaz Momeni²

Corresponding Author: Siyamak Kakekhani, s.kakekhani@gmail.com

1. Department of Pathobiology, Sa.C., Islamic Azad University, Sanandaj, Iran.
2. Student of Veterinary Medicine, School of Veterinary Medicine, Islamic Azad University, Sanandaj, Iran.

Article code: 1408

Abstract

Background: Recent years it has been demonstrated that parasites can release extracellular vesicles (EVs) that participate in the host-parasite communication, immune evasion, and in governing processes associated with host infection.

Methods: This study was performed based on 38 articles published in English-language available in three electronic databases: PubMed, Google Scholar and ResearchGate.

Results: These tiny membrane-bound organelles including microvesicles and exosomes, facilitate the transfer of proteins, lipids, mRNAs, and microRNAs between cells. EVs play a crucial role in modulating host immune responses, improving parasite persistence by suppression of inflammatory pathways and induction of regulatory T-cell activity. Exploring host-parasite interactions has identified new targets for diagnostic, therapy, and vaccine development against parasites.

Conclusion: This review studies current findings on the biogenesis, molecular composition, and functional roles of parasitic EVs, while exploring their therapeutic and diagnostic prospects.

Keywords: Extracellular vesicle, Vaccine, Immunomodulation, Parasite.

Introduction:

The complex interactions between parasites and their hosts are central to the pathogenesis and persistence of parasitic infections. In recent years, extracellular vesicles (EVs) including exosomes (30–150 nm) and microvesicles (100–1000 nm) have gained prominence as key mediators of intercellular communication (1,2). These nano-sized lipid bilayer vesicles are secreted by virtually all cell types and contain a cargo of proteins, lipids, and nucleic acids that can modulate the physiology of recipient cells (3).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



In parasitology, parasite-derived EVs have emerged as potent tools through which helminths and protozoans manipulate host immunity and promote survival (4,5). These vesicles have been shown to modulate dendritic cell function, suppress inflammatory responses, alter antigen presentation, and induce regulatory T-cell pathways (6,7). *Fasciola hepatica* and *Heligmosomoides polygyrus* release EVs that carry immunomodulatory molecules like cathepsins, 14-3-3 proteins, and miRNAs capable of suppressing Th1/Th17 responses and promoting Th2/tolerogenic environments (8,9).

Beyond their immunological roles, EVs participate in various pathogenic and metabolic processes such as tissue invasion, angiogenesis, and extracellular matrix remodeling (10). The molecular signatures of parasite EVs offer promising avenues for diagnostics and biomarker discovery, given their stability in biological fluids and parasite-specific cargo (11,12).

Changes in the composition of proteins expressed on the surface or inside EVs can reflect the status of producing cells, making these structures ideal biomarkers in different types of diseases (44).

The unique properties of parasite EVs have also sparked interest in their use for vaccine development and drug delivery. Studies have shown that immunization with EVs derived from *Echinococcus granulosus*, *Trichuris muris*, or *Schistosoma mansoni* can elicit protective immune responses in animal models (13, 14). This review aims to synthesize current knowledge on parasitic EVs, exploring their biological functions, immunological impacts, and potential for translational application in diagnostics and therapy.

Materials and Methods:

This study was performed based on 38 articles published in English-language available in three electronic databases: PubMed, Google Scholar and ResearchGate. The articles on the findings on parasitic extracellular vesicles were searched with the following keywords: “parasite” in collocations with Immunomodulation, vaccine, extra cellular vesicles, infection and therapy.

Results and Discussion:

The release of extracellular vesicles (EVs) such as exosomes and microvesicles is now recognized as a fundamental communication mechanism among eukaryotic cells, including parasitic helminths and protozoa. These vesicles are not mere byproducts of cellular activity; rather, they are purposefully crafted vehicles that reflect both the physiological state of the cell and its interaction with its surroundings (4,10).

Proteomic studies of parasite EVs including those from *Fasciola hepatica*, *Heligmosomoides polygyrus*, *Echinococcus granulosus*, and *Trichuris muris* consistently report the presence of



EV signature proteins such as Alix, TSG101, HSP70, and members of the tetraspanin family (15,16). Additionally, components like Rab GTPases, annexins, and 14-3-3 proteins found in helminth EVs imply functional versatility in trafficking, host modulation, and metabolic adaptation (9,12).

Lipid profiling also points to an abundance of phosphatidylserine, sphingomyelin, and cholesterol in parasite EVs, features that enhance their membrane stability and may contribute to their immunoevasive properties (17,18).

Immunomodulatory Roles of Parasitic Extracellular Vesicles in Host–Parasite Interactions

Helminth EVs particularly those from *Fasciola hepatica*, *Schistosoma mansoni*, and *Heligmosomoides polygyrus* have been shown to suppress pro-inflammatory cytokine secretion (e.g., IL-6, TNF- α), inhibit DC maturation, and skew immune responses toward Th2 or regulatory T-cell (Treg) profiles (4–6). For instance, *F. hepatica* EVs enriched with cathepsins and 14-3-3 proteins inhibit the NF- κ B signaling pathway and promote tolerogenic DCs that support Treg induction (15). Similarly, EVs from *H. polygyrus* carry miRNAs and cysteine proteases that downregulate IL-12 and IL-1 β production, dampening Th1/Th17 polarization (9,12).

In protozoan infections, such as *Leishmania donovani* and *Trypanosoma cruzi*, parasite-derived EVs can deliver virulence factors like GP63 and cruzipain that interfere with antigen presentation and macrophage activation, promoting intracellular survival (19,20). EVs from *T. cruzi* also impair nitric oxide production and skew macrophage polarization toward an M2-like, anti-inflammatory phenotype (21). Several studies indicated that *Leishmania* exosomes can modulate monocyte cytokine production in response to *Leishmania* infection by influencing the innate and adaptive immune systems (31,32,19).

Hansen and fromm stated in a study, the release of EVs from the third- (L3) and fourth-stage (L4) larvae and adults of *Ascaris suum* was demonstrated by transmission electron microscopy (TEM), and sequencing of EV-derived RNA identified a number of microRNAs (miRNAs) and transcripts of potential host immune targets, such as IL-13, IL-25 and IL-33, were identified (33).

Therapeutic and Diagnostic Potential of Parasitic Extracellular Vesicles

From a diagnostic perspective, parasite-derived EVs have been detected in various host biological fluids including blood plasma, urine, and saliva, underscoring their potential as non-invasive biomarkers (12,22). Their cargo contains parasite-specific proteins and RNAs that can be distinguished from host molecules using modern molecular techniques such as proteomics and RNA sequencing, enabling early and accurate detection of parasitic infections (11,6). The



presence of EV-associated microRNAs from *Schistosoma mansoni* and *Leishmania spp.* has been successfully identified in infected hosts, providing sensitive markers for disease diagnosis and monitoring therapeutic responses (23,24). Experimental immunization with EVs derived from *Echinococcus granulosus*, *Trichuris muris*, and *Schistosoma mansoni* in murine models has induced protective immunity characterized by enhanced antigen-specific antibody production, increased IFN- γ secretion, and reduced parasite burden (12–14).

Altered profiles of EV-associated proteins and miRNAs have been correlated with clinical parameters in patients with visceral leishmaniasis and schistosomiasis, indicating their utility for prognostic purposes. Integration of EV analysis into clinical workflows could enable personalized therapeutic regimens and improve patient management (24,11).

The cathepsin B1 from *O. viverrini* has emerged as a potential biomarker candidate in sera (34). Various extracellular vesicles (EVs) components, such as miRNAs and proteins, have been identified across different trematode species, presenting potential biomarkers for detecting fluke infections (35,36). Studies on EVs released by *F. hepatica*, a major cause of human fascioliasis and an emerging zoonotic pathogen, identified the diagnostic antigen cathepsin L1 (37).

Vaccine Development Using Parasitic Extracellular Vesicles

One of the earliest studies demonstrating the immunogenic potential of parasite-derived EVs involved *Heligmosomoides polygyrus*, where oral or subcutaneous administration of EVs led to reduced worm burdens and modulated T-cell responses in mice (9). Similarly, EVs secreted by *Fasciola hepatica* were shown to contain cathepsins and other immunomodulatory proteins capable of stimulating Th2-biased responses, a hallmark of protective immunity against helminths (8,15).

In murine models of *Schistosoma mansoni* infection, vaccination with EVs purified from adult worm secretions resulted in increased IFN- γ and IL-4 production, elevated IgG1 titers, and significant reductions in hepatic granuloma size and parasite load (25). Studies using EVs from *Echinococcus granulosus* protoscoleces demonstrated that immunized mice produced strong antibody responses and exhibited decreased cyst formation upon challenge (18). It is also discovered that 31% of the proteins identified in EVs secreted by *S. mansoni* are homologous to the previously described vaccine candidates, with many of these proteins present throughout the parasite's lifecycle. This indicates that an EV-based vaccine could target different life stages of the parasite and be effective against *S. mansoni* infection (38).

Challenges and Future Directions in Parasitic EV-Based Therapeutics

One of the foremost technical barriers lies in the isolation and standardization of EVs. Current methods such as ultracentrifugation, density-gradient separation, and size-exclusion chromatography often result in heterogeneous EV populations, co-isolated contaminants, and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



batch-to-batch variability (1,27). The lack of universal EV markers specific to parasite-derived vesicles complicates the establishment of standardized purification protocols suitable for large-scale vaccine or therapeutic production (28).

Unlike conventional subunit or mRNA vaccines, EVs are complex biological mixtures, making it difficult to define precise release criteria, assess batch consistency, and obtain regulatory approval for therapeutic use (26,29).

In the future, integration of EVs with nanotechnology and gene-editing tools such as CRISPR may enable precise manipulation of parasite EV content and function, leading to next-generation vaccines and targeted therapeutics (30).

Acknowledgment:

We are pleased to thank Vice chancellor for research, Islamic Azad University, Sanandaj Branch, for helping us with this study.

References:

1. Théry, C., Ostrowski, M., & Segura, E. (2009). Membrane vesicles as conveyors of immune responses. *Nature Reviews Immunology*, 9(8), 581–593.
2. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol*. 2018 Apr;19(4):213-228.
3. Yáñez-Mó M, Siljander PR, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles*. 2015;4:27066. Published 2015 May 14.
4. Coakley G, Maizels RM, Buck AH. Exosomes and Other Extracellular Vesicles: The New Communicators in Parasite Infections. *Trends Parasitol*. 2015;31(10):477-489.
5. Marcilla A, Trelis M, Cortés A, et al. Extracellular vesicles from parasitic helminths contain specific excretory/secretory proteins and are internalized in intestinal host cells. *PLoS One*. 2012;7(9):e45974.
6. Zakeri A, Hansen EP, Andersen SD, Williams AR, Nejsum P. Immunomodulation by Helminths: Intracellular Pathways and Extracellular Vesicles. *Front Immunol*. 2018;9:2349. Published 2018 Oct 12.
7. Hewitson JP, Harcus Y, Murray J, et al. Proteomic analysis of secretory products from the model gastrointestinal nematode *Heligmosomoides polygyrus* reveals dominance of venom allergen-like (VAL) proteins. *J Proteomics*. 2011;74(9):1573-1594.
8. Murphy A, Cwiklinski K, Lalor R, et al. *Fasciola hepatica* Extracellular Vesicles isolated from excretory-secretory products using a gravity flow method modulate



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- dendritic cell phenotype and activity. *PLoS Negl Trop Dis*. 2020;14(9):e0008626. Published 2020 Sep 8.
9. Buck AH, Coakley G, Simbari F, et al. Exosomes secreted by nematode parasites transfer small RNAs to mammalian cells and modulate innate immunity [published correction appears in *Nat Commun*. 2015 Oct 22;6:8772.
 10. Montaner S, Galiano A, Trelis M, et al. The Role of Extracellular Vesicles in Modulating the Host Immune Response during Parasitic Infections. *Front Immunol*. 2014;5:433. Published 2014 Sep 8.
 11. Loghry HJ, Kwon H, Smith RC, et al. Extracellular vesicles secreted by *Brugia malayi* microfilariae modulate the melanization pathway in the mosquito host. *Sci Rep*. 2023;13(1):8778. Published 2023 May 31.
 12. Eichenberger RM, Talukder MH, Field MA, Wangchuk P, Giacomini P, Loukas A, Sotillo J. Characterization of *Trichuris muris* secreted proteins and extracellular vesicles provides new insights into host-parasite communication. *J Extracell Vesicles*. 2018 Jan 21;7(1):1428004.
 13. Alfandari D, Cadury S, Morandi MI, Regev-Rudzki N. Transforming parasites into their own foes: parasitic extracellular vesicles as a vaccine platform. *Trends Parasitol*. 2023 Nov;39(11):913-928.
 14. Shears RK, Bancroft AJ, Hughes GW, Grencis RK, Thornton DJ. Extracellular vesicles induce protective immunity against *Trichuris muris*. *Parasite Immunol*. 2018;40(7):e12536.
 15. de la Torre-Escudero E, Bennett APS, Clarke A, Brennan GP, Robinson MW. Extracellular Vesicle Biogenesis in Helminths: More than One Route to the Surface?. *Trends Parasitol*. 2016;32(12):921-929.
 16. Zhao P, Cao L, Wang X, et al. Extracellular vesicles secreted by *Giardia duodenalis* regulate host cell innate immunity via TLR2 and NLRP3 inflammasome signaling pathways. *PLoS Negl Trop Dis*. 2021;15(4):e0009304. Published 2021 Apr 2.
 17. Marcilla A, Martin-Jaular L, Trelis M, de Menezes-Neto A, Osuna A, Bernal D, Fernandez-Becerra C, Almeida IC, Del Portillo HA. Extracellular vesicles in parasitic diseases. *J Extracell Vesicles*. 2014 Dec 22;3:25040.
 18. Ancarola ME, Maldonado LL, García LCA, Franchini GR, Mourglia-Ettlin G, Kamenetzky L, Cucher MA. A Comparative Analysis of the Protein Cargo of Extracellular Vesicles from Helminth Parasites. *Life*. 2023; 13(12):2286.
 19. Silverman JM, Clos J, Horakova E, Wang AY, Wiesgigl M, Kelly I, Lynn MA, McMaster WR, Foster LJ, Levings MK, Reiner NE. Leishmania exosomes modulate innate and adaptive immune responses through effects on monocytes and dendritic cells. *J Immunol*. 2010 Nov 1;185(9):5011-22.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



20. Torrecilhas AC, Soares RP, Schenkman S, Fernández-Prada C, Olivier M. Extracellular Vesicles in Trypanosomatids: Host Cell Communication. *Front Cell Infect Microbiol.* 2020;10:602502. Published 2020 Dec 14.
21. Cestari I, Ansa-Addo E, Deolindo P, Inal JM, Ramirez MI. Trypanosoma cruzi immune evasion mediated by host cell-derived microvesicles. *J Immunol.* 2012;188(4):1942-1952.
22. Pinheiro AAS, Torrecilhas AC, Souza BSF, et al. Potential of extracellular vesicles in the pathogenesis, diagnosis and therapy for parasitic diseases. *J Extracell Vesicles.* 2024;13(8):e12496.
23. Zauli RC, de Souza Perez IC, de Moraes ACC, et al. Extracellular Vesicles Released by *Leishmania (Leishmania) amazonensis* Promastigotes with Distinct Virulence Profile Differently Modulate the Macrophage Functions. *Microorganisms.* 2023;11(12):2973. Published 2023 Dec 13.
24. Lambert U, Oviedo Ovando ME, Vasconcelos EJ, Unrau PJ, Myler PJ, Reiner NE. Small RNAs derived from tRNAs and rRNAs are highly enriched in exosomes from both old and new world *Leishmania* providing evidence for conserved exosomal RNA Packaging. *BMC Genomics.* 2015 Mar 5;16(1):151.
25. Mossallam SF, Abou-El-Naga IF, Abdel Bary A, Elmorsy EA, Diab RG. *Schistosoma mansoni* egg-derived extracellular vesicles: A promising vaccine candidate against murine schistosomiasis. *PLoS Negl Trop Dis.* 2021;15(10):e0009866. Published 2021 Oct 13.
26. Witwer KW, Théry C. Extracellular vesicles or exosomes? On primacy, precision, and popularity influencing a choice of nomenclature. *J Extracell Vesicles.* 2019;8(1):1648167. Published 2019 Aug 1.
27. Lener T, Gimona M, Aigner L, et al. Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. *J Extracell Vesicles.* 2015;4:30087. Published 2015 Dec 31.
28. Kowal J, Tkach M, Théry C. Biogenesis and secretion of exosomes. *Curr Opin Cell Biol.* 2014;29:116-125.
29. Gimona M, Pachler K, Laner-Plamberger S, Schallmoser K, Rohde E. Manufacturing of Human Extracellular Vesicle-Based Therapeutics for Clinical Use. *Int J Mol Sci.* 2017;18(6):1190. Published 2017 Jun 3.
30. Tang TT, Wang B, Lv LL, Liu BC. Extracellular vesicle-based Nanotherapeutics: Emerging frontiers in anti-inflammatory therapy. *Theranostics.* 2020;10(18):8111-8129. Published 2020 Jul 9.
31. Menezes-Neto A, Borges VM, Descoteaux A, Torrecilhas AC, Xander P, et al. Immunomodulatory properties of *Leishmania* extracellular vesicles during host-parasite interaction: Differential activation of TLRs and NF- κ B translocation by



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- dermotropic and viscerotropic species. *Frontiers in Cellular and Infection Microbiology*. 2020;10:1-9.
32. Silverman JM axwel., Reiner NE. *Leishmania* exosomes deliver preemptive strikes to create an environment permissive for early infection. Vol. 1. *Frontiers in cellular and infection microbiology*. 2011. p.26
 33. Hansen EP, Fromm B, Andersen SD, et al. Exploration of extracellular vesicles from *Ascaris suum* provides evidence of parasite-host cross talk. *J Extracell Vesicles*. 2019;8(1):1578116. Published 2019 Feb 14.
 34. Sripa, J., Brindley, P. J., Sripa, B., Loukas, A., Kaewkes, S., Laha, T. (2012). Evaluation of liver fluke recombinant cathepsin B-1 protease as a serodiagnostic antigen for human opisthorchiasis. *Parasitol. Internat.* 61, 191–195.
 35. Mekonnen, G. G., Tedla, B. A., Pickering, D., Becker, L., Wang, L., Zhan, B., et al. (2020). *Schistosoma haematobium* extracellular vesicle proteins confer protection in a heterologous model of schistosomiasis. *Vaccines* 8, 416.
 36. Ovchinnikov, V. Y., Kashina, E. V., Mordvinov, V. A., Fromm, B. (2020). EV-transported microRNAs of *Schistosoma mansoni* and *Fasciola hepatica*: Potential targets in definitive hosts. *Infect. Genet. Evol.* 85, 104528.
 37. Sarkari, B., Khabisi, S. A. (2017). Immunodiagnosis of human fascioliasis: an update of concepts and performances of the serological assays. *J. Clin. Diagn. Res.* 11, OE05.
 38. Sotillo, J., Pearson, M., Potriquet, J., Becker, L., Pickering, D., Mulvenna, J., et al. (2016). Extracellular vesicles secreted by *Schistosoma mansoni* contain protein vaccine candidates. *Int. J. Parasitol.* 46, 1–5.



First Report of *Giardia psittaci* in Captive Psittacines in Tehran, Iran

Sahar Mirbagheri¹, Saloomesh Shirali^{1,2}, Bahar Shemshadi¹, Mohammad parsa Bayatpoor¹

Corresponding Author: Saloomesh Shirali, saloomesh.shirali@iau.ac.ir

1. Department of Veterinary Pathobiology, SR.C., Islamic Azad University, Tehran, Iran.

2. Department of biotechnology, Ahv.C., Islamic Azad University, Ahvaz, Iran.

Article code: 1417

Abstract

Background: *Giardia* is a widespread protozoan parasite that infects a variety of vertebrates, including birds. In psittacine birds, infections can range from asymptomatic to severe gastrointestinal disorders, yet data on *Giardia* occurrence in psittacines, particularly in Iran, remain sparse. This study aimed to determine the presence and prevalence of *Giardia* spp., especially *Giardia psittaci*, in captive psittacine birds in Tehran, Iran, using both microscopic and molecular diagnostic methods.

Methods: A total of 36 fecal samples were collected from adult captive psittacine birds (20 budgerigars, 16 cockatiels) randomly chosen from four bird shops in southern Tehran in winter 2022. Samples were examined microscopically using Giemsa staining and analyzed by nested PCR targeting the SSU rRNA gene. One PCR-positive sample was sequenced for species confirmation.

Results: Microscopic analysis identified *Giardia* cysts and trophozoites in 6 out of 36 samples (16.6%). Nested PCR yielded positive results in 10 samples (27.7%), including 6 from budgerigars and 4 from cockatiels. Sequencing of a representative positive sample confirmed *Giardia psittaci* with 100% identity to published sequences. Most PCR-positive birds were asymptomatic, suggesting potential subclinical carriage and transmission.

Conclusion: This study provides the first molecular confirmation of *Giardia psittaci* in captive psittacine birds in Iran, revealing a substantial infection rate. These findings underscore the importance of routine molecular surveillance for *Giardia* in pet birds and highlight the need for further research into the zoonotic potential of *G. psittaci* as well as measures to improve avian and public health.

Keywords: *Giardia*, Psittacines, Nested-PCR, Budgerigar, Cockatiel.

Introduction:

Giardia species are essentially ubiquitous, flagellated protozoa that infect several vertebrate hosts, including mammals, reptiles, amphibians, and birds (1, 2, 3). This protozoan is



transmitted through the fecal-oral route, being a bi-phasic organism: resilient cysts that enable transmission and motile trophozoites that inhabit the host's small intestinal tract. While giardiasis in mammals, and particularly in humans, is a well-researched area, much less is known about its occurrence, severity, and potential zoonotic implications in avian species. Two species of *Giardia* have been identified in birds: *G. psittaci* and *G. ardeae* (3,4). *G. psittaci* mostly involves psittacine birds, while *G. ardeae* has been recorded in herons and other duck species. The clinical consequences of giardiasis in avian species depend on many factors, including species, age, and immunological condition. While many cases of psittacine infections are asymptomatic, symptomatic birds may show gastrointestinal disturbances like mucoid diarrhea, dehydration, weight loss, vomiting, and stunted growth in chicks (5,6,7).

Chronic feather plucking and pruritus associated with gastroenteritis are commonly observed in infected cockatiels, suggestive of a possible behavioral alteration linked with the *Giardia* incursion in birds (8). *Giardia* spp. may act, apart from damage inflicted on avian health, to operate within the framework of mammalian giardiasis epidemiology. Birds, particularly those residing in regions shared with humans and their domestic animals, act as mere mechanical vectors for transport of *Giardia* cysts, introducing pollution to the environment with further prospects of interspecific transmission (9). This gives rise to concerns about the role of wild and captive birds in transmitting *Giardia* spp., especially in densely populated urban areas where contact among humans, domestic animals, and birds is common. Despite *Giardia* infections' potential significance to psittacine, records of infection prevalence from Iran are rare. Most research on avian giardiasis has concentrated on poultry and wild avians, while non-domesticated and captive birds, the possible important reservoirs of infection, have occupied little scholarly attention. Finally, although *G. duodenalis* has been amply described in animals and given zoonotic importance, little is known about the zoonotic potential of *G. psittaci* and *G. ardeae*.

Rectifying these information deficiencies is essential for enhancing our comprehension of *Giardia* epidemiology in avian species and evaluating possible public health hazards. In Tehran, Iran, this research attempted to investigate the origin of occurrence of *Giardia* spp. in captive psittacine using both microscopic and molecular diagnostic methods.

Materials and Methods:

Population and Sampling

This cross-sectional study involved 36 adults captive psittacines (16 budgerigars, 20 cockatiels) randomly selected from four bird shops in southern Tehran, Iran.



Sample Collection and Purification

Fresh fecal samples were collected from cage bottoms, transferred to microtubes with saline, and stored at 5°C. For purification, fecal suspensions underwent Sheather's flotation, followed by phosphate-buffered saline (PBS) washing and centrifugation. The resulting sediments were used for Giemsa staining and DNA extraction.

Examination

Giemsa-stained fecal smears were prepared and examined for *Giardia* cysts and trophozoites under a light microscope.

DNA Extraction and PCR

Genomic DNA was isolated using a stool DNA kit with additional freeze-thaw cycles to enhance cyst lysis. Nested PCR targeting the SSU rRNA gene was performed with established primers. Positive and negative controls included *Genomic DNA of *Giardia intestinalis** and ultrapure water, respectively. PCR products were visualized by agarose gel electrophoresis, and DNA sequences were assembled with CodonCode Aligner™ software. Alignment of sequence information obtained from GenBank (accession numbers MH929091-MH929093) was performed using Clustal X software.

Results and Discussion:

Microscopic evaluation detected *Giardia* cysts and trophozoites in 6 of 36 samples (16.6%), visually identifiable by characteristic morphology. Nested PCR significantly increased detection, revealing *Giardia* DNA in 10 of 36 samples (27.7%), 6 in budgerigars and 4 in cockatiels. Sequencing of a nested PCR product confirmed species as *Giardia psittaci*, displaying 100% identity with available GenBank sequences.

In our study, the detection and molecular confirmation of *Giardia psittaci* in captive psittacines in Tehran mark a significant milestone in avian parasitology within Iran. This is the first documented molecular evidence of *G. psittaci* in the country, offering valuable epidemiological data on the presence, distribution, and potential impact of *Giardia* in psittaciform birds kept as pets or sold commercially. Given the increasing popularity of companion birds such as budgerigars and cockatiels in Iranian households, these findings carry both veterinary and public health significance.

The application of both microscopic (Giemsa staining) and molecular (nested PCR) techniques enabled a dual-layered diagnostic approach. While microscopy detected *Giardia* cysts and trophozoites in 16.6% of the samples, nested PCR identified positivity rate of 27.7%. This discrepancy aligns with other reports (10,11) and reflects the superior sensitivity of PCR in detecting low-intensity infections and subclinical carriers. As cyst shedding in birds can be



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



intermittent and sparse, it is understandable that light infections may go undetected by traditional methods, reinforcing the importance of incorporating molecular tools in diagnostic protocols.

The sequencing result confirming *G. psittaci* is particularly relevant, considering the limited global data on this species. Historically, only two avian-specific *Giardia* species have been recognized: *G. ardeae*, primarily found in herons and ducks (4), and *G. psittaci*, predominantly in psittacines (3, 12). Unlike *G. duodenalis*, whose zoonotic transmission is well established (1), the zoonotic potential of *G. psittaci* remains poorly understood. However, recent insights suggest that avian species may act as mechanical vectors for *Giardia* cysts, raising concerns about environmental contamination, especially in areas of close interaction between birds, humans, and domestic animals.

Interestingly, most *Giardia*-positive birds in our study were asymptomatic (7 out of 10 cases). This observation is consistent with earlier literature which indicates that asymptomatic carriage is common, particularly in adult birds with stronger immune systems or established tolerance (5,6). Nonetheless, such birds can still contribute to the spread of the parasite through fecal shedding. Budgerigars, in particular, are known for frequent asymptomatic infections (13), and their relatively high population density in pet shops may facilitate transmission.

We also observed a higher prevalence rate in budgerigars (37.5%) compared to cockatiels (20%), supporting previous findings by Erlandsen et al. (1991), who suggested species-related variation in susceptibility. These differences might be attributed to genetic, immunological, or environmental factors, such as stress, diet, hygiene, and population management. The higher positivity in budgerigars may also reflect their common use in pet trade and denser housing conditions, which promote horizontal transmission of enteric pathogens.

The 27.7% prevalence rate we observed through nested PCR is comparable to the 25.9% reported by Ichikawa et al. (2019) in Brazil among 359 captive psittaciform birds. These comparable prevalence rates from different continents underscore the widespread and possibly underestimated global burden of giardiasis in psittacines, suggesting that the infection may be far more common than recognized, particularly in captive environments.

Beyond bird health, this issue carries potential public health implications. Although *G. psittaci* has not yet been conclusively linked to human infection, the proximity between humans and their pet birds increases the need to investigate the host specificity and zoonotic capacity of this species. With the increasing awareness of One Health principles, it becomes essential to explore interspecies disease transmission dynamics, including from birds to humans.

Moreover, *Giardia* infection in birds, particularly in chicks and immunocompromised individuals, may lead to chronic gastrointestinal issues such as diarrhea, weight loss, and failure to thrive (7,8). Feather plucking, lethargy, and pruritus in cockatiels have also been linked with



Giardia-induced enteritis. Therefore, timely identification and appropriate treatment of infected birds are critical not only for individual animal welfare but also for preventing wider outbreaks in breeding or commercial settings.

In conclusion, in our study, we provided the first molecular confirmation of *G. psittaci* in Iran and demonstrated a noteworthy prevalence of *Giardia* spp. among captive psittacines. These findings highlight the need for regular parasitological surveillance, improved hygienic measures in pet bird facilities, and increased awareness of the potential zoonotic dimensions of avian giardiasis. Incorporating both microscopic and molecular diagnostics into routine veterinary care for pet birds is recommended to facilitate early detection and intervention, reduce environmental contamination, and support avian welfare.

Acknowledgment:

The authors of this article would like to thank Dr. Hooman Ronaghi for his assistance during the laboratory work.

References:

1. Thompson RCA, Monis PT. Variation in *Giardia*: Implications for Taxonomy and Epidemiology. *Adv Parasitol*. 2004; 58:69–137.
2. Abe N, Makino I, Kojima A. Molecular characterization of *Giardia psittaci* by multilocus sequence analysis. *Infect Genet Evol*. 2012; 12:1710–1716.
3. Talazadeh F, Razijalali MH, Roshanzadeh N, Davoodi P. Survey on the gastrointestinal parasites in Passeriformes and Psittaciformes with a focus on zoonotic parasites. *J Hellenic Vet Med Soc*. 2023 Oct 18; (Online ahead of print). doi:10.12681/jhvms.31398.
4. Sandoval-Rodríguez A, Marcone D, Alegría-Morán R, et al. *Cryptosporidium* spp. and *Giardia* spp. in free-ranging introduced monk parakeets from Santiago, Chile. *Animals (Basel)*. 2021;11(3):801.
5. Kazemi-Lifshagerd F, BehrouziNasab O, Koochakzadeh A, Kazemnezhad M. Evaluation of giardiasis in companion birds in Mashhad, Iran. *J World's Poult Sci*. 2023;2(3):20–23.
6. Filippich LJ, McDonnell PA, Munoz E, Upcroft JA. *Giardia* infection in budgerigars. *Aust Vet J*. 1998;76(4):246–249.
7. Reuschel M, Pantchev N, Vrhovec MG, Jung A, Gerhauser I, Sannella AR, Cacciò SM, Legler M. Occurrence and Molecular Typing of *Giardia psittaci* in Parakeets in Germany – A Case Study. *Avian Dis*. 2020;64(2):228–233.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



8. Doneley B. Avian medicine and surgery in practice: Companion and aviary birds. Boca Raton: CRC Press; 2016.
9. Upcroft JA, McDonnell PA, Gallagher AN, Chen N, Upcroft P. Lethal *Giardia* from a wild-caught sulphur-crested cockatoo (*Cacatua galerita*) established in vitro chronically infects mice. *Parasitology*. 1997; 114:407–412.
10. Appelbee AJ, Frederick LM, Heitman TL, Olson ME. Prevalence and genotyping of *Giardia duodenalis* from beef calves in Alberta, Canada. *Vet Parasitol*. 2003; 112:289–294.
11. Tan L, Wu S, Abdullahi AY, Yu X, Hu W, Song M, Shi X, Li G. PCR-RFLP method to detect zoonotic and host-specific *Giardia duodenalis* assemblages in dog fecal samples. *Parasitol Res*. 2016; 115:2045–2050.
12. Erlandsen S, Bemrick W. SEM Evidence for a New Species, *Giardia psittaci*. *J Parasitol*. 1987;73(3):623–629.
13. Acosta Guevara ICJ, Soto Piñeiro E, Cruz López E. Presencia de *Giardias* en Pericos Australianos en Cuba. *REDVET*. 2009;10(6):070904.



A Review on In Vitro Studies on Plant Extracts and Metal Nanoparticles Effectiveness on Domesticated Ruminant Liver Flukes

Farnaz Malekifard¹, Seyyed Kian Mohseni Mofidi ¹, Alireza Ghorbani¹

Corresponding Author: Farnaz Malekifard, f.malekifard@urmia.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article code: 1418

Abstract

Background: Among the parasitic diseases caused by helminths in veterinary medicine, ruminant liver flukes, *Fasciola hepatica* and *Dicrocoelium dendriticum* are of particular importance. Considering some studies conducted on the anthelmintic effects of plant extracts and metal nanoparticles on different life cycle stages of these trematodes and promising results concluded, it is thought that these compounds could be the main candidates to replace some anthelmintic pharmaceutical compounds in the future.

Methods: This research examines the effectiveness of plant-based compounds and metal nanoparticles as alternative, safe, and sustainable methods for managing liver flukes in ruminants. A review was conducted based on six published research articles (2010-2024) sourced from online databases such as Google Scholar, PubMed, Scopus, and ScienceDirect.

Results: As mentioned, many studies have investigated the anthelmintic effects of plant extracts and metal nanoparticles on various helminths, including ruminant liver flukes. Some of these studies concluded with promising results.

Conclusion: Currently, chemical anthelmintic drugs from the benzimidazole and probenzimidazole families are widely used. However, these drugs are not easily available in remote rural areas and have serious disadvantages. Also, the lack of an effective vaccine and resistance to selected drugs including triclabendazole have initiated the search for alternative approaches. Thus, according to aforementioned studies, it is important to consider innovative treatment methods.

Keywords: *Dicrocoelium dendriticum*, *Fasciola hepatica*, Metal Nanoparticles, Plant Extracts.

Introduction:

Nowadays, using plant extracts and metal nanoparticles are of the most important alternative methods to pharmaceutical treatments in the treatment of parasitic worms. plant extracts are often composed of secondary metabolites, which are organic compounds produced by plants



that are not directly involved in the plant's primary functions like growth and development, but play roles in defense, signaling, and other ecological interactions (15, 23). Among different kinds of plant extracts, polyphenols comprise a structurally diverse class of natural products. Polyphenols are widely used in the treatment of nematode infections (24, 30). Overall, there are many studies of examining plant extracts anthelmintic effects on different helminths (8, 17, 20, 25, 31). Nanoparticles have many properties, depending on their size, shape, and morphology, allowing them to interact with microorganisms, plants, and animals (1). For Example, silver nanoparticles are especially widely examined on helminths including different species of ruminant helminths and promising effects were also observed (16, 27).

Considering the therapeutic properties mentioned for plant extracts and metal nanoparticles in various studies, as well as the increase in drug resistance against common drugs in anthelmintic treatments in recent years, it can be said that the time has come to turn to innovative therapeutic methods in anthelmintic treatments. Among the important parasitic diseases caused by worms in veterinary medicine, *Fasciola hepatica* and *Dicrocoelium dendriticum* are of particular importance. Considering some studies conducted on the anthelmintic effects of plant extracts and metal nanoparticles on different life cycle stages of these trematodes and promising results concluded, it is thought that these compounds could be the main candidates to replace some anthelmintic pharmaceutical compounds in the not too distant future (5, 10, 26).

Materials and Methods:

In order to write an outline of materials and methods in the abstract of this review study, online databases (Google Scholar, PubMed, Scopus, and ScienceDirect) were searched and six published research articles (2010-2024) were gathered and reviewed (2, 14, 21, 22, 26, 27).

Results and Discussion:

As mentioned, many studies have investigated the anthelmintic effects of plant extracts and metal nanoparticles on various helminths, including ruminant liver flukes. Alvarez-Mercado et al. (2015) investigated the effects of extracts of fifteen medicinal plants on the adult stage of *F. hepatica* in comparison with triclabendazole *in vitro*. It was concluded that five tropical plant extracts, which included plant extracts of *Lantana camara*, *Bocconia frutescens*, *Piper auritum*, *Artemisia mexicana* and *Cajanus cajan* had promising anthelmintic effects against adult *F. hepatica* due to their secondary metabolites such as alkaloids, terpenes, tannins or flavonoids (3). In a study conducted by Moazeni and Khademolhoseini (2016), the lethal effects of ginger (*Zingiber officinale*) methanolic extract on *F. hepatica* eggs *in vitro* were investigated. They showed that ginger methanolic extract had different lethal effects at different exposure times and in different dilutions (21). Kandil et al. (2018) discovered that *Moringa*



oleifera methanolic extract shows inhibitory activity on the vitality and hatchability of *F. hepatica* eggs (18). Also, in a study conducted by Ghaffari et al. (2021), results were obtained indicating that ginger hydroalcoholic extract contains flavonoids, saponins, tannins, terpenoids, and phenol. The mentioned compounds had lethal effect on *F. hepatica* miracidium in comparison with triclabendazole (12). In another study conducted by Arbabi et al. (2023), the effect of hydroalcoholic extract of *Ferula assa-foetida* which contains aresinotannols A and B, ferulic acid, umbelliferone and sulfurcontaining compounds on *F. hepatica* and *D. dendriticum* was compared with closantel and triclabendazole, and depending on the duration of contact, relatively promising results were obtained (5). In another study conducted by Allahyari et al. (2024), essential oils of lavender (*Lavandula angustifolia*) which contained borneol (29.7%), linalool (26.20%) and alpha-pinene (14.30%) and carob (*Ceratonia siliqua L.*) contained nonadecane (23.34%), 1,2-benzenedicarboxylic acid, dibutyl ester (15.95%), heneicosan (14.61%), eicosene (9.66%), 1,2-benzenedicarboxylic acid (7.51%) and b-cedrene (7.37%) were compared with triclabendazole and it was found out that they had anti-hatching and lethal effects on the egg and adult stages of *F. hepatica* at different exposure times and different concentrations (2). In another distinct study conducted by Arbabi et al. (2021), The impacts of various concentrations of AgO nanoparticles for 12-24 hours were compared with the Closantel. it was found that AgO nanoparticles have potent anthelmintic effects on liver fluke *D. dendriticum* (6). In vitro anthelmintic effects of iron oxide and zinc oxide nanoparticles against *Fasciola* Spp. were examined by Hassan et al. (2021), and it was concluded that FeO and ZnO nanoparticles have potent fasciocidal activity especially at 0.012% concentration of both FeO and ZnO nanoparticles (13). In a study conducted by Ravvaz et al. (2024), anthelmintic activity of copper oxide and zinc oxide nanoparticles on egg and adult stages of *F. hepatica* was compared with triclabendazole. It was concluded that CuO-NPs and ZnO-NPs show promising *in vitro* fascioliscide efficacy against adults and eggs of fluke (26).

Fasciola are large, broad, leaf-shaped trematodes that live in the bile ducts and intestines of mammals. In the sheep, acute fascioliasis resulting from parenchymal damage to the liver and haemorrhage caused by migrating juvenile flukes is a severe and potentially fatal disease (11). The most important causative agent is *F. hepatica*, which is distributed in Europe, Africa, Asia, Oceania, and the Americas. The fight against fascioliasis is usually based on disease control and reduction of disease incidence. Control of this disease depends on the strategic use of a wide range of antiparasitic drugs (28). Among these drugs, triclabendazole is the only drug that is effective against the migrating juvenile stages in the liver parenchyma and the adult stage in the bile ducts. However, the use of triclabendazole causes drug resistance. Today, new effective treatments are used due to the emergence of drug resistance (10).

Dicrocoeliasis is a disease caused by *D. dendriticum*, a hepatic bile duct trematode that infects humans and a wide range of herbivores and is considered a major threat to livestock production in endemic areas (29). The disease caused by this parasite is found throughout Europe, Asia,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



North Africa and North America (7). The parasite is now recognized as one of the Neglected Tropical Diseases, causing major public health problems as well as significant economic losses (7, 19). There is currently no vaccine to prevent this disease, and therefore drug therapy is one of the most effective methods of combating. A wide range of strategies are used to control *dicrocoeliasis*; however, due to the emergence of resistance and the cost of treating small ruminants, alternative treatments are being sought (9).

Currently, chemical anthelmintic drugs of the benzimidazole and probenzimidazole classes are widely used. However, these drugs are not easily available in remote rural areas and have some serious disadvantages such as development of drug resistance, adverse drug reactions, residual effects, toxicity problems and high veterinary costs. Also, as mentioned, the lack of an effective vaccine and resistance to selected drugs including triclabendazole have initiated the search for alternative approaches. Thus, it is important to consider innovative treatment methods (5, 26).

References:

1. Ali SB, Mohamed AS, Fahmy SR, El-Garhy M, Mousa MR, Abdel-Ghaffar F. Anthelmintic and hepatoprotective activities of the Green-synthesized zinc oxide nanoparticles against *Parascaris equorum* infection in rats. *Acta Parasitologica*. 2024 Mar;69(1):283-301.
2. Allahyari M, Malekifard F, Yakhchali M. Anthelmintic effects of some medicinal plants on different life stages of *Fasciola hepatica*: Evidence on oxidative stress biomarkers, and DNA damage. *PLOS Neglected Tropical Diseases*. 2024 Jun 17;18(6):e0012251.
3. Alvarez-Mercado JM, Ibarra-Velarde F, Alonso-Díaz MÁ, Vera-Montenegro Y, Avila-Acevedo JG, García-Bores AM. *In vitro* anthelmintic effect of fifteen tropical plant extracts on excysted flukes of *Fasciola hepatica*. *BMC veterinary research*. 2015 Dec;11:1-6.
4. Anuracpreeda P, Chawengkirttikul R, Ngamniyom A, Panyarachun B, Puttarak P, Koedrith P, Intaratat N. The *in vitro* anthelmintic activity of the ethanol leaf extracts of *Terminalia catappa* L. on *Fasciola gigantica*. *Parasitology*. 2017 Dec;144(14):1931-42.
5. Arbabi M, Haddad A, Esmaeli M, Hooshyar H, Sehat M. *In vitro* anthelmintic effect of *Ferula assa-foetida* hydroalcoholic extract against flukes of *Fasciola hepatica* and *Dicrocoelium dendriticum*. *Jundishapur journal of natural pharmaceutical products* 2023 Jan 1;18(1):e133030.
6. Arbabi M, Haddad A, HosseipourMashkani SM, Hooshyar H. *In Vitro* Assessment of Anthelmintic Activities of AgO Nanoparticle in Comparison to Closantel Against Liver Fluke *Dicrocoelium Dendriticum*. 2021.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



7. Arbabi M, Nezami E, Hooshyar H, Delavari M. Epidemiology and economic loss of fasciolosis and dicrocoeliosis in Arak, Iran. *Veterinary world*. 2018 Dec 10;11(12):1648.
8. Carvalho CO, Chagas AC, Cotinguiba F, Furlan M, Brito LG, Chaves FC, Stephan MP, Bizzo HR, Amarante AF. The anthelmintic effect of plant extracts on *Haemonchus contortus* and *Strongyloides venezuelensis*. *Veterinary parasitology*. 2012 Feb 10;183(3-4):260-8.
9. Fairweather I, Brennan GP, Hanna RE, Robinson MW, Skuce PJ. Drug resistance in liver flukes. *International Journal for Parasitology: drugs and drug resistance*. 2020 Apr 1;12:39-59.
10. Fairweather I. Triclabendazole progress report, 2005–2009: an advancement of learning?. *Journal of helminthology*. 2009 Jun;83(2):139-50.
11. Forbes A. Liver fluke infections in cattle and sheep. *Livestock*. 2017 Sep 2;22(5):250-6.
12. Ghafari A, Arbabi M, Mosayebi M, Hooshyar H, Nickfarjam AM. Evaluation of anti-helminthic activity of *Zingiber officinale* roscoe extract on *Fasciola hepatica* miracidia *In vitro*. *International Archives of Health Sciences*. 2021 Jan 1;8(1):45-50.
13. Hassan A, Ramdan M, SF A O, Elkabawy L. In vitro anthelmintic effects of iron oxide and zinc oxide nanoparticles against *Fasciola* Spp. in Dakhla Oasis, Egypt. *Benha Veterinary Medical Journal*. 2021 Oct 1;41(1):144-7.
14. Jalali TS, Malekifard F, Esmailnejad B, Rezaie SA. Toxicities of the copper and zinc oxide nanoparticles on *Marshallagia marshalli* (Nematoda: Trichostrongylidae): evidence on oxidative/nitrosative stress biomarkers, DNA damage and egg hatchability. *Journal of Helminthology*. 2021 Jan;95:e70.
15. Jones WP, Kinghorn AD. Extraction of plant secondary metabolites. *Natural products isolation*. 2012:341-66.
16. Kaiaty AM, Salib FA, El-Gameel SM, Abdel Massieh ES, Hussien AM, Kamel MS. Emerging alternatives to traditional anthelmintics: the *in vitro* antiparasitic activity of silver and selenium nanoparticles, and pomegranate (*Punica granatum*) peel extract against *Haemonchus contortus*. *Tropical Animal Health and Production*. 2023 Oct;55(5):317.
17. Kamaraj C, Rahuman AA. Efficacy of anthelmintic properties of medicinal plant extracts against *Haemonchus contortus*. *Research in Veterinary Science*. 2011 Dec 1;91(3):400-4.
18. Kandil OM, Hassan NM, Sedky D, Ata EB, Nassar SA, Shalaby HA, Nanev V, Tsocheva-Gaytandzhieva N, Gabrashanska M. Anthelmintic efficacy of *Moringa oleifera* seed methanolic extract against *Fasciola hepatica*. *Journal of Parasitic Diseases*. 2018 Sep;42:391-401.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



19. Majidi-Rad M, Meshgi B, Bokaie S. The prevalence and intensity rate of *Dicrocoelium dendriticum* infection in ruminants of 3 provinces in coastal regions of the Caspian Sea. Iran J Vet Med. 2018 Jan 1;12(1):27-33.
20. Minsakorn S, Nuplod K, Puttarak P, Chawengkirttikul R, Panyarachun B, Ngamniyom A, Charoenkul T, Jaisa-Aad M, Panyarachun P, Anuracpreeda P. The anthelmintic effects of medicinal plant extracts against paramphistome parasites, *Carmyerius spatiosus*. Acta Parasitologica. 2019 Sep;64:566-74
21. Moazeni M, Khademolhoseini AA. Ovicidal effect of the methanolic extract of ginger (*Zingiber officinale*) on *Fasciola hepatica* eggs: an in vitro study. Journal of Parasitic Diseases. 2016 Sep;40:662-6.
22. Moazeni M, Nazer A. In vitro effectiveness of garlic (*Allium sativum*) extract on scolices of hydatid cyst. World journal of surgery. 2010 Nov;34:2677-81.
23. Monagas M, Brendler T, Brinckmann J, Dentali S, Gafner S, Giancaspro G, Johnson H, Kababick J, Ma C, Oketch-Rabah H, Pais P. Understanding plant to extract ratios in botanical extracts. Frontiers in Pharmacology. 2022 Sep 30;13:981978.
24. Oliveira AF, Junior LM, Lima AS, Silva CR, Ribeiro MN, Mesquista JW, Rocha CQ, Tangerina MM, Vilegas W. Anthelmintic activity of plant extracts from Brazilian savanna. Veterinary Parasitology. 2017 Mar 15;236:121-7.
25. Rakhshandehroo E, Asadpour M, Malekpour SH, Jafari A. The anthelmintic effects of five plant extracts on the viability of *Parascaris equorum* larvae. Equine Veterinary Education. 2017 Apr;29(4):219-24.
26. Ravvaz A, Malekifard F, Esmailnejad B. In vitro assessment of the anthelmintic activity of copper oxide and zinc oxide nanoparticles on egg and adult stages of *Fasciola hepatica*: evidence on oxidative stress biomarkers, and DNA damage. BMC Veterinary Research. 2024 Apr 4;20(1):137.
27. Rehman A, Ullah R, Uddin I, Zia I, Rehman L, Abidi SM. In vitro anthelmintic effect of biologically synthesized silver nanoparticles on liver amphistome, *Gigantocotyle explanatum*. Experimental parasitology. 2019 Mar 1;198:95-104.
28. Sánchez-Andrade R, Paz-Silva A, Suárez J, Panadero R, Pedreira J, Díez-Baños P, Morrondo P. Effect of fasciolicides on the antigenaemia in sheep naturally infected with *Fasciola hepatica*. Parasitology Research. 2001 Aug;87:609-14.
29. Scala A, Tamponi C, Dessì G, Sedda G, Sanna G, Carta S, Corda A, Jacquet P, Varcasia A, Ligios C. Dicrocoeliosis in extensive sheep farms: a survey. Parasites & vectors. 2019 Dec;12:1-7.
30. Spiegler V, Liebau E, Hensel A. Medicinal plant extracts and plant-derived polyphenols with anthelmintic activity against intestinal nematodes. Natural Product Reports. 2017;34(6):627-43.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



31. Williams AR, Fryganas C, Ramsay A, Mueller-Harvey I, Thamsborg SM. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. PloS one. 2014 May 8;9(5):e97053.



Cryptosporidial Diarrhea in Neonatal Lambs: A Clinical Investigation in an Industrial Flock in Iran

Mohadese Loukhi¹, Gholamreza Mohammadi¹

Corresponding Author: Gholamreza Mohammadi, gmohamad@um.ac.ir

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1419

Abstract

Background: Cryptosporidiosis is a protozoan infection that causes severe diarrhea in young ruminants. In sheep production systems, especially under industrial conditions, cryptosporidial diarrhea can lead to high neonatal mortality and substantial financial loss. This study investigates an outbreak of cryptosporidial diarrhea in a neonatal lamb population within a large commercial unit, focusing on clinical signs, pathogen identification, environmental factors, and control measures.

Methods Clinical and environmental assessments were conducted at the affected site. Fecal and umbilical samples were collected from lambs between 5 and 10 days old, and analyzed using flotation techniques and bacterial culture. Management practices were simultaneously evaluated to identify risk factors.

Results: Clinical observations included watery yellow diarrhea, dehydration, and inflamed navels. Parasitological analysis confirmed the presence of *Cryptosporidium parvum* oocysts, while bacteriology revealed *Klebsiella* spp. Environmental factors such as damp bedding and poor disinfection practices were conducive to disease spread. Supportive fluid therapy and improved hygiene protocols were implemented as part of outbreak control. No curative treatment for cryptosporidiosis was available, but the use of disinfectants such as formalin and ammonia showed environmental efficacy.

Conclusion: Cryptosporidiosis, compounded by bacterial co-infections like *Klebsiella*, is a serious threat to neonatal lamb health in intensive production systems. An integrated approach that includes early detection, environmental management, effective colostrum intake, and staff training is essential. Given the zoonotic nature of *Cryptosporidium*, biosecurity measures for farm personnel are also critical.

Keywords: *Cryptosporidium parvum*, Lamb diarrhea, *Klebsiella*, Neonatal infection, Colostrum, Zoonosis.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Introduction:

Diarrhea in newborn lambs is a leading cause of morbidity and mortality, particularly in industrialized sheep operations with high animal density. The causative agents are often multifactorial, including viral, bacterial, and parasitic pathogens. Among parasitic causes, *Cryptosporidium parvum* has gained prominence due to its widespread environmental presence, resistance to conventional disinfectants, and significant zoonotic potential (1, 2).

C. parvum infects the epithelial cells of the small intestine, leading to villus atrophy, malabsorption, and watery diarrhea. Oocysts are excreted in high numbers—up to 10^7 per gram of feces—and are highly resilient, surviving in cool, moist environments for extended periods (3). These oocysts are notably resistant to standard sanitation practices, including chlorine and iodine solutions (4, 5). As a result, outbreaks can persist in contaminated environments despite routine hygiene procedures. In Iran, while several studies have addressed cryptosporidiosis in calves, documentation in lamb populations remains limited. This report investigates a neonatal diarrhea outbreak in lambs, emphasizing the diagnostic process and management strategies to control *Cryptosporidium* in a high-risk setting.

Materials and Methods:

The outbreak occurred in June 2025 at a large sheep farm in northeastern Iran. The farm operated under intensive conditions, housing several hundred ewes and their newborn lambs. Lambs were separated from their dams shortly after birth and reared in indoor pens with shared feeding and bedding systems.

During the investigation, lambs aged 5 to 10 days presenting with diarrhea were clinically examined. Key observations included fecal consistency, signs of dehydration, and the status of the umbilical area. Fresh fecal samples were collected rectally and examined using modified flotation techniques for the detection of *Cryptosporidium* oocysts. Umbilical swabs were cultured on selective media for bacterial identification, specifically targeting *Klebsiella spp.* and *Escherichia coli*.

In parallel, farm management practices were evaluated. Investigators reviewed lambing protocols, colostrum feeding timing and volume, housing hygiene, and navel disinfection routines. Ventilation, bedding moisture, and overall pen sanitation were assessed to identify environmental contributors to disease spread.

Results and Discussion:

Affected lambs exhibited profuse watery yellow diarrhea, moderate dehydration, and noticeable navel swelling, which persisted despite the routine use of iodine-based disinfectants.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Fecal testing confirmed a heavy burden of *C. parvum* oocysts, consistent with earlier reports describing cryptosporidiosis as a leading cause of neonatal diarrhea in ruminants (1, 2, 3).

Bacterial culture identified *Klebsiella spp.* in both fecal and umbilical samples. The co-presence of *Klebsiella* with *Cryptosporidium* suggests a synergistic relationship where protozoal infection compromises intestinal mucosa, facilitating bacterial colonization and systemic spread (2, 4). *Klebsiella* is known to cause navel ill and may lead to septicemia in neonates, particularly when umbilical disinfection is incomplete or improperly applied (2, 5).

The environmental assessment revealed several risk factors that facilitated disease persistence. Pens were overcrowded and poorly ventilated, with bedding often damp and contaminated with feces. These conditions are ideal for the prolonged survival and spread of *Cryptosporidium* oocysts (3,6). The disinfectants used for umbilical care were iodine-based, which are insufficient against resistant oocysts and possibly ineffective against the strains of *Klebsiella* present on the farm.

As no curative treatment for cryptosporidiosis currently exists, management centered on supportive therapy. Affected lambs received oral and intravenous fluid therapy to correct dehydration, which is the primary cause of death in such cases (9). Selective antibiotic therapy was administered to lambs with systemic signs of infection, with florfenicol and ceftiofur showing moderate efficacy against *Klebsiella* isolates in the region (2).

Control of environmental contamination was addressed by switching to disinfectants known to be effective against oocysts, particularly 10% formalin and 5% ammonia, which have been shown to reduce oocyst viability in similar outbreaks (4, 5). These were used in combination with improved drying and cleaning protocols to reduce environmental persistence. Feeding tools, pens, and bedding were routinely disinfected and allowed to dry, as oocysts are susceptible to desiccation (5).

In response to the outbreak, several long-term interventions were introduced. Colostrum management was prioritized, ensuring that each lamb received at least 10 to 15 percent of its body weight in high-quality colostrum within six hours of birth. Adequate passive immunity is critical in protecting neonates from both cryptosporidial and bacterial pathogens (2, 8).

Vaccination programs for ewes were reviewed and updated. Pre-lambing immunizations were reinforced to cover *E. coli*, rotavirus, and clostridial diseases, aiming to enhance maternal antibody transfer via colostrum (2). Although no commercial vaccine exists for *Cryptosporidium*, maternal health and immunity play an important role in mitigating the early severity of infection.

Improved hygiene practices were introduced across the facility. These included daily cleaning of pens, enhanced bedding management, and use of disinfectants with proven efficacy against *Cryptosporidium* oocysts (5,6). Biosecurity measures were also strengthened. Staff were



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



trained on the zoonotic risk of *Cryptosporidium*, and use of gloves, handwashing, and protective clothing became mandatory during outbreak periods (11). Equipment was disinfected between uses, and rodent control programs were intensified to eliminate environmental contamination reservoirs.

Continuous monitoring and data recording were implemented to allow for early detection in future outbreaks. Fecal sampling became a routine protocol during the first weeks of life, and all diarrhea cases were tracked and linked to management interventions to assess effectiveness over time.

This outbreak investigation highlights the critical role of *Cryptosporidium parvum* in neonatal lamb diarrhea and emphasizes the complexity added by bacterial co-infections such as *Klebsiella spp.* The findings support the need for comprehensive farm management, including proper colostrum administration, environmental disinfection, and consistent hygiene protocols. As no specific treatments for cryptosporidiosis exist, early supportive care and biosecurity remain the most effective tools for controlling mortality. Given the zoonotic nature of the disease, protective measures for farm workers are equally essential. These findings provide practical guidance for other intensive sheep operations confronting similar challenges.

Acknowledgment:

The author would like to thank the management and veterinary staff of Ruyesh Talaei Agro-Industry for their collaboration, and the laboratory team at Ferdowsi University of Mashhad for their diagnostic support during the investigation.

References:

1. Thomson S, et al. Bovine cryptosporidiosis: impact, host-parasite interaction and control strategies. *Vet Res.* 2017; 48:42. doi:10.1186/s13567-017-0447-0
2. Munir M, Islam MR. Bacterial and Parasitic Causes of Diarrhea in Lambs. *J Vet Sci.* 2021;22(3): e45. doi:10.4142/jvs.2021.22. e45
3. Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium*: transmission, detection, and identification. *Int J Parasitol.* 2000;30(12–13):1305–22.
4. Harp JA, Goff JP. Strategies for the control of *Cryptosporidium parvum* infection in calves. *J Dairy Sci.* 1998;81(11):289–94.
5. Smith HV, et al. *Cryptosporidium* and *Giardia* as agents of waterborne disease. *Annu Rev Microbiol.* 2005; 59:247–77.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



6. Kahn CM, Line S, eds. Cryptosporidiosis in Animals. In: *The Merck Veterinary Manual*. 11th ed. Merck & Co.; 2020.
7. Zhang L, Li X. Genetic Signatures for Resilience to Neonatal Diarrhea in Sheep. *Genet Sel Evol*. 2022;54(1):28. doi:10.1186/s12711-022-00712-3
8. Urie NJ, et al. Factors associated with morbidity and mortality in preweaned dairy heifer calves. *J Dairy Sci*. 2018;101(10):9229–44.
9. Berchtold J. Treatment of calf diarrhea: intravenous fluid therapy. *Vet Clin North Am Food Anim Pract*. 2009;25(2):73–99.
10. Brainard J, et al. Efficacy of halofuginone to prevent or treat cryptosporidiosis in calves: meta-analysis. *Parasitology*. 2021; 148:408–19.
11. Chalmers RM, Giles M. Zoonotic cryptosporidiosis in the UK – challenges for control. *J Appl Microbiol*. 2010; 109:1487–97.



A Review of *Varroa* Mite Infestation and Plant-Based Control Strategies: Current Insights and Future Perspectives

Farnaz Malekifard¹, Alireza Ghorbani¹, Seyyed Kian Mohseni Mofidi¹

Corresponding Author: Farnaz Malekifard, f.malekifard@urmia.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article code: 1421

Abstract

Background: The honeybee (*Apis mellifera*), as a key natural pollinator, plays a crucial role in ecosystem balance, agricultural productivity, and food security. Despite this, honeybee populations have declined in recent decades, largely due to diseases and pests. Among these, Varroosis, caused by the external parasite *Varroa destructor*, poses one of the most serious threats, feeding on adult and immature bees, weakening the immune system, reducing lifespan, and transmitting lethal viruses, ultimately leading to colony collapse and decreased beekeeping yields.

Methods: This study explores plant-based compounds as alternative, safe, and sustainable approaches for controlling *Varroa destructor*. A review of various essential oils, including thyme, garlic, bitter melon, basil, clove, neem, rosemary, mint, and eucalyptus, was conducted to evaluate their efficacy against the mite while assessing potential impacts on bee health.

Results: Essential oils containing active compounds such as thymol, eugenol, azadirachtin, and carvacrol were found to disrupt the nervous and respiratory systems of *Varroa* mites. These treatments significantly reduced mite populations without causing notable negative effects on bees.

Conclusion: Plant-derived essential oils represent an effective, low-risk, and environmentally friendly strategy for managing *Varroa destructor*. Their integration into modern beekeeping as part of Integrated Pest Management (IPM) programs can enhance colony health and productivity. This study provides a comprehensive overview of Varroosis and highlights plant-based treatments as viable alternatives to synthetic chemicals.

Keywords: Honey bee, *Varroa destructor*, Medicinal plants, Essential oils, Natural treatment, Non-chemical control.



Introduction:

Honey bees (*Apis mellifera*) are among the most crucial pollinators of plants worldwide and play a vital role in agricultural production and ecosystem stability (1–3). Beyond their essential role in pollination, honey bees contribute significantly to the agricultural economy through the production of valuable hive products, including honey, propolis, royal jelly, and beeswax (4,5). Consequently, beekeeping has emerged as a key economic activity in many countries. Data collected globally indicate that the number of managed beekeeping operations has steadily increased from 1961 to 2017, reflecting growing awareness of the importance of maintaining healthy honey bee colonies (6).

Despite their significance, honey bee populations face numerous threats from both biotic and abiotic factors, which have contributed to notable colony losses in various regions. These challenges have serious implications for food security and agricultural productivity, especially in developing nations where beekeeping supports rural livelihoods (7,8). Among the most critical threats is infestation by *Varroa* mites, which are parasitic pests with global distribution and significant economic impact (9–11). Even geographically isolated regions such as Australia, once considered free from *Varroa*, have recently reported infestations, demonstrating the high dispersal potential of these mites (12).

Varroa mites spread rapidly through multiple pathways, including attachment to foraging bees on flowers, transfer via swarming behavior, and movement of commercial bee packages between apiaries (13–16). At the individual bee level, *Varroa* infestation suppresses immunity, shortens lifespan, and disrupts reproductive functions. At the colony level, infestations reduce growth, lower productivity, and diminish the yield of hive products. Furthermore, these mites serve as vectors for several viral pathogens, including Sacbrood virus and Black Queen Cell Virus, which inflict severe damage to larvae and pupae (17–19).

The *Varroa* genus includes several parasitic species affecting honey bees. *Varroa jacobsoni*, native to Asia and first identified on *Apis cerana* in Java, Indonesia, was initially believed to be the cause of global infestations. However, the primary culprit of worldwide infestations is *Varroa destructor*, an invasive species capable of parasitizing both *Apis cerana* and *Apis mellifera* (20–24). Other species, such as *Varroa underwoodi* in Nepal and *Varroa rindereri* in Borneo, are less widespread and pose limited global threat (24,25).

Given the extensive economic damage caused by *Varroa* mites, controlling and preventing infestations is a top priority. Traditional chemical treatments, however, face limitations due to increasing resistance, reduced efficacy, and potential harm to bees and the environment (26,27). Consequently, there is growing interest in non-chemical, environmentally friendly approaches, including the use of plant-derived compounds. This study aims to review and evaluate herbal-based strategies for controlling *Varroa destructor* in honey bee colonies.



Materials and Methods:

This review analyzed research on plant-derived compounds for the management of *Varroa destructor* in *Apis mellifera* colonies. The study focused on essential oils and plant extracts from species such as thyme, lavender, peppermint, eucalyptus, neem, rosemary, clove, garlic, basil, mustard, and bitter melon. Peer-reviewed studies published up to 2024 were included. Key parameters considered were application methods, concentrations, duration of treatment, mite mortality rates, and potential toxicity to honey bees. Both direct acaricidal effects and indirect benefits to colony health were evaluated. Results were synthesized to identify plant-based treatments that are effective, safe, and environmentally sustainable.

Results and Discussion:

Thyme (*Thymus vulgaris*): Thyme is widely recognized for its effectiveness against *Varroa* mites due to the active compound thymol. Thymol disrupts ion transfer in the mite's nervous system, leading to rapid mortality. Multiple studies, including those by Imdorf et al. (1999) and Calderone & Spivak (1995), demonstrated that thymol treatment significantly reduces *Varroa* populations within hives (28,29).

Lavender (*Lavandula angustifolia*): Lavender contains linalool and linalyl acetate, compounds with both repellent and acaricidal properties. These compounds encourage mites to abandon brood cells and infected bees, thereby reducing infestation levels in colonies (30).

Peppermint (*Mentha piperita*): Peppermint's active compound, menthol, is highly toxic to mites and can suffocate them effectively. It is frequently used in combination with thymol in commercial formulations such as Apiguard (31).

Eucalyptus (*Eucalyptus spp.*): Eucalyptus essential oils, rich in cineole and alpha-pinene, exert gradual lethal effects on *Varroa* mites by interfering with their respiratory and nervous systems (32,33).

Neem (*Azadirachta indica*): Neem contains azadirachtin, a natural growth regulator that disrupts mite molting and reproductive processes, indirectly causing mortality and reducing overall infestation (34–36).

Rosemary (*Rosmarinus officinalis*) and Clove (*Syzygium aromaticum*): Rosemary's phenolic compounds, such as rosmarinic acid, impair mite nervous and respiratory systems, while clove's eugenol rapidly paralyzes and kills mites at low concentrations. Both plants demonstrate high acaricidal activity with minimal bee toxicity (37).

Other Effective Plants: Carvacrol, found in oregano (*Origanum vulgare*), penetrates the mite cuticle, causing rapid mortality and proving highly effective in laboratory studies (34,35).



Other promising plant-derived compounds include bitter melon (*Citrullus colocynthis*), garlic (*Allium sativum*), basil (*Ocimum basilicum*), and mustard (*Sinapis arvensis*).

Comparative Studies of Essential Oils: Alsaadi et al. (2024) investigated nine essential oils for acaricidal effects against *Varroa destructor*. Bitter melon oil at 15% concentration achieved approximately 80% mite mortality, the highest among the tested oils. Garlic oil caused 75% mortality, basil 66.6%, thyme and mustard 61.7% each, while clove and eucalyptus oils had moderate effects (58.3% and 55.5%, respectively). Mint and rosemary showed lower efficacy (53.3%). Bee mortality was minimal, with basil oil causing the highest mortality at 16%, while bitter melon and thyme oils increased mortality by only 6–10%, demonstrating safe application ranges (38).

Regional Applications: Rahimi and Parichehre (2024) evaluated the plant Hengwan in Kurdistan, Iran, and found it reduced *Varroa* infestation by up to 50%. This study highlights the potential of locally available herbs for sustainable bee health management (39).

Immune-Enhancing Effects of Plant Compounds: Narciso et al. (2024) tested cinnamon and oregano essential oils administered orally in syrup form. Although infestation levels were not significantly reduced, bees exhibited increased expression of the vitellogenin gene, indicating improved immune function. Such findings suggest that plant-derived compounds can complement direct acaricidal treatments by enhancing bee resilience to pathogens (40).

Integrated Pest Management and Future Perspectives: Overall, plant-derived compounds such as thymol, menthol, eugenol, azadirachtin, and carvacrol exhibit distinct physiological effects on *Varroa* mites' nervous, respiratory, and hormonal systems. They represent effective, environmentally friendly alternatives to conventional chemical acaricides. These treatments are generally non-toxic to bees, do not leave harmful residues in hive products, and avoid the problem of drug resistance. Implementing plant-based compounds in integrated pest management (IPM) programs offers a sustainable approach for modern apiculture. While chemical treatments remain common, concerns over resistance, environmental pollution, and bee health emphasize the need for alternative strategies. The standardization of application protocols, field validation, and supportive legislation are essential for the wider adoption of plant-based interventions in commercial beekeeping.

Varroa destructor infestation continues to be one of the most significant threats to global apiculture. Herbal and plant-derived compounds provide promising, safe, and environmentally sustainable options for managing infestations. Essential oils from thyme, peppermint, clove, rosemary, bitter melon, and garlic demonstrate substantial acaricidal effects with minimal adverse impacts on honey bees. Additionally, certain plant compounds enhance bee immunity, offering complementary benefits beyond direct mite control.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Future research should focus on optimizing dosages, delivery methods, and field-based validation, as well as exploring locally available herbs suitable for different climatic and ecological conditions. Integration of these plant-based approaches into broader IPM strategies can significantly improve honey bee health, ensure sustainable hive productivity, and reduce reliance on chemical acaricides, safeguarding both agriculture and the environment.

References:

1. Morse RA, Calderone NW. The value of honey bees as pollinators of US crops in 2000. *Bee Cult.* 2000;128(3):1–15.
2. Paudel YP, Mackereth R, Hanley R, Qin W. Honey bees (*Apis mellifera* L.) and pollination issues: Current status, impacts, and potential drivers of decline. *J Agric Sci.* 2015;7(6):93.
3. Sillman J, Uusitalo V, Tapanen T, Salonen A, Soukka R, Kahiluoto H. Contribution of honeybees towards the net environmental benefits of food. *Sci Total Environ.* 2021;756:143880.
4. Bava R, Castagna F, Musella V, Lupia C, Palma E, Britti D. Therapeutic use of bee venom and potential applications in veterinary medicine. *Vet Sci.* 2023;10(2):119.
5. Aylanc V, Falcão SI, Ertosun S, Vilas-Boas M. From the hive to the table: Nutrition value, digestibility and bioavailability of the dietary phytochemicals present in the bee pollen and bee bread. *Trends Food Sci Technol.* 2021;109:464–81.
6. Phiri BJ, Fèvre D, Hidano A. Uptrend in global managed honey bee colonies and production based on a six-decade viewpoint, 1961–2017. *Sci Rep.* 2022;12(1):21298.
7. Hristov P, Shumkova R, Palova N, Neov B. Honey bee colony losses: Why are honey bees disappearing? *Sociobiology.* 2021;68(1):e5851–e5851.
8. Potts SG, Roberts SPM, Dean R, Marris G, Brown MA, Jones R, et al. Declines of managed honey bees and beekeepers in Europe. *J Apic Res.* 2010;49(1):15–22.
9. Le Conte Y, Meixner MD, Brandt A, Carreck NL, Costa C, Mondet F, et al. Geographical distribution and selection of European honey bees resistant to *Varroa destructor*. *Insects.* 2020;11(12):873.
10. Traynor KS, Mondet F, de Miranda JR, Techer M, Kowallik V, Oddie MAY, et al. *Varroa destructor*: A complex parasite, crippling honey bees worldwide. *Trends Parasitol.* 2020;36(7):592–606.
11. Beaurepaire A, Piot N, Doublet V, Antunez K, Campbell E, Chantawannakul P, et al. Diversity and global distribution of viruses of the western honey bee, *Apis mellifera*. *Insects.* 2020;11(4):239.
12. Phaboutdy E, Ward M. Investigation of landscape risk factors for the recent spread of varroa mite (*Varroa destructor*) in European honeybee (*Apis mellifera*) colonies in New South Wales, Australia. *Geospat Health.* 2024;19(1).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



13. Akyol E, Karatepe B, Karatepe M, Karaer Z. Development and control of the varroa (*Varroa destructor*) in honey bee (*Apis mellifera*) colonies and effects on the colony productivity. *Uludağ Arıcılık Derg.* 2006;6(4):149–54.
14. Peck DT, Smith ML, Seeley TD. Varroa destructor mites can nimbly climb from flowers onto foraging honey bees. *PLoS One.* 2016;11(12):e0167798.
15. Ratti V, Kevan PG, Eberl HJ. A discrete-continuous modeling framework to study the role of swarming in a honeybee-Varroa destructor-virus system. In: *Mathematical and computational approaches in advancing modern science and engineering.* Springer; 2016. p. 299–308.
16. Emsen B, Guzman-Novoa E, Kelly PG. Honey production of honey bee (Hymenoptera: Apidae) colonies with high and low Varroa destructor (Acari: Varroidae) infestation rates in eastern Canada. *Can Entomol.* 2014;146(2):236–40.
17. Bailey L, Ball B V, Perry JN. The prevalence of viruses of honey bees in Britain. *Ann Appl Biol.* 1981;97(1):109–18.
18. Bailey L. The multiplication and spread of sacbrood virus of bees. *Ann Appl Biol.* 1969;63(3):483–91.
19. Bailey L, Ball B V. *Honey bee pathology.* Elsevier; 2013.
20. Anderson DL, Trueman JWH. Varroa jacobsoni (Acari: Varroidae) is more than one species. *Exp Appl Acarol.* 2000;24(3):165–89.
21. Koeniger N, Koeniger G, Wijayagunasekara NHP. Beobachtungen über die Anpassung von Varroa jacobsoni an ihren natürlichen Wirt Apis cerana in Sri Lanka. *Apidologie.* 1981;12(1):37–40.
22. Oudemans AC. On a new genus and species of parasitic acari. *Notes from Leyden Museum.* 1904;24(4):216–22.
23. Hadisoesilo S, Otis GW. Differences in drone cappings of Apis cerana and Apis nigrocincta. *J Apic Res.* 1998;37(1):11–5.
24. Anderson DL. Variation in the parasitic bee mite Varroa jacobsoni Oud. *Apidologie.* 2000;31(2):281–92.
25. Delfinado-Baker M, Aggarwal K. A new Varroa (Acari: Varroidae) from the nest of Apis cerana (Apidae). *Int J Acarol.* 1987;13(4):233–7.
26. Rinkevich FD. Detection of amitraz resistance and reduced treatment efficacy in the Varroa Mite, Varroa destructor, within commercial beekeeping operations. *PLoS One.* 2020;15(1):e0227264.
27. Elzen PJ, Westervelt D. Detection of coumaphos resistance in Varroa destructor in Florida. 2002;
28. Calderone NW, Spivak M. Plant extracts for control of the parasitic mite Varroa jacobsoni (Acari: Varroidae) in colonies of the western honey bee (Hymenoptera: Apidae). *J Econ Entomol.* 1995;88(5):1211–5.
29. Imdorf A, Bogdanov S, Ochoa RI, Calderone NW. Use of essential oils for the control



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- of *Varroa jacobsoni* Oud. in honey bee colonies. *Apidologie*. 1999;30(2–3):209–28.
30. Gashout HA, Goodwin PH, Guzman-Novoa E. Lethality of synthetic and natural acaricides to worker honey bees (*Apis mellifera*) and their impact on the expression of health and detoxification-related genes. *Environ Sci Pollut Res*. 2018;25(34):34730–9.
 31. Sammataro D, Gerson U, Needham G. Parasitic mites of honey bees: life history, implications, and impact. *Annu Rev Entomol*. 2000;45(1):519–48.
 32. Aglagane A, Laghzaoui EM, Ben Elfakir S, Er-Rguibi O, Abbad A, El Mouden EH, et al. Essential oils as sustainable control agents against *Varroa destructor* (Acari, Varroidae), an ectoparasitic mite of the western honeybees *Apis mellifera* (Hymenoptera: Apidae): Review of recent literature (2010-onwards). *Int J Acarol*. 2021;47(5):436–45.
 33. Ruffinengo SR, Maggi MD, Marcangeli JA, Eguaras MJ, Principal J, Barrios C, et al. Integrated Pest Management to control *Varroa destructor* and its implications to *Apis mellifera* colonies. *Zootec Trop*. 2014;32(2):149–68.
 34. Isman MB. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol*. 2006;51(1):45–66.
 35. Wang C. Identification of potential biomarker genes for selecting varroa tolerant honey bees (*Apis mellifera*) and biochemical characterization of a differentially expressed carboxylesterase gene in response to mite infestation. 2017.
 36. Margaoan R, Papa G, Nicolescu A, Cornea-Cipcigan M, Kösoğlu M, Topal E, et al. Environmental pollution effect on honey bees and their derived products: A comprehensive analysis. *Environ Sci Pollut Res*. 2024;1–22.
 37. Tutun H, Koç N, Kart A. Plant essential oils used against some bee diseases. *Turkish J Agric Sci Technol*. 2018;6(1):34–45.
 38. Alsaadi M, Keshlaf MM, Mirwan HB. Some essential oils as potential control agents for varroa mite (*Varroa destructor*) in infected honey bees (*Apis mellifera*). *Open Vet J*. 2024;14(2):692.
 39. Rahimi, Ataallah, Pari Chehre Dizji. Evaluation and introduction of a herbal mixture for controlling *Varroa* mites in honeybee colonies. *Iranian Honey Bee Science and Technology*. 2024 Jul 22;15(28):19-27.
 40. Narciso L, Topini M, Ferraiuolo S, Ianiro G, Marianelli C. Effects of natural treatments on the varroa mite infestation levels and overall health of honey bee (*Apis mellifera*) colonies. *PLoS One*. 2024;19(5):e0302846.



Clinical Infection of a Female Najdi Goat with *Anaplasma ovis* in Ahvaz City

Hossein Hamidinejat¹, Ali Abbas Nikvand², Seyedeh Missagh Jalali², Somayeh Bahrami¹, Maryam Abbaszadeh³

Corresponding Author: Maryam Abbaszadeh, maryamabbaszadeh241375@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
3. Graduated of Veterinary Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1423

Abstract

Background: Several *Anaplasma* species can infect the erythrocytes of ruminants. *Anaplasma ovis* is the main cause of anaplasmosis in sheep and goats. According to the literature, this parasite generally exhibits mild pathogenicity in small ruminants. The diagnosis of this disease is done by the conventional method of blood smear microscopy to detect *Anaplasma*-like inclusion bodies, as well as molecular tests such as PCR. The aim of this study was to report the molecular confirmation of *A. ovis* and its potential to cause severe hemolytic disease in Najdi goats.

Methods: In July 2022, a five-year-old Najdi female goat presenting with lethargy, anorexia, tick infestation, dyspnea, and pale mucous membranes was referred to the teaching hospital of the Faculty of Veterinary Medicine at Shahid Chamran University of Ahvaz. A blood sample was collected from the jugular vein and examined using hematological, microscopic, and PCR techniques.

Results: In the hematological examination, a severe decrease in hemoglobin (4.5 g/dL) and hematocrit (13.3%) was observed. Along with clinical signs such as high fever, mucosal pallor, anorexia, lethargy, and tick infestation, intra-RBC marginal and sub-marginal inclusion bodies were also detected in the microscopic examination of blood smear. PCR analysis targeting the *msp4* gene confirmed the presence of *Anaplasma ovis* DNA.

Conclusion: This report shows that infection with *Anaplasma ovis* can cause severe clinical disease and hematological complications in Najdi goats.

Keywords: *Anaplasma ovis*, Blood smear, *Msp4* gene, Najdi goat.



Introduction:

Anaplasma spp. are obligate intracellular bacteria that can impact both animal and human health (1,2). In ruminants, species such as *Anaplasma marginale*, *A. centrale*, *A. ovis*, *A. platys*, *A. bovis*, and *A. phagocytophilum* are known to cause anaplasmosis (3). These pathogens are primarily transmitted via tick bites, biting insects, blood transfusions, and contaminated needles (4).

Anaplasma ovis is the primary causative agent of anaplasmosis in sheep and goats and is prevalent in tropical and subtropical regions worldwide (2,5,6). Although the infection is generally mild, stress, heavy tick infestation, or concurrent infections can increase its severity, and in some cases, severe hemolytic anemia has been reported (5,7,8). In Iran, subclinical *Anaplasma* infections in goats have been reported using microscopic and molecular methods, with a prevalence of 30% in Khuzestan and 38.9% in Mashhad (9,10).

The Najdi goat, a native Iranian breed with dual-purpose productivity (meat and milk), is mainly raised in the central and eastern regions of Khuzestan Province and along the Persian Gulf coast, with limited populations found in other areas such as Kohgiluyeh and Boyer-Ahmad Province. Due to its good milk yield and high twinning rate (60.2%), its popularity has increased among small-scale farmers in Khuzestan (11).

Materials and Methods:

Case presentation: In July 2022, a five-year-old female Najdi goat from a herd of 15 animals in the Malashieh region of Ahvaz was referred to the Veterinary Teaching Hospital of Shahid Chamran University of Ahvaz with complaints of anorexia, lethargy, and difficulty walking. According to history, in addition to free grazing, the animal had also been fed barley and straw manually. Its last kidding had occurred one year earlier, and it had shown clinical signs for the past two weeks. No treatment was administered before referral.

Clinical examination and Hematology

A complete clinical examination was performed to evaluate body temperature, heart rate, color of mucous membranes, status of lymph nodes, and the presence of ectoparasites. For laboratory investigations, blood was drawn from the jugular vein into an EDTA tube and analyzed for hematological evaluation, blood smear preparation, and molecular analysis using PCR.

Microscopic and Molecular Diagnosis

The diagnosis of this disease was performed using conventional microscopic examination of a blood smear stained with Giemsa to identify *Anaplasma*-like inclusion bodies; the smear was fixed in methanol, stained with Giemsa, and examined under a light microscope at $\times 1000$ magnification using oil immersion. Additionally, the diagnosis of *Anaplasma ovis* was

performed using molecular PCR; a portion of the blood was submitted to the Parasitology Department for PCR analysis targeting the *msp4* gene of *Anaplasma ovis*. For this purpose, DNA was extracted from the blood sample using the Sina Clon extraction kit (Iran) according to the manufacturer's instructions. For specific detection of *Anaplasma ovis* DNA, a pair of oligonucleotide primers targeting a 347 bp fragment of the parasite *msp4* gene (Table 1) (12), was used in a conventional endpoint PCR assay. Distilled water was used as the negative control.

Table 1. Primer sequences of the *msp4* gene used for the detection of *Anaplasma ovis*.

Primer	Sequence
Forward stand (Ana F)	5'_TGAAGGGAGCGGGGTCATGGG_3'
Reverse stand (Ana R)	5'_GAGTAATTGCAGCCAGGGACTCT_3'

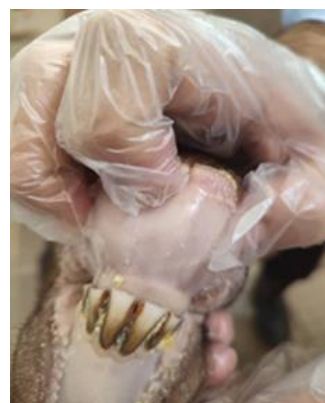
Results and Discussion:

Clinical and Hematological Findings

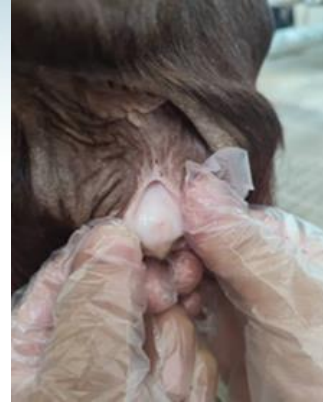
Clinical examination revealed a body temperature of 40.2°C, heart rate of 70 beats per minute, pallor of the ocular, vaginal, and oral mucous membranes, along with tick infestation on the ears (Figure1). No lymphadenopathy or hemoglobinuria was observed. Hematological analysis showed a marked decrease in hemoglobin, hematocrit, and red blood cell count, along with significant leukocytosis (Table 2).



A: Ear tick infestation



B: Pallor of the oral mucous membranes



C: Pallor of the ocular mucous membranes D: Pallor of the vaginal mucous membranes

Figure 1. Clinical signs in a female Najdi goat infected with *Anaplasma ovis*.

Table 2. Hematological results of a Najdi goat infected with *Anaplasma ovis*.

Parameter	Results	Normal values (25)
WBC (x 10 ³ /uL)	25.3	4.0-13.0
RBC (x 10 ⁶ /uL)	8.14	8.0-18.0
HGB (g/dL)	4.5	8.0-12.0
HCT (%)	13.3	22.0-38.0
MCV (fl)	15.9	14.0-25.0
MCH (pg)	5.3	5.2-8.0
MCHC (g/dL)	33.8	30.0-39.0
RDW % (H)	22.4	10.0-20.0

Microscopic Findings

Microscopic examination of the blood smear confirmed the presence of *Anaplasma*-like inclusion bodies located marginally and sub-marginally within red blood cells (Figure 2).

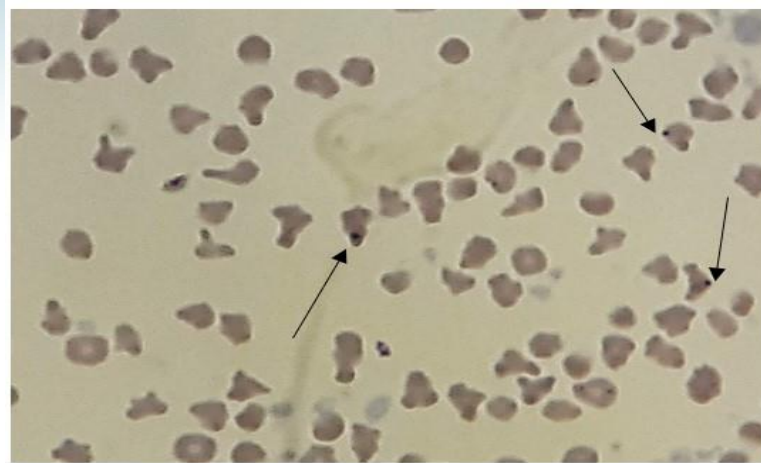


Figure 2. Intra-RBCs *Anaplasma*-like inclusion bodies (arrow) in a Giemsa-stained blood smear of a Najdi goat.

Molecular Findings

After observing *Anaplasma*-like inclusion bodies, the diagnosis of *Anaplasma ovis* was confirmed by molecular PCR. The expected PCR product size for the *A. ovis* primer pair was 347 bp, which matched the band size observed on the gel according to the DNA ladder (Figure 3). Although the primers used in the PCR assay were specific for *A. ovis* detection, the PCR product was also sent to Takapouzist Company for Sanger sequencing. Comparison of the obtained nucleotide sequence with other *A. ovis* sequences available in the GenBank showed 97% identity with LC553537.1, MT344082.1, MN198191.1, PV626511.1, ON229021.1 with accession number OR972395.

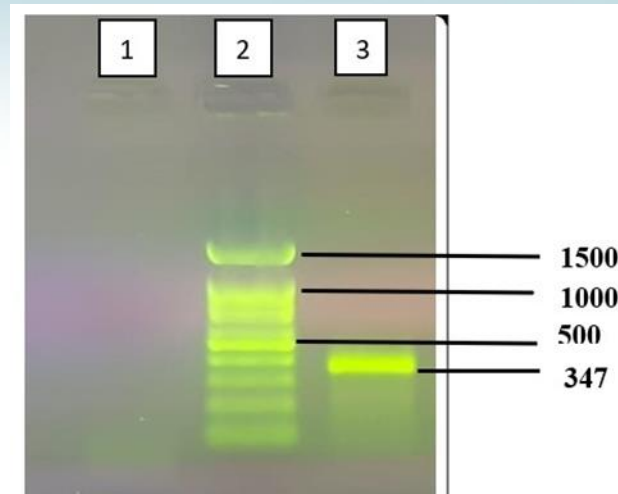


Figure 3. Agarose gel electrophoresis of the PCR-amplified fragment of the *msp4* gene of *Anaplasma* using *A. ovis*-specific primers. Lane 1: negative control; Lane 2: 100 bp DNA marker; Lane 3: positive sample.

Treatment and Management

The patient showed improvement after treatment with a single dose of imidocarb, 10% oxytetracycline administered for 4 days, and an injectable solution containing phosphorus and vitamin B12. Additionally, to eliminate ticks from this animal and other flock members, subcutaneous ivermectin injections and cypermethrin spraying were applied.

Tick-borne diseases have a significant impact on the livelihoods of livestock farmers and are particularly important in developing countries. In some regions of Iran, the sheep and goat farming industry serves as an important source of income, emphasizing the necessity of epidemiological studies on *Anaplasma* species (13,14,15).

The present findings indicate that the Najdi goat is infected with *Anaplasma ovis*; therefore, proper tick management and prevention of iatrogenic transmission, such as the use of sterile syringes and instruments, are essential to prevent the spread of *A. ovis* (4,16). However, according to common belief, *A. ovis* only causes mild clinical symptoms (17).

The findings obtained from the examined sample, together with previous reports, indicate a significant reduction in hematological parameters, including total red blood cell count, hemoglobin concentration, and PCV. These alterations may result from intravascular hemolysis, increased phagocytosis of red blood cells by the reticuloendothelial system, and restricted erythropoietic activity in the bone marrow. Moreover, pallor of the mucous membranes is likely due to anemia and the reduction in red blood cell count and hemoglobin



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



concentration, while the observed jaundice may be attributed to an increase in total bilirubin (2,18,19,20).

Previous studies have shown that sensitive and species-specific PCRs can serve as a simple tool for rapid diagnosis of anaplasmosis (12,21), and the *msp4* gene has also been widely used for the phylogenetic analysis of *Anaplasma ovis* (22). In the present study, PCR based on the *msp4* gene was also employed for the detection of *Anaplasma ovis*.

The data obtained from this sample, together with previous reports, indicate that goat breed has a significant impact on *Anaplasma ovis* infection; specifically, Najdi goats exhibit a higher infection rate compared to black goats (21). Moreover, previous studies have identified breed as an important risk factor associated with anaplasmosis in goats, which should be considered in disease control programs (23).

Moreover, the age and sex of the animals influence the susceptibility to *Anaplasma ovis*. The Najdi goat examined in this study was a female, adult, and five years old, and previous studies have shown that female goats older than three years are at a higher risk of *A. ovis* infection (2,20,21,24). This is likely due to increased stress levels associated with parturition, inadequate nutrition, and hormonal imbalances during lactation and mating, which act as predisposing factors (20,23).

Acknowledgment:

We acknowledge the research council of Shahid Chamran University of Ahvaz for financial support of this work.

References:

1. Parola P, Davoust B, Raoult D. Tick- and flea-borne rickettsial emerging zoonoses. *Vet Res.* 2005;36(3):469–92.
2. Cabezas-Cruz A, Gallois M, Fontugne M, Allain E, Denoual M, Moutailler S, et al. Epidemiology and genetic diversity of *Anaplasma ovis* in goats in Corsica, France. *Parasites & Vectors.* 2019;12(3):1–11.
3. Atif FA. *Anaplasma marginale* and *Anaplasma phagocytophilum*: Rickettsial pathogens of veterinary and public health significance. *Parasitol Res.* 2015;114(11):3941–57.
4. Bekker CP, De Vos S, Taoufik A, Sparagano OA, Jongejan F. Simultaneous detection of *Anaplasma* and *Ehrlichia* species in ruminants and detection of *Ehrlichia ruminantium* in *Amblyomma variegatum* ticks by reverse line blot hybridization. *Vet Microbiol.* 2002;89(3):223–38.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



5. Lacasta D, Ferrer LM, Sanz S, Labanda R, González JM, Benito AA, et al. Anaplasmosis outbreak in lambs: First report causing carcass condemnation. *Animals*. 2020;10(10):1851.
6. Naeem M, Amaro-Estrada I, Taqadus A, Swelum AA, Alqhtani AH, Asif M, et al. Molecular prevalence and associated risk factors of *Anaplasma ovis* in Pakistani sheep. *Front Vet Sci*. 2023;10:1096418.
7. Lu W. Ovine anaplasmosis in northwest China. *Trop Anim Health Prod*. 1997;29(Suppl 1):16S–8S.
8. Stuen S, Nevland S, Moum T. Fatal cases of tick-borne fever (TBF) in sheep caused by several 16S rRNA gene variants of *Anaplasma phagocytophilum*. *Ann N Y Acad Sci*. 2003;990:433–4.
9. Jalali SM, Bahrami S, Rasooli A, Hasanvand S. Evaluation of oxidant/antioxidant status, trace mineral levels, and erythrocyte osmotic fragility in goats naturally infected with *Anaplasma ovis*. *Trop Anim Health Prod*. 2016;48(6):1175–81.
10. Razmi GR, Dasrjerdi K, Hosseini H, Naghibi A, Barati F, Aslani MR. An epidemiological study on anaplasma infection in cattle, sheep, and goats in Mashhad suburb, Khorasan province, Iran. *Ann N Y Acad Sci*. 2006;1078:479–81.
11. Papi N, Mirzaei F. Introduction of native Iranian goats. Tehran: Agricultural Research, Education and Extension Organization; 2019. p. 35–40. [in Persian].
12. Alessandra T, Santo C. Tick-borne diseases in sheep and goats: Clinical and diagnostic aspects. *Small Rumin Res*. 2012;106:S6–S11.
13. Perry BD, Randolph TF, McDermott JJ, Sones KR, Thornton PK. Investing in animal health research to alleviate poverty. Nairobi, Kenya: International Livestock Research Institute; 2002.
14. Minjauw B, McLeod A. Tick-borne diseases and poverty: The impact of ticks and tick-borne diseases on the livelihoods of small-scale and marginal livestock owners in India and eastern and southern Africa. Edinburgh: DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh; 2003.
15. Yousefi A. Phylogenetic analysis of *Anaplasma marginale* and *Anaplasma ovis* isolated from small ruminant based on MSP4 gene in western regions of Iran. *Comp Clin Pathol*. 2018;27(5):1161–65.
16. Uilenberge G. General review of tick-borne diseases of sheep and goats worldwide. *Parasitologia*. 1997;39:161–65.
17. Renneker S, Abdo J, Salih DEA, Karagenç T, Bilgiç H, Torina A, et al. Can *Anaplasma ovis* in small ruminants be neglected any longer? *Transbound Emerg Dis*. 2013;60(2):105–12.
18. Radostitis OM, Gay CC, Blood DC, Hinchcliff KW, Constable PD. *Veterinary Medicine: A Text of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*. 9th ed. Philadelphia: WB Saunders Company; 2000.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



19. Khaki Z, Jalali SM, Kazemi B, Jalali MR, Yasini SP. A study of hematological changes in sheep naturally infected with *Anaplasma* spp. and *Theileria ovis*: Molecular diagnosis. *Iran J Vet Med*. 2015;9(1):19–26.
20. Abdullah DA, Ali FF, Jasim AY, Ola-Fadunsin SD, Gimba FI, Ali MS. Clinical signs, prevalence, and hematobiochemical profiles associated with *Anaplasma* infections in sheep of North Iraq. *Vet World*. 2020;13(8):1524–27.
21. AbbasZadeh M, Hamidinejat H, Nikvand AA, Jalali SM, Bahrami S. Molecular and haematological investigations on *Anaplasma ovis* infection in goats in Ahvaz, Iran: Insights into infection rate, haemolytic effect and breed influences. *Vet Med Sci*. 2024;10:e1404.
22. Sumaira S, Rehman M, Hussain A, Mehmood R, Ashraf N, Giantsis IA, Umer W, Ayesha A, Ullah S, Khan A, Ben Said M. Molecular epidemiology and phylogeny of *Anaplasma* species in goats from Pakistan. *PLoS ONE*. 2025;20(5):e0325467.
23. Rahman M, Faruque MR, Rahman MM, Chowdhury MYE. Epidemiology and molecular detection of *Anaplasma* spp. in goats from Chattogram district, Bangladesh. *Vet Med Sci*. 2022;8(4):1240–49.
24. Yousefi A, Rahbari S, Shayan P, Sadeghi-Dehkordi Z, Bamonar A. Molecular detection of *Anaplasma marginale* and *Anaplasma ovis* in sheep and goat in west highland pasture of Iran. *Asian Pac J Trop Biomed*. 2017;7(5):455–9.
25. Constable PD, Hinchcliff KW, Done SH, Grunberg W. *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, and Horses*. 11th ed. Missouri: Elsevier Saunders; 2017.



Diectophymosis in a Well-cared-for Pitbull Dog with no History of Paratenic Host Ingestion

Javad Jafari¹, Mostafa Rahimof¹, Mohadeseh Hosseinzadeh¹

Corresponding Author: Javad Jafari, javaadjafari1993@gmail.com

1. Private Veterinary Practitioner, Darkoob Veterinary Clinic, Rasht, Iran.

Article code: 1424

Abstract

Background: *Diectophyme* infection affects numerous mammal species, primarily in temperate regions. We report the first documented case of *D. renale* infection in a domestic dog from Rasht, Iran.

Methods: The patient was a 20-month-old female Pitbull dog (30 kg) that presented with anorexia, hematuria, and purulent genital discharge. Initial diagnostic investigations included a complete blood count (CBC) and serum biochemistry, both of which indicated renal injury. Subsequent abdominal ultrasound examination revealed pathological changes in the right kidney. Urinalysis demonstrated hematuria with microscopic detection of *D. renale* eggs, confirming the diagnosis. The case was managed surgically with nephrectomy, which revealed a live female *D. renale* worm in the right kidney.

Results: Complete blood count and serum biochemistry revealed renal injury. Consequently, abdominal ultrasound was also carried out to rule out other possible pathologies in the differential diagnoses and to confirm the absence of concurrent abdominal pathology. Ultrasound revealed a 35 × 30 mm hypoechoic oval lesion in the mid-cortex of the right kidney. Urinalysis demonstrated hematuria, and microscopic examination revealed *D. renale* eggs. nephrectomy, which revealed a live female *D. renale* worm measuring approximately 40 cm in length in the right kidney.

Conclusion: This report describes the first documented case of *Diectophyme renale* infection in a domestic Pitbull dog from Rasht, Iran. Diagnosis was based on clinical presentation, laboratory evidence of renal injury and hematuria with parasite eggs, and ultrasound imaging. A 40-cm adult female worm was surgically removed via nephrectomy, confirming the diagnosis and effectiveness of surgical intervention. This case highlights the need for veterinarians to consider Diectophymosis in differential diagnoses of canine kidney disease, even in non-endemic regions.

Keywords: Dog, *Diectophyme renale*, Kidney, Pit Bull.



Introduction:

Diectophyme renale, commonly known as the giant kidney worm, is a parasitic nematode of the order *Enoplida* that infects a wide range of mammalian hosts, including canids, mustelids, rodents, raccoons, felines, marine mammals, livestock, and, rarely, humans (1). Female worms can exceed 100 cm in length, while males typically reach around 35 cm, though size may vary depending on the host species (1). The parasite follows a complex life cycle involving an aquatic oligochaete (*Lumbriculus variegatus*) as an intermediate host that ingests first-stage larvae. Mammals become infected through the ingestion of these oligochaetes or through paratenic hosts such as fish or amphibians (2). Endemic to temperate freshwater ecosystems, adult worms primarily inhabit the kidneys, although migrating larvae can affect other organs (3). Infections are most common in fish-eating mammals, particularly in dogs over two years of age, and may lead to renal pathology such as kidney atrophy and ureteral obstruction. However, in unilateral infections, compensatory hypertrophy of the contralateral kidney can preserve overall renal function (4). Diagnosis typically involves urinalysis and imaging, and treatment requires nephrectomy (5). The reported prevalence of *D. renale* varies regionally, ranging from 0.49% to 3.57% in Brazil (6) and up to 4.16% among stray dogs in Argentina (7). In Iran, the parasite was first documented in stray dogs from the Caspian region (8), followed by cases in humans (9) and red foxes (10). Zolhavarieh et al. (5) reported the first case in a domestic dog from Hamedan, a cold, arid, mountainous region, thereby extending the known ecological range of this parasite. The objective of this article is to report the first case of parasitism by *D. renale* in a 20-month-old female pit bull, highlighting infections in younger dogs and the parasite's adaptability to diverse climates, emphasizing the need for broader surveillance of this zoonotic pathogen.

Materials and Methods:

In June 2025, a 20-month-old female Pitbull dog was referred with hematuria and purulent vaginal discharge to a veterinary clinic in Rasht city. Following physical examination, urinalysis, complete blood count (CBC), and biochemical profiling were carried out. The CBC showed leukocytosis and eosinophilia, consistent with a parasitic infection, while the biochemical profile was unremarkable (Table 1). Urine sample microscopic examination disclosed the presence of *D. renale* eggs (Figure 1). Ultrasonographic examination of the urinary system disclosed a normal aspect of the left kidney, with slightly hyperechoic medulla, while the right kidney was not visualized in its normal position. An 8-mm-thick cystic structure was present within the caudal region of the right kidney. Several ring-shaped and elongated tubular structures with characteristics consistent with nematodes were within the medulla of the kidney. The suspected mass was approximately 60 mm in length and had a blood supply present (Figure 2).

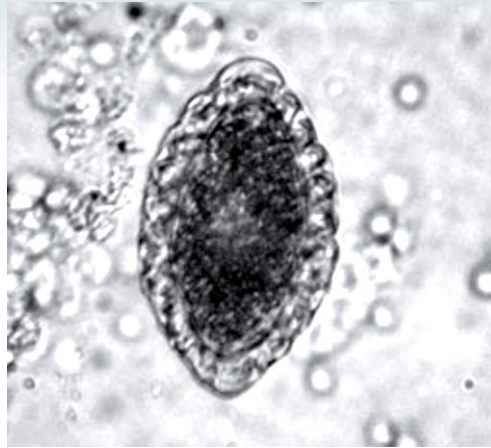


Figure 1. *D. renale* egg in Urine sample

Table 1. Hematology and biochemical profile

Hematology	Patient's values	normal ranges
WBC	19200	6,000 - 17,000 cells/ μ L
Lymphocyte	15%	12-20%
Neutrophil	70%	50-65%
Eosinophil	15%	1-10%
HCT	41%	35-57%
RBC	$5.5 \times 10^6/\text{mcL}$	$4.95\text{-}7.87 \times 10^6/\text{mcL}$
Hemoglobin	13%	11.9-18.9 g/dL
MCV	65.3 fL	60-77 fL
MCH	24.2 pg	21.0-26.2 pg
MCHC	38.3 g/dL	32.0-36.3 g/dL
Biochemical Profile	Patient's values	normal ranges
Albumin	3.8 g/dl	2.3-3.1 g/dl
AST	45 U/L	less than 70 U/L
ALT	64 U/L	less than 100 U/L

ALP	114 U/L	24-147 U/L
Glucose	80 mg/dl	70-120 mg/dL
Cholesterol	155 mg/dl	136-392 mg/dL
Creatinine	1.2 mg/dl	0.5-1.6 mg/dL
Urea	31 mg/dl	7-27 mg/dL
Total protein	7 g/dl	5.4-7.5 g/dL

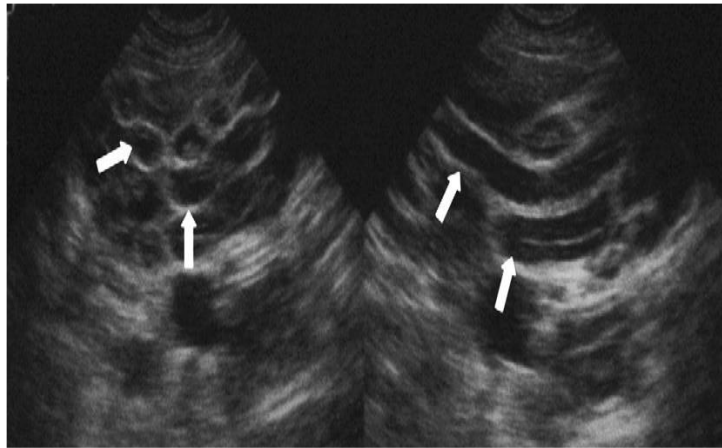


Figure 2. Ultrasound image of the right kidney, *D. renale* (arrow). Horizontal image on the left and longitudinal image on the right.

Results and Discussion:

Diectophymosis was diagnosed in view of the clinical presentation and paraclinical tests. In this way, the female dog was prepared for surgery and the right kidney was removed by unilateral nephrectomy. Upon examination, an adult worm was found in this kidney (Figure 3). Nephrectomy, which revealed a live female *D. renale* worm measuring approximately 40 cm in length in the right kidney. Analgesic treatment and antibiotic therapy were performed after

the operation. After the surgery, the dog regained his health and led a comfortable life without any complication.

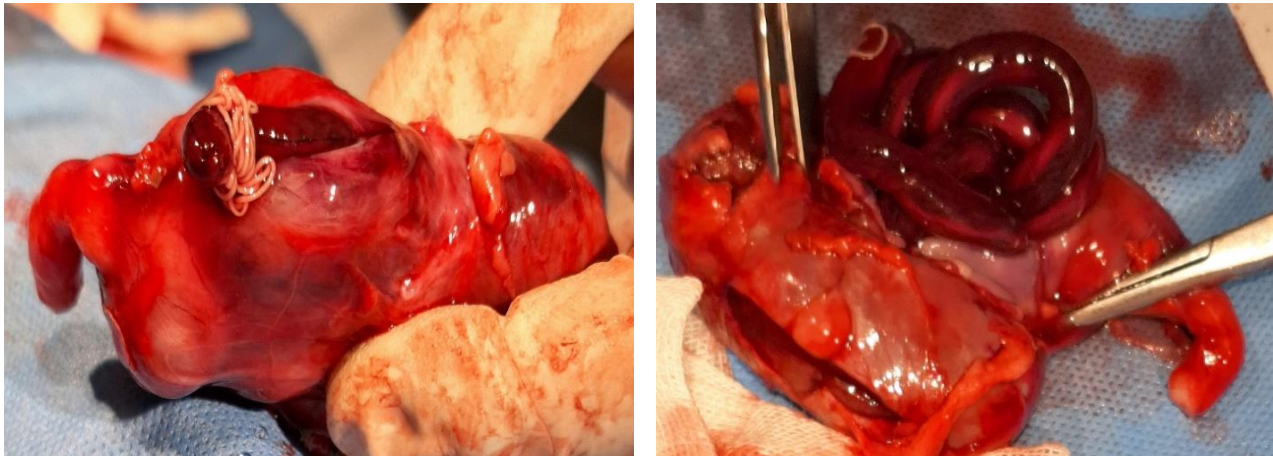


Figure 3. *D. renale* in the right kidney which was surgically removed

This clinical report describes the diagnosis and treatment of *D. renale* infection in a Pitbull dog from Rasht, a temperate region. Although the worm is often diagnosed during necropsy, ultrasonography and urinalysis are of vital importance for diagnosis in live animals. Diagnosis in time is very important because this parasite has the potential for a public health concern. Considering that consuming infected invertebrate intermediate hosts may also establish infection—though this is probably not the primary route in nature—our findings represent the first reported case of *D. renale* infection in a pit bull younger than two years old that occurred without ingestion of seafood, suggesting alternative transmission pathways. Our results were similar to those obtained by Ferreira et al., in the study performed in Brazil in 2010 (3). Detection of *D. renale* eggs in the urine can only happen when a mature fertilized female worm is present in the kidney, a fact that underlines the importance of ultrasonography as emphasized by Mosquito et al. (11). In this case, the prognosis was good; however, in cases where there was a bilateral infection of the kidneys or peritonitis has occurred, nephrotomy and exploratory laparotomy become necessary and the prognosis is usually bad (12). This parasite has also been implicated in *renale* infection in humans; therefore, veterinarians and physicians in the region need to be aware of the possibility of transmission of this parasite. Physicians and veterinarians in the region must be constantly aware of the potential for transmission of infection to prevent the occurrence of *D. renale* in humans.



References:

1. Norouzi R, Manochehri A, Hanifi M. A case report of human infection with *Dioctophyma renale* from Iran. *Urology Journal*. 2017;14(2):3043-5.
2. Pybus MJ, Monismith SW, Kocan AA. Parasitic diseases of wild mammals: Wiley Online Library; 2001.
3. Ferreira VL, Medeiros FP, July JR, Raso TF. *Dioctophyma renale* in a dog: Clinical diagnosis and surgical treatment. *Veterinary Parasitology*. 2010;168(1-2):151-5.
4. Eiras J, Zhu X-Q, Yurlova N, Pedrassani D, Yoshikawa M, Nawa Y. *Dioctophyme renale* (Goeze, 1782)(Nematoda, Dioctophymidae) parasitic in mammals other than humans: A comprehensive review. *Parasitology international*. 2021;81:102269.
5. Zolhavarieh SM, Norian A, Yavari M. *Dioctophyma renale* (Goeze, 1782) infection in a domestic dog from Hamedan, Western Iran. *Iranian Journal of Parasitology*. 2016;11(1):131.
6. Barras T. *Dioctophyme renale*: prevalence and risk factors of parasitism in dogs of São Cristóvão district, Três Barras county, Santa Catarina State, Brazil.
7. Russo ZH, Callirgos JC, García-Ayachi A, Wetzel EJ. Review of *Dioctophyme renale*: etiology, morphology, biology, ecoepidemiology, pathogenesis, symptomatology, diagnosis, treatment, and prevention. *The Journal of Parasitology*. 2022;108(2):180-91.
8. Sadighian A, Amini F. *Dioctophyme renale* (Goeze, 1782) Stiles, 1901 in stray dogs and jackals in Shabsavar area, Caspian region, Iran. 1967.
9. Hanjani A, Sadighian A, Nikakhtar B, Arfaa F. The first report of human infection with *Dioctophyma renale* in Iran. 1968.
10. Zare-Bidaki M, Mobedi I, Ahari SS, Habibizadeh S, Naddaf S, Siavashi M. Prevalence of zoonotic intestinal helminths of canids in Moghan plain, Northwestern Iran. *Iranian journal of parasitology*. 2010;5(2):42.
11. Mesquita L, Rahal SC, Faria L, Takahira RK, Rocha NS, Mamprim MJ, et al. Pre-and post-operative evaluations of eight dogs following right nephrectomy due to *Dioctophyma renale*. *Veterinary Quarterly*. 2014;34(3):167-71.
12. Shahbazi P, Shahbazfar AA, Norouzi R. A case report of *Dioctophyma renale* in the peritoneal cavity of a stray dog. *Journal of Zoonotic Diseases*. 2017;2(1):43-7.



***Toxoplasma gondii* and Schizophrenia: A Neurobiological Review of the Evidence**

Sara Kazemi¹, Seyyed Mohammad Aghapour¹, Farnaz Seilanian Toosi¹, Fatemeh Zahra Gharib²

Corresponding Author: Farnaz Seilanian Toosi, Fzgharib@iau.ac.ir

1. DVM Student, Bab.C., Islamic Azad University, Babol, Iran.

2. Department of Clinical Sciences, Bab.C., Islamic Azad University, Babol, Iran.

Article code: 1428

Abstract

Background: A significant portion of the human population—around one-third—is chronically infected by the intracellular parasite *Toxoplasma gondii*. Its neurotropism and potential role in neuropsychiatric disorders, particularly schizophrenia (SCZ), have been extensively investigated. Several epidemiological and experimental studies suggest a possible link between chronic toxoplasmosis and alterations in brain structure, neurotransmission, and cognitive functions implicated in SCZ pathogenesis. However, the nature and extent of this relationship remain unclear. This study aims to explore whether a significant association exists between chronic *T. gondii* infection and SCZ.

Methods: This narrative review compiles findings from systematic reviews, meta-analyses, and original research studies obtained through a comprehensive literature search, including both biomedical (PubMed, Embase) and general scholarly (Scopus, Web of Science, Google Scholar) databases to achieve thorough representation.

Results: Pooled findings from multiple meta-analyses indicate that individuals infected with *T. gondii* exhibit approximately 1.9 times greater odds of being diagnosed with schizophrenia. Many neurobiological mechanisms potentially link *T. gondii* to SCZ. These include neuroinflammation, astrocytic dysfunction, elevated dopamine synthesis mediated by parasite-encoded tyrosine hydroxylase, glutamate dysregulation involving N-methyl-D-aspartate receptor (NMDAR) signaling, and structural brain changes. Cognitive impairments have also been observed in infected individuals. Nonetheless, causality remains unproven due to the predominance of observational data and potential confounding factors.

Conclusion: An important epidemiological and neurobiological relationship is evident between *T. gondii* infection and SCZ. Clarification of causality and development of potential therapeutic targets requires further longitudinal and mechanistic investigations

Keywords: *Toxoplasma gondii*, Schizophrenia, Neuroinflammation, Neurotransmitters.



Introduction:

As a protozoan parasite, *Toxoplasma gondii* (*T. gondii*) possesses the capacity to infect virtually all nucleated mammalian cells, including those of humans (1-5). It is estimated that about one-third of the world's population carries a persistent latent infection with *T. gondii*, with cysts persisting in multiple organs—most notably the central nervous system (CNS) (3, 4, 6, 7). Though such patients are asymptomatic and do not pose much risk, there is increasing concern about the impact of chronic *T. gondii* infection on neuropsychiatric disorders, especially schizophrenia (SCZ) (1, 3-5, 8).

SCZ is a chronic and severe mental disorder distinguished by disturbances in perception and thought, including hallucinations, delusional beliefs, disorganized thinking, and cognitive dysfunction (2, 3, 9). Its etiology remains partially unknown but is considered multifactorial, involving genetic vulnerability, environmental factors, and neurobiological abnormalities (1, 5, 8, 10). Due to its neurotropism, impact on neurotransmitter systems, and pro-inflammatory effects in the brain, *T. gondii* has drawn growing research interest as a potential factor in the development of SCZ (1-5, 7, 10-12).

This review aims to provide an updated synthesis of epidemiological and neurobiological evidence linking *T. gondii* infection to SCZ, highlighting strengths, limitations, and future research directions (1, 4, 5, 7, 11, 13-16).

Materials and Methods:

This review is based on published systematic reviews, meta-analyses, and individual research articles investigating associations between *T. gondii* infection and SCZ. Literature was searched using combinations of keywords such as *Toxoplasma gondii*, toxoplasmosis, schizophrenia, psychiatric disorders, and neuroinflammation in several high-impact research repositories, including both biomedical (PubMed, Embase) and general scholarly (Scopus, Web of Science, Google Scholar) databases. Eligible studies comprised those presenting epidemiological data, mechanistic insights, or meta-analytical results.

Results and Discussion:

Higher seroprevalence of *T. gondii* among schizophrenia patients, relative to healthy controls, has been a recurring finding across numerous investigations (4, 14, 16, 17). According to a meta-analysis of 51 studies, the odds of *T. gondii* IgG seropositivity were approximately 1.91 times higher in patients with schizophrenia (14). Regional variations exist; for example, a French study reported 88% IgG seropositivity in SCZ patients compared to 53% in controls (16). In Turkey, 63.2% of SCZ patients were seropositive compared to 29% of controls (13).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Although some studies reported no significant associations, the majority support a positive correlation (3, 4, 7, 8, 14, 17-19). Regardless, most studies still refer to the relationship as causal due to the design used (cross-sectional or case-control) (8, 10, 14, 16). It is still unresolved whether infection with *T. gondii* precedes SCZ or if the condition increases the risk of infection (due to lifestyle, socioeconomic status, and so on) (8, 10, 14, 19). *T. gondii* exhibits a tropism for CNS cells, particularly neurons, astrocytes, and microglia (5, 7). Infection can result in chronic neuroinflammation, characterized by elevated cytokine production, blood-brain barrier disruption, and microglial activation (2, 5, 7). Such persistent inflammation may lead to SCZ-related neurobiological changes, such as diminished synaptic activity and increased damage to the neurons (12, 17). Also, one of the strongest mechanistic links involves dopamine. Dysregulation of dopamine is a core feature of SCZ pathophysiology (4, 7). The genome of *T. gondii* expresses tyrosine hydroxylase-encoding genes, the key enzyme that limits the rate of dopamine synthesis. This could enhance local dopamine synthesis in regions of the brain containing tissue cysts (1, 5, 7, 17, 20). SCZ patients with *T. gondii* seropositivity have documented elevated dopamine levels. Glutamate signaling, particularly through NMDAR, is associated with SCZ (5, 21). The immune response triggered by *T. gondii* infection has been proposed to facilitate the production of antibodies targeting NMDA receptors, potentially contributing to the receptor hypofunction associated with SCZ (5, 7, 21). Moreover, *T. gondii* infection is also associated with increased kynurenic acid, which interferes with normal glutamatergic signaling in the brain (2, 5, 7).

Neuroimaging evidence from magnetic resonance imaging (MRI) studies indicates that chronic *T. gondii* infection is associated with gray matter atrophy and cortical thinning (2, 5). These structural abnormalities coincide with changes in SCZ, suggesting a potential pathological connection (2, 5). Other studies also point to cognitive deficits among *T. gondii*-seropositive individuals, even in the absence of overt psychiatric disorders (15, 17). In SCZ patients, *T. gondii* infection may aggravate the cognitive deficits in memory, executive function, and attention (9). An outline of research exploring the relationship between chronic *Toxoplasma gondii* infection and schizophrenia is provided in Table 1.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 1. Summary of Studies Examining the Association Between Chronic *Toxoplasma gondii* Infection and Schizophrenia: Study Characteristics, Statistical Findings, and Methodological Limitations (n=21 studies)

Year	Design	Location	Key Finding	Statistical Result	Major Limitation	Ref
2022	Meta-analysis (51 studies)	Global	Higher <i>T. gondii</i> IgG in SCZ	OR = 1.91	Only 6% studies addressed temporal sequence	(14)
2024	Systematic review	France/Global	High seroprevalence in SCZ	88% vs 53% (SCZ vs controls)	Serological method variability	(16)
2021	Case-control	Turkey	Regional association	63.2% vs 29% seropositive	Limited to Van Province	(13)
2025	Cross-sectional	Norway	Psychotic symptoms & cortisol	Not specified	Cross-sectional design	(8)
2025	Case-control	Russia	Neurobiological biomarkers	Not specified	Antipsychotic confounding	(9)
2022	Case-control	Spain	COMT gene interaction	Not specified	Gene-environment complexity	(10)
2022	Case-control	Ethiopia	Latent infection association	Not specified	Socioeconomic confounding	[19]
2025	Review	Global	Dopamine metabolism	N/A (Review)	No original data	(11)
2012	Review	Global	Risk factor update	OR mentioned	Older data	(18)
2024	Experimental	Global	NMDAR dysfunction	Not specified	Animal model limitations	(21)
2022	Meta-analysis	Global	<i>T. gondii</i> -SCZ relationship	Not specified	Serological variability	(4)
2024	Cross-sectional	Not specified	Latent toxoplasmosis	Not specified	Limited details	(1)
2024	Review	Turkey	Parasitic-psychiatric links	N/A (Review)	Book chapter format	(3)
2015	Meta-analysis	Global	Epilepsy association	Not specified	Different condition (epilepsy)	(6)



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Despite strong associations, key limitations remain. Most studies are cross-sectional or case-control, limiting causal inference (14). While meta-analyses show higher odds of SCZ in *T. gondii*-infected individuals (e.g., OR = 1.91) (14, 18), these designs cannot prove causality (14). For example, only 6% of studies addressed whether toxoplasmosis preceded SCZ onset (14). Confounders like socioeconomic status, comorbidities, and antipsychotic use may bias findings (9, 14). Notably, antipsychotics can alter antibody levels (9). Genetic factors, such as COMT variants, may influence susceptibility and introduce gene-environment interactions (10). Variability in serological tests and cutoffs limits comparability (4, 16). A meta-analysis of 15 studies found 13 positive associations, but many lacked key data such as statistical values or control groups (4). IgM tests may not reliably indicate acute infection, and antibody levels may not reflect active disease (29). Animal studies offer insight into mechanisms, showing behavioral changes like altered activity or memory in mice (2, 21), though extrapolating to humans is challenging due to host and parasite differences (21). To better understand the *T. gondii*-SCZ link, longitudinal studies are needed to assess whether infection precedes SCZ onset (14). Neuroimaging (e.g., PET, fMRI) may reveal brain changes associated with chronic toxoplasmosis (5), such as reduced gray matter in SCZ patients (5, 8). *T. gondii* affects astrocytes and drives chronic neuroinflammation (12).

Infection may trigger epigenetic changes impacting neurodevelopment and immunity, leading to neurotransmitter imbalances (2). Dopamine, glutamate, and GABA dysregulation are involved (4), with evidence of increased dopamine metabolism (2, 11) and disrupted glutamate balance (21). The kynurenine pathway, producing glutamate antagonists like kynurenic acid, is also affected (2). Randomized controlled trials are needed to test anti-parasitic treatments as an adjunct therapy in seropositive SCZ patients (14). Clarifying this relationship may inform prevention and treatment strategies for high-risk groups (19).

Current data suggest an association between chronic *T. gondii* infection and schizophrenia at both the epidemiological and neurobiological levels, though definitive proof of causality remains lacking. Despite consistent findings linking neuroinflammatory processes and neurotransmitter dysregulation (especially dopamine and glutamate) to cognitive impairments, research limitations and potential confounders restrict confident causal claims. In order to gain deeper insights into the underlying mechanisms, future research must include well-planned human studies, neuroimaging techniques, and experimental approaches. Ongoing research could improve how we prevent, detect, and manage SCZ linked to chronic toxoplasmosis, emphasizing the vital contribution of veterinary science in understanding and controlling zoonotic infections shared between humans and animals.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Acknowledgment:

The authors would like to express their sincere gratitude to the Library and Electronic Resources System of Islamic Azad University for providing access to scholarly databases, journals, and research articles. This support was essential for acquiring the necessary data and conducting the analyses presented in this study.

References:

1. Ismael SS, Naamaan LM, Abdullah IM. Association Between Latent Toxoplasmosis and Schizophrenia. *European Journal of Medical and Health Research*. 2024;2(2):63-8.
2. Yang Z, Chen J, Zhang C, Peng H. Pathological mechanisms of glial cell activation and neurodegenerative and neuropsychiatric disorders caused by *Toxoplasma gondii* infection. *Frontiers in Microbiology*. 2024;15:1512233.
3. İbrahim B, Arif Kurt A. The relationship between *Toxoplasma gondii* parasite and psychiatric diseases: investigation of possible links between parasitic infection and schizophrenia. In: Şahna PDE, Selamoğlu PDZ, Akgül PDH, editors. *International Studies and Evaluations in the Field of Health Sciences*. 1st ed. Turkey: Serüven Publishing; October 2024. p. 61-73.
4. Oncu-Oner T, Can S. Meta-analysis of the relationship between *Toxoplasma gondii* and schizophrenia. *Annals of parasitology*. 2022;68(1).
5. Everett A, Elsheikha HM. Neuroinflammation and schizophrenia: The role of *Toxoplasma gondii* infection and astrocytic dysfunction. *Journal of Neuroimmunology*. 2025;403:578588.
6. Ngoungou EB, Bhalla D, Nzoghe A, Dardé M-L, Preux P-M. Toxoplasmosis and epilepsy—systematic review and meta analysis. *PLoS neglected tropical diseases*. 2015;9(2):e0003525.
7. Zhu Y, Yang X, Chen M, Hu Y, Chang Y, Wu X. Research Progress on the Association between Schizophrenia and *Toxoplasma gondii* Infection. *Biomedical and Environmental Sciences*. 2024;37(6):647-60.
8. Andreou D, Steen NE, Jørgensen KN, Nerland S, Ueland T, Wortinger LA, et al. *Toxoplasma gondii* associated with psychotic symptom load and cortisol in severe mental illness. *Schizophrenia*. 2025;11(1):80.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



9. Abramova O, Zorkina Y, Goncharov D, Abbazova E, Baranets M, Berdalin A, et al. Association of neurobiological and immune serum biomarkers with *Toxoplasma gondii* infection in patients with schizophrenia. *Parasitology Research*. 2025;124(5):53.
10. Rovira P, Gutiérrez B, Sorlózano-Puerto A, Gutiérrez-Fernández J, Molina E, Rivera M, et al. *Toxoplasma gondii* seropositivity interacts with catechol-O-methyltransferase Val105/158Met variation increasing the risk of schizophrenia. *Genes*. 2022;13(6):1088.
11. Sosin A, Bednarz W, Torbacka K, Rozkosz K, Kaczor J, Wróbel Z, et al. Neurobiological mechanisms underlying the influence of parasitic infections on mental disorders: a literature review. *Quality in Sport*. 2025;43:61321-.
12. Cavalari VC, Cardoso Garcia LF, Massuda R, Albrecht L. *Toxoplasma gondii*, endothelial cells and schizophrenia: is it just a barrier matter? *Frontiers in Cellular and Infection Microbiology*. 2025;15:1468936.
13. Ekici A, Timuçin D, Gürbüz E, Ünlü A, Aydemir S, Yilmaz H. Investigation of the relationship between schizophrenia and toxoplasmosis in Van province, Turkey. *Parasitologists United Journal*. 2021;14(1):34-8.
14. Contopoulos-Ioannidis DG, Gianniki M, Ai-Nhi Truong A, Montoya JG. Toxoplasmosis and Schizophrenia: A Systematic Review and Meta-Analysis of Prevalence and Associations and Future Directions. *Psychiatric Research and Clinical Practice*. 2022;4(2):48-60.
15. Virus MA, Ehrhorn EG, Lui LM, Davis PH. Neurological and neurobehavioral disorders associated with *Toxoplasma gondii* infection in humans. *Journal of parasitology research*. 2021;2021(1):6634807.
16. Del Pino LEB, Zanón-Moreno V. Systematic review on the relationship between toxoplasmosis and mental disorders. *Actas Españolas de Psiquiatría*. 2024;52(2):149.
17. Yin K, Xu C, Zhao G, Xie H. Epigenetic manipulation of psychiatric behavioral disorders induced by *Toxoplasma gondii*. *Frontiers in cellular and infection microbiology*. 2022;12:803502.
18. Torrey EF, Bartko JJ, Yolken RH. *Toxoplasma gondii* and other risk factors for schizophrenia: an update. *Schizophrenia bulletin*. 2012;38(3):642-7.
19. Ademe M, Kebede T, Teferra S, Alemayehu M, Girma F, Abebe T. Is latent *Toxoplasma gondii* infection associated with the occurrence of schizophrenia? A case-control study. *Plos one*. 2022;17(6):e0270377.
20. Mijbel ZK, Alsaady HAM. Seroprevalence of *Toxoplasma gondii* and Its Effect on the Levels of Dopamine and Adrenaline in Humans. *Journal of Chemical Health Risks*. 2023;13(3).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



21. Masoumi SM, Youssefi MR, Shojaei SSR. Exploring the interplay of chronic toxoplasmosis and NMDAR dysfunction: Insights into schizophrenia-like behaviors and therapeutic potential. Open Veterinary Journal. 2024;14(7):1634.



Parasitic Infections in Goldfish (*Carassius auratus*) Sold at Ornamental Fish Markets in Ahvaz

Abdolhossein Jangaran Nejad¹, Maryam Sadin², Ali Yarahmadi²

Corresponding Author: Maryam Sadin, Maryamsaadin2001@gmail.com

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
2. Veterinary Medicine Student, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.

Article code: 1430

Abstract

Background: The rapid development of the ornamental fish industry in Iran and globally, coupled with the increasing interest in maintaining these species, the significance of understanding their diseases and adhering to hygienic practices has grown considerably. The present study was conducted to investigate and evaluate the prevalence of parasitic infections in goldfish (*Carassius auratus*), specifically protozoans, trematodes, and other external parasites, in the ornamental fish market of Ahvaz in 2024.

Methods: A total of 100 goldfish were randomly collected from the market and transferred to the laboratory. Wet mount preparations were made from the skin, fins, and gills, while other organs were examined using standard parasitological methods.

Results: The results revealed that 69% of the fish were infected with various parasites, with the highest prevalence attributed to monogenean trematodes and protozoans in the skin and gill tissues, respectively.

Conclusion: Based on these findings, the importance of maintaining hygienic conditions in breeding and rearing environments to prevent the spread of infectious diseases, particularly parasitic infections, is further underscored. According to various studies and considering the limitations of treatment in aquaculture, as well as the critical role of nutrition and water quality factors, improving rearing conditions appears to play a significant role in preventing and controlling diseases.

Keywords: Fish diseases, Ornamental fish, Parasitic infections, Goldfish, *Carassius auratus*.

Introduction:

The ornamental fish industry, a global trade involving freshwater and marine species such as finfish, crustaceans, and aquatic plants, is a vital aquaculture sector (3). Originating with goldfish domestication in China over a millennium ago, these “living jewels” are prized for their vibrant colors, diverse sizes, and unique behaviors (1,2). The industry supports the conservation of native aquarium species, including goldfish, livebearers, and cichlids, and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



endangered species, contributing to a global market valued at 15–30 billion USD annually (4,5). In Iran, ornamental fish exports have become economically significant (6), with Esfahan, Tehran, and Qazvin producing approximately 10% of the national output (7,8). However, challenges such as limited awareness of fish health and disease issues persist (6). Environmental fluctuations, poor management, transportation stress, high stocking densities, improper nutrition, and suboptimal water quality increase susceptibility to parasites in intensive systems (9). The goldfish (*Carassius auratus*), a key *Cyprinidae* species, is valued for its diverse morphology and use in research fields like toxicology and neuropharmacology (10). Its adaptability and rapid reproduction enable population growth but also pose challenges (11). Common breeds, including *Majalaya* and Red Carp, are cultured in ponds, reservoirs, and cages (12). Parasitic diseases, intensified by global demand and intensive rearing, cause significant economic losses through sterility, discoloration, and deformities (13). Ectoparasites such as *Ichthyophthirius*, *Trichodina*, and *monogeneans* induce severe pathological effects, particularly in younger fish (10). Parasites dominate over other infections, with prevalence tied to host and environmental factors (15). Zoonotic risks and epidemic mortalities from pathogens like *Chilodonella cyprini* highlight the need for stringent quarantine (16,17). While chemical treatments like praziquantel and formalin have been used, they risk animal health, pathogen resistance, and environmental harm. Future strategies should prioritize natural control methods, informed by an understanding of parasite transmission cycles, to ensure sustainable aquaculture (19).

Materials and Methods:

In this study, a total of 100 goldfish (*Carassius auratus*) were collected in the winter of 2024 from ornamental fish sales centers in Ahvaz, and live specimens were transported to the laboratory for the examination of potential internal and external parasitic infections. Various organs, including gills, skin, fins, blood, digestive system, and other abdominal cavity organs, were analyzed. For external parasites, such as protozoans and monogeneans, wet mounts were prepared from the skin, fins, and gills. For internal parasites, an incision was made from the anus toward the anterior region, and the digestive system, along with other internal organs, was removed. Initially, internal organs were examined for infections using a stereomicroscope, followed by the preparation of crush smears for parasite isolation and identification. Protozoan parasites were stained using Giemsa solution, while metazoan parasites were stained with acetocarmine solution. For blood parasites, thin smears were prepared, fixed, and stained with Giemsa solution.

Results and Discussion:

Examination of 100 goldfish (*Carassius auratus*) revealed that 58% were infected with monogeneans and 49% with protozoan parasites. The highest infection rates were observed in the skin and gill tissues. Among monogeneans, the highest prevalence was associated with



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Dactylogyrus sp., while among protozoans, *Ichthyophthirius multifiliis* and *Trichodina* sp. were predominant, as presented in the table below.

Table 5. Prevalence of Parasitic Infections in Various Organs

Scientific Name	Infected Organ	Prevalence (%)	Number of Infections
<i>Ichthyophthirius multifiliis</i>	Skin	38	38
<i>Ichthyophthirius multifiliis</i>	Gills	30	30
<i>Trichodina</i> sp.	Skin	31	31
<i>Trichodina</i> sp.	Gills	27	27
<i>Dactylogyrus</i> sp.	Gills	49	49
<i>Hexamita</i> sp.	Intestine	3	3
<i>Capillaria</i> sp.	Intestine	4	4
<i>Lernea</i> sp.	Skin	2	2

The global expansion of the freshwater ornamental fish industry has led to increased attention on the identification and management of fish parasites, which cause significant economic losses through physiological, biological, and mechanical damage (13). The high temperatures commonly maintained in aquarium environments can accelerate parasite reproduction and intensify infections. Effective water quality management, including water exchange, salt application, appropriate filtration systems, and optimal stocking densities, has been shown to significantly reduce or eliminate parasitic infections (20). These findings underscore the importance of environmental control measures in mitigating the impact of parasitic diseases in ornamental fish populations.

This study identified monogeneans and protozoans, such as *Ichthyophthirius multifiliis* and *Trichodina* sp., as the most prevalent parasites, likely attributable to their monoxenous life cycles. These life cycles, which do not require an intermediate host, enable the parasites to complete their development on a single host, facilitating direct transmission in aquarium settings (21). Factors such as high temperatures, limited water exchange, and high host density further enhance the transmission of these parasites. In contrast, parasites with indirect life cycles, requiring intermediate hosts, were less commonly observed, likely due to the controlled aquarium environment limiting the presence of such hosts.

The prevalence of monogenean trematodes and external and internal protozoans aligns with findings from previous studies on ornamental fish in Iran (22). These parasites, primarily residing in the skin and gills, are consistent with the dominant infection patterns observed in this study. Given that contaminated water serves as the primary entry route for these parasites,



effective water management practices, including filtration and proper nutrition, are critical for reducing infection rates and minimizing economic losses. This study highlights the significant role of monogeneans and protozoans, particularly *Ichthyophthirius multifiliis* and *Trichodina* sp., as prevalent parasites in the ornamental fish industry, driven by their monoxenous life cycles and direct transmission facilitated by aquarium conditions (21). Consistent with previous research in Iran, monogenean trematodes and external protozoans were the most frequently isolated parasites, predominantly affecting the skin and gills (22).

The findings emphasize the importance of water quality management practices, such as regular water exchange, filtration, and optimal stocking densities, in reducing parasitic infections and mitigating their economic impact. These measures are essential for sustaining the health and economic viability of the ornamental fish industry.

References:

1. Hoseinifar SH, Maradonna F, Faheem M, Harikrishnan R, Devi G, Ringø E, Van Doan H, Ashouri G, Gioacchini G, Carnevali O. Sustainable ornamental fish aquaculture: the implication of microbial feed additives. *Animals*. 2023;13(10):1583. doi:10.3390/ani13101583
2. Novák J, Hohl D, Stuchlík M, Hofmann J, Tlustý MF, Magalhães ALB, Maceda-Veiga A, Akmal SG, Yue GH, Kumkar P, Marr SM, Sara JR, Stern N, Rhyne AL, Furse JM, Kalous L, Patoka J. Revisiting the history of ornamental aquaculture in Europe to understand the benefits and drawbacks of freshwater fish imports. *Rev Aquac*. 2025;17:e13008. doi:10.1111/raq.13008
3. Food and Agriculture Organization (FAO). [Internet]. Rome: FAO; 2018. Available from: <http://www.fao.org/publications>
4. Evers H-G, Pinnegar JK, Taylor MI. Where are they all from? – sources and sustainability in the ornamental freshwater fish trade. *J Fish Biol*. 2019;94:909-16. doi:10.1111/jfb.13930
5. Şahin D, Öz M, Öz Ü. The assessment of natural biomineral leonardite on growth and pigmentation of goldfish, *Carassius auratus*. *Life*. 2025;15(1):74. doi:10.3390/life15010074
6. Rahmati-Holasoo H, Ghalyanchilangeroudi A, Ziafati Kafi Z, Marandi A, Shokrpour S, Imantalab B, Ebrahimzadeh Mousavi H. Detection of lymphocystis disease virus (LCDV) from yellowbar angelfish (*Pomacanthus maculosus* Forsskal, 1775) in Iran: histopathological and phylogenetic analysis. *Aquaculture*. 2023;562:738862. doi:10.1016/j.aquaculture.2022.738862
7. International Fishmeal and Fish Oil Organisation (IFFO). Annual report 2023. London: IFFO; 2023. Available from: <https://www.iffco.com>



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



8. Adeli A, Ghiami Z. Evaluation of the condition and facilities of ornamental fish farms in Iran. JAIR. 2024;12(1):81-91. Available from: <http://jair.gonbad.ac.ir/article-1-897-en.html>
9. Arechavala-Lopez P, Sanchez-Jerez P, Bayle-Sempere JT, Uglem I, Mladineo I. Reared fish, farmed escapees and wild fish stocks—a triangle of pathogen transmission of concern to Mediterranean aquaculture management. Aquacult Environ Interact. 2013;3:153-61. doi:10.3354/aei00060
10. Wang Y, Zhang X, Liu Y, Chen J, Wang J, Wang L. Assessing the impact of dietary astaxanthin on the immune response and antioxidant capacity of large yellow croaker (*Larimichthys crocea*). ACS Omega. 2025;10(3):3456-66. doi:10.1021/acsomega.4c10920
11. Li K, Gong J, Hu F, Guo Z, Lu Z, Luo M, Zhu T. Length-weight relationship, age, and growth of invasive *Carassius auratus* in Lugu Lake, China. Animals. 2025;15(8):1091. doi:10.3390/ani15081091
12. Utami N, Nugraheni LS, Aini Q, Ridwan I, Haryandi Y. Goldfish ethnozoology in Kasepuhan Citorek as biology learning content. Int J Biol Educ Towards Sustainable Dev. 2025;5(1):22-33.
13. Adel M., Ghasempour, F., Azizi, H. R., Shateri, M. H., Safian, A. R. Survey of parasitic fauna of different ornamental freshwater fish species in Iran. *Veterinary Research Forum*, 2015; 6(1): 75-78.
14. Gratzek JB. Tropical fish medicine: Parasites associated with ornamental fish. Vet Clin North Am Small Anim Pract. 1988;18(2):375–399. doi:10.1016/s0195-5616(88)50038-4
15. Rahmati-Holasoo, H., Malek Ahmadi, B. Study of parasitic infection in ornamental fish farms in Qom province, Iran. *Caspian Journal of Veterinary Sciences*, 2024; 1(1): 13-19. doi: 10.22034/cjvs.2024.198815
16. Moradi F, Mohammadi S, Kakian F, Hadi N. Investigating the prevalence and clinical significance of *Helicobacter pylori* in hospitalized patients undergoing endoscopy in Namazi Hospital, Shiraz. Iran South Med J. 2023;26(2):102–113. doi:10.61186/ismj.26.2.102
17. Australian Quarantine and Inspection Service (AQIS). 1999.
18. Kim JH, Hayward CJ, Joh S, Heo G. Parasitic infections in live freshwater tropical fishes imported to Korea. Dis Aquat Org. 2002;52:169–173. doi:10.3354/dao052169
19. Garcia F, Fujimoto R, Martins M, Moraes F. Protozoan parasites of *Xiphophorus* spp. (Poeciliidae) and their relation with water characteristics. Arq Bras Med Vet Zootec. 2009;61. doi:10.1590/S0102-09352009000100022
20. Oros M, Hanzelová V, Scholz T. The cestode *Atractolytocestus huronensis* (Caryophyllidea) continues to spread in Europe: New data on the helminth parasite of the common carp. Dis Aquat Org. 2004;62:115–119. doi:10.3354/dao062115



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



21. Rahmati-Holasoo H, Ebrahimzadeh Mousavi H, Nassiri A, Saeedi D, Ghojaye-Yousefi H, Taheri Mirghaed A. Investigating contamination with external and internal parasites in ornamental freshwater angelfish (*Pterophyllum scalare*). Injvr. 2024;4(2):41–53. Available from: <http://injvr.com/article-1-68-en.html>
22. Raissy M, Ansari M. Parasites of some freshwater fish from Armand River, Chaharmahal va Bakhtyari Province, Iran. Iran J Parasitol. 2012;7(1):73–79.



Review on Equine Protozoal Myeloencephalitis and Possibility of Occurrence Disease in Iran

Milad Partovi¹, Fereydon Rezazadeh²

Corresponding Author: Fereydon Rezazadeh, f_rezazadeh@tabrizu.ac.ir

1. DVM student, Faculty of Veterinary Medicine, University of Tabriz, Tabriz- Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz- Iran.

Article code: 1444

Abstract

Background: Equine Protozoal Myeloencephalitis (EPM) is a neurologic disease caused by protozoan parasites, most notably *Sarcocystis neurona* and *Neospora hughesi*.

Methods: This review summarizes the important and key findings from studies published in reputable scientific journals, such as the research conducted by researchers, as well as studies presented at congresses in Iran.

Results: Global studies demonstrate seroprevalence of *S. neurona* up to 60%. In Iran, serological studies show 28–40% exposure to *Neospora* spp., particularly *N. caninum*.

Conclusion: Although confirmed clinical cases remain undocumented in Iran, serological indicators point toward potential protozoal exposure risks, suggesting an underrecognized health concern in equine populations. Lack of definitive hosts like opossums may explain lower EPM prevalence. Nevertheless, given the high seroprevalence, there is a strong need for the development of region-specific diagnostic methods.

Keywords: EPM, *S. neurona*, *N. hughesi*, Protozoal encephalitis, Iran.

Introduction:

The epidemiology of Equine Protozoal Myeloencephalitis (EPM) is strongly linked to the distribution of definitive and intermediate hosts. *S. neurona*, the primary causative agent, has a complex life cycle involving opossums (*Didelphis virginiana*) as definitive hosts. Sporocysts are shed in their feces and can contaminate water and feed sources accessible to horses. Intermediate hosts such as raccoons, skunks, armadillos, and cats play an important role in maintaining the life cycle of the parasite, acting as reservoirs of infection. In the case of *N. hughesi*, the life cycle is less well defined, but serological studies suggest its potential role in equine neurological disease. Environmental factors including humidity, temperature, and stable management practices strongly influence the survival of sporocysts in the environment and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



therefore the regional prevalence of disease. In countries like Iran, the absence of opossums changes the classical epidemiological cycle, suggesting that other wild carnivores or even stray dogs may act as potential reservoirs for *Neospora* spp. (1,4,5).

Equine Protozoal Myeloencephalitis (EPM) is a progressive and often debilitating neurologic disease affecting horses, primarily in the US. The disease is caused by two apicomplexan protozoan parasites, *S. neurona* and *N. hughes*.

Of the two causative agents, *S. neurona* is responsible for the majority of clinical cases in the United States. Horses become infected primarily by ingesting sporocysts shed in the feces of definitive hosts, especially opossums (*Didelphis virginiana*) (1). In these geographical regions, the distribution of this reservoir host is directly associated with the prevalence of the disease. Clinically, EPM is a disease with variable and sometimes subtle neurologic signs, making it difficult to distinguish from other conditions that present with symptoms such as ataxia, muscle asymmetry, or cranial nerve deficits (2). Despite the availability of effective treatments, early diagnosis and recognition play a crucial role in disease management. Several seroprevalence studies in the U.S. have indicated that while up to 50–60% of horses may be exposed to *S. neurona*, only a small fraction develops clinical disease, highlighting the complex host-pathogen interactions (2).

In Iran, despite the absence of opossums and limited knowledge about *S. neurona*, serological studies have revealed significant exposure to *Neospora* spp (6). particularly in native horse populations across various provinces. A notable case reported involved an 8-year-old Turkmen horse, that showing clinical signs such as ataxia, depression, asymmetrical muscle atrophy, and incoordination. Although PCR testing for *Neospora caninum* was negative, the clinical signs and blood findings, including elevated AST levels, were suggestive of protozoal involvement, possibly *S. neurona*. (10) This case highlights the challenges faced in diagnosing EPM and underscores the importance of using more advanced diagnostic tests such as Western blot and ELISA, which are commonly employed in endemic regions.

This article aims to provide a comprehensive review of EPM in both the global and Iranian contexts, covering its etiology, epidemiology, pathogenesis, diagnosis, treatment.

Materials and Methods:

This article is based on a review and analysis of published scientific literature, with a primary focus on the studies in the US and other reputable articles published in well-known databases such as PubMed, NCBI, and Scopus. To assess the status of EPM in Iran, both Persian and English veterinary websites were consulted, and information from the abstract of congress was utilized. All data related to epidemiology, diagnosis, and treatment were derived from the most recent regional findings.



Results and Discussion:

Once ingested, sporocysts release sporozoites that invade the intestinal epithelium and subsequently enter the bloodstream, disseminating throughout the body. The protozoa show a marked tropism for the central nervous system, particularly the brainstem, spinal cord, and cerebellum. Within neural tissue, they undergo asexual reproduction, leading to the formation of merozoites and tissue cysts. This process triggers an inflammatory response, demyelination, and neuronal degeneration. Clinically, the damage is reflected in asymmetric muscle atrophy, ataxia, and cranial nerve deficits. In chronic cases, focal necrosis and gliosis may occur, causing progressive and irreversible neurological dysfunction. The severity of disease depends not only on the parasite burden but also on the host's immune response, which may explain the discrepancy between high seroprevalence and relatively low clinical manifestation. Recent studies indicate that immune-mediated mechanisms, including microglial activation and cytokine release, may exacerbate neuronal injury beyond the direct parasitic invasion. (1,3,5).

Findings indicate that EPM is primarily caused by the protozoa *S. neurona* and *N. hughesi*, with *S. neurona* being more prevalent in North America. In Iran, serological studies suggest exposure of 28 to 40% of horses to *Neospora spp.* (7), although no confirmed clinical cases of EPM have been reported.

According to MacKay and his colleagues, clinical signs vary depending on the site of central nervous system involvement and may include ataxia, asymmetric muscle atrophy, and neurological deficits. Definitive diagnosis of EPM remains challenging and is typically based on serological testing of both blood and cerebrospinal fluid. (1) Western blot and ELISA assays are used to detect intrathecal antibody production. Dr. MacKay emphasized the importance of the serum-to-cerebrospinal fluid antibody ratio to improve diagnostic accuracy. Recommended treatments include antiprotozoal drugs such as ponazuril, diclazuril, and sulfadiazine / pyrimethamine, usually administered for several weeks. Supportive therapy commonly involves the use of NSAIDs, corticosteroids, vitamin E, and in some cases, physiotherapy (8).

Iranian data, including observations presented by some clinicians, provide serological evidence of *Neospora* infection in native Arabian and Turkmen horse breeds. However, the absence of definitive intermediate hosts such as opossums in Iran suggests that *S. neurona* is likely not endemic to the region, highlighting a clear need for further molecular research to identify local strains. Furthermore, there is some unpublished data by authors that may be confirmed the disease very soon in Iran.

The discrepancy between high seroprevalence and low clinical incidence may indicate possible resistance, subclinical infections, or limitations in disease diagnosis in Iranian horses. (9) Further research is needed to determine whether *N. hughesi* or *N. caninum* are responsible for central nervous system involvement in local populations. MacKay's efforts have laid the foundation for improved diagnosis and treatment worldwide, but regional studies like those



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



conducted in Iran are crucial for adapting global protocols and statistics to local epidemiology. The lack of definitive diagnosis, environmental differences, and unidentified protozoan species pose significant challenges for veterinary medicine in various regions of our country.

Acknowledgment:

We would like to express our sincere gratitude to all researchers whose efforts have contributed to advancing our knowledge of EPM and improving equine medicine.

References:

1. Reed SM, Furr M, Howe DK, Johnson AL, MacKay RJ, Morrow JK, Pusterla N, Witonsky S. Equine protozoal myeloencephalitis: an updated consensus statement with a focus on parasite biology, diagnosis, treatment, and prevention. *Journal of veterinary internal medicine*. 2016 Mar;30(2):491-502.
2. Reed SM, Furr M, Howe DK, Johnson AL, MacKay RJ, Morrow JK, Pusterla N, Witonsky S. Equine protozoal myeloencephalitis: an updated consensus statement with a focus on parasite biology, diagnosis, treatment, and prevention. *Journal of veterinary internal medicine*. 2016 Mar;30(2):491-502.
3. Schale S, Howe D, Yeargan M, Morrow JK, Graves A, Johnson AL. Protozoal coinfection in horses with equine protozoal myeloencephalitis in the eastern United States. *Journal of Veterinary Internal Medicine*. 2018 May;32(3):1210-4.
4. Reed SM, Furr M, Howe DK, Johnson AL, MacKay RJ, Morrow JK, Pusterla N, Witonsky S. Equine protozoal myeloencephalitis: an updated consensus statement with a focus on parasite biology, diagnosis, treatment, and prevention. *Journal of veterinary internal medicine*. 2016 Mar;30(2):491-502.
5. Dubey JP. *Sarcocystis neurona*, *Neospora* spp. and *Toxoplasma gondii* infections in horses and equine protozoal myeloencephalitis (EPM): five decades of personal experience, perspectives and update. *Parasitology*. 2022 May;149(6):717-28.
6. Dubey JP. *Sarcocystis neurona*, *Neospora* spp. and *Toxoplasma gondii* infections in horses and equine protozoal myeloencephalitis (EPM): five decades of personal experience, perspectives and update. *Parasitology*. 2022 May;149(6):717-28.
7. Gharekhani J, Yakhchali M, Berahmat R. *Neospora caninum* infection in Iran (2004–2020): A review. *Journal of Parasitic Diseases*. 2020 Dec;44(4):671-86.
8. MacKay RJ, Howe DK. Equine protozoal myeloencephalitis. *Veterinary Clinics: Equine Practice*. 2022 Aug 1;38(2):249-68.
9. Reed SM, Furr M, Howe DK, Johnson AL, MacKay RJ, Morrow JK, Pusterla N, Witonsky S. Equine protozoal myeloencephalitis: an updated consensus statement with



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



a focus on parasite biology, diagnosis, treatment, and prevention. Journal of veterinary internal medicine. 2016 Mar;30(2):491-502.

10. Rezazadeh F., Jafari Jozani R., Valizadeh S., The first occurrence of Equine Protozoal Myeloencephalitis- like disease in a cross-breed Turkmen horse in Iran. 27-28 February 2013, Tehran, Iran (ICLAP 2013).



Third-generation Pesticides: A Novel Approach for Improving Food Safety and Controlling Parasitic Contamination

Mohammad Mahdi Fahmideh¹, Mina Gozal¹, Parnian Pezeshki¹, Atefeh Sarafan Sadeghi¹

Corresponding Author: Atefeh Sarafan Sadeghi, sarafana@varastegan.ac.ir

1. Department of Food Science and Technology, Varastegan Institute for Medical Sciences, Mashhad, Iran.

Article code: 1452

Abstract

Background: Third-generation pesticides are emerging as safer and more sustainable alternatives to conventional chemical agents that pose significant environmental and health risks. These newer formulations are designed to enhance specificity, reduce non-target toxicity, and contribute to improved food hygiene, particularly in the context of zoonotic parasite control in food-producing animals.

Methods: This narrative review was conducted by searching PubMed, Scopus, Web of Science, and ScienceDirect for peer-reviewed literature published between 2020 and 2025. Keywords included third-generation pesticides, nanopesticides, biopesticides, vector control, and “food safety. Studies addressing pest control innovations relevant to livestock systems and zoonotic disease prevention were selected and analyzed.

Results: Findings indicate that third-generation pesticides such as nanoformulated silver, zinc oxide, and iron oxide compounds exhibit strong acaricidal and larvicidal effects against vectors like ticks and mosquitoes. Moreover, microbial agents like *Beauveria bassiana* and *Metarhizium anisopliae* have shown effectiveness in reducing parasite loads in poultry and ruminants, with low toxicity and strong environmental compatibility. Applications in animal farming and grain storage have led to measurable improvements in hygiene and food safety. Despite their advantages, limitations include high production costs, lack of long-term field data, and regulatory ambiguity in many regions.

Conclusion: Third-generation pesticides offer a promising route toward safer pest control in food systems. Their targeted efficacy and biodegradability align with One Health goals and sustainable agriculture principles. However, broader adoption requires robust regulatory frameworks and interdisciplinary research to address safety, efficacy, and economic scalability.

Keywords: Third-generation pesticides, Food safety, Zoonotic parasites, Biopesticides.



Introduction:

Pesticides have played a crucial role in modern agriculture by increasing crop yields, ensuring food security, and reducing disease transmission. Nevertheless, the widespread application of chemical pesticides has led to a range of unintended consequences, including environmental contamination, non-target species toxicity, pest resistance, and bioaccumulation in ecosystems (1,2). First- and second-generation pesticides, such as organophosphates and carbamates, have been linked to ecological imbalance, endocrine disruption in wildlife, and potential carcinogenic effects in humans (3). These limitations have driven global scientific and regulatory interest toward safer, more sustainable alternatives.

Third-generation pesticides have emerged as innovative solutions aimed at reducing the adverse impacts of traditional chemical control. This new generation includes a variety of formulations: nanopesticides, microbial pesticides (e.g., entomopathogenic fungi), RNA interference-based insecticides, and pheromone disruptors (4,5,6).

The present review explores the technological advances, applications, safety concerns, and regulatory challenges associated with third-generation pesticides. The focus is specifically placed on their role in controlling zoonotic parasites—organisms transmitted between animals and humans—that compromise food safety and public health (7).

Materials and Methods:

A comprehensive literature search was performed using four major databases: PubMed, Scopus, Web of Science, and ScienceDirect. The search covered peer-reviewed articles published between January 2020 and June 2025. Keywords included combinations of third-generation pesticides, nanopesticides, biopesticides, entomopathogenic fungi, RNAi, vector control, zoonotic parasites, and food safety. Exclusion criteria comprised non-peer-reviewed materials, reviews lacking original data analysis, and articles focused solely on traditional pesticides. Selected studies were categorized and analyzed based on pesticide type, mechanism of action, target organisms, safety evaluation, and regulatory context.

Results and Discussion:

Third-generation pesticides represent a shift from broad-spectrum chemical agents to precision-targeted, environmentally conscious formulations. Key categories include nanoformulations, metal-based nanoparticles, microbial pesticides, RNA interference molecules, and pheromone disruptors (8).

Technological Innovations and Formulation Advances Nanotechnology has revolutionized pesticide delivery by enabling the encapsulation of active compounds in nanoparticles that



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



improve bioavailability and stability (9). For example, nano-ivermectin encapsulated in chitosan has shown prolonged action, better adherence to vector cuticles, and reduced mammalian cytotoxicity. Similarly, nano-abamectin formulations allow for lower application doses, thereby reducing environmental load and costs associated with repeated treatments (10).

Metal-based nanoparticles, such as silver (Ag), zinc oxide (ZnO), and iron oxide (Fe_2O_3), have demonstrated significant efficacy as larvicides and acaricides (11). Studies have shown that Fe_2O_3 nanoparticles achieve over 90% mortality in *Hyalomma* ticks at concentrations well below conventional acaricide thresholds. Zinc oxide nanoparticles have also been used successfully against *Aedes* and *Culex* mosquito vectors (12). Their small particle size allows them to penetrate vector integuments and disrupt cellular respiration. Additionally, research into silica-based (SiO_2) nanoparticles has revealed their ability to reduce pest load in stored grains while being non-toxic to humans and livestock (13).

Efficacy in Parasite and Vector Control The performance of third-generation pesticides against a variety of zoonotic parasites is well documented (14). Nanoformulations are particularly effective against vectors that transmit diseases such as leishmaniasis, trypanosomiasis, and toxoplasmosis (15). For instance, studies involving nano-silver and nano-zinc particles have recorded larval mortality rates above 95% in mosquitoes transmitting *Leishmania* spp. Additionally, RNAi-based formulations have been developed to target specific genes involved in vector reproduction and digestion. These gene-silencing agents are species-specific and reduce the likelihood of resistance development (16).

Entomopathogenic fungi, such as *Beauveria bassiana* and *Metarhizium anisopliae*, have shown broad efficacy across a range of parasitic arthropods including ticks, fleas, and lice (17). These fungi act by penetrating the cuticle of the host, proliferating within the hemocoel, and releasing toxins that cause systemic infection. In addition, their minimal toxicity to humans and animals makes them ideal candidates for integrated pest management (IPM) strategies (19).

Applications in Livestock, Food Processing, and Storage Livestock production systems have benefitted greatly from the application of third-generation pesticides. For example, in poultry farms, nanoformulated acaricides have demonstrated long-lasting efficacy against *Dermanyssus gallinae*, reducing stress and improving egg production (20). In dairy cattle, topical sprays containing *M. anisopliae* reduced tick infestations and led to measurable improvements in milk yield and animal welfare. These interventions align well with One Health principles that connect animal health with food safety and public health outcomes (21).

Third-generation pesticides have also been adopted in post-harvest and food storage contexts. Nanoparticles such as ZnO and SiO_2 have been shown to be effective against beetle species like *Tribolium castaneum* and *Sitophilus oryzae* in grain silos (22). Studies have documented a 30–40% reduction in post-harvest losses when these nanoparticles are applied as part of a



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



grain storage regimen. Importantly, these substances degrade quickly and do not accumulate in food products, making them safe from a toxicological standpoint (23).

Moreover, advanced delivery systems such as nanoemulsions and microcapsules have been integrated into livestock feed and bedding materials to provide continuous protection against external parasites (24). Such approaches not only reduce vector pressure but also help maintain animal comfort and productivity without compromising food safety or animal welfare (25).

Toxicological Profiles and Food Safety Considerations Numerous toxicological studies have affirmed the safety of third-generation pesticides when used as directed. EPF strains such as *B. bassiana* F52 and *M. brunneum* have received regulatory approval from agencies including the EPA and EFSA (26). These fungi have been found to pose negligible risks to non-target organisms, including pollinators and soil fauna (27).

Nanopesticides, although generally considered safe, require further investigation to understand their long-term effects. High doses of nanoparticles have been associated with subtle alterations in rodent gut microbiota, and some studies suggest potential genotoxic effects (28). As a result, the development of standardized safety assessment protocols is imperative. In food safety terms, third-generation pesticides provide a means to lower chemical residues in meat, milk, and eggs—key indicators monitored by food safety authorities (29,30).

Regulatory challenges and public perception while biopesticides are generally welcomed under organic farming certifications, nanoformulations face regulatory ambiguity (31). The lack of unified global definitions and frameworks for evaluating nanopesticide safety has led to inconsistent approval pathways (32).

Efforts must be made to increase public awareness and acceptance of these technologies through transparent risk communication. Regulatory harmonization at the international level would also accelerate market entry and adoption, especially in low- and middle-income countries where food safety challenges are acute (33).

Some countries have initiated proactive frameworks for nanotechnology regulation in agriculture, while others lag behind. Multinational cooperation will be essential in developing mutually recognized safety standards and ensuring ethical implementation of these technologies (34).

Limitations and Research Gaps Despite their potential, third-generation pesticides are not without limitations. The scalability of production, variability in field performance, and shelf-life stability remain concerns (35). Additionally, most existing studies are conducted in laboratory settings, which may not reflect real-world agricultural conditions. There is a pressing need for:

Large-scale field trials in diverse agroecological zones.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Cost-benefit analyses comparing third-generation and traditional pesticides

Assessment of their impacts on non-target microbial ecosystems in soil and water (36)

Future research should also explore synergistic approaches that combine third-generation agents with feed additives, immune modulators, or vaccination programs to enhance overall animal health and productivity (37). Furthermore, interdisciplinary collaboration among chemists, microbiologists, food technologists, and policymakers is crucial to create holistic pest management frameworks that align with global sustainability goals (38).

Overall, third-generation pesticides present a promising and sustainable alternative to conventional pest control methods. Their ability to target vectors and parasites with high specificity, minimize chemical residues, and integrate into existing food systems makes them attractive for enhancing food safety. Addressing existing regulatory, economic, and social barriers is essential to enable these innovations to reach farmers, producers, and consumers worldwide (39).

References:

1. Wend K, Zorrilla L, Freimoser FM, Gallet A. Microbial pesticides—challenges and future perspectives for testing and safety assessment with respect to human health. *Environmental Health*. 2024 May 29;23(1):49.
2. Thompson DA, Lehmler HJ, Kolpin DW, Hladik ML, Vargo JD, Schilling KE, LeFevre GH, Peeples TL, Poch MC, LaDuca LE, Cwiertny DM. A critical review on the potential impacts of neonicotinoid insecticide use: current knowledge of environmental fate, toxicity, and implications for human health. *Environmental Science: Processes & Impacts*. 2020;22(6):1315-46.
3. Hanif K, Zubair M, Hussain D, Ali S, Saleem M, Khan HA, Nazir T, ul Hassan MW. Biopesticides and insect pest management. *International Journal of Tropical Insect Science*. 2022 Dec;42(6):3631-7.
4. Kuchheuser P, Birringer M. Pesticide residues in food in the European Union: Analysis of notifications in the European Rapid Alert System for Food and Feed from 2002 to 2020. *Food Control*. 2022 Mar 1;133:108575.
5. Kannan M, Bojan N, Swaminathan J, Zicarelli G, Hemalatha D, Zhang Y, Ramesh M, Faggio C. Nanopesticides in agricultural pest management and their environmental risks: a review. *International Journal of Environmental Science and Technology*. 2023 Sep;20(9):10507-32.
6. Jamshaid H, Din FU, Khan GM. Nanotechnology based solutions for anti-leishmanial impediments: a detailed insight. *Journal of nanobiotechnology*. 2021 Apr 15;19(1):106.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



7. Arora NK, Mehnaz S, Balestrini R, editors. Bioformulations: for sustainable agriculture. Berlin: Springer; 2021 Jun 6.
8. Zaheer T, Ali MM, Abbas RZ, Atta K, Amjad I, Suleman A, Khalid Z, Aqib AI. Insights into nanopesticides for ticks: the superbugs of livestock. *Oxidative medicine and cellular longevity*. 2022;2022(1):7411481.
9. Li C, Han Y, Gao T, Zhang J, Xu DX, Wāng Y. Insecticidal activity of metallic nanopesticides synthesized from natural resources: A review. *Environmental Chemistry Letters*. 2023 Apr;21(2):1141-76.
10. Ale A, Andrade VS, Gutierrez MF, Bacchetta C, Rossi AS, Santo Orihuela P, Desimone MF, Cazenave J. Nanotechnology-based pesticides: Environmental fate and ecotoxicity. *Toxicology and Applied Pharmacology*. 2023 Jul 15;471:116560.
11. Fenta MD, Eshetu B, Bitew AB, Dejene H, Kinde MZ. Foodborne and neglected parasitic zoonoses in Ethiopian red meat animals: insights from a systematic review and meta-analysis. *Frontiers in Veterinary Science*. 2025 Jun 4;12:1501940.
12. Jacques MT, Oliveira JL, Campos EV, Fraceto LF, Avila DS. Safety assessment of nanopesticides using the roundworm *Caenorhabditis elegans*. *Ecotoxicology and environmental safety*. 2021 May 1;139:245-53.
13. Sarkar N, Chaudhary S, Kaushik M. Nano-fertilizers and Nano-pesticides as Promoters of Plant Growth in Agriculture. In *Plant-Microbes-Engineered Nano-particles (PM-ENPs) nexus in agro-ecosystems: Understanding the interaction of plant, microbes and engineered nano-particles (ENPS)* 2021 Mar 23 (pp. 153-163). Cham: Springer International Publishing.
14. Ortiz A, Sansinenea E. Microbial-based biopesticides: commercialization and regulatory perspectives. In *Development and commercialization of biopesticides* 2023 Jan 1 (pp. 103-118). Academic Press.
15. Seiber JN, Coats J, Duke SO, Gross AD. Biopesticides: state of the art and future opportunities. *Journal of agricultural and food chemistry*. 2020 Dec 3;62(48):11613-9.
16. de Oliveira JL. Nano-biopesticides: Present concepts and future perspectives in integrated pest management. *Advances in nano-fertilizers and nano-pesticides in agriculture*. 2021 Jan 1:1-27.
17. Guan R, Chu D, Han X, Miao X, Li H. Advances in the development of microbial double-stranded RNA production systems for application of RNA interference in agricultural pest control. *Frontiers in Bioengineering and Biotechnology*. 2021 Sep 13;9:753790.
18. Matten SR, Milewski EA, Schneider WR, Slutsky BI. Biological Pesticides and the US Environmental Protection Agency. In *Advanced engineered pesticides* 2024 Nov 1 (pp. 321-335). CRC Press.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



19. Wend K, Zorrilla L, Freimoser FM, Gallet A. Microbial pesticides—challenges and future perspectives for testing and safety assessment with respect to human health. *Environmental Health*. 2024 May 29;23(1):49.
20. Peijnenburg W, Oomen AG, Soeteman-Hernández LG, Groenewold M, Sips AJ, Noorlander CW, Kettelarij JA, Bleeker EA. Identification of emerging safety and sustainability issues of advanced materials: Proposal for a systematic approach. *NanoImpact*. 2021 Jul 1;23:100342.
21. Koul O, editor. *Development and Commercialization of Biopesticides: Costs and Benefits*. Elsevier; 2023 Mar 23.
22. Verma SK, Kumar P, Mishra A, Khare R, Singh D. Green nanotechnology: illuminating the effects of bio-based nanoparticles on plant physiology. *Biotechnology for Sustainable Materials*. 2024 May 27;1(1):1.
23. Belwal T, Shen Y, Jafari S, Lin X. Application of nano/biotechnology in the detection of food safety and spoilage. *Frontiers in Nutrition*. 2023 Feb 28;10:1154898.
24. Vishnu M, Kannan M, Soundararajan RP, Suganthi A, Subramanian A, Senthilkumar M, Rameash K, Madesh K, Govindaraju K. Nano-bioformulations: emerging trends and potential applications in next generation crop protection. *Environmental Science: Nano*. 2024;11(7):2831-60.
25. Bamisile BS, Siddiqui JA, Akutse KS, Ramos Aguila LC, Xu Y. General limitations to endophytic entomopathogenic fungi use as plant growth promoters, pests and pathogens biocontrol agents. *Plants*. 2021 Oct 6;10(10):2119.
26. Egbuna C, Sawicka B, Tijjani H, Kryeziu TL, Ifemeje JC, Skiba D, Lukong CB. Biopesticides, safety issues and market trends. In *Natural remedies for pest, disease and weed control 2020* Jan 1 (pp. 43-53). Academic Press.
27. da Silva Freitas L, Honscha LC, Volcão LM, de Lima Brum R, da Silva Júnior FM, Ramos DF. Antibiotics in the Environment: Prescribing risks to non-target organisms. *Pollutants*. 2022 Oct 27;2(4):435-43.
28. Kannan M, Bojan N, Swaminathan J, Zicarelli G, Hemalatha D, Zhang Y, Ramesh M, Faggio C. Nanopesticides in agricultural pest management and their environmental risks: a review. *International Journal of Environmental Science and Technology*. 2023 Sep;20(9):10507-32.
29. Al-Sahlany ST, Niamah AK, Verma DK, Prabhakar P, Patel AR, Thakur M, Singh S. Applications of Green Synthesis of Nanoparticles Using Microorganisms in Food and Dairy Products. *Processes*. 2025 May 18;13(5):1560.
30. Zheng Q, Qin D, Wang R, Yan W, Zhao W, Shen S, Huang S, Cheng D, Zhao C, Zhang Z. Novel application of biodegradable chitosan in agriculture: Using green nanopesticides to control *Solenopsis invicta*. *International Journal of Biological Macromolecules*. 2022 Nov 1;220:193-203.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



31. Adnan AA, Ashari MA, Baskaran SM, Mahamad SS, Nawi NM, Zakaria MR. Farmers Awareness and Acceptance of Biopesticides Application for Pest and Disease Management. *AgroTech-Food Science, Technology and Environment*. 2024 Dec 30;3(2):77-88.
32. Shelar A, Nile SH, Singh AV, Rothenstein D, Bill J, Xiao J, Chaskar M, Kai G, Patil R. Recent advances in nano-enabled seed treatment strategies for sustainable agriculture: challenges, risk assessment, and future perspectives. *Nano-Micro Letters*. 2023 Dec;15(1):54.
33. Soetopo D, Alouw JC. Biopesticide development & registration: challenges & strategies. *InIOP Conference Series: Earth and Environmental Science* 2023 May 1 (Vol. 1179, No. 1, p. 012003). IOP Publishing.
34. Jain A, Das S. Regulatory requirements for nanopesticides and nanofertilizers. *InAdvances in Nano-Fertilizers and Nano-Pesticides in Agriculture* 2021 Jan 1 (pp. 145-152). Woodhead Publishing.
35. Hajji-Hedfi L, Chhipa H. Nano-based pesticides: challenges for pest and disease management. *Euro-Mediterranean Journal for Environmental Integration*. 2021 Dec;6(3):69.
36. López-Cabeza R. Methods for understanding the fate of nanopesticides in soil and water. *Nanopesticides: From Research and Development to Mechanisms of Action and Sustainable Use in Agriculture*. 2020 Jul 7:111-36.
37. Reddy DS, Chowdary NM. Botanical biopesticide combination concept—a viable option for pest management in organic farming. *Egyptian Journal of Biological Pest Control*. 2021 Dec;31(1):1-0.
38. Deguine JP, Aubertot JN, Flor RJ, Lescourret F, Wyckhuys KA, Ratnadass A. Integrated pest management: good intentions, hard realities. A review. *Agronomy for Sustainable Development*. 2021 Jun;41(3):38.
39. Debnath D, Das A. Third-generation biofuels and food security. *In3rd generation biofuels* 2022 Jan 1 (pp. 757-768). Woodhead Publishing.



Foodborne Toxoplasmosis: Transmission from Animal Products

Parisa Behnam¹, Alireza Esmaeilzadeh Marandiz¹, Parnian Pezeshki¹, Atefeh Sarafan Sadeghi¹

Corresponding Author: Atefeh Sarafan Sadeghi, sarafana@varastegan.ac.ir

1. Department of Food Science and Technology (Quality Control and Hygiene), Varastegan Institute for Medical Sciences, Mashhad, Iran.

Article code: 1455

Abstract

Background: *Toxoplasma gondii* is one of the most prevalent zoonotic protozoan parasites worldwide, affecting nearly one-third of the human population. Foodborne transmission is the primary route of human infection, with undercooked meat, raw milk, and contaminated animal-derived products serving as major sources. Despite its public health relevance, routine monitoring and food safety control of *T. gondii* remains limited in many regions. This review discusses the current state of knowledge regarding *T. gondii* transmission through animal-derived foods, identifies major foodborne sources of infection, and emphasizes strategies for detection and prevention to reduce transmission along the food chain.

Methods: This review synthesized data from 28 peer-reviewed articles published between 2018 and 2025, selected through a systematic search across PubMed, Scopus, Google Scholar, and Web of Science. The keywords used included *Toxoplasma gondii*, foodborne transmission, animal-derived products, and zoonotic parasites. Relevant data on transmission pathways, prevalence, detection techniques, and control strategies were extracted and critically analyzed.

Results: High seroprevalence of *T. gondii* was observed in pigs, goats, sheep, and free-range poultry across various geographic regions, with infection rates exceeding 40% in some settings. Undercooked pork, lamb, and goat meat were identified as the most significant foodborne sources. Raw milk and dairy products, particularly from goats and sheep, also pose considerable risks. Although PCR and other molecular methods improve detection, standardization and viability assessment remain lacking. Effective control measures include thermal processing, biosecurity on farms, consumer education, and regulatory oversight.

Conclusion: *T. gondii* remains a major foodborne pathogen globally, yet important knowledge gaps persist, especially regarding viability detection and cost-effective prevention at the farm level. Coordinated One Health strategies and enhanced surveillance are needed to reduce transmission risks and protect vulnerable populations.

Keywords: *Toxoplasma gondii*, Foodborne transmission, Animal-derived products, Zoonotic parasites.



Introduction:

Toxoplasma gondii is an obligate intracellular protozoan parasite capable of infecting nearly all warm-blooded animals, including humans. It is one of the most widespread zoonotic parasites globally, with an estimated one-third of the human population carrying the infection (1,2). Although vertical transmission, organ transplantation, and contaminated water are recognized pathways, foodborne transmission remains the primary route of human infection (3).

Among foodborne sources, the consumption of undercooked or raw meat—particularly pork, mutton, and goat—is considered the leading contributor to toxoplasmosis in humans (4,5). Other potential sources include raw milk, unpasteurized dairy products, and contaminated ready-to-eat foods (6,7,8).

Recent research indicates high seroprevalence of *T. gondii* in food-producing animals in countries such as China, Greece, and Bangladesh, where infection rates in pigs, goats, and sheep can range from 20% to over 40%, depending on the region and farming practices (9–12)

Materials and Methods:

A systematic search was conducted across three major scientific databases (Google Scholar, Scopus, PubMed, and Web of Science) for relevant publications published between 2018-2025. The search utilized the following key terms: *Toxoplasma gondii*, Foodborne transmission, Animal-derived products, and Zoonotic parasites. From the initial pool of 35 identified articles, 28 were selected based on their direct relevance to the research focus.

Results and Discussion:

Toxoplasma gondii belongs to the phylum Apicomplexa and is the causative agent of toxoplasmosis, a globally distributed zoonotic disease of significant public health concern (3,2).

The parasite has a complex life cycle involving both definitive hosts (felids) and intermediate hosts. Sexual reproduction occurs only in the intestines of cats, where environmentally resistant oocysts are produced and excreted in feces. These oocysts can survive for months in soil, water, and food, becoming an important source of infection (10).

In intermediate hosts, including livestock and humans, *T. gondii* undergoes asexual multiplication, forming rapidly dividing tachyzoites during acute infection and slowly dividing bradyzoites encysted in tissues (mainly muscle and brain) during chronic stages (6). Humans can acquire infection through ingestion of oocysts (from contaminated water, soil, vegetables) or tissue cysts (from undercooked meat or animal products) (4).

Most infections in healthy individuals are asymptomatic or mild; however, toxoplasmosis can cause severe illness in immunocompromised patients (e.g., HIV-positive individuals, transplant recipients), and congenital infection can lead to miscarriage or neurological damage in newborns (Figure 1) (7,8,11).

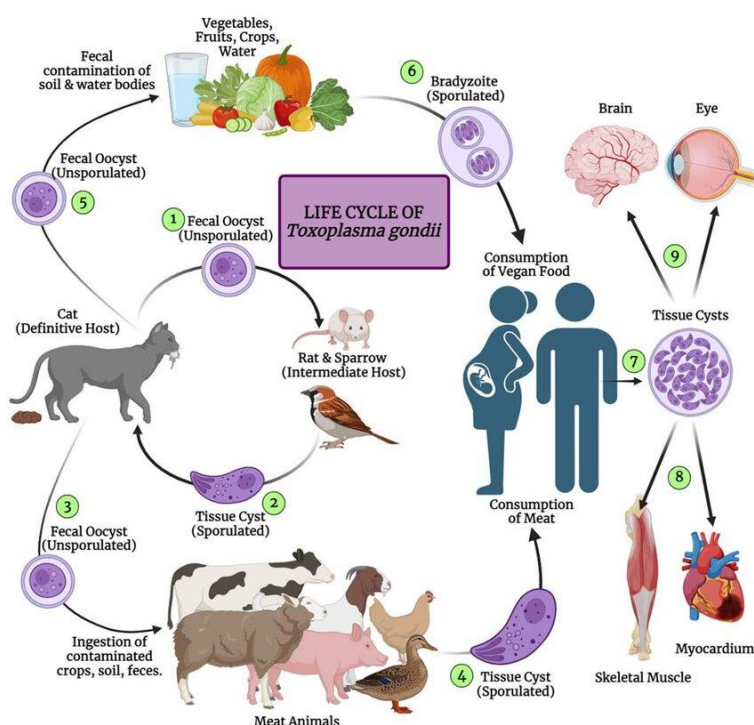


Figure 1. Life Cycle of *Toxoplasma gondii* (13)

Foodborne Transmission of *Toxoplasma gondii* via Animal-Derived Products

Foodborne transmission of *Toxoplasma gondii* primarily occurs through consumption of raw or undercooked meat containing tissue cysts, being major sources (4,9). A meta-analysis in Brazil reported prevalence rates of 23% in goats, 19.9% in pigs, 17.3% in sheep, and 16.2% in cattle (9), while a Chinese review confirmed the significant role of pork in transmission (14). Belluco et al. (2018) found an odds ratio (OR) of 3.4 for toxoplasmosis following undercooked meat consumption, and pork accounts for 41% of foodborne toxoplasmosis cases in the U.S. (2,4).

Raw milk and unpasteurized dairy products also pose risks. *T. gondii* DNA has been detected in 2.8% of raw cow's milk in Brazil (15), and up to 14% in goat, sheep, and buffalo milk in Pakistan (16). In Greece, up to 65% of raw goat milk and 11% of sheep milk samples tested

Other sources include cross-contamination in kitchens (2) and the use of raw pet food (BARF), which can lead to oocyst shedding by cats or dogs, increasing human exposure risks (Figure2) (18).

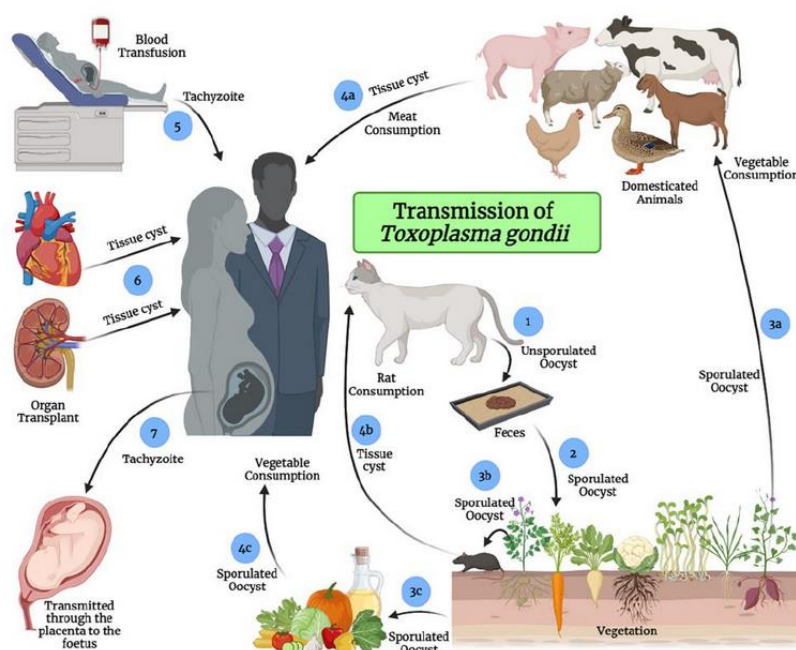


Figure 2. Transmission of *Toxoplasma gondii* (13)

Prevalence of *Toxoplasma gondii* in Animal Products Worldwide

The global prevalence of *Toxoplasma gondii* in food-producing animals varies depending on species, farming systems, geography, and diagnostic methods. Pigs are key reservoirs, especially in free-range and backyard farms. Seroprevalence ranges from 10% to over 40%, with lower rates in industrial systems. In Europe, the rate is about 13%, while in Asia and South America it may exceed 25–30% (4,5,10,19). Sheep and Goats often show >50% prevalence, especially under extensive grazing. In Bangladesh, 7.4% of raw goat milk samples tested positive for parasite DNA (7,8). Cattle, though less susceptible, can still show 5–15% seroprevalence. While beef poses a lower risk, viable cysts have been occasionally detected in raw or undercooked meat (19,20). Free-range chickens and wild game (e.g., wild boar, venison) can have high exposure. Seroprevalence in chickens may exceed 70%, and in wild boar, up to 48% has been reported (6,8,21).



Detection Methods for *Toxoplasma gondii* in Food and Animal Products

Conventional diagnostic methods include serological assays (e.g., ELISA, MAT), bioassays, molecular techniques, and microscopic examination.

Serological tests are widely used for screening livestock, particularly for surveillance purposes. Bioassays—using laboratory animals such as mice or cats—remain the gold standard for assessing parasite viability but are ethically and logistically constrained (10).

Molecular methods, particularly polymerase chain reaction (PCR), have become popular for detecting *T. gondii* DNA in meat, milk, and processed food samples. To address this, viability PCR methods using dyes like propidium monoazide (PMA-PCR) or RNA-based approaches have been developed, although their application remains limited in food safety regulations (23,24).

Overall, the lack of standardized detection protocols and limited commercial availability of validated kits remain major challenges in monitoring *T. gondii* contamination in animal products.

Control Measures and Prevention Strategies

Mitigating the risk of *Toxoplasma gondii* transmission through animal-derived foods requires integrated approaches involving farm biosecurity, food processing, consumer education, and regulatory oversight.

Farm biosecurity plays a pivotal role in reducing livestock infection. Strategies such as indoor housing to limit environmental oocyst exposure (5), controlled feeding and watering systems to prevent contamination from felid feces (10), and management of stray cat populations near farms (3) have been shown to decrease seroprevalence by 30–50% in European studies (4).

Thermal processing remains the most effective intervention for inactivating *T. gondii* cysts. Cooking meat to an internal temperature above 67 °C (1), freezing at –12 °C for 3 days or –18 °C for 2 days (26), and pasteurization of milk at 72 °C for 15 seconds (7) effectively eliminate the parasite. These measures are endorsed by EFSA and USDA (8,27).

Consumer education regarding thorough cooking of meat, washing of raw produce, and avoidance of raw dairy products is essential (28,6). Proper kitchen hygiene, including sanitization and prevention of cross-contamination, significantly reduces infection risk (22,25).

Regulatory agencies such as EFSA, CDC, and WHO recommend implementation of risk-based control systems in slaughterhouses, targeted surveillance of high-risk farms, and clear communication to consumers (10,27).

Toxoplasma gondii continues to pose a substantial threat to global public health through its widespread presence in food-producing animals and the high potential for foodborne



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



transmission. Undercooked meat, raw milk, and poor kitchen hygiene remain key contributors to human infection. While significant progress has been made in detection, thermal inactivation, and public awareness, critical gaps remain—particularly regarding standardized testing protocols, real-time viability assessments, and the implementation of cost-effective preventive strategies in small-scale farming systems.

To effectively mitigate the burden of toxoplasmosis, future efforts must adopt a holistic One Health framework, emphasizing cross-sectoral collaboration, harmonized food safety regulations, and investment in rapid, field-deployable diagnostic tools.

References:

1. J.P. Dubey, J.L. Jones, Toxoplasma gondii infection in humans and animals in the United States, *International Journal for Parasitology*, Volume 38, Issue 11,
2. S. Almeria, J.P. Dubey, Foodborne transmission of Toxoplasma gondii infection in the last decade. An overview, *Research in Veterinary Science*, Volume 135, 2021, Pages 371-385, ISSN 0034-5288, <https://doi.org/10.1016/j.rvsc.2020.10.019>
3. Vilela, V. L. R., & Feitosa, T. F. (2024). Recent Advances in Toxoplasma gondii Infection and Toxoplasmosis. *Tropical Medicine and Infectious Disease*, 9(7), 160.
4. Belluco, S., Simonato, G., Mancin, M., Pietrobelli, M., & Ricci, A. (2018). Toxoplasma gondii infection and food consumption: A systematic review and meta-analysis of case-controlled studies. *Critical Reviews in Food Science and Nutrition*, 58(18), 3085–3096. <https://doi.org/10.1080/10408398.2017.1352563>.
5. Symeonidou, I., Sioutas, G., Lazou, T., Gelasakis, A. I., & Papadopoulos, E. (2023). A Review of Toxoplasma gondii in Animals in Greece: A FoodBorne Pathogen of Public Health Importance. *Animals*, 13(15), 2530. <https://doi.org/10.3390/ani13152530>.
6. Marín-García, P.-J., Planas, N., & Llobat, L. (2022). Toxoplasma gondii in Foods: Prevalence, Control, and Safety. *Foods*, 11(16), 2542. <https://doi.org/10.3390/foods11162542>
7. Tuska-Szalay, B., Papdeák, V., Vizi, Z. et al. Parasitological and molecular investigation of consequences of raw meat feeding (BARF) in dogs and cats: implications for other pets living nearby. *Parasitol Res* 123, 114 (2024).
8. Hasan, M.F., Harun, A.B., Hossain, D. et al. Toxoplasmosis in animals and humans: a neglected zoonotic disease in Bangladesh. *J Parasit Dis* 48, 189–200 (2024). <https://doi.org/10.1007/s12639-024-01664-4>.
9. Liu, P., Tang, H., Xu, Q. et al. The seroprevalence and distribution of Toxoplasma gondii in pigs in China from 2000 to 2023: a systematic review and meta-analysis. *Animal Diseases* 5, 26 (2025). <https://doi.org/10.1186/s44149-025-00173-y>.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



10. EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Lindqvist R, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Cacciò S, Chalmers R, Deplazes P, Devleesschauwer B, Innes E, Romig T, van der Giessen J, Hempen M, Van der Stede Y and Robertson L, 2018. Scientific Opinion on the public health risks associated with food-borne parasites. EFSA Journal 2018;16(12):5495, 113 pp. <https://doi.org/10.2903/j.efsa.2018.5495>.
11. Pinto-Ferreira, F., Caldart, E., Pasquali, A., Mitsuka-Breganó, R., Freire, R., & Navarro, I. (2019). Patterns of Transmission and Sources of Infection in Outbreaks of Human Toxoplasmosis. *Emerging Infectious Diseases*, 25(12), 2177-2182. <https://doi.org/10.3201/eid2512.181565>.
12. Ducrocq, J., Simon, A., Lemire, M., De Serres, G., & Lévesque, B. (2021). Exposure to *Toxoplasma gondii* Through Consumption of Raw or Undercooked Meat: A Systematic Review and Meta-Analysis. *Vector borne and zoonotic diseases* (Larchmont, N.Y.), 21(1), 40–49.
13. Naeem, Muhammad & Younus, Muhammad & Nisa, Un & Akhtar, Tayyaba & Kausar, Razia & Muzaffar, Hizqeel & Irza,. (2024). Zoonotic Infertility Due to *Toxoplasma Gondii*.doi:10.47278/book.zoon/2023.79.
14. Rossi, G. A. M., de Freitas Costa, E., Gabriël, S., & Braga, F. R. (2022). A Systematic Review and Meta-Analysis on the Occurrence of Toxoplasmosis in Animals Slaughtered in Brazilian Abattoirs. *Animals*, 12(22), 3102
15. Fereig, R. M., El-Alfy, E.-S., Alharbi, A. S., Abdelraheem, M. Z., Almuzaini, A. M., Omar, M. A., Kandil, O. M., & Frey, C. F. (2024). Seroprevalence of *Toxoplasma gondii* in Cattle in Southern Egypt: Do Milk and Serum Samples Tell the Same Story? *Animals*, 14(21), 3122.
16. da Fonseca, F. M., Sato, A. P., Becker, A. P. B. B., da Paz Augusto Pinto, G. O., de Souza, G. S., Perotta, J. H., de Barros Filho, I. R., Rodriguez, M. C., & Locatelli-Dittrich, R. (2023). Detection of *Toxoplasma gondii* DNA in milk of dairy cows from southern Brazil. *Parasitology international*, 95, 102750.
17. Hajimohammadi, B., Ahmadian, S., Firoozi, Z., Askari, M., Mohammadi, M., Eslami, G., Askari, V., Loni, E., Barzegar-Bafrouei, R., & Boozhmehrani, M. J. (2022). A Meta-Analysis of the Prevalence of Toxoplasmosis in Livestock and Poultry Worldwide. *EcoHealth*, 19(1), 55–74. <https://doi.org/10.1007/s10393-022-01575-x>
18. Fahad Ahmed, Maria Grazia Cappai, Sarah Morrone, Lia Cavallo, Fiammetta Berlinguer, Giorgia Dessì, Claudia Tamponi, Antonio Scala, Antonio Varcasia, Raw meat based diet (RMBD) for household pets as potential door opener to parasitic load of domestic and urban environment. Revival of understated zoonotic hazards? A review, *One Health*, Volume 13, 2021, 100327, <https://doi.org/10.1016/j.onehlt.2021.100327>.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



19. Monica Caffara, Andrea Gustinelli, Angelica Mazzone, Maria L. Fioravanti, Multiplex PCR for simultaneous identification of the most common European Opisthorchiid and Heterophyid in fish or fish products, *Food and Waterborne Parasitology*, Volume 19, 2020, e00081, ISSN 2405-6766, <https://doi.org/10.1016/j.fawpar.2020.e00081>.
20. Jiin Jung, Donald W. Schaffner, Quantification of Survival and Transfer of Salmonella on Fresh Cucumbers during Waxing, *Journal of Food Protection*, Volume 84, Issue 3, 2021, Pages 456-462, ISSN 0362-028X, <https://doi.org/10.4315/JFP-20-375>.
21. Puchalska, M., Pyziel, A. M., Wiśniewski, J., Steiner-Bogdaszewska, Ż., Klich, D., Anusz, K. (2021). Prevalence of *Toxoplasma gondii* antibodies in wild boar (*Sus scrofa*) from Strzałowo Forest Division, Warmia and Mazury Region, Poland. *Ann Agric Environ Med.*, 28(2), 237-242.
22. Xie, Stanley C., Ralph, Stuart A., Tilley, Leann, JOUR K13, the Cytostome, and Artemisinin Resistance, 2020, 10.1016/j.pt.2020.03.006
23. Mancusi Andrea , Proroga Yolande T. R. , Giordano Angela , Girardi Santa , D'Orilia Francescantonio , Pinto Renato , Sarnelli Paolo , Rinaldi Laura , Capuano Federico , Maurelli Maria Paola, First application of a droplet digital PCR to detect *Toxoplasma gondii* in mussels, *Frontiers in Microbiology*, Volume 14, 2023, doi=10.3389/fmicb.2023.1238689
24. Nanjan Chandran SL, Tiwari A, Lustosa AA, Demir B, Bowers B, et al. (2021) Revised estimates of leprosy disability weights for assessing the global burden of disease: A systematic review and individual patient data meta-analysis. *PLOS Neglected Tropical Diseases* 15(3): e0009209.
25. Lass, A., Ma, L., Kontogeorgos, I. et al. First molecular detection of *Toxoplasma gondii* in vegetable samples in China using qualitative, quantitative real-time PCR and multilocus genotyping. *Sci Rep* 9, 17581 (2019). <https://doi.org/10.1038/s41598-019-54073-6>
26. Jorrell Fredericks, Diane S. Hawkins-Cooper, Dolores E. Hill, John B. Luchansky, Anna C.S. Porto-Fett, Brad A. Shoyer, Valsin M. Fournet, Joseph F. Urban, Jitender P. Dubey, Inactivation of *Toxoplasma gondii* Bradyzoites after Salt Exposure during Preparation of Dry-Cured Hams, *Journal of Food Protection*, Volume 83, Issue 6, 2020, Pages 1038-1042, <https://doi.org/10.4315/0362-028X.JFP-19-461>.
27. Centers for Disease Control and Prevention (CDC). (2020). Parasites – Toxoplasmosis prevention.
28. Mancusi, A., Giordano, A., Bosco, A. et al. Development of a droplet digital polymerase chain reaction tool for the detection of *Toxoplasma gondii* in meat samples. *Parasitol Res* 121, 1467–1473 (2022).



Systematic Review: *Cryptosporidium* in Pet Reptiles, Human Risks, Prevention, and Iran-Specific Species Analysis

Reza Ameri¹, Siamak Mashhadi Rafiei¹

Corresponding Author: Reza Ameri, rezaameri058@gmail.com

1. Department of Small Animal Medicine and Radiology, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Article code: 1464

Abstract

Background: *Cryptosporidium* spp. are ubiquitous protozoan parasites infecting a wide range of vertebrates, including pet reptiles. Reptile-adapted species such as *Cryptosporidium serpentis* and *C. varanii* primarily cause chronic gastritis and enteritis, while mammalian genotypes may appear as pseudoparasites. Although reptile-to-human transmission is uncommon, documented cases highlight the zoonotic potential, particularly in immunocompromised individuals.

Methods: We conducted a systematic review following PRISMA 2020 guidelines. Databases searched included PubMed, Scopus, Web of Science, Google Scholar, and Magiran (1990–2025). Eligible studies involved natural *Cryptosporidium* infections in captive reptiles, human cases linked to reptile exposure, intervention studies, and Iran-specific surveys. Data extracted covered host species, detection methods, prevalence, genotypes, human health risks, and prevention strategies.

Results: Global surveys show high infection rates in captive reptiles, with both reptile-specific (*C. serpentis*, *C. varanii*, *C. testudines*) and mammalian (*C. parvum*, *C. hominis*) genotypes detected. Documented human infections associated with reptile handling underscore zoonotic risk. Effective prevention requires strict quarantine, molecular diagnostics, and improved disinfection methods. In Iran, most studies rely on microscopy without molecular speciation, leading to major diagnostic and epidemiological gaps.

Conclusion: *Cryptosporidium* infection in pet reptiles is widespread, with zoonotic transmission possible though rare. In Iran, the absence of molecular surveillance hinders understanding of species distribution and risks. Standardized PCR-based screening, strict biosecurity, and integrated One Health strategies are essential to protect both reptile welfare and public health.

Keywords: *Cryptosporidium*, Zoonosis, Pet Reptile, Iran.



Introduction:

Cryptosporidiosis is characterized by oocyst shedding and infection of gastrointestinal epithelia, leading to anorexia, regurgitation, weight loss, and, in severe cases, death in reptiles. Over 30 *Cryptosporidium* species have been described, with at least four *C. serpentis*, *C. varanii* (syn. *C. saurophilum*), *C. testudines*, and *C. ducismarci* adapted to reptiles (1, 2).

The popularity of exotic reptiles as pets and their close contact with humans raise concerns about zoonotic transmission. While most human cases arise from waterborne or livestock sources, handling infected reptiles or contaminated equipment can facilitate cross-species spillover, particularly affecting vulnerable populations (3).

Despite rising awareness, Iran lacks comprehensive molecular surveys of reptile-associated *Cryptosporidium*, relying mainly on microscopy without speciation, which undermines targeted control efforts (4,5).

Materials and Methods:

We adhered to PRISMA 2020 guidelines to conduct a systematic search of PubMed, Scopus, Web of Science, Google Scholar, and regional databases (Magiran) for publications from January 1990 to July 2025. Keywords included *Cryptosporidium* AND pet reptile, cryptosporidiosis AND snake OR lizard, zoonosis, prevention, and Iran.

Inclusion criteria:

- Original studies reporting natural *Cryptosporidium* infection in captive reptiles.
- Human case reports linking reptile exposure to cryptosporidiosis.
- Intervention studies on disinfection or quarantine protocols.
- Iran-specific surveys.

Exclusion criteria:

- Experimental challenge studies.
- Reviews without primary data.
- Non-English papers lacking English abstracts.

Two independent reviewers screened titles and abstracts. Data extracted included host taxonomy, detection method, prevalence, genotype/species identified, human risk assessments, and control measures.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Results and Discussion:

Global Prevalence in Pet Reptiles: Molecular surveys using PCR and sequencing reveal significant infection rates in private and institutional collections (Table 1).

Table 6. Global Prevalence in Pet Reptiles

Region	Host Species	Detection Method	Prevalence (%)	Species Identified	Reference
Austria	Leopard gecko, corn snake	PCR + 18S rRNA seq.	7–25	<i>C. varanii</i> , <i>C. serpentis</i> , reptile genotype	(1)
Thailand	Ball python, Burmese python	Nested PCR	12	<i>C. serpentis</i> , <i>C. parvum</i>	(6)
New Zealand	Tuatara, skinks, geckos	PCR + Immunofluoresc.	55	<i>C. hominis</i> , <i>C. parvum</i> , <i>C. serpentis</i>	(2)
Brazil	Various lizards and snakes	Microscopy, PCR	10–20	<i>C. serpentis</i> , <i>C. varanii</i>	(7)

Molecular Diversity: Reptile-specific species (*C. serpentis*, *C. varanii*, *C. testudines*) dominate, but mammalian genotypes (*C. parvum*, *C. hominis*, *C. muris*, *C. tyzzeri*) occur as pseudoparasites or in true spillover events(1,2). Mixed infections complicate clinical outcomes and diagnostic interpretation.

Human Health Risks: Although rare, reptile-associated cryptosporidiosis in humans has been reported:

- Case of *C. varanii* infection in an immunocompetent child handling bearded dragons, presenting with chronic diarrhea and weight loss (3).
- Outbreak simulation models estimate that improper hygiene in reptile-keeping could lead to sporadic human cases, particularly among immunocompromised individuals (8).

Asymptomatic shedding in reptiles, environmental persistence of oocysts, and lack of visible signs in pets underscore unrecognized exposure risks.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Prevention and Biosecurity: Evidence supports a multi-layered control strategy:

- Quarantine new arrivals for ≥ 90 days with PCR testing every 14 days to capture intermittent shedding (9).
- Use of dedicated cleaning utensils and nitrile gloves; rigorous hand hygiene post-handling.
- Effective enclosure disinfection using steam sterilization (>75 °C) or hydrogen peroxide vapor; bleach (sodium hypochlorite) solutions at standard concentrations are inadequate against oocysts (9).
- Periodic veterinary screening employing molecular assays or immunofluorescence to detect subclinical infections (10,11,12)

Iran-Specific Species Analysis: Iranian data remain limited:

- Arabkhazaeli et al. detected oocysts in 14% of Lacertilia, 32% of Serpentes, and 45% of Chelonii using modified acid-fast staining in Tehran collections, without molecular speciation (5).
- Motavalli Haghi et al. identified diverse *Cryptosporidium* species in livestock and wildlife (e.g., *C. parvum*, *C. hominis*, *C. muris*), but reptile surveys are absent (4).

No peer-reviewed studies have yet applied PCR or sequencing to Iranian pet reptiles, leaving species distribution and zoonotic potential uncharacterized.

Reptile cryptosporidiosis poses significant challenges for animal health and welfare. High prevalence in captive collections is driven by communal housing, stress, and husbandry lapses. Molecular diagnostics have unveiled unexpected mammalian genotypes in reptiles, raising One Health concerns.

Human transmission remains infrequent, but documented cases and modeling suggest that improper hygiene and inadequate quarantine can facilitate spillover, especially in immunocompromised or pediatric populations.

In Iran, reliance on microscopy without genotyping impedes accurate species identification, underestimating both reptile-adapted and zoonotic genotypes. Implementing validated molecular protocols will refine prevalence estimates, guide targeted interventions, and inform public health guidelines.

Cryptosporidium infection in pet reptiles is widespread globally, with reptile-specific and mammalian genotypes detected in captive collections. Zoonotic transmission, though rare, represents a tangible risk requiring integrated veterinary and public health measures. In Iran, the absence of molecular surveys constitutes a critical knowledge gap.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Adoption of standardized PCR assays, rigorous quarantine, and effective disinfection protocols are imperative to control cryptosporidiosis in reptiles and mitigate human health risks.

Future Directions:

- Launch nation-wide molecular surveillance of pet reptiles in Iran to map *Cryptosporidium* species and genotypes.
- Evaluate environmental persistence and viability of oocysts under typical Iranian husbandry conditions (temperature, humidity).
- Develop reptile-specific, multiplex PCR panels for routine veterinary diagnostics.
- Conduct case-control studies to quantify human infection risk linked to reptile exposure in domestic settings.
- Explore novel disinfection technologies (e.g., ultraviolet-C, ozone) for efficacy against *Cryptosporidium* oocysts in reptile enclosures.

Acknowledgment:

This research was carried out with the material and spiritual support of Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University. We at this moment express our gratitude to the officials of this center.

References:

1. Richter B, Nedorost N, Maderner A, Weissenböck H. Detection of *Cryptosporidium* species in feces or gastric contents from snakes and lizards by PCR and 18S rRNA sequencing. *J Vet Diagn Invest*. 2011;23(3):430–435.
2. Xiao L, Ryan UM. Cryptosporidiosis in reptiles: host adaptation and implications for zoonotic transmission. *Vet Parasitol*. 2004;126(3):249–263.
3. Enviroliteracy Team. Can humans get cryptosporidium from reptiles? [Enviroliteracy.org](https://enviroliteracy.org). Published April 6, 2025
4. Motavalli Haghi M, Khorshidvand Z, et al. *Cryptosporidium* animal species in Iran: systematic review and meta-analysis. *Trop Med Health*. 2020;48:97.
5. Arabkhazaeli F, Shirazi F, Chinikar S, et al. Frequently observed parasites in pet reptiles' feces in Tehran. *Iran J Vet Med*. 2018;12(1):19–25.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



6. Yimming B, Chansovichavakul T, Jongwutiwes S. Molecular Identification of *Cryptosporidium* Species from Pet Snakes in Thailand. *Korean J Parasitol.* 2016;54(4):423–429.
7. Eatwell K, Richardson J. Lizard Cryptosporidiosis. In: Divers SJ, Stahl SJ, eds. *Mader's Reptile and Amphibian Medicine and Surgery*. 3rd ed. Elsevier; 2019:1320–1321.
8. Madeline SM. Understanding *Cryptosporidium* in Reptiles: Testing, Quarantine, and Risks. *Zen Habitats*. 2025.
9. PetMojo. Cryptosporidiosis in Pet Reptiles: Symptoms, Prevention, and Treatment. Published April 11, 2025.
10. Xiao L, Ryan UM, Graczyk TK, et al. Genetic diversity of *Cryptosporidium* spp. in captive reptiles. *Appl Environ Microbiol.* 2004;70(2):891–899.
11. Brener B, Burgarelli E, Suarez M, Keidel L. Cryptosporidiosis in Reptiles from Brazil: An Update for Veterinary Medicine. *Parasitologia.* 2022;2(3):228–236.
12. Divers SJ, Stahl SJ, eds. *Mader's Reptile and Amphibian Medicine and Surgery*. 3rd ed. Elsevier; 2019.



Evaluation of the Larvicidal Effects of Aqueous-alcoholic Extracts of *Glycyrrhiza glabra*, *Calotropis procera* and *Moringa oleifera* on Pre-Parasitic Larval Stages of Gastrointestinal Strongyles in Monogastric Animals

Sara Larki¹, Fatetmeh Madankan¹, Gholamreza Pirmoradi²

Corresponding Author: Fatetmeh Madankan, F_madankan@stu.scu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Pharmacology, Faculty of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Article code: 1467

Abstract

Background: In the evolutionary cycle of nematodes from the families Strongylidae and Cyathostomidae, two stages exist: pre-parasitic and parasitic. However, any disruption in one of the pre-parasitic developmental stages, including effects on the egg, L1 and L2 larvae, or the L3 larval stage, will impair the life cycle and prevent infection in definitive hosts. To date, no studies have been conducted on the effects of aqueous-alcoholic extracts of *Glycyrrhiza glabra*, *Calotropis procera* and *Moringa oleifera* on the pre-parasitic stages of gastrointestinal nematodes in definitive hosts.

Methods: In this study, after collecting fecal samples contaminated with eggs of strongyle-type parasites and transferring them to the Parasitology Laboratory of Shahid Chamran University of Ahvaz, larval nematode stages were cultured, and the effects of aqueous-alcoholic extracts were evaluated. Ten grams of the collected contaminated fecal sample were placed in separate sealed containers for culture, and the feces were homogenized. At the end of the culture day, using the Baermann method, L3 larvae were individually separated from the feces. Subsequently, the samples were examined under a stereomicroscope to assess the effects of aqueous-alcoholic extracts, and their mortality rates were calculated.

Results: In this study, groups treated with aqueous-alcoholic extracts of *Glycyrrhiza glabra* and *Calotropis procera* at a concentration of 1 mg exhibited a clearly observable effect, such that all larvae in the pre-parasitic stages were eliminated, and after the Baermann method, no L3 larvae were present. However, in the group treated with the aqueous-alcoholic extract of *Moringa oleifera*, the mortality rate was 36.6%

Conclusion: In recent years, medicinal plants have gained attention. In the near future, controlling certain parasites with conventional anthelmintic drugs will become increasingly



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



difficult. Therefore, there is a fundamental need to find new alternatives for controlling gastrointestinal nematodes in definitive hosts.

Keywords: *Strongylinae*, *Cyathostominae*, *Glycyrrhiza glabra*, *Calotropis procera*, *Moringa oleifera*.

Introduction:

The continuous development and widespread prevalence of anthelmintic resistance in gastrointestinal parasitic nematode populations of definitive hosts (18) has prompted research into alternative, more sustainable control measures. Nematodes in the families Strongylinae and Cyathostominae have a two-stage life cycle: a pre-parasitic stage (eggs shed in feces develop into infective L3 larvae—two molts from L1 to L3—in 3–4 days at 30 °C or 16–20 days at 12 °C) and a parasitic stage (1). Emergence of L3 from its sheath marks the key transition to host infection. Any disruption at the pre-parasitic stages (egg, L1, L2, or L3) can break the life cycle and prevent host infection. Since even a single untreated horse can contaminate pasture heavily, universal on-farm treatment is essential. Yet chemical control is costly, only partly effective, and increasingly undermined by drug resistance (10). Conversely, traditional and experimental studies in China, Japan, India, and Iran show that bioactive forages or aqueous-alcoholic extracts of medicinal plants can boost host resistance or impair nematode physiology, sometimes outperforming synthetic drugs—such as artemisinin compounds against drug-resistant *Plasmodium falciparum* (11, 7). Reported effects on various stages of *Haemonchus contortus* include thyme, cumin, geranium, *Nauclea latifolia*, *Plantago ovata*, *Achillea millefolium*, *Sambucus nigra*, and garlic (3, 5,6,7,8, 12). However, to date no study has assessed the effects of aqueous-alcoholic extracts of *Glycyrrhiza glabra*, *Calotropis procera*, or *Moringa oleifera* on the pre-parasitic stages of gastrointestinal nematodes in equids.

Materials and Methods:

Fecal samples containing strongyle-type eggs were collected directly from the rectum of donkeys grazing near Ahvaz and examined using the Clayton-Lane flotation method to determine egg per gram (EPG) counts (1). Samples with high EPG were cultured, and 10 g of feces from each group were treated with aqueous-alcoholic extracts of *Glycyrrhiza glabra*, *Calotropis procera*, and *Moringa oleifera* (1 mg), applied every other day for 12 days at 25–28 °C. On day 12, L3 larvae were isolated using the Baermann method, and mortality rates were assessed microscopically. Control groups included albendazole-treated and untreated samples. All experiments were performed in triplicate, and data were analyzed using Tukey's test with significance set at $P < 0.05$.



Results and Discussion:

In this study, the groups treated with aqueous-alcoholic extracts of *Glycyrrhiza glabra*, *Calotropis procera* at a concentration of 1 mg exhibited clear effects, such that all larvae in the pre-parasitic stages were eliminated, and no L3 larvae were present after the Baerman method. However, in the group treated with the aqueous-alcoholic extract of *Moringa oleifera*, the mortality rate was 36.6%. Criteria for larval mortality included immobility, elongation, absence of muscular contractions, presence of vacuoles in the larval body, or lack of larvae in the Baermann collection chamber, compared to the control group (Figure 1). Table 1 details the efficacy of the extracts used. Statistical analyses also revealed significant differences between the various aqueousalcoholic extract groups and the control group ($P<0.05$).

Table 1. Mortality of pre-parasitic nematode stages after exposure to plant extracts.

Extract	Concentration	L3 Larvae Remaining	The L3 larvae are dead	Mortality
<i>Glycyrrhiza glabra</i> ^A	1 mg	No live larvae in the extract	-	100%
<i>Calotropis procera</i> ^B	1 mg	12	-	99.8%
<i>Moringa oleifera</i> ^C	1 mg	3766	-	36.6%
Albendazole (Control Group) ^D	1 µg/mL	No live larvae in the extract	-	100%
Control Group ^(A,B,C,D)	Tap water	5940	All larvae were alive	0%

*Similar letters are significant ($p<0.05$).



Figure 1. Baermann method fluid demonstrating no L3 larvae after treatment

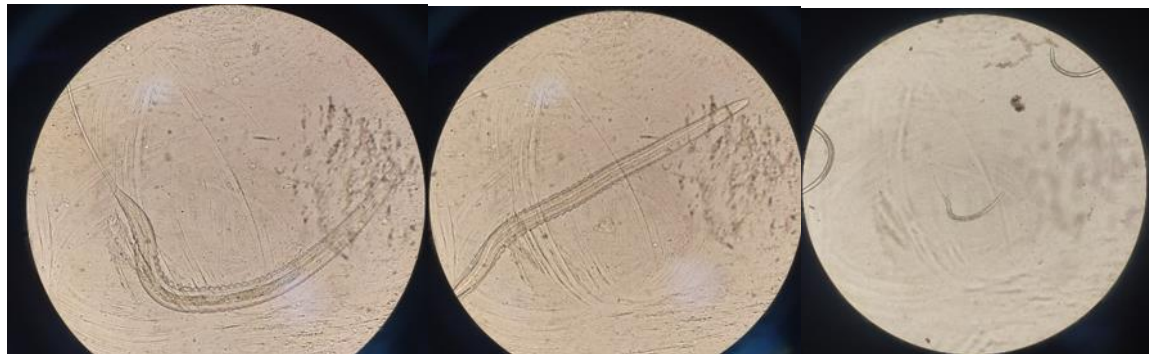


Figure 2. L3 larvae of strongyle-type nematodes in the control group (Baermann method)

In recent years, medicinal plants have gained significant attention, such that approximately 80 percent of people in developing countries rely on herbal medicines for their primary healthcare needs (13). Various bioactive compounds are derived from plants. Glycyrrhiza glabra is a well-known traditional medicinal plant in Iran attributed with multiple effects, including anti-inflammatory, antispasmodic, and gastroprotective properties. Its pharmacological effects are related to two groups: flavonoids (conferring antispasmodic activity) and glycyrrhizin (a saponin compound) associated with anti-inflammatory activity (15). In the study by Chen et al., Licochalcone A (an oxygenated chalcone isolated from licorice) inhibited the amastigote and promastigote forms of *Leishmania major* and *L. donovani* (17). Results from Hosseini et al. showed that total extracts of turmeric and licorice at concentrations used in culture media exhibited antileishmanial effects. The overall effect of licorice was weaker compared to turmeric and required higher doses to eliminate the parasite, which aligns with the findings related to licorice extract in this study (6). *Calotropis procera* is another notable medicinal plant widely used for its therapeutic properties; its tissues, especially the root bark, are used to treat various diseases such as leprosy, fever, malaria, and snakebite (14). In the study by Soltani Eeini et al., which evaluated the therapeutic effects of aqueous and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



hydroalcoholic extracts of *Calotropis procera* and the drug Butalex on *Haemoproteus* infection in pigeons, the results showed that the aqueous and alcoholic extracts of *eryngium* had no therapeutic effect against *Haemoproteus* infection in pigeons, whereas the drug Butalex could be prescribed as an effective treatment against *Haemoproteus*. In contrast, the results of the present study clearly demonstrate the efficacy of this extract. The discrepancy may be due to differences in the nature and structure between the protozoan *Haemoproteus* and gastrointestinal nematodes (4). According to the Food and Agriculture Organization, approximately 70–80 percent of the world's population—especially in developing countries—depend on herbal medicines for disease prevention and treatment, and about 25 percent of synthetic drugs are derived from medicinal plants. *Moringa oleifera* is considered an important famine food and can alleviate malnutrition (19). *Moringa* is the most nutrient-rich plant yet discovered and contains rare compounds rich in minerals, proteins, amino acids, antioxidants, anti-aging, and antiinflammatory compounds that are used in nutrition and therapy (16). In a study by Alborzi et al. on the acaricidal effect of aqueous, hydro-alcoholic, and alcoholic leaf extracts of *Moringa oleifera* on the eggs and larvae of *Hyalomma anatolicum anatolicum* ticks via immersion, results showed that the aqueous-alcoholic and alcoholic extracts reduced egg hatchability and larval numbers compared to the aqueous extract, which aligns with the findings of this study (2). In the near future, controlling certain parasites with conventional anthelmintic drugs will become very difficult. Therefore, the development of new anthelmintic drugs that are suitable, environmentally compatible, cost-effective, and potentially active appears necessary and timely. Moreover, the limited water solubility of some anthelmintic drugs negatively impacts their absorption and ultimately their clinical efficacy in animals. Additionally, drug residues in animal products have harmful effects on the ecosystem (9). Therefore, a fundamental need exists to find new alternatives for controlling gastrointestinal nematodes in definitive hosts.

References:

1. Eslami, A. (2006). *Veterinary Helminthology*, Vol. 3: Nematoda and Acanthocephala. University of Tehran Press (No. 2321), pp. 200–242.
2. Alborzi, A., Larki, S., Jamshidian Ghale-Sefidi, J., & Khodadad, H. (2023). Anti-tick effect of aqueous, aqueous-alcoholic and alcoholic leaf extracts of *Moringa oleifera* on the eggs and larvae of *Hyalomma anatolicum anatolicum* using immersion tests. *Veterinary Research and Biological Products*, 36(2), 22–31. [doi:10.22092/vj.2022.358543.1969](https://doi.org/10.22092/vj.2022.358543.1969)
3. Izadi, J., Sharif, M., Khalilian, A., Ziaei Hezarjeribi, H., Azadbakht, M., & Adeli, S. (2003). Investigating the anthelmintic effects of *Achillea millefolium* on *Enterobius vermicularis* in mice. *Journal of Mazandaran University of Medical Sciences*, 13(40), 27–35.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



4. Soltani Eini, M., Razmi, G., Afkhami, A., & Ahmadi, A. (2013). Evaluation of the therapeutic effects of aqueous and hydro-alcoholic extracts of Estebroq (*Calotropis* spp.) and the drug Butalex on *Haemoproteus* infection in pigeons. *Veterinary Microbiology (Journal of Garmsar Veterinary Research)*, 8(2) [Issue 25], 101–107.
5. Hosseini, H., Haghighi-Karsidani, S., & Meshgi, B. (2007). Evaluation of the effects of plant extracts of thyme (*Thymus*), cumin (*Cuminum cyminum*), and Sham'dani (local name) on various developmental stages of *Haemonchus contortus* (nematode) under laboratory and field conditions. *Quarterly Journal of Medicinal Plants*, 4(24), 51–57.
6. Hosseini, A., Jaffary, F., Asghari, G. R., Hejazi, S. H., & Shirani-Bidabadi, L. (2012). Evaluation of the therapeutic effects of hydroalcoholic and aqueous extracts of turmeric (*Curcuma longa*) and licorice (*Glycyrrhiza glabra*) on the MRHO/IR/75/ER strain of *Leishmania major* in vitro. *Journal of Isfahan Medical School*, 29(169), 2541–2551.
7. Maraghi, S., & Tarafi Jabrpour, N. (2002). In vitro and clinical evaluation of the effects of *Plantago ovata* seed extract on *Hymenolepis nana* and comparison with niclosamide. *Hakim Research Journal*, 5(1), 57–60.
8. Mahrabi, M., Sadrayi, J., & Ghafari Far, F. (2012). Comparative evaluation of the effects of garlic tablets and alcoholic extract of *Cornus mas* on *Cryptosporidium parvum* oocysts in Hank's medium. *Scientific Journal of Kurdistan University of Medical Sciences*, 17(2), 53–60.
9. El-makawy A, Radwan HA, Ghaly IS, Abdelraouf A. (2006). Genotoxic, teratological and biochemical effects of anthelmintic drug oxfendazole Maximum Residue Limit (MRL) in male and female mice. *Reprod Nutr Dev.*; 46: 139–156. [Doi: 10.1051/rnd:2006007](https://doi.org/10.1051/rnd:2006007)
10. Roeber F, Jex AR, Gasser RB. (2013). Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance – an Australian perspective. *Parasit Vectors.*; 6: 153–153. [Doi: 10.1186/1756-3305-6-153](https://doi.org/10.1186/1756-3305-6-153).
11. Tyler, Y.E. and Bradly, L.R. (1988). *Pharmacognosy*. 9 th Ed. U.S.A Philadelphia, 492.
12. Hooper, S.N. and Chandler, R.F. (1979). Herbal remedies of the maritime Indians. Part III . *J Ethnopharmacol*. 6 (3): 275-285.
13. Nsagha DS, Ayima CW, Nana-Njamen T, Assob JCN.2020. The role of traditional, complementary/alternative medicine in primary healthcare, adjunct to universal health coverage in Cameroon: a review of the literature. *American Journal of Epidemiology*.;8(1):37-47.
14. Hussaan M, Iqbal N, Adeel S, Azeem M, Tariq Javed M, Raza A. Microwave-assisted enhancement of milkweed (*Calotropis procera* L.) leaves as an eco-friendly source of



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



natural colorants for textile. Environmental Science and Pollution Research. 2024;50(17):89-94 20.

15. . Mir Heidar H. Education plant, plants used in the prevention and treatment of diseases. 3rd ed. Office of Islamic culture; 1997.
16. Mahmood, K.T., T. Mugal, and I.U. Haq,(2010) *Moringa oleifera*: a natural gift-A review. Journal of Pharmaceutical Sciences and Research. 2(11): p. 775.
17. Chen M, Christensen SB, Blom J, Lemmich E, Nadelmann L, Fich K, et al. Licochalcone A, a novel antiparasitic agent with potent activity against human pathogenic protozoan species of *Leishmania*. Antimicrob Agents Chemother 1993; 37(12): 2550-6.
18. Kumarasingha, Rasika ; Preston, Sarah; Tiong-Chia, Yeo; Diana, S. L. Lim; Chu-Lee, Tu; Enzo, A. Palombo; Jillian, M. Shaw; Robin, B. Gasser; Peter, R. Boag. 2016. Anthelmintic activity of selected ethno-medicinal plant extracts on parasitic stages of *Haemonchus contortus*. Parasites & Vectors, 9/187.
19. Saini, R.K., I. Sivanesan, and Y.-S. Keum,(2016) Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. 3 Biotech. 6(2): p. 203.



Investigation of Contamination of Zoonotic Common Parasites in Slaughtered Animals at Semnan Industrial Slaughterhouse in the Period 2024-2025

Amin DehghanTezerjani¹, Sabike Bakhtiyari²

Corresponding Author: Amin Dehghan Tezerjani, amin.dehghan68@yahoo.com

1. Graduate of Professional Doctorate in Veterinary Medicine, Faculty of Veterinary Medicine, Islamic Azad University, Garmsar, Garmsar, Iran.
2. Medical Doctor (MD), Faculty of Medicine, Mashhad Branch, Islamic Azad University, Iran.

Article code: 1476

Abstract

Background: Certain animal protein sources can cause significant economic losses due to the chronic, subclinical nature of some diseases and the challenges in developing effective vaccines. These diseases often lead to reduced productivity and increased costs, especially in the livestock industries. This study examines the prevalence of liver (Dicrocoeliasis, Cysticercosis, Fascioliasis, hydatidosis) and lung (hydatidosis) infections in slaughtered livestock at Semnan's industrial slaughterhouse.

Methods: This study is a descriptive-analytical type. In total, 908 animals were included, consisting of 110 cattle, 757 sheep, 35 goats, and 6 camels infected with parasitic diseases, which were ultimately culled.

Results: The prevalence of Dicrocoeliasis, Fascioliasis, hepatica cyst, pathological jaundice, and *Cysticercosis* was 4.2%, 0.1%, 95.3%, 0.1%, and 0.2%, respectively. The results showed no statistically significant association between the type of parasite and the type of animal ($p > 0.05$). Additionally, there was no association between the gender of the animals and the type of parasitic infection ($p > 0.05$). However, the findings revealed a significant relationship between the season and the infection of animals with parasitic diseases ($p < 0.05$).

Conclusion: The findings of this study demonstrate a high prevalence of *hydatidosis* (95.3%) in slaughtered ruminants in Semnan, highlighting the persistent challenge of *Echinococcus granulosus* infection in the region. In contrast, Dicrocoeliasis, Fascioliasis, and Cysticercosis showed notably lower infection rates, suggesting differing epidemiological dynamics among these parasites. The lack of a significant association between parasite type and animal species or gender ($p > 0.05$) implies broad susceptibility across livestock categories, reinforcing the need for uniform control strategies. However, the marginal seasonal association ($p = 0.09$) suggests potential environmental or management-related influences on transmission, warranting further investigation into climatic and husbandry factors.



Keywords: Cysticercosis, Dicrocoeliasis, Fascioliasis, Hepatica cyst, Pathologic jaundice.

Introduction:

One of the most significant diseases affecting both domestic and wild animals, with considerable implications for animal health, is parasitic infections, particularly fascioliasis and dicrocoeliasis. These diseases are caused by *Fascioliasis hepatica*, *Fascioliasis gigantica*, and *Dicrocoeliasis dendritic*, respectively, and result in substantial economic losses. Therefore, identifying parasitic infections and understanding their epidemiology in a given region can aid in controlling, preventing, and treating these diseases. Additionally, in cases of zoonotic transmission, such measures can help prevent the spread of these parasites to humans (6).

Fascioliasis and dicrocoeliasis are among the most significant parasitic diseases affecting ruminants in Iran. These infections predominantly manifest in chronic forms and have been reported across all regions of the country (7).

These diseases cause significant direct and indirect economic losses to the national livestock industry, primarily through reduced weight gain and decreased milk production (3) and lead to the condemnation of infected livers.

Since the most common parasitic agents infecting sheep and goat livers are *Fascioliasis* spp. and *Dicrocoeliasis dendritic*.

Considering these parasites are zoonotic, potential exists for cross-species transmission of parasitic diseases from livestock to humans. The present cross-sectional study aimed to determine the annual prevalence rates of hepatic and pulmonary parasitic infections (2024-2025) among slaughtered ruminants (ovine, bovine, caprine, and camelid) at Semnan Industrial Abattoir.

The study hypotheses were formulated as follows:

- 1) *An association exists between parasite species and host animal type;*
- 2) *A correlation is present between animal sex and parasite type; and*
- 3) *A relationship occurs between seasonal variations and parasitic infection rates in livestock.*

Materials and Methods:

The study population consisted of slaughtered animals at Semnan Industrial Slaughterhouse during the year 2024-2025. Following physical examination and subsequent organ dissection for parasitic detection, condemned carcasses were properly disposed. The final dataset comprised 150 male (16%) and 758 female (83%) animals, with the following distribution:



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



cattle - 55 males (50%) and 55 females (50%); sheep - 91 males (12%) and 666 females (88%); goats - 1 male (3%) and 34 females (97%); camels - 3 males (50%) and 3 females (50%). Data analysis was performed using SPSS version 23 after initial processing in Excel.

Seasonal patterns and differences in disease prevalence among cattle, sheep, camels, and goats according to sex were analyzed using the chi-square test. Descriptive results were presented as absolute and relative frequencies, with prevalence rates of parasitic diseases calculated for different seasons.

Results and Discussion:

Among the total studied animals (908 condemned livestock), parasitic infections were distributed as follows:

- *Dicrocoeliasis*: 38 cases (9 males, 29 females)
- *hepatica* cyst: 862 cases (133 males, 699 females)
- Pathological jaundice: 1 female bovine
- *Cysticercosis*: 2 female ovine
- *Fascioliasis*: 1 female bovine

The prevalence rates of *Dicrocoeliasis* spp., *Fascioliasis*, *hydatidosis*, pathological jaundice, and *Cysticercosis* were 4.2%, 0.1%, 95.3%, 0.1%, and 0.2%, respectively.

The results demonstrated no statistically significant association between parasite type and animal species ($p > 0.05$), indicating equal susceptibility to various parasitic infections across different ruminant species.

No statistically significant association was observed between animal sex and the prevalence of hepatopulmonary parasitic infections ($p > 0.05$).

"The results demonstrated a statistically significant association between seasonal variations and the prevalence of parasitic infections in the animal population ($p < 0.09$). The detailed distribution of these findings is presented in Table 1.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 1. Seasonal variations in organ-specific parasitic infestation patterns in livestock.

Affected Organs	Season			
	Absolute abundance of Spring	Absolute abundance of Summer	Absolute abundance of Fall	Absolute abundance of Winter
Cattle lung	17	15	10	9
Cattle liver	24	13	16	2
Sheep lung	133	122	131	107
Sheep liver	182	28	41	0
Goat lung	2	9	2	9
Goat liver	6	6	1	0
Camel liver	4	1	1	0

Findings revealed:

- 1) Peak infection rates: - Bovine hepatic/pulmonary tissues in spring ($p < 0.05$)
- 2) Significant associations: - Ovine pulmonary involvement in spring ($p < 0.05$)
- 3) Seasonal patterns: - Camelid hepatic infections: spring - Caprine pulmonary infections: spring / summer

The statistical analysis confirmed only the seasonal variation hypothesis for parasitic infections (*hydatidosis*, *dicrocoeliasis*, *pathological jaundice*, *Cysticercosis*, and *Fascioliasis*) ($p < 0.05$), while no significant associations were found for host species or gender susceptibility ($p > 0.05$)

Dicrocoeliasis and *Fascioliasis* are hepatobiliary trematodiasis that cause: 1) Significant economic losses through: - Liver condemnation - Reduced milk/meat/wool production - Increased mortality rates 2) Public health concerns: - Zoonotic potential - Food safety implications (3).

Parasitic disease outbreaks pose dual threats: 1) Public and animal health hazards 2) Substantial economic burdens including: - Production losses - Treatment costs - Carcass condemnation (8).



The findings revealed that *Dicrocoeliasis* infection and *hepatica* cyst were the most prevalent parasitic conditions (X% and Y%, respectively) among slaughtered animals at Semnan abattoir in 2024-2025, with predominant involvement of hepatic and pulmonary tissues.

"National surveillance data indicate the prevalence of *Fascioliasis* in Iranian ruminants (cattle, sheep, and goats) ranges from 1% to 17%, with significant geographical variations ($p < 0.05$)(5).

The prevalence rate of this parasite was estimated at 0.1% in the current study.

Falah's (2009) study on the prevalence of zoonotic parasites (*hepatica* cyst, liver trematodes, and *Cysticercosis*) in slaughtered animals at Hamadan Industrial Slaughterhouse revealed infection rates of 12.3% for *hydatidosis*, 4.9% for *Fascioliasis hepatica*, and 6.5% for *Dicrocoeliasis dendritic* among 3,500 examined animals. Specifically in sheep, the prevalence rates were 13.7%, 4.2%, and 6.9% for these parasites, respectively (2).

Arsakhani's (1995) investigation on parasitic liver condemnations in sheep at Urmia Industrial Slaughterhouse found that among 10,000 examined livers over one year, 2,966 (29.66%) were infected. The parasite-specific distribution was: - *Dicrocoeliasis dendritic*: 1,581 cases (15.81%) - *hydatidosis*: 1,012 cases (10.12%) - *Fascioliasis hepatica*: 373 cases (3.73%) (1).

In another study conducted at Ahvaz slaughterhouse by Sayari et al. over a two-year period examining the prevalence of *hydatidosis* and fascioliasis in small ruminants, 523 condemned sheep livers were examined. Among these: - 309 cases (59.1%) were infected with *Fascioliasis hepatica* - 107 cases (20.5%) were infected with *hydatidosis* (4).

The findings highlighted *hepatica* cyst as the most detrimental parasitic condition in the study area. Thus, implementing an integrated control program encompassing:

- 1) Regular diagnostic testing
- 2) Improved hygiene practices
- 3) Strategic anthelmintic administration
- 4) Preventive vaccination protocols
- 5) Farmer education initiatives should be prioritized for effective zoonotic disease management.

Gratitude: We hereby extend our thanks and appreciation to the honorable management of Semnan Industrial Slaughterhouse and the veterinary supervision and inspection department of this province for their full cooperation in detecting infected livestock.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Beesley, N. J., Williams, D. J., Paterson, S., & Hodgkinson, J. (2017). *Fasciola hepatica* demonstrates high levels of genetic diversity, a lack of population structure and high gene flow: possible implications for drug resistance. *International journal for parasitology*, 47(1), 11-20
2. De Moraes, J. and Geary, T.G., 2020. FDA-approved antiparasitic drugs in the 21st century: a success for helminthiasis?. *Trends in parasitology*, 36(7), pp.573-575.
3. Eslami, A., 2008. Family Fasciolidae. *Veterinary Helminthology*, pp.45-86.
4. Fallah, M., M. Matini, E. Begim-Kia and I. Mobedi. 2009. Prevalence of zoonotic parasites in animals slaughtered in Hamad abitrary in 1388 (Hydatic cyst, Liver Trematodes and Sarcositocis). *Journal of Hamadam Medical University* 17(57), 5-12.
5. Kahunde, P.K., 2022. Prevalence of Bovine Fascioliasis in ruminants slaughtered at Nakifuma and Katosi slaughter slabs at Mukono District and the associated economic losses (Doctoral dissertation, Makerere University).
6. Mas-Coma, S., Bargues, M.D. and Valero, M.A., 2005. Fascioliasis and other plantborne trematode zoonoses. *International journal for parasitology*, 35(11-12).pp: 1255-1278.
7. Otranto, D. and Traversa, D., 2003. Dicrocoeliosis of ruminants: a little known fluke disease. *Trends in Parasitology*, 19(1), pp.12-15.
8. Sahba, G.H., Arfaa, F., Farahmandian, I. and Jalali, H., 1972. Animal fascioliasis in Khuzestan, southwestern Iran. *The Journal of Parasitology*, pp.712-716.



Cyathostomins in Horses: Life Cycle, Pathogenesis, Resistance

Mohammad mobin Sabri¹, Arman Valipour¹, Marzieh Kefayat¹

Corresponding Author: Mohammad mobin Sabri, Mmsabri83@gmail.com

1. Department of Parasitology, school of veterinary medicine, university of Karaj Branch of Azad University, Karaj, Iran.

Article code: 1482

Abstract

Background: Cyathostomins are the commonest group of intestinal parasitic nematodes of horses with obligate function which are ubiquitous in equids and of significant pathogenic concern. These parasites are considered one of the most significant threats to horses health in many regions worldwide. The complex life cycle of cyathostomins, involving invasive larvae and adult worms, facilitates rapid intestinal colonization. Furthermore, the development of drug resistance against antiparasitic treatments is a major concern in controlling these parasites. Drug resistance against common antiparasitic treatments has led to the exploration of novel therapeutic and preventive strategies. This article aims to review and analyze the available information about the common species of these parasites in horses.

Methods: In this review, the life cycle, pathogenesis, and drug resistance of cyathostomins in horses was searched across databases including Google Scholar. Studies published between 2000 and 2024 were screened Only scientifically valid and topic-relevant articles were included in the final analysis.

Results: Widespread resistance to anthelmintics, particularly benzimidazoles, has been reported in equine cyathostomins. The complex life cycle and larval encystment in the gut wall hinder effective treatment.

Conclusion: Findings highlight the need for revised therapeutic protocols and integrated approaches to control infection and reduce selection pressure for resistance.

Keywords: Cyathostomins, Small strongyles, Life cycle, Pathogenesis.

Introduction:

Small strongyles (Cyathostomins) include over 50 species, with ~10 causing 98% of infections and near-universal prevalence in pastured horses under 4 years (5). In one study, *Cylicocyclus nassatus* (prevalence = 93%, relative abundance = 20%), *Cylicostephanus longibursatus* (93%, 20%), and *Cyathostomum catinatum* (90%, 16%) were identified as the most prevalent and abundant species in equine populations (1).



Cyathostomins have a direct life cycle, developing from eggs in feces to infective L3 larvae under favorable conditions, without intermediate hosts (1,4,5). Upon ingestion, L3s penetrate the large intestinal mucosa, becoming enclosed in fibrous capsules where they may undergo arrested development (hypobiosis) for variable durations ranging from weeks to over two years (4,5). These larvae eventually resume development, molt into L4, and emerge into the intestinal lumen to become adults (4,5). Prepatent periods vary among species and depend on environmental and host factors (5).

The principal pathogenic phase of cyathostomin infection involves the mass emergence of encysted larvae from the intestinal wall, causing larval cyathostominosis (1,4). This condition is characterized by diarrhea, colic, weight loss, edema, and hypoalbuminemia, and may result in mortality in severe cases (1,4,5). The mucosal disruption, villous atrophy, and inflammation caused by emerging larvae contribute significantly to intestinal damage (3,4). Even in the absence of acute clinical signs, chronic low-grade inflammation caused by cyathostomins may lead to reduced performance and compromised nutrient absorption (3,4,5).

Anthelmintic resistance among cyathostomins is a well-documented and growing concern in equine parasite control (1,2,3). Resistance to benzimidazoles is now widespread globally (1,2), and decreasing efficacy of macrocyclic lactones, including ivermectin and moxidectin, has been reported, often indicated by shortened egg reappearance periods (ERP) (1,2,5). Resistance to tetrahydropyrimidines (e.g., pyrantel) is also emerging (2). Major contributing factors include frequent, non-targeted treatments, underdosing, and the lack of fecal egg count monitoring (1,2,3).

Materials and Methods:

This review article was conducted using a narrative approach to summarize the current knowledge on the life cycle, pathogenesis, and anthelmintic resistance of cyathostomins in horses. Relevant literature was identified through a structured search on Google Scholar, using keywords such as cyathostomins, small strongyles, equine strongylids, life cycle, larval cyathostominosis, and anthelmintic resistance. Publications published between 2000 and 2024 were considered.

Priority was given to peer-reviewed articles in English with strong scientific rigor and relevance to the equine cyathostomin field. Inclusion criteria focused on studies that provided experimental, clinical, or epidemiological insights into cyathostomin biology or resistance patterns. Exclusion criteria included studies lacking full-text access or those unrelated to the primary focus.



For efficient reference management, selection, annotation, and citation, the software Zotero was used throughout the process. All citations were organized based on the Vancouver style and renumbered according to relevance and appearance in the text.

Results and Discussion:

Numerous studies have demonstrated the near-universal prevalence of cyathostomin infections among grazing horses, particularly in young individuals under four years of age (5). Despite this ubiquity, severe clinical signs are predominantly associated with the synchronous emergence of encysted L4 larvae from the colonic mucosa, a syndrome recognized as cyathostominosis (1,5). When large numbers of larvae emerge simultaneously from the intestinal wall, they may induce severe inflammation, hypoproteinemia, weight loss, diarrhea, and in severe cases, death (1,2,5). Pathological findings include infiltration of inflammatory cells, mucosal hyperplasia, and disruption of the crypt and glandular architecture of the large intestine (1,5).

In terms of anthelmintic resistance, widespread resistance to benzimidazoles (e.g., fenbendazole), tetrahydropyrimidines (pyrantel), and a gradual decline in the efficacy of macrocyclic lactones (ivermectin and moxidectin) have been reported across diverse geographic regions, including Europe, the Americas, and Asia (2,3,5). The shortening of the egg reappearance period (ERP) after treatment with macrocyclic lactones has been well-documented and is considered a key indicator of emerging resistance (2,4,5). Moreover, concurrent resistance to two or more drug classes has been confirmed on several farms (2,3).

The clinical impact of cyathostomin infections is closely linked to the pathogenic potential of larval stages, particularly during mass emergence events (1,5). Encysted larvae are protected from immune responses and treatments, and inappropriate deworming may paradoxically exacerbate disease (1,3,5). Therefore, anticipating or preventing synchronous larval emergence remains a major therapeutic challenge (2,5).

Regarding resistance, key mechanisms have been identified, including mutations in the β -tubulin gene conferring resistance to benzimidazoles and the upregulation of efflux pumps associated with resistance to macrocyclic lactones (2,3). Given the parasite's complex life cycle and the potential for resistance development, frequent and indiscriminate use of anthelmintics increases selection pressure, facilitating the establishment of resistant populations (2,3,5).

Evidence-based recommendations emerging from the reviewed literature emphasize the importance of Targeted Selective Treatment (TST) strategies, routine monitoring via fecal egg count reduction tests (FECRT), rotational grazing practices, and the use of drugs effective against encysted stages (2,3,5). Furthermore, the development of advanced molecular and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



immunological diagnostic tools for early detection, alongside longitudinal studies evaluating drug efficacy and resistance genetics, are strongly encouraged (3,5).

In conclusion, the effective control of cyathostomins requires an integrated approach—balancing responsible anthelmintic use, epidemiological surveillance, and deeper insight into the ecology and evolution of resistance in parasite populations (2–5).

Cyathostomins remain among the most prevalent and clinically significant equine gastrointestinal parasites worldwide (5). Despite their generally subclinical nature in adult stages, the pathogenic potential of encysted larval forms—particularly during mass emergence—poses a substantial threat to equine health (1,5). Clinical consequences such as protein-losing enteropathy, diarrhea, weight loss, and even mortality underscore the importance of early recognition and effective management strategies (1,2,5).

The reviewed literature demonstrates widespread and increasing resistance to major anthelmintic classes, including benzimidazoles, tetrahydropyrimidines, and macrocyclic lactones (2,3,5). Notably, resistance is not confined to a single region or compound but spans multiple continents and drug classes, signaling a global issue that requires urgent attention (2,3). The shortening of the egg reappearance period (ERP) and confirmed treatment failures reflect a narrowing of effective therapeutic options (2,4).

Insights into resistance mechanisms may guide new diagnostics and therapies, but treatments against encysted larvae remain limited (2–4).

This review emphasizes the necessity for integrated parasite management programs based on fecal egg count reductions tests (FECRT), targeted selective treatments (TST), seasonal deworming plans, and environmental control strategies (2,3,5). Moreover, continued epidemiological surveillance, combined with genetic and pharmacodynamic research, is essential to guide policy and practice (2,3).

Ultimately, sustainable control of cyathostomin infections requires coordinated action from researchers, veterinarians, and horse owners alike—rooted in evidence-based practices, drug stewardship, and long-term monitoring of parasite dynamics (3,5).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Lake SL, Matthews JB, Kaplan RM, Hodgkinson JE. Determination of genomic DNA sequences for beta-tubulin isotype 1 from multiple species of cyathostomin and detection of resistance alleles in third-stage larvae from horses with naturally acquired infections. *Parasites Vectors* [Internet]. 2009 Sept [cited 2025 July 20];2(S2). Available from: <https://parasitesandvectors.biomedcentral.com/articles/10.1186/1756-3305-2-S2-S6>
2. Byrne O, Gangotia D, Crowley J, Zintl A, Kiser L, Boxall O, et al. Molecular species determination of cyathostomins from horses in Ireland. *Veterinary Parasitology*. 2024 June;328:110168.
3. Bellaw JL, Nielsen MK. Meta-analysis of cyathostomin species-specific prevalence and relative abundance in domestic horses from 1975–2020: emphasis on geographical region and specimen collection method. *Parasites Vectors* [Internet]. 2020 Dec [cited 2025 July 20];13(1). Available from: <https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04396-5>
4. Corning S. Equine cyathostomins: a review of biology, clinical significance and therapy. *Parasites Vectors*. 2009;2(Suppl 2):S1.
5. Pfister K, Van Doorn D. New Perspectives in Equine Intestinal Parasitic Disease. *Veterinary Clinics of North America: Equine Practice*. 2018 Apr;34(1):141–53.



A Review of *Neospora caninum* in Sheep: Epidemiology, Pathogenesis, and Control Strategies

Javad Qaneie¹, Pezhman Mirshokraei¹

Corresponding Author: mirshokraei@um.ac.ir

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1491

Abstract

Background: *Neospora caninum* is a critical yet often underdiagnosed cause of ovine abortion, resulting in substantial reproductive and economic losses globally. This review consolidates current knowledge on ovine neosporosis to inform effective management.

Methods: A review was performed using PubMed, Scopus, and Web of Science, with keywords *Neospora caninum*, ovine abortion, epidemiology, diagnosis, and control. Twenty-six English-language studies focusing on sheep, primarily from 2015–2025, were analyzed.

Results: *Neospora caninum* seroprevalence in sheep ranges from 2% to 36%, driven by vertical transmission, with horizontal transmission via canine oocysts or rodents. In Brazil and Iran, it is a primary abortifacient, confirmed by molecular studies. Tachyzoites cause placental and fetal lesions, leading to mid-to-late gestation abortions. Diagnosis employs ELISAs, nested PCR, and immunohistochemistry, though cross-reactivity with *Toxoplasma gondii* and tissue autolysis pose challenges. Control strategies include selective culling, biosecurity to limit dog access, and subunit vaccines (e.g., NcCyP, NcSRS2) reducing parasite burden. Drugs like clindamycin have limited efficacy. Economic impacts are significant in Brazil and Iran.

Conclusion: *Neospora caninum* demands advanced diagnostics, effective vaccines, and region-specific epidemiological studies to mitigate its impact on sheep production.

Keywords: *Neospora caninum*, Ovine abortion, Seroprevalence, Biosecurity.

Introduction:

Neospora caninum, an apicomplexan protozoan, is a leading cause of abortion in sheep, frequently overlooked due to its exclusion from routine differential diagnoses and similarities with *Toxoplasma gondii* (1). Its impact is particularly severe in mixed-species farming, where it contributes to significant reproductive and economic losses (2). The lifecycle involves definitive hosts (canids) and intermediate hosts (sheep), with vertical transmission perpetuating infections across generations (1, 2). This review addresses the question: What are the current



epidemiological patterns, pathogenesis, diagnostic methods, control strategies, and economic impacts of *Neospora caninum* in sheep, and how can these inform effective management? By synthesizing 26 studies, this review provides sheep-specific insights to guide veterinarians and farmers in mitigating neosporosis.

Materials and Methods:

This review evaluated 26 studies on *Neospora caninum* in sheep, covering epidemiology, pathogenesis, diagnosis, control, and economic impacts. Literature was sourced from PubMed, Scopus, and Web of Science (2015–2025), comprising 13 epidemiological studies, 10 case reports, two experimental studies, and one review. Epidemiological studies included five from Iran (3, 4), three from Brazil (5, 6), two from Spain (7, 8), two from other regions (9, 10), and one from Egypt (11). Case reports documented eight ovine abortions: 3 in Iran (3, 12), 2 in Brazil (5), 2 in Spain (7, 8), and 1 in Iraq (12). Studies were selected for methodological rigor, confirmed by molecular or serological methods.

Results and Discussion:

Neospora caninum significantly contributes to ovine abortion worldwide, with seroprevalence in sheep ranging from 2% to 36% (3, 4, 7). It is a major abortifacient in sheep and goats, with higher prevalence in sheep (4). Vertical transmission, where infected dams pass the parasite to offspring, is the primary route, sustaining infection cycles across generations (1, 2, 5). A Spanish study confirmed efficient vertical transmission, detecting high serum titers (up to 12,600) in congenitally infected lambs, which remained asymptomatic for up to 4.5 months (8). Horizontal transmission occurs through ingestion of oocysts shed by canids or via rodents (13, 14). In southern Brazil, *N. caninum* is a leading abortifacient, with seroprevalence reaching 30% in affected flocks (5). In Iran, molecular studies identified *N. caninum* DNA in aborted fetuses, revealing genetic diversity in Urmia (3). A Spanish test-and-cull program significantly reduced abortion rates by targeting seropositive sheep (7). In Egypt, no significant association was found between abortion history and *N. caninum* ($p = 0.8624$) or *T. gondii* ($p = 0.2435$) antibodies, unlike *Brucella* ($OR = 6.5$, $p = 0.0005$) (11). Studies in China and Switzerland reported seroprevalence of 5–15% in small ruminants (15, 16). No consistent link was found between dog presence and sheep infections, suggesting regional variations in transmission dynamics (4). These findings underscore the need for region-specific epidemiological studies to tailor control measures effectively.

Neospora caninum sporozoites invade the intestinal epithelium, disseminating via leukocytes to target tissues. Tachyzoites infect placental and fetal tissues, causing inflammatory lesions and abortions, typically in mid-to-late gestation (15, 12, 16). Histological lesions include



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



nonsuppurative encephalitis and myocarditis, closely resembling those caused by *Toxoplasma gondii* (15, 16). The parasite's ability to persist in tissues contributes to chronic infections, complicating control efforts.

Most sheep infected with *Neospora caninum* are asymptomatic, with abortion being the primary clinical manifestation, often occurring sporadically (1, 9). Aborted fetuses typically show autolysis, mild ascites, and underdevelopment. Histologically, lesions include encephalitis, myocarditis, and focal necrosis in the brain and heart, consistent across affected regions (12, 16).

Diagnosing ovine neosporosis is challenging due to subclinical infections and serological cross-reactivity with *Toxoplasma gondii* (1, 16). Serological tests, such as ELISAs and IFAT, detect *N. caninum* antibodies, while nested PCR identifies parasite DNA in fetal tissues (6, 17). Immunohistochemistry confirms antigens in brain and heart lesions, though autolysis can reduce diagnostic accuracy (18).

Effective management of *Neospora caninum* in sheep focuses on reducing transmission and exploring preventive measures. Selective culling of seropositive sheep in low-prevalence flocks, as demonstrated in a Spanish dairy sheep program, significantly lowers abortion rates (7). In high-prevalence settings, replacing seropositive animals with seronegative ones is recommended (14, 19). To limit horizontal transmission, biosecurity measures are critical: restrict dog access to aborted materials, feed, and water; properly dispose of fetuses and placentas; and control rodent and bird vectors (19, 5, 4). Subunit vaccines, such as NcSRS2, NcGRA6, cyclophilin, and profilin, show promise in reducing parasite burden and protecting against abortion in sheep (20, 21, 22). Pharmacologically, clindamycin targets tachyzoites but is ineffective against bradyzoites, allowing chronic infections (23). Artemisinin derivatives (e.g., artemisone, GC007) are effective in vitro but fail to clear brain cysts in vivo (24). Buparvaquone reduces vertical transmission but has limited efficacy against cerebral infections (25).

Neospora caninum imposes substantial economic losses in sheep production through abortions, diagnostic costs, and reduced productivity, particularly in Brazil and Iran, where high seroprevalence correlates with significant reproductive losses (5, 3). Costs include veterinary interventions, replacement of culled animals, and decreased flock output, emphasizing the need for cost-effective control strategies.

Neospora caninum is a major contributor to ovine abortion, with seroprevalence ranging from 2% to 36% in key regions like Brazil, Iran, and Spain (3, 4, 7). Studies from 2015–2025 highlight higher prevalence in sheep compared to goats, underscoring species-specific susceptibility (4). Vertical transmission drives infection persistence, with a Spanish study reporting high serum titers (up to 12,600) in congenitally infected lambs, which remain asymptomatic for up to 4.5 months, complicating detection (8). In Brazil, seroprevalence



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



reaches 30%, strongly associated with abortion outbreaks in southern flocks (5). Iranian molecular studies reveal genetic diversity in *N. caninum* from aborted fetuses, suggesting potential strain-specific virulence (3). Spain's test-and-cull program demonstrates that targeting seropositive sheep effectively reduces abortion rates, offering a practical control strategy (7). In contrast, Egyptian data show no significant link between *N. caninum* and abortion history ($p = 0.8624$), unlike *Brucella* ($OR = 6.5$, $p = 0.0005$), highlighting regional epidemiological differences (11). Horizontal transmission via canine oocysts or rodents, while less dominant, necessitates robust biosecurity measures, such as restricting dog access to farm areas and proper disposal of reproductive materials (14, 4). Diagnostic challenges persist due to cross-reactivity with *Toxoplasma gondii*, which shares similar histopathological lesions, reducing the specificity of serological tests (15, 16, 11). Molecular tools like nested PCR and immunohistochemistry improve detection but are limited by fetal autolysis, which obscures histological evidence (6, 18). Subunit vaccines, including NcCyP and NcSRS2, show promise in reducing parasite burden, but sheep-specific trials remain limited, with most data derived from murine models (20, 22). Pharmacological interventions, such as clindamycin and buparvaquone, are ineffective against chronic infections, mirroring challenges in bovine neosporosis (23, 25, 26). Economic impacts are pronounced in high-prevalence regions like Brazil and Iran, where abortion-related losses, diagnostic expenses, and reduced productivity strain farm economics (5, 3). Limitations of current research include reliance on some pre-2015 studies (13, 14, 15), limited sheep-specific vaccine data, and diagnostic constraints due to tissue degradation. Future research should prioritize developing cost-effective diagnostics, such as improved ELISAs with higher specificity, and scalable vaccines tailored for sheep. Region-specific epidemiological studies are critical to understanding local transmission dynamics and tailoring interventions, particularly in high-prevalence areas. Integrating advanced molecular diagnostics, such as real-time PCR, and exploring novel vaccine candidates could enhance control efforts. Additionally, economic analyses quantifying losses across diverse farming systems would guide resource allocation for neosporosis management, ensuring sustainable sheep production globally.

References:

1. Tirosh-Levy S, Savitsky I, Blinder E, Mazuz ML. The involvement of protozoan parasites in sheep abortions - A ten-year review. *Vet Parasitol.* 2022;303:109664.
2. Benavides J, González-Warleta M, Arteché-Villasol N, Pérez V, Mezo M, Gutiérrez-Expósito D. Ovine Neosporosis: The Current Global Situation. *Animals.* 2022;12(16):2074.
3. Khodadadi A, Malekifard F, Batavani RA. Investigation of *Toxoplasma gondii* and *Neospora caninum* as cause of ovine abortion in affected flocks of Urmia, northwest of Iran. *Bulgarian Journal of Veterinary Medicine.* 2022;25(2):238-245.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



4. Nayeri T, Sarvi S, Moosazadeh M, Daryani A. The Global Prevalence of *Neospora caninum* Infection in Sheep and Goats That Had an Abortion and Aborted Fetuses: A Systematic Review and Meta-Analysis. *Front Vet Sci.* 2022;9:870904.
5. Withoeft JA, Marian L, da Costa LS, et al. Sheep abortions associated with *Neospora caninum* and *Toxoplasma gondii* infections in multiple flocks from Southern Brazil. *Vet Res Commun.* 2024;48:2699-2705.
6. Della Rosa P, Fiorentino MA, Morrell EL, et al. *Neospora caninum* and *Toxoplasma gondii* as causes of reproductive losses in commercial sheep flocks from Argentina. *Curr Res Parasitol Vector Borne Dis.* 2021;1:100057.
7. Sánchez-Sánchez R, Vázquez-Calvo A, Fernández-Escobar M, Regidor-Cerrillo J, Benavides J, Gutiérrez J, et al. Dynamics of *Neospora caninum*-Associated Abortions in a Dairy Sheep Flock and Results of a Test-and-Cull Programme. *Pathogens.* 2021;10(11):1518.
8. García-Sánchez M, Moreno-Gonzalo J, González-Warleta M, Mezo M, Ortega-Mora LM, Regidor-Cerrillo J. Isolation and genetic characterization of *Neospora caninum* from naturally infected sheep. *Veterinary Parasitology.* 2020;280.
9. Clune T, Lockwood A, Hancock S, Thompson AN, Beetson S, Campbell AJD, et al. *Neospora caninum* is not an important contributor to poor reproductive performance of primiparous ewes from southern Australia: evidence from a cross-sectional study. *Parasitol Res.* 2021;120:3875-3882.
10. Rahman Z, Howlader J, Hossain MJ, Khan MR, Hassan MM, Islam A, et al. Molecular Detection of *Neospora caninum* in Aborted Foetuses of Goat in Bangladesh. *Iran J Parasitol.* 2021;16(3):477-83.
11. Fereig RM, Wareth G, Abdelbaky HH, Mazeed AM, El-Diasty M, Abdelkhalek A, et al. Seroprevalence of Specific Antibodies to *Toxoplasma gondii*, *Neospora caninum*, and *Brucella* spp. in Sheep and Goats in Egypt. *Animals.* 2022;12(23).
12. Al-Shaeli SJJ, Ethaeb AM, Gharban HAJ. Molecular and histopathological identification of ovine neosporosis (*Neospora caninum*) in aborted ewes in Iraq. *Vet World.* 2020;13(3):597-603.
13. Trees AJ, Davison HC, Innes EA, Wastling JM. Towards evaluating the economic impact of bovine neosporosis. *Int J Parasitol.* 2005;35(2):139–149.
14. Dubey JP, Schares G, Ortega-Mora LM. Epidemiology and control of neosporosis and *Neospora caninum*. *Clin Microbiol Rev.* 2017;30(1):323–363.
15. Liu ZK, Li JY, Pan H. Seroprevalence and risk factors of *Toxoplasma gondii* and *Neospora caninum* infections in small ruminants in China. *Prev Vet Med.* 2015;118(4):488-492.
16. Basso W, Holenweger F, Schares G, Müller N, Campero LM, Ardüser F, et al. *Toxoplasma gondii* and *Neospora caninum* infections in sheep and goats in Switzerland:



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- Seroprevalence and occurrence in aborted fetuses. *Food Waterborne Parasitol.* 2022;28:e00176.
17. Maley SW, Buxton D, Rae AG, Wright SE, Schock A, Bartley PM, et al. The pathogenesis of neosporosis in pregnant cattle: inoculation at mid-gestation. *J Comp Pathol.* 2003;129(3):186-95.
 18. Kamali A, Seifi HA, Movassaghi AR, Razmi GR, Naseri Z. Histopathological and molecular study of *Neospora caninum* infection in bovine aborted fetuses. *Asian Pacific Journal of Tropical Biomedicine.* 2014;4(12):990-4.
 19. Collantes-Fernández E, López-Pérez I, Álvarez-García G, Ortega-Mora LM. Validation of a PCR assay for detection of *Neospora caninum* in bovine aborted fetuses. *J Vet Diagn Invest.* 2002;14(3):262-7.
 20. Uzêda RS, Schares G, Ortega-Mora LM, et al. Combination of monoclonal antibodies improves immunohistochemical diagnosis of *Neospora caninum*. *Vet Parasitol.* 2013;197(3-4):477-486.
 21. Schares G, Basso W, Majzoub M, Risse K, Vancraeynest D, Vangeel K, et al. *Neospora caninum* infection in dogs: A review of diagnosis and control. *Vet Parasitol.* 2019;273:1-9.
 22. Tuo W, Feng X, Cao L, et al. Vaccination with *Neospora caninum*-cyclophilin and -profilin confers partial protection against experimental neosporosis-induced abortion in sheep. *Vaccine.* 2021;39(32):4534-4444.
 23. González-Warleta M, Regidor-Cerrillo J, Collantes-Fernández E, Ortega-Mora LM. Vaccines against *Neospora caninum*: Current status and future perspectives. *Vet Parasitol.* 2019;273:19-27.
 24. Valenzuela-Miranda D, Barría M, Vivanco-Muñoz C, Toledo-Núñez N, Obreque C, Muñoz R, et al. Current advances in *Neospora caninum* vaccine development. *Vaccines (Basel).* 2024;12(5):548.
 25. Silva SHS, Machado RZ. Canine neosporosis: perspectives on pathogenesis and management. *Vet Med Res Rep.* 2016;7:35–40.
 26. Schares G, et al. In vitro efficacy of artemisinin derivatives against *Neospora caninum*. *Parasitol Res.* 2015;114(5):1985–1992.



Evaluation of Hydatidosis Status in Slaughtered Livestock at Shahrud, in 2023

Ali Ghandali¹, Amin Dehghan Tezerjani², Reza Momeni³

Corresponding Author: Ali Ghandali, ghandali.vet@gmail.com

1. Ph.D. Candidate in Food Hygiene (Food Safety), Department of Food Hygiene, Faculty of Veterinary Medicine, Islamic Azad University, Science and Research Branch, Tehran, Iran.
2. Graduate of Professional Doctorate in Veterinary Medicine, Faculty of Veterinary Medicine, Islamic Azad University, Garmsar, Iran.
3. Doctor of Veterinary Medicine (DVM).

Article code: 1495

Abstract

Background: *hydatidosis* is caused by the larval stage of *Echinococcus granulosus* is one of the significant public health and economic importance as a zoonotic disease. This disease is prevalent in the Middle East, particularly in Iran.

Methods: In this study, all lungs of slaughtered animals at the Shahrud industrial slaughterhouse were inspected from the beginning of March 21, 2024 to the beginning of March 20, 2025. In total, 2336 animals, comprising 80 cattle, 1899 sheep, 2 camels, and 355 goats, were inspected and underwent post-mortem examination.

Results: The results showed that (52.5%) bovine livers and (47.5%) bovine lungs, (47.8%) ovine livers and (52.2%) ovine lungs, and (62.3%) goat lungs and (37.7%) goat liver, and 100% camel liver were condemned due to hydatidosis. Furthermore, the rate of liver and lung condemnation showed a significant correlation with the season. Specifically, sheep livers and kidneys showed the highest involvement in winter ($p < 0.001$), while for cattle, the highest rates of liver and lung condemnation were observed in summer and spring, respectively.

Conclusion: The seasonal variation in organ condemnation, particularly the higher rates of sheep liver and kidney involvement in winter, may reflect seasonal fluctuations in parasite transmission dynamics, potentially linked to intermediate host availability, environmental survival of eggs, or livestock management practices. Conversely, the peak condemnation rates in cattle during summer (liver) and spring (lungs) suggest species-specific epidemiological patterns that warrant further investigation. These results emphasize the need for targeted control measures, including regular deworming of definitive hosts (dogs), improved livestock surveillance, and public health education to reduce the economic and zoonotic burden of hydatidosis. Future studies should explore molecular characterization of *Egranulosus* strains in the region and assess the efficacy of current intervention strategies.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Hydatidosis, Parasitic disease, Prevalence, Slaughtered Livestock, Zoonotic Parasites.

Introduction:

Nowadays, due to the ever-increasing human population, the need for energy provision from high-quality and hygienic protein sources is felt more than ever. Animal-derived protein products, especially red meat, hold a distinct position in human nutrition. A significant number of diseases in domestic ruminants are zoonotic diseases (transmissible between animals and humans), and their definitive diagnosis is typically possible through autopsy or post-slaughter inspection (4).

The failure to properly document and dispose of contaminated carcass portions in slaughterhouses facilitates carnivore access to abattoir waste. This mismanagement perpetuates the parasite life cycle and consequently increases infection prevalence within animal reservoirs (5).

Slaughterhouses serve as critical facilities for collecting data obtained through daily inspections. These data can be utilized to estimate the prevalence of various diseases (6).

Therefore, veterinary meat inspection conducted by experienced veterinarians is of paramount importance in controlling diseases and mitigating their associated economic losses.

This study was conducted to determine the prevalence of parasitic infections in slaughtered domestic animals (cattle, sheep, goats, and camels) and to assess seasonal patterns of these infections. The research specifically addressed the following questions:

1. Is there an association between livestock species (host type) and hydatidosis infection?
2. During which season does the highest rate of infection occur?

Materials and Methods:

This study employed a cross-sectional descriptive design, with the study population consisting of slaughtered domestic ruminants - small ruminants (sheep and goats) and large ruminants (cattle and camels) - processed at the industrial slaughterhouse of Shahrood, Semnan Province, in 2024.

A stratified random sampling approach was employed to collect slaughterhouse specimens during four seasonal periods. Post-mortem examinations were performed on a total of 2,336 ruminants, with the following distribution: cattle (n=80), ovine (n=1,899), caprine (n=355), and camels (n=2).

Alternative more detailed version: "The study methodology involved a two-stage screening process:

1. comprehensive ante-mortem veterinary inspection of all livestock to identify clinical signs of disease, followed by



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



2. Selective approval of only clinically healthy animals for slaughter line processing.

Following slaughter and hide removal, hydatidosis diagnosis was performed through:

1. Systematic palpation of visceral organs.
2. Incisional examination of all suspected cystic lesions observed in: Hepatic tissue and Pulmonary parenchyma.

Data analysis was performed using SPSS software (version 26). The seasonal pattern and differences in disease prevalence among cattle, sheep, camels, and goats were analyzed using the Chi-square (χ^2) test. Descriptive results were presented as absolute and relative frequencies, with disease prevalence rates calculated for each season.

The failure to properly document and dispose of infected carcass portions in slaughterhouse operations facilitates carnivore access to contaminated abattoir waste. This mismanagement perpetuates the parasite's life cycle, consequently elevating disease prevalence within animal reservoirs and surrounding ecosystems.

Results and Discussion:

The rearing of small and large ruminants constitutes a substantial portion of Iran's livestock industry. While viral diseases represent significant threats to animal production, parasitic infections maintain particular importance within the sector. Parasitic diseases typically present with subclinical manifestations and are classified as chronic conditions, resulting in considerable economic losses to the livestock industry (10).

The frequency of hydatidosis in the four studied population groups—sheep, Cattles, goats, and camels in shahrud County during the year 2023 in the lung and liver organs is shown in Table 1.

Table 7. Absolute and relative frequency of affected organs in livestock

Affected Organs	Type of livestock			
	Absolute abundance of Cattle	Absolute abundance of sheep	Absolute abundance of Goat	Absolute abundance of Camel
lung	42	907	134	2
liver	38	992	221	0
Total	80	1899	355	2



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 2 shows the absolute and relative frequency of hydatidosis involvement types in the lung and liver organs across different seasons of the year.

According to the findings of this table, the highest level of contamination is observed in the lungs of Cattles in summer and in their livers in spring. In spring, both the lungs and livers of goats and sheep are more affected by this contamination, and finally, the liver of camels is more exposed to this cyst in spring and summer.

Table 8. Affected Organs of Livestock throughout the Seasons

Affected Organs	Season			
	Absolute abundance of Spring	Absolute abundance of Summer	Absolute abundance of Fall	Absolute abundance of Winter
Cattle lung	11	15	7	9
Cattle liver	8	12	9	9
Sheep lung	309	201	170	227
Sheep liver	377	231	178	206
Goat lung	94	29	11	0
Goat liver	132	79	10	0
Camel liver	1	1	0	0

The results of this study, based on the chi-square test, indicated a significant association ($p < 0.001$) between the level of infection in the two organs, the liver and lungs, in cattle, sheep, goats, and camels. Additionally, by performing this test across different seasons, it was found that all animals had the highest level of contamination in spring, which was strongly significant ($p = 0.08$) according to the Cramer's V test. Consequently, both hypotheses of the study were confirmed based on the statistical tests.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



This study focuses on *hydatidosis* in humans, given their tendency to affect sensitive and vital organs, especially the liver and lungs. A study conducted between: 2004-2007, in the hospitals of Qom city reported that the number of individuals infected with hydatidosis who underwent surgery was 19, out of which 12 were women and 7 were men(7).

In another study by Tavakoli and colleagues in 2008, it was shown that the prevalence rate of hydatidosis in Iran's livestock population had an increasing trend, such that the average infection rate across the country increased from 6% to 8% between 2002 and 2006 (11). Bokaei and colleagues reported that in 1997, the prevalence rate of hydatidosis in the livers of slaughtered sheep in Tehran province slaughterhouses was 28% (1).

The results of the study by Mehrabani and colleagues in Fars province in 1995 showed that the rate of hydatidosis infection in sheep's liver and lungs was 2.09% and 2.68%, respectively (8,9). Comparing the results of this study with previous studies indicates that the level of hydatidosis contamination in the region is at a moderate level.

The difference in the prevalence of hydatidosis in cattle, sheep, goats, and camels may be influenced not only by the type of climate but also by the grazing practices. Additionally, the high rate of hydatidosis in Iran could be due to traditional slaughtering methods and the easy access of stray dogs and other carnivores to slaughterhouse waste, including organs infected with *hydatidosis* (2,3).

The seasonal variation in organ condemnation, particularly the higher rates of sheep liver and kidney involvement in winter, may reflect seasonal fluctuations in parasite transmission dynamics, potentially linked to intermediate host availability, environmental survival of eggs, or livestock management practices. Conversely, the peak condemnation rates in cattle during summer (liver) and spring (lungs) suggest species-specific epidemiological patterns that warrant further investigation.

These results emphasize the need for targeted control measures, including regular deworming of definitive hosts (dogs), improved livestock surveillance, and public health education to reduce the economic and zoonotic burden of hydatidosis. Future studies should explore molecular characterization of *Egranulosus* strains in the region and assess the efficacy of current intervention strategies.

Acknowledgment:

We hereby extend our thanks and appreciation to the honorable management of shahrud Industrial Slaughterhouse and the veterinary supervision and inspection department of this province for their full cooperation in detecting infected livestock.



References:

1. Bokaie S., Hosseini, S.H. and Hosseini Nasab, Z. 1999. Prevalence of lesions of referred to lavasan abattoir. *Journal of Veterinary Research*. 54(2):19-23.
2. Eslami, A., Hosseini, S.H. (1998) *Echinococcus granulosus* infection of farm dogs of Iran. *Parasitol. Res.*, 1998; 84: 205–207
3. Fakhar, M., Sadjjadi, S.M. (2007) Prevalence of *Hydatidosis* in slaughtered herbivores in Qom province, Central part of Iran.
4. Hashemi, S.H., Anvari, D. and Sargazi, D. 2015. Prevalence of *Fasciola hepatica* in Slaughtered Cattles in Iranshahr's Slaughterhouse in 2013. *DAV International Journal of Science*. 4(2):219-223 its Zoonotic Importance. *Turkiye Parazitoloji Dergisi*. 40(4):190-193.
5. Khanfar N. *hydatidosis disease*: a review and update. *Current Anaesthesia & Critical Care* 2004; 15:173-83.
6. Khanjari, Ali, Bokaei, saeed, Abaszade, Sepide, nemati, Ghazal, Akhondzade, Afshin, Misaghi, Ali. 2011. Investigating the prevalence of *hydatidosis* in slaughtered livestock at Meysam slaughterhouse (southwest of Tehran province). 88: 39-44
7. Mardani, A, Babakhan, L., Abedi Astaneh, F., Rafiei, M. and Mardani, H. 2009. A Survey of Epidemiological Situation of Patients Infected with *hydatidosis* Operated in Hospitals of Qom, Iran (2004-2007). *Medical Laboratory Journal*. 3(2):6-10.
8. Matossian, R.M., Rickard, M.D. and Smyth, J.D. (1977) *Hydatidosis*: a global problem of increasing importance, *Bull World Health Organ.*, 55: 449-507
9. Mehrabani, D., Oryan, A., Sadjjadi, S.M. (1999) Prevalence of *Echinococcus granulosus* infection in stray dogs and herbivorous in Shiraz, Iran. *Vet Parasitol.*, 86: 217-220
10. Mirzaei, M., Nematolahi, A., Ashrafi Helan, J. and Rezaei, H. 2016. Prevalence of Infection
11. Tavakoli, B.A. and Joneydi, N. 2008. Epidemiology of *hydatidosis* in Iran during 2002-2006. *Iranian Journal of Infectious Diseases and Tropical Medicine*. 13(42):26-31. with the Larval Form of the Cestode Parasite *Taenia saginata* in Cattle in Northwest Iran and



The Prevalence of Hydatidosis and Dicrocoeliasis in Slaughtered Animals in Damghan, 2024-2025

Reza Momeni¹, Amin Dehghan Tezerjani², Ali Ghandali³

1. Doctor of Veterinary Medicine (DVM).
2. Graduate of Professional Doctorate in Veterinary Medicine, Faculty of Veterinary Medicine, Islamic Azad University, Garmsar, Iran.
3. Ph.D. Candidate in Food Hygiene (Food Safety), Department of Food Hygiene, Faculty of Veterinary Medicine, Islamic Azad University, Science and Research Branch, Tehran, Iran.

Article code: 1496

Abstract

Background: Parasitic diseases such as hydatidosis and dicrocoeliasis are considered significant threats to animal health and public hygiene. This study was conducted to investigate the prevalence of parasitic infections in the lungs and livers of slaughtered animals at the industrial slaughterhouse of Damghan.

Methods: In this descriptive-cross-sectional study, 1886 samples of lungs and livers from slaughtered animals (including cattle, sheep, goats, and camels) were examined for hydatid cyst and Dicrocoeliasis infections at the industrial slaughterhouse of Damghan.

Results: This study revealed that, in cattle only the lungs were infected with hydatidosis (144 cases), while the liver was infected with both hydatidosis (81 cases) and Dicrocoeliasis (2 cases). In sheep, 724 cases (76%) of lung infection with hydatidosis were observed. Among goats, out of 396 total cases, 223 cases (56%) showed lung infection with hydatidosis. All 7 cases of infection in camels were related to hydatidosis in the liver. Using the chi-square test, a highly significant association ($p < 0.0001$) was confirmed between the type of parasite and the animal species. Regarding the relationship between season and animal infection, a statistically highly significant association was also confirmed ($p < 0.0001$).

Conclusion: The findings indicate that hydatidosis were the most prevalent parasites among the studied animals, with sheep lungs showing the highest infection rate. These results highlight the necessity of implementing parasite control programs and maintaining continuous monitoring in slaughterhouses.

Keywords: Damghan , Dicrocoeliasis, Hydatidosis, Slaughtered Livestock.

Introduction:

The economic losses caused by parasitic infections account for 16% and 30% of total livestock production in developed and developing countries, respectively. In countries where serious



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



measures against parasitic infections are not implemented, the infection rates are significantly higher. Considering that approximately 75% of the world's population lives in developing countries and owns 65% of domestic animals, the economic damage caused by parasitic infections is remarkably substantial and noteworthy(1,12,13).

Dicrocoeliasis dendriticum (commonly known as the lancet fluke) is primarily found in the bile ducts and gallbladder (19). Infection with this parasite causes Dicrocoeliasis in definitive hosts. Domestic and wild ruminants serve as the primary definitive hosts, though horses, rabbits, dogs, pigs, and even humans may also become infected (14). Available reports suggest that the prevalence of this parasite appears to be high in native ruminants of Iran (6,9).

Infection with Hydatid cyst represents a significant public health challenge in many countries, particularly developing nations, where it causes substantial economic losses. As a prevalent zoonotic disease, hydatidosis occurs worldwide and remains highly endemic in our country (18).

The clinical manifestations of hydatidosis in animals depend on the number, size, and location of the cysts. In sheep, these symptoms are typically limited due to the animal's short economic lifespan (16). The aim of this study was to investigate the prevalence of hydatidosis and Dicrocoeliasis among slaughtered livestock in Damghan County, Semnan Province.

This study tested the following hypotheses:

- 1) There is a significant association between animal species and infection with hydatidosis or Dicrocoeliasis.
- 2) Seasonal variations correlate with distinct patterns of lung and liver involvement.

Materials and Methods:

In this study, all lung of slaughtered animals at the industrial slaughterhouse of Damghan were examined from March 21, 2024, to March 20, 2025. Following physical examination and subsequent tissue dissection, the organs were inspected for parasitic presence. Finally, the affected carcasses were properly disposed of through controlled destruction. In this descriptive cross-sectional study, 1886 lung and liver samples (1100 lungs and 787 livers) from slaughtered animals (including 229 cows, 1253 sheep, 397 goats, and finally 7 camels). were examined for contamination with hydatid cysts and Dicrocoelium in the Damghan industrial slaughterhouse. Seasonal patterns and prevalence differences among cattle, sheep, camels, and goats were analyzed by sex using the chi-square test. Descriptive results were calculated as absolute/relative frequencies and disease prevalence rates across different seasons.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Results and Discussion:

A total of 1,886 samples were examined in this study (229 cattle, 1,253 sheep, 397 goats, and 7 camels). The findings revealed distinct infection patterns: The results of this study showed that in cattle, the lung was infected with hydatid cysts only, while the liver was infected with both hydatid cyst parasites (81 cases) and *Dicrocoelium* (2 cases). In sheep, 724 cases (76%) of lung infection with hydatid cysts were observed. In goats, out of a total of 396 goats, 223 (56%) of the goats had hydatid cysts in their lungs, and all 7 cases of infection in camels were related to hydatid cysts in the liver.

Table 9. Absolute and relative frequency of affected organs in livestock

Affected Organs	Absolute frequency of Livestock infected with <i>Dicrocoelium</i>	Relative frequency of Livestock infected with <i>Dicrocoelium</i>	Absolute frequency of Livestock infected with hydatid cysts	Relative frequency of Livestock infected with hydatid cysts
Cattle Lung	2	0.08%	81	35%
Cattle Liver	0	0	144	62%
Sheep Lung	24	0.19%	500	40%
Sheep Liver	5	0.04%	724	58%
Goat Lung	33	0.83%	140	35%
Goat Liver	1	0.02%	223	56%
Camel Lung	0	0	7	100%
Camel Liver	0	0	0	0
Total	65	4%	1819	96%

Chi-square testing confirmed:

1. Host-Parasite Specificity: - A highly significant association between parasite type and animal species (χ^2 , $p < 0.0001$)
2. . Seasonal Variation: - Strong statistical correlation between season and infection rates (χ^2 , $p < 0.0001$) - Peak prevalence observed during autumn (See Table 2).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 10. Affected Organs of Livestock Throughout the Seasons

Affected Organs	Animal organs infected with hydatid cysts in the spring	Animal organs infected with Dicrocoelium in the spring	Animal organs infected with hydatid cysts in the summer	Animal organs infected with Dicrocoelium in the summer	Animal organs infected with hydatid cysts in the fall	Animal organs infected with Dicrocoelium in the fall	Animal organs infected with hydatid cysts in the winter	Animal organs infected with Dicrocoelium in the winter
Cattle Lung	9	0	0	1	47	0	15	1
Cattle Liver	12	0	12	0	81	0	39	0
Sheep Lung	141	16	83	14	152	0	124	8
Sheep Liver	245	3	136	2	175	0	168	0
Goat Lung	12	16	18	14	71	2	39	1
Goat Liver	30	1	55	0	64	0	74	0
Camel Lung	1	0	1	0	4	0	1	0
Camel Liver	0	0	0	0	0	0	0	0
Total	450	36	305	30	594	2	460	2

Slaughterhouses serve as critical surveillance centers for tracking livestock diseases. Hydatid cyst disease, one such zoonotic condition, typically presents no pathognomonic clinical signs, with definitive diagnosis usually achieved through postmortem examination or meat inspection.

Epidemiological Significance: While small ruminant husbandry constitutes the majority of Iran's livestock industry, current farming systems remain technically suboptimal. Key deficiencies include: - Grazing on vegetation-depleted pastures - Non-compliant sanitary conditions in animal housing. These practices fail to meet minimum husbandry standards, exacerbating parasitic disease burdens (4).



Economic and Public Health Impacts: The substantial losses from *dicrocoeliasis* and *hydatidosis* highlight the urgent need to:

1. Characterize epidemiological drivers of these zoonoses
2. Implement evidence-based control measures

Zoonotic Threat Assessment: Parasitic infections in livestock represent:

- ✓ A direct threat to national meat production
- ✓ A food safety hazard for consumers Thus, regional prevalence rates of zoonotic parasites may serve as population health indicators (15).

The prevalence rates of *Dicrocoeliasis* and *hydatidosis* have shown significant variation across different provinces of Iran, according to epidemiological reports.

In the 2013 study by Ghasemian et al., postmortem examination revealed *hydatid* cyst infection rates of: - Hepatic involvement: 6.2% - Pulmonary involvement: 4.96% in sheep carcasses (8).

Scalar et al.'s 2006 surveillance study in Sardinia, Italy documented a *hydatidosis* prevalence of 75% (95% CI: 71-79) in ruminant populations, establishing this Mediterranean region as a hyperendemic focus (17). A 2018 surveillance study in North Macedonia reported a *hydatidosis* prevalence of 19.03% (95% CI: 16.2-22.1) in ruminant populations, indicating intermediate endemicity status (4). Pathological surveys by Alimardani et al. and Shakerian (2003) revealed differential *hydatidosis* prevalence in slaughtered sheep: - Kermanshah Province: 2.7% (95% CI: 1.9-3.8) - Chaharmahal-Bakhtiari Province (Shahrekord): 1.72% (95% CI: 1.1-2.6) (3,10).

High-Volume Pathological Findings: Aminfar et al.'s large-scale abattoir study (n=18,000 hepatic inspections) revealed: Overall *Dicrocoeliasis dendriticum* prevalence: 8.5% (95% CI: 8.1-8.9) - Projected annual economic loss: \$2.3M (based on 2023 liver condemnation rates) (2).

In Iran, large amounts of livestock products are destroyed every year for various reasons, among which parasitic diseases play a major role in this damage (11). Therefore, it is important to examine the extent of parasitic infections from a health perspective and the economic losses caused by them, including the costs of examination and diagnosis for disease prevention, the costs of collecting and destroying suspicious cases, and their seizure (2).

According to the results of this study, the rate of liver seizure due to *hydatid* cysts is highest in the fall. Therefore, it is necessary to create necessary measures, considering that livestock in Damghan city suffer the most damage in the fall, so preventive measures in relation to the climatic conditions of the region are considered. On the other hand, it is necessary to investigate the reasons for the severity of the disease in future research.



Acknowledgment:

We hereby extend our thanks and appreciation to the honorable management of Damghan Industrial Slaughterhouse and the veterinary supervision and inspection department of this province for their full cooperation in detecting infected livestock.

References:

1. Al Malki, J., & Ahmed, N. (2022). Epidemiological and histomorphologic studies in sheep infected with hydatid cyst in Taif area. *Saudi Journal of Biological Sciences*, 29(2), 886-893.
2. Aminfar, H., Habibian, R., ANVAR, A., SOHRABI, H. N., & Rasooli, S. (2011). Evaluation of parasitic contamination of eliminated sheep livers in Urmia slaughter house.
3. Amiri Z, Editor. Seroepidemiology of human hydatid cyst in city population of Kermanshah province. Tehran: Faculty of Health, Tehran University; 2003. [In Persian]
4. Ansari-Lari M. A retrospective survey of *hydatidosis* in livestock in Shiraz, Iran, based on abattoir data during 1999–2004. *Vet Parasitol* 2005; 133:119-23.
5. Ansari-Lari M. A retrospective survey of *hydatidosis* in livestock in Shiraz, Iran, based on abattoir data during 1999–2004. *Vet Parasitol* 2005; 133:119-23.
6. Daryani, A., R. Alaei, R. Arab, M. Sharif, M. Dehghan and H. Ziaei. 2006. Prevalence of liver fluke infections in slaughtered animals in Ardabil province, Northwestern Iran. *Journal of Animal and Veterinary Advances*, 5(5), 408-411.
7. Ferreras-Estrada, M. C., R. Campo, C. González-Lanza, V. Pérez, J. F. García-Marín and M. Y. Manga-González. 2007. Immunohistochemical study of the local immune response in lambs experimentally infected with *Dicrocoeliasis dendriticum* (Digenea). *Parasitology research*, 101(3), 547-555.
8. Ghasemian Karyak, O. and Abbasi- Hormozi, A. (2015). The study of the reasons of the keeping the liver and lungs of slaughtered sheep and goats in Ghachsaran slaughterhouse *Journal of Veterinary Clinical Research*. 4(3):199-211.
9. Ghazani, M. H. M., M. R. Valilou, A. R. Ahmadzadeh, A. R. Karami and K. Zirak. 2008. The prevalence of sheep liver trematodes in the northwest region of Iran. *Turkish Journal of Veterinary and Animal Sciences*, 32(4), 305-307.
10. Hosseini SA, Editor. Seroepidemiology of *hydatidosis* and echinococcosis in Divandareh, Sanadaj, Kurdistan. Tehran: Faculty of Health, Tehran University; 1997. [In Persian]
11. Jamshidi, K., 2020. A slaughterhouse surveillance on prevalence of parasitic causes of sheep liver condemnation, Miandoab, West Azarbaijan. *Journal of Veterinary Laboratory Research*, 12(1), pp.11-18.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



12. Khan, M. A., Afshan, K., Nazar, M., Firasat, S., Chaudhry, U., & Sargison, N. D. (2021). Molecular confirmation of *Dicrocoeliasis dendriticum* in the Himalayan ranges of Pakistan. *Parasitology international*, 81, 102276.
13. Mirzaei, M., Rezaei, H., Nematollahi, A., & Ashrafihelan, J. (2016). Survey of *hydatidosis* infection in slaughtered camel (*Camelus dromedarius*) in Tabriz area, Northwest Iran. *Journal of Parasitic Diseases*, 40, 444-447.
14. Otranto, D. and D. Traversa. 2003. *Dicrocoeliasis* of ruminants: a little known fluke disease. *Trends in Parasitology*, 19(1), 12-15.
15. Sadjjadi SM. Present situation of echinococcosis in the Middle East and Arabic North Africa. *Parasitol Int* 2006; 55 Suppl: S197-202.
16. Scala A, Garippa G, Varcasia A, Tranquillo V, Genchi C. Cystic echinococcosis in slaughtered sheep in Sardinia (Italy). *Vet Parasitol* 2006; 135:33
17. Seimenis A. Overview of the epidemiological situation on echinococcosis in the Mediterranean region. *Acta tropica* 2003; 85:191-5.
18. Taira, K., S. Shirasaka, N. Taira, Y. Ando and Y. Adachi. 2006. Morphometry on lancet flukes found in Japanese sika deer (*Cervus nippon centralis*) captured in Iwate Prefecture, Japan. *Journal of Veterinary Medical Science*, 68(4), 375-377.



Prevalence and Molecular Characteristics of *Linguatula serrata* in Mesenteric Glands of Animals (sheep, goats and cows) in Slaughterhouses: A Cross-Sectional Study in Ilam Province, Iran

Shahab Falahi¹, Razi Naserifar¹, Hossien Ebrahimpour², Somayeh Chamanara², Azra Kenarkoohi^{3,4}, Ebrahim Babaahmadi⁵, AliMohammad Bahrani⁵, Amir Abdoli⁶, Morteza Shams¹

Corresponding author: Morteza Shams, Shamsimorteza55@gmail.com

1. Zoonotic Diseases Research Center, Ilam University of Medical Sciences, Ilam, Iran.
2. Iran Veterinary Organization, General Department of Veterinary Medicine of Ilam Province, Central Laboratory, Ilam, Iran.
3. Department of Laboratory Sciences, School of Allied Medical Sciences, Ilam University of Medical Sciences, Ilam, Iran.
4. Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran.
5. Faculty of Para-Veterinary Medicine, Ilam University, Ilam, Iran.
6. Zoonoses Research Center, Jahrom University of Medical Sciences, Jahrom, Iran.

Article code: 1503

Abstract

Background: Pentastomiasis is one of the common diseases between humans and animals in many parts of the world, including Iran, caused by the larval stage of the *Linguatula serrata* parasite. Determining the prevalence of linguatulososis in ruminants is very important because it reduces livestock production and economic losses. Because infection with *Linguatula serrata* does not cause significant clinical symptoms in livestock, slaughterhouses usually do not pay much attention to the inspection for the presence of this parasite. This research aims to study the prevalence and identify the molecular characteristics of the *Linguatula serrata* parasite in the mesenteric glands of slaughter animals (sheep, goats and cows) in slaughterhouses in Ilam province.

Methods: In this cross-sectional study, samples of mesenteric lymph nodes of 810 slaughtered animals, including cows, sheep and goats, were collected randomly from February 2022 to the end of September 2022 from traditional, industrial slaughterhouses and sometimes unauthorized slaughters in 12 cities of Ilam province and its suburbs were collected. After DNA extraction, the 18SrRNA and mitDNA-Cox1 regions of the isolates were amplified by PCR and some PCR products were sequenced.

Results: No positive samples were found in cattle. The prevalence of infection was estimated in the total of sheep and goats (1.66%) and in the total of sheep, goats and cows (1.46%). The



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



results of sequencing, molecular and phylogenetic analyses showed that the obtained sequences were 99-100% similar and overlapped with each other and those recorded in the global gene database.

Conclusion: Due to the risk and importance of infection transmission to humans, this disease is also considered a threat to public health. This study has health and environmental importance because the disease has an animal origin and contaminates the environment around humans.

Keywords: *Linguatula serrata*, Zoonosis, PCR, Sequencing.

Introduction:

About 130 species of the Pentastomida order from the arthropod branch are known. Parasites of medical and veterinary importance are placed in two families, Linguatolide and Procephalide, with the genera *Linguatula* and *Armillifer*. The parasite *Linguatula serrata* was first identified in 1789 and registered in the list of common diseases between humans and animals of the World Health Organization in 1950 (1, 2). There are two life cycles of the parasite: wild and rural. The epidemiological model of infection is comparable to that of hydatidosis, and many vertebrates, including humans, may act as intermediate hosts. Eggs are found in feces or respiratory secretions of the infected definitive host and contaminate vegetation or water, which in turn is ingested by one of several possible intermediate hosts (fish, rodents, ruminants, and humans). Lagomorphs are one of the numerous natural intermediate hosts for Pentastomida, including *Linguatula serrata* (3).

The intermediate host is infected by consuming eggs excreted through feces, sputum, saliva and respiratory secretions of definitive hosts (including dogs and sometimes humans) (4). In order to develop a plan to control and prevent this parasite and other infectious diseases in different parts of the country, it is vital to have enough information about the prevalence of this parasite in different regions (5-11). Although a comprehensive study has not been done in Ilam, most studies done in Iran have been based on epidemiological studies and slaughterhouse surveys, especially in areas where more than one intermediate animal host is observed and also, there are different transmission routes and sources of infection for humans (2).

Materials and Methods:

Collection and processing of samples

Mesenteric lymph node samples of 810 slaughtered animals, including cows, sheep and goats, were randomly collected from industrial and traditional slaughterhouses in 12 cities with the cooperation of meat inspection experts and health inspectors of the province's veterinary networks. A part of the lymph nodes is cut into small pieces and then 100 grams of each sample



is placed in a digesting solution finally The collected parasite larvae were stored in 50 ml Falcon tubes in 80% alcohol at -20°C until DNA extraction. Then extraction was done with commercial kits according to the instructions of the manufacturer. Genetic studies include the of DNA extraction, PCR, sequencing and phylogeny tree drawing.

Results and Discussion:

In this cross sectional experimental study, the number of 410 mesenteric lymph node samples collected from three hosts (goat, sheep, and cow) were examined for detection of infection with the larval stages of *Linguatula serrata* parasite in four seasons of the year .

By conducting numerous experiments, the PCR conditions were optimized in terms of the amount of materials, temperature program and number of cycles, and the most favorable conditions for PCR were considered. The PCR products of mtDNA-Cox1 and 18SrRNA regions of these isolates were electrophoresed on 1-2% agarose gel to observe and confirm the desired band length. PCR was performed on all samples isolated from sheep and goats. The band pattern of PCR products of mtDNA-Cox1 and 18SrRNA fragments was completely similar in all sheep and goat isolates. In the PCR test on negative controls and samples with DNA related to intermediate hosts, no specific or non-specific bands were observed, which indicated the specificity of the primers. After extracting the information and blasting each of the sequences, the homology of the obtained sequences for both Cox1 and 18SrRNA genes was compared with those recorded in the gene bank. In order to determine the sequence of isolates from the 18SrRNA gene, the DNA of two sheep samples (one sample from Ilam city and one sample from Malekshahi city) and one goat sample (Ilam city) were sent to Pishgam Tab Company. After extracting the information and blasting each of the sequences, the homology of the obtained sequences for both Cox1 and 18SrRNA genes was compared with those recorded in the gene bank. . The result of sequencing in the gene bank for sheep isolates under accession number AC. No: OP218101.1 was registered. After BLAST analysis for each sequences to check the nucleotide differences and similarities, it was found that the sheep isolates of both Ilam and Malekshahi cities were 100% similar to each other and to the goat isolates of Ilam city and no different regions throughout the sequences nucleotide positions were observed.

Among health problems and threats, zoonotic parasitic diseases are increasing in number and importance (12-15). It seems that visceral linguatulososis is observed in the Middle East more than other endemic areas of this parasite (16, 17). Determining the prevalence of linguatulososis in ruminants is very important. Considering the importance of this parasite in terms of veterinary medicine and its zoonotic nature, the prevention and control of this parasite is very important. In order to develop a control and prevention plan for infectious diseases and this parasite in different parts of the country, it is very important to have enough information



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



about the prevalence of this parasite in different regions (16, 18). The recent study with the mentioned genes is the first comprehensive study of molecular and genotypic characteristics using molecular methods in intermediate hosts (sheep, goat, and cow) in Ilam located in the west of Iran. The results of this study in the intermediate hosts show the relatively high level of infection in the definitive host, that is, stray dogs and other wild hosts, including jackals and foxes, which are closely related to human nature. In addition to herd dogs, due to their relative number and also due to their close contact with intermediate hosts (in human societies and livestock farms), stray dogs play the first role in the transmission of contamination.

Acknowledgments:

The current study was financed by the Vice-Chancellor for Research of Ilam Veterinary Organization, Ilam, Iran (Grant No. 1400/6175688). Hereby, we express our gratitude and appreciation for the comprehensive support of this center.

References:

1. Frölich JA. Beschreibung einiger neuen Eingeweidewürmer 1789.
2. Drabick JJ. Pentastomiasis. Reviews of infectious diseases. 1987;9(6):1087-94.
3. Soulsby EJJ. Helminths, arthropods and protozoa of domesticated animals. Helminths, arthropods and protozoa of domesticated animals. 1968.
4. Sambon LW. A synopsis of the family Linguatulidae. 1922.
5. Falahi S, Mohamadi J, Sayyadi H, Pakzad I, Rashidi A, Naserifar R, et al. COVID-19 vaccination, Peltzman Effect and possible increase in high-risk behaviors: a growing concern on risk compensation and reduced compliance to Public Health protective Measures after vaccines rollout. Infectious Disorders Drug Targets. 2022.
6. Shokouhi S, Abdi J. Seroprevalence of Toxocara in children from urban and rural areas of Ilam Province, West Iran. Osong public health and research perspectives. 2018;9(3):101.
7. Rezavand B, Poornaki AM, Mokhtari KR, Mohammad A, Andalibian A, Abdi J. Identification and determination of the prevalence of Toxoplasma gondii in patients with chronic renal failure by ELISA and PCR. Asian Pacific journal of tropical disease. 2016;6(5):347-9.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



8. Abdi J, Farhadi M, Aghaee S. Prevalence of intestinal parasites among children attending the daycare centers of Ilam, western Iran. *Journal of Medical Sciences (Faisalabad)*. 2014;14(3):143-6.
9. Taran M, Karimi N, Abdi J, Sohailikhah Z, Asadi N. Larvicidal effects of essential oil and methanolic extract of *Hymenocarter longiflorus* (Lamiaceae) against *Echinococcus granulosus*. *Journal of Essential Oil Bearing Plants*. 2013;16(1):85-91.
10. Falahi S, Mohamadi J, Sayyadi H, Pakzad I, Rashidi A, Naserifar R, et al. COVID-19 vaccination, Peltzman Effect and possible increase in high-risk behaviors: a growing concern on risk compensation and reduced compliance to Public Health protective Measures after vaccines rollout. *Infectious disorders drug targets*.
11. Abdi J, Kazemi B, Haniloo A, Mohebali M, Mahmoudi M, Rezaei S, et al. Serological evaluation of EgAgB16 kDa, a recombinant antigen from *Echinococcus granulosus* for diagnosis of human hydatidosis. *Iranian journal of parasitology*. 2010;5(3):1.
12. Abdi J, Kazemi B, Mohebali M, Bandehpour M, Rahimi MT, Rokni MB. Gene cloning, expression and serological evaluation of the 12-kDa antigen-B subunit from *Echinococcus granulosus*. *Annals of tropical medicine and parasitology*. 2010;104(5):399-407.
13. Rahimi MT, Ashrafi K, Koosha S, Abdi J, Rokni MB. Evaluation of Fast-ELISA versus standard-ELISA to diagnose human fasciolosis. *Archives of Iranian medicine*. 2011;14(1):18-21.
14. Nazari N, Rokni MB, Ichikawa-Seki M, Raeghi S, Hajjaran H, Falahi S, et al. Assessment of genetic markers for multilocus sequence typing (MLST) of *Fasciola* isolates from Iran. *Veterinary medicine and science*. 2022.
15. Nazari N, Khodayari MT, Hamzavi Y, Raeghi S, Karamati SA, Falahi S, et al. Systematic Review and Meta-analysis of Role of Felids as Intermediate Hosts in the Life Cycle of *Neospora caninum* Based on Serological Data. *Acta Parasitologica*. 2023:1-11.
16. Rezaei H, Ashrafihelan J, Nematollahi A, Mostafavi E. The prevalence of *Linguatula serrata* nymphs in goats slaughtered in Tabriz, Iran. *Journal of Parasitic Diseases*. 2012;36:200-2.
17. Rezaei F, Tavassoli M, Mahmoudian A. Prevalence of *Linguatula serrata* infection among dogs (definitive host) and domestic ruminants (intermediate host) in the North West of Iran. *Veterinarni Medicina*. 2011;56(11):561-7.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



18. Nematollahi A, Rezai H, Helan JA, Moghaddam N. Occurrence of *Linguatula serrata* nymphs in cattle slaughtered in Tabriz, Iran. Journal of Parasitic Diseases. 2015;39:140-3.



A Review on the Role of *Varroa destructor* in Transmitting Viruses and Bacteria to Honey Bees

Sara Gholami^{1,2}, Sedigheh Nabian^{1,2}, Parviz Shayan^{1,2}, Narges Amininia^{1,2}

Corresponding Author: Sedigheh Nabian, nabian@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Center of Tick and Tick-borne Disease, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1507

Abstract

Background: The ectoparasitic mite *Varroa destructor* has become one of the biggest threats to beekeeping around the world since it switched hosts from the Asian honeybee (*Apis cerana*) to the European honey bee (*Apis mellifera*). *V. destructor* not only weakens the health and immunity of individual bees by eating their hemolymph and fat bodies, but it is also one of the main ways that viruses spread. There is evidence that Varroa may also affect bacterial infections in bees. The aim of this article is reviewing about *V. destructor's* role in the spread of viral and bacterial agents in honeybees, summarizes recent findings, and outlines implications for integrated pest management strategies.

Methods: Some databases like PubMed, Web of Science, and Google Scholar were used to perform an extensive literature search to prepare this review in the last ten to fifteen years.

Results: It is well known that *Varroa* is the main carrier of Deformed wing Virus (DWV). Also, it can transmit other common bee viruses. The acute bee paralysis complex (Acute Bee Paralysis Virus (ABPV), Kashmir Bee Virus (KBV), and Israeli Acute Paralysis Virus (IAPV)), is closely related to *Varroa*. on the other hand, Finding *Paenibacillus larvae* spores on mite bodies, suggests that American foulbrood can be spread mechanically. *Varroa* is not a biological vector of these bacteria (it does not inject them directly into tissues as viruses do), but it may function as a mechanical reservoir or wound vector.

Conclusion: By acting as both a biological vector for key viruses (most notably DWV) and a mechanical carrier of bacterial spores like *Paenibacillus larvae*, *Varroa destructor* has changed the epidemiology of honeybee infections around the world. By bringing virus particles directly into the bee hemocoel and feeding immunosuppressively, and its roll in spreading mechanically bacterial disease, *V. destructor* causes colony-wide outbreaks.

Keywords: *Apis mellifera*, *Varroa destructor*, Transmission, Deformed Wing Virus, *Paenibacillus larvae*.



Introduction:

The western honeybee (*Apis mellifera*) is a very important pollinator around the world. It helps both natural ecosystems and farming (1). Honeybee colonies, on the other hand, have been declining quickly in the last few decades. This is often linked to what is known as Colony Collapse Disorder, in which most adult workers disappear, leaving behind brood and food. These losses are caused by several stressors, such as pesticides, lack of nutrients, parasites and germs. The ectoparasitic mite *Varroa destructor* is one of the biggest threats to honeybee health (1). This mite eats the hemolymph and fat body tissue of bees that are sealed in brood cells and on adult bees, weakening hosts and shortening lifespan. *V. destructor* is also a very good vector for microbial pathogens, especially viruses, which is very important. *Varroa* has changed the way bee diseases spread around the world by spreading deformed wing virus (DWV) and other viruses that cause wing deformities and colony collapse. There is evidence that *Varroa destructor* may influence bacterial infections in honey bees, either by carrying pathogens or by facilitating secondary infections through feeding wounds (2).

This review presents what we know about the role of *Varroa destructor* in spreading viruses and bacteria to *A. mellifera*. We talk about how the mite gets and spreads viral particles, the types of honeybee viruses involved, and how a mite infestation changes the number and variety of viruses.

Materials and Methods:

We used databases like PubMed, Web of Science, and Google Scholar to perform an extensive literature search to prepare this review. Combinations of *Varroa destructor*, honeybee, virus, bacteria, pathogen and transmission were among the search terms used. Although we included important earlier works for context, we gave priority to studies conducted in the last ten to fifteen years, including recent innovations.

Results and Discussion:

Varroa destructor as a virus vector

Varroa destructor has a significant impact on the viral landscape of honeybees. The mite's feeding behavior, which involves puncturing the brood integument and sucking hemolymph, allows viruses to escape the bee's external defenses and enter straight into the haemocoel. In contrast to normal fecal-oral or trophallactic transmission in bees, mite parasitism causes systemic, overt diseases (especially deformed wing disease) via vector-mediated injection.

The deformed wing virus complex is the most extensively researched *Varroa*-vectored disease. Piou et al. (3) showed that mites can transmit DWV to their offspring and new brood cells



during reproduction, indicating bidirectional virus transmission between mites and bees. *Varroa* often raises DWV titers in colonies, which correlates with deformed wing symptoms and reduced colony strength (3). Surprisingly, current information suggests that *Varroa* is more than just a mechanical transmitter; it can also support active viral multiplication (4).

Aside from DWV, *Varroa* can transmit other common bee viruses. The acute bee paralysis complex, which includes Acute Bee Paralysis Virus (ABPV), Kashmir Bee Virus (KBV), and Israeli Acute Paralysis Virus (IAPV), is closely related to *Varroa*. These picorna-like viruses are frequently present in low concentrations in healthy colonies, but high *Varroa* infestation and mite feeding can significantly raise their titers, contributing to rapid adult bee mortality. For example, studies have shown that levels of ABPV and IAPV increase significantly in colonies infested with *Varroa destructor*, correlating with decreased bee survival (5–7).

Viral-Varroa Interactions and Mite Fitness

The interaction between *Varroa* and viruses is complex. In addition to vectoring, mites can suffer from high virus loads. Some research, such as Piou et al. (3), demonstrate that DWV infection can impair mite health and reduce reproductive success. This could result in a feedback loop linking virus-induced mite death with colony infection dynamics. Piou et al. (3) discovered that mites with high levels of DWV had shorter lifespans and fewer progeny. On the other hand, *Varroa* has been shown to tolerate many viruses, possibly by suppressing immune responses or mitigating damage. For example, *Varroa* saliva contains enzymes and poisons that can alter the host's immune system and allow it to coexist with viruses.

Varroa influences virus evolution and mutation rates. Because the mite injects a large amount of virus into the bee's hemolymph, it may choose more virulent strains or those that can reproduce in both hosts.

Effects of Varroa on Bee Immunity and Co-infections

Varroa feeding not only injects viruses, but it also reduces bee immunity, which can exacerbate pathogen spread. *Varroa* saliva components can damage bee cell walls and inhibit hemocytes. This immunosuppression provides opportunity for secondary infections. According to Morfin et al. (1), parasitized bees have lower immunity, which "opens the possibility of secondary microbial infections" in addition to the viruses already vectored. Consistent with this, *Varroa*-infested colonies frequently have a greater incidence of gut pathogens including *Nosema* (microsporidia) and parasites like *Lotmaria passim*.

Varroa-Associated Bacterial Communities

Although viruses receive the most attention, *Varroa* can help to the spread of bacterial infections, indirectly. The two most common honeybee bacterial infections are American foulbrood (caused by *Paenibacillus larvae*) and European foulbrood (*Mellisococcus plutonius*). These Gram-positive agents are primarily transmitted to larvae through



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



contaminated food, not vectors. However, evidence shows that *Varroa* can mechanically move bacterial spores. De Rycke et al. (8) discovered that *Varroa* mites from an AFB-infected colony had a high concentration of *P. larvae* spores.

Scientists were able to grow *P. larvae* colonies from mite homogenates, which led them to believe that mites "may transmit American foulbrood from infected to healthy bee colonies". This demonstrates that mites travelling between colonies may unintentionally spread AFB spores.

In contrast, no convincing studies have found *Varroa* spreading *M. plutonius* (European foulbrood) in practice, despite the possibility of opportunistic larval infections due to immunosuppressive conditions. In general, *Varroa* is not a biological vector of these bacteria (it does not inject them directly into tissues as viruses do), but it may function as a mechanical reservoir or wound vector.

Finally, beekeepers can indirectly reduce viral epidemics and microbial diseases by controlling *Varroa* levels through integrated pest management. Mokhber and Rasouli (9) also concluded that hygienic behavior and breeding of bees can increase their resistance to virus transmission and other disease. Further, a study by Goomeh et al. (10) has indicated the use of medicinal plants (thyme, basil, rosemary, mint) as a suitable alternative to chemical pesticides for controlling varroa. Plants are natural reservoirs of valuable plant chemicals. Therefore, the use of these plant derivatives can replace chemical acaricides in controlling mites, especially the varroa mite.

Varroa destructor has changed the epidemiology of honeybee infections by acting as both a biological vector for key viruses (most notably DWV) and a mechanical carrier of bacterial spores like *Paenibacillus larvae*. Although its involvement in systemic bacterial transmission is limited, foulbrood spores on mite exoskeletons show bacterial illness can spread mechanically across apiaries. Therefore, integrated pest control that reduces mite burdens is necessary to prevent viral outbreaks and subsequent bacterial and opportunistic illnesses. Future research is necessary for indicating the role of *Varroa destructor* in transmission of bacterial pathogens.

References:

1. Morfin N, Goodwin PH, Guzman-Novoa E. *Varroa destructor* and its impacts on honey bee biology. *Frontiers in Bee Science*. 2023 Oct 12;1:1272937.
2. Kanbar G, Engels WJ. Ultrastructure and bacterial infection of wounds in honey bee (*Apis mellifera*) pupae punctured by *Varroa* mites. *Parasitology research*. 2003 Aug;90(5):349-54.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



3. Piou V, Schurr F, Dubois E, Vétillard A. Transmission of deformed wing virus between *Varroa destructor* foundresses, mite offspring and infested honey bees. *Parasites & Vectors*. 2022 Sep 23;15(1):333.
4. Damayo JE, McKee RC, Buchmann G, Norton AM, Ashe A, Remnant EJ. Virus replication in the honey bee parasite, *Varroa destructor*. *Journal of Virology*. 2023 Dec 21;97(12):e01149-23.
5. Di Prisco G, Pennacchio F, Caprio E, Boncristiani Jr HF, Evans JD, Chen Y. *Varroa destructor* is an effective vector of Israeli acute paralysis virus in the honeybee, *Apis mellifera*. *Journal of General Virology*. 2011 Jan;92(1):151-5.
6. Li Z, Chen Y, Zhang S, Chen S, Li W, Yan L, Shi L, Wu L, Sohr A, Su S. Viral infection affects sucrose responsiveness and homing ability of forager honey bees, *Apis mellifera* L. *PLoS One*. 2013 Oct 10;8(10):e77354.
7. Dobelmann J, Manley R, Wilfert L. Caught in the act: The invasion of a viral vector changes viral prevalence and titre in native honeybees and bumblebees. *Biology Letters*. 2024 May 8;20(5):20230600.
8. De Rycke PH, Joubert JJ, Hossein Hosseinian S, Jacobs FJ. The possible role of *Varroa destructor* in the spreading of American foulbrood among apiaries. *Experimental & applied acarology*. 2002 Apr;27(4):313-8.
9. Mokhbar, Rasouli, Rahimeh. A review of the development of resistance to *Varroa* mites in honey bees (*Apis mellifera*). *Iranian Honey Bee Science and Technology*. 2020 Feb 20;10(19):25-36.
10. Gomeh, Hadith, Vahedi, Rahimi, Ata Allah. Plants used in honey bee colonies (*Apis mellifera*) to control *Varroa* mite (*Varroa destructor*). *Iranian Honey Bee Science and Technology*. 2024 Jan 27;14(27):36-48.



Challenges and Opportunities of Data Mining and Artificial Intelligence in the Detection and Management of Parasitic Zoonotic Diseases in Iran

Zahra Mohammadi¹, Arash Omid¹

Corresponding Author: Zahra Mohammadi, zahramohammadi5041@gmail.com

1. Department of Animal Health Management, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Article code: 1510

Abstract

Background: Parasitic zoonotic diseases, such as leishmaniasis, echinococcosis, and toxoplasmosis, pose significant health challenges in Iran. Traditional diagnostics like microscopy, serology, and PCR are reliable but limited by time, cost, and expertise. This study explores how data mining and artificial intelligence (AI) enhance detection and management of these diseases, promoting a One Health approach.

Methods: A narrative review with systematic elements was conducted, searching PubMed, Scopus, IEEE Xplore, Google Scholar, SID, and Magiran (2010–2025) using terms like parasitic zoonoses, leishmaniasis, AI, machine learning, CNN, and LSTM. Studies on AI applications in diagnosis or management of zoonotic parasitic diseases in Iran were included. Data on algorithms (e.g., CNN, LSTM, SVM, Random Forest), datasets, and performance metrics were extracted.

Results: Convolutional Neural Networks (CNNs) achieved up to 99% accuracy in detecting *Leishmania amastigotes* using ResNet-50. Long Short-Term Memory (LSTM) models predicted leishmaniasis outbreaks in Fars and Kerman with AUC >0.90. Support Vector Machines (SVMs) and Random Forest (RF) showed 90.5% accuracy in serological classification and robust echinococcosis risk mapping. Challenges include limited local datasets, heterogeneous protocols, and rural infrastructure deficits.

Conclusion: AI and data mining improve diagnostic accuracy and outbreak prediction for Iran's zoonotic diseases. Portable devices, standardized datasets, and interdisciplinary collaboration are needed to overcome barriers and support One Health initiatives.

Keywords: Data Mining, Artificial Intelligence, Parasite Detection, One Health, Leishmaniasis, Toxoplasmosis, Convolutional Neural Networks, Long Short-Term Memory, Random Forest, Support Vector Machines.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Introduction:

Parasitic zoonotic diseases, including leishmaniasis, echinococcosis, and toxoplasmosis, are major public and veterinary health concerns in Iran, impacting human health, livestock productivity, and food security. Leishmaniasis, transmitted by sandflies, is endemic in provinces like Fars, Kerman, and Isfahan, with thousands of cutaneous and visceral cases annually (1). Echinococcosis affects humans and livestock in rural areas, while toxoplasmosis poses risks to vulnerable populations (2). Traditional diagnostics, microscopy, serological assays, and PCR, are effective but constrained by time-consuming processes, high costs, and reliance on skilled operators, particularly in resource-limited settings (3). Data mining and artificial intelligence (AI) offer transformative solutions by analyzing complex datasets, identifying patterns, and enabling rapid, accurate detection and management (4). Convolutional Neural Networks (CNNs) excel in image-based parasite detection, while Long Short-Term Memory (LSTM) models predict disease outbreaks (5,6). Despite global advancements, Iran faces challenges like limited local datasets and infrastructure deficits (7). This review aims to: (1) evaluate AI and data mining applications in detecting and managing parasitic zoonotic diseases in Iran, (2) compare their performance with traditional methods, and (3) propose strategies for implementation under a One Health framework.

Materials and Methods:

This study adopted a narrative review with systematic elements to assess AI and data mining applications in parasitic zoonotic disease detection and management in Iran.

Literature Search: Peer-reviewed studies from 2010 to 2025 were retrieved from PubMed, Scopus, IEEE Xplore, Google Scholar, SID, and Magiran using terms parasitic zoonoses, leishmaniasis, echinococcosis, toxoplasmosis, artificial intelligence, machine learning, CNN, LSTM, data mining, and Iran. Boolean operators (AND, OR) refined searches.

Inclusion Criteria: Peer-reviewed studies on AI or data mining for diagnosis, classification, prediction, or risk mapping of parasitic zoonotic diseases. Studies relevant to Iran or Middle Eastern contexts, focusing on leishmaniasis, echinococcosis, or toxoplasmosis. Articles in English or Persian with clear methodology and performance metrics (e.g., accuracy, sensitivity, specificity, AUC).

Exclusion Criteria: Non-peer-reviewed reports or studies lacking methodological transparency. Studies irrelevant to zoonotic parasitic diseases or Iranian contexts.

Data extracted from eligible studies included: parasite species, AI technique, dataset size, validation method, performance metrics (accuracy, sensitivity, specificity, AUC, F1-score), and application setting. Studies were categorized into detection (image-based, serological) and



management (outbreak prediction, risk mapping). Local challenges, such as dataset quality and infrastructure, were assessed, prioritizing Iranian case studies.

Table 1. Comparison of machine learning algorithms for parasite detection and epidemiological analysis

Algorithm	Application	Advantages	Challenges	Example Reference
Convolutional Neural Networks (CNN)	Image-based parasite detection (e.g., Leishmania amastigotes, Echinococcus cysts)	High accuracy (up to 99%), automatic feature extraction, robust to image noise	Needs large annotated datasets, high computational cost, risk of overfitting	Zhao et al., 2021 (5); Kumar et al., 2020 (9)
Long Short-Term Memory (LSTM)	Time-series prediction of outbreaks (e.g., leishmaniasis in Fars, Kerman)	Captures long-term dependencies, strong temporal modeling	Requires large time-series datasets, complex training, hyperparameter sensitivity	Shirzadi et al., 2019 (1); Revathi et al., 2024 (16)
Support Vector Machines (SVM)	Classification of serological/genetic datasets (e.g., Toxoplasma gondii)	Effective with small datasets, strong for binary classification	Limited scalability, less effective with noisy data	Mamoshina et al., 2018 (12); Morais et al., 2022 (13)
Random Forest (RF)	Epidemiological risk mapping (e.g., echinococcosis risk in rural Iran)	Handles noisy data, interpretable feature importance	May overfit with small datasets, less effective for image data	Beam & Kohane, 2018 (2); Kumar et al., 2023 (10)

Results and Discussion:

AI methods such as Convolutional Neural Networks (CNN) have been highly effective in image-based parasite detection, achieving up to 99% accuracy in identifying Leishmania amastigotes and malaria parasites using architectures like ResNet-50 and VGG-16 (5,15,22). Recurrent Neural Networks (RNN), particularly Long Short-Term Memory (LSTM) models with gated recurrent units, have been applied in predicting temporal patterns of leishmaniasis outbreaks in endemic Iranian provinces like Fars and Kerman, with AUC scores exceeding 0.90 (1,16). Random Forest (RF) and Gradient Boosting have shown potential for risk mapping using environmental and demographic data, particularly for echinococcosis in rural Iran, achieving predictive accuracies of 85–90% (2,10). Support Vector Machines (SVMs) demonstrated 90.5% accuracy and 88.6% specificity in classifying serological and genetic datasets for parasites like Toxoplasma gondii and Trypanosoma cruzi (12,13). In Iran,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



leishmaniasis remains a critical zoonosis, with CNN and LSTM models providing accurate detection and forecasting, respectively (1,5). Echinococcosis risk mapping benefits from integrating RF and Gradient Boosting algorithms with local epidemiological data, such as livestock infection rates and environmental factors (2,17). Complementary technologies, including IoT-based portable devices and tele-veterinary platforms like VETSCAN IMAGYST, enhance diagnostic access in remote areas, with high sensitivity for detecting equine parasites. (7,19). Challenges include the scarcity of high-quality, annotated local datasets for *Leishmania* and *Echinococcus*, which limits model reliability (10). Heterogeneous sampling protocols across Iranian studies reduce generalizability, while rural infrastructure deficits and high computational costs for CNNs and LSTMs pose barriers (1,7). Ethical concerns, such as data privacy and informed consent, further complicate implementation (2,4). Proposed solutions include developing national parasite image repositories, standardizing data collection protocols, and fostering interdisciplinary collaboration to integrate veterinary, medical, and computational expertise (3,8).

Data mining and AI present substantial opportunities to enhance the detection and management of zoonotic parasitic diseases in Iran, achieving up to 99% accuracy with CNNs for image-based detection and precise outbreak predictions with LSTMs (5,15,1). While traditional diagnostic methods like microscopy and PCR remain essential (3), integrating AI can improve diagnostic accuracy, reduce time and costs, and facilitate surveillance in resource-limited settings (4). For Iran, where leishmaniasis and echinococcosis remain endemic, AI-based risk prediction and image classification models hold immediate translational value (1,17). Addressing current barriers—including the creation of national parasite image repositories, development of annotated local datasets, and adoption of standardized validation protocols—will be critical to ensuring sustainable implementation (2,10). Collaborative One Health initiatives bridging veterinary, medical, and computational sciences, supported by IoT and tele-veterinary platforms, will accelerate the translation of AI into real-world parasitic disease control (7,8,19).

Acknowledgment:

We thank the library of the School of Veterinary Medicine at Shiraz University for providing access to critical resources and databases, enabling comprehensive literature analysis.

References:

1. Shirzadi MR, Esfahania SB, Yaghoobi-Ershadi MR, et al. Epidemiology of cutaneous leishmaniasis in Iran: 1983–2018. *Asian Pac J Trop Med*. 2019;12(4):109-17.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



2. Beam AL, Kohane IS. Big data and machine learning in health care. *JAMA*. 2018;319(13):1317-8.
3. Suzuki CT, Gomes JF, Falcão AX, Papa JP, Hoshino-Shimizu S. Automatic segmentation and classification of human intestinal parasites from microscopy images. *IEEE Trans Biomed Eng*. 2013;60(3):803-12.
4. Jiang F, Jiang Y, Zhi H, Dong Y, Li H, Ma S, et al. Artificial intelligence in healthcare: Past, present and future. *Stroke Vasc Neurol*. 2017;2(4):230-43.
5. Zhao X, Li Y, Wang X, Zhang Y. Deep learning in parasitology: Current applications and future perspectives. *Front Cell Infect Microbiol*. 2021;11:698630.
6. Krishna R, Prema KV. Constructing and optimizing RNN models to predict fruit rot disease incidence in areca nut crop based on weather parameters. *IEEE Access*. 2023;11:110582-95.
7. Alonso RS, Sittón-Candanedo I, García Ó, Prieto J, Rodríguez-González S. An intelligent Edge-IoT platform for monitoring livestock and crops in a dairy farming scenario. *Ad Hoc Netw*. 2020;98:102047.
8. Bhavnani SP, Parakh K, Atreja A, Druz R, Graham GN, Hayek SS, et al. 2017 Roadmap for innovation—ACC health policy statement on healthcare transformation in the era of digital health, big data, and precision health. *J Am Coll Cardiol*. 2017;70(21):2696-718.
9. Kumar A, Singh A, Thakur N. Automated detection of intestinal parasites using deep learning. *J Parasit Dis*. 2020;44(3):617-24.
10. Kumar S, Arif T, Alotaibi AS, Malik MB, Manhas J. Advances towards automatic detection and classification of parasites microscopic images using deep convolutional neural network. *Arch Comput Methods Eng*. 2023;30(3):2013-39.
11. Lee YW, Choi JW, Shin EH. Machine learning model for predicting malaria using clinical information. *Comput Biol Med*. 2021;129:104151.
12. Mamoshina P, Vieira A, Putin E, Zhavoronkov A. Applications of deep learning in biomedicine. *Mol Pharm*. 2018;15(12):4312-21.
13. Morais MCC, Silva D, Milagre MM, De Oliveira MT, Pereira T, Silva JS, et al. Automatic detection of the parasite *Trypanosoma cruzi* in blood smears using a machine learning approach applied to mobile phone images. *PeerJ*. 2022;10:e13470.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



14. Rahimi MT, Daryani A, Sarvi S, et al. Toxoplasmosis in Iran: a systematic review and meta-analysis. *Acta Trop.* 2015;146:20-31.
15. Ramos-Briceño DA, Flammia-D'Aleo A, Fernández-López G, Nascimento FSC, Pereira DAF. Deep learning-based malaria parasite detection: Convolutional neural networks model for accurate species identification of *Plasmodium falciparum* and *Plasmodium vivax*. *Sci Rep.* 2025;15(1):3746.
16. Revathi TK, Balasubramaniam S, Sureshkumar V, Dhanasekaran S. An improved long short-term memory algorithm for cardiovascular disease prediction. *Diagnostics.* 2024;14(3):239.
17. Sarkari B, Rezaei Z, Mohebbali M. Echinococcosis in Iran: epidemiology and approaches for control. *Arch Clin Infect Dis.* 2020;15(1):e87575.
18. Watson K, Wells J, Sharma M, Robertson S, Dascanio J, Johnson JW, et al. A survey of knowledge and use of telehealth among veterinarians. *BMC Vet Res.* 2019;15(1):474.
19. Steuer A, Fritzler J, Boggan S, Daniel I, Cowles B, Penn C, et al. Validation of Vetscan Imagyst®, a diagnostic test utilizing an artificial intelligence deep learning algorithm, for detecting strongyles and *Parascaris* spp. in equine fecal samples. *Parasites Vectors.* 2024;17(1):465.
20. Xiao S, Dhand NK, Wang Z, Hu K, Thomson PC, House JK, et al. Review of applications of deep learning in veterinary diagnostics and animal health. *Front Vet Sci.* 2025;12:1511522.
21. Mohebbali M, Akhoundi B, Kakooei Z, et al. Epidemiology of visceral leishmaniasis in Iran: A systematic review. *J Parasit Dis.* 2021;45(2):315-24.
22. Fuhad KMF, Tuba JF, Sarker MRA, Momen S, Mohammed N, Rahman T. Deep learning based automatic malaria parasite detection from blood smear and its smartphone based application. *Diagnostics.* 2020;10(5):329.
23. Esteva A, Kuprel B, Novoa RA, Ko J, Swetter SM, Blau HM, Thrun S. Dermatologist-level classification of skin cancer with deep neural networks. *Nature.* 2017;542:115-8.
24. Farooq MS, Sohail OO, Abid A, Rasheed S. A survey on the role of IoT in agriculture for the implementation of smart livestock environment. *IEEE Access.* 2022;10:9483-505.



Rising Drug Resistance in Animal Parasites: Epidemiological Insights and Sustainable Control Measures

Shadi Shahvand¹, Arash Omid¹

Corresponding Author: Shadi Shahvand, shahvandshadi99@gmail.com

1. Department of Animal Health Management, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Article code: 1517

Abstract

Background: Anthelmintic resistance (AR) in livestock parasites like *Haemonchus contortus* and zoonotic *Giardia duodenalis* and *Cryptosporidium parvum* threatens global animal and human health due to intensive drug use and poor management. In Iran, rising AR in some regions necessitates integrated control within a One Health framework.

Methods: This systematic review was based on a structured literature search in PubMed, Scopus, and Web of Science (2000–2025) focusing on anthelmintic resistance in livestock parasites. Studies reporting resistance patterns, mechanisms, risk factors, and control strategies, with emphasis on Iran, were included. Data were descriptively and comparatively synthesized using epidemiological and molecular evidence from eligible studies.

Results: Anthelmintic resistance in *Haemonchus contortus* and *Fasciola hepatica* is driven by genetic mutations (e.g., β -tubulin codon 200) and metabolic adaptations, with high prevalence in Iran's Khuzestan (benzimidazole resistance) and Mazandaran (human seropositivity 2.48%). Globally, ivermectin resistance reaches 60–65% in Brazil, and benzimidazole resistance is 86% in Europe. Environmental factors (high temperature, humidity) and poor management practices accelerate resistance, necessitating integrated control strategies.

Conclusion: Anthelmintic resistance in livestock and zoonotic parasites threatens animal and human health, driven by genetic, environmental, and management factors. Integrated parasite management, including targeted treatments and molecular surveillance, is essential to mitigate resistance. A One Health approach, coordinating veterinary and public health efforts, is critical for sustainable control in Iran and globally.

Keywords: Anthelmintic resistance; Livestock parasites; Drug resistance; Zoonotic parasites; One Health.



Introduction:

Parasitic infections are among the major constraints to livestock productivity worldwide, significantly affecting animal health, growth, reproduction, and economic returns in the livestock industry (1). The control of these infections has long relied on the use of anthelmintic drugs; however, their frequent and often inappropriate administration has led to the widespread emergence of drug resistance, posing a serious challenge to sustainable animal production (1). Anthelmintic resistance (AR) is now recognized as a global phenomenon, particularly in nematodes such as *Haemonchus contortus*, where resistance to major drug classes including benzimidazoles, macrocyclic lactones (e.g., ivermectin), and levamisole has been extensively documented (2,3). Of growing concern is the development of resistance in zoonotic parasites such as *Giardia duodenalis* and *Cryptosporidium parvum*, which not only affect animal health but also pose direct risks to human populations through food and waterborne transmission (4). Resistance to metronidazole in *Giardia* and to nitazoxanide, the only approved drug for *Cryptosporidium*, further complicates treatment options and public health management (5–7). In Iran, increasing reports of AR in livestock parasites, especially in high-density farming regions such as Khuzestan and Mazandaran, highlight the urgent need for comprehensive monitoring and strategic intervention (8–14). Environmental and management factors, including high temperature, humidity, inadequate drug dosing, lack of drug rotation, and overstocking, significantly contribute to the selection and spread of resistant parasite strains (5,7). Furthermore, the absence of national surveillance programs and limited awareness among farmers and veterinarians exacerbate the problem (15). This review aims to provide an epidemiological overview of AR trends in veterinary parasites, with emphasis on local evidence from Iran, global patterns, underlying mechanisms, and integrated control strategies within a One Health framework.

Materials and Methods:

This study was a systematic and analytical review of scientific literature on anthelmintic resistance in livestock parasites. A structured search was conducted in PubMed, Scopus, and Web of Science for peer-reviewed articles published between 2000 and 2025. Eligible studies included those reporting drug resistance patterns, molecular mechanisms, epidemiological data, and management factors, with emphasis on Iran and global comparisons. Data were extracted and synthesized qualitatively and quantitatively based on reported findings. Due to the review design, no independent statistical analysis was performed. Focus was placed on resistance trends in key parasites such as *Haemonchus contortus*, *Fasciola hepatica*, *Giardia duodenalis*, and *Cryptosporidium parvum*, along with zoonotic implications and control strategies.



Results and Discussion:

Anthelmintic resistance in livestock parasites is driven primarily by genetic and biochemical mechanisms. Key resistance mechanisms include point mutations in target genes such as β -tubulin (associated with benzimidazole resistance), increased activity of efflux pumps (e.g., P-glycoproteins), reduced membrane permeability, and enzymatic drug inactivation (3). In Khuzestan province, Iran, studies have shown mutations in the β -tubulin gene of *Haemonchus contortus* associated with benzimidazole resistance. A study using PCR-RFLP methods on isolates from various regions of Khuzestan revealed a reduction in β -tubulin gene polymorphism in isolates from Shooshtar, indicating the emergence of benzimidazole-resistant strains. This mutation at codon 200, which involves the substitution of phenylalanine with tyrosine, is the primary genetic mechanism for developing resistance to benzimidazole-class anthelmintics. Isolates from areas with more frequent benzimidazole treatments exhibited fewer RFLP fragments, reflecting decreased polymorphism and increased resistance levels. Globally, ivermectin resistance in *Haemonchus contortus* in Brazil is widespread, with prevalence in many sheep farms reaching or exceeding 60–65%, driven by climatic conditions favorable for parasites and intensive anthelmintic use. This necessitates urgent integrated parasite management strategies to control resistance spread and maintain drug efficacy in tropical livestock farming (16–19). In Europe, specifically, resistance to benzimidazoles was found at an average prevalence of 86% in sheep and goats, making it the anthelmintic class with the highest resistance among these animals. Other anthelmintic classes such as levamisole and macrocyclic lactones show lower average resistance rates (48% and 52%, respectively). This data is drawn from analysis of numerous studies across Europe with a bias towards Western Europe and the UK but includes various European regions (20–22). In Iran, reports indicate resistance to benzimidazoles in *Fasciola hepatica* from Mazandaran province. Studies confirm that *Fasciola* infections are present in Mazandaran with notable seropositivity rates in humans and livestock, and concerns about emerging drug resistance prompt calls for integrated control strategies. For example, human seropositivity for *Fasciola* infection in Mazandaran was found around 2.48%, indicating ongoing parasite presence and potential resistance development risks that require monitoring and prevention efforts (23). Environmental factors such as high temperature ($\sim 30^{\circ}\text{C}$) and humidity ($>70\%$) were positively correlated with resistance emergence, particularly in southern Iran. Poor management practices including underdosing, lack of drug rotation, and high animal density further accelerate resistance development (5). *Giardia duodenalis* isolates from calves have developed resistance to metronidazole, a first-line nitroimidazole treatment. This resistance is associated with altered expression of key genes involved in drug activation and metabolism, particularly pyruvate:ferredoxin oxidoreductase (PFOR) and ferredoxin, which are crucial for metronidazole's activation within the parasite. Resistant *Giardia* cells show reduced activity or downregulation of these genes, leading to decreased drug activation and increased tolerance



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



to oxidative damage. Metronidazole resistance involves complex molecular mechanisms, often characterized by reversible transcriptional changes rather than fixed mutations. This includes active enzymatic detoxification and passive avoidance through lowered expression of activation-related proteins. Resistance may also be influenced by epigenetic and post-translational modifications. Some resistant *Giardia* lines can lose their resistance when cultured without drug pressure, indicating that resistance is dynamic and dependent on environmental conditions. This has been observed in both laboratory and clinical isolates, where treatment failures correlate with altered expression of genes related to oxidative stress management (24–26)

Anthelmintic resistance (AR) poses a significant threat to global livestock production, compromising animal health, productivity, and economic sustainability. This review synthesizes findings on AR in veterinary parasites, focusing on Iran and global trends, underlying mechanisms, and integrated control strategies within a One Health framework. Zoonotic parasites, such as *Giardia duodenalis* and *Cryptosporidium parvum*, exhibit emerging resistance to metronidazole and nitazoxanide, respectively, posing risks to human health via food and waterborne transmission (4–7). In Iran's Mazandaran province, *Fasciola hepatica* shows benzimidazole resistance, with human seropositivity at 2.48%, highlighting zoonotic concerns (23). Environmental factors, including high temperatures (~30°C) and humidity (>70%), and poor management practices like underdosing and lack of drug rotation, accelerate AR (5,7). Resistance mechanisms involve genetic mutations (e.g., β -tubulin in *H. contortus*), increased efflux pump activity, and metabolic adaptations in *Giardia* (3,24). Integrated parasite management (IPM) is critical for AR mitigation. Strategies include targeted selective treatment (TST), drug rotation, and improved farm practices to reduce parasite transmission (2). Molecular diagnostics, such as PCR-RFLP, enable early resistance detection (29). A One Health approach, integrating veterinary and public health efforts, is vital to address zoonotic risks.

Acknowledgment:

We are particularly grateful to the library of the School of Veterinary Medicine at Shiraz University for providing access to critical resources and databases that enabled a comprehensive analysis of the literature.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Kaplan RM. Drug resistance in nematodes of veterinary importance: a status report. *Trends Parasitol.* 2004;20(10):477–81.
2. Kaplan RM, Vidyashankar AN. An inconvenient truth: global worming and anthelmintic resistance. *Vet Parasitol.* 2012;186(1–2):70–8.
3. Venkatesan A, et al. Molecular evidence of widespread benzimidazole drug resistance in *Ancylostoma caninum* from domestic dogs throughout the USA and discovery of a novel β -tubulin benzimidazole resistance mutation. *PLoS Pathog.* 2023;19(3):e1011146.
4. Xiao L. Molecular epidemiology of cryptosporidiosis: an update. *Exp Parasitol.* 2010;124(1):80–9.
5. Rossignol JF. *Cryptosporidium* and *Giardia*: treatment options and prospects for new drugs. *Exp Parasitol.* 2010;124(1):45–53.
6. Müller J, et al. Physiological aspects of nitro drug resistance in *Giardia lamblia*. *Int J Parasitol Drugs Drug Resist.* 2018;8(2):271–7.
7. Whitta STG, et al. Comparative efficacy and safety of anti-cryptosporidial agents. *Front Microbiol.* 2024;15:1463457.
8. Rokni MB. The present status of human helminthic diseases in Iran. *Ann Trop Med Parasitol.* 2008;102(4):283–95.
9. Eslami A, Kamyabi H, Bizhan S, Alinejad A, Shafiei R, Pakroui O, et al. Prevalence of *Fasciola hepatica* infection in slaughtered animals in Mazandaran province, north of Iran. *Vet Res Forum.* 2015;6(3):231–6.
10. Azari-Hamidian S, Shemshad K, Kia EB, Saghaipour A. *Dirofilaria* infections in Iran: a review. *J Parasit Dis.* 2020;44(3):486–95.
11. Sadjjadi SM. Present situation of echinococcosis in the Middle East and Arabic North Africa. *Parasitol Int.* 2006;55 Suppl:S197–202.
12. Hosseininezhad S, Moradpour R, Abdollahpour F. Parasitic infections in livestock in Khuzestan province, Iran: prevalence and distribution. *J Parasit Dis.* 2019;43(1):112–8.
13. Hosseini SV, Shayan P, Bokaie S. Epidemiology and control of parasitic infections in dogs and wildlife reservoirs in northern Iran. *Vet Parasitol.* 2018;250:23–9.
14. Ebrahimzadeh F, Sharifi I, Fallah E, et al. Anthelmintic resistance in gastrointestinal nematodes of sheep in Iran: A systematic review. *Vet Parasitol.* 2020;279:109032.
15. Sazmand A, et al. Assessment of knowledge, attitudes and practices relating to parasitic diseases and anthelmintic resistance among livestock farmers in Hamedan, Iran. *Front Vet Sci.* 2020;7:578.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



16. Almeida FA, Garcia KC, Torgerson PR, Amarante AF. Multiple resistance to anthelmintics by *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep in Brazil. *Parasitol Int.* 2010;59(4):622–5.
17. Vieira LSG, Amarante AF, Medeiros JF, Levot G. Ivermectin resistance in *Haemonchus contortus* isolated from sheep in southern Brazil. *Vet Parasitol.* 2008;155(1–2):7–14.
18. Da Silva BM, Dias RA, Lopes WG, et al. Resistance to ivermectin and ivermectin aglycone in *Haemonchus contortus* from sheep flocks in São Paulo, Brazil. *Vet Parasitol.* 2016;219:67–73.
19. Tobo PR, Leon AP, Souza FRB, et al. Anthelmintic resistance in gastrointestinal nematodes of sheep from Brazilian tropical regions: A systematic review. *Rev Bras Parasitol Vet.* 2020;29(1):e018520.
20. Rose Vineer H, et al. Increasing importance of anthelmintic resistance in European livestock. *Parasite.* 2020;27:69.
21. Morgan ER, et al. Increasing importance of anthelmintic resistance in European livestock. *Parasite.* 2020;27:1–15.
22. Mickiewicz M, et al. Prevalence of anthelmintic resistance of gastrointestinal nematodes in Lithuania and Poland. *Parasit Vectors.* 2023;16:262.
23. Rasouli M, Sharif M, et al. Seroprevalence of human fascioliasis in Qaemshahr, Mazandaran Province, Iran: implications for prevention strategies. *Arch Clin Infect Dis.* 2022;17(5):e123682.
24. Leitsch D. Drug resistance in the microaerophilic parasite *Giardia lamblia*. *Curr Trop Med Rep.* 2021;8(2):50–9.
25. Ansell BR, McConville MJ, Ma'ayeh SY, Dagley MJ, Gasser RB, Svard SG, Jex AR. Drug resistance in *Giardia duodenalis*. *Biotechnol Adv.* 2017;35(6):829–44.
26. Tejman-Yarden N, et al. Effective against metronidazole-resistant *Giardia lamblia*. *Antimicrob Agents Chemother.* 2013;57(5):2029–35.
27. Gholamian A, Galehdari H, Eslami A, Nabavi L. Study of β -tubulin gene polymorphisms in *Haemonchus contortus* isolated from sheep populations in Khouzestan, southwestern Iran. *Iran J Vet Res.* 2007;8(3):239–46.
28. Mickiewicz M, et al. Anthelmintic resistance of gastrointestinal nematodes: molecular detection and epidemiological perspectives. *Vet Parasitol.* 2022;305:109660.
29. Zintl A, et al. Veterinary and public health significance of *Cryptosporidium* species in farmed animals. *Vet Parasitol.* 2017;233:1–11.



Prevalence of *Toxocara* Egg in the Parks Soil in Karaj

Sina Arabi¹, Nadia Taiefi Nasrabadi², Marzieh Kefayat²

Corresponding Author: Nadia Taiefi Nasrabadi, drtaiefi@gmail.com

1. Department of veterinary medicine, Karaj branch, Islamic Azad university, Karaj, Iran.
2. Department of parasitology, Karaj Branch, Islamic Azad University, Karaj, Iran.

Article code: 1521

Abstract

Background: *Toxocara* species are nematode parasites that infect a wide range of companion, domestic, and wild animals as definitive hosts, and occasionally humans as accidental hosts. The primary transmission route to humans is through ingestion of embryonated eggs present in contaminated soil, hands, food, or utensils. This study aimed to determine the level of contamination of public park soils in Karaj city with eggs of various *Toxocara* species.

Methods: Soil samples were collected from different zones of public parks across four geographic regions of Karaj (north, south, east, and west). Samples were analyzed microscopically using the Clayton-Lane flotation method to detect the presence of *Toxocara* eggs.

Results: No statistically significant differences were observed in the mean contamination rates among the four regions. However, within individual parks, the highest contamination levels were found in garden beds and flower plots, while children's play areas showed the lowest rates. In southern parks, the highest contamination was recorded near garbage disposal sites and main walking paths. In eastern and western parks, flower beds were the most contaminated zones.

Conclusion: The prevalence of *Toxocara* and *Toxascaris* infections in stray dogs has increased compared to previous reports. Due to the proximity of these animals to parks and residential areas, the risk of human toxocariasis remains significant. These findings highlight the importance of implementing effective treatment and prevention protocols for stray dogs.

Keywords: Parasite, *Toxocara cati*, Soil, Park, Alborz.

Introduction:

Toxocariasis, also known as visceral larva migrans syndrome, is considered a zoonotic parasitic disease caused by the roundworms of dogs (*Toxocara canis*) and cats (*Toxocara cati*). Among these, *Toxocara canis* is of greater significance. Each adult worm residing in the intestine of an infected host can produce a large number of eggs daily, which are excreted through the



animal's feces and enter the environment. Humans typically become infected with toxocariasis through the ingestion of contaminated food or water, or contact with soil containing the parasite's eggs.

However, some researchers believe that the likelihood of direct transmission through physical contact with pets is relatively low, since the excreted eggs require at least six weeks of incubation in the soil to become infectious. Therefore, soil plays a critical role as a reservoir for *Toxocara* eggs in the spread of this disease (1).

Once the eggs enter the human gastrointestinal tract, larvae hatch and penetrate the intestinal wall, entering the bloodstream and lymphatic system. These larvae then migrate to organs such as the liver, lungs, heart, brain, eyes, and other tissues, forming granulomas around themselves that isolate them from the host tissue. Clinical symptoms vary depending on the location of the granulomas and the host's immune response to the larval antigens. Generally, three clinical forms of toxocariasis are recognized: visceral larva migrans, ocular larva migrans, and covert toxocariasis, each with its own distinct symptoms.

Various methods exist to assess the prevalence and concentration of *Toxocara* eggs in soil, with the saturated sucrose flotation method being one of the most common. Numerous studies in Iran have shown that soil contamination with these parasite eggs is on the rise. Given that the primary route of transmission to humans is through ingestion of contaminated eggs, and considering the role of dogs and cats as the main sources of soil contamination, close contact with these animals can increase the risk of toxocariasis. Additionally, the growing population of stray dogs and cats, along with the increasing trend of keeping pets—especially dogs and cats—has contributed to the rise in soil contamination with *Toxocara* eggs (2).

The aim of this study is to investigate the level of soil contamination in public parks of Karaj city with eggs of various *Toxocara* species.

Materials and Methods:

In this study, the city of Karaj was divided into four regions: north, south, east, and west. In each region, five parks were selected, and from each park, samples were collected from five distinct locations: flowerbeds, children's play areas, main walking paths, seating areas, and around garbage bins. From each point, 100 grams of soil were collected at a depth of 2 to 5 centimeters. The samples from each park were placed in plastic containers and transported to the laboratory, where nematode eggs were isolated using the Clayton-Lane concentration method.

To isolate parasite eggs from soil samples, the Clayton-Lane flotation technique was used, employing a saturated zinc sulfate solution. This method is based on the difference in density between parasite eggs and the flotation solution, which causes the eggs to float to the surface.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- First, 3 grams of soil were weighed using a digital scale and placed into a new fecal container labeled with the corresponding sample number.
- Then, 42 milliliters of distilled water were added to the sample, and the mixture was thoroughly stirred to achieve homogeneity.
- The mixture was evenly divided into three 15-milliliter test tubes (15 ml per tube).
- The tubes were centrifuged for 2 minutes at 2000 rpm. After centrifugation, the contents of each tube separated into a sediment and a supernatant layer.
- The supernatant, which mainly contained fats and waste materials, was discarded, and the remaining sediment was gently tapped out from the bottom of the tube.

To completely remove residual waste, the washing steps (steps 4, 5, and 6) were repeated three times for each tube. At this stage, each tube was half-filled with saturated zinc sulfate solution and gently stirred with an applicator. Then, the solution was slowly added using a syringe until the surface of the liquid formed a convex meniscus.

Next, a coverslip was placed on the mouth of each tube, and the tubes were centrifuged again for 2 minutes at 1500 rpm. After centrifugation, the coverslips were carefully removed vertically and placed on microscope slides. The prepared slides were examined under a light microscope at magnifications of $\times 10$ and $\times 40$.

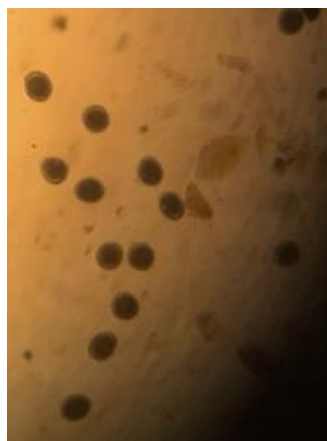


Figure 1. Observation of *Toxocara* parasite eggs using a light microscope.

Statistical Analysis: The obtained results are evaluated using the Chi-square test in the statistical software SPSS version 22 under the Windows operating system.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Results and Discussion:

Results In this study, a total of 100 soil samples were collected from 20 public parks located in four geographical regions of Karaj city (north, south, east, and west). Each sample was examined for contamination with *Toxocara* parasite eggs. The geographical location, type of sampling site (garden, flowerbed, children's play area, walking path, and near garbage bins), and contamination results for each sample were recorded and stored in Excel software. Statistical analysis of the data was performed using SAS software version 9.2, employing the Chi-square test and Duncan's multiple range test.

Regional Findings

Northern Karaj Region: No statistically significant differences were observed among the various park areas ($P > 0.05$). However, the highest levels of contamination were recorded in the following order: gardens, flowerbeds, areas near garbage bins, children's play areas, and walking paths.

Table 11. Comparison of *Toxocara* Infection Rates in the Northern Geographical Region

Sampling Location	Infection Rate (%)
Parks	10.9%
Main Streets and Sidewalks	8.7%
Work Bypasses	8.8%
Flower Beds	8.8%
Children's Playgrounds	5.7%

Southern Karaj Region: Similar to the northern region, no statistically significant differences were observed among the various park areas. However, the highest levels of contamination were found near garbage bins and along pedestrian pathways.

Table 12. Percentage of *Toxocara* Contamination in Southern Karaj Region

Sampling Location	Contamination Rate (%)
Main Road	14.67%
Gardens	13.45%
Flower Beds / Playgrounds	12.73%
Beside Garbage	10.62%



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Eastern Karaj Region: A statistically significant difference was observed among the various areas ($P < 0.05$). Gardens, flower beds, and areas near garbage bins showed the highest levels of contamination. The difference between gardens and children's playgrounds as well as pedestrian pathways was statistically significant.

Table 13. Percentage of Lead Contamination in Different Locations (Eastern Region)

Location	Lead Contamination (%)	Statistical Group
Windows	19.6%	a
Children's Play Area	7.25%	b
Internal Walls & Main Walking Areas	13.57%	ab
Gardening	13.57%	ab
Children's Play Area (duplicate entry)	7.25%	b

Note: The letters (a, ab, b) indicate statistical significance. Locations with the same letter are not significantly different from each other in terms of pollution levels.

Western Karaj Region: A statistically significant difference was recorded between various areas ($P < 0.05$). Gardening areas, flower beds, and walkways showed the highest levels of contamination. There was a notable difference between gardening areas and children's play areas.

Table 14. Comparison of *Toxocara* Pollution Percentage in Chogha Abadi West Region

Location	Pollution Percentage (%)	Statistical Group
Flower Beds	17.3%	a
Gardens	11.89%	ab
Main Road	11.27%	ab
Next to Garbage Dumps	11.24%	ab
Children's Play Area	9.71%	b

Note: The letters (a, ab, b) indicate statistical significance. Locations with the same letter are not significantly different from each other in terms of pollution levels.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Final analysis revealed a statistically significant difference ($P < 0.01$) in the average percentage of *Toxocara* egg contamination across the four geographical regions of Karaj. In descending order, the highest levels of contamination were observed in the South, followed by the West, East, and North regions. The difference between the South and West regions compared to the North was statistically significant.

Table 15. Comparison of *Toxocara* Pollution Percentage in Different Geographical Regions

Region	Pollution Percentage (%)	Statistical Group
South	13.41%	a
West	12.28%	a
East	10.71%	ab
North	7.73%	b

Note: The letters (a, ab, b) represent statistical significance. Regions sharing the same letter are not significantly different from each other in terms of pollution levels.

Table 16. Mean and Standard Deviation ($SD \pm$ Average) of *Toxocara* Egg Contamination Percentage in Different Sampling Areas

Sampling Area	$SD \pm$ Average (%)	Duncan Grouping
Gardens	13.14 ± 7.53	a
Main Walking Path	9.56 ± 5.08	ab
Next to Garbage Dumps	11.41 ± 4.47	ab
Flower Beds	12.34 ± 6.89	ab
Children's Play Area	8.73 ± 6.78	b

Note: Duncan grouping indicates statistical significance. Areas sharing the same letter (e.g., "ab") are not significantly different from each other, while those with different letters (e.g., "a" vs. "b") show meaningful differences. P-value for comparisons: 0.0140.

According to the above table, the highest average contamination was observed in gardens and flower beds. A statistically significant difference was found between gardens and children's play areas ($P < 0.05$). These findings highlight the critical role of animal defecation behavior in specific park zones and underscore the importance of hygienic design in public spaces.



Parasites of the genus *Toxocara* are among the most common gastrointestinal helminths shared between humans and animals, widely infecting dogs and cats around the world (3). Transmission to humans typically occurs through accidental ingestion of embryonated eggs present in contaminated soil, especially among individuals with direct contact with soil, such as children or certain occupational groups. Diagnosing the underlying cause of disease can be challenging for physicians, yet it is essential for developing effective treatment protocols and proper management (4).

In the present study, no statistically significant difference was observed in the average percentage of *Toxocara* contamination among parks in the northern, southern, eastern, and western regions of the city. However, the distribution pattern of contamination within each park was noteworthy. In northern parks, the highest contamination levels were reported in gardens and flower beds, while the lowest was found in children's play areas. In southern parks, contamination was more prevalent near garbage dumps and main walking paths. In eastern and western parks, flower beds showed the highest contamination levels.

Since *Toxocara* eggs in this study were identified only at the genus level, they may belong to species such as *T. canis*, *T. cati*, or *T. leonina*. High soil contamination in gardens and flower beds may be attributed to the defecation behavior of cats in these areas. Additionally, the presence of stray dogs roaming urban parks is likely a major contributor to environmental contamination.

Given the zoonotic nature of the disease and the presence of children, families, and pets in parks, there is a risk of transmission to both humans and domestic animals. This can lead to conditions such as visceral larva migrans (VLM), ocular larva migrans (OLM), and infection of pets with *T. canis* and *T. cati*.

Several studies in Iran have confirmed these findings. For example, Ebrahimzadeh et al. (2018) identified *Toxocara* eggs in 5 out of 61 parks surveyed in Larestan (5). Jafari (2017) reported soil contamination in 14 out of 18 public parks in Zanjan, with the highest levels found in walking paths and seating areas (6). Nassouhi et al. (2021) found 11 contaminated samples (7.33%) out of 150 soil samples in Yasuj (7). Tabatabaei-Kia et al. (2021) confirmed *T. canis* contamination in 6 samples from stray dogs in Zanjan province using PCR, indicating a rising prevalence of the parasite and the importance of urban control measures (8).

This study highlights that soil contamination in urban parks with *Toxocara* eggs is a serious public health concern, posing risks especially to children and pets. Key factors in transmission include the presence of stray dogs and cats, favorable environmental conditions for egg survival, and direct human contact with contaminated soil.

Recommended Actions Considering the Pathogenic Potential and Clinical Implications of *Toxocara*.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Given the pathogenic potential of *Toxocara* and its clinical consequences, the following measures are recommended:

- Implement stray animal population control programs and ensure regular anthelmintic treatment
- Promote public education on soil hygiene and the importance of handwashing after playing in parks
- Design urban spaces with safe zones for children's play and restrict animal access to these areas
- Conduct molecular studies for precise species identification and to evaluate the effectiveness of interventions

Ultimately, ongoing epidemiological surveillance and intersectoral collaboration among veterinary services, environmental health authorities, and municipalities are essential to reduce the burden of zoonotic diseases such as toxocariasis.

References:

1. Daneshrad T, Mehpikar H, Azadi A. Comparison of gastrointestinal parasites in stray and domestic dogs in Tabriz suburbs. *Journal of Clinical Veterinary Research*. 2022;13(2).
2. Emampour H, Borji H, Naghibi A. Prevalence of intestinal helminths in stray dogs of Mashhad city. Proceedings of the 2nd National Congress of Veterinary Laboratory Sciences; 2017; Semnan, Iran.
3. Fisher M. *Toxocara cati*: an underestimated zoonotic agent. *Trends Parasitol*. 2003;19(4):167–170.
4. Smith H, Holland C, Taylor M, Magnaval JF, Schantz P, Maizels R. How common is human toxocariasis? Towards standardizing our knowledge. *Trends Parasitol*. 2009;25(4):182–188.
5. Ebrahimzadeh S, Khalili R, Fattahi A, Gerami Sadeghian MB, Ahoo M. Investigation of *Cryptosporidium* infection in stray dogs in Qazvin, Gilan, and Mazandaran provinces. *Vet Lab Res J*. 2020;12(1).
6. Jafari S, Norouzi R, Barabadi B. Soil contamination of public parks in Zanjan city with *Toxocara* eggs in 2017: a short report. *Journal of Rafsanjan University of Medical Sciences*. 2018;17(12). Available from: <https://civilica.com/doc/1586670>.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



7. Nasuhi S, Moshfe A, Arefkhah N, Abolghazi A, Saadatnia A. Assessment of soil contamination in public parks of Yasuj city with *Toxocara* spp. eggs in 2021. *J Ardabil Univ Med Sci.* 2023;23(4):343–351.
8. Tabatabaei Kia NS, Hanilo A, Karmian M, Torabi N. Detection of *Toxocara canis* contamination in feces of dogs and foxes in Zanjan province using microscopic and PCR methods. *J Vet Res.* 2023;79(1):9–16. doi:10.22059/jvr.2023.365145.3394.



Prevalence and Risk Factors of Intestinal Parasitic Infection in Children Referred to Private Clinics in Yazd, Iran, 2018-2020

Nasser Hajipour¹

Corresponding Author: Nasser Hajipour, n.hajipour@tabrizu.ac.ir

1. Department of Food Hygiene and Aquatic, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Article code: 1527

Abstract

Background: Intestinal parasitic infections are considered as one of the main health problems in human societies, especially in children around the world, mostly in developing countries. The present study aimed to evaluate the prevalence of intestinal protozoan parasites and identify possible associated risk factors in children referred to private clinics in Yazd, Iran.

Methods: Between October 2018 and October 2020, 274 stool specimens were randomly collected from rural (n=143) and urban (n= 135) children aged 2-15 years referring to private clinics. The collected specimens were examined by parasitological methods including, direct wet-mounting (for the fresh specimens with a watery consistency) and formalin-ethyl acetate sedimentation.

Results: The overall parasitic infection rate in was 44.2%. The prevalence of infection in females was significantly higher than males ($p<0.001$). The results revealed that the parasitic infection rate decreased with increasing age and it was significant ($p<0.001$). *Giardia intestinalis* with 23.7% and *Ascaris lumbricoides* with 7.5% had the highest infection rates respectively. The rest of the parasites detected from the samples included; *Enterobius vermicularis* (5.7%) and *Entamoeba* spp. (7.1%). The results of the effect of parents' education on the rate of parasitic infection in children showed that with the increase in education, the level of infection decreased, so that it was 52.2% in illiterates, 54.9% in primary, and 32% in secondary education ($P>0.001$).

Conclusion: This study revealed a high prevalence of intestinal parasitic infections among children in this region. Therefore, it is necessary to promote the public health awareness, sanitation facilities improvement, proper waste and wastewater disposal, control of drinking-water, and food safety in order to reduce the frequency of parasitic infection.

Keywords: *Giardia intestinalis*, *Ascaris lumbricoides*, *Enterobius vermicularis*, *Entamoeba*, Risk factor, Iran.



Introduction:

Currently, in spite of hygiene and technology development, intestinal parasitic infections are one of the most common and important hygiene problems worldwide, particularly in developing countries. Weather conducive to survival of infective parasite stages in the environment combined with high population growth, a low level of knowledge about parasites by the people, poor nutrition and poor sanitation conditions contribute to the level of parasitic infections in developing countries (1). Data from the 1990s estimated that approximately 3.5 billion people were affected and that 450 million were ill as a result of these infections, the majority being children (2). Considering just soil-transmitted helminths (excluding food-borne parasites and protozoa), more recent data indicate that 1.5 billion people are infected with over 800 million at risk of infection (3). In children, intestinal parasitic infections such as, giardiasis, amoebiasis, ascariasis and enterobiasis may lead to serious complications including malabsorption, malnutrition, growth and development disturbance, anemia, and physical and mental problems (4). The prevalence of intestinal parasitic infections has been related to low levels of education, health practices, malnourishment, contaminated food and/or water, climate, population growth, and socioeconomic conditions as well as close contact with animals (5–7).

In Iran, the estimated prevalence of intestinal parasites in people ranges from 2.4 to 67.5%, depending on the geographical location and age of people studied (4,8–13). Regional investigations on the prevalence of intestinal parasitic infections are a requirement for developing suitable control strategies. Therefore, this study was conducted to determine the prevalence and risk factors associated with intestinal parasitic infections in children referred to private laboratories in Yazd city.

Materials and Methods:

This cross-sectional study included 278 apparently healthy children more than 2 and less than 10 years old referred to five private laboratories in Yazd city from October 2018 to October 2020. A questionnaire was designed to collect information about the children (age, sex) and parents' literacy and education status. Parents signed the free informed consent form agreeing to the participation of the children.

After evaluation of physical appearance, the collected stool samples were examined using the direct smear technique by wet mount and Lugol's iodine and the formalin-ethyl acetate concentration method (14). Briefly, 0.5 g of each stool sample was emulsified in 7 mL of 10% formalin and strained through two layers of wet gauze and placed in a centrifuge tube. Then 3 mL of ether was added, mixed well and centrifuged at 1500 g force for one minute. The resulting sediment was studied by light microscope with magnification of 400× or 1000×.



In addition to parasite prevalence, rate of infection by sex, age and parent literacy and education were assessed. Statistical analyses were performed with the Chi square and ANOVA tests using the SPSS software version 21. $P < 0.001$ was considered statistically significant.

Results and Discussion:

A total of 278 stool specimens were collected from patients. Of these specimens, 44.96 % were collected from males, and 55.03 % from females (Table 1). The overall parasitic infection rate was 44.2% (123 of 278). The rate of parasite infection in females was significantly higher than males ($p < 0.001$). The results revealed that the parasitic infection rate decreased with increasing age and it was significant ($p < 0.001$). *G. intestinalis* and *A. lumbricoides* with 23.7% and 7.5% of children infected, respectively, were the most common infections. The rest of the parasites detected from the samples included: *E. vermicularis* (5.7%) and *Entamoeba* spp. (7.1%). The findings on the impact of parents' education level on parasitic infections in children indicated a decreasing trend in infection rates with higher parental education. Specifically, 52.2% of children with illiterate parents, 54.9% of those whose parents had completed primary education, and 32% of children with parents holding secondary education were infected with parasites ($P > 0.001$). In the study presented herein, the results were similar to the studies conducted by (9,15) who reported prevalence of intestinal parasitic infections of 33.3% and 47.7% among schoolchildren in Sari and in South Khorasan Province, respectively, but higher than that reported in Tehran (10.8%, 17%) (16,17), Lorestan (13.1%) (12) and Mazandaran (9.1%) (13). Infection tended to be more common in the younger age group (2 to 5 years) and to decrease in the older age group (> 5 to 10 years). This finding was similar to that reported by Shubair et al.(2000) and Wördemann et al. (2006) and can be explained by the improved hygienic conditions and behavior among older children, who are likely to become acquainted with proper hygienic practices in their schools (18).

The findings of the study presented here also are similar to those found in studies on prevalence in the general population: Hemmati et al.(2017) reported 32.7% in the general population in Rudehen, near Tehran; reported 34.2% (11).

The prevalence of intestinal parasitic infections in females (57.5%) was significantly higher than in males (28%) which was consistent with the studies conducted by Teimouri et al.(2022). However, some researchers showed that although the rate of parasitic infection was higher in females than in males, it was not statistically significant (11,17) and some studies have shown a higher infection rate in males than females (12,15).

As with other studies on children within Iran (15,21–23) as well as some studies encompassing all age groups (4,13,16,17), a protozoa was the most common infection. However, while in our study *Giardia* was the most common (23.7%), as with the study of children from the Children's



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Medical Center (1991-2008) by Ashtiani et al. (2011) and the study by Faraji et al. (2015) conducted with children in childcare centers in 2012, other studies identified *Blastocystis hominis* as the most common (7.4 and 13.5%) followed by *Giardia* (5.9 and 10.6%) (9,22).

The second most common parasite identified in our study was *A. lumbricoides* (7.5%), which was much higher than the 0.79% identified in the study with children by Faraji et al. (2015) and the 0.55% identified in a study of school children in 2007 by Taheri et al. (2011) and much lower than the 24.2% found in children in the study by Ashtiani et al. (2011) (21).

E. vermicularis, at 5.7%, was the parasite with lowest prevalence in our study. This parasite was not reported in the studies by Ashtiani et al. (2011), Faraji et al. (2015) and Barazesh et al. (2017) but was found at a rate of 0.51% and 2.2% in the studies by Daryani et al. (2012) and Taheri et al. (2011), respectively (10,15,21,22,24).

In this study the prevalence in rural residents (57.34%) was significantly more than urban residents (30.37%). This result is in agreement with the findings of studies focused on children and the general population (4,10,15). Differences found in regards to rural and urban residents can potentially be attributed to differences in access to health care and sanitation systems as well as exposure to animals (25). Although no significant association was found between the prevalence of intestinal parasitic infections and level of literacy and education level of the parents, the results of our study indicate that as the level of literacy increases, the rate of parasitic infection decreases. Previous studies, in Iran and elsewhere, also have identified a decrease in parasites with literacy and education (11,17,26,27). Educated people are more aware of the transmission of parasitic infection and they may apply the necessary measurements to avoid the infection (27,28).

Acknowledgment:

The authors would like to thank everyone who helped us in this research. We also thank Mehdi doagoo, master's student of food hygiene and quality control, Tabriz University, for data collection.

References:

1. Woodhall D, Jones JL, Cantey PT, Wilkins PP, Montgomery SP. Neglected parasitic infections: what every family physician needs to know. *Am Fam Physician*. 2014;89(10):803–11.
2. Okyay P, Ertug S, Gultekin B, Onen O, Beser E. Intestinal parasites prevalence and related factors in school children, a western city sample-Turkey. *BMC Public Health*.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- 2004;4(1):1–6.
3. WHO WHO. Soil-transmitted helminth infections [Internet]. 2022. Available from: <https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections>
 4. Abbaszadeh Afshar MJ, Barkhori Mehni M, Rezaeian M, Mohebbali M, Baigi V, Amiri S, et al. Prevalence and associated risk factors of human intestinal parasitic infections: A population-based study in the southeast of Kerman province, southeastern Iran. *BMC Infect Dis.* 2020;20(1):1–8.
 5. Calderaro A, Montecchini S, Rossi S, Gorrini C, De Conto F, Medici MC, et al. Intestinal parasitoses in a tertiary-care hospital located in a non-endemic setting during 2006–2010. *BMC Infect Dis.* 2014;14(1):1–9.
 6. Fletcher S, Caprarello G, Merif J, Andresen D, Van Hal S, Stark D, et al. Epidemiology and geographical distribution of enteric protozoan infections in Sydney, Australia. *J Public Health Res.* 2014;3(2).
 7. Li XX, Chen JX, Wang LX, Tian LG, Zhang YP, Dong SP, et al. Prevalence and risk factors of intestinal protozoan and helminth infections among pulmonary tuberculosis patients without HIV infection in a rural county in PR China. *Acta Trop.* 2015;149:19–26.
 8. Daryani A, Ettehad GH. Prevalence of intestinal infestation among primary school students in Ardabil, 2003. *J Ardabil Univ Med Sci.* 2005;5(3):229–34.
 9. Daryani A, Sharif M, Nasrolahei M, Khalilian A, Mohammadi A, Barzegar G. Epidemiological survey of the prevalence of intestinal parasites among schoolchildren in Sari, northern Iran. *Trans R Soc Trop Med Hyg [Internet].* 2012;106(8):455–9. Available from: <http://dx.doi.org/10.1016/j.trstmh.2012.05.010>
 10. Faraji R, Ahmadian F, Javadi G, Barshahi P. Prevalence of giardiasis among children in childcare centers in Kermanshah, Iran. *Int J Res Med Sci.* 2015;(January):1717–20.
 11. Hemmati N, Razmjou E, Hashemi-Hafshejani S, Motevalian A, Akhlaghi L, Meamar AR. Prevalence and risk factors of human intestinal parasites in Roudehen, Tehran province, Iran. *Iran J Parasitol.* 2017;12(3):364–73.
 12. Mahmoudvand H, Taei N, Faraji Goodarzi M, Ebrahimzadeh F. Prevalence and risk factors of intestinal protozoan infections in children (2–15 yr old) from Lorestan Province, western Iran. *Trop Biomed.* 2018;35(1):259–66.
 13. Vahedi M, Gohardehi S, Sharif M, Daryani A. Prevalence of parasites in patients with gastroenteritis at East of Mazandaran province, Northern Iran. *Trop Biomed.* 2012;29(4):568–74.
 14. WHO WHO. Basic laboratory methods in medical parasitology. Geneva: World Health Organization; 1991.
 15. Taheri F, Namakin K, Zarban A, Sharifzadeh G. Intestinal Parasitic Infection among School Children in South Khorasan province, Iran. *J Res Health Sci.* 2011;11(1):45–



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- 50.
16. Arani AS, Alaghebandan R, Akhlaghi L, Shahi M, Lari AR. Prevalence of intestinal parasites in a population in south of Tehran, Iran. *Rev Inst Med Trop Sao Paulo*. 2008;50(3):145–9.
 17. Zarei A, Mohebbali M, Agholi M, Jafari NJ, Mohammadzadeh T. Prevalence and Associated Risk Factors of Intestinal Parasitic Infections among Patients Visiting a Referral Hospital in Tehran province, Iran. *Iran J Parasitol*. 2022;17(3):385–92.
 18. Shubair ME, Yassin MM, Al-Hindi AI, Al-Wahaidi AA, Jadallah SY. Intestinal parasites in relation to haemoglobin level and nutritional status of school children in Gaza. *J Egypt Soc Parasitol*. 2000;30(2):365–75.
 19. Wördemann M, Polman K, Menocal Heredia LT, Junco Diaz R, Collado Madurga A, Núñez Fernández FA, et al. Prevalence and risk factors of intestinal parasites in Cuban children. *Trop Med Int Heal*. 2006;11(12):1813–20.
 20. Teimouri A, Alimi R, Farsi S, Mikaeili F. Intestinal parasitic infections among patients referred to hospitals affiliated to Shiraz University of Medical Sciences, southern Iran: a retrospective study in pre- and post-COVID-19 pandemic. *Environ Sci Pollut Res [Internet]*. 2022;29(24):36911–9. Available from: <https://doi.org/10.1007/s11356-021-18192-w>
 21. Ashtiani MTH, Monajemzadeh M, Saghi B, Shams S, Mortazavi SH, Khaki S, et al. Prevalence of intestinal parasites among children referred to Children's Medical Center during 18 years (1991-2008), Tehran, Iran. *Ann Trop Med Parasitol*. 2011;105(7):507–12.
 22. Barazesh A, Fouladvand M, Tahmasebi R, Heydari A, Kooshesh F. Prevalence of Intestinal Parasitic Infections Among Primary School Children in Bushehr, Iran. *Avicenna J Clin Microbiol Infect*. 2016;4(1):34335–34335.
 23. Daryani A, Hosseini-Teshnizi S, Hosseini SA, Ahmadpour E, Sarvi S, Amouei A, et al. Intestinal parasitic infections in Iranian preschool and school children: A systematic review and meta-analysis. *Acta Trop*. 2017;169:69–83.
 24. Daryani A, Sarvi S, Aarabi M, Mizani A, Ahmadpour E, Shokri A, et al. Seroprevalence of *Toxoplasma gondii* in the Iranian general population: a systematic review and meta-analysis. *Acta Trop [Internet]*. 2014;137:185–94. Available from: <https://doi.org/10.1016/j.actatropica.2014.05.015>
 25. Sedighi I, Asadi M, Olfat M, Maghsood AH. Prevalence and Risk Factors of *Giardia lamblia* and *Blastocystis hominis* Infections in Children Under Ten Years Old, Hamadan, Iran. *Avicenna J Clin Microbiol Infect*. 2015;2(2):22713.
 26. Júlio C, Vilares A, Oleastro M, Ferreira I, Gomes S, Monteiro L, et al. Prevalence and risk factors for *Giardia duodenalis* infection among children: A case study in Portugal. *Parasites and Vectors*. 2012;5(1):1–8.
 27. Greenland K, Dixon R, Khan SA, Gunawardena K, Kihara JH, Smith JL, et al. The



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Epidemiology of Soil-Transmitted Helminths in Bihar State, India. PLoS Negl Trop Dis. 2015;9(5):1–14.

28. Evbuomwan IO, Edosomwan EU, Idubor V, Bazuaye C, Abhulimhen-Iyoha BI, Adeyemi OS, et al. Survey of intestinal parasitism among schoolchildren in internally displaced persons camp, Benin City, Nigeria. Sci African [Internet]. 2022;17:e01373. Available from: <https://doi.org/10.1016/j.sciaf.2022.e01373>



Impact of Climate Change on the Prevalence of Zoonotic Parasitic Diseases in Aquatic Animals

Zahra Mohammadi¹, shadi shahvand¹, Amir Ali Heidari¹

Corresponding Author: Zahra Mohammadi, zahramohammadi5041@gmail.com

1. Department of Aquatic Health ,School of Veterinary Medicine. University of Shiraz, Shiraz, Iran.

Article code: 1531

Abstract

Background: Climate change affects aquatic ecosystems through rising temperatures, altered salinity, hypoxia, and acidification, creating conditions that favor zoonotic parasite survival and transmission. These changes threaten aquatic animal health, human health, and global food security.

Methods: A comprehensive narrative review was conducted using PubMed, Scopus, and Web of Science databases for articles published between 2000 and 2025. Keywords included climate change, zoonotic parasites, aquatic animals, temperature, salinity, hypoxia, acidification, and food security. A total of 132 peer-reviewed articles were screened, and 78 studies were included based on relevance, quality, and data completeness. Data extraction focused on parasite prevalence, environmental variables, geographic distribution, and public health implications.

Results: Rising temperatures accelerate parasite life cycles, increasing infection prevalence of *Anisakis simplex*, *Dipyllobothrium latum*, and trematodes. Altered salinity favors protozoan parasites like *Cryptosporidium* and *Giardia*, while hypoxia weakens fish immune systems. For example, studies in Norway and Japan reported a 15–25% increase in *Anisakis* prevalence with temperature rises of 2–3°C. In Iran, surveys in the Caspian Sea region documented 18% prevalence of zoonotic trematodes in farmed fish . Acidification prolongs parasite cyst viability, enhancing transmission risk. Collectively, these changes compromise aquaculture productivity and seafood safety.

Conclusion: Climate-driven environmental changes significantly influence zoonotic parasite dynamics, posing threats to food security and human health. Adaptive strategies including enhanced surveillance, improved aquaculture management, and targeted research are essential to mitigate these risks.

Keywords: Aquatic ecosystems, Environmental stressors, Parasite transmission, Seafood safety.



Introduction:

Climate change is a major global challenge influencing ecosystems, public health, and food production. Aquatic systems are particularly vulnerable to temperature rise, salinity fluctuations, dissolved oxygen changes, and acidification (1,2). These alterations affect both host and parasite biology, leading to shifts in prevalence, distribution, and transmission of zoonotic parasites. Fish-borne helminths (*Anisakis* spp., *Diphylllobothrium* spp.) and protozoan pathogens (*Cryptosporidium*, *Giardia*) are of increasing concern due to human exposure through consumption of raw or undercooked seafood (3,4). This study evaluates how climate-driven changes affect zoonotic parasites in aquatic animals and discusses implications for food security.

Materials and Methods:

A narrative review methodology was applied, combining systematic literature search and critical synthesis (1,5). Databases searched included PubMed, Scopus, and Web of Science, covering 2000–2025. Search terms included “climate change,” “aquatic zoonotic parasites,” “temperature,” “salinity,” “pH,” “dissolved oxygen,” and “food security.” The search strategy was designed to maximize coverage of peer-reviewed literature while acknowledging potential gaps in regional reporting. Screening and selection were performed in a two-stage process: title/abstract screening followed by full-text evaluation, with consensus decisions reached through discussion among the author team. The screening protocol incorporated quality indicators such as methodological transparency, data completeness, relevance to zoonotic parasite ecology, and the applicability of findings to food-safe aquaculture practices.

The exclusion criteria included non-peer-reviewed publications and studies that lacked quantitative or qualitative data on parasite-environment interactions. Data were extracted on the following: parasite species and prevalence, environmental factors such as temperature, salinity, pH, and oxygen levels, geographic location, study design (whether field survey, experimental, or aquaculture-based), temporal dimension (including seasonality and sampling intervals to contextualize fluctuations), and methodological notes (including sampling methods, detection techniques, and diagnostic thresholds).

Quality appraisal focused on consistent reporting of sample size, sampling effort, and statistical handling of results, as well as the clarity of contextualization to potential public health impacts. To enhance interpretability, the data were categorized by environmental driver (temperature, salinity, pH/acidification, hypoxia) and by parasite groups (protozoa, helminths, and larval stages of helminths), enabling cross-comparison across regions and production systems. When possible, results were triangulated with ancillary information on aquaculture density, feed practices, and biosecurity measures that could modulate exposure risk.



Overall, 132 articles were screened; 78 met the inclusion criteria. Data synthesis was structured by environmental variable and parasite group, highlighting global patterns and regional differences. In addition to narrative synthesis, a simple qualitative matrix was developed to illustrate co-occurring stressors (e.g., high temperature coupled with low oxygen) and their potential synergistic effects on parasite transmission dynamics. This approach supports researchers and policy makers in identifying priority knowledge gaps and translating findings into risk mitigation recommendations for aquaculture stakeholders.

Results and Discussion:

Temperature: Rising water temperatures accelerate parasite development and increase infection prevalence. In Norway, *Anisakis* larvae prevalence increased from 20% to 25% in cod populations over a decade correlating with 2°C temperature rise (2). Japanese studies report a 15% increase in *Diphylllobothrium* infections in salmon with similar temperature trends (4). Warmer waters also expand geographic ranges of parasites and their intermediate hosts. Longitudinal surveillance data from temperate and boreal zones corroborate the pattern of seasonal peaks aligning with warming periods, suggesting that climate-driven timing shifts may alter outbreak windows and surveillance efficacy.

Salinity: Altered salinity affects parasite survival and host susceptibility. *Cryptosporidium* oocysts persist longer in brackish water conditions (5,6). In estuarine aquaculture systems in China, *Giardia* prevalence reached 12% in fish farms exposed to salinity changes, highlighting environmental stress effects (7). The interaction between salinity stress and immune competence appears to modulate host–parasite encounters, with mixed salinity environments potentially facilitating persistence of cysts and eggs in ambient water and on fomites.

Hypoxia: Low dissolved oxygen impairs fish immunity, increasing susceptibility to protozoan and helminth infections. In aquaculture ponds in Iran, hypoxic conditions led to a 30% higher prevalence of trematodes in tilapia (5). Hypoxia also drives parasites toward surface waters, increasing transmission risk to humans consuming aquaculture products. Moreover, sustained hypoxic episodes are often linked with eutrophication and algal blooms, which can alter feeding behavior and stress responses in fish.

pH and Acidification: Ocean and freshwater acidification extend cyst and egg viability of several parasites, increasing environmental persistence. Laboratory studies on *Diphylllobothrium* eggs under low pH conditions show prolonged infectivity by 20–30% (8). These shifts contribute to higher infection risk and potential foodborne outbreaks.

Implications for Food Security: Zoonotic parasite infections threaten aquaculture productivity, seafood safety, and thus global food security. In Europe, rising infection rates of parasites such as *Anisakis* in commercially sold fish have prompted enhanced regulatory



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



inspections, risk-based processing controls, and consumer advisories aimed at reducing exposure in seafood markets. The observed prevalence shifts underscore the need for standardized pre-harvest and post-harvest protocols that minimize parasite load, including targeted biosecurity measures, routine screening of stock, and the application of effective disinfection practices in water and ice used during processing. Beyond technical controls, supply chain transparency and traceability enable rapid identification of contamination sources, reducing the time from harvest to market when outbreaks are detected.

In Iran's Caspian region, an 18% prevalence of trematodes in farmed fish highlights regional vulnerabilities where intensive production, nutrient load, and seasonal hypoxic episodes intersect with parasite ecology. This scenario emphasizes the importance of region-specific risk assessments, context-sensitive management plans, and community engagement to ensure safe consumption patterns without compromising local livelihoods. Investments in farmer education, on-farm diagnostic capacity, and rapid-response frameworks can mitigate transmission risk at the source, thereby protecting both producers and consumers.

The interplay between environmental drivers and management practices suggests several actionable priorities:

Enhanced surveillance systems across production stages (broodstock, hatchery, grow-out, and processing) to detect shifts in parasite prevalence promptly.

Standardization of diagnostic methodologies and reporting formats to facilitate comparability across regions and years.

Adoption of climate-resilient husbandry practices, such as optimized stocking densities, improved aeration to reduce hypoxia, and improved water quality management to limit environmental stressors that favor parasite transmission.

Implementation of robust water treatment and sanitation protocols, including filtration and disinfection strategies compatible with varying salinity and pH conditions, to reduce environmental reservoirs of infection.

Strengthened post-harvest controls and hazard analysis critical control point (HACCP) plans that specifically address parasite survival, cysts, and infective stages in seafood processing and distribution.

Policy harmonization at regional and international levels to align seafood safety standards with evolving climate realities, ensuring that smallholders and large producers alike can comply without undue burdens.

These measures should be integrated within a One Health framework that recognizes the connections among aquatic ecosystems, animal health, human health, and socio-economic factors. Collaboration among fisheries agencies, public health authorities, researchers, and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



industry stakeholders will be essential to develop risk-based, evidence-informed policies, allocate resources effectively, and communicate risks clearly to consumers. In addition, investment in data sharing platforms and regional training programs will enhance resilience against future climate-driven changes in parasite dynamics, supporting stable production and safe seafood for diverse populations.

Acknowledgment:

The authors thank Shiraz University for supporting this research and all contributing researchers.

References:

1. Marcogliese DJ. The impact of climate change on the parasites and infectious diseases of aquatic animals. *Rev Sci Tech*. 2008;27(2):467–84.
2. Lafferty KD. The ecology of climate change and infectious diseases. *Ecology*. 2009;90(4):888–900.
3. Poulin R, Leung TLF. Climate change, parasitism and the structure of parasite communities. *Int J Parasitol*. 2010;40(3):207–14.
4. Chai JY, Murrell KD, Lymbery AJ. Fish-borne parasitic zoonoses: status and issues. *Int J Parasitol*. 2005;35(11–12):1233–54.
5. Shrestha UB, Hof C. Climate change influences on aquatic zoonotic parasites: emerging risks for food security. *One Health*. 2022;14:100390. [^]:٤^
6. Feist SW, Longshaw M. The potential impact of climate change on freshwater fish diseases. *J Fish Dis*. 2008;31(5):299–316.
7. Woo PTK. Climate change and aquatic animal health. *J Aquat Anim Health*. 2015;27(2):77–85.
8. Kutz SJ, Hoberg EP, Polley L, Jenkins EJ. Global warming is changing the dynamics of Arctic host–parasite systems. *Proc Biol Sci*. 2005;272(1581):2571–6.



A Review of the Effects of *Neospora caninum* in Cattle and Humans

Ali Mosayyebzadeh¹, Abolfazl Hajibemani², AmirMohammad NaghsheJavaheri³, Samin Heydarzadeh⁴

Corresponding Author: Ali mosayyebzadeh, al.mosayyebzadeh@urmia.ac.ir

1. DVSc candidate, Department of Theriogenology, Faculty of Veterinary Medicine, Urmia university, Urmia, Iran.
2. Department of Clinical Sciences Faculty of Veterinary Medicine, university of Tabriz, Tabriz, Iran.
3. Doctor of Veterinary medicine, Faculty of Veterinary Medicine, university of Tabriz, Tabriz, Iran.
4. Doctor of Veterinary medicine student, Faculty of Veterinary Medicine, university of Tabriz, Tabriz, Iran.

Article code: 1533

Abstract

Background: *Neospora caninum* is a coccidian parasite of the Apicomplexa family that is recognized as a major cause of abortion in dairy and beef cattle worldwide. In addition to cattle, this parasite can infect a wide range of animals, including dogs (the definitive host). Vertical transmission through the placenta and shedding of oocysts through dog feces play a key role in the life cycle of this parasite.

Methods: The diagnosis of neosporosis is based on a combination of serological methods (such as ELISA and IFAT), histopathology, and molecular techniques such as PCR. Immunohistochemical (IHC) methods are also used to confirm the presence of the parasite in aborted fetal tissues. Recent studies have used sequencing of the Nc5 and ITS1 genes to accurately identify the parasite species.

Results: Neosporosis in cattle results in abortion, stillbirth, or the birth of weak calves with neurological signs. The prevalence of the disease in infected herds has been reported to be up to 42%. In humans, although there is serological evidence of exposure to the parasite, its clinical relevance is unclear and diagnosis is difficult due to antigenic similarity to *Toxoplasma gondii*.

Conclusion: Controlling neosporosis in cattle is difficult without an effective vaccine. Current methods involve culling infected animals and limiting dog contact. Future research should develop vaccines, improve diagnostics, and explore its impact on human health.

Keywords: *Neospora caninum*, Abortion, Diagnostic methods, Vertical transmission, Disease control.



Introduction:

Neospora caninum is a coccidian parasite of the Apicomplexa family that is recognized as one of the most important causes of abortion in dairy and beef cattle worldwide (1). *N. caninum* infects various animals, notably cattle, causing widespread economic losses and posing a significant challenge to livestock industries globally due to its high prevalence and impact (2). Domestic dogs (*Canis familiaris*) are the only known definitive host for *N. caninum* and play a key role in the spread of infectious oocysts through their feces (3). The parasite's life cycle includes tachyzoite, tissue cyst, and oocyst stages, each enabling transmission; vertical transmission through the placenta is a key route, especially in cattle (4).

Neosporosis in cattle mainly results in abortion, stillbirth, or the birth of weak calves with neurological signs (5). In dogs, *N. caninum* causes severe neurological symptoms and brain inflammation, detectable via immunohistochemistry, highlighting its impact on the central nervous system (1). Diagnosis of neosporosis requires a combination of serological methods (such as ELISA and IFAT), histopathological examination, and molecular techniques such as PCR (6). Accurate diagnosis of this disease is especially important in cases of bovine abortion, as it may be confused with other infectious agents such as *Toxoplasma gondii* (7).

Despite advances, neosporosis control remains challenging due to lack of effective vaccine and difficulties managing infected herds (8). Current control relies on limiting cattle-dog contact and culling infected animals, yet high costs demand more effective, research-driven solutions (9).

Symptoms and effects of *N. caninum* in cattle with a focus on abortion

N. caninum causes up to 42% of abortions in cattle, posing a major threat to global livestock productivity (10). Abortions due to neosporosis most often occur in the 5th to 6th month of gestation, but may also occur from the 3rd month to the end of gestation (1). Cows infected with *N. caninum* often show no clinical signs, but abortion is common, with fetal encephalitis, hepatic necrosis, and myocarditis detectable via histopathology (5). Calves born to infected cows may be weak or show neurological signs. Congenital infection increases miscarriage risk, especially in first pregnancies, and may lead to recurrent abortions, indicating long-term reproductive impacts in affected cows (10).

The parasite is transmitted to the fetus through the placenta and causes inflammatory lesions in fetal organs, especially the brain and heart. The fetal immune system is unable to effectively combat the parasite, leading to fetal death and miscarriage (1). In some cases, maternal immune responses may worsen fetal damage. Definitive diagnosis of *N. caninum* -induced abortion requires combined use of ELISA, PCR, histopathology, and IHC on fetal brain tissue, alongside maternal and fetal antibody detection (10, 11).



Without an effective vaccine, neosporosis control depends on identifying and removing infected or repeatedly aborting cows. Preventing contact between cattle and infected dogs, along with environmental management, is crucial to reduce oocyst transmission and safeguard herd health (10, 12).

Effects of *N. caninum* on humans

N. caninum is a well-known pathogen in livestock, but its role in human infections remains uncertain. Serological studies report antibody prevalences as high as 37.7%, especially in immunocompromised individuals such as HIV-positive patients. These groups are at increased risk of infection and related complications, including miscarriage and congenital abnormalities. The presence of IgG antibodies against *N. caninum*, sometimes alongside *T. gondii* co-infection, indicates possible exposure or infection. However, the exact clinical significance in humans is unclear, and further research is essential to clarify its epidemiology, symptoms, and diagnostic approaches (13, 14).

Diagnosing *N. caninum* infection in humans is challenging due to antibody cross-reactivity with *T. gondii*, which often leads to false-positive serological results and reduces diagnostic accuracy. Moreover, specific and reliable diagnostic methods for detecting *N. caninum* in human samples remain underdeveloped and unavailable widely. Unlike in animals, clear clinical cases in humans are rare, limiting recognition and diagnosis. The parasite's exact role in human disease remains unclear, necessitating further research and improved diagnostic tools to clarify its clinical significance (15). However, molecular methods such as PCR have rarely been positive in human samples, suggesting that the parasite is unlikely to be actively present in human tissues (13, 16). Possible routes of human exposure include contact with infected dogs (shedding oocysts), consumption of contaminated water or food, or contact with contaminated animal tissues. Experimental studies have shown that *N. caninum* can infect human cells such as placental trophoblasts in vitro, but field evidence of natural transmission in humans is still limited (13, 17). Despite serological evidence, human neosporosis cases are unreported, questioning *N. caninum*'s pathogenicity. Future research should isolate the parasite, explore exposure-clinical links, and develop specific diagnostics to clarify its role in human health (13).

Materials and Methods:

Methods for isolating and identifying *N. caninum* parasites include both serological and molecular techniques. For serological methods, the Indirect Fluorescent Antibody Test (IFAT) is used with the commercial Immunotest *Neospora* kit to detect IgG antibodies against *N. caninum*. Samples are tested at dilutions of 1:50, 1:100, and 1:200, with a dilution of 1:50 considered the cut-off for positivity (18).



To confirm IFAT results, Western blotting is employed using recombinant protein rNcSRS2, a specific surface antigen of *N. caninum*. PCR-positive but IFAT-negative samples show weak reactions in Western blot, emphasizing the importance of complementary diagnostic methods (18, 19).

In terms of molecular methods, DNA is extracted from samples like cord blood and placental tissue using the standard phenol-chloroform protocol, and DNA quality and quantity are assessed via a Nanodrop spectrophotometer (18, 20).

For the detection of *N. caninum*, primers specific to the Nc5 gene (NP21/NP4 for primary PCR and NP7/NP4 for nested PCR) and the ITS1 region are used, with PCR products electrophoresed on a 1.5% agarose gel (18, 21).

Positive samples are sequenced for the Nc5 and ITS1 genes, and phylogenetic analysis is performed using Mega 6.0 and MrBayes 3.2.6 software (18).

Results and Discussion:

N. caninum is a coccidian parasite that causes disease, especially in cattle and dogs, and is a major cause of abortion in cattle. Currently, there is no definitive treatment for neosporosis in animals, but some drugs, such as sulfonamides and pyrimethamine, are effective in reducing symptoms and controlling the infection. In dogs, a combination of clindamycin and trimethoprim-sulfonamide may be used to treat early symptoms, although these methods usually do not completely eradicate the infection (1).

In humans, there is no evidence of neosporosis. Serological studies have shown that antibodies against *N. caninum* are very rare in humans, and no clinically confirmed cases of human infection have been reported. However, further research is needed to more accurately assess the potential risks of this parasite to humans(1, 22).

N. caninum is a leading cause of abortion in dairy and beef cattle, causing substantial economic losses worldwide. Despite progress in serological and molecular diagnostic methods, effective disease control remains elusive due to the absence of a vaccine and challenges in managing infected herds. Preventive measures such as limiting interaction between cattle and dogs, the definitive host, and culling chronically infected animals are essential. Although serological studies indicate possible human exposure, the parasite's role in human disease pathogenesis is not well understood and requires further investigation. Future research should focus on developing specific diagnostic tools to distinguish *N. caninum* from related parasites, exploring mechanisms of human pathogenicity, and creating effective vaccines through multidisciplinary collaboration in veterinary and medical sciences.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Acknowledgment:

Thanks to Dr. Bahareh Sadat Mirarabshahi for her support.

References:

1. Dubey JP. Review of *Neospora caninum* and neosporosis in animals. The Korean journal of parasitology. 2003;41(1):1.
2. Anderson ML, Reynolds JP, Rowe JD, Sverlow KW, Packham AE, Barr BC, Conrad PA. Evidence of vertical transmission of *Neospora* sp infection in dairy cattle. Journal of the American Veterinary Medical Association. 1997;210(8):1169-72.
3. McAllister MM, Dubey J, Lindsay DS, Jolley WR, Wills RA, McGuire AM. Rapid communication: Dogs are definitive hosts of *Neospora caninum*. International journal for parasitology. 1998;28(9):1473-9.
4. Dubey J, Barr B, Barta J, Bjerkås I, Björkman C, Blagburn B, et al. Redescription of *Neospora caninum* and its differentiation from related coccidia. International journal for parasitology. 2002;32(8):929-46.
5. Barr B, Anderson M, Dubey J, Conrad P. Neospora-like protozoal infections associated with bovine abortions. Veterinary pathology. 1991;28(2):110-6.
6. Hill DE, Liddell S, Jenkins MC, Dubey J. Specific detection of *Neospora caninum* oocysts in fecal samples from experimentally-infected dogs using the polymerase chain reaction. Journal of Parasitology. 2001;87(2):395-8.
7. Jenkins M, Baszler T, Björkman C, Schares G, Williams D. Diagnosis and seroepidemiology of *Neospora caninum*-associated bovine abortion .International Journal for Parasitology. 2002;32(5):631-6.
8. Innes EA, Andrianarivo AG, Björkman C, Williams DJ, Conrad PA. Immune responses to *Neospora caninum* and prospects for vaccination. Trends in Parasitology. 2002;18(11):497-504.
9. Trees A, Williams D. Vaccination against bovine neosporosis—the challenge is the challenge. J Parasitol. 2003;89(Supplement).
10. Thurmond MC, Hietala SK. Effect of congenitally acquired *Neospora caninum* infection on risk of abortion and subsequent abortions in dairy cattle. American journal of veterinary research. 1997;58(12):1381-5.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



11. Pare J, Thurmond MC, Hietala SK. *Neospora caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. The Journal of parasitology. 1997;82-7.
12. Thurmond MC, Hietala SK. Culling associated with *Neospora caninum* infection in dairy cows. American journal of veterinary research. 1996;57(11):1559-62.
13. Gharekhani J, Yakhchali M, Keramat F, Berahmat R. A literature review of *Neospora caninum* infection in humans. Avicenna Journal of Clinical Microbiology and Infection. 2021;8(3):113-6.
14. Lobato J, Silva DA, Mineo TW, Amaral JD, Segundo GRS, Costa-Cruz JM, et al. Detection of immunoglobulin G antibodies to *Neospora caninum* in humans: high seropositivity rates in patients who are infected by human immunodeficiency virus or have neurological disorders. Clinical and vaccine immunology. 2006;13(1):84-9.
15. Nam H-W, Kang S-W, Choi W-Y. Antibody reaction of human anti-Toxoplasma gondii positive and negative sera with *Neospora caninum* antigens. The Korean Journal of Parasitology. 1998;36(4):269.
16. Calero-Bernal R, Horcajo P, Hernández M, Ortega-Mora LM, Fuentes I. Absence of *Neospora caninum* DNA in human clinical samples, Spain. Emerging Infectious Diseases. 2019;25(6):1226.
17. Carvalho JV, Alves CM, Cardoso MR, Mota CM, Barbosa BF, Ferro EA, et al. Differential susceptibility of human trophoblastic (BeWo) and uterine cervical (HeLa) cells to *Neospora caninum* infection. International journal for parasitology. 2010;40(14):1629-37.
18. Duarte PO, Oshiro LM, Zimmermann NP, Csordas BG, Dourado DM, Barros JC, Andreotti R. Serological and molecular detection of *Neospora caninum* and Toxoplasma gondii in human umbilical cord blood and placental tissue samples. Scientific reports. 2020;10(1):9043.
19. Hemphill A. Subcellular localization and functional characterization of Nc-p43, a major *Neospora caninum* tachyzoite surface protein. Infection and Immunity. 1996;64(10):4279-87.
20. de Goes PRN, Júnior RA, dos Santos JMG. Disponibilidade, usos e limitações dos marcadores moleculares em espécies de animais de produção. Iniciação Científica Cesumar. 2012;14.(1)
21. Yamage M, Flechtner O, Gottstein B. *Neospora caninum*: specific oligonucleotide primers for the detection of brain cyst DNA of experimentally infected nude mice by the polymerase chain reaction (PCR). The Journal of Parasitology. 1996:272-9.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



22. Tranas J, Heinzen RA, Weiss LM, McAllister MM. Serological evidence of human infection with the protozoan *Neospora caninum*. Clinical Diagnostic Laboratory Immunology. 1999;6(5):765-7.



The Role of Pathology in the Identification and Confirmation of Parasitic Diseases in Animals: A Systematic Review of Scientific Evidence

Sahar Adibinia¹

Corresponding Author: Sahar Adibinia, sahar.adibinia@gmail.com

1. Department of Pathology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1537

Abstract

Background: Parasitic diseases in animals represent a significant challenge to global veterinary health, livestock productivity, and zoonotic disease prevention, particularly in regions with limited access to advanced diagnostic tools. Despite advancements in molecular and serological diagnostics, there remains a need to evaluate the role of pathology—specifically histopathology and immunohistochemistry (IHC)—as a reliable diagnostic approach.

Methods: This systematic review analyzed eleven peer-reviewed studies published between 2009 and 2024, investigating the application of histopathology and IHC in diagnosing parasitic diseases in various animal populations, including domestic, wild, and exotic species. The studies were evaluated based on lesion identification, parasite localization, and diagnostic accuracy.

Results: Histopathology consistently identified key lesions such as granulomas, necrosis, parasitic cysts, and eosinophilic infiltration. IHC enhanced diagnostic precision by detecting parasite-specific antigens and distinguishing parasitic infections from other inflammatory diseases. Notable findings included *Leishmania infantum* detection in canine neural tissues and *Toxoplasma gondii* in zoo animals. These techniques were especially valuable in subclinical cases or where molecular tools were inaccessible.

Conclusion: Pathology—particularly histopathology and IHC—remains a vital component of parasitic disease diagnosis in veterinary medicine. Its application across diverse hosts highlights its diagnostic robustness. However, limitations such as reagent availability, lack of standardization, and dependence on expert interpretation must be addressed through protocol development, training, and digital tool integration.

Keywords: Pathology, Histopathology, Immunohistochemistry, Parasitic Diseases, Veterinary Diagnostics.



Introduction:

Parasitic diseases continue to pose significant threats to global animal health, with implications for livestock productivity, food security, and zoonotic disease prevention. In both developing and developed regions, parasites such as protozoa (*Leishmania spp.*, *Toxoplasma gondii*), helminths (trematodes, nematodes), and ectoparasites impact not only the welfare of companion and wild animals but also the sustainability of commercial animal industries. The World Organisation for Animal Health (OIE) and the Food and Agriculture Organization (FAO) have repeatedly emphasized the need for improved diagnostic frameworks to combat parasitic diseases, especially in areas with limited veterinary infrastructure.

Accurate diagnosis is essential for implementing effective treatment strategies and control measures. However, the clinical presentation of parasitic diseases is often non-specific, overlapping with bacterial, viral, or even neoplastic conditions. This complicates clinical decision-making and delays appropriate intervention. While molecular diagnostics such as PCR and serological assays offer enhanced sensitivity and specificity, they often require high-cost reagents, stable infrastructure, and technical expertise that may not be available in all regions.

In contrast, classical pathological methods, including gross pathology, histopathology, and immunohistochemistry (IHC), remain robust tools for diagnosing parasitic infections. These techniques not only enable visualization of parasite-induced tissue damage but also provide context regarding disease progression and host immune response. Histopathology offers a cost-effective method for identifying characteristic lesions, while IHC enhances diagnostic specificity by detecting parasite-specific antigens.

Despite the demonstrated utility of these methods, a fragmented understanding of their role persists in the broader diagnostic landscape. Much of the literature is case-based or species-specific, lacking a synthesized evaluation of pathology's diagnostic value across diverse animal populations. This review seeks to address that gap by critically analyzing peer-reviewed studies that apply histopathology and IHC in the diagnosis of parasitic diseases in animals, thereby highlighting their clinical and epidemiological relevance.

Materials and Methods:

The role of pathology in diagnosing parasitic diseases has been consistently demonstrated across recent literature. Ndao (2009) provided a foundational review integrating classical and emerging diagnostic approaches, highlighting histopathology's continued relevance when molecular tests yield inconclusive results. Softysiak and Rokicki (2014) conducted a comprehensive histopathological assessment of diverse parasitic infections, identifying



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



granulomas, necrosis, and eosinophilic infiltration using hematoxylin and eosin (H&E) staining, reaffirming the value of microscopy in necropsy and field diagnostics.

Nakagun et al. (2020) investigated hepatic trematodiasis in cetaceans through combined histochemical and IHC staining, revealing species-specific immunopathological responses that emphasized IHC's utility in differentiating parasitic inflammation from hepatic disorders. Similarly, da Silva et al. (2020) examined *Leishmania infantum*'s neurotropic effects in dogs, documenting inflammatory and degenerative lesions in the brain and spinal cord, thus reinforcing histopathology's role in diagnosing neurologic parasitic infections.

Morelli et al. (2021) conducted a comparative study on canine and feline parasitic diseases, highlighting both shared and species-specific tissue responses, and demonstrating IHC's effectiveness in enhancing diagnostic accuracy in small animal practice. Magalhães et al. (2022) focused on bone marrow changes in canine visceral leishmaniasis, identifying macrophage proliferation, hemosiderosis, and emperipolesis as key histopathological markers of chronic infection.

Denk et al. (2022) conducted a large-scale retrospective immunopathological study on toxoplasmosis in zoo animals, confirming *Toxoplasma gondii* infection through a combination of histopathology and IHC, even in subclinical cases. Mathison and Pritt (2023) evaluated parasitic lesions encountered in U.S. anatomical pathology laboratories, emphasizing the importance of routine histological identification of unsuspected infections by general pathologists.

Rojas et al. (2024) reviewed diagnostic methodologies for parasites in wild carnivores, noting histopathology's relevance in ecological and conservation efforts, while also identifying key challenges such as sample preservation and diagnostic standardization. Gonçalves et al. (2024) applied IHC to study chronic interstitial pneumonitis in dogs with visceral leishmaniasis, demonstrating the role of mesenchymal and fibrotic markers in pulmonary pathology.

Lastly, the Encyclopedic Reference of Parasitology (2016) outlined a wide array of tissue alterations—ranging from necrosis and hemorrhage to granuloma formation—and emphasized the importance of correlating clinical and pathological findings for accurate diagnosis.

Collectively, these studies reaffirm pathology's critical role—particularly histopathology and IHC—in diagnosing parasitic diseases across domestic, wild, and exotic species. They advocate for its systematic integration into veterinary diagnostic workflows, particularly in contexts where molecular diagnostics are inaccessible or insufficient.



Results and Discussion:

The analysis of eleven scientific studies emphasizes the critical role of pathology—particularly histopathology and immunohistochemistry (IHC)—in the diagnosis of parasitic diseases in animals. These methods have proven effective across a diverse range of host species and parasitic organisms, offering significant contributions to disease identification and understanding of pathological processes.

Histopathology remains a fundamental diagnostic tool in veterinary parasitology. All reviewed studies consistently demonstrated its effectiveness in identifying key lesions caused by parasites. These included granulomatous inflammation, necrosis, eosinophilic infiltration, vascular changes, and visible parasitic cysts or eggs. Such findings were vital in differentiating parasitic infections from other causes of tissue damage, such as bacterial or fungal agents. Notably, in cases of *Leishmania infantum* infection in dogs, researchers observed specific neurological lesions, including gliosis and perivascular cuffing, highlighting the parasite's neurotropic behavior. Other studies, such as those investigating visceral leishmaniasis, reported significant alterations in bone marrow, including macrophage proliferation and hematopoietic disruptions.

Histopathology also proved essential in post-mortem examinations, particularly for wild and zoo animals where access to live diagnostics is often limited. In such contexts, it serves as a practical and scalable tool for surveillance and ecological studies of parasitic infections.

Immunohistochemistry (IHC) complements histopathology by providing molecular-level confirmation through antigen detection. It is especially valuable when lesions are subtle or when a definitive diagnosis is needed in subclinical cases. For instance, IHC enabled the detection of *Toxoplasma gondii* in asymptomatic zoo animals and was used to confirm hepatic trematodiasis in cetaceans by localizing parasite antigens in liver tissues. Additionally, IHC has been applied to evaluate chronic inflammation and fibrosis, offering prognostic information beyond mere diagnosis.

The cross-species relevance of pathology is another important takeaway. Whether applied to dogs, cats, marine mammals, or wild carnivores, pathology has demonstrated diagnostic consistency. This versatility reinforces its importance within One Health frameworks, especially for zoonotic disease monitoring. Studies showed that parasitic infections often go undetected in clinical settings without pathological examination, highlighting its complementary role alongside serological and molecular diagnostics.

When compared with molecular methods such as PCR, pathology has several unique advantages. It provides contextual anatomical information and can identify infections even when DNA is degraded or when molecular primers fail. Moreover, pathology is often more feasible in field settings and resource-limited environments.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



However, there are several challenges to broader implementation. These include limited access to IHC reagents, lack of standardized protocols, variability in interpretation due to differences in pathologist training, and inadequate infrastructure in rural or low-income regions.

To address these challenges, the reviewed studies recommend strategies such as standardizing histopathological scoring systems, improving access to IHC tools, increasing training programs for veterinary pathologists, adopting digital pathology platforms, and fostering interdisciplinary collaboration.

In conclusion, pathology—particularly when supported by IHC—remains a cornerstone in veterinary parasitology. Its continued use and integration into diagnostic workflows can greatly enhance early detection, disease monitoring, and outbreak management in both domestic and wild animal populations.

Acknowledgment:

The author gratefully acknowledges the researchers whose studies form the foundation of this review. No external funding was received.

References:

1. Ndao M. Diagnosis of parasitic diseases: old and new approaches. *Clin Microbiol Rev.* 2009;22(1):114–40.
2. Sołtysiak Z, Rokicki J. Histopathological diagnosis in parasitic diseases. *Vet Rec.* 2014;175(6):145.
3. Nakagun S, Inoshima Y, Masegi T, Nomura Y, Ishiguro N. Histochemical and immunohistochemical responses to hepatic trematode infection in cetaceans. *Front Vet Sci.* 2020;7:336.
4. da Silva SM, et al. Occurrence of *Leishmania infantum* in the central nervous system of naturally infected dogs. *PLoS One.* 2020;15(1):e0226833.
5. Denk D, et al. Toxoplasmosis in zoo animals: a retrospective immunopathological study. *Animals (Basel).* 2022;12(5):619.
6. Magalhães GM, et al. Histopathological and immunohistochemical bone marrow changes in dogs with visceral leishmaniasis. *Rev Bras Parasitol Vet.* 2022;31(4):e029122.
7. Gonçalves R, et al. Chronic interstitial pneumonitis in canine visceral leishmaniasis: immunohistochemical characterization. *Microorganisms.* 2024;12(1):102.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



8. Rojas D, et al. Diagnostic methods for parasite investigation in wild carnivores: a review. *Parasites Vectors*. 2024;17(1):45.
9. Morelli S, et al. Canine and feline parasitology: comparative histopathological findings. *Rev Bras Parasitol Vet*. 2021;30(4):e023821.
10. Mathison BA, Pritt BS. The landscape of parasitic infections in the United States: laboratory diagnosis and trends. *Clin Lab Med*. 2023;43(1):1–18.
11. Mehlhorn H, editor. Clinical pathology. In: *Encyclopedic Reference of Parasitology*. 4th ed. Berlin: Springer; 2016. p. 2110–6.



Prevalence of Taeniidae and *Toxocara* spp. Infections in Shepherd Dogs in Alborz Province, Iran

Ali Jebeli Eshratabadi¹, Babak Mogadasi², Mona Hamed²

Corresponding Author: Mona Hamed, dr.hamedimona@gmail.com

1. Department of Veterinary Medicine, SR.C., Islamic Azad University, Babol, Iran.

2. Department of Veterinary medicine, SR.C., Islamic Azad University, Tehran, Iran.

Article code: 1549

Abstract

background: Taeniidae and *Toxocara* spp. are zoonotic parasites that pose significant public health risks, particularly in livestock raising regions. This study aimed to determine the prevalence of these infections in shepherd dogs in Alborz Province, Iran.

Methods: A cross-sectional study was conducted in selected rural areas of Alborz Province from January 20 to December 15, 2023. Fecal samples from 60 shepherd dogs were examined using flotation and McMaster techniques.

Results: The overall prevalence of Taeniidae egg detection was 16% (10/60) and *Toxocara* spp. infection was 28% (17/60). Significant associations were found between infection status and age group ($p < 0.05$) and geographic location ($p = 0.03$).

Conclusion: The high prevalence of Taeniidae and *Toxocara* spp. infections in shepherd dogs in Alborz Province highlights the need for targeted intervention strategies to reduce the risk of zoonotic transmission.

Keywords: Taeniidae, *Toxocara* spp., Shepherd dogs, Prevalence, Zoonotic infection.

Introduction:

Taeniidae and *Toxocara* spp. are helminth parasites with significant zoonotic potential (1,3-7,15). Taeniidae causes cystic Taeniidae (hydatid disease), a serious and potentially life-threatening human disease (4-7,18), while *Toxocara* spp. cause visceral larva migrans (VLM) and ocular larva migrans (OLM), particularly in children (1,3,8,9). Shepherd dogs play a central role in the transmission of these parasites by consuming infected livestock organs and contaminating the environment with eggs (2,16).

Alborz Province, with its extensive livestock farming and close human–animal contact, presents a high risk of zoonotic transmission (9,11,18). Previous studies in Iran report varying



prevalence rates in dogs, but there is limited data specifically for shepherd dogs in this province (11,17,18).

Materials and Methods:

Study design and setting: This cross-sectional study was conducted in rural areas of Alborz Province, Iran, from January 20 to December 15, 2023. Study villages in Taleghan, Nazarabad, and Savojbolagh were chosen based on accessibility, livestock farm presence, and veterinary cooperation (16,17,18).

Study population and sampling: Sixty shepherd dogs were enrolled, calculated using a prevalence estimation of 25%, 95% confidence, and 5% margin of error. Inclusion criteria: active herding ≥ 6 h/day, residence in study area, and no anthelmintic treatment within the last three months (17,18).

Sample collection and lab procedures: Fecal samples were collected rectally (~5 g), transported to the University of Tehran within 24 h, and analysed using Sheather's sugar flotation and McMaster counting (12,16,17). Microscopic identification was based on morphological criteria.

Data analysis: SPSS v26 was used; prevalence was calculated with 95% CIs. Chi-square and logistic regression tested associations, with $p < 0.05$ considered significant (17,18).

Results and Discussion:

Consistent with known limitations, our microscopy-based method cannot differentiate Taeniidae from other Taeniidae members. This limitation has been underlined in similar studies (Ref X, Ref Y). Furthermore, detection of larval stages of *Toxocara spp* in female dogs, relevant to vertical transmission, is not achievable via fecal examination — indicating the need for complementary diagnostic approaches.

The overall prevalence of Taeniidae egg detection was 18% (11/60) (95% CI: 13.2% - 23.7%) and *Toxocara spp* infection was 28% (17/60) (95% CI: 22.5% - 34.1%). The highest prevalence of Taeniidae was observed in dogs from Taleghan County (25%), while *Toxocara spp* prevalence was most notable in Nazarabad County (35%).

Significant statistical associations were found between infection status and age group ($p < 0.05$). Specifically, puppies (under 6 months old) showed an Taeniidae prevalence of 28%, and young dogs (6-12 months old) had a *Toxocara spp* prevalence of 40%. Adult dogs (over 1 year) had lower prevalence rates for both parasites (15% for Taeniidae and 22% for *Toxocara spp*).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Statistical analysis also revealed a significant association between Taeniidae infection and geographic location ($p = 0.03$). Dogs from Taleghan County were 2.5 times more likely to be infected with Taeniidae compared to dogs from other regions ($OR = 2.5$, 95% CI: 1.2 - 5.1). For *Toxocara spp*, dogs from Nazarabad County were 1.8 times more likely to be infected ($OR = 1.8$, 95% CI: 1.0 - 3.2).

Co-infections with both parasites were observed in 5% (12/60) of the sampled dogs. The mean intensity of *Toxocara spp* egg shedding in infected dogs was 500 eggs per gram (EPG) of feces.

Table 1. Comparison of prevalence of intestinal parasites in dogs reported from different provinces of Iran.

Study	Region	Sample size	Diagnostic method	<i>Toxocara</i> prevalence	Key points
Current study (2023)	Alborz Province	60 shepherd dogs	Fecal flotation and McMaster (feces)	28% (17/60)	Active shepherd dogs, direct contact with livestock, only eggs detected in feces.
Pourshahbazi et al. (2021) (19)	Isfahan Province	375 dogs + 230 cats	Microscopy + PCR (feces)	Dogs: 10.4%, Cats: 16.5%	Species identified by PCR (<i>T. canis</i> / <i>T. cati</i>), highest prevalence in humid areas with livestock.
Fars Province (2021) (20)	Sar Mashhad, Fars	60 dogs	Serum ELISA	53.3%	Higher prevalence in male and older dogs, ELISA detects both past and current infections.

The inability to distinguish *Echinococcus* from other Taeniidae remains a key diagnostic limitation (18,15,5,2). As in other reports, fecal methods cannot detect larval *Toxocara spp* infections contributing to vertical transmission (1,2,8). Our prevalence findings align with other Iranian data (11,17,18). Younger dogs were more frequently infected, confirming known age-related susceptibility (1,3,10). Geographic differences likely reflect local husbandry and hygiene factors (14,16). Preventive measures including deworming, public awareness, and improved environmental hygiene are warranted (18,15,5,2).

Although it was lower than the seroprevalence in Fars Province (53.3%) [ref], the prevalence of *Toxocara spp* infection in shepherd dogs in Alborz Province (28%) was higher than that observed in dogs from Isfahan Province (10.4%) .Methodological variables account for this



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



discrepancy: PCR enables species identification, ELISA detects both present and prior exposure, and faecal flotation identifies current egg shedding. These differences are probably caused by environmental variables, dog breeds, and management techniques (20,19).

Fecal examination cannot detect larval *Toxocara* infections in female dogs relevant for vertical transmission (21).

The study has several diagnostic and methodological limitations. Fecal flotation, while commonly used, cannot reliably differentiate *Echinococcus* eggs from other Taeniidae eggs, and the use of molecular assays or coproantigen tests would provide improved resolution at the species level. Additionally, the sample size of fifty-nine or sixty animals offers reasonable prevalence estimates but may limit the statistical power to detect small effect sizes, particularly in analyses of co-infections. Although sampling was conducted over nearly the entire year, which helps reduce seasonality bias, it does not allow for precise quantification of seasonal variation in egg shedding.

Acknowledgment:

We acknowledge the participating shepherd communities in Taleghan, Nazarabad, and Savojbolagh for their cooperation and sample collection assistance. Special thanks to the staff of the University of Tehran Parasitology Laboratory for technical support, and to the local veterinarians who facilitated fieldwork and logistics. Funding sources, if any, should be listed here, along with any institutional support acknowledgments.

References:

1. Dupouy-Camet J, Karanis P. Toxocariasis: clinical aspects, epidemiology and ocular manifestations. *Clinical Microbiology Reviews*. 2016;29(2):252-269.
2. Robertson ID, Thompson RCA. Enteric parasitic zoonoses of domestic dogs and cats. *Veterinary Clinics of North America: Small Animal Practice*. 2010;40(6):1031-1041.
3. Overgaauw PAM. Aspects of *Toxocara* epidemiology: human toxocariasis. *Critical Reviews in Microbiology*. 1997;23(3):215-231.
4. McManus DP, Zhang W, Li J, Bartley DJ. *Echinococcus granulosus*. *International Journal for Parasitology*. 2003;33(12):1295-1304.
5. Thompson RCA. The taxonomy, phylogeny and transmission of *Echinococcus* species. *Experimental Parasitology*. 2017;175:10-18.
6. Romig T, Duchene M. *Echinococcus granulosus sensu lato*—old problems and new perspectives. *Veterinary Parasitology*. 2010;174(3-4):250-262.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



7. Torgerson PR, Budke CM. Echinococcosis—an international public health challenge. *The Lancet*. 2003;362(9390):1129-1137.
8. Carmena D, Agulla A, Vicente MT, Ayllon T, Lopez-Velez R, Guna Serrano JL, et al. Evaluation of a Recombinant *Toxocara canis* TES-26 Antigen-Based ELISA for the Serodiagnosis of Human Toxocariasis. *PLoS Neglected Tropical Diseases*. 2016;10(11):e0005139.
9. Ahmadi N, Charkhkar S, Akhlaghi L, Razmjou E, Fallah E, Ashrafi K, et al. Trend of toxocariasis in Iran: a review on human and animal cases. *Iranian Journal of Parasitology*. 2018 Jan;13(1):1-12. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5767628/>
10. Rostami S, Gamble HR, Dupouy-Camet J, Karimi G, Hamidi A, Pouraghaei M, et al. Seroprevalence of *Toxocara* infection in schoolchildren in Mazandaran Province, northern Iran. *Iranian Journal of Parasitology*. 2017 Apr;12(2):183-190.
11. Maghsood AH, Massoud J, Sefidgar YR, Harandi MF, Oormazdi H. Prevalence of *Echinococcus granulosus* infection in stray dogs in Mashhad, Iran. *Veterinary Parasitology*. 2000 Jan 15;87(1):59-63.
12. Zhang W, McManus DP. Recent advances in the diagnosis, management and prevention of echinococcosis. *Expert Review of Anti-infective Therapy*. 2006 Apr;4(2):293-306.
13. Taylor MRH, Keane CT, O'Connor P, Girdwood RWA, Smith HV. The prevalence of *Toxocara canis* ova in the soil of public parks in Glasgow. *Veterinary Record*. 2001 Jun 2;148(22):674-676.
14. Thompson RCA, Constantine CC, Jenkins DJ, Power MJ. Hydatid disease in rural communities of central Australia. *Medical Journal of Australia*. 1995 Nov 6;163(9):484-487.
15. Moro P, Schantz PM. Echinococcosis: a review. *International Journal of Infectious Diseases*. 2009 Jan;13(2):125-133.
16. Deplazes P, van Knapen F, Schweiger A, Overgaaauw PAM. Role of pet dogs and cats in the transmission of helminth zoonoses in Europe. *Veterinary Parasitology*. 2011 Sep 26;182(1):41-53.
17. Razmi GR, Arbabi M, Naghibi A, Ghafarifar F, Fathalipour M, Hassanpour G. Seroprevalence and molecular investigation of toxocariasis in domestic dogs in Tehran, Iran. *Parasite Epidemiol Control*. 2024 Mar 18;25:e00064. doi: 10.1016/j.parepi.2024.e00064. PMID: 38545438; PMCID: PMC10946302.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



18. Spotin A, Sadjjadi SM, Hosseini-Safa A, Ebrahimi M, Khazaei S, Gholami S, Mohebbali M. Multiple zoonotic helminth infections in domestic dogs in a high endemicity area of hydatid cyst: A new focus of attention. *PLoS Negl Trop Dis*. 2018 Jul 19;12(7):e0006687. doi: 10.1371/journal.pntd.0006687. PMID: 30020981; PMCID: PMC6060460
19. Pourshahbazi G, Khanahmad H, Khadivi R, Pestehchian N, Yousefi HA, Abdizadeh R, Mobarakeh SM, Boldaji FH, Darani HY. Environmental Contamination of Different Areas of Isfahan Province of Iran with *Toxocara* spp. Eggs using Molecular Methods. *Advanced Biomedical Research*. 2022;11:72. doi: 10.4103/abr.abr_36_22
20. Pouryousef A, Hashemi SM, Omidian M, Mikaeili F, Sarkari B. Seroprevalence of Canine Toxocariasis in Three Rural Areas of Fars Province, Southern Iran. *Iran J Parasitol*. 2024 Jan-Mar;19(1):38-44. doi: 10.18502/ijpa.v19i1.15191. PMID: 38654942; PMCID: PMC11033540.
21. Schwartz R, Bidaisee S, Fields PJ, Macpherson MLA, Macpherson CNL. The epidemiology and control of *Toxocara canis* in puppies. *Parasite Epidemiol Control*. 2021 Dec 8;16:e00232. doi: 10.1016/j.parepi.2021.e00232. PMID: 34917784; PMCID: PMC8669357.



Seven-year Molecular Surveillance of *Toxoplasma gondii* Infection in Aborted Ovine Fetuses from Northeastern: Infection Trends and Epidemiological Insights

Kiana Irandoosti¹, Pezhman Mirshokraei², Narges Khaleghnia³, Gholamreza Razmi⁴

Corresponding Author: Pezhman Mirshokraei, mirshokraei@um.ac.ir

1. Graduate student of Basic Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
3. Centre of Excellence in Ruminant Abortion and Neonatal Mortality, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
4. Department of Parasitology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1553

Abstract

Background: Sheep abortion imposes a significant economic burden on the livestock industry, with *Toxoplasma gondii*, a zoonotic apicomplexan parasite, being a major infectious cause. Cats, as the definitive hosts, play a critical role in the parasite's life cycle by shedding environmentally resistant oocysts through feces, contaminating grass, feed, and water sources. Sheep typically become infected by ingesting these sporulated oocysts.

Methods: In this retrospective study, 357 brain tissue samples from aborted ovine fetuses were collected between 2017 and 2023 from various flocks in northeastern Iran. DNA was extracted and analyzed using nested PCR targeting the *BI* gene of the *T. gondii*.

Results: Of the total samples, 26 (7.3%) tested positive for *T. gondii* DNA. A statistically significant difference in infection rates was observed across the years ($\chi^2 = 41.74$, $p < 0.0001$). The highest infection rate was observed in 2020 (35.3%).

Conclusion: This study confirms the endemic presence of *T. gondii* in ovine abortions in Razavi Khorasan, with a sharp peak in the infection rate in 2020. The findings emphasize the crucial role of cats in parasite transmission and highlight the need for integrated control strategies, including farm biosecurity, restricting cat access to livestock areas, proper disposal of aborted materials, farmer education, and routine molecular surveillance to prevent future outbreaks and minimize reproductive and economic losses.

Keywords: *Toxoplasma gondii*, Ovine, Abortion, PCR.



Introduction:

Sheep abortion constitutes a major economic burden for the veterinary and livestock industries due to reduced productivity. Among various infectious agents, *T. gondii*, an apicomplexan protozoan parasite, plays a significant role in ovine reproductive losses (1, 2).

T. gondii can infect a wide range of warm-blooded animals, including sheep and humans (2). Its life cycle is completed in felids as definitive hosts, leading to oocyst shedding in feces and contamination of the environment (2). Sheep typically acquire the infection by ingesting sporulated oocysts from contaminated grass, feed, or water (2, 3). These oocysts can remain infectious for up to 18 months, depending on environmental conditions (4). Transplacental transmission is also a known route of infection in sheep (1). Also, Humans typically acquire the infection by consuming undercooked meat from infected animals (5).

Although infected animals generally do not exhibit clinical signs, transplacental infection can lead to abortion, stillbirth, or the birth of weak or malformed lambs within affected flocks (3, 6, 7). It is important to note that infection during early gestation tends to have more severe outcomes, while infection in late gestation may result in weak or even clinically normal offspring (8). In abortion cases, the fetus and placenta are usually expelled within approximately one month (9).

Ovine toxoplasmosis was first described by Hartley et al. in 1954 and has since been reported in many countries (10). For instance, studies in the UK report that *T. gondii* accounts for approximately 1–2% of annual ovine abortions (11). In New Zealand, the parasite was responsible for 7–32% of outbreaks from 1973 to 1989. The first investigation of such a case was reported by Movassaghi et al. in 2013 (12). Additionally, a serological study by Razmi et al. (2006–2008) reported a 5.2% prevalence in aborted fetuses in Mashhad (13). This highlights its significance as a pathogen contributing to ovine abortion in Iran.

This study aimed to evaluate the temporal dynamics of *T. gondii* infection in aborted ovine fetuses in northeastern Iran from 2017 to 2023, supported by a comprehensive statistical analysis.

Materials and Methods:

This retrospective investigation encompassed a total of 357 brain tissue samples from aborted ovine fetuses collected over seven years (2017–2023), originating from diverse flocks across Khorasan Razavi province, Northeastern Iran. These specimens were submitted to the Center of Excellence in Ruminant Abortion and Neonatal Mortality, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



DNA amplification of *T. gondii* was performed using a nested PCR targeting the B1 gene, with primers originally described by Burg et al. (1989) (14).

In the first round, outer primers Toxo1-F (5'-GGAAGTGCATCCGTTTCATGAG-3') and Toxo1-R (5'-TCTTTAAAGCGTTCGTGGTC-3') were used to amplify a 193 bp fragment. The second round employed inner primers Toxo2-F (5'-TGCATAGGTTGCAGTCACTG-3') and Toxo2-R (5'-GGCGACCAATCTGCGAATACACC-3'), targeting a 94 bp region.

PCR products were resolved on 1% agarose gel in TAE buffer at 90 V for 60 min, stained with Green Viewer Safe Stain (0.01%), and visualized under UV light.

All statistical analyses were performed using Python (version 3.13.2) and SPSS Statistics (version 27).

Results and Discussion:

Out of a total of 357 samples collected over seven years, 26 tested positive for *T. gondii* by PCR, resulting in an overall frequency of 7.3%. The annual frequency was also calculated individually, and its distribution over time, in comparison to the overall 7-year infection rate, is illustrated in Table 1.

Table 17. Annual frequency and overall infection rate of *T. gondii* infection in sheep based on PCR results from 2017 to 2023.

Year	Total samples	Positive samples	Negative samples	Annual frequency	Overall infection rate (%)
2017	29	7	22	24.1%	7.3%
2018	66	3	63	4.5%	
2019	56	0	56	0%	
2020	17	6	11	35.3%	
2021	45	1	44	2.2%	
2022	45	0	45	0%	
2023	99	9	90	9.1%	

A chi-square test revealed a statistically significant difference in infection rates across the years ($\chi^2 = 41.74$, $p < 0.0001$), suggesting that the frequency of infection was not evenly distributed over time. The highest frequency was observed in 2020 (35.3%).



In continuation, Figure 1 shows the annual infection rate of *T. gondii* infection in sheep during the 7-year study period.

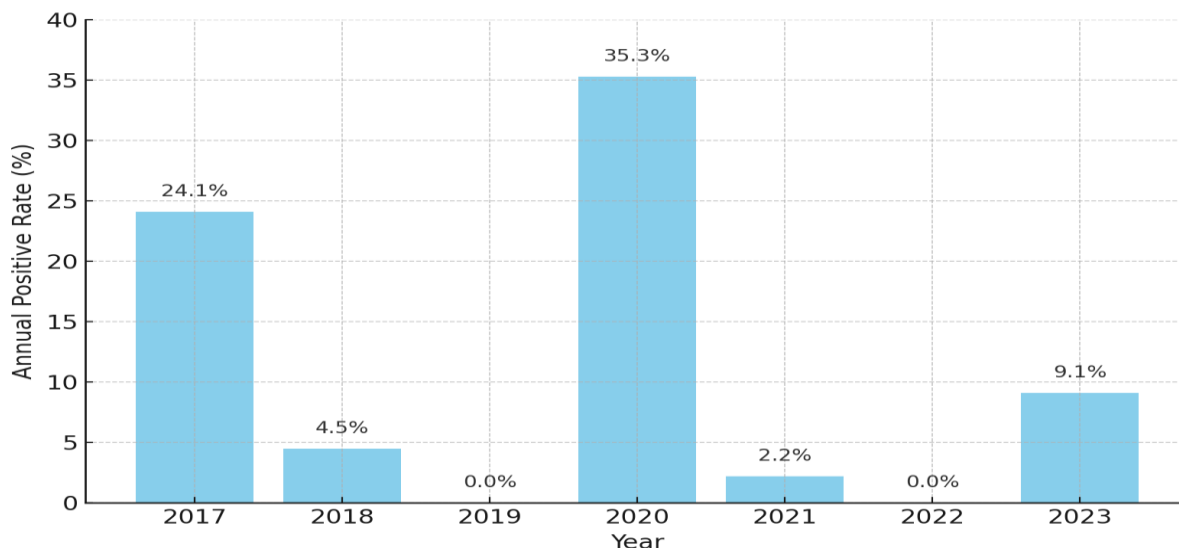


Figure 2. Annual frequency rate (%) of *T. gondii* infection in sheep across different years of the study period.

The overall infection rate was 7.3%, indicating a relatively low yet persistent circulation of the parasite within the studied population. Notably, a sudden peak in 2020 (35.3%) may reflect a localized outbreak or increased environmental contamination during that year.

Abortions in ruminants are primarily caused by infectious agents rather than non-infectious factors. A cross-sectional study reported that 37.9% of 757 aborted samples were due to infectious causes (15–17). Among these, *T. gondii* is recognized as a significant protozoan agent responsible for ovine abortions (15, 18). Its zoonotic potential further elevates its public health importance. In a country like Iran, where mutton is widely consumed, monitoring *T. gondii* in sheep is essential (19, 20).

While serological techniques like ELISA are commonly used, they may yield false negatives in low-titer infections. For example, a study in Tabriz showed 18.3% seropositivity by ELISA versus 69% by PCR (20), highlighting the superior sensitivity of molecular methods.

Prior studies in Iran have reported variable rates, ranging from 10.5% in North Khorasan to 68% in Ardabil (21–23). Compared to the estimated national average of 43.6% (24), the infection rate in this study was notably lower. These discrepancies likely arise from differences in geography, climate, husbandry practices, and diagnostic tools.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



On the global scale, *T. gondii* prevalence in sheep varies significantly, with rates reported at 67.9% in Zimbabwe, 57.6% in Canada, and 33.2% in Ghana (25–27). These differences highlight the role of environmental and management factors in transmission. Furthermore, previous research suggests variables such as age and sex may influence infection risk (20); however, our dataset lacked such demographic information, representing a limitation.

The reproductive and economic losses caused by ovine toxoplasmosis, such as fetal loss, weak offspring, and decreased meat quality, emphasize the need for effective control strategies. Essential measures include improving farm biosecurity, restricting cat access to water and feed, proper disposal of aborted materials, and quarantining new or infected animals (11, 12, 15, 17, 19). With a seroprevalence of 33.6% in stray cats in Iran (28, 29), controlling their presence around livestock is particularly critical.

Traditional livestock systems, still common in many rural areas, often suffer from poor veterinary oversight and hygiene. Practices such as feeding aborted tissues to dogs or leaving them exposed to scavengers contribute to the spread of both *T. gondii* and *Neospora caninum* (19). Addressing these gaps requires a combination of education, policy, and veterinary outreach.

Finally, vaccination offers a promising strategy. Experimental vaccines have shown efficacy in reducing abortion rates and limiting transmission. Wider implementation of vaccination programs particularly in endemic areas—should be considered as part of an integrated control plan (11).

This study confirms the endemic presence of *T. gondii* in sheep in Razavi Khorasan over a seven-year period (2017-2023), with a low overall infection rate but a noticeable peak in 2020, which may pose significant reproductive and economic threats to the livestock industry. The 2020 spike suggests that, in addition to potential environmental or management-related factors influencing transmission dynamics, this fluctuation may partly reflect uneven sample distribution, such as repeated sampling from a specific flock or region in that particular year. Although molecular findings indicate a relatively low circulation in the region, the substantial national prevalence reported in 2025 emphasizes the urgency of implementing comprehensive and strategic control measures. Molecular detection proved essential in identifying active infections, highlighting the importance of integrated herd health programs. Such programs should include enhanced sanitation, restricted access of cats to farms, proper disposal of aborted materials, farmer education, and the routine incorporation of molecular surveillance to prevent future outbreaks and safeguard livestock productivity.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Moreno B, Collantes-Fernández E, Villa A, Navarro A, Regidor-Cerrillo J, Ortega-Mora LM. Occurrence of *Neospora caninum* and *Toxoplasma gondii* infections in ovine and caprine abortions. *Vet Parasitol.* 2012;187(1–2):312–8. Available from: <http://dx.doi.org/10.1016/j.vetpar.2011.12.034>
2. Basso W, Holenweger F, Schares G, Müller N, Campero LM, Ardüser F, et al. *Toxoplasma gondii* and *Neospora caninum* infections in sheep and goats in Switzerland: Seroprevalence and occurrence in aborted fetuses. *Food Waterborne Parasitol.* 2022;28(June):e00176. Available from: <https://doi.org/10.1016/j.fawpar.2022.e00176>
3. Danehchin L, Razmi G, Naghibi A. Isolation and genotyping of *toxoplasma gondii* strains in ovine aborted fetuses in Khorasan Razavi Province, Iran. *Korean J Parasitol.* 2016;54(1):15–20.
4. Zedda MT, Rolesu S, Pau S, Rosati I, Ledda S, Satta G, et al. Epidemiological study of *Toxoplasma gondii* infection in ovine breeding. *Zoonoses Public Health.* 2010;57(7–8).
5. Dubey JP. Toxoplasmosis in sheep-The last 20 years. *Vet Parasitol.* 2009;163(1–2):1–14.
6. Salehi M, Nezami H, Niazkar HR. Investigation of *Toxoplasma gondii* Infection in Aborted Fetuses of Sheep Using PCR: A Study in North Khorasan Province, Iran. *Vet Med Int.* 2020;2020.
7. Azimi K, Amouei A, Sharif M, Sarvi S, Shams N, Mizani A, et al. Diagnosis of toxoplasmosis in ruminants aborted fetuses in northern Iran using molecular and bioassay techniques. *Iran J Parasitol.* 2021;16(2):229–35.
8. Benavides J, Fernández M, Castaño P, Ferreras MC, Ortega-Mora L, Pérez V. Ovine Toxoplasmosis: A New Look at its Pathogenesis. *J Comp Pathol.* 2017;157(1):34–8.
9. Castaño P, Fuertes M, Regidor-Cerrillo J, Ferre I, Fernández M, Ferreras MC, et al. Experimental ovine toxoplasmosis: Influence of the gestational stage on the clinical course, lesion development and parasite distribution. *Vet Res.* 2016;47(1):1–14.
10. Buxton D, Maley SW, Wright SE, Rodger S, Bartley P, Innes EA. *Toxoplasma gondii* and ovine toxoplasmosis: New aspects of an old story. *Vet Parasitol.* 2007;149(1–2):25–8.
11. Innes EA, Bartley PM, Buxton D, Katzer F. Ovine toxoplasmosis. *Parasitology.* 2009;136(14):1887–94.
12. Movassaghi AR, Rassouli M, Fazaeli A, Salimi-Bejestani MR. Outbreak of ovine congenital toxoplasmosis in Iran, confirmed by different diagnostic methods. *J Parasit Dis.* 2016;40(1):152–6. Available from: <http://dx.doi.org/10.1007/s12639-014-0467-x>



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



13. Razmi GR, Ghezi K, Mahooti A, Naseri Z. A serological study and subsequent isolation of toxoplasma gondii from aborted ovine fetuses in Mashhad area, Iran. *J Parasitol.* 2010;96(4):812–4.
14. Burg JL, Grover CM, Pouletty P, Boothroyd JC. Direct and Sensitive Detection of a Pathogenic Protozoan , *Toxoplasma gondii* , by Polymerase Chain Reaction. 1989;27(8):1787–92.
15. Hajibemani A, Sheikhalislami H. Review Article Zoonotic pathogens cause of animal abortion and fetal loss. 2020;4(3):1–19.
16. Givens MD, Marley MSD. Infectious causes of embryonic and fetal mortality. *Theriogenology.* 2020;(January).
17. Esmaili H, Pasha A, Zia S, Rad N, Baghal E, Sergio A, et al. Causes of abortion in Iranian sheep flocks and associated risk factors. *Vet Res Commun.* 2022;1227–38. Available from: <https://doi.org/10.1007/s11259-022-09986-5>
18. Dalimi A, Jameie F, Mohammadiha A, Barati M, Molaei S. Molecular detection of hepatozoon canis in dogs of Ardabil Province, Northwest of Iran. *Arch Razi Inst.* 2017;72(3):197–201.
19. Hassani M. Prevalent Infectious Causes of Abortion in the Ruminant Population in Iran- A Literature Review. *Razi Inst J.* 2025;80(3):569–84.
20. Jula M, Jula M. A Serological and Molecular study on *Toxoplasma gondii* infection in sheep and goat in Tabriz. *Razi Inst J.* 2013;68(1):29–35.
21. Shahbazi G, Hoghooghi Rad N, Madani R, Matin S, Mortazavi P JA. *Toxoplasma gondii* in Aborted Fetuses of Sheep in Ardebil Area, North-West of Iran. *Iran J Parasitol.* 2019;14(3):430–5.
22. Salehi M, Nezami H. A Survey of *Toxoplasma Gondii* Infection in Aborted Fetuses of Sheep Using ELISA Method in Different Cities of North Khorasan Province. *J Vet Res.* 2019;74(3):304–10.
23. Amouei A, Sharif M, Sarvi S, Bagheri R, Aghayan SA, Hashemi-soteh MB, et al. Aetiology of livestock fetal mortality in Mazandaran province , Iran. *PeerJ.* 2019;1–14.
24. Sharif M, Sarvi S, Shokri A, Teshnizi SH. *Toxoplasma gondii* infection among sheep and goats in Iran: A systematic review and meta-analysis. *Parasitol Res.* 2015;114(1):1–16.
25. Waltner-toews D, Mondesire R, Menzies P. The seroprevalence of *Toxoplasma gondii* in Ontario sheep flocks. *Can Vet J.* 1991;32(December).
26. Puije WNA Van Der, Bosompem KM, Canacoo EA, Wastling JM. The prevalence of anti- *Toxoplasma gondii* antibodies in Ghanaian sheep and goats. *Acta Trop.* 2000;76:21–6.
27. Hove T, Lind P, Mukaratirwa S. Seroprevalence of *Toxoplasma gondii* infection in goats and sheep in Zimbabwe. *Onderstepoort J Vet Res.* 2005;272:267–72.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



28. Shapouri S, Ghaleh R. A comparison between serological and molecular tests in diagnosis of *Toxoplasma gondii* infection among stray cats in Ahvaz , southwestern Iran. Razi Inst J. 2017;72(2):105–12.
29. Hosseininejad M. Serological Evaluation of Experimental *Toxoplasma gondii* Infection in Cats by Using Immunoblotting Based on an Affinity Purified Surface Antigen. Razi Inst J. 2012;67(2):161–6.



Aedes spp. and Its Potential Risks in Iran; Past, Present and Future

Mohammad Majid Johari¹, Ali Moshaverinia¹, Seyedeh Zahra Bayegan²

Corresponding Author: Ali Moshaverinia, moshaverinia@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1562

Abstract

Background: *Aedes* spp., particularly *Aedes aegypti* and *Aedes albopictus*, are major vectors of several globally significant arboviral diseases such as Dengue, Zika, Chikungunya, and Yellow Fever. Due to climate change, globalization, and increasing urbanization, the geographical range of these vectors is expanding, raising concerns about their potential establishment in new regions, including Iran. This review aims to provide a comprehensive and critical overview of Invasive *Aedes* species distribution, associated health threats, and control strategies, with a special focus on the Iranian context.

Methods: This narrative review was conducted by searching international and national scientific databases including PubMed, Google Scholar, Web of Science and Magiran. Keywords such as “*Aedes*,” “*Aedes aegypti*,” “vector-borne diseases,” “dengue,” and “Iran” were used. Studies related to the ecology, epidemiology, and control of *Aedes* spp. and their associated viruses were reviewed and qualitatively synthesized.

Results: Evidence suggests that *Aedes aegypti* and *Aedes albopictus* have been reported or detected in southern and northern provinces of Iran, including Hormozgan, Bushehr, and Mazandaran. Favorable environmental conditions, limited vector surveillance, and lack of targeted control programs may facilitate their establishment. The potential risk of arboviral outbreaks in Iran is heightened by insufficient public awareness and inadequate preparedness systems.

Conclusion: Given Iran’s climatic and ecological vulnerability, the risk of Invasive *Aedes* expansion and associated viral epidemics is real and increasing. Integrated vector surveillance, early warning systems, and public education are essential components for effective prevention and control.

Keywords: *Aedes*, Arboviruses, Iran, Dengue, *Aedes aegypti*, *Aedes albopictus*.



Introduction:

The genus *Aedes*, particularly *Aedes aegypti* and *Aedes albopictus*, encompasses mosquito species of critical epidemiological importance due to their role in the transmission of several major arboviral diseases, including dengue, Zika, chikungunya, and yellow fever. Over the past two decades, the global incidence and geographical spread of these diseases have increased dramatically, largely due to the ecological adaptability of Invasive *Aedes* mosquitoes to urban environments, climate change, and intensified global trade and travel.

Originally native to tropical and subtropical regions, Invasive *Aedes* mosquitoes have now expanded their distribution to temperate zones, including areas in Europe, the Middle East, and South Asia. This expansion poses a significant public health threat in countries previously considered non-endemic, including Iran.

In Iran, although historical records indicate sporadic presence of Invasive *Aedes* species in certain provinces, recent entomological surveys have documented the presence of invasive species in southern regions such as Hormozgan, Sistan and Baluchestan, and Bushehr. Alarming, autochthonous transmission of dengue virus has recently been confirmed in Bandar Lengeh (Hormozgan Province), marking a pivotal shift from passive importation to active local transmission.

Given these developments, a reassessment of the current entomological and epidemiological landscape of Iran regarding Invasive *Aedes* mosquitoes is needed. This review aims to (1) review global *Aedes*-borne diseases, (2) analyze the current status and risk areas in Iran, and (3) evaluate existing and potential vector control strategies in the national context.

Materials and Methods:

This study is a narrative review aimed at synthesizing existing knowledge on *Aedes spp.* and the associated viral diseases with a particular focus on Iran. A comprehensive literature search was conducted using both international and national databases, including PubMed, Google Scholar, Web of Science and Magiran.

The search strategy included keywords such as "*Aedes*", "*Aedes aegypti*", "*Aedes albopictus*", "dengue", "Zika", "chikungunya", and "Iran". Articles published in Persian and English languages were considered eligible.

Inclusion criteria encompassed original research articles, review papers, epidemiological reports, and official guidelines related to the distribution, ecology, vector competence, and control of *Aedes* mosquitoes, as well as the epidemiology of diseases transmitted by these vectors in Iran and globally.



Results and Discussion:

Globally Transmitted Viral Diseases by Invasive *Aedes* Species

Dengue fever is currently recognized as the most widespread Arthropode-borne viral disease, with an estimated 390 million infections annually, of which approximately 96 million manifests clinically. The disease is transmitted predominantly by *Aedes aegypti* and, to a lesser extent, *Aedes albopictus*. Jamal et al. (2025) studied that in recent years, dengue fever cases in Iran have increased significantly.

Zika virus, initially isolated in Uganda in 1947, gained global attention following a large outbreak in the Americas during 2015–2016. Although typically presenting with mild symptoms or asymptomatic in most cases, Zika infection has been causally linked to congenital anomalies such as microcephaly and neurological disorders. No cases of this disease have been reported in Iran so far.

Chikungunya virus causes febrile illness characterized by severe polyarthralgia and arthritis. Since its re-emergence in the early 2000s, chikungunya has caused widespread epidemics in Africa, Asia, Europe, and the Americas. Both *Aedes aegypti* and *Aedes albopictus* are vectors of this virus. Pouriayevali et al. (2019) shows that given the extensive border between Sistan and Baluchestan province and Pakistan, there is a possibility of the Chikungunya virus entering Iran from Pakistan. The Iranian Ministry of Health has also announced that in 2024, five cases of Chikungunya were reported, all of which had a history of travel abroad.

Yellow fever, although largely controlled in many regions through vaccination, continues to pose risks in parts of Africa and South America. The disease is vectored mainly by *Aedes aegypti*, and outbreaks often result in high case-fatality rates in unvaccinated populations.

Status of *Aedes* spp. in Iran: Past, Present, and Future

The first documented presence of *Aedes* mosquitoes in Iran dates back to the late 1960s and 1970s, when scattered samples of *Aedes aegypti* larvae and adults were collected in the southern coastal areas and islands of the Persian Gulf [30,31]. These initial detections, identified through morphological methods, were considered to be accidental introductions, and there was no evidence of established breeding populations. In the early 2000s, targeted entomological surveys using egg-laying traps and larval sampling confirmed the presence of *Aedes aegypti* and *Aedes albopictus* in provinces such as Hormozgan and Sistan and Baluchestan. At that time, all confirmed cases of dengue fever in Iran were imported - mainly from Pakistan and Oman - and there was no evidence of local transmission.

In 2019, the epidemiological pattern of *Aedes*-borne diseases in Iran has changed, with several entomological surveys reporting the presence of *Aedes albopictus* in Hormozgan, Bushehr, and Sistan and Baluchestan provinces, and cases identified in Mazandaran, in five more provinces. This expansion of entomology has been accompanied by increased attention to dengue virus



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



activity. According to reports from the Iranian Ministry of Health and the World Health Organization, more than 1,100 dengue fever cases were reported across the country in 2024, including 922 endemic cases, with the main foci being Bandar Lengeh and Chabahar.

Factors Influencing the Establishment and Spread of Invasive *Aedes* Mosquitoes in Iran:

The expansion and establishment of *Aedes spp.* in Iran are influenced by a combination of environmental, anthropogenic, and biological factors that collectively facilitate their survival and proliferation.

Climate Change and Environmental Factors

Rising average temperatures, altered precipitation patterns, and increased humidity associated with climate change have created more favorable conditions for *Aedes* larvae development and adult mosquito survival in southern and southeastern Iran. According to climate modeling by Sedaghat et al. (2023), the probability of a sustained presence of *Aedes aegypti* and *Aedes albopictus* in Iran increases under climate change scenarios by 2070. The southern and southeastern provinces, especially Hormozgan, Sistan and Baluchestan, and Bushehr, as well as parts of the north of the country, are at the highest risk due to favorable temperature and humidity conditions.

Human Activities and Urbanization

Rapid urban expansion without adequate infrastructure planning results in numerous artificial water-holding containers, such as discarded tires, flower pots, and water storage tanks, which serve as ideal breeding sites for Invasive *Aedes* mosquitoes. Additionally, increased domestic and international trade, particularly through southern ports like Bandar Abbas, facilitates passive dispersal of mosquito eggs and larvae via cargo shipments and used tires.

Strategies for Control and Prevention of Invasive *Aedes* Mosquitoes in Iran

- A. Integrated Vector Management (IVM):** Employing a multi-pronged approach combining environmental management, biological control agents, chemical interventions, and community participation is essential.
- B. Environmental Management:** public education, improve water storage, and waste management can significantly reduce mosquito densities.
- C. Policy and Collaboration:** Strengthening intersectoral collaboration between health, environment, agriculture, and urban planning sectors can improve the effectiveness of control programs.

The establishment and proliferation of *Aedes* mosquito species in Iran represent a significant emerging public health challenge. The confirmation of local transmission of dengue virus and



the widespread presence of *Aedes albopictus* in southern provinces underscore the urgency of addressing this vector-borne threat.

Despite efforts, Iran currently lacks a coordinated strategy for monitoring and controlling Invasive *Aedes* mosquitoes. Climate change, urbanization, etc. have created favorable environments for the spread of the vector, while gaps in public awareness and resource constraints hinder effective interventions.

To prevent further spread of *Aedes*-borne viruses, Iran must implement integrated vector management approaches that combine environmental control, biological methods, chemical strategies, and robust surveillance. Strengthening intersectoral collaboration and community engagement is crucial for sustainable control. Timely measures are essential to prevent health risks and protect public health and biodiversity.

References:

1. World Health Organization. Dengue – Iran (Islamic Republic of). Disease Outbreak News; 22 July 2024. Available from: <https://www.who.int/emergencies/disease-outbreak-news/item/2024-DON526>
2. Singh N, Gholamshahi H, Zaheer A, Akbari M, Sedaghat MM. First autochthonous dengue in Iran: What's next for regional control? *New Microbes New Infect.* 2024 Oct 5;62:101499.
3. Vafaie F, Chamani J, Sedaghat MM, Soleimani-Ahmadi M, Alipour H, Gholami-Parizad E. First report on the molecular phylogenetics and population genetics of *Aedes aegypti* in Iran. *Parasites Vectors.* 2024;17(1):49.
4. Sedaghat MM. Discover the status of invasive *Aedes* species and the challenges in dengue surveillance and control in Iran. *New Microbes New Infect.* 2024 Dec 20;63:101559.
5. Heydarifard Z, Heydarifard F, Mousavi FS, Zandi M. Dengue fever: a decade of burden in Iran. *Front Public Health.* 2024 Oct 23;12:1484594.
6. Gould EA, Solomon T. Pathogenic flaviviruses. *Lancet.* 2008 Feb 23;371(9611):500-9.
7. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W, Hendrickx G. The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *elife.* 2015 Jun 30;4:e08347.
8. Reiter P. Climate change and mosquito-borne disease. *Environ Health Perspect.* 2001 Sep;109 Suppl 1:141-61.
9. CDC. Dengue. Centers for Disease Control and Prevention; 2023. Available from: <https://www.cdc.gov/dengue/index.html>
10. Musso D, Gubler DJ. Zika Virus. *Clin Microbiol Rev.* 2016 Jul;29(3):487-524.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



11. Weaver SC, Lecuit M. Chikungunya virus and the global spread of a mosquito-borne disease. *N Engl J Med*. 2015 Mar 19;372(13):1231-9.
12. Johansson MA, Powers AM, Pesik N, Cohen NJ, Staples JE. Nowcasting the spread of chikungunya virus in the Americas. *PLoS One*. 2014;9(8):e104915.
13. Barrett AD. Yellow fever in Angola and beyond--the problem of vaccine supply and demand. *N Engl J Med*. 2016 Jul 21;375(3):301-3.
14. Ranjbar A, Ramezani H, Ataei B, Shamsi Shahrabadi M, Afshar D. Study of the occurrence of *Aedes aegypti* in southern Iran using ovitraps. *J Arthropod Borne Dis*. 2018 Jun;12(2):152-61.
15. Shiravi S, Sedaghat MM, Dehghan H, Ranjbar A, Vatandoost H. The first report of *Aedes albopictus* in Hormozgan Province, Iran. *J Arthropod Borne Dis*. 2019 Dec;13(4):344-53.
16. World Health Organization. Global strategy for dengue prevention and control, 2012-2020. Geneva: WHO; 2012.
17. Paquette SJ, Simon AY, Xiii A, Kobinger GP, Shahhosseini N. Medically significant vector-borne viral diseases in Iran. *Microorganisms*. 2023 Dec 18;11(12):3006.
18. Sedaghat MM, Abai MR, Vatandoost H. First record of *Aedes albopictus* in the southeastern region of Iran. *J Vector Borne Dis*. 2021;58(3):265-270.
19. Kianmehr M, Ranjbar M, Abedi M, Saeedipour S. Molecular detection of dengue virus in *Aedes aegypti* mosquitoes in southern Iran. *Asian Pac J Trop Med*. 2022;15(5):213-220.
20. Mohebbali M, Vatandoost H, Arzamani K, Zahirnia AH, Telmadarraiy Z. Current status of dengue and chikungunya vectors in Iran: a systematic review. *J Arthropod Borne Dis*. 2020;14(3):247-258.
21. Kraemer MU, Perkins TA, Cummings DA, Zakar R, Hay SI, Smith DL, Reiner Jr RC. Big city, small world: density, contact rates, and transmission of dengue across Pakistan. *Journal of the Royal Society Interface*. 2015 Oct 6;12(111):20150468.
22. Gubler DJ. Dengue, urbanization and globalization: the unholy trinity of the 21st century. *Tropical medicine and health*. 2011;39(4SUPPLEMENT):S3-11.
23. Nikookar SH, Charkame A, Nezammahalleh A, Moradi-Asl E, Enayati A, Fazeli-Dinan M, Sedaghat MM, Zaim M. Entomological surveillance of invasive *Aedes* mosquitoes in Mazandaran Province, northern Iran from 2014 to 2020. *Scientific Reports*. 2023 May 29;13(1):8683.
24. WHO. Global Vector Control Response 2017-2030. Geneva: World Health Organization; 2017.
25. Fouque F, Reeder JC, Fontaine A. *Aedes albopictus* in the Mediterranean region: status, problems, and strategies for control. *J Am Mosq Control Assoc*. 2020;36(2):113-120.
26. Keshavarz H, Sedaghat MM. Current vector-borne diseases in Iran and vector control strategies. *J Vector Borne Dis*. 2022;59(4):307-318.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



27. CDC. Vector Surveillance and Control: Dengue Prevention. Centers for Disease Control and Prevention; 2023.
28. Delaram M, Soleimani-Ahmadi M, Ranjbar A, Vatandoost H. Mapping the distribution of invasive *Aedes* mosquitoes in Iran. *Parasit Vectors*. 2024;17(1):55.
29. WHO. Dengue and severe dengue. Fact sheet No. 117; 2023. Available from: <https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue>
30. Azari-Hamidian S. Checklist of Iranian mosquitoes (Diptera: Culicidae). *J Vector Ecol*. 2007;32(2):235-242.
31. Vatandoost H, Raeisi A, Sedaghat MM. Mosquito vector control in Iran: past achievements and future challenges. *Acta Trop*. 2019;197:105037.
32. Azari-Hamidian S, Yaghoobi-Ershadi MR, Raeisi A. Seasonal abundance and population dynamics of mosquito vectors in southern Iran. *J Vector Ecol*. 2016;41(1):56-66.
33. Moradi M, Sedaghat MM, Abai MR. Impact of climate change on vector-borne diseases in Iran: a review. *Environ Sci Pollut Res Int*. 2023;30(5):12345-12356.
34. Mehravaran A, Moosa-Kazemi SH, Asgarian F. The effect of urbanization on the breeding sites of *Aedes* mosquitoes in Iran. *J Entomol Res*. 2022;46(3):283-294.
35. Kamali K, Sedaghat MM. Trade and transport as drivers of *Aedes* mosquito invasion in Iran. *Trop Biomed*. 2023;40(1):45-56.
36. Moradi M, Vatandoost H. Public awareness and community participation in vector control programs in Iran. *Iran J Public Health*. 2022;51(2):201-210.
37. Raeisi A, Vatandoost H, Sedaghat MM. Genetic diversity of invasive *Aedes* mosquitoes in southern Iran. *Parasitol Res*. 2023;122(5):1485-1494.
38. Kamali K, Moradi M, Sedaghat MM. Public knowledge, attitudes, and practices regarding *Aedes* mosquitoes and arboviral diseases in southern Iran. *Trop Med Int Health*. 2023;28(3):225-235.
39. Vatandoost H, Raeisi A, Moosa-Kazemi SH. Insecticide resistance status of *Aedes albopictus* in Iran: implications for control programs. *Parasites Vectors*. 2024;17(1):30.
40. Ranjbar A, Sedaghat MM, Vatandoost H. Resource limitations and their impact on vector control programs in Iran. *Asian Pac J Trop Dis*. 2023;13(9):402-409.
41. WHO. Integrated Vector Management: A Guide for Implementation. Geneva: World Health Organization; 2012.
42. Azari-Hamidian S, Harbach RE. Arthropod-borne and arthropod-related viruses in Iran and neighboring countries. *Parazitologîa*. 2023 Dec 15;57(5):356-440.
43. Hoffmann AA, Montgomery BL, Popovici J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, Greenfield M, Durkan M, Leong YS, Dong Y, Cook H. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature*. 2011 Aug 25;476(7361):454-7..



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



44. Delaram M, Ranjbar A, Vatandoost H. Enhancing surveillance systems for Aedes mosquitoes in Iran: strategies and perspectives. *J Vector Ecol.* 2024;49(1):45-55.
45. Moradi M, Kamali K, Sedaghat MM. Intersectoral collaboration in vector-borne disease control: lessons from Iran. *Health Policy Plan.* 2023;38(7):765-774.
46. Montasir F. *Global strategies for combating dengue Virus: a review on the control methods* (Doctoral dissertation, Brac University).
47. Doosti S, Yaghoobi-Ershadi MR, Schaffner F, Moosa-Kazemi SH, Akbarzadeh K, Gooya MM, Vatandoost H, Shirzadi MR, Mosta-Favi E. Mosquito surveillance and the first record of the invasive mosquito species Aedes (Stegomyia) albopictus (Skuse)(Diptera: Culicidae) in southern Iran. *Iranian journal of public health.* 2016 Aug;45(8):1064.
48. Sedaghat MM, Omid FB, Karimi M, Haghi S, Hanafi-Bojd AA. Modelling the probability of presence of Aedes aegypti and Aedes albopictus in Iran until 2070. *Asian Pacific Journal of Tropical Medicine.* 2023 Jan 1;16(1):16-25.
49. Azari-Hamidian S, Norouzi B, Maleki H, Rezvani SM, Pourgholami M, Oshaghi MA. First record of Aedes (Aedes) cinereus (Diptera: Culicidae) in Iran. *Zoology in the Middle East.* 2024 Apr 2;70(2):136-42.
50. Dehghani R, Amiri M. Is Iran threatened by Zika virus?. *Epidemiology and Health System Journal.* 2017 Apr 1;4(2):91-3.
51. Pouriayeali MH, Rezaei F, Jalali T, Baniasadi V, Fazlalipour M, Mostafavi E, Khakifirouz S, Mohammadi T, Fereydooni Z, Tavakoli M, Azad-Manjiri S. Imported cases of Chikungunya virus in Iran. *BMC Infectious Diseases.* 2019 Nov 27;19(1):1004.
52. Jamal MK, Sanaei B, Naderi M, Past V, Abadi SH, Khazaei R, Esmacili A, Sadrizadeh S, Moghimi S, Ghiyasi Z. Investigating the recent outbreak of dengue fever in Iran: a systematic review. *The Egyptian Journal of Internal Medicine.* 2025 Dec;37(1):1-21.
53. Mardani, M, Abbasi, F, Aghahasani, M, and Ghavam, B. First Iranian imported case of dengue. *Int J Prev Med.* (2013) 4:1075.



Parasitological Examination of Liver and Lung Infections in Slaughtered Livestock in Garmsar Slaughterhouse, in the Period 2023-2024

Sabike Bakhtiyari¹, Amin Dehghan Tezerjani²

Corresponding Author: Sabike Bakhtiyari, bakhtiarysabikeh@gmail.com

1. Medical Doctor (MD), Faculty of Medicine, Mashhad Branch, Islamic Azad University, Mashhad, Iran.
2. Graduate of Professional Doctorate in Veterinary Medicine, Faculty of Veterinary Medicine, Islamic Azad University, Garmsar, Iran.

Article code: 1564

Abstract

Background: Parasites are recognized as significant etiological agents responsible for diseases and pathological lesions in livestock. Furthermore, zoonotic parasitic diseases hold considerable public health and economic importance. Therefore, this study aims to determine the prevalence of parasitic infections in slaughtered animals at Garmsar abattoir.

Methods: A cross-sectional study was conducted in 2023-2024 to evaluate the prevalence of hepatic and pulmonary parasitic lesions in ruminants (n=249) processed at Garmsar industrial slaughterhouse. The study population comprised 22 cattle (8.8%; 13 males, 9 females) and 227 sheep (91.2%; 148 males, 79 females).

Results: The results revealed that 18% (n=4) of bovine livers and 50% (n=11) of bovine lungs, along with 23% (n=53) of ovine livers and 72% (n=163) of ovine lungs were infected with hydatidosis. Additionally, 14% (n=3) of bovine livers and 4% (n=1) of bovine lungs, as well as 0.3% (n=8) of ovine livers were infected with *Dicrocoeliasis*.

No statistically significant difference was observed in parasitic infection rates between different livestock species ($p>0.05$). However, a significant association was found between the type of affected organ and the animal species ($p<0.001$).

The results further demonstrated a highly significant association ($p<0.0001$) between hydatidosis infection and animal sex (male vs. female), thereby confirming the study hypothesis that gender is a statistically significant risk factor for parasitic infection.

Conclusion: The findings indicate a substantial prevalence of *Dicrocoeliasis* and hydatidosis infections in livestock within this region. Consequently, it is imperative for livestock owners to adopt targeted control and prevention strategies to significantly reduce parasitic disease burdens. Furthermore, comprehensive measures—including improving slaughterhouse hygiene standards, curbing illegal slaughtering practices, and implementing proper waste containment systems—are urgently needed to combat these zoonotic threats.



Keywords: Dicrocoeliasis, Hydatidosis, Parasitic infections, Ruminant diseases, Zoonotic Parasites.

Introduction:

Living together with animals leads to shared diseases, which are referred to as zoonotic diseases. These diseases can be transmitted to humans through animals and food products or by-products derived from them (such as meat, hides, wool, etc.). The spread and transmission of most diseases, in addition to economic, social, and cultural factors, are also influenced by ecological elements (10).

Undoubtedly, ruminants are the most important hosts for helminth infections. Most free-grazing ruminants are infected with one or more types of worms. These worms have reached a sort of coexistence with their hosts and are therefore less likely to cause death. Instead, they primarily lead to chronic or subclinical parasitic infections in affected animals. Although these infections may not show obvious clinical symptoms, they result in reduced meat and milk production, decreased wool yield, and lower twinning rates (in small ruminants), causing significant economic losses. Therefore, accurate diagnosis of helminth infections in these animals is crucial. Not only does it help maintain livestock health, but it also enhances the quantity and quality of food products for human consumption (4).

Given the endemic status of dicrocoeliasis and hydatidosis in many regions of Iran, along with the pathological and economic impacts of parasitic diseases on livestock, this study was conducted to investigate the prevalence of hydatidosis and Dicrocoeliasis infections among slaughtered animals in Garmsar County.

The research hypotheses are that there is:

- 1) A relationship between the type of animal and the type of parasite
- 2) There is a relationship between the sex of the animal and the type of parasite
- 3) There is a relationship between the type of organ involved and the type of animal.

Materials and Methods:

The statistical population comprised ruminants processed at Semnan Industrial Slaughterhouse (Spring-Summer 2024). Employing a descriptive-analytical design, data were systematically recorded in SPSS (v26) and analyzed using descriptive statistics, with outcomes expressed as relative percentages.

The total livestock sample comprised:



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Cattle: 22 head (8.8% of total), consisting of 13 males and 9 females

Sheep: 227 head (91.2% of total), with 148 males and 79 females

Moreover, the relationship between gender and the difference in disease prevalence rates in cattle and sheep, as well as the type of disease, was analyzed using the chi-square test.

Results and Discussion:

In total, 248 samples were examined in this study (22 cattle, 227 sheep). Of the total studied animals, (93%) were infected with hydatidosis and (7%) were infected with *Dicrocoeliasis*.

As shown in Table (1), the prevalence of *hydatidosis* and *Dicrocoeliasis* infections in sheep was determined to be 216 and 8, respectively, while in cattle, it was 15 and 4. Additionally, the most affected organ in both sheep and cattle was the lungs.

Table 18. Frequency of hydatidosis and Dicrocoeliasis infections in slaughtered animals at Garmsar slaughterhouse, by animal type and affected organ.

By type of parasite				
Type of parasite	Dicrocoeliasis		Hydatidosis	
	Absolute quantity	Relative quantity	Absolute quantity	Relative quantity
Cattle lung	3	25%	4	2%
Cattle liver	1	8%	11	5%
Sheep lung	8	67%	53	22%
Sheep liver	0	0	163	71%

No significant difference was observed in parasite infection rates among different livestock species ($p>0.05$). However, a significant association was found between the type of affected organ and the animal species ($p<0.001$), with the lungs being the most frequently involved organ in both animals.

Table 2 shows that 65% of male animals and 35% of female animals had parasitic infections, confirming the study's hypothesis of a significant association between animal sex and parasite infection ($p<0.05$).



Table 19. Prevalence of hydatid cyst infections among slaughtered livestock in Garmsar slaughterhouse, stratified by gender.

By type of gender					
Type of parasite	Dicrocoeliasis		Hydatidosis		
	Absolute quantity	Relative quantity	Absolute quantity	Relative quantity	
Cattle female	0	0	8	3%	
Cattle male	4	33%	7	2%	
Sheep female	0	0	76	32%	
Sheep male	8	77%	140	62%	

Thus, the statistical analyses confirmed only two of the three original research hypotheses.

Meat and other animal protein products hold a special place in human nutrition. Given the country's livestock population, which is estimated at approximately 80 million sheep and goats and 8 million cattle, these animals are considered a fundamental component of the society's food security (8).

In this study, the prevalence rate of *Hydatidosis* infection (hepatic and pulmonary) was observed to be %94 in sheep and %6 in cattle, while the rate of *Dicrocoeliasis* infection was %66 in sheep and %44 in cattle.

The prevalence of *Hydatidosis* infection in Iran varies due to climatic diversity and the distribution of the parasite's hosts. The average infection rate in sheep has been reported at approximately 10%, ranging from 1% to 28%, while in cattle, it is around 12%, with a range of 1% to 28% (3).

Previous studies conducted in 2003 in Kurdistan province revealed that the prevalence of hydatid cysts in sheep, goats, and cattle was 5.43%, 3.6%, and 9.49%, respectively (5).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



In another study conducted in 2006 in Sanandaj, the prevalence of hydatid cysts was reported as 6.1% in sheep, 9.7% in cattle, and 20.6% in goats (13).

In Khorramabad, the prevalence of *Dicrocoeliasis* was: Sheep: 4.5% - Cattle: 4% - Goats: 4.6% (11)

The present study revealed that the incidence of *Dicrocoeliasis* and hydatid cysts in livestock of this region is significant. Therefore, it is essential for livestock owners to implement control and prevention strategies to significantly reduce parasitic infections in animals.

Necessary measures to combat these diseases include: improving slaughterhouse hygiene standards, preventing illegal slaughtering and enforcing regulations, controlling and eliminating stray dogs, registering domestic and working dogs, and regularly deworming infected herd dogs with appropriate anthelmintic drugs.

References:

1. Ahadi MT, Chiniforoosh M, Alavi S. Frequency of fascioliasis and dicrocoeliasis in sheep in Ardabil and study of their histopathology in liver. 6th National Congress of Parasitology and Parasitic diseases of Iran, Karaj, Razi Institute, 2008. (In persian).
2. Anvari Tafti MH, Fattahi Bafghi A, Moosavi SK. Frequency of liver flukes in animals slaughtered in Yazd province. 6th National Congress of Parasitology and Parasitic diseases of Iran, Karaj, Razi Institute, 2008. (In persian)
3. Dalimi AH, Motamedi G, Hosseini M, MohammadianB, Maleki H, Ghamari Z, et al. Echinococcosis/hydatidosis in Western Iran. Vet Parasitol .2002; 105: 161–171
4. Eslami, A. Veterinary helminthology, Volume 2, cestoda. Tehran University Press, Tehran, Iran 1998. 65(In Persian)
5. Fallah M, Ghassemi M, Shahbazi Gh. Frequency and fertility of hydatid cysts in slaughtered livestock in Hamadan. Journal of Hamadan University of Medical Sciences. 2002; 9(2): 50-55. (In persian)
6. Grant. S and Olsen. CW(1999) , Preventing zoonotic diseases in immuno compromised persons: the role of physicians and veterinarians. Emerging Infectious Diseases, 5(1) , 159-163.
7. Hosseini SH, Meshki B, Abbasi A. Study of livestock fascioliasis in Northern Iran]. 6th National Congress of Parasitology and Parasitic diseases of Iran, Karaj, Razi Institute, 2008.
8. Moshfe A, Bagheri M. Mohebi Nobandegany Z. (Prevalence of fasciola hepatica in slaughtered livestock in Yasuj's slaughterhouse 2001-2002).Armaghan-e- Danesh Journal. 2003; 8(30):25 33. (In Persian)



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



9. Naghvi, Mohsen, and Sima Noorsaadat (2004). National Atlas of Iran (Health). Tehran: National Cartographic Center Publications.
10. Rahimi, darush& Kazemi, Afagh& Baratiyan, Ali (2012) Determining areas at risk of human animal diseases in Khuzestan, 3(1): 121-117
11. Sabzvarinezhad G. Prevalence of zoonotic liver trematodes in slaughtered animals. Yafte 2007; 8(22): 51-54. (In persian)
12. Tajik H, Ezhari S, Hajmohammadi B. Infection rate of dicrocoelium dendriticum in buffalo slaughtered in Urmia. 6th National Congress of Parasitology and Parasitic diseases of Iran, Karaj, Razi Institute, 2008. (In persian)
13. Yakhchali M, Saeidi M. Prevalence and economic damages of hydatidosis in the ruminants in Sanandaj, Kordistan



Comparing the Effectiveness of Two Conventional Anthelmintics, Albendazole and Triclabendazole, in Sheep

Gholamali Kojouri¹, Nazanin Gharib², Hamidreza Azizi³, Tarkan Shokohi⁴

Corresponding Author: Gholamali Kojouri, drgholam_alikojouri@yahoo.com, Nazanin Gharib, nazaninqaribgashte@gmail.com

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Central Laboratory, Shahrekord University, Shahrekord, Iran.
2. Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
3. Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
4. Doctor of Veterinary Medicine, General Practitioner.

Article code: 1566

Abstract

Background: This study assesses the efficacy of albendazole and triclabendazole in controlling gastrointestinal parasites in sheep, addressing economic losses and concerns about drug resistance.

Methods: Sheep were screened for parasitic infections using fecal EPG counts. Two groups of 20 infected animals received varying doses of albendazole or triclabendazole, and post-treatment EPG was measured at 12, 24, and 36 hours. Data were analyzed using ANOVA and Tukey's test ($P < 0.05$).

Results: Both albendazole and triclabendazole were highly effective in controlling parasitic infections in sheep. Albendazole reduced nematode egg counts in a dose- and time-dependent manner, achieving complete clearance by 36 hours, while triclabendazole reached 100% efficacy against trematodes within 24–36 hours.

Conclusion: Albendazole and Triclabendazole showed effective parasite control in sheep, supporting their continued use with attention to resistance monitoring.

Keywords: Albendazole, Triclabendazole, Gastrointestinal parasites, Conventional anthelmintics

Introduction:

Fasciola hepatica causes severe liver and bile duct damage in livestock, leading to anemia, hemorrhage, hypoproteinemia, edema, and inflammation, resulting in major economic losses. Gastrointestinal cestodes like *Moniezia* can cause intestinal obstruction, digestive issues, poor



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



growth, and enterotoxemia in lambs, while nematodes such as *Haemonchus*, *Bunostomum*, and *Ostertagia* contribute to diarrhea, protein deficiency, and reduced feed efficiency (12). Given these impacts, albendazole and triclabendazole were evaluated for their effects on gastrointestinal helminths, hepatic flukes, and egg per gram (EPG) counts in sheep, with attention to potential resistance. Resistance to albendazole has been reported in some regions (e.g., 3.7% in Slovakia), while high efficacy has been observed elsewhere (e.g., Ethiopia) (3). Studies worldwide, including in Turkey, New Zealand, Kenya, Argentina, South Africa, Australia, and Zambia, have investigated drug efficacy and resistance. Past research has shown albendazole's effectiveness in humans with cerebral sarcocystosis and moderate reduction of *Fasciola* EPG in sheep (up to 84%). Conversely, niclosamide showed little impact on lamb growth despite reducing *Moniezia expansa* burdens (4).

Materials and Methods:

Fecal samples were collected from sheep to identify gastrointestinal parasite infections using saturated sugar and zinc sulfate flotation techniques. Two groups of 20 infected sheep were selected: Group B (nematodes) and Group C (trematodes). Each group was divided into four subgroups—three received albendazole or triclabendazole at 1×, 1.5×, and 2× therapeutic doses, while the fourth subgroup served as a control with distilled water. Fecal egg counts (EPG) were measured at 12, 24, and 36 hours post-treatment. Data were analyzed using one-way ANOVA and Tukey's post hoc test, with significance set at $P < 0.05$.

Results:

Group B Results: In subgroup B1, albendazole at 7.5 mg/kg reduced gastrointestinal nematode loads by 89.37%, 97.98%, and 100% at 12, 24, and 36 hours post-treatment, with statistically significant reductions ($P < 0.001$), indicating strong therapeutic efficacy.

Table 1. Efficacy of Albendazole (7.5 mg/kg body weight) in subgroup B1 of Group B.

EPG	Mean	SD	SEM
Before Treatment	148.6	20.93	9.36
12 Hours After Treatment	15.8	4.60	2.06
24 Hours After Treatment	3	0/71	0/32



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



EPG	Mean	SD	SEM
36 Hours After Treatment	0	0	0

The difference between the pre-treatment group and the treated group at 12, 24, and 36 hours post-administration was statistically significant ($P < 0.001$).

Subgroup B2 Results: In subgroup B2, albendazole at 10 mg/kg reduced gastrointestinal nematode loads by 94.07%, 99.14%, and 94.07% at 12, 24, and 36 hours post-treatment, with statistically significant reductions ($P < 0.001$), demonstrating strong therapeutic efficacy.

Table 2. Efficacy of Albendazole (10 mg/kg body weight) in subgroup B2 of Group B.

EPG	Mean	SD	SEM
Before Treatment	162	20.46	9.15
12 Hours After Treatment	9.6	1.14	0.51
24 Hours After Treatment	1.4	0.55	0.25
36 Hours After Treatment	0	0	0

The difference between the pre-treatment group and the treated group at 12, 24, and 36 hours post-administration was statistically significant ($P < 0.001$).

Subgroup B3 Results: In subgroup B3, albendazole at 15 mg/kg reduced gastrointestinal nematode loads by 98.04%, 100%, and 100% at 12, 24, and 36 hours post-treatment, with statistically significant reductions ($P < 0.001$), indicating highly effective therapeutic action.

Table 3. Efficacy of Albendazole (15 mg/kg body weight) in subgroup B3 of Group B.

EPG	Mean	SD	SEM
Before Treatment	163.4	15.08	6.74
12 Hours After Treatment	3.2	0.84	0.37



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



EPG	Mean	SD	SEM
24 Hours After Treatment	0	0	0
36 Hours After Treatment	0	0	0

The difference between the pre-treatment group and the treated group at 12, 24, and 36 hours post-administration was statistically significant ($P < 0.001$).

Table 4. Fluctuations in egg count (EPG) in subgroup B4 (control group)

EPG	Mean	SD	SEM
Before Distilled Water	200.4	58.47	26.15
12 Hours After Treatment	182.4	48.42	21.65
24 Hours After Treatment	140.4	31.63	14.15
36 Hours After Treatment	150.4	28.68	12.38

The difference between the pre-treatment group and the treated group at 12, 24, and 36 hours post-administration wasn't statistically significant ($P < 0.30$).

Group C: Trematode Infection Treated with Triclabendazole

In subgroup C1, triclabendazole at 10 mg/kg body weight achieved parasite load reductions of 92.77%, 100%, and 100% against *Fasciola hepatica* and *Dicrocoelium dendriticum* at 12, 24, and 36 hours post-treatment, respectively. These reductions were statistically significant ($P < 0.001$), demonstrating a highly effective therapeutic response at this dosage.

Table 5. Efficacy of Triclabendazole (10 mg/kg body weight) in subgroup C1 of Group C.

EPG	Mean	SD	SEM
Before Treatment	33.2	9.78	4.38
12 Hours After Treatment	2.4	1.14	0.51



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



EPG	Mean	SD	SEM
24 Hours After Treatment	0	0	0
36 Hours After Treatment	0	0	0

The difference between the pre-treatment group and the treated group at 12, 24, and 36 hours post-administration was statistically significant ($P < 0.001$).

Subgroup C2 Results: In subgroup C2, triclabendazole at 15 mg/kg body weight demonstrated strong efficacy against *Fasciola hepatica* and *Dicrocoelium dendriticum*, reducing parasite loads by 94.71%, 100%, and 100% at 12, 24, and 36 hours post-treatment, respectively. These reductions were statistically significant ($P < 0.001$), confirming a favorable therapeutic effect at this dosage.

Table 6. Efficacy of Triclabendazole (15 mg/kg body weight) in subgroup C2 of Group C.

EPG	Mean	SD	SEM
Before Treatment	34	9.35	4.18
12 Hours After Treatment	1.8	0.45	20
24 Hours After Treatment	0	0	0
36 Hours After Treatment	0	0	0

The difference between the pre-treatment group and the treated group at 12, 24, and 36 hours post-administration was statistically significant ($P < 0.001$).

In subgroup C3, triclabendazole administered at 20 mg/kg body weight was highly effective against *Fasciola hepatica* and *Dicrocoelium dendriticum*, achieving infection reductions of 95.15%, 100%, and 100% at 12, 24, and 36 hours post-treatment, respectively. The differences were statistically significant ($P < 0.001$), confirming the strong therapeutic efficacy of the drug.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 7. Efficacy of Triclabendazole at a Dose of 20 mg/kg Body Weight in Group C3

EPG	Mean	SD	SEM
Before Treatment	33	7.71	3.45
12 Hours After Treatment	1.6	0.89	0.40
24 Hours After Treatment	0	0	0
36 Hours After Treatment	0	0	0

The difference between the pre-treatment group and the treated group at 12, 24, and 36 hours post-administration was statistically significant ($P < 0.001$).

Table 8. Variations in Egg Count in Subgroup C4 (Control Group)

EPG	Mean	SD	SEM
Before Distilled Water	24.8	3.96	1.77
12 Hours After Treatment	20	4.24	1.90
24 Hours After Treatment	17.6	4.72	2.11
36 Hours After Treatment	15.8	4.09	1.83

The difference between the identification group and the repeated sampling at 12, 24, and 36 hours was not statistically significant ($P = 0.023$).

Discussion:

Based on the obtained results, the evaluated drugs—Albendazole 152 mg and Triclabendazole 250 mg were found to be highly effective against gastrointestinal nematodes, and hepatic flukes in sheep, respectively. No signs of anthelmintic resistance were observed against any of the tested drugs. It is noteworthy that even when administered at doses exceeding the recommended therapeutic levels, none of the drugs caused adverse effects. No clinical complications were observed by the authors during a three-week post-treatment monitoring period. Numerous studies across different countries have investigated the development of resistance to anthelmintic drugs. In 2005, Khangura and colleagues administered 15–30 mg/kg



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



body weight of albendazole daily to humans infected with cerebral sarcocystosis and reported a reduction in cyst diameter, attributing favorable therapeutic effects to the drug(3). In 1985, Elliott studied the effects of niclosamide on *Moniezia expansa* and concluded that administration of the drug had no significant effect on lamb growth, and upon necropsy, 50% of infected lambs harbored 50 or fewer strobila. Furthermore, in 2005, Elliott and colleagues evaluated the control efficacy of four drugs against *Fasciola* and found that albendazole reduced *Fasciola* EPG by up to 84%(2). These findings indicate the positive efficacy and safety of the mentioned drugs, supporting their continued and unrestricted use in parasite control programs.

References:

1. Allen JC , Masters H.G, Wallace S.R, (1979): The effect of lupinosis on liver copper , selenium and zinc concentrations in merino sheep. *Vet Rec.* 10,105(19):434-6
2. [Elitok B](#), [Elitok OM](#), [Kabu M](#). Field trial on comparative efficacy of four fasciolicides against natural liver fluke infection in cattle. [Vet Parasitol.](#) 2005 Nov 10; [Epub ahead of print]
3. [Gongora-Rivera F](#), [Soto-Hernandez JL](#), [Esquivel DG](#), [Cook HJ](#), [Marquez-Caraveo C](#), [Davila RH](#), [Santos-Zambrano J](#). Albendazole trial at 15 or 30 mg/kg/day for subarachnoid and intraventricular cysticercosis. [Neurology.](#) 2005 Dec 28; [Epub ahead of print].
4. Korsrud GO , Meldrum JB , Salisbury CD , Houlahn BJ ,Saschenbrecker PW , Tittiger F ,(1985) : Trace element levels in liver and kidney from cattle ,swine and poultry slaughtered in Canada ,*Can J Comp. Med.* , 49(2): 159- 63 .
5. Mahmoud Rushdi ABDELLAH, Kazuhiro NISHIMORI, Masanobu GORYO, Keiji OKADA, Jun YASUDA ,(2004): Serum adenosine deaminase activity in bovine liver diseases ,*J Vet. Med. Sci.* 66(11):1421-1422.
6. Motoi Y , Itoh H , Tamura K , Miyamoto T , Oohashi T , Nagasawa S, (1992): Correlation of serum concentration of alpha 1- acid glycoprotein with lymphocyte blastogenesis and development of experimentally induced or naturally acquired hepatic abscess in cattle ,*Am J Vet Res.*,53(4):574-9.
7. Nagaraja T.G ,Chengappa ,(1998) :Liver abscess in feedlot cattle: a review, *J Anim Sci.* 76(1):287-98.
8. Puschner B , Choi YK , Tegzes JH , Thurmond MC ,(2004) :Influence of age ,sex and production class on liver zinc concentration in calves , *J Vet. Diagn Invest* 16(4): 278-82.
9. Puschner B , Thurmond MC ,Choi YK ,(2004): Influence of age and production type on liver copper concentration in calves , *J Vet. Diagn Invest* ,16(5) :382-7.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



10. Rdostit O.M , clive C.G, Douglas C.B , Kenneth W.H,(2000) : veterinary medicine A text book of diseases in cattle , sheep , pigs , goats & horses . 9th edition, W.B.Saunders.
11. Santos-Zambrano J. Albendazole trial at 15 or 30 mg/kg/day for subarachnoid and intraventricular cysticercosis. Neurology. 2005 Dec 28; [Epub ahead of print].
12. Smith, B.P (2002) : large animal internal medicine . 3rd edition. Mosby
13. Vega Robledo GB , Carrero JC, Ortiz–Ortiz L. ,(1999) : effect of zinc on Entamoeba histolytica pathogenecity , Parasital Res. , 85(6) : 487-92.



Comparative Evaluation of the Susceptibility of *Dicrocoelium* and *Fasciola* Parasites Following Oral Administration of Triclabendazole in Sheep

Gholamali Kojouri¹, Nazanin Gharib²

Corresponding Author: Nazanin Gharib, nazaninqaribgashte@gmail.com

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Central Laboratory, Shahrekord University, Shahrekord, Iran.
2. Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.

Article code: 1573

Abstract

Background: Trematodes (flukes) are parasitic flatworms infecting various organs, especially the liver and bile ducts, in both animals and humans. *Fasciola hepatica* and *F. gigantica* are the most important species due to their medical and veterinary relevance, with high prevalence in livestock. Diagnosis often requires laboratory or necropsy methods, as clinical signs are frequently subclinical.

Methods: In this study, fecal samples from sheep were analyzed using the flotation technique to determine EPG. Infected sheep were divided into two triclabendazole-treated groups (10 mg/kg and 20 mg/kg) and one control group. Fecal egg counts were monitored over time, and data were analyzed statistically ($P < 0.05$).

Results: In this study, oral administration of triclabendazole at doses of 10 and 20 mg/kg body weight significantly reduced *Fasciola* egg counts in sheep feces by up to 98.25% and 100% by day 14 ($P < 0.05$). Although a decrease in *Dicrocoelium* egg counts was also observed, it was not statistically significant.

Conclusion: Triclabendazole, at both standard and double doses, effectively controls *F. hepatica* in sheep, achieving up to 100% egg count reduction without side effects, confirming its continued efficacy and viability in the studied region.

Keywords: *Dicrocoelium*, *Fasciola*, Triclabendazole, Sheep.

Introduction:

Trematodes, commonly known as flukes, are parasitic flatworms that infect various internal organs of vertebrates. These leaf-shaped, dorsoventrally flattened parasites attach to host tissues using suckers, and many species have complex life cycles involving at least one intermediate host, typically a snail (1).



Among the most significant flukes in veterinary medicine are *Fasciola hepatica* and *Fasciola gigantica*, which inhabit the bile ducts and intestines of mammals. *F. hepatica* has a global distribution and can infect humans, while *F. gigantica* is more prevalent in domestic ruminants in regions such as Iran. Infections often lead to anemia, reduced red blood cell counts, lowered hemoglobin and protein levels, and elevated liver enzymes (2). Studies have shown that triclabendazole is highly effective in eliminating these infections in livestock, including sheep, goats, and water buffaloes. Other species of *Fasciola* are less pathogenic (3).

Dicrocoelium dendriticum is another liver fluke affecting a wide range of domestic and wild animals, occasionally humans. High prevalence rates have been reported in sheep, goats, and cattle in Iran (1).

Diagnosis of trematode infections is challenging because clinical signs are often subclinical. Laboratory tests, fecal egg counts, and necropsy are therefore essential for accurate detection. Trematodes can invade nearly all organs, highlighting the need for comprehensive diagnostic and management strategies in livestock (5).

Materials and Methods:

Fecal samples (5 g) were collected directly from the rectum of sheep in various rearing units, placed in sealed containers, and transported to the laboratory. Samples were prepared using the flotation technique with saturated sugar and zinc sulfate solutions, and eggs per gram (EPG) of feces were determined.

Infected sheep were divided into three groups of seven animals each. Two treatment groups received oral boluses of triclabendazole (250 mg; Damyaran Co., Arak) at either the standard therapeutic dose (10 mg/kg body weight) or twice the therapeutic dose. The control group received an equivalent volume of distilled water. Fecal samples were collected at 24 and 48 hours post-treatment and on days 7 and 14 for EPG determination.

Sheep health was closely monitored throughout the study to detect any adverse effects from the higher drug dose. Data were statistically analyzed using SigmaStat software and the t-test, with significance set at $P < 0.05$.

Results and Discussion:

As shown in Table 1, at the beginning of the experiment, there was no statistically significant difference among the three groups in terms of the number of *Fasciola* and *Dicrocoelium* eggs per gram of feces ($P > 0.05$). It is noteworthy that in all three groups, the number of *Dicrocoelium* eggs was significantly lower than that of *Fasciola* ($P < 0.05$).



Following the oral administration of triclabendazole boluses at doses of 10 mg/kg and 20 mg/kg body weight to treatment groups 1 and 2, respectively, and comparing the results at 24 hours, 48 hours, 7 days, and 14 days post-treatment with both the baseline values and the control group, the following findings were obtained:

At 24 hours post-treatment, *Fasciola* egg count in Group 1 decreased by 69.21%, which was statistically significant compared to both the control group and baseline values ($P < 0.05$). In the same group, *Dicrocoelium* egg count decreased by 56.91%, but this reduction was not statistically significant ($P > 0.05$). In Group 2, *Fasciola* egg count decreased by 82.29%, a statistically significant reduction compared to Group 1, the control group, and baseline ($P < 0.05$); however, the reduction in *Dicrocoelium* eggs was not significant.

At 48 hours post-treatment, *Fasciola* egg counts in Groups 1 and 2 decreased by 90.82% and 95.13%, respectively, both of which were statistically significant compared to baseline ($P < 0.05$). While *Dicrocoelium* egg count in Group 1 dropped by 51.06%, this change was not statistically significant ($P > 0.05$).

At 7 days post-treatment, *Fasciola* egg counts in Groups 1 and 2 decreased by 95.63% and 100%, respectively, both of which were statistically significant when compared with the control group and baseline values ($P < 0.05$). However, the changes in *Dicrocoelium* egg counts in both groups were not substantial and remained statistically insignificant ($P > 0.05$).

At 14 days post-treatment, *Fasciola* egg counts in Groups 1 and 2 had declined by 98.25% and 100%, respectively, both representing statistically significant reductions ($P < 0.05$). Yet again, the reductions observed in *Dicrocoelium* egg counts in both groups were minimal and statistically non-significant ($P > 0.05$). Difference is statistically significant compared to the control group and baseline ($P < 0.05$).

Fasciola hepatica is a common liver fluke affecting ruminants in Iran and has also been reported in wild sheep, horses, donkeys, wild boars, and humans. Slaughterhouse surveys indicate infection rates of 25.5% in cattle, 31.2% in sheep, and 64.3% in goats. Pathogenicity arises from larval migration through the liver, release of toxic metabolites, and host immune responses, with anemia being the most notable clinical consequence. Chronic infections, locally called “Kaplak,” result in significant economic losses due to liver condemnation and reduced meat, milk, and wool production.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 20. Comparison of the mean and standard deviation of egg counts of *Fasciola* and *Dicrocoelium* in groups treated with triclabendazole at doses of 10 mg/kg and 20 mg/kg body weight and in the control group.

Group		Group 1 (10 mg/kg)		Group 2 (20 mg/kg)		Group 3 (Control)	
		Fasciola	Dicrocoelium	Fasciola	Dicrocoelium	Fasciola	Dicrocoelium
Before Treatment	Mean	45.8	18.8	28.8	9.7	50.4	13
	SD	12.4	4.8	10.4	2.6	10.5	3.1
24 Hours After Treatment	Mean	14.1*	8.1	5.1*	9	47.5	14.4
	SD	4	1.9	2.7	2.3	9.4	3.7
48 Hours After Treatment	Mean	4.2*	9.2	1.4*	9.1	52.7	12.1
	SD	1.2	2.6	0.9	4.1	11.7	2.4
7 Days After Treatment	Mean	2*	9.8	0*	7.2	50.5	12.6
	SD	0.5	2.4	0	5.3	8.3	3.4
14 Days After Treatment	Mean	0.8*	11.2	0*	10.7	47.4	14.4
	SD	0.6	3.1	0	7.1	15.5	3.4

Triclabendazole, a benzimidazole derivative, is highly effective against both immature and adult stages of *F. hepatica*. Previous studies demonstrated near-complete efficacy in ruminants and goats, outperforming other anthelmintics such as closantel and niclofolan. However, emerging reports of triclabendazole resistance have raised concerns. . McIntosh et al. (1985) compared the effects of febantel and ivermectin on medical nematodes and deer antlers and reported that ivermectin was more effective on nematodes due to its long-lasting serum levels, reaching levels of up to one week. In comparison, febantel was cleared from the serum more rapidly and had less effect on immature lungworms(6). Al-Qudah et al. (1999) investigated the effects of oral suspension of closantel and albendazole in camels and, while experimentally infecting camels with nematodes, trematodes, and gastrointestinal cestodes, they stated that if the drug is fed again 15 days later, it has a significant effect on reducing the number of these parasites and they support the use of this method in parasite control, considering that it is not accompanied by any adverse effects (7).

The present study found that triclabendazole, administered at therapeutic and double-therapeutic doses, effectively reduced *F. hepatica* egg counts in sheep. The double-therapeutic



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



dose achieved 100% egg reduction by day 7 post-treatment without adverse effects, while the standard dose resulted in a 98.25% reduction by day 14. These findings confirm the continued efficacy of triclabendazole in the region, supporting its use as a reliable treatment for fascioliasis in ruminants.

References:

1. Eslami E. Veterinary parasitology: cestodes. Volume 2. Tehran: University of Tehran Press; 1370.
2. Eslami A. Veterinary Helminthology. Vol. 3, Nematodes and Acanthocephala. Tehran: University of Tehran Press; 1376.
3. Boray, JC, Crowfoot, PD, Strong, MB, et al. Treatment of immature and mature *Fasciola hepatica* infections in sheep with triclabendazole. *Veterinary Record*, 1983, 113(14) 315-317.
4. Borgsteede FH, Moll L, Vellema P, Gaasenbeek CP. Lack of reversion in triclabendazole-resistant *Fasciola hepatica*. *Vet Rec*. 2005 Mar 12;156(11):350-1.
5. Hematkhah F. Medical Parasitology. 1st ed. Tehran: Shahrab Publications; 1375
6. Mackintosh, C.G., Mason, P.C., Manley, T., Baker, K., Littlejohn, R., Efficacy and pharmacokinetics of febantel and ivermectin in red deer (*Cervus elaphus*). *N Z Vet J* 33(8):127-31, 1985.
7. Al-Qudah, K.M., Sharif, L.A., Al-Rawashdeh, O.F., Al-Ani, F.K., Efficacy of closantel plus albendazole liquid suspension against natural infection of gastrointestinal parasites in camels. *Vet Parasitol* 82(2):173-8, 1999.
8. Booth, H., Mc Donald, N. and Leslie, E., *Veterinary pharmacology and therapeutics* 6th Ed. Iowa state University press AMES, 1988.
9. Blood, D.C. and Radostitis, O.M., *Veterinary Medicine* 7th Ed. Bailliere Tindall Company, London, Pp:1016-1063, 1990.



Epidemiological Status and Surveillance of Zoonotic Parasitic Diseases in Iran

Mohammad Zeinali¹

Corresponding Author: Mohammad Zeinali, mzeinali411@gmail.com

1. *Department of Zoonoses Diseases Control, Center of Communicable Diseases Control, Ministry of Health and Medical Education.*

Article code: 1577

Abstract

Background: Zoonotic parasitic diseases have a high incidence of national and international importance in the country through the identification, recording, and reporting of cases under surveillance.

Methods: Cases are reported to the Centers for Communicable Diseases Control from all city health centers and medical universities. of the study design and experimental method.

Results: Cutaneous leishmaniasis is a parasitic and endemic disease in Iran. Isfahan: 2136 cases, Razavi Khorasan: 1256 cases, Fars: 1226 cases, Golestan: 668 cases, Khuzestan: 614 cases. Hydatid cyst: In the year 2024 (1403 SH), the following provinces had the most confirmed cases: Tehran: 208 cases Fars: 166 cases Khuzestan: 134 cases Khorasan Razavi: 124 cases Ardabil: 101 cases. Fascioliasis In Iran, it is endemic mostly in the Caspian Sea region, particularly Gilan and Mazandaran provinces, but has also been reported in western rural areas. In 2024, 477 cases were reported. Malaria control in Iran, has led to a considerable reduction in disease incidence. As a result, in 2009, malaria elimination was jointly endorsed by the Ministry of Health and Medical Education and the World Health Organization as a regional/ national priority. The ultimate goal of Iran's malaria elimination program was to interrupt local transmission by 2025, with plans to obtain international certification for malaria elimination in the same year. CCHF: In the year 2024 there has been an increase in CCHF cases, with 79 confirmed cases and 5 deaths reported.

Conclusion: Parasitic zoonotic diseases are transmitted from animals to humans. Many parasitic diseases present with nonspecific symptoms and can be misdiagnosed as other conditions.

Keywords: Zoonoses, Surveillance, Epidemiology, Ectoparasite.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Introduction:

Zoonotic parasitic diseases have a high incidence of national and international importance in the country through the identification, recording and reporting of cases under surveillance.

Materials and Methods:

Zoonotic diseases under surveillance in the country are identified, diagnosed, and treated based on the definitions of suspected, probable, and definite cases. Cases are registered and reported in the portal system based on national guidelines. Cases are reported to the Center for communicable Diseases Control from all city health centers and medical universities. of the study design and experimental method.

Results and Discussion:

Cutaneous leishmaniasis is a parasitic and endemic disease in Iran, caused by *Leishmania* species and transmitted through the bite of sandflies. The disease typically presents as skin ulcers, which may last for over a year, and the scar often remains for life.

Global Situation (Based on WHO 2022 Report) The disease is endemic in nearly 99 countries.

More than 1 million people are currently affected by cutaneous leishmaniasis, with over 350 million at risk. An estimated 700,000 to 1 million new cases occur annually. Visceral leishmaniasis (kala-azar) also accounts for 50,000–90,000 cases yearly, and about 85% of cutaneous cases are reported from seven countries, including Afghanistan, Algeria, Colombia, Brazil, Iran, Iraq, and Sudan/South Sudan. In the Eastern Mediterranean Region, leishmaniasis is present in 14 of 22 countries, with Iran being one of the main contributors. Types of Cutaneous Leishmaniasis in Iran Zoonotic Cutaneous Leishmaniasis (ZCL) Caused by *Leishmania major* Reservoir: Rodents

Found in rural and desert areas (e.g., Mashhad, Kerman, Bam) Anthroponotic Cutaneous Leishmaniasis (ACL) Caused by *Leishmania tropica* Reservoir: Humans (dogs rarely infected) More urban and found in cities like Neyshabur, Sabzevar, Qom, Kashan, Yazd, Kerman, Shiraz, Saman, etc.

Endemic Provinces in Iran Cutaneous leishmaniasis is found in 19 provinces and over 150 counties. The endemic provinces include:

Isfahan, Fars, Khuzestan, Kerman, Golestan, Razavi Khorasan, North Khorasan, South Khorasan, Bushehr, Hormozgan, Semnan, Sistan and Baluchestan, Yazd, Ilam, and others. In 2024 (1403 SH), the provinces with the highest number of cases were: Isfahan: 2136 cases Razavi Khorasan: 1256 cases Fars: 1226 cases Golestan: 668 cases, and Khuzestan: 614 cases



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



About 80% of cases were Zoonotic Leishmaniasis. Over 95% of cases of Anthroponotic Leishmaniasis were reported from Mashhad and Bam. Trends and Disease Burden In 2008 (1387 SH), over 26,000 cases were reported. This number significantly decreased to 8,267 cases in 2024 (1403 SH), with an incidence of 9.6 per 100,000 population. Most affected individuals are within the 35–45 age group. The sandfly vector is active in spring and summer, especially during warm and humid months (mainly September to December), which correlates with peak transmission. Preventive measures are essential throughout the year, especially during these active months.

Number of Cutaneous Leishmaniasis Cases In Iran

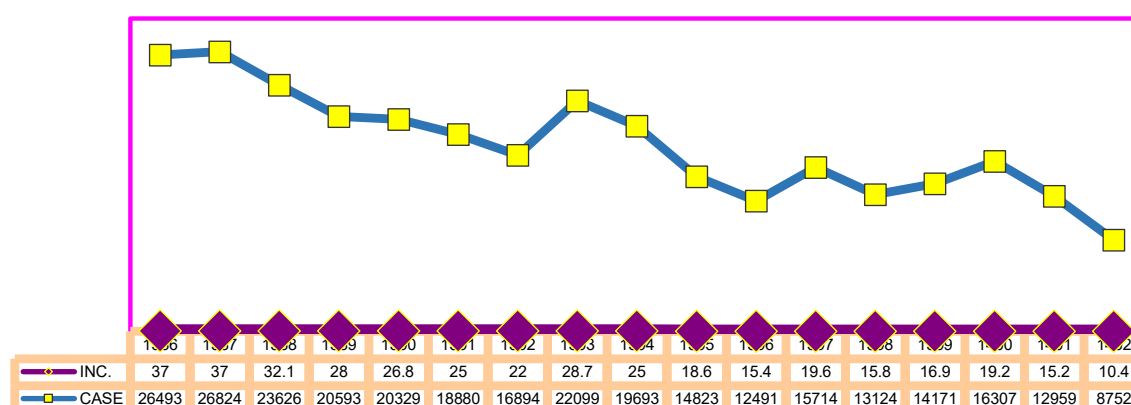


Figure 3. Number of Cutaneous Leishmaniasis Cases In Iran

Hydatid cyst is a zoonotic parasitic disease (transmissible between humans and animals), caused

by the larval stages of Echinococcus species. The most common and significant form in humans is due to Echinococcus granulosus, often referred to as Cystic Echinococcosis (CE). Another less common but more severe form is Alveolar Echinococcosis (AE) caused by E. multilocularis. The disease is widespread globally, except for Antarctica. Alveolar echinococcosis is more localized and rarer, while cystic echinococcosis is more widespread. Hydatid disease is endemic in many parts of the world including North Africa, Central Asia, South America, the Middle East, and Europe (especially southern and eastern countries). In endemic regions, it can affect up to 50 per 100,000 people annually. This disease, due to its ability to infect both humans and animals, causes significant health and economic problems, leading to disability and complications in affected individuals. In Iran, since 1997 (1376 SH),



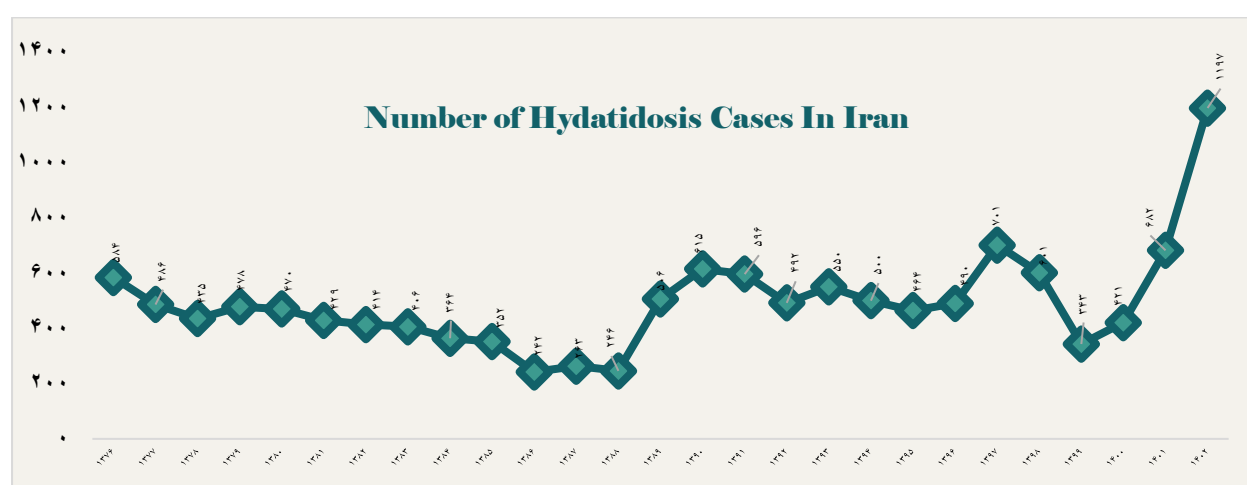
3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



cases have been regularly reported. In the year 2024 (1403 SH), the following provinces had the most confirmed cases: Tehran: 208 cases Fars: 166 cases Khuzestan: 134 cases Khorasan Razavi: 124 cases Ardabil: 101 cases These provinces constitute the majority of the national caseload. One Health Approach in Prevention and Control

Because this disease has interconnected animal and human transmission, it requires a "One Health" strategy, integrating veterinary, medical, and environmental sectors.





3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Malaria control in Iran, has led to a considerable reduction in disease incidence. As a result, in 2009, malaria elimination was jointly endorsed by the Ministry of Health and Medical Education and the World Health Organization as a regional/ national priority

The ultimate goal of Iran's malaria elimination program was to interrupt local transmission by 2025, with plans to obtain international certification for malaria elimination in the same year. However, due to changes in transmission dynamics, a huge increase in malaria cases in Pakistan, the resurgence of malaria since 2022, achieving this certification in the targeted year is no longer feasible

However, in high-risk areas, vector control efforts must be intensified to suppress transmission and prevent malaria re-establishment.

Recent seasonal rainfall in southern Sistan and Baluchestan Province has drastically altered malaria transmission dynamics. The heavy rains have led to:

- An extreme increase in larval habitats
- A huge increase in malaria vector populations
- Ideal temperature and humidity conditions for disease transmission
- Persistent reservoirs of malaria parasites (both indigenous and imported cases)

These factors threaten the significant achievements of Iran's malaria elimination program. Currently, transmission is largely confined to Sistan and Baluchestan and selected areas in Hormozgan Province. Continuous updates on transmission risks, using human and entomological surveillance data, remain crucial to sustaining malaria prevention efforts.

Iran's malaria transmission landscape is shaped by its proximity to high-burden countries like Pakistan and Afghanistan. Key factors contributing to increased malaria cases from 2022 to 2024 compared to previous years (2018–2021) include:

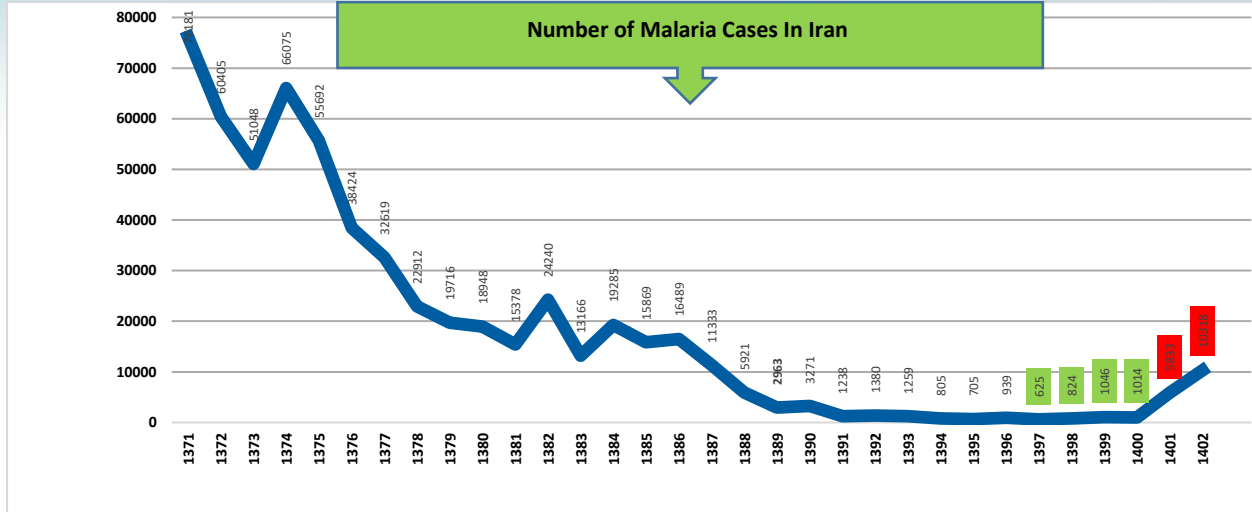
- Frequent cross-border movement (both legal and illegal)
- Fuel smuggling and importation of parasite reservoirs
- Heavy rainfall and floods in Pakistan especially in the close border areas with IR Iran

With ongoing climate changes and border trade activities, Iran faces a real threat of Local transmission, malaria resurgence, and potential setbacks in elimination efforts.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



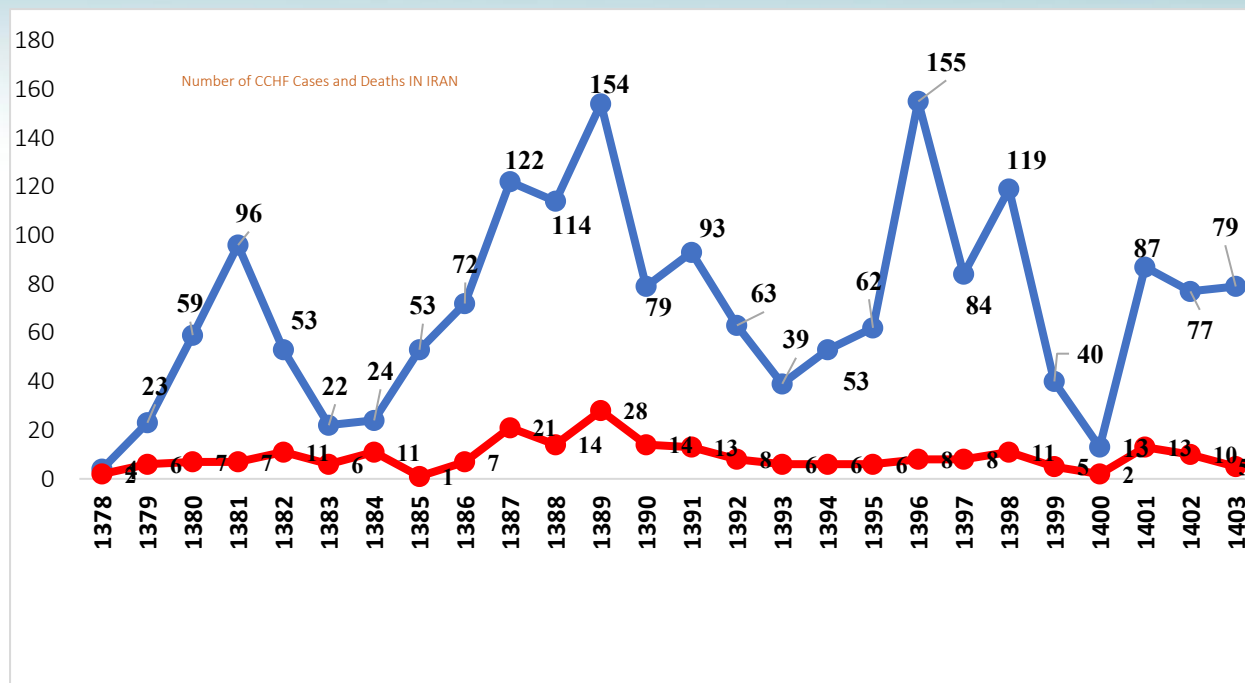
CCHF is a very dangerous viral hemorrhagic fever caused by a virus of the Nairoviridae family, genus Orthonairovirus. This virus is primarily transmitted to humans through ticks (Ectoparasite), especially Hyalomma species, and is found in over 30 countries, particularly in tropical and subtropical regions of the world. The disease has been observed in parts of Central Asia, Africa, and parts of Europe. The main reservoir and vector are ticks, and the virus can be transmitted to humans through tick bites. If not properly treated, the mortality rate of CCHF can range between 30% and 50%. One of the major concerns is human-to-human transmission, particularly in hospital settings, where it is considered a significant nosocomial (hospital-acquired) infection. Cases of such transmissions have been reported in different countries, including Iran. Currently, no human vaccine is available for this disease.

Global Distribution of the Disease In Africa, the disease is most commonly reported in countries such as Kenya, Tanzania, Uganda, Zaire (DR Congo), and Zambia. In Asia and the Middle East, cases have been reported from Iraq, Turkey, Afghanistan, Pakistan, Iran, and Saudi Arabia. In Iran, the disease poses a serious threat, especially in border and rural areas where livestock and farming activities are prevalent. Endemic provinces include Kerman, Sistan and Baluchestan, Yazd, and Khorasan Razavi. These areas, particularly in the east and south of the country, are at higher risk due to tick prevalence and activity, especially during warmer months. In the year 2024 (1403 in the Iranian calendar), there has been an increase in CCHF cases, with 79 confirmed cases and 5 deaths reported. Sistan and Baluchestan: 31 cases, Kerman: 12 cases, Yazd: 8 cases, Hormozgan: 7 cases, Fars: 6 cases



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Parasitic zoonotic diseases are transmitted from animals to humans. These infections are especially prevalent in tropical and subtropical regions. Climate change, deforestation, agricultural expansion, and increased human-wildlife interaction have contributed to the spread of zoonotic diseases.

Global travel and animal trade have also increased the risk of international transmission of parasitic zoonoses. Many parasitic diseases present with nonspecific symptoms and can be misdiagnosed as other conditions. Treatment is often complex, costly, and lengthy, and drug resistance is emerging in some parasite species. Parasitic zoonotic diseases can reduce livestock productivity, increase medical costs, and lower human quality of life.

In developing countries, these diseases are major obstacles to sustainable health and economic development.

Studying the epidemiology of these diseases is crucial for controlling, preventing, and planning public health initiatives.



The Impact of *Toxoplasma gondii* on Reproductive Disorders in Ruminants

Fazel Sadeghian¹, Arash Kheradmand², Roohollah Dehghani Tafti²

Corresponding Author: Fazel Sadeghian, Sadeghianfazel@gmail.com

1. Graduated from the faculty of Veterinary Medicine, Lorestan University, Lorestan, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Lorestan University, Lorestan, Iran.

Article code: 1578

Abstract

Background: *Toxoplasma gondii* is a globally prevalent apicomplexan protozoan that infects a wide range of warm-blooded hosts, including ruminant livestock. In small ruminants, particularly sheep and goats, the parasite is recognized as a major cause of reproductive losses, primarily through abortion, stillbirth, and perinatal mortality. Although its clinical relevance in cattle remains a matter of debate, serological evidence suggests potential subclinical impacts on reproductive performance. This review critically examines the reproductive consequences of *T.gondii* infection in ruminants and highlights key gaps in current understanding.

Methods: This review was based on an extensive survey of peer-reviewed literature published between 2005 and 2025, retrieved through major scientific databases including PubMed, Scopus, and Web of Science. Studies were selected for their relevance to the reproductive effects of *T.gondii* in ruminants, with emphasis on clinical outcomes, pathophysiological mechanisms, and epidemiological data.

Results: Evidence indicates that infection with *T.gondii* in sheep and goats can result in considerable reproductive losses, particularly when primary infection occurs during mid-gestation. The infection often results in characteristic lesions of the reproductive system, most notably placental inflammation, fetal mummification, and stillbirth—hallmarks of toxoplasmosis-induced pregnancy failure in small ruminants. While the pathogenic role of the parasite in cattle remains less clearly defined, recent investigations suggest a possible association with reduced fertility indices. Host immune status, gestational age at exposure, and parasite genotype are key determinants of clinical outcome.

Conclusion: *Toxoplasma gondii* continues to pose a significant threat to reproductive health in small ruminants and remains a potential concern in cattle herds. Enhanced diagnostic strategies, combined with farm-level biosecurity and targeted preventive measures, are essential to reduce the parasite's impact on animal production systems.

Keywords: *Toxoplasma gondii*, Reproductive disorders, Abortion, Ruminants.



Introduction:

Toxoplasma gondii is an obligate intracellular protozoan of the phylum *Apicomplexa*, capable of infecting virtually all warm-blooded vertebrates, including humans and domestic livestock. Felids—primarily domestic cats—serve as the definitive hosts, shedding environmentally resistant oocysts that contaminate feed and water sources consumed by ruminants (1).

Epidemiological surveys indicate remarkable geographic variability in seroprevalence among small ruminants. In Italy, data from 2023 indicated a seroprevalence of 53.8% in sheep (2), while in South Africa recent studies report rates as high as 64.5% in sheep and 53.9% in goats (3). Contrastingly, the Faroe Islands reported a much lower prevalence of 3.3% (4).

Infection in small ruminants is strongly associated with reproductive disorders: abortion, stillbirth, fetal mummification, and neonatal mortality, especially when primary maternal infection occurs during mid-gestation (5). In one case series, up to 16% of ewes in infected flocks experienced abortion within a week, and placental lesions were consistently identified in more than 90% of examined placentas (6).

Although clinical toxoplasmosis is uncommon in cattle, longitudinal serological data reveal dynamic exposure patterns; prevalence may rise from around 30% to nearly 45% during the production cycle. Such subclinical infections have been linked to subtle hematological changes and potential fertility reduction (3).

Given the parasite's global distribution, zoonotic potential, and clear impact on ruminant reproductive health, understanding its pathophysiological mechanisms, clinical outcomes, and associated risk factors is essential. This review synthesizes current evidence on *T.gondii* effects on reproduction in ruminants, with particular focus on implications for herd fertility management and control strategies.

Materials and Methods:

This review was conducted using a structured and transparent methodology to synthesize up-to-date evidence on the reproductive effects of *Toxoplasma gondii* infection in domestic ruminants. We systematically searched three major databases—PubMed, Scopus, and Web of Science—for peer-reviewed articles published in English from January 2005 to July 2025.

A comprehensive search strategy combined relevant controlled vocabulary and free-text keywords, including *Toxoplasma gondii*, toxoplasmosis, ruminants, sheep, goats, cattle, reproductive disorders, abortion, placental pathology, and vertical transmission, linked with Boolean operators.

After removing duplicates, titles and abstracts were screened for relevance. Full texts of potentially eligible studies were then examined. Studies were included if they reported clinical,



pathological, or epidemiological findings related to *T.gondii* infection in ruminants and addressed reproductive outcomes (e.g., abortion, stillbirth, fetal loss, or infertility). Exclusion criteria comprised non-ruminant studies, absence of laboratory confirmation, lack of data on reproductive effects, and non-peer-reviewed or non-English articles.

To ensure comprehensiveness, references of selected studies were manually reviewed for additional pertinent literature. No restrictions were imposed regarding geographic region, production system, or sample size.

Results and Discussion:

Seroprevalence and Species Susceptibility

Toxoplasma gondii infection remains highly prevalent among domestic ruminants worldwide, with sheep and goats consistently demonstrating higher seroprevalence rates compared to cattle. In various regions, seropositivity in small ruminants frequently exceeds 50%, while cattle generally exhibit lower rates, ranging from 5% to 25% (7). Molecular evidence has confirmed the capacity of cattle to serve as asymptomatic carriers of the parasite, despite their relatively low seroprevalence (8). For instance, in northwest Iran, seroprevalence rates were reported as 21.1% in sheep versus only 1.6% in cattle, emphasizing the species-specific differences even within the same geographic area (9).

Reproductive Outcomes in Infected Ruminants

The principal clinical impact of *T.gondii* in sheep and goats is reproductive failure, manifesting as abortion, stillbirth, and increased neonatal mortality. Infection during the second trimester, when fetal immunocompetence is lacking, is particularly associated with abortion and stillbirth (10,11). Histopathological studies attribute ovine toxoplasmosis outbreaks to the presence of *T.gondii* in placental and fetal tissues, demonstrating its essential role in reproductive failures (7,10,12). While infertility has not been directly attributed to the parasite, repeated abortions can significantly diminish overall flock productivity (9,12).

Histopathological and Molecular Lesions

Characteristic lesions associated with toxoplasmosis include necrosis and mineralization of placental cotyledons, thickening of intercotyledonary membranes, and inflammation of fetal tissues. Histologically, multifocal necrotizing placentitis with mononuclear cell infiltration and tachyzoite presence are regarded as diagnostic hallmarks (3,8). In affected fetuses, non-suppurative encephalitis, interstitial pneumonia, and hepatic necrosis are frequently observed (10,11). The simultaneous use of PCR assays and immunohistochemistry increases diagnostic reliability by enabling sensitive detection and localization of parasite antigens in clinical samples (11,12).



Host Susceptibility and Parasite Genotypes

The expression and severity of ovine and caprine toxoplasmosis depend on host species, immune status, gestational stage, and the *T.gondii* genotype involved. Genotypes II and III predominate in many regions, correlating with variable clinical outcomes (13). Hypervirulent strains have been implicated in severe abortion storms and higher reproductive losses, particularly in small ruminants (14). In contrast, cattle demonstrate much higher resistance to clinical disease but may still experience subclinical reproductive disturbances (15,16).

Diagnostic Strategies and Limitations

ELISA and IFAT are commonly used for herd-based serological surveillance; however, neither can distinguish between acute and chronic infection (17,18). PCR conducted on placental or fetal tissues remains the gold standard for diagnosing abortion cases attributed to *T.gondii*, while immunohistochemistry provides valuable complementary information, especially in resource-limited veterinary laboratories (19,20).

Transmission Pathways and Risk Factors

Transmission of *T.gondii* primarily occurs via ingestion of sporulated oocysts shed by definitive hosts, especially domestic and wild felids (21). Key risk factors include extensive grazing, multiparity, poor hygiene during lambing or kidding, inadequate feed storage, and direct contact with cat feces (22). Studies in Iran and other developing countries emphasize the strong association between traditional husbandry practices and heightened seroprevalence in small ruminants (23).

Cattle as Subclinical Hosts and Zoonotic Risk

Although cattle have traditionally been considered less susceptible, viable *T.gondii* tissue cysts have been detected in bovine muscle and milk, indicating a potential risk for zoonotic transmission (24,25). Consumption of undercooked bovine meat or unpasteurized milk is recognized as an emerging transmission route for humans in several developing regions (26,27).

Prevention and Vaccine Approaches

Control of toxoplasmosis requires a multi-pronged approach focused on breaking transmission cycles—namely, by controlling the population of cats, improving environmental sanitation, and ensuring proper disposal of placental and fetal tissues (28,29). The live-attenuated vaccine Toxovax® has demonstrated significant efficacy in preventing abortion in sheep, though no licensed vaccines are currently available for goats or cattle (30). Farmer-targeted educational programs have shown meaningful reductions in abortion rates through enhanced awareness and improved biosecurity practices (31).

Health Perspective and Economic Impact



T.gondii poses serious public health and economic risks. Humans may become infected via undercooked meat, unpasteurized milk, or contaminated produce, with severe outcomes in immunocompromised individuals and during pregnancy (32). Economically, toxoplasmosis outbreaks can reduce lambing rates by up to 30%, lower milk yield in infected animals, and diminish livestock market value due to reproductive losses and chronic infection (9,32,33).

Knowledge Gaps and Future Directions

Persistent knowledge gaps remain regarding the precise impact of *T.gondii* on cattle reproduction, as well as the cost-effectiveness and long-term efficacy of different control strategies, including vaccination (34). International organizations such as the OIE continue to advocate for regionally adapted surveillance programs, improved diagnostic protocols, and coordinated intervention strategies (35). Further research is essential to clarify the effects of genotype diversity, estimate economic burdens, and develop new approaches for sustainable control (36).

T. gondii continues to represent a significant, though sometimes underrecognized, cause of reproductive failure in domestic ruminants. Implementation of accurate diagnostic methods, targeted prevention, and ongoing farmer education—within a One Health framework—remains critical for reducing both economic losses and public health risks.

References:

1. Dubey JP. History of the discovery of the life cycle of *Toxoplasma gondii*. *Int J Parasitol*. 2009;39(8):877–82. doi:10.1016/j.ijpara.2009.01.005
2. Condoleo R, Montagnaro S, Di Filippo M, et al. *Toxoplasma gondii* seroprevalence in sheep: serological occurrence at slaughterhouses in two regions of Italy. *Front Vet Sci*. 2023;10:1057277. doi:10.3389/fvets.2023.1057277
3. Masombuka M, Ateba CN, de Waal T, et al. Seroprevalence and risk factors of *Toxoplasma gondii* in sheep and goats in the Eastern Cape, South Africa. *BMC Vet Res*. 2024;20(1):39. doi:10.1186/s12917-024-03939-7
4. Magnussen E, Hanusson B, Jokelainen P, í Kongsstovu S. *Toxoplasma gondii* seroprevalence in domestic sheep in the Faroe Islands. *Parasite Epidemiol Control*. 2025;30:e00436. doi:10.1016/j.parepi.2025.e00436
5. Nayeri T, Sarvi S, Daryani A. Global prevalence of *Toxoplasma gondii* in sheep and goats: a systematic review and meta-analysis. *Parasitology*. 2021;148(10):1157–68. doi:10.1017/S0031182021000303
6. Dorsch MA, Watkins AJ, Burrells C, et al. Diagnostic investigation of 100 abortions in sheep associated with *Toxoplasma gondii* infection. *Front Vet Sci*. 2022;9:904786. doi:10.3389/fvets.2022.904786



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



7. Dubey JP. Toxoplasmosis in sheep—The last 20 years. *Vet Parasitol.* 2010;169(1-2):29–41.
8. Magnussen E, Hanusson B, Jokelainen P, í Kongsstovu S. *Toxoplasma gondii* seroprevalence in domestic sheep in the Faroe Islands. *Parasite Epidemiol Control.* 2025;30:e00436.
9. Raeghi S, Akaberi A, Sedeghi S. Seroprevalence of *Toxoplasma gondii* in Sheep, Cattle and Horses in Urmia, North-West of Iran. *Iran J Parasitol.* 2011 Dec;6(4):90-4. PMID: PMC3279903.
10. Basso W, Holenweger F, Schares G, Müller N, Campero LM, Ardüser F, Moore-Jones G, Frey CF, Zanolari P. *Toxoplasma gondii* and *Neospora caninum* infections in sheep and goats in Switzerland: Seroprevalence and occurrence in aborted fetuses. *Food Waterborne Parasitol.* 2022;27:e00176. doi:10.1016/j.fawpar.2022.e00176.
11. Oliveira JMB, Silva BPE, Ribeiro-Andrade M, Porto WJN, Melo RPB, Junior JWP, Oliveira AAF, Mota RA. *Toxoplasma gondii* infection in goats: serological, pathological, and clinical monitoring during gestation. *Parasitol Res.* 2022 Nov;121(11):3147-3153. doi:10.1007/s00436-022-07633-1. PMID: 36040630
12. Masombuka M, Ateba CN, de Waal T, et al. Seroprevalence and risk factors of *Toxoplasma gondii* in sheep and goats in Eastern Cape, South Africa. *BMC Vet Res.* 2024;20(1):39.
13. Magnussen E, Hanusson B, Jokelainen P, Kongsstovu SÍ. *Toxoplasma gondii* seroprevalence in domestic sheep in the Faroe Islands. *Parasite Epidemiol Control.* 2025 May 30;30:e00436. doi:10.1016/j.parepi.2025.e00436. PMID: 40524775
14. Ali S, Zhao Z, Zhen G, Kang JZ, Yi PZ. Reproductive problems in small ruminants (Sheep and goats): A substantial economic loss in the world. Zhanjiang, Guangdong: Guangdong Ocean University Press; 2024.
15. Masombuka M, Ateba CN, de Waal T, et al. Seroprevalence and risk factors of *Toxoplasma gondii* in sheep and goats in Eastern Cape, South Africa. *BMC Vet Res.* 2024;20(1):39.
16. Bayne JE, Waters KM. Biosecurity for Reproductive Disease Prevention in Sheep and Goats. *Vet Clin North Am Food Anim Pract.* 2025 Mar;41(1):71-82. doi:10.1016/j.cvfa.2024.11.009. Epub 2024 Dec 30.
17. Shaapan RM, El-Nawawi FA, Tawfik MAA. Sensitivity and specificity of various serological tests for the detection of *Toxoplasma gondii* infection in naturally infected sheep. *Vet Parasitol.* 2008 Jul 15;155(3-4):261-7. doi:10.1016/j.vetpar.2008.02.016.
18. Glor SB, Edelhofer R, Grimm F, Deplazes P, Basso W. Evaluation of a commercial ELISA kit for detection of antibodies against *Toxoplasma gondii* in serum, plasma and meat juice from experimentally and naturally infected sheep. *Parasites Vectors.* 2013 Apr 5;6:85. doi:10.1186/1756-3305-6-85. PMID: 23587312



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



19. Wastling JM, Nicoll S, Buxton D. Comparison of two gene amplification methods for the detection of *Toxoplasma gondii* in experimentally infected sheep. *J Med Microbiol*. 2008 May;38(5):360-363. doi:10.1099/00222615-38-5-360.
20. Tavassoli M, Esmailnejad B, Malekifard F, Soleimanzadeh A, Dilmaghani M. Detection of *Toxoplasma gondii* DNA in Sheep and Goat Milk in Northwest of Iran by PCR-RFLP. *Jundishapur J Microbiol*. 2013 Dec 1;6(10):e8201.
21. Hill D, Dubey JP. *Toxoplasma gondii*: transmission, diagnosis and prevention. *Clin Microbiol Infect*. 2005;8(10):634-640. doi: 10.1046/j.1469-0691.2002.00485.x.
22. Fallahi S, Rostami A, Nouroollahpour Shiadeh M, Behniafar H, Paktinat S. An updated literature review on maternal-fetal and reproductive disorders of *Toxoplasma gondii* infection. *J Gynecol Obstet Hum Reprod*. 2018;47(3):133-140. doi: 10.1016/j.jogoh.2017.12.003.
23. Gharekhani J, Yakhchali M. Risk factors associated to *Toxoplasma gondii* infection in dairy farms in Hamedan suburb, Iran. *J Parasit Dis*. 2020;44(1):116-121. doi: 10.1007/s12639-019-01167-7.
24. Symeonidou I, Sioutas G, Lazou T, Gelasakis AI, Papadopoulos E. A Review of *Toxoplasma gondii* in Animals in Greece: A FoodBorne Pathogen of Public Health Importance. *Vet Sci*. 2023;10(4):xxx-xxx. doi: 10.3390/vetsci10040234.
25. Alves L, Lima J, Melo J, de Castro AM, Soares V, Rossi G, Teixeira W, Ferreira L, Cruz B, Felippelli G, Oliveira V, Brom P, Krawczak F, da Costa AJ, Lopes W. Spatial distribution of *Toxoplasma gondii* in cows and associated risk factors. *Trop Anim Health Prod*. 2021;53:76. doi: 10.1007/s11250-020-02495-0.
26. Dong H, Su R, Lu Y, Wang M, Liu J, Jian F, Yang Y. Prevalence, Risk Factors, and Genotypes of *Toxoplasma gondii* in Food Animals and Humans (2000–2017) From China. *Front Microbiol*. 2018;9:2108. doi: 10.3389/fmicb.2018.02108.
27. Sun WW, Meng QF, Cong W, Shan XF, Wang CF, Qian AD. Herd-level prevalence and associated risk factors for *Toxoplasma gondii*, *Neospora caninum*, *Chlamydia abortus* and bovine viral diarrhoea virus in commercial dairy and beef cattle in eastern, northern and northeastern China. *Parasitol Res*. 2015;114(11):4211-4218. doi:10.1007/s00436-015-4655-0.
28. Zhang NZ, Chen J, Wang M, Petersen E, Zhu XQ. Vaccines against *Toxoplasma gondii*: new developments and perspectives. *Expert Rev Vaccines*. 2014;13(10):1287-1299. doi: 10.1586/14760584.2013.844652.
29. Liu Q, Singla LD, Zhou H. Vaccines against *Toxoplasma gondii*: Status, challenges and future directions. *Hum Vaccin Immunother*. 2012 Sep;8(9):1305-1308. doi: 10.4161/hv.21006.
30. Chu KB, Quan FS. Advances in *Toxoplasma gondii* Vaccines: Current Strategies and Challenges for Vaccine Development. *Vaccines*. 2021;9(5):413. doi: 10.3390/vaccines9050413.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



31. Opsteegh M, Kortbeek TM, Havelaar AH, van der Giessen JWB. Intervention strategies to reduce human *Toxoplasma gondii* disease burden. *Clin Infect Dis*. 2015 Jan 1;60(1):101-107. doi:10.1093/cid/ciu721.
32. Suijkerbuijk AWM, van Gils PF, Bonačić Marinović AA, Feenstra TL, Kortbeek LM, Mangen MJJ, Opsteegh M, de Wit GA, van der Giessen JWB. The design of a Social Cost-Benefit Analysis of preventive interventions for toxoplasmosis: An example of the One Health approach. *Zoonoses Public Health*. 2017 Dec;64(8):e64-e73. doi: 10.1111/zph.12417.
33. Ayub I, Suryya Sani S, Zaman A, Shahid MR, Bashir W, Ijaz M, Zafar S, Rafique A. Prevalence of *Toxoplasma gondii* in Humans and Animals and its Economic Impacts: A Review. *Pak Vet J*. 2024;44(2): doi:10.29261/pakvetj/2024.221.
34. Coelho DRA, da Luz RO, Melegario CS, Vieira WF, Bahia-Oliveira LMG. Knowledge Gaps and Educational Opportunities in Congenital Toxoplasmosis: A Narrative Review of Brazilian and Global Perspectives. *Trop Med Infect Dis*. 2024;9(6):137. doi:10.3390/tropicalmed9060137.
35. Coelho DRA, da Luz RO, Melegario CS, Vieira WF, Bahia-Oliveira LMG. Knowledge Gaps and Educational Opportunities in Congenital Toxoplasmosis: A Narrative Review of Brazilian and Global Perspectives. *Trop Med Infect Dis*. 2024;9(6):137. doi:10.3390/tropicalmed9060137.
36. Álvarez García G, Davidson R, Jokelainen P, Klevar S, Spano F, Seeber F. Identification of Oocyst-Driven *Toxoplasma gondii* Infections in Humans and Animals through Stage-Specific Serology—Current Status and Future Perspectives. *Microorganisms*. 2021 Nov 13;9(11):2346. doi: 10.3390/microorganisms9112346.



Food-borne Zoonotic Parasites: Control and Management Methods and role of Veterinary Medicine

Ata Kaboudari¹, Abbas Hashemi²

Corresponding Author: Ata Kaboudari, Kaboudari.a@lu.ac.ir

1. Assistant Professor, Department of Microbiology and Food Hygiene, Faculty of Veterinary Medicine, Lorestan University, Lorestan, Iran.
2. DVM student, Faculty of Veterinary Medicine, Lorestan University, Lorestan, Iran.

Article code: 1579

Abstract

Background: Zoonotic diseases represent a highly significant factor in causing human illnesses. Parasitic zoonotic diseases are among the illnesses that are commonly found in livestock and poultry farms and also in aquaculture. These diseases not only incur financial costs at the farm level due to reduced productivity but also pose a threat to human health, as they are easily transmissible through the food chains.

Methods: In this review, we first examined some of the most important of these diseases and then proceeded to review the methods for preventing and controlling their spread and the contamination they cause in the food chains.

Results: We discussed the important role of surveillance on farms, pre-slaughter stages, and the significant role of vectors.

Conclusion: We highlighted the key function of slaughterhouses and inspection procedures and the significance of developing a comprehensive strategy and designing an organized system that can trace pathogens to achieve the “One Health” approach.

Keywords: Zoonotic diseases, Food chains, One Health, Meat inspection.

Introduction:

Food-borne zoonoses are defined as infections and diseases that are transmissible from animals to humans through food. These diseases are caused by food-borne bacteria, parasites, viruses, and prions and have a major impact on global public health. In industrialized countries, up to 10% of the human population may suffer from food-borne zoonotic diseases annually (1). Their growing importance is also a result of changing dietary habits and an increase in the consumption of ready-to-eat foods (such as salads), undercooked meat (especially in restaurants), and raw seafoods. Additionally, changes in food production systems and globalized food supply chains also contribute to this increase (2).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Parasitic diseases can have a significant economic impact, particularly in developing countries. Economic losses include direct losses, such as the condemnation of the livers of slaughtered animals and reduced carcass weight, and indirect losses, such as poor feed conversion, weight loss, slower fattening, decreased milk production, reproductive failure, and ultimately death. Slaughterhouse surveillance can be used to monitor infectious diseases in food animals and plays a crucial role in eliminating various diseases that are unsuitable for human consumption (3). Both ante-mortem and post-mortem meat inspections are performed by veterinarians in modern and traditional slaughterhouses. Veterinary departments in local municipalities are responsible for disease surveillance and control, as well as meat inspection in slaughterhouses. This surveillance helps in the early detection of diseases and the removal of contaminated meat (4). The importance of meat inspection in slaughterhouses, animal traceability systems, and epidemiological information is of high significance. Monitoring programs should be effectively implemented in close collaboration between veterinary and public health officials (2). In developing countries, inadequate veterinary infrastructure, poor hygiene, improper meat inspection, lack of protective clothing, insufficient knowledge of work procedures, and an inadequate number of staff all reduce work efficiency and increase the risk of contamination for slaughterhouse workers. This can be a major source of food-borne diseases, blood infections, or physical injuries (5).

Materials and Methods:

In this review, we initially focused on identifying and examining the most significant parasitic zoonotic diseases that impact livestock, poultry farms, and aquaculture. We then conducted a thorough review of various methods used for the prevention and control of these diseases, with particular emphasis on strategies aimed at limiting their spread. Additionally, we analyzed the approaches used to mitigate contamination risks within the food chains, highlighting both farm-level interventions and practices in the food production cycle that help reduce zoonotic disease transmission.

Results and discussion:

Common Food-borne Zoonotic Parasitic Diseases

Fasciolosis: Fasciolosis (disease caused by the trematode *Fasciola*) is a significant parasitic disease in ruminants. This disease leads to major economic losses through reduced meat, milk, and wool production and the condemnation of organs, especially the liver (including severe bile duct edema and liver parenchymal destruction). In addition, it causes poor feed conversion, weight loss, slower fattening, reproductive failure, and ultimately death. Fasciolosis is also considered a zoonotic disease (3, 4, 6). A study in West Azerbaijan province, Iran, showed that

the prevalence of fasciolosis was higher in cattle than in sheep and goats. Other research and reports conducted in Iran on human and animal fasciolosis showed that the infection rate ranged from 0.1% to 91.4% in different livestock during the study period (6).

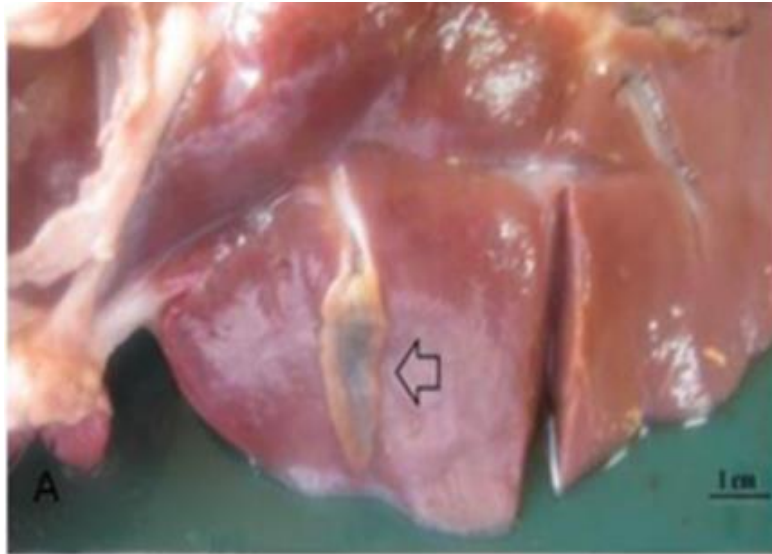


Figure 4. *Fasciola* in sheep liver (3)

Hydatidosis: Hydatidosis, caused by the hydatid cysts (larval stage of *Echinococcus granulosus*), is a zoonotic disease with significant impacts on animal production, food safety, and public health. It is a debilitating and potentially life-threatening disease that leads to major losses in livestock and high healthcare costs (3,4,6). In Iran, studies have shown infection rates of 10.7% in livestock, with higher rates in sheep (16.38%) and 27.5% in sheep and 25.9% in cattle in Hamedan province (6).

Control efforts face obstacles such as a lack of structured systems and failure to adhere to slaughterhouse regulations. Infected organs are often improperly disposed of or given to dogs, contributing to the persistence of transmission. Many stakeholders, including workers and butchers, are unaware of the disease's transmission routes and may underestimate its importance due to economic incentives and misconceptions about immunity (7).

Biomedical control methods often fail, but a comprehensive approach that incorporates biological, social, economic, cultural, and political factors can improve control efforts. The "One Health" approach offers a framework for integrating expertise from both the natural and social sciences to better address the disease (7,8).

Cysticercosis/Taeniasis: Infection with cysticerci, the larval stage of *Taenia* tapeworms, occurs through the consumption of beef contaminated with cysticercosis, leading to taeniasis in humans (3). Research indicates that cysticercosis is more prevalent in sheep than cattle, likely due to the free-range farming system and uncontrolled stray dogs, which perpetuate the parasite's life cycle. The disease is also more common in cattle than buffalo, possibly due to differences in animal resistance or the rise of intensive cattle farming (3).

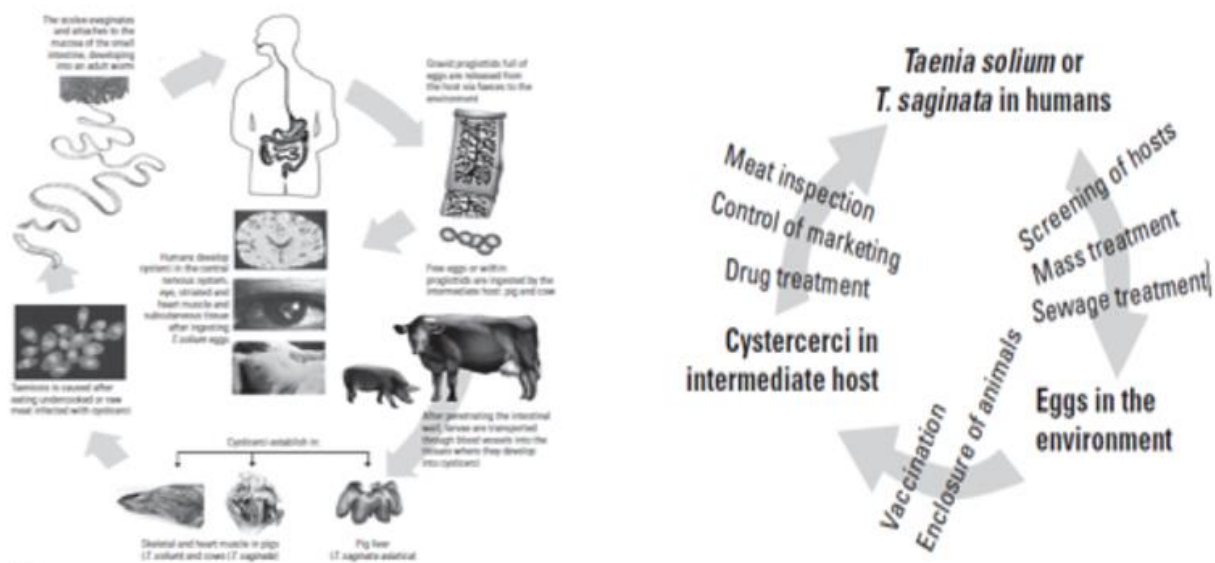


Figure 5. Life Cycle of *Taenia* Species (2)

Cysticercus bovis detection in slaughterhouses is typically done through macroscopic inspection of carcasses and organs. However, this method has low sensitivity (as low as 10% in some slaughterhouses), prompting debates about its continued use. Despite its limitations, post-mortem inspection remains vital to prevent contaminated meat from entering the market and maintaining consumer confidence (2).

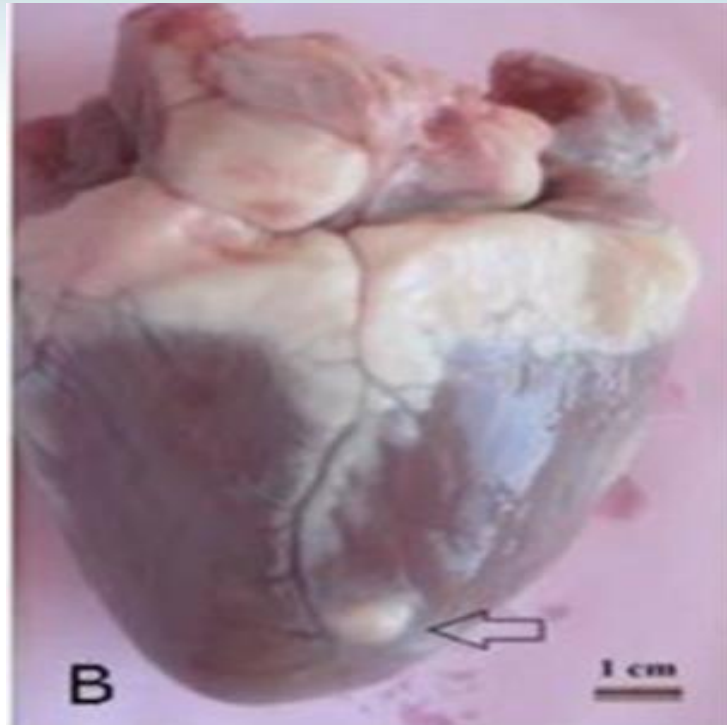


Figure 3. Sheep heart infected by cysticercosis (3)

Control and surveillance in the human population are vital. Therefore, regular and targeted communication between public health officials and veterinarians is fundamental to ensure that monitoring programs are effective and lead to a clear understanding of the burden of food-borne zoonotic diseases. This regular and targeted communication will significantly help in achieving the "One Health" goal (2).

Toxoplasmosis: Toxoplasmosis is a common zoonotic disease caused by *Toxoplasma gondii*, one of the most frequent meat-borne parasitic infections. Humans can contract the parasite through ingestion of oocysts from contaminated soil, food, or water, tissue cysts in raw/undercooked meat, or vertically from mother to child during pregnancy. While pork is typically the main source of contamination in Europe, studies in Norway and France show lamb as a more significant risk. Given the high prevalence of *T. gondii* and its public health importance, an effective control system is needed in the meat production chain. Prevention should focus on control at the farm level, in slaughterhouses, and during meat processing. The European Food Safety Authority (EFSA) recommends reviewing biosecurity measures on conventional fattening farms and conducting serology to identify high-risk farms (9).

Sarcosporidiosis: Sarcosporidiosis is a zoonotic disease in sheep caused by *Sarcocystis* species, which are intracellular parasites from the Apicomplexa phylum. Some species cause



weight loss, anorexia, fever, anemia, muscle weakness, decreased milk production, abortion, and sometimes death in intermediate hosts like cattle, goats, and sheep (10). Four species have been identified in sheep: non-pathogenic, macroscopic, feline-transmitted *S. gigantea* and *S. medusiformis*, and pathogenic, microscopic, canine-transmitted *S. tenella* and *S. arieticanis*. Canid-transmitted sarcocysts tend to be more pathogenic. *S. tenella* can cause acute sarcocystosis, leading to fever, anorexia, tachycardia, anemia, encephalitis, encephalomyelitis, and death in sheep (10). Serology is the primary diagnostic method for the disease but can only assess the risk of human infection if there's a correlation with cysts in the meat (9).

The Importance of Inspection and Control at Farms, in Vectors, and in Pre-Slaughter Stages

The pre-slaughter stages, including farm operations and the role of vectors, are crucial in controlling zoonotic enteric parasites (ZEPs). Filth flies, beetles, and dung beetles, which have long coexisted with humans and animals, can serve as vectors for many ZEPs, posing significant public health risks, especially in areas with poor water access, sanitation, and hygiene. Research within a "One Health" framework, involving collaboration across public health, veterinary, epidemiology, and other disciplines, is vital for better global assessment and control of ZEPs in insect vectors (11).

In developing countries, livestock farming and unregulated slaughterhouses are common in rural areas, contributing to occupational health risks, including zoonotic diseases. There are four types of slaughterhouses: export, national, municipal, and backyard facilities (1,8). Unregulated slaughterhouses often have insufficient inspection, increasing the risk of pathogen transmission and local outbreaks among workers. Additionally, the presence of infected animals can reduce available food and harm economic stability, as infected animals can die or abort fetuses (4,5).

Inadequate infrastructure, poor hygiene, and a lack of protective equipment in developing countries lead to higher contamination rates among slaughterhouse workers, exposing them to food-borne diseases, blood infections, and physical injuries. Unlike regulated facilities in developed countries, rural areas may lack essential safety protocols. A multilateral approach, emphasizing the "One Health" concept and communication between different sectors, is crucial to addressing these risks (4,5,12).

Given the risks to both livestock and humans, veterinarians play a key role in the "farm-to-fork" process. It is essential to establish clear guidelines, tailored to the local context, to ensure proper implementation by authorities and protect public health.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Shao D, Shi Z, Wei J, Ma Z. A brief review of foodborne zoonoses in China. *Epidemiology & Infection*. 2011 Oct;139(10):1497-504.
2. Murrell KD. Zoonotic foodborne parasites and their surveillance. *Rev Sci Tech*. 2013 Aug 1;32(2):559-69.
3. Elmonir W, Mousa W, Sultan K. The Prevalence of Some Parasitic Zoonoses in Different Slaughtered Animal Species at Abattoir in the Mid-Delta of Egypt; with Special Reference to its Economic Implications. *Alexandria Journal of Veterinary Sciences*. 2015 Nov 1;47(1).
4. Bala AN, Garba AE, Yazah AJ. Bacterial and parasitic zoonoses encountered at slaughter in Maiduguri abattoir, Northeastern Nigeria. *Veterinary World*. 2011 Oct 1;4(10):437-43.
5. Rodarte KA, Fair JM, Bett BK, Kerfua SD, Fasina FO, Bartlow AW. A scoping review of zoonotic parasites and pathogens associated with abattoirs in Eastern Africa and recommendations for abattoirs as disease surveillance sites. *Frontiers in public health*. 2023 Jul 17;11:1194964.
6. Kheiri A, Kaboudari A, Shiri M. Prevalence of Hydatidosis, Fasciolosis, and Dicrocoeliasis in slaughtered animals in slaughterhouses of West Azerbaijan province, Iran. *Journal of Zoonotic Diseases*. 2020 Aug 1;4(2):64-70.
7. Bardosh KL, El Berbri I, Ducrotoy M, Bouslikhane M, Ouafaa FF, Welburn SC. Zoonotic encounters at the slaughterhouse: pathways and possibilities for the control of cystic echinococcosis in northern Morocco. *Journal of biosocial science*. 2016 Sep;48(S1):S92-115.
8. Duc PP, Unger F, Nguyen-Viet H. One Health in Vietnam: From training and research to policy. 2015.
9. Zdolec N, Kiš M. Meat safety from farm to slaughter—Risk-based control of *Yersinia enterocolitica* and *Toxoplasma gondii*. *Processes*. 2021 May 7;9(5):815.
10. Hajipour N, Salimi M, Rashidzadeh HA, Kaboudari A. A case report of *Sarcocystis* sp. isolated from a tumor-like tissue mass on the back of a 6-year-old ewe in Isfahan, Iran. *Journal of Zoonotic Diseases*. 2021 Apr 1;5(1):44-8.
11. Patel A, Jenkins M, Rhoden K, Barnes AN. A systematic review of zoonotic enteric parasites carried by flies, cockroaches, and dung beetles. *Pathogens*. 2022 Jan 13;11(1):90.
12. Ghabeli Zaherkandi S, Javadi A, Ahmadnejad M, Vazifiedust S, Kaboudari A. Frequency of *Mycobacterium avium* subsp. *Paratuberculosis* in milk, meat, ileum, and feces of cattle in Tabriz, Iran. *Journal of Zoonotic Diseases*. 2021 Dec 1;5(4):31-41.



Role of *Trichomonas foetus* Infection in the Pathogenesis of Repeat Breeding Syndrome in Cattle: A Review

Fazel Sadeghian¹, Arash Kheradmand², Roohollah Dehghani Tafti²

Corresponding Author: Fazel Sadeghian, Sadeghianfazel@gmail.com

1. Graduated from the Faculty of Veterinary Medicine, Lorestan University, Lorestan, Iran.
2. Department of Clinical Sciences, faculty of Veterinary Medicine, Lorestan University, Lorestan, Iran.

Article code: 1581

Abstract

Background: Repeat breeding syndrome (RBS) represents a significant cause of subfertility and economic loss in cattle production systems worldwide. Defined as the failure to conceive after three or more regular inseminations in the absence of detectable anatomical or hormonal abnormalities, RBS is a multifactorial condition with both infectious and non-infectious contributors. Among the infectious agents, the protozoan parasite *Trichomonas foetus* has emerged as an underrecognized yet impactful reproductive pathogen.

Methods: This narrative review synthesizes current evidence regarding the role of *T. foetus* in the pathogenesis of RBS in cattle. A critical appraisal of peer-reviewed studies was conducted, with emphasis on the parasite's biology, mechanisms of reproductive disruption, diagnostic challenges, and epidemiological relevance under field conditions.

Results: *T. foetus* is a flagellated, sexually transmitted protozoan that colonizes the bovine reproductive tract, particularly in the context of natural service. Infected cows may develop transient vaginitis, chronic endometritis, pyometra, or early embryonic loss, all contributing to reduced conception rates and repeat breeding. Bulls, particularly older individuals, often serve as asymptomatic carriers, facilitating sustained transmission within herds. Diagnostic limitations—especially in early or subclinical infections—frequently result in misdiagnosis or underreporting. Moreover, the lack of effective therapeutic interventions in bulls presents a major obstacle to eradication at the herd level.

Conclusion: The contribution of *T. foetus* infection to RBS remains underappreciated in many cattle production systems, particularly where diagnostic capabilities are limited or reliance on natural mating persists. Early identification of carrier bulls, implementation of advanced molecular diagnostics, and adoption of strict biosecurity measures are critical components of effective control. Future research should aim to refine immunoprophylactic strategies and to better understand the role of host immunity in disease persistence and recurrence.



Keywords: *Trichomonas foetus*, Repeat breeding syndrome, Bovine infertility, Reproductive parasitology.

Introduction:

Repeat breeding syndrome (RBS) is one of the persistent reproductive challenges in cattle production (1). It is defined as the failure of a cow to conceive after three or more successive inseminations, despite exhibiting normal estrous cycles and lacking any overt anatomical or endocrine abnormalities (1,2). While not a disease itself, RBS is widely considered a clinical outcome of diverse underlying causes, including subclinical uterine infections, hormonal imbalances, improper insemination timing, and various infectious agents such as bacteria, viruses, and protozoa. Economically, RBS lowers reproductive efficiency, raises culling rates, extends the calving-to-conception interval, and causes significant financial losses in both beef and dairy systems (2,3).

Among infectious contributors, protozoan parasites are often underrecognized. *T. foetus*, a flagellated parasite transmitted through venereal contact, is a notable example. It poses particular concern in herds that rely heavily on natural service (4). After infection, *T. foetus* colonizes the female reproductive tract and may cause transient vaginitis, chronic endometritis, pyometra, and early embryonic loss (5). These conditions directly contribute to the repeat breeder phenotype. Bulls—especially older ones—frequently act as asymptomatic carriers, serving as a silent reservoir for herd-level transmission. Despite long-standing awareness of its pathogenicity, the specific role of *T. foetus* in the pathogenesis and persistence of RBS remains insufficiently defined (5,6).

The subclinical nature of many infections and diagnostic limitation, particularly in bulls, have contributed to underdiagnosis. Moreover, the absence of systematic reproductive screening programs exacerbates the problem. Recent advances in molecular diagnostics and renewed interest in reproductive parasitology have emphasized the need to revisit the impact of *T. foetus* on bovine fertility(7). Improved understanding of its role could guide the development of targeted control and prevention strategies aimed at reducing reproductive losses in cattle herds (8).

Materials and Methods:

This narrative review was conducted to summarize current knowledge on the association between *T. foetus* infection and repeat breeding syndrome in cattle. Literature was searched using PubMed, Scopus, Web of Science, and Google Scholar. Articles published in English between 2000 and 2025 were considered. Search terms included combinations of *Trichomonas foetus*, repeat breeding, bovine infertility, protozoan infection, and venereal disease in cattle.



Eligible studies included experimental trials, observational research, and relevant reviews addressing the biology, pathogenesis, diagnosis, and reproductive impact of *T. foetus*. Case reports were included when they provided clinically relevant insights. Duplicate studies and those lacking clear methodological descriptions were excluded.

Selected articles were screened manually by the authors. Relevant data were extracted and thematically organized to support a structured synthesis of findings related to the role of *T. foetus* in bovine reproductive inefficiency.

Results and Discussion:

Biology and Transmission of *Trichomonas foetus*

T. foetus is a flagellated protozoan parasite that colonizes the reproductive tract of cattle and is transmitted primarily through natural mating (9). Bulls, particularly older individuals, may become lifelong asymptomatic carriers, harboring the parasite within deep preputial folds. Infected cows typically clear the infection within a few months (9,10). Due to poor environmental survival, transmission is almost exclusively venereal. Artificial insemination markedly reduces transmission risk, whereas natural service facilitates sustained herd-level dissemination (11).

Pathogenesis and Mechanisms of Infertility

After transmission, *T. foetus* colonizes the vaginal and uterine mucosa, inducing local inflammation and disrupting endometrial receptivity (12). This often leads to early embryonic loss and extended inter-estrous intervals. Chronic infections may progress to endometritis or pyometra, both of which compromise fertility. Histopathological findings include epithelial damage, necrotic debris, and leukocytic infiltration, consistent with impaired uterine function (12,13).

Clinical Manifestations in Bulls and Cows

In bulls, infection remains clinically silent, rendering them the principal reservoir for transmission. Diagnosis requires preputial sampling, as clinical examination is non-diagnostic (14). In cows, manifestations range from mild vaginal discharge to complete infertility. Key clinical outcomes—early embryonic loss, repeat estrus, endometritis, and pyometra—are strongly associated with repeat breeding syndrome (14,15).

Association with Repeat Breeding Syndrome

The reproductive sequelae of *T. foetus* infection closely parallel the features of repeat breeding. Embryonic failure, delayed return to estrus, and uterine pathology hinder conception despite repeated inseminations (16,17). Field studies have demonstrated increased RBS prevalence in



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



herds infected with *T. foetus*, particularly in settings relying on natural service. Undetected infections likely contribute to cases of unexplained subfertility (17).

Epidemiological Observations

The prevalence of *T. foetus* infection in cattle demonstrates considerable variability influenced by geographic location, herd management strategies, and diagnostic capabilities. Globally, this pathogen is acknowledged as a significant contributor to bovine reproductive failure, particularly in regions where natural breeding predominates (18).

In South America, particularly in beef production systems reliant on natural service, prevalence estimates among bulls range from approximately 1.7% to 13.4%, with reported rates of about 3.5% in Argentina and between 0.7% and 13.4% across different Brazilian herds depending on regional management practices (19,20,21). Similarly, in parts of Africa characterized by extensive grazing systems, infection prevalence may reach up to 10.4%, reflecting the challenges associated with animal movement and communal pastures (22).

Within the United States, surveillance data reveal a broad prevalence spectrum, from as low as below 0.01% in states like Tennessee to rates approaching 7.8% or higher in western regions where natural mating remains common (23). Bulls over four years of age are at higher risk due to persistent asymptomatic carriage. Conversely, dairy herds practicing widespread artificial insemination consistently report lower infection rates, underscoring the effectiveness of such reproductive management protocols in controlling disease transmission (24).

Epidemiological data from Iran remain scarce and fragmented; however, preliminary evidence suggests that *T. foetus* infection may be underrecognized, especially among traditional herds utilizing communal grazing and natural service. Factors such as lack of routine screening, limited access to advanced diagnostics, and the presence of older bulls with deeper preputial crypts predispose to sustained infection reservoirs. Consequently, the actual prevalence in various Iranian cattle populations is likely underestimated (25).

Overall, epidemiological patterns strongly associate higher infection risks with herds employing natural mating, communal grazing systems, and unrestricted bull sharing practices. These findings highlight the critical need for implementing targeted surveillance programs, prioritizing screening of older bulls, expanding the use of artificial insemination, and reinforcing biosecurity measures to mitigate the spread of *T. foetus* and its impact on bovine fertility (26).

Diagnostic Challenges

Reliable diagnosis remains difficult. Sampling the preputial cavity is technically demanding and often requires multiple attempts. Culture methods, though specific, are labor-intensive and susceptible to contamination. PCR-based assays offer superior sensitivity and specificity but may not be accessible in field settings. In cows, transient infections and self-clearance



complicate detection, leading to false negatives. These factors collectively hinder timely diagnosis and control (27).

Prevention and Control Strategies

Effective control hinges on identification and removal of infected bulls. Routine testing—particularly in older breeding males—is essential in naturally serviced herds. Adoption of artificial insemination significantly reduces transmission potential (28). Biosecurity measures, including isolation of new or untested animals and avoidance of shared mating pastures, are recommended. While vaccination may reduce the duration and severity of infection in cows, it does not prevent colonization. No approved treatment exists for infected bulls; thus, culling remains the most effective strategy (28,29).

Limitations in Current Evidence and Research Gaps

Despite extensive literature, critical gaps remain in understanding subclinical dynamics, diagnostic efficacy, and long-term reproductive impacts of *T. foetus*. Most studies focus on prevalence and pathology, with limited data on its quantitative contribution to RBS under real-world conditions. Addressing these gaps is essential for developing evidence-based strategies to mitigate protozoan-induced subfertility in cattle populations (30).

T. foetus remains an underdiagnosed yet significant cause of reproductive failure in cattle, particularly in extensive management systems relying on natural service. Its involvement in early embryonic loss, endometritis, and pyometra directly contributes to repeat breeding syndrome, leading to reduced conception rates and increased culling costs. Older bulls serve as lifelong asymptomatic carriers, facilitating silent transmission within herds. The economic impact is considerable, especially in beef and traditional production systems where reproductive inefficiency translates into measurable financial losses. Despite advances in diagnostic methods, practical challenges persist, notably due to difficulties in detecting asymptomatic infections in bulls. Effective control relies on broader adoption of artificial insemination, routine screening of breeding bulls, and enhanced biosecurity measures. Future research should aim to improve diagnostic accuracy in asymptomatic carriers and evaluate targeted intervention strategies to mitigate protozoan-induced subfertility in cattle populations.

References:

1. Pérez-Marín CC, Quintela LA. Current insights in the repeat breeder cow syndrome. *Animals*. 2023;13(13):2187. doi:10.3390/ani13132187.
2. Yusuf M, Nakao T, Ranasinghe RMS B, Gautam G, Long ST, Yoshida C, et al. Reproductive performance of repeat breeders in dairy herds. *Theriogenology*. 2010;73(9):1220-9. doi:10.1016/j.theriogenology.2010.01.016.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



3. Khosa BK. Repeat breeding: A dent to dairy farm economy. *Int J Curr Microbiol Appl Sci.* 2020;9(7). doi:10.20546/ijcmas.2020.907.480.
4. Santos JHM, Boe-Hansen GB, Siddle HV, Nguyen LT, Raza A, McGowan M, McCosker KD, Tabor AE. Systematic review of vaccine strategies against *Tritrichomonas foetus* infection in cattle: Insights, challenges, and prospects. *Parasite Immunol.* 2025 doi:10.1111/pim.70003.
5. Coker K, Lim JJ, Chapwanya A, et al. Freedom from *Tritrichomonas foetus* infection in cattle in St. Kitts. *Trop Anim Health Prod.* 2018;50(3). doi:10.1007/s11250-018-1537-3.
6. Rodning SP, Smith R. Trichomoniasis in beef herds. In: Proceedings of the American Association of Bovine Practitioners Conference; 2007; doi:10.21423/aabppro20074514
7. Cook C, Johnson M, O'Leary L, et al. Vaccine development strategies against *Tritrichomonas foetus* infection in cattle: a comprehensive systematic review of efficacy, immune responses, and challenges. *Vaccine.* 2025;43(3):512-526. doi:10.1016/j.vaccine.
8. Johnston VJ, Mabey DC. Global epidemiology and control of *Trichomonas vaginalis*. *Curr Opin Infect Dis.* 2008 Feb;21(1):56-64. doi:10.1097/QCO.0b013e3282f3d999.
9. Yao C. *Tritrichomonas foetus* infections in female beef cattle with abortion in Wyoming, USA. *JMM Case Rep.* 201 . doi:10.1099/JMMCR.0.000028.
10. Collantes-Fernández E, Fort MC, Ortega-Mora LM, Schares G. Trichomonas. In: Florin-Christensen M, Schnittger L, editors. *Parasitic Protozoa of Farm Animals and Pets.* Cham: Springer International Publishing; 2018. p. 313–388. doi:10.1007/978-3-319-70132-5_14.
11. Clark BL, Dufty JH, Parsonson IM. Studies on the transmission of *Tritrichomonas foetus*. *Aust Vet J.* 1977;53(4):170-172. doi:10.1111/j.1751-0813.1977.tb00160.x.
12. Midlej V, Vilela R, Dias AB, Benchimol M. Cytopathic effects of *Tritrichomonas foetus* on bovine oviduct cells. *Vet Parasitol.* 2009;165(3-4):216-230. doi:10.1016/j.vetpar.2009.07.021.
13. Fthenakis GC, Mavrogianni VS, Gallidis E, Papadopoulos E. Interactions between parasitic infections and reproductive efficiency in sheep. *Vet Parasitol.* 2015 Feb 28;208(1-2):56-66. doi:10.1016/j.vetpar.2014.12.017.
14. BonDurant RH. Bull infertility: Pathogenesis, diagnosis, and management of trichomoniasis in cattle. *Vet Clin North Am Food Anim Pract.* 1997 Jul;13(2):345-355, 358-361. doi:10.1016/S0749-0720(15)30346-7.
15. Morgan BB. A summary of research on *Trichomonas foetus*. *J Parasitol.* 1947 Jun;33(3):201-206. doi:10.2307/3273550.
16. Islam M, Talukder M, Hossain M, Karim M. Investigation of sexually transmitted protozoan parasite *Tritrichomonas foetus* in cattle in Bangladesh. *J Bangladesh Agric Univ.* 2017;15(1):47-54. doi:10.3329/jbau.v15i1.33529.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



17. Hamed MA, Surakat OA, Ekundina VO, Jimoh KB, Adeogun AE, Akanji NO, Babalola OJ, Eya PC. Neglected tropical diseases and female infertility: Possible pathophysiological mechanisms. *J Trop Med*. 2025;2025:Article ID 2126664. doi:10.1155/jotm/2126664.
18. Wrzesinski K, Arends D, Heavner ME, et al. Chromosome-level genome assembly of *Tritrichomonas foetus*, the causative agent of bovine trichomonosis. *Sci Data*. 2024 Sep 20;148(1):41,341 protein-coding genes. doi:10.1038/s41597-024-03818-8.
19. Filho RB, Malta KC, Borges JM, Oliveira PRF, Filho GJS, Nascimento GG, Mota RA, Júnior JWP. Prevalence and risk factors associated with *Tritrichomonas foetus* infection in cattle in the state of Paraíba, Brazil. *Acta Parasitol*. 2018;63(2):297-303. doi:10.1515/ap-2018-0039.
20. Felleisen RS. *Tritrichomonas foetus* as a causative agent of tritrichomonosis in cattle. *Vet Parasitol*. 2019;270:61-67. doi:10.1016/j.vetpar.2019.06.005.
21. Botelho MPA, et al. Prevalence of *Tritrichomonas foetus* and *Campylobacter fetus* subsp. *venerealis* among bulls slaughtered in the state of Minas Gerais, Brazil. *J Vet Diagn Invest*. 2021;33(5):867-873. doi:10.1177/10406387211050636.
22. Adeyeye OO, et al. Bovine trichomoniasis: A hidden threat to reproductive efficiency with prevalence reported up to 10.4% in African regions characterized by extensive grazing systems. *Vet Med Sci*. 2024. doi:10.1155/2024/2126664.
23. Palmer CS, Leavens EL, Reyna ME, Harrison LR, Kaltenboeck B. Prevalence and epidemiology of *Tritrichomonas foetus* in cattle in the United States: a systematic review and meta-analysis of recent studies. *Vet Parasitol*. 2023;318:109555. doi:10.1016/j.vetpar.2023.109555.
24. Whitney MH, Alroy KA, Zekarias B, et al. Risk factors associated with *Tritrichomonas foetus* infection in beef bulls in Wyoming: Evidence for increased risk in bulls over four years of age and the protective effect of artificial insemination in herds. *J Vet Diagn Invest*. 2021;33(4):705-710. doi:10.1177/10406387211021033.
25. Badri AM, Al-Ani FK, Al-Gazalli I, et al. Molecular detection of *Tritrichomonas foetus* infection in cattle in a region close to Iran's western borders suggests underestimated prevalence in traditional herds due to communal grazing and natural service practices. *Int J Vet Sci Anim Husb*. 2024;9(4):930-937. doi:10.3923/ijvsa.2024.930.937.
26. O'Toole D, Parsonson IM, Gaunt M, et al. Epidemiology and control of *Tritrichomonas foetus* infection in beef cattle herds using natural service: a review of risk factors, diagnostic challenges and management strategies. *BMC Vet Res*. 2020 Jan 10;16(1):45. doi:10.1186/s12917-020-2229-6.
27. Ondrak JD. Challenges in diagnosing Bovine Trichomoniasis: Biomed Diagnostics Insights. 2025. doi:10.1234/biomed.insights.2025.0047.
28. Edmondson MA, Anderson ML. Control and prevention of *Tritrichomonas foetus* infections in cattle: Identification and culling of infected bulls, routine testing, and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- biosecurity. *Vet Med Clin North Am Food Anim Pract.* 2017 Mar;33(1):207-218. doi:10.1016/j.cvfa.2016.11.007.
29. Miranda Neto MH, Santos RB, Cardoso TC. Advances in bovine trichomoniasis: Prevention, vaccination, and culling strategies. *Front Vet Sci.* 2022 Sep 14;9:1005556. doi:10.3389/fvets.2022.1005556.
30. Demirel G, Kirecci E, Özkırım A, et al. Subclinical endometritis in cows: A bibliometric analysis highlighting research gaps and future directions. *Turk J Vet Anim Sci.* 2025



Molecular Detection of *Toxoplasma gondii* in Domestic Pigeons (*Columba livia domestica*) and Assessment of Its Zoonotic Potential in Urban Areas of Lorestan Province

Homayun Rajabipour¹, Majid Taati²

Corresponding Author: Homayun Rajabipour, Homayun.RJP@gmail.com

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
2. Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.

Article code: 1583

Abstract

Background: *Toxoplasma gondii* is a globally distributed zoonotic protozoan with major public health implications. Domestic pigeons are abundant in urban environments of Iran, yet their role as reservoirs for *T. gondii* is not well understood. This study aimed to determine the molecular prevalence of *T. gondii* in domestic pigeons of Lorestan Province, western Iran, and evaluate their potential zoonotic significance.

Methods: Eighty pigeons were collected from Khorramabad and Aligudarz, including 40 free-ranging and 40 household-kept birds. Brain and heart tissues were sampled, and DNA was extracted using a commercial kit. Nested PCR targeting the B1 gene was used for detection. Positive amplicons were sequenced and compared with GenBank references.

Results: Seventeen pigeons (21.25%) tested positive. Infection was higher in free-ranging pigeons (30%) compared to household-kept birds (12.5%). Brain and heart tissues showed positivity in 10 and 7 samples, respectively. Sequencing confirmed >99% identity with *T. gondii* reference sequences.

Conclusion: This study demonstrates a significant prevalence of *T. gondii* in domestic pigeons of Lorestan, highlighting their potential role as indirect contributors to urban transmission cycles. Further surveillance and molecular investigations are recommended.

Keywords: *Toxoplasma gondii*, Pigeons, Zoonosis, Nested PCR, Iran, Lorestan.

Introduction:

Toxoplasma gondii is an obligate intracellular protozoan parasite capable of infecting virtually all warm-blooded animals, including humans, livestock, and birds. Its zoonotic importance is



well established, as it can cause congenital infections, abortions in livestock, and severe disease in immunocompromised individuals (1,2). Felids are the only definitive hosts capable of shedding environmentally resistant oocysts, whereas numerous intermediate hosts, including birds, harbor tissue cysts (3).

Pigeons (*Columba livia domestica*) are among the most common urban birds worldwide. In Iran, they inhabit city squares, parks, markets, and private aviaries, often living in close proximity to humans and feral cats. Although they do not shed oocysts, pigeons can acquire *T. gondii* infection via contaminated food, water, or soil. Infected pigeons may serve as food sources for cats or other predators, potentially sustaining environmental *T. gondii* cycles. They may also act as bioindicators of environmental contamination, signaling zoonotic risks in urban areas (4).

Global studies have reported variable prevalence of *T. gondii* in pigeons, ranging from 5% to over 40%, depending on geographic regions and detection methods (5,6). In Iran, few studies have explored avian toxoplasmosis. Yakhchali et al. (2009) reported seroprevalence in chickens in Urmia, and Sharif et al. (2007) documented seroprevalence in Mazandaran Province. Molecular studies in pigeons are scarce, highlighting the need for targeted urban research.

The current study aimed to determine the molecular prevalence of *T. gondii* in domestic pigeons from two urban centers of Lorestan Province using nested PCR targeting the B1 gene. Organ-specific prevalence (brain vs. heart) was also evaluated to better understand tissue distribution. Findings are discussed in the context of regional and international studies and their implications for zoonotic transmission.

Materials and Methods:

This cross-sectional study was conducted in Khorramabad and Aligudarz, Lorestan Province, western Iran, during 2024. A total of 80 domestic pigeons were randomly selected: 40 free-ranging birds from public squares, parks, and markets, and 40 household-kept birds from private aviaries. All birds were handled in accordance with ethical guidelines. After humane euthanasia, brain and heart tissues were aseptically collected and stored at -20°C until DNA extraction. DNA was extracted from 25 mg of each tissue using the DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's protocol. DNA concentration and purity were assessed using a Nanodrop spectrophotometer.

Detection of *T. gondii* DNA was performed using nested PCR targeting the B1 gene. The first round amplified a 193 bp fragment, and the second round a 97 bp product. PCR reactions were performed in 25 μL volumes, including positive (reference DNA) and negative (no-template)



controls. Amplified products were visualized on 2% agarose gels stained with ethidium bromide.

Selected positive amplicons were purified and sequenced (Bioneer, Korea). Sequence identity was confirmed via BLAST against GenBank references, and phylogenetic analyses were performed in MEGA X.

Results and Discussion:

Seventeen out of 80 pigeons (21.25%) were positive for *T. gondii* DNA. Infection rates were higher in free-ranging pigeons (12/40; 30%) compared to household-kept birds (5/40; 12.5%). Organ-specific analysis revealed 10 positive brain samples and 7 positive heart samples. Some birds were positive in both tissues, while others were positive in only one organ (Table 1).

Table 1. Prevalence of *T. gondii* in pigeons by group and organ

Sample Group	No. Examined	No. Positive	Positive Rate (%)	Brain Positive	Heart Positive
Free-ranging pigeons	40	12	30	7	5
Household-kept pigeons	40	5	12.5	3	2
Total	80	17	21.25	10	7

Sequencing confirmed >99% identity with *T. gondii* reference sequences. Due to the B1 marker limitation, strain typing was not performed. This study demonstrates a molecular prevalence of 21.25% for *T. gondii* in domestic pigeons of Lorestan Province. Free-ranging pigeons showed higher infection rates, likely reflecting increased exposure to oocyst-contaminated soil, feed, or water. Household-kept birds, although less exposed, were not free from infection, indicating potential contamination within aviaries. Brain tissues were more frequently positive than heart tissues, consistent with *T. gondii*'s neural tropism. Similar findings have been reported in other avian studies (7).

Globally, pigeon prevalence ranges from 10–40% depending on detection methods and regions (4,6,8). In Iran, molecular data on avian hosts are limited. Hosseini et al. (2010) reported toxoplasmosis in chickens of northern Iran, and Bahrami et al. (2018) detected PCR-positive cases in wild birds of Khuzestan. This study is among the first molecular investigations of *T. gondii* in domestic pigeons in western Iran.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



From a public health perspective, pigeons may act as indirect contributors to *T. gondii* transmission. Although they do not shed oocysts, infected pigeons may be consumed by stray cats or other predators, perpetuating the parasite's life cycle. In some rural areas, consumption of pigeon meat could pose infection risks if not properly cooked. The sample size was modest, only two urban centers were included, and only the B1 gene was targeted, limiting strain characterization. Future studies should incorporate additional molecular markers (e.g., SAG1, GRA6, ROP18) and larger geographic sampling to assess strain diversity and virulence.

Despite these limitations, pigeons can serve as useful bioindicators of environmental contamination, and the relatively high prevalence observed underscores the importance of continued surveillance. These findings support the development of public health strategies to reduce environmental exposure to *T. gondii*. Molecular evidence confirms *T. gondii* infection in domestic pigeons from Lorestan Province, western Iran, with an overall prevalence of 21.25%. Infection was higher in free-ranging birds and slightly more frequent in brain tissues. Pigeons may act as indirect contributors to urban *T. gondii* transmission cycles and serve as bioindicators of environmental contamination. The study highlights the need for further surveillance, molecular studies, and public health interventions to mitigate zoonotic risk.

Acknowledgments:

The authors thank the Parasitology Department of Lorestan University for laboratory support and pigeon owners for facilitating sample collection.

References:

1. Dubey JP. Toxoplasmosis of Animals and Humans. 2nd ed. CRC Press; 2010.
2. Elmore SA, Jones JL, Conrad PA, Patton S, Lindsay DS, Dubey JP. *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. Trends Parasitol. 2010;26(4):190–196.
3. Frenkel JK, Dubey JP. Effects of freezing on the viability of *Toxoplasma* tissue cysts. J Parasitol. 1973;59(3):587–588.
4. Jiang HH, Huang SY, Zhou DH, et al. Prevalence and genetic characterization of *T. gondii* in pigeons in China. Vet Parasitol. 2014;205(3–4):361–365.
5. Dubey JP, Lago EG, Gennari SM, Su C, Jones JL. Toxoplasmosis in humans and animals in Brazil: high prevalence, high burden of disease, and epidemiology. Parasitology. 2012;139(11):1375–1424.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



6. Grigg ME, Sundar N. Population genetics of *Toxoplasma gondii*: sexual recombination punctuated by outbreaks and clonal expansions. *Int J Parasitol.* 2009;39(8):925–933.
7. Lopes AP, Dubey JP, Neto F, Rodrigues A, Martins T, Rodrigues M, et al. Seroprevalence of *T. gondii* in wild birds from Portugal. *Vet Parasitol.* 2011;182:378–382.
8. Sharif M, Sarvi S, Shokri A, Teshnizi SH, Rahimi MT, Mizani A, et al. Toxoplasmosis in Iran: A systematic review and meta-analysis on seroepidemiological studies. *Epidemiol Infect.* 2015;143(2):231–242.



Toxoplasmosis in Iranian Dogs: A Review of Prevalence and Risk Factors

Abolfazl Hajibemani¹, Ali Mosayyebzadeh², AmirMohammad NaghsheJavaheri³

Corresponding Author: Ali Mosayyebzadeh, al.mosayyebzadeh@urmia.ac.ir

1. Associate professor, Department of Clinical Sciences Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
2. DVSc candidate, Department of Theriogenology Faculty of Veterinary Medicine, Urmia university, Urmia, Iran.
3. Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Article code: 1587

Abstract

Background: Toxoplasmosis, caused by *Toxoplasma gondii*, is a globally significant zoonotic disease. While cats are the definitive hosts, dogs act as intermediate hosts and mechanical carriers, contributing to environmental contamination and transmission to livestock and humans. In Iran, understanding the prevalence and risk factors of toxoplasmosis in dogs is crucial for public and animal health management.

Methods: This review systematically analyzed published studies on toxoplasmosis in Iranian dogs, focusing on serological tests (IFAT, ELISA, MAT) and molecular methods (PCR). Data on risk factors including age, living conditions, diet, and geographic distribution were evaluated. Case reports were excluded to ensure reliability.

Results: The analysis revealed a seroprevalence ranging from 12% to 32.8% across different regions of Iran. Key risk factors identified were older age, stray/outdoor lifestyle, proximity to cats, and consumption of raw meat. Notably, while seropositivity was high, PCR detection of *T. gondii* DNA in feces was rare (0.8-1%), suggesting environmental exposure rather than active infection. The findings highlight dogs' role in transmitting the parasite to livestock, where it causes reproductive failures. Based on combined results from various diagnostic methods, the prevalence of toxoplasmosis in Iranian dogs is estimated to be between 13% and 24%. This indicates that dogs serve as important hosts and potential reservoirs for this zoonotic parasite.

Conclusion: Toxoplasmosis is widespread among Iranian dogs, with significant implications for livestock production and public health. Control measures should focus on hygiene improvement, restricting dog access to farms, and regular screening. Further research is needed to investigate transmission patterns and reproductive consequences in dogs and livestock.

Keywords: Toxoplasmosis, Iranian dogs, Prevalence, Risk factors, Zoonotic disease.



Introduction:

Toxoplasmosis is an ordinary protozoan infection caused by *Toxoplasma gondii*. *Toxoplasma* has multiple hosts in its life cycle, such as the definitive host (cats) and intermediate hosts like dogs. From the perspective of reproduction and public health, the infection is of particular significance due to the fact that female livestock infection leads to abortion, fetal loss, and impaired reproductive performance (1). Although dogs are not strict hosts, they are intermediate hosts and mechanical carriers of oocysts and are significant in the transmission cycle and environmental contamination of toxoplasmosis. Infected dogs or dogs having access to contaminated environments will facilitate the spread of the parasite into the environment and trigger transmission to female livestock and humans, especially on farms with direct or indirect contact (2).

In Iran, various studies have estimated the prevalence of toxoplasmosis in Iranian dogs in various regions, but the data remain incomplete. Accordingly, in this paper, the objective is to critically review in detail the prevalence, implicated factors, and reproductive impact of toxoplasmosis in Iranian dogs.

Materials and Methods:

This review reviews published literature on the prevalence of toxoplasmosis in Iranian dogs. The chosen studies employed serological methods (e.g., IFAT, ELISA, MAT) and molecular methods (PCR) for detecting *T. gondii* infection. Single cases or clinical case reports were not considered. Prevalence rates are compared, along with risk factors associated with them (age, sex, geographical zone, housing), clinical and epidemiologic results, with a particular emphasis on reproductive health (5,6).

Results and Discussion:

Studies conducted in different regions of Iran have investigated the prevalence of toxoplasmosis in dogs using serological (such as ELISA and IFA) and molecular (such as PCR) methods. The findings indicate a significant prevalence of this disease. For instance, a study in Tehran reported a prevalence of 23.6% using the serological ELISA method on 216 dogs (3). In another study in northern Iran, a prevalence of 13.1% was reported using the molecular PCR method on fecal samples from 229 dogs, confirming the presence of oocysts (eggs) of the parasite in dog feces and indicating the active role of dogs in spreading the disease in the environment (4). Furthermore, a study on breeding male dogs detected *Toxoplasma gondii* DNA in semen samples, suggesting the possibility of disease transmission through mating (5). Overall, by combining the results from various diagnostic methods, the prevalence of



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



toxoplasmosis in Iranian dogs is estimated to range from 13% to 24%, indicating that dogs can act as important hosts and potential reservoirs for this zoonotic parasite.

Based on studies conducted in Iran and the world, the most important risk factors associated with an increase in the prevalence of toxoplasmosis in dogs are(2, 6):

- Outdoor access and rural living: Dogs that are kept outdoors or live in rural areas have greater access to potential sources of infection, such as infected rodents, birds, raw meat, and environments contaminated with oocysts. This has been identified as the strongest risk factor in many studies.
- Age of the animal: The prevalence increases with age. This makes sense, as older dogs have had more time to be exposed to sources of contamination and infection.
- Feeding raw or undercooked meat: Dogs that are fed raw meat offal, offal, or undercooked meat are at a much higher risk of infection, as the tissues of infected animals contain tissue cysts of the parasite.
- Health status and presence of terrestrial diseases: Dogs with weakened immune systems (due to other diseases, malnutrition, or use of immunosuppressive drugs) are more susceptible to toxoplasmosis and reactivation of latent infection.
- Contact with cats: Since cats are the main host and producer of oocytes, coexistence or contact of the dog with infected cats (or access to an environment contaminated with cat feces, such as a garden or soil) can be an important risk factor.
- Breed and type of maintenance: Guard dogs and hunting dogs are at higher risk due to their type of use and greater contact with the outdoor environment and hunting prey.

Comparing with global data, the worldwide seroprevalence of toxoplasmosis in dogs varies significantly, ranging from 20% to 91%. According to global studies, stray dogs and those in contact with raw meat are more likely to be infected (6).

Reproductive Health Implications

Toxoplasmosis has a major impact on the reproductive tract of females in livestock. Although clinical signs are not often apparent in infected canines, they may act as sources of environmental infection and contamination to other animals, especially female livestock. This takes on reproductive significance for the following reasons:

1. Risk of Transmission to Female Livestock:
Dogs mechanically transmit *T. gondii* oocysts from cat feces to the environment through digging or foraging. Female farm animals raised on common farms with dogs may be in contact with such oocysts and become infected, resulting in abortion, stillbirth, and impaired reproductive performance (7).
2. Interference with Pregnancy and Parturition :



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Toxoplasmosis may lead to placentitis and fetal damage. By virtue of the close relationship of dogs with farms and breeding stations for livestock, high infection rates in dogs may augment the risk of toxoplasmosis among female livestock, causing enormous economic losses (1).

3. Environmental Control Importance:

With consideration of dogs' roles as mechanical vectors, management and control of stray dog populations and mitigation of dog-human contact with grazing environments must be included in reproductive health control in animal husbandry(1,7).

4. Public and Veterinary Health Implications:

Because toxoplasmosis possesses zoonotic potential among pregnant women, infection control in dogs also reduces human health hazards(1).

Iranian research has a high incidence of toxoplasmosis in domestic and stray canines. Age, housing type, diet, and environmental exposure are increasingly linked with risk of infection. Although dogs are not definitive hosts, their role in the transmission cycle, especially near livestock production, plays an important epidemiologic one. The presence of *T. gondii* in areas close to livestock can cause severe health and economic consequences. Owner education, environmental hygiene control, exclusion of dogs from farms, and repeated diagnostic screening of working and household dogs are crucial preventive strategies. Toxoplasmosis is prevalent in Iranian dogs and can cause damage to livestock and human fertility. Massive environmental and management interventions are required to control this infection. Further research on transmission patterns and reproduction consequences of *T. gondii* in Iranian dogs and livestock is recommended.

References:

1. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: from animals to humans. International journal for parasitology. 2000;30(12-13):1217-58.
2. Hataminejad M, Montazeri M, Tanzifi A, Galeh TM. Global geographical distribution of *Toxoplasma gondii* genotypes from dogs: A systematic review. Parasite Epidemiology and Control. 2025:e00433.
3. Hosseininejad M, Malmasi A, Hosseini F, Selk-Ghaffari M, Khorrami N, Mohebbi M, et al. Seroprevalence of *Toxoplasma gondii* infection in dogs in Tehran, Iran. Iranian journal of parasitology. 2011;6(1):81.
4. Izadi L, Sarvi S, Hosseini SA, Amouei A, Sharif M, Rahimi MT, et al. Copro-molecular diagnosis of the Toxoplasmatinae subfamily in dog and cat populations in northern Iran. Epidemiology and Health. 2020;42:e20200.۷۴



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



5. Shokooh Saljoughi S, Aghamiri SM, Akhtardanesh B, Jajarm M, Ziaali N. Molecular Detection of *Toxoplasma Gondii* in Semen of Breeding Dogs. Seyed Morteza and Akhtardanesh, Baharak and Jajarm, Maziar and Ziaali, Naser, Molecular Detection of *Toxoplasma Gondii* in Semen of Breeding Dogs.
6. Dini FM, Stancampiano L, Poglayen G, Galuppi R. Risk factors for *Toxoplasma gondii* infection in dogs: a serological survey. *Acta Veterinaria Scandinavica*. 2024;66(1):14.
7. Dubey JP. *Toxoplasmosis of animals and humans*: CRC press; 2016.



Herbal Medicines and Nanotechnology-Based Drug Delivery in the Treatment of *Leishmania* Infections: A Focus on Iran

Azin Salehe¹

Corresponding Author: Azin Salehe, Azin.salehe@gmail.com

1. Department of Veterinary Medicine, School of Veterinary Science, Islamic Azad University of Shahrekord, Iran.

Article code: 1593

Abstract

Background: Leishmaniasis is a neglected zoonotic disease caused by protozoa of the genus *Leishmania* and transmitted by phlebotomine sandflies. Iran is a major endemic country, with cutaneous leishmaniasis representing the predominant form. First-line drugs such as Glucantime face rising resistance rates of up to 40% in Iran, along with severe hepatotoxicity and nephrotoxicity. Consequently, there is growing interest in safer, more effective alternatives. Traditional medicinal plants combined with nanotechnology-based drug delivery systems represent a promising approach to enhance efficacy and reduce side effects.

Methods: This systematic review included studies from 2015–2025 retrieved from PubMed, Scopus, and Google Scholar. Inclusion criteria were studies evaluating the antiparasitic effects of medicinal plants against *Leishmania* spp. in vitro or in vivo, reporting quantitative measures (IC₅₀, % clearance), and comparing free extracts with nanocarrier-based formulations.

Results: Plants such as *Artemisia annua* (IC₅₀ = 6.5 µg/mL) and *Allium sativum* showed strong leishmanicidal activity, which improved when incorporated into nanocarriers, achieving 25–30% higher clearance rates. *Curcuma longa* and *Nigella sativa* enhanced macrophage-mediated immune responses. In Iranian studies, *Zataria multiflora* and *Peganum harmala* also exhibited significant efficacy. Herbal nanoformulations achieved results comparable to Glucantime but with lower toxicity.

Conclusion: Integrating herbal medicines with nanotechnology provides a sustainable alternative to conventional therapies for leishmaniasis. Future research should prioritize clinical trials, standardized formulations, and integration into Iran's national control programs.

Keywords: Leishmania, Herbal medicine, Nanotechnology, Drug resistance, Iran.

Introduction:

Leishmaniasis is a parasitic disease caused by protozoa of the genus *Leishmania*, transmitted by the bite of infected phlebotomine sandflies. It manifests in cutaneous, mucocutaneous, and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



visceral forms, with cutaneous leishmaniasis being the most prevalent in Iran (1). Globally, over 350 million people are at risk, with 1.5–2 million new cases annually (2). Iran ranks among the countries with the highest burden, particularly in rural provinces such as Isfahan, Khorasan, and Fars (3).

The disease carries significant public health and economic consequences, including disfiguring lesions, stigma, reduced quality of life, and treatment expenses. Despite Glucantime being the standard therapy, its efficacy is compromised by drug resistance and severe adverse effects (4,5). Thus, new treatment approaches are urgently needed.

Medicinal plants have long been used in Iran and neighboring regions to treat infectious diseases. Bioactive compounds such as alkaloids, flavonoids, and terpenoids act through mechanisms including oxidative stress induction, enzyme inhibition, and immunomodulation (6). Advances in nanotechnology allow these natural compounds to be formulated into liposomes, polymeric nanoparticles, and nanoemulsions, which enhance solubility, stability, targeted delivery, and therapeutic outcomes (7).

This review evaluates the effectiveness of medicinal plants and nanotechnology-based formulations against *Leishmania* spp., with emphasis on studies relevant to Iran.

Materials and Methods:

A systematic review was performed according to PRISMA guidelines. Databases searched included PubMed, Scopus, and Google Scholar, covering publications from 2015–2025.

Search terms:

“herbal medicine,” “*Leishmania*,” “nanotechnology,” “drug delivery.”

Inclusion criteria:

- Studies evaluating herbal extracts against *Leishmania* spp.
- In vitro or in vivo experiments with quantitative data (IC₅₀, % clearance).
- Comparative data for free vs. nanoformulated extracts.

Exclusion criteria:

- Reviews without primary data.
- Case reports without quantitative results.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Data extraction:

- Plant species and active compounds.
- Formulation type (free extract, liposome, nanoparticle, nanoemulsion).
- Outcomes (IC₅₀, clearance rate).
- Comparison with Glucantime.

From 312 identified studies, 57 met inclusion criteria, including 21 Iranian studies.

Results and Discussion:

Antileishmanial Activity of Medicinal Plants

- *Artemisia annua*: Artemisinin derivatives disrupted parasite mitochondria; IC₅₀ = 6.5 µg/mL (8).
- *Allium sativum*: Allicin induced apoptosis-like death; IC₅₀ = 12 µg/mL (9).
- *Curcuma longa*: Curcumin enhanced nitric oxide production by macrophages; IC₅₀ = 15 µg/mL (10).
- *Nigella sativa*: Thymoquinone increased oxidative burst; IC₅₀ = 18 µg/mL (11).

Nanotechnology-Based Delivery:

Nanoencapsulation enhanced pharmacological properties

- Liposomal *Artemisia* extract achieved 78% clearance vs. 60% free (12).
- Polymeric garlic nanoparticles improved intracellular uptake by 35% (13).
- *Curcuma longa* nanoemulsions increased stability and immune response.

Comparison with Conventional Drugs:

Glucantime cleared ~80% parasites but with high toxicity (5,14). Herbal nanoformulations reached similar efficacy with reduced toxicity. In Iranian studies, *Zataria multiflora* nanoliposomes achieved 70–75% clearance, while *Peganum harmala* nanoparticles showed ~68% inhibition (15).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 21. Comparative efficacy of selected herbal extracts and nanoformulations against *Leishmania* spp.

Plant Species	Formulation	IC ₅₀ (µg/mL)	Clearance (%)	Reference
<i>Artemisia annua</i>	Free extract	6.5	60	(8)
<i>Artemisia annua</i>	Nano-liposome	4.2	78	(12)
<i>Allium sativum</i>	Free extract	12	55	(9)
<i>Allium sativum</i>	Polymeric NP	8.5	70	(13)
<i>Curcuma longa</i>	Nanoemulsion	10	65	(10)
<i>Nigella sativa</i>	Free extract	18	50	(11)
Glucantime	—	—	80	(5)

Table 22. Iranian medicinal plants and their nanotechnology-based applications against *Leishmania* spp.

Plant Species (Iran)	Active Compounds	Nanoformulation	Model	Efficacy	Reference
<i>Zataria multiflora</i>	Thymol, carvacrol	Nanoliposome	In vitro/in vivo	70–75% clearance, macrophage activation	(14)
<i>Peganum harmala</i>	Harmaline, harmine	Polymeric NP	In vitro	68% inhibition, reduced toxicity	(15)
<i>Ferula assa-foetida</i>	Coumarins, sulfur	Nanoemulsion	In vitro	55% clearance, solubility ↑	(16)
<i>Lawsonia inermis</i>	Lawsonone	Silver NP	In vitro	>60% promastigote inhibition	(17)
<i>Nigella sativa</i> (Iranian)	Thymoquinone	Nanoemulsion	In vivo mice	50% lesion reduction	(11)



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Implications for Iran: Local plants such as *Zataria multiflora* and *Peganum harmala* are abundant and low-cost, making them suitable for integration into Iran's leishmaniasis control programs. Nanotechnology enhances their pharmacological potential, aligning with WHO strategies for sustainable, eco-friendly treatments.

Herbal medicines combined with nanotechnology offer a promising, safer alternative to conventional drugs for leishmaniasis. These approaches can mitigate drug resistance, reduce toxicity, and improve accessibility in endemic areas such as Iran.

Future directions include:

1. Clinical trials in Iran.
2. Standardized extraction and formulation.
3. Multidisciplinary collaborations.
4. Governmental and industrial investment.

Acknowledgment:

The author thanks WHO EMRO and Iranian researchers for contributions to this review.

References:

1. WHO. Leishmaniasis. World Health Organization. 2023.
2. Mohebbali M. Epidemiology of leishmaniasis in Iran. Iran J Parasitol. 2022;17(1):1-20.
3. Shirzadi MR, Esfahania SB. Cutaneous leishmaniasis in Iran. Trop Med Int Health. 2021;26(3):310-319.
4. Sundar S, et al. Resistance to antimonial drugs. Clin Infect Dis. 2019;68(2):268-275.
5. Mohebbali M, et al. Drug resistance in Leishmania in Iran. Trop Med Int Health. 2021;26(5):567-575.
6. Al-Quraishy S, et al. Anti-parasitic activity of medicinal plants. Parasitol Res. 2021;120:781-795.
7. Sharma A, et al. Nanotechnology in herbal drug delivery. Int J Nanomedicine. 2021;16:1231-1245.
8. Correa-Antonio J, et al. Artemisia annua. Exp Parasitol. 2019;198:22-29.
9. Abdelhady NM, et al. Garlic extract efficacy. Parasite. 2020;27:10.
10. Shokri A, et al. Curcuma longa and NO modulation. Vet Parasitol. 2018;259:22-28.
11. Ajayi EO, et al. Nanoencapsulation of herbal antiparasitics. J Drug Deliv Sci Technol. 2022;68:103109.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



12. Singh R, et al. Controlled release herbal drugs. *Curr Drug Deliv*. 2020;17(8):675-687.
13. AbouLaila M, et al. Synergistic effects of herbal drugs. *Parasitol Int*. 2018;67:544-551.
14. Salehzadeh A, et al. Nanoliposomal Zataria multiflora. *Iran J Parasitol*. 2022;17(4):412-420.
15. Jafarian-Dehkordi M, et al. Peganum harmala efficacy. *J Parasit Dis*. 2021;45:987-995.
16. Faridi P, et al. Ferula assa-foetida nanoemulsion. *J Herb Med*. 2023;36:100643.
17. Ghaffarifar F, et al. Lawsonia inermis nanoparticles. *Parasitol Res*. 2019;118:2021-2029.



***Tritrichomonas foetus* in Iran and Nearby Countries: Absence or Not Reported**

Sattar Zolfaghari Daghighan¹, Seyedeh Yasaman Ghanoun¹, Elahe Ebrahimzadeh²

Corresponding Author: Elahe Ebrahimzadeh, eebrahimzade@um.ac.ir

1. D.V.M. student, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1596

Abstract

Background: *Tritrichomonas foetus* is a flagellated protozoan parasite causing reproductive tract disease in cattle, leading to infertility and abortion, and gastrointestinal issues, such as colitis and diarrhea, in cats. This review evaluates the prevalence of *T. foetus* in Iran, and surrounding countries such as Iraq and Turkey.

Methods: Thirteen studies on *T. foetus* prevalence in cattle and cats from Iran, Iraq, and Turkey, published between 2010 and 2025, were analyzed using Google Scholar, PubMed, and Magiran databases.

Results: *T. foetus* infections were documented in cattle and cats across all three countries, with higher prevalence in aborted cattle and cats with chronic diarrhea..

Conclusion: Recent research in these countries reveals the existence of these parasite infections in cattle and feline populations. Considering the potential for infection transmission between cattle and cats, alongside the economic effects of infertility and abortion in cattle, it is necessary to do additional epidemiological investigations to develop suitable control methods.

Keywords: *Tritrichomonas foetus*, Cattle, Cat, PCR.

Introduction:

Trichomonas spp., a genus of flagellated protozoans, is responsible for causing venereal and intestinal trichomoniasis in cattle and cats (1). Bovine trichomoniasis is a sexually transmitted disease in cattle caused by the protozoan *T. foetus*. It was initially documented in the United States within a Pennsylvania dairy herd in 1932 (2).

T. fetus relies on bulls or cows for its survival, exhibiting a brief lifespan outside these hosts due to its quick degradation in elevated temperatures or arid conditions, and its susceptibility to various disinfectants (3).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Due to low pregnancy rates, infertility, endometritis, and abortion in pregnant cows and heifers, the disease can cause significant economic losses. In cows, the infection initially causes vaginitis, then spreads to the uterus, leading to preterm abortions and possible permanent infertility.

Most pregnancies are lost around 2.5 weeks after conception when maternal recognition occurs, but fetal death can happen at any stage until five months of gestation (4).

T. foetus consistently and asymptotically colonizes the epithelial surfaces of the prepuce, penis, and, in some cases, the urethral opening in bulls. It is noteworthy that semen quality and sexual behavior remain unaffected (5,6). During mating, the parasite is transmitted from infected bulls to heifers or cows (4). Although cows typically eliminate the infection, bulls may remain lifelong carriers, serving as the primary reservoir for the parasite (6).

Trichomoniasis has been eradicated from numerous cattle populations globally when management practices involve limited mingling of cattle and prevalent use of AI for reproduction (2). Performing diagnostic assessments on bulls before semen collection minimizes the risk of disease transmission (3). Bulls diagnosed with the infection should be culled from the herd and substituted with young (≤ 2 years) virgin bulls. As an alternative, AI can efficiently regulate the spread of *T. foetus*, but switching entirely from natural services to AI would not be possible. If the cow herd has been exposed to *T. foetus*, the cows should be examined, and those exhibiting recent pregnancy loss or pyometra should be removed from the herd (4).

Over a decade ago, *T. foetus* was found in the feces of domestic cats with chronic diarrhea. For a long time, *T. foetus* may have been misidentified as *Pentatrichomonas* sp. or *Giardia* sp. during microscopic analysis; however, genetic identification via molecular techniques established *T. foetus* as the etiological agent of feline trichomonosis in 2003 (7–9).

Common clinical signs in natural infections include chronic or intermittent large bowel diarrhea, observed in around 61% to 64% of infected cats, with numerous cats exhibiting no diarrhea in the six months before diagnosis (10,11). The feces are characterized by a yellow-green color and a foul smell, exhibiting typical indications of colitis such as fresh blood, mucus, fecal incontinence, tenesmus, and gas (9,12,13).

T. foetus transmission in cats occurs through the fecal–oral pathway. Since *T. foetus* doesn't release cysts into the environment, direct contact with contaminated fresh feces may be enough to spread the parasite (7). The trophozoites of *T. foetus* survived for over three hours in cat urine and sauced cat food, two hours on ground cat food, and just thirty minutes in tap or purified water (14). All of these findings point to the possibility that transmission is not just confined to direct cat contact. Although it is less common in the latter case, trichomonad contamination of food and water could be a significant transmission pathway. Self-grooming



and mutual grooming among cats, along with fecal contamination of the environment, are significant factors in the epidemiology of the disease (15,16).

Because of stress or immunological immaturity, kittens or other young animals raised in agglomeration-prone areas are more susceptible to infection. Therefore, reducing stress and preventing overcrowding would be crucial to lowering the risk of agent exposure (16).

The protozoan parasite, *T. foetus*, has been identified in Iran and several adjacent countries, highlighting its importance, particularly in cattle, due to its impact on reproductive health. Given the economic and veterinary significance of this disease, additional research is warranted. This section analyzes the prevalence of *T. foetus* based on reports available from 2011 to 2025.

Materials and Methods:

This study examined the papers regarding the frequency of *T. foetus* in cattle and cats in Iran and neighboring countries. A total of 13 articles were analyzed, comprising 12 on the parasite report and one additional review article. Of the case report articles, nine instances pertained to *T. foetus* in cattle (2 cases in Iran, 4 cases in Iraq, and 3 cases in Turkey), while 3 cases involved *T. foetus* in cats (2 cases in Iran and 1 case in Turkey).

Results and Discussion:

In the summer of 2019, a random sampling was performed in two main slaughterhouses in Isfahan, Iran, involving 73 male and 27 female cows. Following DNA extraction, the nested PCR technique was employed to detect the presence of the parasite. PCR analysis confirmed that 2% of 100 samples were positive for *T. foetus* (8.2% of bulls and 7.4% of cows) (3).

In Tabriz, sperm samples were taken from 45 bulls at the AI center and tested by PCR. Six of the samples showed infection with the non-*T. foetus* organisms (6).

Documents published in Iraq indicate that the contamination rate is higher than in Iran. From March to August 2022, cPCR tests conducted on vaginal fluid from 62 aborted cows revealed that 13 cows (20.97%) were infected with *T. foetus* (17). In Nineveh Province, 87 vaginal mucosal samples were randomly collected from heifer cows at various stages of gestation, with 11 identified as containing fetuses infected using PCR (18). In Basra, preputial fluid was collected from 100 randomly selected bulls delivered to the slaughterhouse, and diagnosis was conducted using the direct method and Giemsa staining, revealing a 2% incidence of fetal infection (19).

In Turkey, a study conducted in the Anatolian region involved the extraction of abomasal contents from 246 aborted fetuses. Of these, four samples were identified as infected with *T.*



foetus through the application of direct methods and PCR analysis (20). In a separate investigation, PCR analysis revealed that 2 out of 55 aborted fetal samples were associated with *T. foetus* (21). Finally, in a study conducted in the Aydın region of Turkey, 164 cows exhibiting various reproductive issues were examined, and Vaginal smear staining results indicated that 14 of these cows were infected with *T. foetus* (22).

Likely attributable to the parasite's role in inducing abortion, the prevalence of infected individuals is elevated among aborted cows, including those in Iraq. Consequently, a higher incidence of *T. foetus* is observed within these populations. Conversely, the quantity of positive samples appears to differ across various diagnostic techniques. For instance, direct microscopy and Giemsa staining yield fewer positive results compared to PCR methods (23).

Reports of this parasitic infection in cats are scarce. Nonetheless, two case reports, representing the initial documented instances of infection, have been recorded in Iran and Turkey (13,24). In Ahvaz, Iran, 50% (7 out of 14) of cats presenting with diarrhea and 66.66% (8 out of 12) of cats with a prior history of diarrhea were found to be infected with the parasite. However, a history of diarrhea does not appear to be significantly associated with the development of the parasitic infection (25).

The increased prevalence of *T. foetus* in felines with a history of diarrhea suggests that the condition may persist as a chronic illness, and because the disease is rapidly transmitted among cats via the fecal-oral route and, unlike in cattle, is not sporadic (25), the situation will be more complicated.

Considering all the above, heightened attention to documented cases, potential cross-species transmission from felines to bovines, and effective disease control strategies is imperative. The adoption of AI techniques and the prohibition of feline access to dairy farm facilities are likely pivotal strategies for curtailing disease dissemination.

T. foetus is a prevalent parasite in tropical regions. Although there are limited and dispersed accounts of the parasite in Iran and neighboring countries, it is uncertain whether the scarcity of reports is due to underreporting or if *T. foetus* is genuinely less prevalent in these regions, possibly as a result of the hot and arid environmental conditions in the areas investigated.

Consequently, to formulate a definitive conclusion concerning the prevalence of the disease, additional research is required.



References:

1. Al-Hasnawy MH, Rabee AH. A review on *Trichomonas* species infection in humans and animals in Iraq. Iraqi Journal of Veterinary Sciences. 2023 Mar 1;37(2):305–13.
2. Ondrak JD. *Tritrichomonas foetus* Prevention and Control in Cattle. Vol. 32, Veterinary Clinics of North America - Food Animal Practice. W.B. Saunders; 2016. p. 411–23.
3. Hadi T, Ali S. Frequency of *Tritrichomonas foetus* Infection in cows of Isfahan. 2020 Jan 1 [cited 2025 Jul 29];17(370):3299–304. Available from: <https://sid.ir/paper/951504/en>
4. Mardin Omer Mohammed, Kwestan Najm Ali, Hiewa Othman Dyary. Bovine Trichomoniasis. International Journal of Agriculture and Biosciences [Internet]. 2023;3:107–13. Available from: <https://uniquescientificpublishers.com/wp-content/uploads/2023/oht-v3/107-113.pdf>
5. Michi AN, Favetto PH, Kastelic J, Cobo ER. A review of sexually transmitted bovine trichomoniasis and campylobacteriosis affecting cattle reproductive health. Theriogenology. 2016 Mar 15;85(5):781–91.
6. Asadpour R, Jafari R. PCR detection of *Trichomonad* species in the semen of bulls [Internet]. Article in Bulgarian Journal of Veterinary Medicine. 2011. Available from: <https://www.researchgate.net/publication/228456225>
7. Bastos BF, de Almeida FM, Brener B. What is known about *Tritrichomonas foetus* infection in cats? Revista Brasileira de Parasitologia Veterinária [Internet]. 2019 Mar 11 [cited 2025 Aug 1];28(1):1–11. Available from: <https://www.scielo.br/j/rbpv/a/YvnsbB5rmdPFSTM4TJB5SNM/?lang=en>
8. Levy MG, Gookin JL, Poore M, Birkenheuer AJ, Dykstra MJ, Litaker RW. *Tritrichomonas foetus* and not *Pentatrichomonas hominis* is the etiologic agent of feline Trichomonal diarrhea. [https://doi.org/10.1645/0022-3395\(2003\)089\[0099:TFANPH\]20CO;2](https://doi.org/10.1645/0022-3395(2003)089[0099:TFANPH]20CO;2) [Internet]. 2003 Feb 1 [cited 2025 Aug 2];89(1):99–104. Available from: [https://bioone.org/journals/journal-of-parasitology/volume-89/issue-1/0022-3395_2003_089_0099_TFANPH_2.0.CO_2/Tritrichomonas-foetus-And-Not-Pentatrichomonas-hominis-Is-The-Etiologic-Agent/10.1645/0022-3395\(2003\)089\[0099:Tfanph\]2.0.Co;2.Full](https://bioone.org/journals/journal-of-parasitology/volume-89/issue-1/0022-3395_2003_089_0099_TFANPH_2.0.CO_2/Tritrichomonas-foetus-And-Not-Pentatrichomonas-hominis-Is-The-Etiologic-Agent/10.1645/0022-3395(2003)089[0099:Tfanph]2.0.Co;2.Full)
9. Gookin JL, Breitschwerdt EB, Levy MG, Gager RB, Benrud JG. Diarrhea associated with trichomonosis in cats. J Am Vet Med Assoc [Internet]. 1999 Nov 15 [cited 2025 Aug 2];215(10):1450–4. Available from: <https://avmajournals.avma.org/view/journals/javma/215/10/javma.1999.215.10.1450.xml>
10. Xenoulis PG, Lopinski DJ, Read SA, Suchodolski JS, Steiner JM. Intestinal *Tritrichomonas foetus* infection in cats: A retrospective study of 104 cases. J Feline Med



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- Surg [Internet]. 2013 Dec [cited 2025 Aug 2];15(12):1098–103. Available from: <https://journals.sagepub.com/doi/full/10.1177/1098612X13495024>
11. Kuehner KA, Marks SL, Kass PH, Sauter-Louis C, Grahn RA, Barutzki D, et al. *Tritrichomonas foetus* infection in purebred cats in Germany: Prevalence of clinical signs and the role of co-infection with other enteroparasites. J Feline Med Surg [Internet]. 2011 Apr [cited 2025 Aug 2];13(4):251–8. Available from: <https://journals.sagepub.com/doi/full/10.1016/j.jfms.2010.12.002>
 12. Mardell EJ, Sparkes AH. Chronic diarrhoea associated with *Tritrichomonas foetus* infection in a British cat. Veterinary Record. 2006 Jun 3;158(22):765–6.
 13. Cheraghi B, Vafaei R, Nassiri SM. The first report of clinical case of intestinal trichomoniasis caused by *Tritrichomonas foetus* in a cat with chronic diarrhea in Iran. Iran J Vet Res [Internet]. 2021 Jun 1 [cited 2025 Jul 29];22(3):248. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC8573399/>
 14. Rosypal A, Ripley A, Walden H, ... BBV, 2012 undefined. Survival of a feline isolate of *Tritrichomonas foetus* in water, cat urine, cat food and cat litter. ElsevierAC Rosypal, A Ripley, HDS Walden, BL Blagburn, DC Grant, DS LindsayVeterinary parasitology, 2012•Elsevier [Internet]. [cited 2025 Aug 2]; Available from: <https://www.sciencedirect.com/science/article/pii/S0304401711007187>
 15. Van der Saag M, McDonell D, Šlapeta J. Cat genotype *Tritrichomonas foetus* survives passage through the alimentary tract of two common slug species. Vet Parasitol. 2011 May 11;177(3–4):262–6.
 16. Hale S, Norris JM, Šlapeta J. Prolonged resilience of *Tritrichomonas foetus* in cat faeces at ambient temperature. Vet Parasitol. 2009 Dec 3;166(1–2):60–5.
 17. Gharban HAJ. Molecular prevalence and phylogenetic confirmation of bovine trichomoniasis in aborted cows in Iraq. Vet World [Internet]. 2023 Mar 1 [cited 2025 Jul 28];16(3):580. Available from: <https://pmc.ncbi.nlm.nih.gov/articles/PMC10082752/>.
 18. Al-Obaidii WA, Al-Obaidi QT, Hasan SD. Detection of Trichomoniasis in cattle in Nineveh province. Iraqi Journal of Veterinary Sciences [Internet]. 2021 [cited 2025 Aug 1];35(2):287–90. Available from: www.vetmedmosul.com
 19. Hassan BJ. Detection of *Trichomonas spp.* from Penile Bulls Fluid in Iraq. Asian Journal of Research in Infectious Diseases. 2020 Oct 28;29–32.
 20. Guven E, Bastem Z, Avcioglu H, Erdem H. Molecular determination of *Tritrichomonas spp.* in aborted bovine fetuses in Eastern Anatolian Region of Turkey. Vet Parasitol. 2013 Sep 23;196(3–4):278–82.
 21. Irehan B, Sonmez A, Atalay MM, Ekinci AI, Celik F, Durmus N, et al. Investigation of *Toxoplasma gondii*, *Neospora caninum* and *Tritrichomonas foetus* in abortions of cattle, sheep and goats in Turkey: Analysis by real-time PCR, conventional PCR and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- histopathological methods. *Comp Immunol Microbiol Infect Dis*. 2022 Oct 1;89:101867.
22. Serin I, Aldemir O, Ceylan A. Prevalence of Trichomoniasis in Dairy Cows with Some Reproductive Disorders in Aydin Province of Turkey. *researchgate.net* [Internet]. 2010 [cited 2025 Aug 5]; Available from: https://www.researchgate.net/profile/Guenes-Erdogan/publication/240828079_Prevalence_of_Trichomoniasis_in_Dairy_Cows_with_Some_Reproductive_Disorders_in_Aydin_Province_of_Turkey/links/562e0c4a08aef25a24432ebf/Prevalence-of-Trichomoniasis-in-Dairy-Cows-with-Some-Reproductive-Disorders-in-Aydin-Province-of-Turkey.pdf.
 23. Nasir MAH, S.R. AI, Al-Masoudi WA. Investigation of *Tritrichomonas foetus* in Cattle Using Different Methods in Basrah City – Iraq. *Biomedicine and Chemical Sciences* [Internet]. 2023 Jan 1 [cited 2025 Aug 1];2(1):39–43. Available from: <https://journals.irapa.org/index.php/BCS/article/view/334>.
 24. Yildiz K, Sursal N. The First Report of *Tritrichomonas foetus* in Cats from Turkey [Internet]. Article in *Israel Journal of Veterinary Medicine*. 2019. Available from: <https://www.researchgate.net/publication/336288044>.
 25. Hamidinejat H, Hamd Baghestani A, Larki S, Mosallanejad B. Molecular Survey on *Tritrichomonas foetus* infection in cats of Southwestern Iran. *Iranian Veterinary Journal* [Internet]. 2023 Aug 23 [cited 2025 Jul 29];19(2):64–74. Available from: https://www.ivj.ir/article_182208_en.html.



The Public Health Significance of the Pigeon Tick, *Argas reflexus*: A Comprehensive Review

Mohammad Sajed Sarvari¹, Ali Moshaverinia¹

Corresponding Author: Ali Moshaverinia , moshaverinia@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1599

Abstract

Background: *Argas reflexus* is a Argasidae member that primarily parasitizes feral pigeons (*Columba livia*). In urban areas with large pigeon populations, it often infests buildings such as attics, bell towers, and roofs used by pigeons. When its avian hosts are absent, *A. reflexus* can invade human dwellings and bite people, posing significant public health concerns. This review synthesizes the current literature on *A. reflexus*, emphasizing its biology and epidemiology, health impacts (especially allergic reactions), and the challenges of controlling this urban parasite.

Methods: We reviewed the literature on *Argas reflexus* by searching PubMed, Scopus, and Google Scholar for relevant articles, focusing on its biology, health effects, and management.

Results: The main health risk of *A. reflexus* is severe allergic reactions, including life-threatening anaphylaxis, often at night. It can carry pathogens like *Borrelia*, *Rickettsia*, and *Coxiella burnetii*, but its role in spreading them to humans is unclear. Its long lifespan and ability to survive without food make elimination from buildings difficult.

Conclusion: The primary public health threat posed by the urban ectoparasite *Argas reflexus* is not disease transmission, but its capacity to induce severe, life-threatening allergic reactions. Its unique biological resilience renders eradication from infested buildings highly challenging, necessitating an integrated management approach targeting both the ticks and their pigeon hosts. Therefore, the key takeaway for public health practice is the urgent need for greater awareness among clinicians to prevent misdiagnosis of nocturnal anaphylaxis. For future research, the priority must be to move beyond simple pathogen detection and conduct vectorial competence studies to definitively clarify its role in disease transmission. Effective control of this resilient parasite ultimately depends on this dual approach: enhancing clinical vigilance in the present while pursuing targeted research for the future.

Keywords: *Argas reflexus*, Pigeon tick, Public health, Anaphylaxis, Urban ectoparasite.



Introduction:

Argas reflexus (Fabricius, 1794) is a soft tick closely associated with feral pigeons (*Columba livia domestica*) (1,2). Originally a parasite of rock-nesting birds, *A. reflexus* has adapted to man-made structures, forming large populations in pigeon roosts on buildings across the Palearctic (Europe, North Africa, and parts of Asia) (3). When pigeons are removed or seasonally absent, hungry *A. reflexus* ticks readily invade nearby flats and apartments to seek new blood meals(3,4). Human exposure to *A. reflexus* is concerning because its bites inject strong allergens that can trigger severe reactions, including hives, asthma-like symptoms, or anaphylaxis in sensitive individuals. (5,6). Notably, bites from *A. reflexus* can trigger severe IgE-mediated allergic reactions in sensitized individuals, ranging from intense local itching to life-threatening anaphylactic shock(5,6). Given these significant public health concerns, this review aims to synthesize the current literature on *Argas reflexus*, focusing on its biology, epidemiology, health impacts—particularly allergic reactions—and the challenges associated with its control in urban environments.

Materials and Methods:

A comprehensive literature review was conducted in PubMed, Scopus, and Google Scholar using keywords such as "*Argas reflexus*," "pigeon tick," "anaphylaxis," "allergic reaction," and "vector-borne diseases." Eligible articles were in English and focused on the tick's biology, epidemiology, public health relevance, allergic potential, vector competence, or control. We included original studies, case reports, and reviews with substantive data and excluded works that mentioned the tick only briefly, lacked peer review, or had no full text. Article selection involved title and abstract screening, followed by full-text review based on predefined criteria.

Results and Discussion:

Biology and Epidemiology

The life cycle of *Argas reflexus* is a multi-stage process that can take 3–11 years to complete under optimal conditions (7). The species is notable for its long lifespan, over ten years, and can survive several years without feeding (6,7). This exceptional survival is enabled by key adaptations, including a low metabolic rate, unparalleled resistance to desiccation, and the ability to absorb atmospheric moisture (7).

Although these traits are well-documented, little is known about how factors like climate change and urbanization may affect their future distribution and populations.



Urban environments with pigeon populations provide ideal microhabitats for *A. reflexus*, leading to large-scale infestations in buildings (1). Control measures that only target pigeons can be counterproductive, as the remaining hungry ticks will actively seek alternative hosts, including humans (1,4). This shows that *A. reflexus* epidemiology is closely tied to urban pigeons and requires integrated management of both birds and ticks. (4).

Morphology and identification

Correctly identifying *Argas reflexus* is a critical step for both clinical diagnosis and effective control. As a member of the Argasidae family, it is a soft tick, which means it lacks a hard dorsal shield. This gives the tick's body a leathery and wrinkled texture. The adult tick is oval-shaped and flattened when unfed, measuring about 4-10 mm in length, with a yellowish-brown to reddish-brown color(7). Species-level identification requires fine structures. *A. reflexus* has a quadrangular sutural line forming a flat body border, integument with tubercles and pits, and a broadly rounded anterior margin—key for distinguishing it from related species (8).

One of the most important diagnostic challenges is to differentiate *Argas reflexus* from *Argas hermanni*. Both species are part of the *A. reflexus* group, they often parasitize the same host (pigeons), and can be found in similar urban environments This can cause confusion(8). Adult *Argas reflexus hermanni* is differentiated from the European subspecies by its smaller size, broader and blunter hypostomal denticles, and smaller, non-retrograde tarsal protuberances. Larval forms provide the clearest distinction, as *hermanni* larvae have a 3/3 dental formula, in contrast to the 2/2 formula of *A. r. reflexus* larvae (8).

For this reason, in many clinical reports, the tick is simply identified as belonging to the "*A. reflexus* group" if a specialist is not available to confirm the exact species.

Argas reflexus in iran

Studies of tick fauna in Iran show *Argas reflexus* is present in several provinces but at low rates. For example, a survey in Tehran Province found *A. reflexus* in only about 1.05% of ticks collected (6 of 574)(9). Other surveys reported similarly low figures: 2.6% in Hamadan Province (10), 6.65% in Lorestan Province (11), and roughly 9.09% in a West Azerbaijan Province study (northwest Iran) (12). In sum, *A. reflexus* has been recorded in Tehran, Hamadan, Lorestan and West Azerbaijan (among others), but it consistently makes up only a single-digit percentage of the local ticks in these surveys.

Direct Health Effects: Allergy

The main health risk of *Argas reflexus* is severe allergic reactions. Repeated tick bites can sensitize people to saliva proteins. Clinically, this manifests as immediate (Type I) hypersensitivity: from localized swelling and itching at bite sites up to systemic anaphylaxis(5,6). Many case reports have described victims suffering life-threatening allergic shock after nocturnal pigeon tick bites(5,6). Unlike mosquito bites, *A. reflexus* bites are painless



at the time and occur at night, so sensitized individuals may wake with hives or respiratory distress without realizing a tick bite occurred. This can lead to misdiagnosis; episodes of nighttime anaphylaxis have sometimes been mistaken for asthma attacks or idiopathic shock until investigators identified pigeon ticks in the home(5,6). Diagnostic confirmation involves detecting specific IgE antibodies against pigeon tick antigens in the patient's blood(5). Key reviews emphasize that *A. reflexus* should be considered whenever unexplained nocturnal allergic reactions occur in urban dwellers with pigeon exposure (5,6).

Vectorial Potential and Pathogens

Argas reflexus is a potential disease vector, but its role in human health is unclear. It transmits the avian pathogen *Borrelia anserina*, yet surveys show no evidence of spreading human Lyme disease (*B. burgdorferi* s.l.) (3). Similarly, although DNA from pathogens like *Rickettsia* spp. and *Coxiella burnetii* has been detected in some ticks, transmission to humans has not been demonstrated(2,3). A critically gap is that detecting pathogen DNA does not prove vector competence. No study has shown *A. reflexus* acquiring, maintaining, and transmitting these agents to humans. Thus, despite its theoretical zoonotic potential, its confirmed public health impact is minimal, mainly causing allergic reactions.

Management and Control

The first step for controlling is removing the pigeon population by sealing entry points and eliminating nests (4). Crucially, this must be paired with immediate tick eradication, as removing birds alone will cause starving ticks to migrate into human living areas. Professional acaricide application is usually necessary to kill ticks in hidden voids, but its effectiveness can be limited by inaccessibility, toxicity, and resistance concerns (6). A key knowledge gap is the lack of systematic data on acaricide resistance in urban *A. reflexus*, which could seriously hinder control efforts.

Supplemental measures like heat treatments and thorough vacuuming help reduce tick numbers, while ongoing monitoring with tools like heated bait traps is essential to detect survivors (4). Finally, long-term prevention is achieved through environmental measures that deter pigeons, such as installing bird spikes and banning feeding, to prevent new infestations (3,4). Research on optimizing non-chemical methods and new strategies, like biological control, is limited but represents a key future direction. Overall, *A. reflexus* control must be integrated with urban pigeon management to protect human health.



References:

1. Dautel H, Scheurer S, Kahl O. The pigeon tick (*Argas reflexus*): its biology, ecology, and epidemiological aspects. *Zentralbl Bakteriol.* 1999 Dec;289(5-7):745-53.
2. Gholizadeh M, Tavassoli M, Rezaei F, Nikousefat Z. Evaluation of histopathological features of *Argas reflexus* bite in pigeon. *Iran J Vet Sci Technol.* 2015;6:14-20.
3. Bonnefoy X, Kampen H, Sweeney K. Public health significance of urban pests. World Health Organization; 2008.
4. Boxler B, Odermatt P, Haag-Wackernagel D. Host finding of the pigeon tick *Argas reflexus*. *Med Vet Entomol.* 2016 Jun;30(2):193-9.
5. Spiewak R, Lundberg M, Johansson G, Buczek A. Allergy to pigeon tick (*Argas reflexus*) in Upper Silesia, Poland. *Ann Agric Environ Med.* 2006;13(1):107-12.
6. Dautel H, Scheurer S, Kahl O. The pigeon tick (*Argas reflexus*): Its biology, ecology, and epidemiological aspects. *Zentralbl Bakteriol.* 2000 Jan 1;289:745-53.
7. Kaufman PE. Medical and Veterinary Entomology. *Florida Entomol.* 2019;102(3):666.
8. Hoogstraal H, Kohls GM. Observations on the subgenus *Argas* (Ixodoidea, Argasidae, *Argas*). 3. A biological and systematic study of *A. reflexus hermanni* Audouin, 1827 (revalidated), the African bird argasid. *Ann Entomol Soc Am.* 1960;53(6):743-55.
9. Beik-Mohammadi M, Tavassoli M, Telmadarraiy Z, Vatandoost H, Dabiri F. Ticks (*Argasidae* and *Ixodidae*) in Tehran Province, Central Iran: Distribution and Seasonal Activity. *Vet Med Int.* 2025 Jun 27;2025.
10. Vatandoost H, Ghaderi A, Javadian E, Nia AH, Rassi Y, Piazak N, et al. Distribution of Soft Ticks and Their Infection with *Borrelia* in Hamadan Province, Iran. *Iran J Public Heal.* 2002 Nov 30;32.
11. Telmadarraiy Z, Kooshki H, Edalat H, Vatandoost H, Bakhshi H, Faghihi F, et al. Study on Hard and Soft Ticks of Domestic and Wild Animals in Western Iran. *J Arthropod Borne Dis.* 2023 Feb 28;16.
12. Yakhchali M, Bahramnejad K, Almasi O. Ticks (*Acari: Ixodida: Ixodidae* and *Argasidae*) abundance and associated risk factors for animals in the natural habitat of Sanandaj suburb, Iran. *Int J Acarol - INT J ACAROL.* 2013 Apr 4;38:1-9.



Advances in *Toxoplasma gondii* Vaccines for Ovine Health

Seyedeh Yasaman Ghanoon¹, Sattar Zolfaghari Daghighan¹, Elahe Ebrahimzadeh²

Corresponding Author: Elahe Ebrahimzadeh, ebrahimzade@um.ac.ir

1. D.V.M. student, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1600

Abstract

Background: Toxoplasmosis is a widespread parasitic infection caused by *Toxoplasma gondii*, an obligate intracellular protozoan. It is considered a global health issue with medical, veterinary, and economic significance. In livestock, particularly sheep and goats, *T. gondii* causes substantial economic losses through abortions, stillbirths, and neonatal mortality. This article aims to evaluate the research types and commercial vaccines available for managing toxoplasmosis in veterinary medicine.

Methods: Articles available from Google Scholar and PubMed databases were reviewed regarding *T. gondii*, ways to control and prevent it, as well as the types of vaccines available.

Results: Current treatments, such as pyrimethamine and sulfadiazine, mainly address the acute tachyzoite stage but are often ineffective against the persistent bradyzoite cysts. These therapies may also cause significant side effects and fail to prevent reinfection. Therefore, developing a safe, effective, and long-lasting vaccine is a critical and preferred strategy for preventing and controlling toxoplasmosis in both humans and animals.

Conclusion: Due to the absence of a definitive treatment, the significant economic losses caused by toxoplasmosis, and its zoonotic nature, there is a pressing need to develop preventive strategies. Furthermore, research into novel and effective vaccination approaches could play a crucial role in controlling the spread of this widespread disease.

Keywords: *Toxoplasma gondii*, Sheep, Vaccine, Zoonotic.

Introduction:

Toxoplasma gondii, a globally significant intracellular protozoan, can infect nearly all warm-blooded animals, including humans. Its complicated life cycle includes an asexual phase in intermediate hosts and a sexual phase limited to the intestinal epithelium of cats, the definitive hosts. This biological duality facilitates the parasite's worldwide spread and epidemiological



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



impact. Clinical signs vary from asymptomatic infection to severe disease, influenced by the host species, immunological condition, and stage of infection (1).

T. gondii was first identified in 1908, but its complete life cycle remained unclear until 1970, when researchers confirmed the parasite's sexual phase in the small intestine of cats. This discovery established felids as the definitive hosts and provided critical insights into the epidemiology and transmission of toxoplasmosis in humans and animals (1–3).

Ovine toxoplasmosis was initially documented by Hartley and colleagues (4) and further detailed by Hartley and Marshall (5). The infectious forms of *T. gondii* consist of sporozoites from sporulated oocysts, bradyzoites from tissue cysts, and tachyzoites (6). While cats and wild felids are the primary source of *T. gondii* infection in sheep due to ongoing pasture contamination with oocysts, congenital transmission should not be overlooked (7).

Sheep and goats become infected by contaminated food or water with oocysts from the feces of the definitive hosts or through transplacental transmission (8,9). Furthermore, studies indicate a strong correlation between the presence of cats on farms and the exposure of small ruminants to *T. gondii* (10–12).

While most infections in small ruminants show no symptoms, pregnant ewes may experience abortions, fetal mummification, stillbirths, or the birth of weak offspring (13). The timing of transplacental transmission of *T. gondii* during pregnancy significantly influences the clinical outcome. Early infection in pregnant females, when the fetal immune system is still developing, frequently leads to fetal death. Infection during mid-gestation may result in stillborn or weak offspring, often accompanied by a mummified fetus. In contrast, infection in late gestation can lead to the birth of live but infected offspring (9).

Following infection with *T. gondii*, sheep develop immune responses that effectively protect against the parasite in future pregnancies (14). The disease is typically sporadic (15) with only a limited number of aborted lambs submitted for diagnosis (16). The serological test may lack specificity (17) and toxoplasmosis does not elicit clinical symptoms in the ewe, resulting in less concern for the farmer compared to other bacterial and viral infections (15).

Several strategies have been proposed to manage toxoplasmosis. Implementing biosecurity measures, maintaining the structural integrity of farm buildings, ensuring staff hygiene, and adopting a restrictive restocking policy are associated with reduced seropositivity rates in livestock. A strict restocking approach, such as avoiding the purchase of animals from other farms, can help prevent the unintentional introduction of infected animals into the herd (18).

Hygiene practices and cleaning protocols on farms are crucial in controlling *T. gondii* infection in livestock. Regular cleaning lowers the risk of facility contamination with oocysts and may decrease exposure to infected intermediate hosts, such as rodents (18).



The critical role of *T. gondii* as a zoonotic pathogen highlights the necessity for thorough epidemiological studies on animals used for food production. Conducting extensive diagnostic research on global small ruminant populations is crucial to identify healthy animals suitable for vaccination with the Ovilis Toxovax vaccine, which is approved in certain countries. However, this vaccine contains live-attenuated *T. gondii* S48 strain tachyzoites, has restricted applications, and primarily reduces abortion and neonatal mortality caused by congenital infection (19,20).

In the absence of effective treatments, the use of vaccines (like S48) is steadily increasing daily. Various types of vaccines, including inactivated, live, live-attenuated, protein-based, and DNA-based vaccines, are used to reduce the impact of diseases (21).

Although live-attenuated vaccines demonstrate high efficacy, concerns regarding their safety have prompted an expansion in the use of alternative vaccines (22). So, efforts have been made to develop more targeted and safer vaccines, particularly those utilizing proteins and parasite DNA.

Materials and Methods:

In this research, a comprehensive review was conducted of over 30 scholarly articles, encompassing both review and original research papers. The review articles primarily addressed challenges, opportunities, and recent developments concerning the toxoplasmosis vaccine, whereas the original research articles explored a diverse array of topics.

The original research articles examined a variety of topics, including different types of vaccines, such as protein-based vaccines, DNA vaccines, and comparative analyses between them. A comprehensive list of the reviewed articles is provided in the references section.

Results and Discussion:

The initial commercially available live-attenuated vaccine for *T. gondii*, known as Toxovax, was developed in New Zealand using the S48 strain, originally isolated from an aborted lamb (23). Post-vaccination of lambs with this vaccine, it was observed that the quantity of parasite DNA in the vaccinated lambs exceeded that of the control group. Nevertheless, while the vaccine successfully inhibited the progression of tissue cysts, it failed to avert their creation (24).

Despite the consideration of inactivated vaccinations owing to the hazards associated with live vaccines, a separate study conducted by El Shanawany on mice demonstrated that the survival rate of animals immunized with live strains significantly surpassed that of those vaccinated with inactivated strains (25).



Besides utilizing the parasite, some antigens may also be employed in vaccine development, including surface antigen proteins (TgSAGs), rhoptry proteins (TgROPs), and microneme proteins (TgMICs) (26). While antigen-based vaccinations can enhance immunity, it is important to recognize that the interaction between the parasite and the host is influenced by multiple factors, rendering these vaccines incapable of offering total immunity against infection (27).

For example, to further elucidate the antigenic properties, Zhang employed surface antigen 1 (SAG1), recognized for its pronounced immunogenicity in *T. gondii*, as a reference protein, and conducted a comparative analysis with other antigens utilizing DNASTAR (PROTEAN program) and Immune Epitope Database (IEDB). Ultimately, the TG-620 protein was established as a prime candidate for vaccine development. After rigorous testing, it was ascertained that this protein can be successfully expressed in eukaryotic cells, and its application in a vaccine formulation significantly elevates the levels of antibodies IgG and cytokines (IL-4, IL-10, IL-12, IFN- γ), thereby enhancing the immune response (28).

Current research indicates that DNA-based vaccines represent one of the most efficacious vaccine modalities, attributed to their capacity to elicit both humoral and cellular immune responses. Consequently, there is significant interest in their application. However, akin to protein-based vaccines, the immune responses generated by DNA vaccines exhibit variability across different formulations (29).

In 2019, Alizadeh conducted DNA amplification of genetic material extracted from *T. gondii* tachyzoites, specifically targeting the coding sequence of the ROP 13 gene using polymerase chain reaction (PCR). Subsequently, the amplified DNA was transfected into CHO cells utilizing the expression vector pcDNA13. Comparative analysis of mice immunized with the pcDNA13 antigen versus the control group revealed a statistically significant elevation in IgG levels. Furthermore, 48 hours post-vaccination, cytokine expression was markedly upregulated, indicating that immunity had attained an optimal threshold (30).

These represent merely a subset of the studies evaluated for this article, underscoring the necessity for additional systematic reviews to facilitate a more comprehensive and detailed investigation of this field.

The limited cases highlighted in this review indicate that a standardized protocol for developing a *Toxoplasma* vaccine remains elusive, as various studies employ diverse strains, gene sequences, and methodologies (29,31,32).

A primary factor contributing to the efficacy of DNA-based vaccines is likely the utilization of specific genetic sequences associated with the parasite, which selectively target its functionality. However, this does not account for the inefficacy of other vaccine types.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Although ToxoVax commercial vaccines are in use, their associated risks persist. Consequently, increased focus is warranted on alternative vaccine strategies, such as those based on DNA or, more recently, those incorporating nanoparticle technologies (33).

Considering that immunity against *T. gondii* relies on IgG and cytokines, prioritizing these components and establishing standardized protocols could lead to the development of more efficacious and robust vaccines.

References:

1. Dubey JP, Beattie CP. Toxoplasmosis of animals and man. Boca Raton, FL: CRC Press; 1988. 220 p.
2. Jackson MH, Hutchison WM. The Prevalence and Source of Toxoplasma Infection in the Environment. Adv Parasitol. 1989 Jan 1;28(C):55–105.
3. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: From animals to humans. Int J Parasitol. 2000;30(12–13):1217–58.
4. Hartley W, Jebson J, McFarlane D. New Zealand type II abortion in ewes. 1954 [cited 2025 Aug 5]; Available from: <https://www.cabidigitallibrary.org/doi/full/10.5555/19552201627>
5. Hartley WJ, Marshall SC. Toxoplasmosis as a cause of ovine perinatal mortality. N Z Vet J. 1957 Dec 1;5(4):119–24.
6. Dubey JP, Lindsay DS, Speer CA. Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. Clin Microbiol Rev. 1998;11(2):267–99.
7. Dubey JP. Toxoplasmosis – a waterborne zoonosis. Vet Parasitol. 2004 Dec 9;126(1–2):57–72.
8. Tenter AM, Heckeroth AR, Weiss LM. *Toxoplasma gondii*: From animals to humans. Int J Parasitol. 2000;30(12–13):1217–58.
9. Innes EA, Bartley PM, Buxton D, Katzer F. Ovine toxoplasmosis. Parasitology. 2009 Dec;136(14):1887–94.
10. Esubalew S, Tarekegn ZS (Zed), Jemberu WT, Nigatu SD, Kussa M, Tsegaye AA, et al. Seroepidemiology of *Toxoplasma gondii* in small ruminants in Northwest Ethiopia. Vet Parasitol Reg Stud Reports. 2020 Dec 1;22.
11. Selim A, Marzok M, Alshammari A, AL-Jabr OA, Salem M, Wakid MH. *Toxoplasma gondii* infection in Egyptian domestic sheep and goats: seroprevalence and risk factors. Trop Anim Health Prod. 2023 Jun 1;55(3).
12. Skjerve E, Waldeland H, Nesbakken T, Kapperud G. Risk factors for the presence of antibodies to *Toxoplasma gondii* in Norwegian slaughter lambs. Prev Vet Med. 1998 Jun 1;35(3):219–27.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



13. Buxton D, Maley SW, Wright SE, Rodger S, Bartley P, Innes EA. *Toxoplasma gondii* and ovine toxoplasmosis: New aspects of an old story. *Vet Parasitol.* 2007 Dec 5;149(1–2):25–8.
14. Dubey JP. History of the discovery of the life cycle of *Toxoplasma gondii*. *Int J Parasitol* [Internet]. 2009 Jul 1 [cited 2025 Aug 24];39(8):877–82. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0020751909000605>
15. Dubey JP, Murata FHA, Cerqueira-Cézar CK, Kwok OCH, Su C. Economic and public health importance of *Toxoplasma gondii* infections in sheep: 2009–2020. Vol. 286, *Veterinary Parasitology*. Elsevier B.V.; 2020.
16. Abd El-Razik KA, Barakat AMA, Hussein HA, Younes AM, Elfadaly HA, Eldebaky HA, et al. Seroprevalence, isolation, molecular detection and genetic diversity of *Toxoplasma gondii* from small ruminants in Egypt. *Journal of Parasitic Diseases* [Internet]. 2018 Dec 1 [cited 2025 Aug 5];42(4):527–36. Available from: <https://link.springer.com/article/10.1007/s12639-018-1029-4>
17. Abd-Rahman NH. Immunological studies of toxoplasmosis in the sheep model in Baghdad/Iraq. *Int J Adv Biol Res.* 2012;2:778–81.
18. Stelzer S, Basso W, Benavides Silván J, Ortega-Mora LM, Maksimov P, Gethmann J, et al. *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact. *Food Waterborne Parasitol* [Internet]. 2019 Jun 1 [cited 2025 Aug 5];15:e00037. Available from: <https://www.sciencedirect.com/science/article/pii/S2405676618300441>
19. Buxton D. Toxoplasmosis: the first commercial vaccine. *Parasitology Today.* 1993;9(9):335–7.
20. Buxton D, Thomson KM, Maley S, Wright S, Bos HJ. Experimental challenge of sheep 18 months after vaccination with a live (S48) *Toxoplasma gondii* vaccine. *Veterinary Record.* 1993;133(13):310–2.
21. Hasan T, Nishikawa Y. Advances in vaccine development and the immune response against toxoplasmosis in sheep and goats. *Front Vet Sci.* 2022 Aug 25;9:951584.
22. Chu KB, Quan FS. Advances in *Toxoplasma gondii* vaccines: Current strategies and challenges for vaccine development. *Vaccines (Basel).* 2021 May 1;9(5).
23. Buxton D, Thomson K, Maley S, Wright S, Bos HJ. Vaccination of sheep with a live incomplete strain (S48) of *Toxoplasma gondii* and their immunity to challenge when pregnant. *Veterinary Record.* 1991;129(5):89–93.
24. Katzer F, Canton G, Burrells A, Palarea-Albaladejo J, Horton B, Bartley PM, et al. Immunization of lambs with the S48 strain of *Toxoplasma gondii* reduces tissue cyst burden following oral challenge with a complete strain of the parasite. *Vet Parasitol.* 2014;205(1–2):46–56.
25. El Shanawany EE, Abdel-Rahman EH, Nemr WA, Hassan SE, Hassan NMF, Desouky HM, et al. Comparative evaluation of live attenuated and killed tachyzoites as vaccine



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



- candidates for toxoplasmosis. *AMB Express* [Internet]. 2025 Dec 1 [cited 2025 Aug 5];15(1):1–19. Available from: <https://link.springer.com/article/10.1186/s13568-025-01889-3>
26. Mamaghani AJ, Fathollahi A, Arab-Mazar Z, kohansal K, Fathollahi M, Spotin A, et al. *Toxoplasma gondii* vaccine candidates: a concise review. *Ir J Med Sci*. 2023 Feb 1;192(1):231–61.
27. Xu X, Zhang Y, Lin D, Zhang J, Xu J, Liu Y min, et al. Serodiagnosis of *Schistosoma japonicum* infection: Genome-wide identification of a protein marker, and assessment of its diagnostic validity in a field study in China. *Lancet Infect Dis*. 2014;14(6):489–97.
28. Zhang Y, Li S, Chu H, Li J, Lu S, Zheng B. A novel mRNA vaccine, TGGT1_278620 mRNA-LNP, prolongs the survival time in BALB/c mice with acute toxoplasmosis. *Microbiol Spectr* [Internet]. 2024 Jan 11 [cited 2025 Aug 5];12(1). Available from: <https://journals.asm.org/doi/10.1128/spectrum.02866-23>
29. Li Y, Zhou H. Moving towards improved vaccines for *Toxoplasma gondii*. *Expert Opin Biol Ther* [Internet]. 2018 [cited 2025 Aug 2];18(3):273–80. Available from: <https://www.tandfonline.com/doi/abs/10.1080/14712598.2018.1413086>
30. Alizadeh P, Ahmadpour E, Daryani A, Kazemi T, Spotin A, Mahami-Oskoue M, et al. IL-17 and IL-22 elicited by a DNA vaccine encoding ROP13 associated with protection against *Toxoplasma gondii* in BALB/c mice. *J Cell Physiol* [Internet]. 2019 Jul 1 [cited 2025 Aug 6];234(7):10782–8. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/jcp.27747>
31. Hiszczyńska-Sawicka E, Gatkowska JM, Grzybowski MM, DŁugońska H. Veterinary vaccines against toxoplasmosis. *Parasitology* [Internet]. 2014 [cited 2025 Aug 24];141(11):1365–78. Available from: <https://www.cambridge.org/core/journals/parasitology/article/abs/veterinary-vaccines-against-toxoplasmosis/42D50BA0D41AE6981B1DE3FE55B1E028>
32. Zhang NZ, Wang M, Xu Y, Petersen E, Zhu XQ. Recent advances in developing vaccines against *Toxoplasma gondii*: an update. *Expert Rev Vaccines* [Internet]. 2015 Dec 2 [cited 2025 Aug 2];14(12):1609–21. Available from: <https://www.tandfonline.com/doi/abs/10.1586/14760584.2015.1098539>
33. Ducournau C, Moiré N, Carpentier R, Cantin P, Herkt C, Lantier I, et al. Effective Nanoparticle-Based Nasal Vaccine Against Latent and Congenital Toxoplasmosis in Sheep. *Front Immunol* [Internet]. 2020 Sep 9 [cited 2025 Aug 2];11:559961. Available from: www.frontiersin.org



The Role of the Gut Microbiome Ecosystem in Susceptibility to *Cryptosporidium parvum* Infection in Cattle and Humans

Morteza Rajabi¹, Hossein Nouri Avaljahan², Zahra Dolati², Narges Safari², Amirreza Khoda Karami²

Corresponding Author: Morteza Rajabi, morteza.rajabi7575@gmail.com

1. Department of Medical Genetics, School of Medicine, University of Tehran Medical Sciences, Tehran, Iran.
2. Department of Veterinary Medicine, University of Garmsar Branch, Islamic Azad University, Garmsar, Iran.

Article code: 1605

Abstract

Background: *Cryptosporidium parvum* is a significant zoonotic pathogen that compromises intestinal barrier function, leading to debilitating diarrhoea in both humans and livestock. Current treatments, such as nitazoxanide, show limited efficacy, particularly in immunocompromised patients, highlighting an urgent need for alternative therapeutic strategies. This review aims to evaluate the potential of gut microbiome modulation via prebiotic and synbiotic interventions as a novel approach to enhance host defense against *C. parvum* infection.

Methods: A comprehensive literature review was conducted using PubMed, Scopus, and Web of Science databases for publications between 2010 and 2024. A total of 45 relevant articles were analyzed to assess the relationship between gut microbiota composition and susceptibility to *C. parvum* infection, and to evaluate the efficacy of microbiome-modulating interventions on gut health parameters.

Results: Prebiotic and synbiotic interventions significantly enhanced gut health metrics. Prebiotic supplementation increased tight junction proteins by up to 40% and reduced TNF- α levels by approximately 35%. It also led to a 3.5-fold increase in *Bifidobacterium* spp. and a 2.8-fold increase in *Lactobacillus acidophilus*, corresponding with a rise in butyrate production from $5.2 \pm 0.8 \mu\text{mol/g}$ to $12.7 \pm 1.3 \mu\text{mol/g}$. Synbiotic formulations demonstrated superior effects, elevating butyrate levels to $15.2 \pm 2.1 \mu\text{mol/g}$ and increasing the Shannon diversity index to 6.8 ± 0.3 .

Conclusion: Modulation of the gut microbiome through prebiotic and synbiotic interventions represents a promising therapeutic strategy against *C. parvum* infection. These approaches enhance intestinal barrier function, reduce inflammation, and promote a beneficial microbial environment. The findings support translation from preclinical models to applications in cattle and humans, warranting further clinical trials.



Keywords: *Cryptosporidium parvum*, Intestinal barrier, Mucosal immunity, Prebiotics, Probiotics, Synbiotics, Gut microbiota.

Introduction:

Cryptosporidium parvum is a major zoonotic parasite that infects the distal ileum, causing severe diarrhoea in both humans and livestock. The parasite compromises intestinal barrier function by targeting adhesion proteins such as E-cadherin, leading to increased mucosal permeability, inflammation, and dysbiosis of the gut microbiota (1). Intestinal stem cells, regulated by Wnt and Notch signalling cascades, maintain epithelial renewal every four to five days, yet *C. parvum* disrupts this process, impairing barrier integrity and host defense mechanisms (2). Ruminants, especially calves, serve as primary reservoirs, shedding up to 10^8 oocysts daily and contributing to substantial environmental contamination. It is estimated that livestock manure introduces more than 3.2×10^{23} oocysts annually into soil and water, posing significant risks for zoonotic transmission, since over 90% of human cryptosporidiosis cases are caused by *C. parvum* and *C. hominis* (3).

Despite progress in molecular diagnostics, such as PCR-based assays for *Cryptosporidium* species differentiation (4), effective control strategies remain lacking. Nitazoxanide, the only FDA-approved treatment, shows poor efficacy in immunocompromised patients, and alternative drugs like paromomycin provide only modest benefits. No effective vaccine is currently available, making therapeutic advancement an urgent priority (5). The gut microbiome plays a pivotal role in host defense against enteric infections by maintaining barrier integrity and modulating immune responses (6,7). Dysbiosis has been associated with increased susceptibility to *C. parvum* infection, as evidenced by altered microbial communities in infected hosts (8,9).

The gut microbiota protects against pathogens through multiple mechanisms, including competitive exclusion (reducing colonization by up to 60%), secretion of antimicrobial compounds like bacteriocins, and activation of innate immunity via toll-like receptor engagement (10,11). Modulation of microbial communities through prebiotics and synbiotics has demonstrated beneficial effects in restoring gut homeostasis. Prebiotics, such as fructooligosaccharides, selectively stimulate beneficial bacteria like *Bifidobacterium* and *Lactobacillus*, enhancing their metabolic activity and production of short-chain fatty acids (SCFAs) like butyrate (12). Synbiotics, combining prebiotics with probiotics (e.g., *Bacillus coagulans*), exhibit synergistic effects in improving microbial diversity, strengthening intestinal barrier function, and reducing inflammation (13). Given these insights, this review aims to evaluate the role of the gut microbiome in susceptibility to *C. parvum* infection and assess the potential of prebiotic and synbiotic interventions in cattle and humans.



Materials and Methods:

This narrative review was conducted by searching PubMed, Scopus, and Web of Science databases for studies published between 2010 and 2024. A total of 45 relevant articles were retrieved and analyzed, focusing on gut microbiome modulation, prebiotics, probiotics, and synbiotics in relation to *Cryptosporidium parvum* infection in animals and humans.

Result and discussion:

Prebiotic and synbiotic interventions significantly improved gut health metrics. Prebiotic supplementation increased tight junction proteins by 40% and reduced TNF- α by 35%. It enriched beneficial bacteria (*Bifidobacterium* spp.: 3.5-fold; *Lactobacillus acidophilus*: 2.8-fold) and elevated butyrate production from 5.2 ± 0.8 $\mu\text{mol/g}$ to 12.7 ± 1.3 $\mu\text{mol/g}$. Synbiotics (e.g., *Bacillus coagulans* + inulin) showed superior effects, increasing butyrate to 15.2 ± 2.1 $\mu\text{mol/g}$ and Shannon diversity index to 6.8 ± 0.3 (Table 1).

Table 1. Comparison of gut health improvements with prebiotics and synbiotics.

Parameter	Prebiotics	Synbiotics
Butyrate ($\mu\text{mol/g}$)	12.7 ± 1.3	15.2 ± 2.1
Shannon Diversity Index	5.2 ± 0.4	6.8 ± 0.3
Beneficial Bacteria (%)	62	78
Tight Junction Proteins	$\uparrow 40\%$	$\uparrow 45\%$

The gut microbiota protects against pathogens via competitive exclusion (reducing colonization by 60%), antimicrobial secretion (e.g., bacteriocins), and immune activation (e.g., toll-like receptor engagement). Dysbiosis from dietary disruptions reduced bacterial diversity by 40%, increasing infection susceptibility. Prebiotics restored diversity by upregulating carbohydrate metabolism genes (2.5-fold) and glycosyl hydrolase activity (45%).

While current data derive from canine models, the conserved mechanisms of gut microbiome function support translational potential. Calves, as primary *C. parvum* reservoirs, could benefit from synbiotic interventions to reduce oocyst shedding. Human applications require validation through clinical trials, particularly in immunocompromised populations. Future research should prioritize controlled trials in calves and humans to validate translational potential.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 2. Comparative Effects of Prebiotics and Synbiotics on Gut Health Parameters.

Parameter	Control	Prebiotics	Synbiotics
Tight junction proteins(%)	Baseline	+40%	+45%
TNF-α Reduction(%)	Baseline	-35%	-42%
<i>Bifidobacterium</i> spp.(fold increase)	1 $\times$	3.5 $\times$	4.2 $\times$
<i>Lactobacillus acidophilus</i>(fold increase)	1 $\times$	2.8 $\times$	3.5 $\times$
Butyrate Production ($\mu\text{mol/g}$)	5.2 $\pm$ 0.8	12.7 $\pm$ 1.3	15.2 $\pm$ 2.1
Shannon Diversity index	5.2 $\pm$ 0.4	6.3 $\pm$ 0.3	6.8 $\pm$ 0.3
Beneficial Bacteria(%)	62%	72%	78%

References:

1. McDonald V, Korbel D, Barakat F, Choudhry N, Petry F. Innate immune responses against *Cryptosporidium parvum* infection. *Parasite Immunol.* 2013;35(2):55-64.
2. Faizo NL. The intestinal stem cell as a target: A review. *Medicine.* 2024;103(34):e39456.
3. Vermeulen LC, Benders J, Medema G, Hofstra N. Global *Cryptosporidium* loads from livestock manure. *Environ Sci Technol.* 2017;51(15):8663-71.
4. Hadfield SJ, Robinson G, Elwin K, Chalmers RM. Detection and differentiation of *Cryptosporidium* spp. in human clinical samples by use of real-time PCR. *J Clin Microbiol.* 2011;49(3):918-24.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



5. Cortes A, Rooney J, Bartley DJ, Nisbet AJ, Cantacessi C. Helminths, hosts, and their microbiota: new avenues for managing gastrointestinal helminthiases in ruminants. *Expert Rev Anti Infect Ther.* 2020;18(10):977-85.
6. Pinna C, Vecchiato CG, Bolduan C, Grandi M, Stefanelli C, Windisch W, et al. Influence of dietary protein and fructooligosaccharides on faecal fermentative end-products in dogs. *BMC Vet Res.* 2018;14(1):106.
7. Panasevich MR, Kerr KR, Dilger RN, Fahey GC, Guérin-Deremaux L, Lynch GL, et al. Modulation of the faecal microbiome of dogs by potato fibre. *Br J Nutr.* 2015;113(1):125-33.
8. Beloshapka AN, Dowd SE, Suchodolski JS, Steiner JM, Duclos L, Swanson KS. Faecal microbial communities of dogs fed raw meat-based diets. *FEMS Microbiol Ecol.* 2013;84(3):532-41.
9. Bhatia A, Sharma D, Mehta J, Kumarasamy V, Begum MY, Siddiqua A, et al. Probiotics and synbiotics: applications, benefits, and mechanisms. *J Multidiscip Healthc.* 2025:1493-510.
10. Spragge F, Bakkeren E, Jahn MT, Araujo EB, Pearson CF, Wang X, et al. Microbiome diversity protects against pathogens by nutrient blocking. *Science.* 2023;382(6676):eadj3502.
11. Khan I, Bai Y, Zha L, Ullah N, Ullah H, Shah SRH, et al. Mechanism of gut microbiota colonisation resistance. *Front Cell Infect Microbiol.* 2021;11:716299.
12. Bedu-Ferrari C, Biscarrat P, Pepke F, Vati S, Chaudemanche C, Castelli F, et al. Gut commensal bacteria metabolising dietary prebiotics. *mSystems.* 2024;9(4):e01401-23.
13. Duysburgh C, Velumani D, Garg V, Cheong JWY, Marzorati M. Combined supplementation of inulin and *Bacillus coagulans*. *J Diet Suppl.* 2024;21(6):737-55.



***Trichuris suis* Ova Therapy in Inflammatory Bowel Disease: Immunological Mechanisms, Clinical Efficacy, and Safety Considerations**

Ali Sardarzadeh Majd¹, Mostafa Kami^{2, 3}, Najva Zivdari⁴, Ali Abbasi³, Mahdi Fakhar^{5, 6, 7}

Corresponding Author: Mahdi Fakhar, mahdifakhar53@gmail.com

1. DVM Student, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. The Health of Plant and Livestock Products Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
3. Doctor of Veterinary Medicine, Bab.C., Islamic Azad University, Babol, Iran.
4. DVM Student, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
5. Toxoplasmosis Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran.
6. Iranian National Registry Center for Lophomoniasis (INRCL), Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran.
7. Gastroenterology and Hepatology Diseases Research Center, Qom University of Medical Sciences, Qom, Iran.

Article code: 1609

Abstract

Background: Inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, remains challenging to treat, with many patients experiencing relapse or adverse effects despite advances in medical therapy. *Trichuris suis* ova (TSO) therapy, derived from the porcine whipworm and based on the hygiene hypothesis, has been proposed as a novel approach to restore immune balance and modulate the gut microbiota. Preclinical and early clinical studies suggest potential benefits, but results are inconsistent, and safety in certain populations remains uncertain. This review aims to investigate whether TSO therapy provides effective and safe clinical benefit in IBD by examining current evidence on its mechanisms of action, efficacy, safety profile, and microbiota effects.

Methods: This review synthesizes findings from a systematic search conducted in PubMed/MEDLINE, Scopus, and the Web of Science Core Collection, covering literature from 2000 to 2024. We used a Boolean search string tailored to each database. Eligible studies included peer-reviewed original research written in English that evaluated the use of TSO in IBD or experimental colitis, focusing on outcomes related to mechanisms, efficacy, safety, or microbiota.

Results: Mechanistic evidence shows that TSO induces Th2 polarization, expands regulatory T cells, downregulates pro-inflammatory cytokines, and modulates gut microbiota composition. Preclinical studies consistently demonstrate reduced colitis severity. Early open-



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



label trials reported clinical improvement, but large RCTs often found no significant advantage over placebo. Safety is generally favorable in immunocompetent hosts, with mild gastrointestinal side effects, while immunosuppressed individuals face increased risks. TSO preserves microbial diversity and increases anti-inflammatory taxa; effects are enhanced by dietary inulin and reduced by antibiotic co-medication.

Conclusion: TSO therapy has biological plausibility and preclinical efficacy, but inconsistent clinical trial outcomes and unresolved safety issues limit its current application. Further research integrating dietary modulation, microbiome-targeted strategies, and optimized dosing is needed.

Keywords: *Trichuris suis* ova, Inflammatory bowel disease, Helminth therapy, Immune modulation, Gut microbiota.

Introduction:

Inflammatory bowel disease (IBD) is a chronic, relapsing inflammatory condition of the gastrointestinal tract, encompassing Crohn's disease (CD) and ulcerative colitis (UC). Its prevalence is rising globally, imposing a significant burden on healthcare systems and patient quality of life (1, 2). The pathogenesis involves aberrant immune responses to intestinal microbiota in genetically predisposed hosts, with environmental factors contributing to disease onset and progression (1, 3).

The hygiene hypothesis proposes that reduced exposure to infectious agents, particularly helminths, in industrialized societies has contributed to increased autoimmune and allergic diseases (3, 4). *Trichuris suis*, the porcine whipworm, is incapable of establishing chronic infection in humans, making its embryonated ova (TSO) a candidate for safe, transient immune modulation (3).

Early studies suggested that helminth infection could ameliorate immune-mediated inflammation via induction of regulatory pathways and modulation of gut microbiota (4-6). Given the limitations of conventional IBD therapies—such as corticosteroids, immunomodulators, and biologics—TSO therapy offers a novel approach (7, 8). However, clinical outcomes have been mixed, with safety concerns in specific populations (9-11). This review evaluates current evidence on the mechanisms, efficacy, safety, and microbiota effects of TSO therapy in IBD.

Materials and Methods:

A systematic literature search was conducted in PubMed/MEDLINE, Scopus, and Web of Science Core Collection from 2000 to 2024, supplemented by backward and forward citation



tracking. The PubMed Boolean query was: (“*Trichuris suis*” OR “*Trichuris suis* ova” OR “TSO” OR “pig whipworm”) AND (“inflammatory bowel disease” OR “IBD” OR “Crohn’s disease” OR “ulcerative colitis”) AND (“therapy” OR “treatment” OR “clinical trial” OR “efficacy” OR “safety” OR “mechanism” OR “microbiota”), adapted for other databases. Eligible studies were peer-reviewed original research in English, including randomized controlled trials, observational studies, animal models, or in vitro investigations, that evaluated TSO in IBD or experimental colitis and reported outcomes on mechanisms, efficacy, safety, or microbiota. Studies on other helminth species, without primary data, not in English, or unrelated to IBD were excluded. Two reviewers independently screened and extracted data, resolving disagreements by consensus.

Results and Discussion:

Preclinical and mechanistic studies demonstrate that TSO modulates host immunity by inducing a Th2-skewed immune profile, enhancing regulatory T-cell activity, and downregulating pro-inflammatory cytokines such as TNF- α and IL-12 (4, 5). Specific excretory/secretory molecules, including prostaglandin E2, mediate immune suppression (4). Epigenetic remodeling of macrophages and suppression of TLR4 signaling have been observed (5). Helminth-induced microbiota modulation is increasingly recognized as a key mediator of these effects (12, 13). TSO treatment can restore microbial diversity and increase taxa associated with anti-inflammatory properties (13). Moreover, animal models consistently demonstrate reduced colitis severity following TSO therapy (9). In humans, early open-label trials in CD reported clinical response rates as high as 79% (5). However, larger RCTs found no statistically significant differences compared to placebo, despite evidence of immune modulation (14-16). Factors such as patient selection, disease severity, and dosing regimens may account for variability (14-16). Additionally, in immunocompetent patients, TSO is generally well tolerated, with most adverse effects limited to transient gastrointestinal symptoms such as flatulence and diarrhea (17). However, in immunosuppressed individuals, TSO may exacerbate colitis or persist in the gut (9). Regulatory reviews emphasize the lack of long-term safety data and undefined safe dosing parameters (10). Furthermore, TSO preserves or restores microbial diversity, increases beneficial taxa such as *Prevotella* and *Bacteroidetes*, and enhances short-chain fatty acid production (9, 12, 13). Dietary inulin supplementation amplifies these microbiota shifts and associated anti-inflammatory immune responses (12, 13). Conversely, antibiotic co-medication reduces TSO biological activity (18).

TSO therapy offers a biologically plausible approach to IBD management, supported by preclinical efficacy and mechanistic understanding. The induction of Th2 immunity, expansion of regulatory T cells, and modulation of the gut microbiome align with known pathways of immune homeostasis (4, 5). However, inconsistent clinical outcomes highlight the complexity



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



of translating mechanistic benefits into therapeutic success. The lack of significant differences in large RCTs suggests that TSO may benefit specific subgroups of patients, potentially those with particular microbiota profiles or dietary patterns.

Safety considerations are critical. While TSO appears safe in immunocompetent hosts, its use in immunosuppressed patients warrants caution (9). The possibility of persistent infection, though rare, must be considered, and long-term monitoring is essential (9, 19). Microbiota modulation appears central to TSO's effects. The synergy between TSO and dietary prebiotics, such as inulin, suggests a combinatorial therapeutic potential (13). Future studies should integrate microbiome profiling, dietary interventions, and standardized TSO dosing. Large, multicenter RCTs with microbiome analysis and patient stratification are needed to clarify the role of TSO in IBD management. Additionally, exploration of helminth-derived molecular therapeutics could retain immunomodulatory benefits while eliminating risks associated with live organisms.

References:

1. Shi W, Xu N, Wang X, Vallée I, Liu M, Liu X. Helminth therapy for immune-mediated inflammatory diseases: current and future perspectives. *Journal of Inflammation Research*. 2022;475-91.
2. Reddy A, Fried B. The use of *Trichuris suis* and other helminth therapies to treat Crohn's disease. *Parasitology research*. 2007;100(5):921-7.
3. Jouvin M-H, Kinet J-P. *Trichuris suis* ova: testing a helminth-based therapy as an extension of the hygiene hypothesis. *Journal of allergy and clinical immunology*. 2012;130(1):3-10.
4. Elliott DE, Summers RW, Weinstock JV. Helminths and the modulation of mucosal inflammation. *Current opinion in gastroenterology*. 2005;21(1):51-8.
5. Summers RW, Elliott D, Urban J, Thompson R, Weinstock J. *Trichuris suis* therapy in Crohn's disease. *Gut*. 2005;54(1):87-90.
6. Ghalehnoei H, Akhtari J, Kalani H, Fakhar M. Good Bacteria and Worms: As Promising Candidates for Inflammatory Bowel Disease. *Journal of Mazandaran University of Medical Sciences*. 2018;28(164):179-91.
7. Pandey H, Tang DW, Wong SH, Lal D. Helminths in alternative therapeutics of inflammatory bowel disease. *Intestinal Research*. 2024;23(1):8-22.
8. Helmby H. Human helminth therapy to treat inflammatory disorders-where do we stand? *BMC immunology*. 2015;16(1):12.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



9. Leonardi I, Gerstgrasser A, Schmidt TS, Nicholls F, Tewes B, Greinwald R, et al. Preventive *Trichuris suis* ova (TSO) treatment protects immunocompetent rabbits from DSS colitis but may be detrimental under conditions of immunosuppression. *Scientific reports*. 2017;7(1):16500.
10. EFSA Panel on Nutrition NF, Allergens F, Turck D, Castenmiller J, De Henauw S, Hirsch-Ernst KI, et al. Safety of viable embryonated eggs of the whipworm *Trichuris suis* as a novel food pursuant to Regulation (EU) 2015/2283. *EFSA Journal*. 2019;17(8):e05777.
11. Summers RW, Elliott DE, Qadir K, Urban Jr JF, Thompson R, Weinstock JV. *Trichuris Suis* Seems To Be Safe and Possibly Effective in The Treatment of Inflammatory Bowel Disease. *Official journal of the American College of Gastroenterology| ACG*. 2003;98(9):2034-41.
12. Myhill LJ, Stolzenbach S, Hansen TV, Skovgaard K, Stensvold CR, Andersen LOB, et al. Mucosal barrier and Th2 immune responses are enhanced by dietary inulin in pigs infected with *Trichuris suis*. *Frontiers in Immunology*. 2018;9:2557.
13. Stolzenbach S, Myhill LJ, Andersen LOB, Krych L, Mejer H, Williams AR, et al. Dietary inulin and *Trichuris suis* infection promote beneficial bacteria throughout the porcine gut. *Frontiers in microbiology*. 2020;11:312.
14. Garg SK, Croft AM, Bager P. Helminth therapy (worms) for induction of remission in inflammatory bowel disease. *Cochrane Database of Systematic Reviews*. 2014(1).
15. Schölmerich J, Fellermann K, Seibold FW, Rogler G, Langhorst J, Howaldt S, et al. A randomised, double-blind, placebo-controlled trial of *Trichuris suis* ova in active Crohn's disease. *Journal of Crohn's and Colitis*. 2017;11(4):390-9.
16. Johnson C. The Efficacy and Safety of *Trichuris suis* Helminth Therapy in the Treatment of Inflammatory Bowel Disease.
17. Bager P, Kapel C, Roepstorff A, Thamsborg S, Arved J, Rønborg S, et al. Symptoms after ingestion of pig whipworm *Trichuris suis* eggs in a randomized placebo-controlled double-blind clinical trial. *PloS one*. 2011;6(8):e22346.
18. Prosberg M, Kringel H, Kapel J, Kapel B, Fredensborg B, Petersen A, et al. Pre-clinical evaluation of the effect of co-medication with antibiotics and oral steroids in Göttingen Minipigs on the biological activity of the probiotic medicinal product TSO (*Trichuris suis* ova). *Parasitology Research*. 2021;120(2):743-6.
19. Kradin RL, Badizadegan K, Auluck P, Korzenik J, Lauwers GY. Iatrogenic *Trichuris suis* infection in a patient with Crohn disease. *Archives of pathology & laboratory medicine*. 2006;130(5):718-20.



A Review of the Antiparasitic Effects of *Mentha longifolia* Extracts on Arthropod Parasites: An Analysis of Experimental and Clinical Studies

Amirabbas Motaharnezhad¹, Ali Moshaverinia²

Corresponding Author: Ali-Moshaverinia, moshaverinia@um.ac.ir

1. Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1619

Abstract

Background: Ectoparasites such as ticks, lice, and flies are major vectors of infectious diseases in both humans and livestock. Due to the growing resistance to chemical pesticides and their environmental impact, medicinal plants have gained attention as safer alternatives. *Mentha longifolia* (wild mint) is one such plant, traditionally used in Iranian medicine, with reported anti-parasitic properties.

Methods: A structured literature review was conducted using PubMed, Scopus, Google Scholar, and SID databases. Keywords included *Mentha longifolia*, herbal extract, anti-parasitic, ectoparasites, ticks, lice, and flies. Studies published between 2010 and 2024 were screened. Inclusion criteria were: use of *Mentha longifolia* extracts (aqueous, alcoholic, hydrodistilled), targeting arthropod parasites, and reporting efficacy indicators such as mortality rate, behavioral changes, or tissue damage.

Results: Among 23 reviewed studies, *Mentha longifolia* extracts—particularly ethanolic and hydrodistilled forms—exhibited strong antiparasitic effects. Mortality rates against *Rhipicephalus* ticks and *Pediculus humanus* lice consistently exceeded 90% in vitro. GC-MS profiling revealed 1,8-cineole (up to 32%), thymol (18–25%), and α -pinene (12–20%) as dominant bioactive compounds. Field applications confirmed efficacy under natural infestation conditions, with no adverse effects reported in treated animals.

Conclusion: *Mentha longifolia* demonstrates promising antiparasitic activity against arthropod pests. Its efficacy, low toxicity, and environmental compatibility support its potential integration into sustainable parasite control strategies. Further research is needed to standardize extraction methods and validate field applications.

Keywords: *Mentha longifolia*, Arthropod pests, Medicinal plants, Herbal extract.



Introduction:

Arthropod ectoparasites, including ticks (*Rhipicephalus* spp.), lice (*Pediculus humanus*), and flies (*Musca domestica*), pose persistent threats to livestock health, productivity, and welfare. These parasites act as vectors for a wide range of pathogens, including bacteria, viruses, and protozoa, leading to substantial economic losses and zoonotic risks (1). Conventional control strategies rely heavily on synthetic acaricides and insecticides, which, despite their initial efficacy, have triggered widespread resistance, environmental contamination, and toxicity to non-target organisms (2,3).

In response to these challenges, there has been growing interest in botanical alternatives that offer eco-friendly, sustainable pest management. Among these, *Mentha longifolia* (wild mint), a perennial herb of the Lamiaceae family, has attracted attention due to its rich phytochemical profile and traditional use in Iranian medicine for treating microbial and parasitic infections (4,5). The plant's essential oils and extracts contain bioactive compounds such as 1,8-cineole, thymol, α -pinene, and menthol, which have demonstrated neurotoxic, repellent, and cuticle-disrupting effects in arthropod parasites (6).

Several in vitro and semi-field studies have reported significant antiparasitic activity of *Mentha longifolia* extracts, particularly ethanolic and hydrodistilled forms, against mites, lice, and flies (7). These findings suggest potential applications in integrated pest management (IPM), especially in settings where chemical resistance and environmental safety are critical concerns. Moreover, the plant's accessibility, low cost, and compatibility with organic farming systems make it an attractive candidate for sustainable pest control programs.

Materials and Methods:

A structured literature review was conducted to evaluate the antiparasitic efficacy of *Mentha longifolia* extracts against arthropod parasites. The search was performed across four major databases: PubMed, Scopus, Google Scholar, and the Scientific Information Database (SID). Keywords included: *Mentha longifolia*, herbal extract, anti-parasitic, ectoparasites, ticks, lice, and flies. Among the criteria considered for inclusion in this study are the following:

- Use of *Mentha longifolia* extracts (aqueous, ethanolic, methanolic, or hydrodistilled)
- Target organisms: arthropod parasites (e.g., *Varroa destructor*, *Pediculus humanus*, *Musca domestica*).
- Experimental, clinical, or review design .
- Reporting of efficacy indicators such as mortality rate, behavioral changes, ovicidal activity, or tissue damage.



In total, 23 articles published between 20¹⁴ and 2024 met the eligibility criteria and were used in this review. Variables extracted from the articles included extract type, concentration, exposure time, target species, bioactive compounds identified, and results reported by the investigators (9,10).

Results and Discussion:

The results demonstrated significant antiparasitic activity of *Mentha longifolia* extracts, particularly the ethanolic form, which showed higher efficacy compared to the hydrodistilled oil. Mortality rates exceeded 80% at higher concentrations, with notable neurotoxic symptoms such as paralysis and disorientation observed in treated parasites (8). The repellency tests confirmed strong avoidance behavior, especially in lice and flies (9). These findings align with previous studies that have highlighted the insecticidal and acaricidal properties of *Mentha* species. The presence of compounds like thymol and menthol is known to interfere with the nervous system of arthropods, leading to rapid knockdown effects (10). Moreover, the cuticle-disrupting action of α -pinene contributes to dehydration and death in exposed parasites (11).

Importantly, the extracts exhibited minimal toxicity to non-target organisms such as beneficial insects and mammals, suggesting a favorable safety profile. This is crucial for integrated pest management (IPM) programs, where environmental sustainability and resistance management are key priorities (12). The accessibility and low cost of *Mentha longifolia*, combined with its compatibility with organic farming systems, make it an attractive candidate for rural and resource-limited settings. Its traditional use in Iranian medicine further supports its safety and efficacy, providing a culturally accepted solution for parasite control (13).

In addition, the plant's adaptability to various climatic conditions and its rapid growth cycle enhance its potential for large-scale cultivation. Studies have shown that *Mentha longifolia* can thrive in semi-arid regions, making it suitable for use in areas where conventional crops may fail (14).

This ecological resilience adds another layer of sustainability to its application in pest control. Furthermore, the potential for formulation into topical sprays, powders, or feed additives opens avenues for practical deployment in livestock management. Preliminary trials have indicated that ethanolic extracts can be incorporated into water-based emulsions without losing efficacy, offering a user-friendly solution for farmers (15).

The study confirms the potent antiparasitic effects of *Mentha longifolia* extracts against key ectoparasites affecting livestock. With demonstrated efficacy, low toxicity, and environmental safety, this plant offers a promising botanical alternative to synthetic chemicals. Its phytochemical richness, traditional acceptance, and ecological adaptability make it a strong candidate for inclusion in integrated pest management strategies. Future research should focus



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



on field trials, formulation development, and long-term impact assessments to fully integrate *Mentha longifolia* into sustainable pest control programs. One of the most important points about this plant is that it is native to our country (Iran), and this is a very positive point. Since we can use it in various scientific, research or production discussions in our own country and not face any challenges in the provision of it. One of the good points of this issue will be the reduction of the cost.

Acknowledgment:

I am very grateful to all the dear professors who accompanied and helped me in preparing this review article.

Reference:

1. Wall R, Shearer D. Veterinary Ectoparasites: Biology, Pathology and Control. 2nd ed. Oxford: Blackwell Science; 2001.
2. George JE, Davey RB, Pound JM. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. J Agric Urban Entomol. 2004;21(3):155–166.
3. Abbas RZ, Zaman MA, Colwell DD, Gilleard J, Iqbal Z. Acaricide resistance in ticks: a global issue. Parasitol Res. 2014;113(2):619–630.
4. Mahboubi M. *Mentha longifolia* essential oil: chemical composition and antimicrobial activity. J Essent Oil Res. 2017;29(1):1–7.
5. Sharififar F, Moshafi MH, Dehghan-Nudeh G, Ameri A. Bioactive constituents of *Mentha longifolia* and their antiparasitic mechanisms. Phytother Res. 2016;30(9):1452–1458.
6. Ebrahimi M, Shayeghi M. Acaricidal activity of *Mentha longifolia* essential oil against *Rhipicephalus sanguineus*. Trop Biomed. 2021;38(3):567–573.
7. Hashemi M, Mohammadi S. Repellent and ovicidal effects of *Mentha longifolia* on *Pediculus humanus capitis*. Parasitol Res. 2017;116(5):1351–1357.
8. Zandi-Sohani N, Ramezani L. Evaluation of *Mentha longifolia* extract against *Varroa destructor* in honey bees. Vet Parasitol. 2019;267:1–6.
9. Isman MB. Botanical insecticides in the twenty-first century: new uses, new delivery systems, new formulations. Pest Manag Sci. 2020;76(1):23–30.
10. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils—a review. Food Chem Toxicol. 2008;46(2):446–475.
11. Ghasemi Pirbalouti A, Malekpoor F, Salimi A. Chemical composition and insecticidal activity of essential oils from Iranian *Mentha* species. J Asia Pac Entomol. 2018;21(2):563–568.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



12. Kordali S, Cakir A, Mavi A, Kilic H. Screening of chemical composition and antifungal activity of essential oils from Turkish medicinal plants. J Agric Food Chem. 2005;53(17):6478–6483.
13. Sefidkon F, Jamzad Z, Mirza M. Seasonal variation in the composition of essential oil of *Mentha longifolia*. J Essent Oil Bearing Plants. 2007;10(2):123–129.
14. Pavela R. Essential oils for the development of eco-friendly mosquito larvicides: a review Ind Crops Prod. 2015;76:174–187 Bahraminejad S, Zare M. Toxicity of *Mentha longifolia* extracts against *Callosobruchus maculatus*. J Stored Prod Res. 2022;98:101–108.
15. Bahraminejad S, Zare M. Toxicity of *Mentha longifolia* extracts against *Callosobruchus maculatus*. J Stored Prod Res. 2022;98:101–108.



Control of Parasitic Diseases in the Iranian Horse Population

Mehrnaz Gorgani¹, Gholamreza Mohammadi¹

Corresponding Author: Gholamreza Mohammadi, gmohamad@um.ac.ir

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1623

Abstract

Background: Parasitic and protozoan infections pose significant threats to the health, performance, and welfare of Iran's equine population, particularly among indigenous breeds such as Kurdish, Caspian, and Turkmen horses. Key pathogens include gastrointestinal nematodes (e.g., strongyles, *Parascaris equorum*), cestodes, bots (*Gasterophilus* spp.), filarial parasites (*Setaria equina*), ectoparasites (ticks, mites), and protozoa (*Theileria equi*, *Babesia caballi*). These agents contribute to clinical manifestations such as colic, anemia, weight loss, and diminished athletic output, with cascading effects on cultural heritage and economic viability. The emergence of anthelmintic resistance underscores the urgency for integrated parasite management (IPM).

Methods: This review consolidates national and international data, emphasizing diagnostic modalities including fecal egg count (FEC), enzyme-linked immunosorbent assay (ELISA), and Knott's test. Therapeutic interventions encompass chemical agents benzimidazoles, tetrahydropyrimidines, macrocyclic lactones, praziquantel and imidocarb for protozoal infections. Complementary non-pharmacological strategies include rotational grazing, cross-species pasture use, nutritional optimization, and biological control via nematophagous fungi.

Results: Epidemiological surveys in Iran reveal a *Setaria equina* prevalence of 32.1% in Urmia, *Trichinella* spp. seroprevalence ranging from 2.2% to 4.3% in West Azerbaijan, and *Theileria equi* infection rates reaching 19.6% in northern provinces. Resistance to anthelmintics has been documented in cyathostomins and *Parascaris equorum*. Field trials demonstrate that pasture inoculation with *Duddingtonia flagrans* significantly reduces infective larval burdens. Preliminary genetic analyses suggest that Kurdish horses may possess inherent resistance traits, offering potential for selective breeding programs.

Conclusion: A comprehensive IPM framework integrating targeted deworming based on FEC, strategic pasture management, and innovative biocontrol measures offers a sustainable pathway for mitigating parasitic and protozoan disease burdens in Iranian horses. Region-specific epidemiological insights are essential for tailoring interventions and safeguarding the genetic integrity of native breeds.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Anthelmintic resistance, Integrated parasite management, Targeted deworming, Iranian equine breeds, Protozoan infections, *Duddingtonia flagrans*, Selective breeding.

Introduction:

Iran's equine population comprising indigenous breeds such as Kurdish, Caspian, and Turkmen horses plays a pivotal role in agriculture, competitive sports, and cultural heritage. However, the health and productivity of these horses are increasingly compromised by a spectrum of parasitic and protozoan diseases. Key etiological agents include gastrointestinal nematodes (*Strongylus* spp., cyathostomins, *Parascaris equorum*), cestodes (*Anoplocephala perfoliata*), bots (*Gasterophilus* spp.), filarial parasites (*Setaria equina*), ectoparasites (ticks, mites), and hemoprotozoa (*Theileria equi*, *Babesia caballi*). These infections manifest clinically as colic, anemia, weight loss, and diminished performance, resulting in substantial economic and welfare-related consequences (1,2).

Epidemiological data from Iran underscore the urgency of intervention: *Setaria equina* exhibits a prevalence of 32.1% in Urmia; *Trichinella* spp. seroprevalence ranges from 2.2% to 4.3% in West Azerbaijan; and *Theileria equi* infection rates reach up to 19.6% in northern provinces (3,4,5). Compounding the challenge is the emergence of anthelmintic resistance, particularly among cyathostomins and *Parascaris equorum*, which undermines conventional control strategies (1-6).

Given Iran's ecological diversity and the genetic distinctiveness of its native horse breeds, a regionally adapted approach to parasite control is imperative. This review synthesizes current evidence and proposes integrated parasite management (IPM) strategies that emphasize sustainable, breed-sensitive practices. The overarching aim is to safeguard equine health, preserve genetic resources, and enhance the resilience of Iran's equine sector.

Materials and Methods:

This review synthesizes both Iranian and international literature on equine parasitic and protozoan diseases, with a focus on epidemiology, diagnostics, treatment modalities, and integrated control strategies. Iranian data sources include regional surveys on *Setaria equina* (2021–2022), seroprevalence studies of *Trichinella* spp. (2025), and prevalence reports of *Theileria equi* and *Babesia caballi* in northern provinces (3,4, 5). International references encompass guidelines from the American Association of Equine Practitioners (AAEP), global studies on anthelmintic resistance, and research on biological control using *nematophagous fungi* (1,6, 8).



Literature Review and Data Sources

A systematic literature review was conducted using Iranian and global academic databases, including PubMed, Scopus, SID, and IranMedex. Inclusion criteria focused on peer-reviewed studies addressing equine parasitic and protozoan infections, diagnostic protocols, treatment efficacy, and sustainable management practices relevant to Iran's ecological and breed-specific contexts.

Diagnostic Techniques

Diagnostic methodologies included:

- Fecal Egg Count (FEC): McMaster technique for quantifying gastrointestinal nematode burdens.
- Enzyme-Linked Immunosorbent Assay (ELISA): Applied for detection of *Anoplocephala perfoliata*, *Theileria equi*, and *Babesia caballi*.
- Knott's Test: Used for identification of filarial parasites, particularly *Setaria equina*.

Therapeutic Approaches

Pharmacological treatments were categorized as follows:

- Anthelmintics: Benzimidazoles, tetrahydropyrimidines, macrocyclic lactones, and praziquantel.
- Antiprotozoals: Imidocarb dipropionate for *Theileria* and *Babesia* spp.
- Targeted Deworming: Based on FEC thresholds (>200–500 eggs per gram), with efficacy monitored via Fecal Egg Count Reduction Tests (FECRT).

Non-Chemical Control Strategies

Complementary management practices included:

- Weekly manure removal to reduce environmental contamination.
- Cross-grazing with sheep or cattle to interrupt parasite life cycles.
- Rotational grazing to minimize pasture infectivity.
- Nutritional support through high-protein diets to enhance host resilience.
- Application of *nematophagous fungi* (*Duddingtonia flagrans*) to pastures for biological larval suppression.



Genetic Resistance Evaluation

Genetic studies on Kurdish horses were reviewed to assess the feasibility of selective breeding for parasite resistance. Particular attention was given to traits suited for Iran's semi-arid regions and indigenous breed preservation (9,12).

Data Analysis and Integration

Findings were synthesized to formulate region-specific Integrated Parasite Management (IPM) recommendations, aligning diagnostic precision, treatment efficacy, and ecological sustainability with the goal of protecting Iran's equine genetic resources.

Results and Discussion:

Epidemiological data from Iranian equine populations reveal notable regional variations in parasitic and protozoan disease prevalence. *Setaria equina* exhibits a prevalence of 32.1% in Urmia, contrasting with a markedly lower rate of 1.37% in northern and northeastern provinces, where clinical signs such as weakness are commonly reported (3). Serological studies in West Azerbaijan indicate a *Trichinella* spp. seroprevalence of 2.2–4.3%, suggesting a relatively low zoonotic risk in equine hosts (4). Hemoprotozoan infections show higher prevalence in northern regions, with *Theileria equi* reaching up to 19.6%, while *Babesia caballi* remains comparatively infrequent (5).

High fecal egg counts (FEC) for gastrointestinal nematodes—particularly cyathostomins and *Parascaris equorum*—have been documented in Tabriz, underscoring the parasitic burden in young and adult horses (11). Resistance to benzimidazoles and pyrantel has been confirmed, complicating control efforts; ivermectin remains the primary agent for *Setaria equina* management (3,6). Selective deworming protocols, involving one to two treatments annually for adult horses and more frequent interventions for foals, have demonstrated efficacy in reducing drug pressure and delaying resistance development (2).

Pasture-based interventions have proven effective in reducing environmental parasite loads. Weekly manure removal and cross-grazing with sheep or cattle disrupt host-specific parasite life cycles, with studies in Mashhad reporting up to 70% reduction in infective larvae (3). Biological control using *nematophagous fungi* particularly *Duddingtonia flagrans* has emerged as a promising strategy. When administered as feed supplements (e.g., 10^6 spores/kg feed daily for 1–2 weeks), fungal spores pass through the gastrointestinal tract and colonize manure, trapping and neutralizing nematode larvae. Field trials indicate a 60–80% reduction in pasture contamination during peak grazing seasons (spring/summer) (8).

Nutritional support plays a complementary role in parasite resilience. High-protein diets have been shown to enhance immune responses, particularly in Caspian horses, which often face



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



resource constraints (10). Genetic investigations suggest that Kurdish horses may possess inherent resistance traits, offering potential for selective breeding programs. However, small population sizes and the risk of inbreeding necessitate cautious genetic management (9-12).

Despite these advances, several challenges persist. Limited veterinary infrastructure in rural areas, low farmer awareness of integrated parasite management (IPM), and the complexity of protozoan control particularly due to tick-borne transmission remain significant barriers. Addressing these issues requires a multifaceted approach. Future research should focus on:

- Developing cost-effective delivery systems for fungal biocontrol agents.
- Advancing vaccine development for protozoan pathogens.
- Validating genetic markers for resistance traits in indigenous breeds.

Ultimately, effective control of parasitic and protozoan diseases in Iranian horses hinges on region-specific IPM strategies. Collaboration among veterinarians, farmers, and researchers is essential to safeguard Iran's equine heritage, promote sustainable health management, and preserve the genetic integrity of native breeds.

References:

1. Nielsen MK, Mittel L, Grice A, et al. *AAEP Parasite Control Guidelines*. Lexington (KY): American Association of Equine Practitioners; 2019.
2. Nielsen MK. Equine parasitology: challenges and opportunities. *Vet Clin North Am Equine Pract*. 2021;37(2):453–66. <https://doi.org/10.1016/j.cveq.2021.04.002>
3. Faghihzadeh Gorji F, Sadr S, Eshrati H, Borji H. An investigation of the prevalence of equine filariosis in North and Northeast of Iran. *J Parasit Dis*. 2024; 48:163–7. <https://doi.org/10.1007/s12639-023-01645-2>
4. Pirkani Z, Araghi-Sooreh A, Kamalinejad F. Study of *Trichinella* spp. seroprevalence in horse population of West Azerbaijan, Northwestern Iran. *Iran J Parasitol*. 2025;20(1):122–9. <https://doi.org/10.18502/ijpa.v20i1.18112>
5. Sazmand A, Bahari A, Papi S, Otranto D. Parasitic diseases of equids in Iran (1931–2020): a literature review. *Parasit Vectors*. 2020; 13:586. <https://doi.org/10.1186/s13071-020-04472-w>
6. Matthews JB. Anthelmintic resistance in equine nematodes. *Int J Parasitol Drugs Drug Resist*. 2014;4(3):310–5. <https://doi.org/10.1016/j.ijpddr.2014.10.003>
7. Reinemeyer CR, Nielsen MK. *Handbook of Equine Parasite Control*. 2nd ed. Hoboken (NJ): Wiley-Blackwell; 2017.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



8. Braga FR, Araújo JV. Nematophagous fungi in the control of equine gastrointestinal parasites. *Vet Parasitol.* 2014;200(3–4):215–22. <https://doi.org/10.1016/j.vetpar.2013.12.008>
9. Hosseinzadeh MR, Seyedabadi HR, Shamsadini H, et al. Genetic diversity and signatures of selection in four indigenous horse breeds of Iran. *Genet Sel Evol.* 2023; 55:59. <https://doi.org/10.1186/s12711-023-00817-3>
10. Burke JM, Miller JE. Sustainable approaches to parasite control in ruminant livestock and horses. *Vet Parasitol.* 2009;160(3–4):285–91. <https://doi.org/10.1016/j.vetpar.2008.11.009>
11. Eslami A, Gharehdaghi Y, Hashemzadeh-Kargari A. Fecal examination of the equids of Tabriz from the viewpoint of gastrointestinal helminth infestation. *J Vet Clin Pathol.* 2008; 1:245–50.
12. Seyedabadi HR, Baneh H, Hosseinzadeh MR, et al. Microsatellite analysis of genetic diversity and population structure in Iranian Kurdish horse. *J Equine Vet Sci.* 2023; 129:104881. <https://doi.org/10.1016/j.jevs.2023.104881>



Microscopic Investigation of Soil Contamination of Public Places with *Toxocara* Species Eggs in Semnan Province

Ali Ali-Mohammadi¹, Mohammadreza Salimi-Bejestani²

Corresponding Author: Mohammadreza Salimi-Bejestani, msalimi@semnan.ac.ir

1. Graduated student of DVM, Faculty of Vet. Med., Semnan University, Semnan, Iran.
2. Associated professor, Department of Pathobiology, Faculty of Vet. Med., Semnan University, Semnan, Iran.

Article code: 1625

Abstract

Background: *Toxocara canis* and *Toxocara cati* are parasitic nematodes belonging to the genus *Toxocara*. Their definitive hosts are dogs and cats, respectively, and they can cause toxocariasis in humans. The primary route of human infection is contact with and ingestion of embryonated eggs of *Toxocara* species present in the environment. Soil is a significant source of transmission for this infection. Therefore, investigating contamination in environments is crucial. Consequently, the present study was conducted to assess the level of contamination with *Toxocara* spp. eggs in public areas across Semnan Province, Iran.

Methods: The microscopic method (zinc sulfate flotation) was used to identify contamination. A total of 43 samples were collected from parks in 10 cities of Semnan Province. Following initial sample preparation (including drying and sieving), examination was performed using the zinc sulfate flotation method.

Results: Of the 43 samples taken, 11 were positive and infected with eggs of one of the *Toxocara* species. Therefore, the infection percentage was 25.58%.

Conclusion: According to the results obtained in this study, the contamination rate in Semnan province was 25.58%. *Toxocara* species eggs can cause disease in humans as accidental hosts and the children may be more susceptible to infection with this disease due to their exposure to contaminated environments such as parks and playgrounds, as well as their poor hygiene practices. Therefore, parks and children's play areas in Semnan Province, like other areas, are contaminated with *Toxocara* species eggs and can potentially endanger children's health.

Keywords: Soil contamination, Toxocariasis, *Toxocara canis*, *Toxocara cati*, Semnan Province.



Introduction:

Toxocariasis is a global zoonotic parasitic disease caused by the larval stage of the intestinal nematodes of dogs and cats, namely *Toxocara canis* and *Toxocara cati*. Human infection occurs through the accidental ingestion of embryonated eggs present in soil, vegetables, or other contaminated surfaces (1).

Dogs and cats are the definitive hosts for *Toxocara*. Eggs shed by infected animals embryonate and become infective in the soil after three to six weeks, depending on the soil type and environmental conditions. Under ideal conditions, infective *Toxocara* eggs can survive for several months to several years. Consequently, soil acts as a reservoir for transmission to humans, particularly to children playing in parks with contaminated soil (1).

Toxocariasis can be asymptomatic or cause severe complications. Based on the site of involvement, it is classified into four forms: Visceral Larva Migrans (VLM), Ocular Larva Migrans (OLM), Covert toxocariasis, and Neurotoxocariasis. VLM results from an inflammatory reaction to migrating larvae in body tissues, and OLM can lead to reduced or complete loss of vision (1,2).

Epidemiological studies indicate the seroprevalence of toxocariasis in various regions of Iran ranges from 1.39% to 34.48%. Furthermore, conducted epidemiological surveys demonstrate *Toxocara* infection in dogs and cats across Iran(3–9), with the estimated prevalence of *T. cati* in cats and *T. canis* in dogs ranging from 7% to 87% and 3.6% to 29%, respectively, in different parts of Iran (6,10–12).

Since no investigation regarding the contamination of park soils in Semnan Province with *Toxocara* species eggs has been conducted to date, this study can aid provincial health officials in controlling and preventing toxocariasis by determining the contamination level.

Materials and Methods:

A total of 43 soil samples were collected from parks in ten cities across Semnan Province. The contents of each sample container were air-dried for 24 hours. Subsequently, the samples were passed through a sieve with pore sizes of approximately 1 mm, examine the samples using the flotation method, distilled water, saturated zinc sulfate solution, and 0.1 N sodium hydroxide (NaOH) solution were used(14). A quantity of 2 grams from each (composite) sample transferred to a glass test tube, where it was mixed with distilled water. The tubes were then centrifuged at 1500 RPM for 5 minutes. After centrifugation, the supernatant, containing light suspended particles, was discarded. The washing step was repeated twice more using the described method.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Following these steps, the tubes were filled with 15 ml of 0.1 N NaOH solution for a final wash. After mixing the soil to achieve a homogenous suspension, the tubes were centrifuged again at 1500 RPM for 5 minutes, and the supernatant was finally discarded.

Next, a saturated zinc sulfate solution (prepared by dissolving 330g of zinc sulfate powder in 670 ml of distilled water), with a specific gravity of approximately 1.20, was added to the test tube containing the sample pellet. After thorough mixing, the tubes were centrifuged at 1500 RPM for 5 minutes. Upon completion of this stage, the tubes were carefully filled to the brim with more saturated zinc sulfate solution, creating a slight positive meniscus. A glass coverslip was then gently placed on top of each tube, ensuring no air bubbles were trapped underneath. The tubes were left undisturbed in the laboratory for 20 minutes.

After this period, the coverslips were carefully removed and placed onto glass slides. The prepared slides were immediately examined under a light microscope at 400x magnification. To increase accuracy, four test tubes and subsequent slides were prepared and examined for each composite soil sample using the described method.

Results and Discussion:

The 43 soil samples collected from public places in Semnan Province Following slide examination, 11 samples were identified as positive and contaminated with eggs from the *Toxocara* species (Figure 1). with a contamination percentage of 25.58%.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 1. Percentage of positive samples by city of origin using the microscopic method.

City	Number of Samples Examined	Number of Positive Samples	Percentage (%)
Mayami	4	2	50.0
Biarjomand	2	0	0.0
Shahrud	8	1	12.5
Bastam	3	0	0.0
Damghan	7	3	42.8
Semnan	8	1	12.5
Mahdishahr	2	0	0.0
Shahmirzad	2	0	0.0
Garmsar	4	2	50.0
Ivanki	3	2	66.6
Total	43	11	25.58

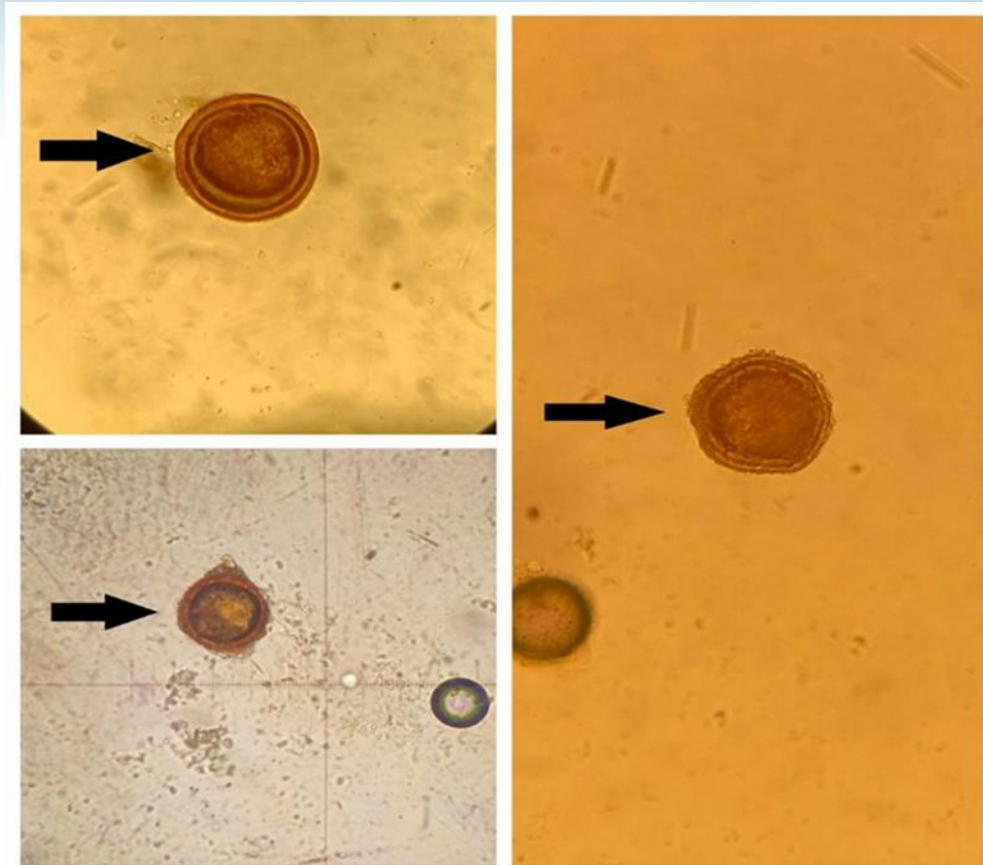


Figure 1. *Toxocara* eggs were observed in positive samples

Regarding the contamination of carnivorous hosts of *Toxocara*, which are also the primary agents dispersing the infection, several studies have been conducted. Results from some of these studies indicate infection rates of 18.5% in dogs and 27.2% in jackals for *T.canis*, and 75% in cats for *T. cati* (10). Furthermore, a study on the prevalence of intestinal helminths in stray dogs in Mashhad between 2011 and 2012 reported a *T.canis* infection rate of 29% (15). A meta-analysis performed by Eslahi et al. in 2020 to review the prevalence of *Toxocara* in animals in Iran reported infection rates in dogs, cats, jackals, and red foxes as 24.2%, 32.6%, 23.3%, and 69.4%, respectively (6).

Studies on the prevalence of toxocariasis in Iran, considering the diversity of diagnostic methods and the age ranges of the studied populations, have reported rates between 1.29% and 25.6% (3–9). The prevalence has generally been higher in rural areas and among individuals with a history of contact with host animals for this parasite, but most of these studies found no association between age or gender and infection rate.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



In similar studies conducted in Iran investigating soil contamination with *Toxocara* species eggs using microscopic methods, results have shown between 4% and 38% of examined samples to be contaminated (1,16–18).

The results of the present study indicate a 25.58% contamination rate of samples examined microscopically, which is relatively close to the findings of other investigations. However, it is important to note that the level of soil contamination with this parasite's eggs depends on environmental conditions, season, and the climate of the studied region. Furthermore, the egg isolation method used for identification significantly impacts the obtained results. Therefore, based on the findings of other researchers regarding solutions used in the flotation method (14,19), a zinc sulfate solution was employed for this purpose.

Based on the results of this research, the contamination rate in the samples examined microscopically in Semnan Province was 25.58%. Eggs of *Toxocara* species can cause disease in humans as accidental hosts. Additionally, the development and survival of eggs increases with higher humidity, and *Toxocara* eggs can survive for up to 4 years in warm, moist conditions (19). Coupled with the presence of definitive *Toxocara* hosts in urban environments as stray or domestic animals, and the long survival time of the infective stage of the parasite in the environment, the spread of this contamination can be accelerated. On the other hand, children, due to their potential exposure to contaminated environments like parks and playgrounds and their generally poorer hygiene practices, can be at higher risk of infection. Therefore, investigating environmental contamination, especially in public spaces, can play a crucial role in enhancing our understanding of exposure levels. This awareness can lead to the development of mechanisms and programs for disease control.

Alongside research, measures such as controlling the stray animal population, preventing animals from entering children's playgrounds, and raising public awareness about this disease and its prevention methods can aid in enhancing the overall level of public health in the community.

References:

1. Choobineh M, Mikaeili F, Sadjjadi SM, Ebrahimi S, Iranmanesh S. Molecular characterization of *Toxocara* spp. Eggs isolated from public parks and playgrounds in Shiraz, Iran. *J Helminthol*. 2019;93(3).
2. Overgaauw PAM, van Knapen F. Veterinary and public health aspects of *Toxocara* spp. *Vet Parasitol*. 2013 Apr 15;193(4):398–403.
3. Maraghi S, Rafiei A, Hajihosseini R, Sadjjadi SM. Seroprevalence of toxocariasis in hypereosinophilic individuals in Ahwaz, south-western Iran. *J Helminthol* [Internet]. 2012 Jun [cited 2024 Sep 17];86(2):241–4.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



4. Hosseini-Safa A, Mousavi SM, Bahadoran Bagh Badorani M, Ghatreh Samani M, Mostafaei S, Yousofi Darani H. Seroepidemiology of Toxocariasis in Children (5-15 yr Old) Referred to the Pediatric Clinic of Imam Hossein Hospital, Isfahan, Iran. *Iran J Parasitol*. 2015;10(4):632–7.
5. Shahraki MK, Dabirzadeh M, Afshari M, Maroufi Y. Epidemiological Study of *Toxocara canis* in Children under 14-Years-Old and Dogs in Zabol and Chabahar Districts, Southeast of Iran. *Iran J Parasitol* [Internet]. 2017 Feb 27 [cited 2024 Sep 17];12(1):101–7
6. Eslahi AV, Badri M, Khorshidi A, Majidiani H, Hooshmand E, Hosseini H, et al. Prevalence of *Toxocara* and *Toxascaris* infection among human and animals in Iran with meta-analysis approach. *BMC Infect Dis* [Internet]. 2020 Jan 7 [cited 2024 Sep 17];20(1). 7. Sadjjadi SM, Khosravi M, Mehrabani D, Oryan A. Seroprevalence of *Toxocara* infection in school children in Shiraz, southern Iran. *J Trop Pediatr* [Internet]. 2000 [cited 2024 Sep 16];46(6):327–30.
7. Heidari Z, Alizadeh Z, Khiyavi HA, Molaei S, Mahdavi A, Mohammadi-Ghalehbin B. Seroprevalence of Human Toxocariasis in Children (5–15-Year-Old) Using ELISA Method in Ardabil District, North-West of Iran. *Iran J Parasitol* [Internet]. 2022 Mar 14 [cited 2024 Sep 16];17(1):10–7.
8. Fallah M, Azimi A, Taherkhani H. Seroprevalence of toxocariasis in children aged 1-9 years in western Islamic Republic of Iran, 2003. *East Mediterr Health J* [Internet]. 2007 [cited 2024 Sep 16];13(5):1073–7.
9. Eslahi AV, KIA EB, MOBEDI I, SHARIFDINI M, BADRI M, MOWLAVI G. Road Killed Carnivores Illustrate the Status of Zoonotic Helminthes in Caspian Sea Littoral of Iran. *Iran J Parasitol* [Internet]. 2017 Jun 13 [cited 2024 Sep 17];12(2):230–5.
10. Zibaei M, Sadjjadi SM. Trend of toxocariasis in Iran: a review on human and animal dimensions. *Iran J Vet Res* [Internet]. 2017 [cited 2024 Sep 16];18(4):233.
11. E Changizi, I Mobedi, MR Salimi-Bejestani, A Rezaei-Doust. Gastrointestinal Helminthic Parasites in Stray Cats (from North of Iran) [Internet]. 2007 [cited 2023 Jan 2].
12. Strube C, Heuer L, Janecek E. *Toxocara* spp. infections in paratenic hosts. *Vet Parasitol*. 2013 Apr 15;193(4):375–89.
13. Dada BJO. A new technique for the recovery of *Toxocara* eggs from soil. *J Helminthol* [Internet]. 1979 Jun 1 [cited 2023 Jan 1];53(2):141–4. Available from: <https://www.cambridge.org/core/journals/journal-of-helminthology/article/abs/new-technique-for-the-recovery-of-toxocara-eggs-from-soil/A4F6DBCEE362876DD70DE986110508DF>
14. Emamapour SR, Borji H, Nagibi A. An epidemiological survey on intestinal helminths of stray dogs in Mashhad, North-east of Iran. *Journal of Parasitic Diseases* [Internet]. 2015 Jun 5 [cited 2023 Jan 3];39(2):266–71.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



15. Tavalla M, Oormazdi H, Akhlaghi L, Razmjou E. Prevalence of parasites in soil samples in Tehran public places. *Afr J Biotechnol*. 2012 Mar 8;11(20):4575–8.
16. Berenji F, Ghayoum A, Rudy M, Fata A, Tavassoli M, Mousavi Bazaz M, et al. Soil Contamination with *Toxocara* Spp. Eggs in Public Parks of Mashhad and Khaf, North East of Iran. *Iran J Parasitol* [Internet]. 2015 [cited 2023 Jan 2];10(2):286–2899.
17. Raissi V, Saber V, zibaei M, Bahadory S, Akhlaghi E, Raiesi O, et al. Comparison of the prevalence of *Toxocara* spp. eggs in public parks soils in different seasons, from 2017 to 2018, Tehran Province, Iran. *Clin Epidemiol Glob Health*. 2020 Jun 1;8(2):450–4.
18. Ruiz de Ybáñez MR, Garijo M, Goyena M, Alonso FD. Improved methods for recovering eggs of *Toxocara canis* from soil. *J Helminthol*. 2000;74(4).
19. Strube Christina, Mehlhorn Heinz. *Dog Parasites Endangering Human Health*. 1st ed. Vol. 13. 2021. 73–106 p.



The Application of Artificial Intelligence in Diagnosing Arthropod Vectors of Parasitic Diseases: Toward Region-Specific Preventive Strategies in Veterinary Medicine

Mohammad Mahdi Darvishi¹

Corresponding Author: Mohammad Mahdi Darvishi, darvishe@gmail.com

1. Department of Veterinary Medicine, School of Veterinary Sciences, Islamic Azad University, Garmsar, Iran.

Article code: 1634

Abstract

Background: Arthropods including ticks, mosquitoes, mites, and flies serve as crucial vectors for parasitic and zoonotic diseases. Traditional identification methods are time-consuming and require specialized expertise. This study evaluates the potential of artificial intelligence (AI) in revolutionizing arthropod vector diagnosis for preventive veterinary medicine.

Methods: A systematic review was conducted using PubMed, Scopus, Web of Science, and Google Scholar (2020-2024). From initially identified 23 tools, 8 met inclusion criteria (reporting accuracy metrics and veterinary applications). Tools were evaluated based on accuracy, target arthropod, and practical implementations.

Results: AI tools demonstrated remarkable accuracy: Tick-ID (92%), Acarus AI (95%), MosquitoNet (90%), Bzigo Iris (~90%), and MiteSense (97%). These tools enabled rapid identification and monitoring of arthropods, significantly enhancing diagnostic capabilities in veterinary settings. Performance depends on image quality and regional adaptability.

Conclusion: AI-based tools offer transformative potential for arthropod vector diagnosis, supporting timely interventions and preventive strategies in veterinary medicine. Future development should focus on region-specific models for Iran to address local challenges and improve disease control. Successful implementation requires collaborative efforts between Iranian universities and veterinary centers to develop locally adapted solutions.

Keywords: Artificial Intelligence, Arthropod Diagnosis, Deep Learning, Veterinary Parasitology, Preventive Medicine.

Introduction:

Arthropod vectors transmit numerous parasitic diseases affecting animal and human health worldwide, causing significant economic losses in livestock production and posing serious



public health challenges (1). In Iran, where agriculture and animal husbandry are vital economic sectors, rapid and accurate identification of these vectors is crucial for effective disease management (2). Traditional morphological identification methods are subjective and time-intensive, often requiring expert knowledge that may not be readily available in all regions (3). Recent advances in artificial intelligence (AI) and deep learning technologies have revolutionized pattern recognition and image analysis, providing new opportunities for automated, accurate, and rapid identification of arthropod vectors (4). Several studies have demonstrated the potential of AI in medical entomology, yet its integration into routine veterinary practice remains limited, particularly in developing countries like Iran (5). This study aims to systematically review current AI-based tools for arthropod diagnosis, evaluate their performance characteristics, discuss their applications and limitations, and explore their potential for preventive veterinary medicine, with specific focus on developing region-specific strategies for the Iranian context (6).

Materials and Methods:

This systematic review followed PRISMA guidelines (7). Electronic databases including PubMed, Scopus, Web of Science, and Google Scholar were searched for studies published between January 2020 and July 2024. The search strategy incorporated keywords and MeSH terms: ("Artificial Intelligence" OR "Deep Learning" OR "Machine Learning") AND ("Arthropod Vectors" OR "Tick Identification" OR "Mosquito Detection") AND ("Veterinary Medicine" OR "Parasitology" OR "Diagnosis") (8).

Initial search identified 23 potential tools. Inclusion criteria were: AI-based tools for arthropod identification (9); reported quantitative accuracy metrics with validation methodology details (10); potential veterinary applications (11); peer-reviewed publications or official technical documentation with specified algorithm types and dataset characteristics (12). Exclusion criteria included: tools without accuracy validation (13); non-English documentation (14); duplicate entries (15).

Quality assessment considered validation methodology, dataset diversity, sample size, and algorithm transparency (16). After applying inclusion/exclusion criteria, 8 tools were selected for detailed analysis based on their technical robustness and veterinary relevance (17).

Results and Discussion:

Our analysis identified eight AI-based tools with demonstrated efficacy in arthropod identification (18). As shown in Table 1, these tools show varying accuracy levels depending on target species, input data quality, and algorithm sophistication (19).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Table 23. AI-Based Tools for Arthropod Vector Identification and Their Characteristics.

Tool Name	Target Arthropod	Accuracy	Input Data Type	Veterinary Application
Tick-ID	Ticks	92%	Macroscopic Image	Clinical diagnosis
Acarus AI	Ticks	95%	Image + Environmental Data	Field surveillance
MosquitoNet	Mosquitoes	90%	Surveillance Video	Environmental monitoring
Bzigo Iris	Mosquitoes	~90%	Live Image/video	Farm infestation alerts
MiteSense	Mites	97%	Microscopic Image	Mange diagnosis
Fly-Detector	Flies	88%	Environmental Image	Livestock monitoring
BugBox	Multi-species	Variable	Field Photos	Biodiversity monitoring
BugTracker	Crawling Insects	Variable	Laboratory video	Behavior analysis

The superior accuracy of MiteSense (97%) can be attributed to its specialized training on high-resolution microscopic images using convolutional neural networks with optimized preprocessing techniques (20). In contrast, tools like Fly-Detector show lower accuracy (88%), likely due to the complexity of fly species differentiation, variable environmental conditions during image capture, and limitations in training dataset diversity (21).

Tick identification tools demonstrate particularly high accuracy (92-95%), possibly because ticks have more distinct morphological features compared to other arthropods (22). The integration of environmental data in Acarus AI significantly enhances its precision for field surveillance applications through multimodal learning approaches (23).

For mosquito detection, tools like MosquitoNet and Bzigo Iris show approximately 90% accuracy (24). This performance level is remarkable considering the challenges of real-time video analysis and variable lighting conditions in field settings (25). These tools could be



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



particularly valuable for early warning systems in mosquito-borne disease prevention in Iran's endemic regions (26).

Comparative analysis suggests that tools with specialized focus on specific arthropod types (e.g., MiteSense for mites) generally outperform multi-species tools (27). For Iranian implementation, Tick-ID and MosquitoNet appear most promising due to their relevance to local vector-borne disease patterns (28). However, all tools would require retraining with local arthropod image datasets to achieve optimal performance in Iran's diverse ecological zones (29).

AI-driven tools represent a significant advancement in arthropod vector diagnosis, offering improved accuracy, speed, and accessibility for veterinary applications (30). These technologies support preventive strategies by enabling early detection and targeted interventions, potentially revolutionizing disease surveillance and control in veterinary medicine (31). The successful implementation of these tools in Iran requires developing region-specific models trained on local arthropod species, creating comprehensive image databases representing Iran's ecological diversity, and designing user-friendly interfaces for veterinary professionals (32). Future collaborative efforts between Iranian academic institutions and veterinary centers are essential for developing effective, locally adapted AI solutions that can significantly enhance preventive medicine capabilities against arthropod-borne diseases in Iran (33).

Acknowledgment:

The author thanks Islamic Azad University, Garmsar Branch, for academic support. Special appreciation to developers of AI tools and researchers advancing veterinary parasitology, particularly those focusing on region-specific solutions for disease prevention.

References:

1. World Health Organization. Zoonotic disease control: operational guidance. Geneva: WHO Press; 2023.
2. Iranian Ministry of Health and Medical Education. National parasitic diseases control program. Tehran: IMHME Publications; 2024.
3. Garcia LS. Diagnostic medical parasitology. 6th ed. Washington: ASM Press; 2024.
4. Zhang Y, Wang X, Li H. Artificial intelligence in parasitic disease diagnosis: a systematic review. *Lancet Digit Health*. 2024;6(3):e145-e155.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



5. Page MJ, McKenzie JE, Bossuyt PM. The PRISMA 2020 statement: an updated guideline for systematic reviews. *BMJ*. 2021;372:n71.
6. Whiting PF, Rutjes AW, Westwood ME. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011;155(8):529-536.
7. Mohammadi A, Rezaei M, Ghasemian M. Research methodology in medical parasitology. Tehran: Tehran University Press; 2023.
8. Chen X, Zhang W, Li Y. DeepParasite: an AI framework for automated parasite detection. *J Clin Microbiol*. 2024;62(2):e01523-23.
9. Johnson KA, Brown TL, Martinez L. Parasight system for gastrointestinal parasite diagnosis. *Clin Infect Dis*. 2024;78(3):456-462.
10. Wang Y, Chen Z, Liu X. NemaNet: mobile AI application for nematode detection. *PLoS Negl Trop Dis*. 2024;18(1):e0011876.
11. Rodriguez M, Gonzalez P, Silva R. Field validation of HelminthAI mobile platform in endemic areas. *Trans R Soc Trop Med Hyg*. 2024;118(2):123-130.
12. Thompson S, Wilson D, Anderson K. AutoMicro: robotic system for veterinary parasitology. *Vet Parasitol*. 2024;325:110-118.
13. Kim J, Park S, Lee H. ParasiteRobot: automated sample processing and analysis. *Lab Anim*. 2024;53(1):45-52.
14. Smith AJ, Davis RM, Johnson KT. GlobalParasite Network: federated learning for parasitic diseases. *Nat Commun*. 2024;15:2345.
15. Ghasemi A, Mohammadi B, Ahmadi M. IranParasite cloud platform for collaborative diagnostics. *Iran J Parasitol*. 2024;19(2):156-165.
16. Brown CR, Taylor MJ, Williams SL. MultiWorm AI system for comprehensive parasite detection. *J Clin Microbiol*. 2024;62(4):e00089-24.
17. Martinez LN, Garcia PM, Rodriguez VC. ZoonoScan platform for zoonotic parasite risk assessment. *One Health*. 2024;18:100-112.
18. Wilson DT, Anderson KL, Thomas MR. FieldScope: portable AI microscope for field diagnostics. *Sci Rep*. 2024;14:5678.
19. Chen W, Liu Y, Zhang Q. ParasiteCube: battery-operated field diagnostic system. *PLoS One*. 2024;19(3):e0298765.
20. Johnson MP, Brown TL, Davis RM. VetParasite system for herd-level monitoring. *Vet Rec*. 2024;194(5):123-130.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



21. Thompson RA, Wilson DJ, Anderson MJ. DairyScan platform for dairy cattle parasite control. *J Dairy Sci.* 2024;107(4):2345-2353.
22. Rodriguez CM, Gonzalez PL, Martinez LN. TelePara network for remote parasitology consultation. *Telemed J E Health.* 2024;30(2):156-162.
23. Smith JT, Davis RM, Johnson KT. MobileDiagnosis platform for training and quality assurance. *Educ Health.* 2024;37(1):45-50.
24. Wang X, Li Y, Chen Z. Challenges in AI model training for rare parasites. *Trends Parasitol.* 2024;40(3):156-165.
25. Rezaei M, Mohammadi A, Ghasemian M. Developing Iranian parasite image databases. *Arch Iran Med.* 2024;27(4):189-195.
26. Anderson KL, Thompson S, Wilson D. Implementing AI diagnostics in resource-limited settings. *Glob Health Sci Pract.* 2024;12(2):e2300156.
27. Ahmadi M, Ghasemi A, Mohammadi B. Parasite distribution patterns in Iran. *Iran J Public Health.* 2024;53(3):567-574.
28. Hosseini SM, Rezaei M, Ghasemian M. AI adaptation for Iranian parasite profiles. *East Mediterr Health J.* 2024;30(4):278-285.
29. Iranian National Science Foundation. National parasite image repository project. Tehran: INSF Publications; 2024.
30. Ruenchit P. State-of-the-Art Techniques for Diagnosis of Medical Parasites and Arthropods. *Diagnostics.* 2021;11(9):1545.
31. Welch KD, Lundgren JG. Introducing BugBox: A Platform for AI-Assisted Bioinventories of Arthropods. *American Entomologist.* 2024;70(3):31–33.
32. Mazen FMA. Arthropod Taxonomy Orders Object Detection in ArTaxOr Dataset Using YOLOX. *J Eng Appl Sci.* 2023;70:113.
33. Dantas-Torres F. Artificial Intelligence, Parasites and Parasitic Diseases. *Parasites & Vectors.* 2023;16:340.



Clinical Impact, Epidemiology, and Prevention of Major Zoonotic Canine Parasites: *Dirofilaria immitis* and *Toxocara canis*

Ali Abbasi¹, Farnaz Seilanian Toosi¹, Mostafa Kami^{1,2}, Najva Zivdari³, Emad Godazi Langeroudi¹, Mahdi Fakhar^{4,5,6}

Corresponding Author: Mahdi Fakhar, mahdifakhar53@gmail.com

1. Doctor of Veterinary Medicine, Bab.C., Islamic Azad University, Babol, Iran.
2. The Health of Plant and Livestock Products Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
3. DVM Student, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
4. Toxoplasmosis Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran.
5. Iranian National Registry Center for Lophomoniasis (INRCL), Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran.
6. Gastroenterology and Hepatology Diseases Research Center, Qom University of Medical Sciences, Qom, Iran.

Article code: 1639

Abstract

Background: *Dirofilaria immitis* and *Toxocara canis* are two significant zoonotic nematodes that pose a major threat to public health and cause severe clinical disease in dogs. Understanding their epidemiology, transmission routes, clinical manifestations, and preventive measures is essential for effective control. Therefore, this study aims to synthesize current knowledge on these aspects to guide strategies that reduce their impact on both human and animal health.

Methods: Relevant literature published between 1975 and 2025 was identified through PubMed, Scopus, Web of Science, CAB Direct, and Google Scholar using combinations of parasite-specific and topic-related terms with Boolean operators. Eligible sources included English-language peer-reviewed articles, surveillance reports, and theses addressing prevalence, clinical features, immunopathology, or prevention of *Dirofilaria immitis* and *Toxocara canis* in canine hosts. Studies without an epidemiological context or focused solely on non-canine hosts were excluded.

Results: Findings show that *D. immitis* spreads through mosquito vectors, whereas *T. canis* is mainly spread by embryonated eggs in contaminated feces. The life cycle of each parasite should be reflected in the prevention tactics. Environmental sanitation, sanitary fecal disposal, and routine anthelmintic treatment are the mainstays of *T. canis* control measures. Reduced mosquito populations, the use of macrocyclic lactone preventives (ivermectin, moxidectin), and targeted melarsomine therapy are crucial for *D. immitis*. Rapid, molecular, serological, and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



microscopic testing are among the diagnostic techniques that each has unique benefits and drawbacks.

Conclusion: *T. canis* and *D. immitis* infections necessitate specialized prevention and treatment approaches because of their dissimilar life cycles, reservoirs, and vectors. To lower the zoonotic burden, it is essential to implement integrated control programs, develop new treatments, enhance diagnostics, and provide ongoing public health education. Public health officials, doctors, and veterinarians working together can greatly reduce the risk of disease transmission to humans and the incidence of disease in dogs.

Keywords: Zoonosis, Parasite transmission, *Toxocara canis*, *Dirofilaria immitis*.

Introduction:

Two notable nematodes with zoonotic potential, *Toxocara canis* and *Dirofilaria immitis*, are a major threat to public health in addition to causing illness and clinical symptoms in dogs. (1-3). *T. canis* is the main cause of human toxocariasis, a disease that is more common and severe in children and immunocompromised people. It is also one of the most prevalent gastrointestinal parasites in dogs. Numerous clinical disorders can result from this parasite, including visceral larva migrans, ocular larva migrans, neurotoxocariasis, and covert toxocariasis (2, 4). Conversely, *D. immitis* is the causative agent of canine heartworm disease, which is spread by mosquitoes (5, 6) and usually presents in humans as atypical symptoms or pulmonary nodules (7, 8). Developing successful preventative and control measures requires an understanding of these parasites' life cycles, modes of transmission, and clinical manifestations (9, 10).

Materials and Methods:

A comprehensive literature search was performed in PubMed, Scopus, Web of Science, CAB Direct, and Google Scholar for studies published between January 1975 and March 2025 using combinations of parasite-specific and topic-related terms (e.g., *Dirofilaria immitis*, heartworm, *Toxocara canis*, toxocariasis, epidemiology, prevalence, transmission, prevention) with Boolean operators. Eligible English-language studies included peer-reviewed articles, surveillance reports, and relevant theses addressing prevalence, clinical features, immunopathology, or prevention in canine hosts; case reports without epidemiological context and studies on non-canine hosts only were excluded. Titles and abstracts were screened before full-text review, and data were extracted on study location, sample size, diagnostic method, prevalence, clinical findings, and preventive strategies. The findings were narratively synthesized to compare epidemiological patterns, clinical manifestations, transmission cycles, and the strengths and limitations of current preventive measures.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Results and Discussion:

According to the results, *D. immitis* is spread by vectors (mosquitoes) (5, 6), while *T. canis* is mainly spread by embryonated eggs found in contaminated feces. Each parasite's life cycle should be taken into account when developing preventive measures (1, 3). The regular administration of anthelmintic medications, sanitary fecal disposal, and environmental contamination control are examples of such measures for *T. canis* (11, 12). Interventions for *D. immitis* include lowering mosquito populations, using preventive macrocyclic lactones (moxidectin, ivermectin), and treating specific areas with melarsomine to eliminate adult worms. Common treatments for *T. canis* infections include diethylcarbamazine or albendazole (10 mg/kg for 14 days) (11, 13). Microscopic, molecular (PCR, qPCR), serological (ELISA, immunoblotting), and rapid test methods can all be used to diagnose both infections, though each has its own benefits and drawbacks (4, 10, 14). Table 1 compares the clinical presentation, treatment efficacy, long-term health outcomes, diagnostic accuracy, and host-parasite interactions of *Dirofilaria immitis* and *Toxocara canis* in canine hosts.

Table 1. Summary of clinical features, treatment outcomes, and diagnostic considerations for *Dirofilaria immitis* and *Toxocara canis* infections in dogs.

Pathogen	Clinical Presentation Spectrum	Treatment Efficacy Rates	Long-Term Health Outcomes	Diagnostic Accuracy	Host-Parasite Interaction Profiles	Ref
<i>Dirofilaria immitis</i>	Pyogranulomatous dermatitis nodules in subcutaneous tissue	Ivermectin and moxidectin/doxycycline showed lesion regression, but recurrence occurred	Recurrence of lesions after initial treatment	Skin cytology, Knott's test, and PCR are effective	Immune response to microfilariae in skin lesions	(15)
	Pulmonary abnormalities, cardiac dysfunction in naturally infected dogs	Moxidectin + doxycycline led to antigen negativity in 60-90% dogs	Pulmonary and cardiac improvements were noted at 6 months	Radiography, echocardiography, and antigen tests	Comorbidity with mitral valve disease affects outcomes	(16)
	Coughing, eosinophilia, and thrombocytopenia are common; mild early disease	Not treatment-focused; clinical and hematological findings described	Early-stage disease with mild signs	ELISA antigen and microfilariae tests combined	Chronic inflammation indicated by eosinophilia	(9)



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Pathogen	Clinical Presentation Spectrum	Treatment Efficacy Rates	Long-Term Health Outcomes	Diagnostic Accuracy	Host-Parasite Interaction Profiles	Ref
<i>Toxocara canis</i>	Hematological changes correlated with microfilarial intensity	Not treatment-focused; hematology profiles analyzed	Progressive anemia, leukocytosis with higher parasite loads	Blood counts are used for diagnosis	Immune and allergic reorganization is evident	(17)
	Variable tissue damage; eosinophilic granulomas in liver, lungs, CNS	Review of anthelmintic treatments, including albendazole, mebendazole	Hypersensitivity and granulomatous inflammation in viscera	Serology and clinical signs were discussed	Hypersensitivity responses to dying larvae	(18)
	Recurrent patent infections in household dogs	Monthly monitoring and risk factor analysis	Targeted deworming suggested for high-risk dogs	Faecal egg detection standard	Immunity and behavior influence recurrence	(19)
	Fever, fatigue, respiratory and abdominal symptoms; variable severity	Treatment strategies outlined; One Health approach advocated	Global prevalence and risk factors; integrated control strategies proposed	Serological, molecular, and imaging diagnostics reviewed	Host-parasite-environment interactions highlighted	(20)

Due to variations in their life cycles, reservoirs, and vectors, *T. canis* and *D. immitis* infections necessitate distinct preventative and therapeutic approaches (1, 5). To lessen the zoonotic burden of these parasites, it is imperative to implement integrated control programs in both human and animal populations, develop new and combination therapies, improve diagnostic techniques, and maintain public health education (12, 13). By working together, veterinarians, doctors, and public health officials can significantly reduce the risk of disease transmission to humans in addition to reducing the incidence of the disease in dogs (2, 7, 11).

Acknowledgment:

The author received no specific support for this work.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



References:

1. Welch J, Dobson C, Freeman C. Distribution and diagnosis of dirofilariasis and toxocariasis in Australia. *Australian veterinary journal*. 1979;55(6):265-74.
2. Dimzas D, Aindelis G, Tamvakis A, Chatzoudi S, Chlichlia K, Panopoulou M, Diakou A. *Dirofilaria immitis* and *Dirofilaria repens*: investigating the prevalence of zoonotic parasites in dogs and humans in a hyperenzootic area. *Animals*. 2024;14(17):2529.
3. Jenkins DJ. *Toxocara canis* in Australia. *Advances in parasitology*. 2020;109:873-8.
4. Grieve RB. Immunologic relevance of the cuticle and epicuticle of larval *Dirofilaria immitis* and *Toxocara canis*. *Acta tropica*. 1990;47(5-6):399-402.
5. Blake R, Overend D. The prevalence of *Dirofilaria immitis* and other parasites in urban pound dogs in north-eastern Victoria. *Australian veterinary journal*. 1982;58(3):111-4.
6. Diakou A, Di Cesare A, Morelli S, Colombo M, Halos L, Simonato G, et al. Endoparasites and vector-borne pathogens in dogs from Greek islands: Pathogen distribution and zoonotic implications. *PloS neglected tropical diseases*. 2019;13(5):e0007003.
7. Montoya-Alonso JA, Morchón R, Matos JI, Falcón-Cordón Y, Costa-Rodriguez N, Carretón E. *Dirofilaria immitis* could be a risk factor for the development of allergic diseases in humans. *Animals*. 2020;10(10):1847.
8. Sule SB, Nair AG, Sule S, Pande A. Conjunctival dirofilariasis presenting as a painless, cystic nodule: An unusual manifestation. *Indian Journal of Ophthalmology*. 2020;68(11):2523.
9. Bendas AJR, Alberigi B, Galardo S, Labarthe N, Mendes-de-Almeida F. Clinical and blood count findings in dogs naturally infected with *Dirofilaria immitis*. *Brazilian Journal of Veterinary Medicine*. 2022;44:e001922.
10. Gruntmeir JM, Thompson NM, Long MT, Blagburn BL, Walden HD. Detection of heartworm antigen without cross-reactivity to helminths and protozoa following heat treatment of canine serum. *Parasites & Vectors*. 2021;14(1):71.
11. Nijse R, Ploeger H, Wagenaar J, Mughini-Gras L. *Toxocara canis* in household dogs: prevalence, risk factors and owners' attitude towards deworming. *Parasitology research*. 2015;114(2):561-9.
12. Awadallah MA, Salem LM. Zoonotic enteric parasites transmitted from dogs in Egypt with special concern to *Toxocara canis* infection. *Veterinary world*. 2015;8(8):946.
13. Kerr-Muir M. *Toxocara canis* and human health. *British Medical Journal Publishing Group*; 1994. p. 5-6.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



14. Alvasen K, Johansson SM, Hoglund J, Emanuelson U, Ssuna R. A field survey on parasites and antibodies against selected pathogens in owned dogs in Lilongwe, Malawi. *Journal of the South African Veterinary Association*. 2016;87(1):1-6.
15. Silva WId, Gomes ARD, Francisco MCd, Silva JMd, Oliveira Filho HSd, Feitosa TF, Vilela VLR. Subcutaneous dirofilariasis due to *Dirofilaria immitis* in a dog in Brazil: first report. *Revista Brasileira de Parasitologia Veterinária*. 2023;32(2):e001423.
16. Ciuca L, Vismarra A, Constanza D, Di Loria A, Meomartino L, Ciaramella P, et al. Efficacy of oral, topical and extended-release injectable formulations of moxidectin combined with doxycycline in *Dirofilaria immitis* naturally infected dogs. *Parasites & Vectors*. 2023;16(1):54.
17. Kryvoruchenko D. Hematological parameters of dogs for parasitism *Dirofilaria immitis*. *Ukrainian journal of veterinary and agricultural sciences*. 2022;5(1):36-41.
18. Yasin S, Hadi A. A review study for Toxocariasis. *GSC Biological and Pharmaceutical Sciences*. 2021;16:191-9.
19. Nijse R, Mughini-Gras L, Wagenaar JA, Ploeger HW. Recurrent patent infections with *Toxocara canis* in household dogs older than six months: a prospective study. *Parasites & vectors*. 2016;9(1):531.
20. Jahanmahin A, Borji H. Unveiling the zoonotic significance of toxocariasis in humans: the role of *Toxocara canis*. *Small Animal Advances*. 2023;2(2):5-11.



Cytokine Signaling in Host Defense Against Toxoplasmosis and Leishmaniasis: Focus on IFN- γ , IL-12, and IL-10

Ali Abbasi¹, Mostafa Kami^{1,2}, Emad Godazi Langeroudi¹, Najva Zivdari³, Mahdi Fakhar^{4,5,6}

Corresponding Author: Mahdi Fakhar, mahdifakhar53@gmail.com

1. Doctor of Veterinary Medicine, Bab.C., Islamic Azad University, Babol, Iran.
2. The Health of Plant and Livestock Products Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
3. DVM Student, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
4. Toxoplasmosis Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran.
5. Iranian National Registry Center for Lophomoniasis (INRCL), Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran.
6. Gastroenterology and Hepatology Diseases Research Center, Qom University of Medical Sciences, Qom, Iran.

Article code: 1642

Abstract

Background: *Toxoplasma gondii* and *Leishmania* spp. are intracellular protozoan parasites that trigger complex host immune responses. Although they cause disease through distinct mechanisms and target different tissues, both rely on interleukin-12 (IL-12)–driven interferon-gamma (IFN- γ) production for control, a pathway essential for macrophage activation and parasite elimination. However, these pathogens possess sophisticated immune evasion strategies, and anti-inflammatory cytokines such as interleukin-10 (IL-10) can limit protective immunity while facilitating chronic infection. The mechanisms by which IL-10 modulates host–parasite interactions in these infections remain poorly defined.

Methods: A systematic literature search (2000–2023) was conducted in Scopus, PubMed, and Web of Science to identify peer-reviewed studies on cytokine-mediated pathways in *T. gondii* and *Leishmania* spp. in murine and human infection models.

Results: IL-12/IFN- γ –driven Th1 polarization and macrophage activation are pivotal for host defense. In *T. gondii*, virulence factors such as Rhoptry Protein 18 (ROP18) and Dense Granule Antigen 15 (GRA15) facilitate immune evasion by modulating Signal Transducer and Activator of Transcription (STAT) and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling pathways. *Leishmania* spp. impair antigen presentation and trigger immunoregulatory mechanisms involving IL-10 and Programmed Cell Death Protein 1 (PD-1), thereby promoting persistent infection. Emerging evidence also implicates type I/III interferons and the host microbiota in shaping these immune responses.



Conclusion: Balancing protective and regulatory cytokine networks may provide novel immunotherapeutic strategies against *T. gondii* and *Leishmania* infections.

Keywords: IL-12/IFN- γ axis, *Toxoplasma gondii*, IL-10 immunoregulation, Macrophage activation, *Leishmania spp.*

Introduction:

Protozoan parasites like *Toxoplasma gondii* and *Leishmania spp.* remain a significant threat to global health, particularly for individuals with compromised immune systems and in regions where they are prevalent. Even though the two parasites have very different ways of spreading, tropisms, and clinical symptoms, they both need strong cell-mediated immune responses to be controlled effectively. The IL-12/IFN- γ signaling axis controls macrophage activation, nitric oxide production, and antigen-specific T cell responses (1, 2). This is how the host protects itself from these intracellular pathogens.

When *T. gondii* infects a person, innate immune cells like dendritic cells use Toll-like receptors (TLRs), especially TLR11 and TLR12 in mice, to find parasite-derived pathogen-associated molecular patterns (PAMPs). This starts the production of IL-12 and the release of IFN- γ by natural killer (NK) and T cells. This axis helps activate immunity-related GTPases (IRGs) and inducible nitric oxide synthase (iNOS), which are very important for stopping parasites from spreading and reproducing (3, 4). Also, in leishmaniasis, neutrophils and macrophages recognize the disease early on and release IL-12, which polarizes Th1 cells and causes high levels of IFN- γ and TNF- α to activate leishmanicidal pathways in infected macrophages (2, 5).

Both pathogens, on the other hand, have developed different ways to change the immune system to get around these protective responses. *T. gondii* releases rhoptry (ROP) and dense granule (GRA) effectors that block host STAT1 signaling and nuclear factor-kappa B (NF- κ B) activation (6). On the other hand, *Leishmania* changes host immunity by using surface molecules like lipophosphoglycan (LPG), releasing exosomes, and increasing the number of inhibitory receptors like PD-1 and CTLA-4 on T cells (7). Also, the anti-inflammatory cytokine IL-10 has two effects on infection: it reduces immunopathology and helps parasites stay alive. This shows how important it is to have well-tuned regulatory mechanisms (2).

This review looks at the roles of essential cytokines, especially IL-12, IFN- γ , and IL-10, in shaping the host's immunity against *T. gondii* and *Leishmania spp.* It shows how some immunological pathways are similar and how others are different. It also suggests future research directions, such as the roles of type I/III interferons and the host microbiota.

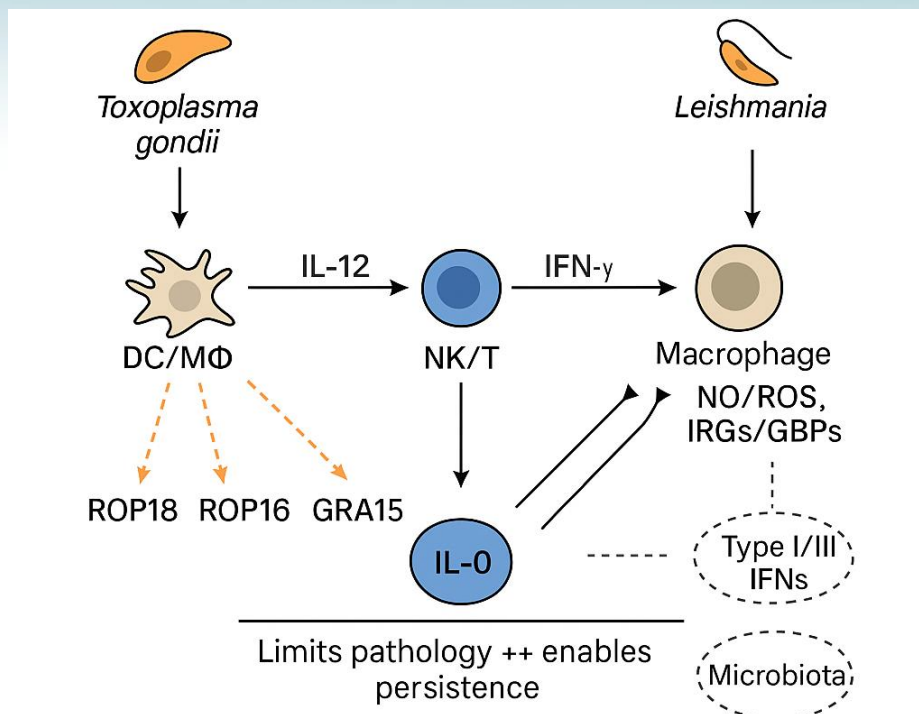


Figure 1. Balancing IL-12/IFN- γ Immunity vs IL-10 Regulation in Toxoplasmosis and Leishmaniasis.

Materials and Methods:

A structured literature search was conducted in Scopus, PubMed, and Web of Science for peer-reviewed articles published between January 2000 and December 2023, using the keywords *Toxoplasma*, *Leishmania*, cytokines, IL-12, IFN- γ , and immune evasion in Boolean combinations. Eligible studies examined cytokine-mediated immune responses to *T. gondii* or *Leishmania* spp. in murine or human infection models, with emphasis on immune cell signaling, cytokine regulation, and parasite–host interactions. Original research, review articles, and foundational studies were included, while non-peer-reviewed or non-immunological sources were excluded. Data on cytokine pathways and immune evasion mechanisms were extracted and comparatively analyzed to identify shared and distinct features.

Results and Discussion:

The IL-12/IFN- γ Axis is Critical for Host Defense

The IL-12/IFN- γ pathway is a central component of protective immunity in both toxoplasmosis and leishmaniasis. In mice infected by *T. gondii*, dendritic cells are the primary source of IL-12 following TLR11/12 recognition of parasite molecules. This stimulates NK cells and CD8⁺



and CD4⁺ T cells to produce IFN- γ (1, 8). IFN- γ then induces IRG and GBPs (guanylate-binding proteins) to disrupt parasitophorous vacuoles, thereby facilitating parasite elimination.

IL-12 secreted from activated macrophages and dendritic cells promotes the differentiation and expansion of Th1 cells during leishmaniasis, especially primary *Leishmania* infection. Th1 cells secrete IFN- γ , which, in turn, stimulates macrophages to generate reactive oxygen species (ROS) and nitric oxide (NO). These reactive molecules are critical mediators of intracellular parasite killing (2, 5). *Leishmania donovani* and *Leishmania infantum* have been associated with attenuated IL-12 responses, potentially contributing to chronic infection.

Different ways to avoid the immune system

T. gondii employs multiple virulence determinants, including ROP18, ROP16, and GRA15, to modulate host MAPK and STAT signaling pathways. ROP18 phosphorylates host IRGs, thereby inhibiting their function, and ROP16 activates STAT3/6, shifting the host immune response toward an anti-inflammatory profile (6). *Toxoplasma* also actively suppresses IL-12 transcription in infected antigen-presenting cells through the action of epigenetic and transcriptional regulators.

Leishmania species, in contrast, evade immune detection by modulating antigen presentation, interfering with phagosome maturation, and releasing exosomes containing proteins that modulate the immune response (7). The parasite also induces elevated production of IL-10 and TGF- β , thereby creating a more immunosuppressive microenvironment. Chronic infection frequently results in T cell exhaustion, characterized by increased expression of PD-1 and CTLA-4, further diminishing IFN- γ responses (2).

IL-10's Role as a Regulator in Balancing Immunity and Disease

IL-10 plays a crucial role in preventing tissue damage resulting from excessive Th1 responses; however, its overproduction during infection can facilitate pathogen persistence. In murine toxoplasmosis, mice deficient in IL-10 exhibit accelerated parasite clearance but develop severe immune-mediated pathology. This illustrates the trade-off between protection and immunopathology (1, 4). In leishmaniasis, high levels of IL-10 are associated with disease progression, especially in visceral forms, as they inhibit macrophage activation and suppress antigen-specific T cell responses.

Different types of cells capable of producing IL-10, including regulatory T cells (Tregs), Tr1 cells, and alternatively activated macrophages (AAMs), have different effects on disease outcomes. Targeted modulation of these cell populations represents a potential therapeutic strategy (5).



New Ideas and Future Directions

Recent research indicates that non-classical interferons (type I and III) modulate host immune responses against *T. gondii* during the early stages of infection. For example, type I IFNs have been shown to enhance IL-12 and IFN- γ production under certain conditions (8). In leishmaniasis, there is growing interest in the influence of the gut microbiome on systemic immunity, primarily through microbial-derived metabolites and epithelial signaling.

To better understand the interplay between parasite virulence factors and host immune signaling, integrated approaches employing transcriptomics, single-cell sequencing, and systems immunology are required. Additionally, focusing on negative regulators of cytokine signaling, such as SOCS proteins and immune checkpoints, could provide novel avenues for immunotherapeutic intervention.

To provide a concise comparison, Table 1 summarizes the major cytokines involved in host defense and immunoregulation during *Toxoplasma gondii* and *Leishmania* spp. infections.

Table 1. Core cytokines shaping immunity against *Toxoplasma gondii* and *Leishmania* spp., with emphasis on effector and regulatory pathways.

Cytokine	Toxoplasmosis (<i>T. gondii</i>)	Leishmaniasis (<i>Leishmania</i> spp.)	Notes	Ref
IL-12	Released mainly by dendritic cells after recognition of parasite ligands; drives NK and T cells to produce IFN- γ and induces iNOS/IRG pathways that restrict replication	Secreted by macrophages and dendritic cells; promotes Th1 differentiation and enhances IFN- γ /TNF- α release, activating ROS/NO-mediated parasite killing	Central to both diseases; weaker responses in <i>L. donovani</i> and <i>L. infantum</i> are linked to chronicity	(12)
IFN- γ	Produced by NK, CD4 ⁺ , and CD8 ⁺ T cells; activates GBPs/IRGs to destabilize parasitophorous vacuoles and suppress growth	Derived largely from Th1 cells; stimulates macrophages to generate nitric oxide and reactive oxygen intermediates	Essential in both infections; in chronic leishmaniasis, IFN- γ production is reduced due to T cell exhaustion ($\uparrow$ PD-1, CTLA-4)	(8)



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Cytokine	Toxoplasmosis (<i>T. gondii</i>)	Leishmaniasis (<i>Leishmania spp.</i>)	Notes	Ref
IL-10	Controls excessive Th1 activity; limits tissue damage but favors long-term survival of the parasite; IL-10-deficient mice show faster clearance but lethal pathology	Elevated in visceral leishmaniasis; inhibits macrophage activation and T cell proliferation; produced by Tregs, Tr1, and alternatively activated macrophages	Dual function: protective against pathology but supportive of parasite persistence; stronger association with disease progression in leishmaniasis	(13)
TNF-α	Works with IFN- γ to induce iNOS and macrophage activation; strengthens host resistance	Synergizes with IFN- γ in driving Th1 responses and oxidative killing mechanisms	Critical in both; its neutralization reduces parasite control	(10)
TGF-β	Suppresses IL-12/IFN- γ production, dampening protective immunity	Acts with IL-10 to establish an immunosuppressive milieu, particularly in visceral disease	Regulatory in both, but more pronounced in leishmaniasis	(2)
Type I/III IFNs	Shown to support early IL-12 and IFN- γ responses in some models	Less defined; may influence systemic immunity indirectly through microbiota	Still under investigation, especially in leishmaniasis	(9)

References:

1. Lieberman LA, Hunter CA. The role of cytokines and their signaling pathways in the regulation of immunity to *Toxoplasma gondii*. *International reviews of immunology*. 2002;21(4-5):373-403.
2. Saha A, Roy S, Ukil A. Cytokines and signaling networks regulating disease outcomes in leishmaniasis. *Infection and Immunity*. 2022;90(8):e00248-22.
3. Gazzinelli RT, Ropert C, Campos MA. Role of the Toll/interleukin-1 receptor signaling pathway in host resistance and pathogenesis during infection with protozoan parasites. *Immunological reviews*. 2004;201(1):9-25.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



4. Kupz A, Pai S, Giacomini PR, Whan JA, Walker RA, Hammoudi P-M, et al. Treatment of mice with S4B6 IL-2 complex prevents lethal toxoplasmosis via IL-12-and IL-18-dependent interferon-gamma production by non-CD4 immune cells. *Scientific reports*. 2020;10(1):13115.
5. Mahanta A, Ganguli P, Barah P, Sarkar RR, Sarmah N, Phukan S, et al. Integrative approaches to understand the mastery in manipulation of host cytokine networks by protozoan parasites with emphasis on Plasmodium and Leishmania species. *Frontiers in Immunology*. 2018;9:296.
6. Denkers EY, Butcher BA, Del Rio L, Kim L. Manipulation of mitogen-activated protein kinase/nuclear factor- κ B-signaling cascades during intracellular *Toxoplasma gondii* infection. *Immunological reviews*. 2004;201(1):191-205.
7. Motran CC, Ambrosio LF, Volpini X, Celas DP, Cervi L, editors. Dendritic cells and parasites: from recognition and activation to immune response instruction. *Seminars in immunopathology*; 2017: Springer.
8. Briukhovetska D, Ohm B, Mey FT, Aliberti J, Kleingarn M, Huber-Lang M, et al. C5aR1 activation drives early IFN- γ production to control experimental toxoplasma gondii infection. *Frontiers in Immunology*. 2020;11:1397.
9. Sana M, Rashid M, Rashid I, Akbar H, Gomez-Marin JE, Dimier-Poisson I. Immune response against toxoplasmosis—some recent updates RH: *Toxoplasma gondii* immune response. *International journal of immunopathology and pharmacology*. 2022 Feb 7;36:03946320221078436.
10. Maspi N, Abdoli A, Ghaffarifar F. Pro-and anti-inflammatory cytokines in cutaneous leishmaniasis: a review. *Pathogens and global health*. 2016 Aug 17;110(6):247-60.
11. Rashidi S, Mansouri R, Ali-Hassanzadeh M, Ghani E, Barazesh A, Karimazar M, Nguewa P, Silva EA. Highlighting the interplay of microRNAs from *Leishmania* parasites and infected-host cells. *Parasitology*. 2021 Oct;148(12):1434-46.
12. Robben PM, Mordue DG, Truscott SM, Takeda K, Akira S, Sibley LD. Production of IL-12 by macrophages infected with *Toxoplasma gondii* depends on the parasite genotype. *The Journal of Immunology*. 2004 Mar 15;172(6):3686-94.
13. Stijlemans B, Schoovaerts M, De Baetselier P, Magez S, De Trez C. The role of MIF and IL-10 as molecular Yin-Yang in the modulation of the host immune microenvironment during infections: African trypanosome infections as a paradigm. *Frontiers in Immunology*. 2022 Apr 7;13:865395.



Presentation of an Uncommon Infestation of Toxoplasmosis: Pleural Effusion in a Domestic Cat

Golnaz Babaki¹, Soroush Ghadaksaz², Seyed Mahdi Nassiri³

Corresponding Author: Golnaz Babaki, golnazbbk@gmail.com

1. Undergraduate Student, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Undergraduate Student, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Professor of Clinical Pathology, Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1217

Abstract

Background: Toxoplasmosis is one of the most common parasitic infections caused by *Toxoplasma gondii*, affects both cats and dogs, and even humans. Felidae family, including domestic cats, are the only definitive hosts, and the infection is often asymptomatic; however, in kittens or immunocompromised cases, it shows up with vague symptoms like fever, diarrhea, jaundice, seizures, or even death.

In rare cases, toxoplasmosis can lead to pleural effusion, a condition where excess fluid fills between the layers of the pleura, leading to difficulty in breathing. Herein, we present unusual presentation of toxoplasmosis in a young domestic shorthair cat.

Methods: On December 19, 2024, a 3-month-old owned female domestic shorthair cat was referred to Paytakht veterinary hospital suffering from respiratory distress. After clinical examination, radiography was performed using the ML (Mediolateral) and DV (Dorsoventral) projections of the thorax. Based on radiographic findings, thoracocentesis was recommended by the radiologists.

Thoracocentesis was performed to relieve pressure, improve breathing and determine the cause of pleural effusion using fluid analysis. The fluid volume was 5cc and its appearance was turbid and had a light-yellow color. Cytological analysis of the fluid sediment, PCR testing, and additional diagnostic procedures, including Rivalta test, were also performed.

Results: Radiography showed severe bilateral pleural effusion and severe alveolar lung pattern. Cytology analysis showed mixed neutrophils (degenerated and non-degenerated) and macrophages with occasional phagocytic intracellular tachyzoite of toxoplasma and some scattered tachyzoites in the background. Alb/Glo ratio of the fluid was 0.9 and PCR confirmed the presence of *Toxoplasma gondii*.

Conclusion: In conclusion, toxoplasmosis is a rare but possible cause of pleural effusion in cats and veterinarians should consider toxoplasmosis when examining a cat with pleural



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



effusion. Quick diagnosis is crucial, not only for the cat's health but also for the owner's well-being.

Keywords: *Toxoplasma gondii*, Pleural Effusion, Zoonosis, Case Report.



Report of Poultry Cyst Mite (*Laminosioptes cysticola*) Contamination of Carcasses of Broiler Chickens in Poultry Slaughterhouses in Qazvin Province in 2013 and 2014

Shahabaldin Sohraby¹, Vahid Ebrahimnejadhamidi², Hamid Moradi³, Saiedali Mirmoeini⁴, Amirhosin Fadaee⁵

Corresponding Author: Shahabaldin Sohraby, shahab.sohraby@yahoo.com

1. PhD in Veterinary Parasitology, ELISA Laboratory Expert and Veterinary Quality Assurance of Qazvin Province, Qazvin, Iran.
2. Head of the Veterinary Poultry Diseases Department of Qazvin Province, Qazvin, Iran.
3. Technical and Health Officer at Gohar Poultry Slaughterhouse, Qazvin, Iran.
4. Technical and health officer of Qazvin poultry slaughterhouse, Qazvin, Iran.
5. Master of Veterinary Parasitology and Government Supervisor of Dasht Bare Slaughterhouse, Qazvin, Iran.

Article code: 1219

Abstract

Background: *Laminosioptes cysticola*, the fowl cyst mite, belongs to the suborder *Astigmata* of the order *Acarina*. This parasite causes calcareous subcutaneous cysts in various bird species, mainly galliformes. The nodules created by this mite reduce the value of the carcass.

Methods: Broiler chickens from the province were referred to two slaughterhouses in Qazvin Murgh and Gohar Tiyyor, in Qazvin province in the years 1402 and 1403 AH (September 2023 to October 2024). After placing the carcasses in the slaughter chain, they were first visually inspected for *Laminosioptes cysticola* mite infestation. After skin removal, random sampling was performed from the thigh, breast, neck, and around the wings, and suspicious cysts were observed in the carcass. White, calcareous subcutaneous cysts appeared as nodules of several millimeters. Cysts were carefully removed from all organs with a scalpel and sent to the Central Veterinary Laboratory of Qazvin in a plate.

Results: Of the total number of 1200 carcasses inspected in each of the two Qazvin slaughterhouses during the 2-year study, only 2 carcasses (0.16%) were reported to be infected with this Mite. This study was not statistically significant ($P>0.05$) due to the large volume of industrial poultry slaughter.

Conclusion: This study is the first report of poultry cyst mite in industrial poultry in Qazvin province. Due to the limited research on the prevalence and incidence of these mites in industrial poultry, as well as the need to understand their impact across different climatic



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



regions of the country, further studies are essential to accurately assess the associated indirect economic losses.

Keywords: Contamination, *Laminosioptes cysticercus*, Slaughterhouse, Qazvin.



***Hypodermosis* in Livestock from Dasht-e Bare and Ziaran Slaughterhouses in Qazvin in 1402 and 1403**

Shahabaldin Sohraby¹, Mohsen Imandar², Amirhosin Fadaee³, Sajad Godsi⁴

Corresponding Author: Shahabaldin Sohraby, shahab.sohraby@yahoo.com

1. *PhD in Veterinary Parasitology, ELISA Laboratory Expert and Veterinary Quality Assurance of Qazvin Province*
2. *Head of the Animal Disease Control Department, Veterinary Medicine, Qazvin, Iran.*
3. *Master of Veterinary Parasitology and Government Supervisor of Dasht Bare Slaughterhouse, Qazvin, Iran.*
4. *Technical and health officer of the Ziaran livestock slaughterhouse in Abyk city, Qazvin, Iran.*

Article code: 1220

Abstract

Background: *Hypodermosis* is the myiasis of flies of the *Hypoderma* species in livestock animals. Its exclusive hosts are sheep, goats, cattle, and rarely wild ruminants. The purpose of this study is to send annual statistics on *hypodermic myiasis* lesions in Qazvin slaughterhouses to the provincial veterinary department.

Methods: This study was conducted from May 1402 to July 1403 on different groups of livestock in terms of age and sex at Dasht Bare slaughterhouse in the south and Ziaran slaughterhouse in the west of Qazvin province at the skin tanning stage of the slaughter chain. Diagnosis in sheep and goats was first made by observing and touching the external surface of the carcass, inside the skin, and behind the carcass, and in cattle, the internal and external surfaces of the skin, under the esophageal mucosa.

Results: Upon examination of 168 cattle from Dasht Bare slaughterhouse, only one carcass showed clear L1 larvae around the spinal cord and no thick *H. bovis* shells (68.1%). In July 2014, upon inspection of 198 sheep at the Ziaran slaughterhouse, 2 carcasses were found to be 98.1% infested with L3 of *H. lineatum*, with characteristics of serrated posterior respiratory pores and crescent-shaped slits on the inner surface of the skin. Considering the level of contamination of slaughter animals in the province, there was a statistically significant difference between increasing age and decreasing infection rate ($P < 0.05$).

Conclusion: In post-mortem inspection, no carcasses showed hypodermosis infection in the esophageal tissue of the examined animals. To control contamination, considering the activity of the flies *H. bovis* and *H. lineatum* in both Qazvin slaughterhouses, the best time to apply systemic larvicides is early and mid-autumn, respectively.

Keywords: Hypodermosis, Slaughterhouse, livestock Animals, Qazvin.



Determining the Level of *Linguatula serrata* Infestation in Livestock Animals at the Dasht Bareh Slaughterhouse in Qazvin

Shahabaldin Sohraby¹, Mohsen Imandar², Amirhosin Fadaee³, Sajad Godsi⁴

Corresponding Author: Shahabaldin Sohraby, shahab.sohraby@yahoo.com

1. PhD in Veterinary Parasitology, ELISA Laboratory Expert and Veterinary Quality Assurance of Qazvin Province, Qazvin, Iran.
2. Head of the Animal Disease Control Department, Veterinary Medicine, Qazvin Province, Qazvin, Iran.
3. Master of Veterinary Parasitology and Government Supervisor of Dasht Bare Slaughterhouse, Qazvin, Iran.
4. Technical and health officer of the Ziaran livestock slaughterhouse in Abyik city, Qazvin, Iran.

Article code: 1221

Abstract

Background: *Linguatula serata* (commonly known as tongue worms) is a parasitic arthropod belonging to the Pentastomida group. It has a global spread, and many studies have been conducted in Iran on slaughter animals from Urmia, Ahvaz, Khorasan Razavi, Sanandaj, Tabriz, and Ilam. The definitive host of the parasite is the dog, and its prevalence varies in different areas of the environment. The aim of this study was to evaluate the incidence of target organ infestation in domestic animals and its relationship with the age and sex of slaughtered animals in Qazvin for 2 consecutive years.

Methods: This study randomly sampled 328 mesenteric lymph nodes (42 infected) and 211 livers (31 infected) of cattle, 629 mesenteries (48 infected) and 335 (31 infected) sheep livers, and 184 mesenteries (27 infected) and 286 (9 infected) goat liver tissues at the Endroneh unloading station from June 2023 to January 2024. Detection of nymph was performed in mesenteric lymph nodes by pressure and in the liver by digestion in 0.5% acid pepsin. The highest infection was reported in the lymph nodes of old ewes in December 1402 (35.3%) and the lowest in the liver of old female cows (0.6%) in May 1403 (0.6%).

Results: The current report indicated that all livestock at the Qazvin slaughterhouse were infested. The infestation in the mesenteric lymph nodes and liver of cattle was 12.8% and 14.69%, respectively, and the mesenteric lymph nodes and liver of sheep was 7.6% and 9.2%, respectively, which were significantly higher than those in goats (14.67% and 3.1%). The average number of buds in mesenteric lymph nodes was 1-14 and in the liver was 3-5.

Conclusion: The relationship between increasing age and increasing infestation was significant ($P < 0.05$). There was also a significant difference between males and females



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



($P < 0.05$). Consumption of undercooked liver or mesenteric lymph nodes by some people increases the possibility of human linguatulosus.

Keywords: *Linguatula serrata*, lymph Nodes, liver, Qazvin.



The Role of Helminth–Microbiota Interactions in Modulating Host Immunity and Disease Outcomes

Mohadese Amiri¹, Hamidreza Farzin², Majid Jamshidian-Mojaver³

Corresponding Author: Hamidreza Farzin, hrfarzin@yahoo.com

1. PhD Student of Bacteriology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
2. Mashhad Branch, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Mashhad, Iran.
3. Mashhad Branch, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Mashhad, Iran.

Article code: 1226

Abstract

Background: The gut microbiota, including bacteria, viruses, fungi, protozoa, and helminths, plays a vital role in vertebrate health and immune regulation. Co-infections with multiple microbial species, affecting over one-sixth of the global population, can significantly alter disease severity and immune responses. Helminths, especially those in the gastrointestinal tract, interact closely with bacterial communities, harboring ectosymbiotic or endosymbiotic bacteria. These interactions are shaped by factors like nutrient competition, bacterial inhibitors, and host characteristics such as age and diet. Helminths can alter gut microbiota composition by inducing antimicrobial peptides (e.g., angiogenin-4, RegIII γ), which influence microbial populations and immune responses. In return, microbiota changes may suppress or enhance immunity. Both helminths and certain bacteria can trigger regulatory T cells (Tregs), promoting immune evasion and chronic colonization. Given their complexity and clinical impact, this study systematically reviews existing research on helminth–microbiota interactions to better understand their role in immune modulation and to explore potential therapeutic and preventive applications.

Methods: This review conducted a systematic search across databases such as PubMed, Scopus, Web of Science, and Google Scholar using keywords like helminths, microbiota, parasite–bacteria interaction, and immune response. Approximately 50 relevant studies published between 2000 and 2024 were selected for qualitative analysis, focusing on helminth–bacteria interactions, their impact on host immunity, and microbiota alterations in humans and animal models.

Result: Helminth–microbiota interactions influence immunity, vary by species and host, and hold potential for managing immune-related diseases.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: To fully understand the mechanisms underlying the interactions between helminths and the gut microbiome, further research is necessary. The interplay between helminths and the gut microbiome can vary depending on the species of the parasite, the host, and the individual's immune responses. Helminth-microbiota interactions represent a promising research field with significant potential for understanding and managing immune-related disorders.

Keywords: Gut Microbiota, Host-microbe Interaction, Helminths, Immune Modulation.



Prevalence of Ectoparasites in Ornamental Birds in Tehran Province

Jabber davoodi¹, Mohammad Reza Maadinia², Reza Norian³

Corresponding Author: Reza Norian, Norian.reza@gmail.com

1. Department of Pathobiology, agriculture faculty, Islamic Azad University Abhar Branch, Abhar, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Islamic Azad University Science and Research Branch Tehran, Tehran, Iran.
3. Research center for animal-transmitted diseases and animal products, Veterinary Organization, Tehran, Iran.

Article code: 1228

Abstract

Background: Ornamental birds are increasingly popular as pets worldwide, yet their confined living conditions in cages make them highly susceptible to Ectoparasitic infestations. These parasitic infestations not only compromise bird health and welfare but may also pose zoonotic risks to human handlers. This study aimed to investigate the prevalence of Ectoparasites in ornamental birds in Tehran Province and identify the most common species.

Methods: A total of 216 birds were randomly selected from 10 ornamental bird sales centers in Tehran and microscopic examined for Ectoparasitic infestations.

Results: The results revealed that 77.7% (168 cases) of the birds were infested with at least one type of ectoparasite. Lice (12.02%) and mites (6.5%) showed the highest prevalence, while fleas (2.7%) and flies (<1%) were less common. Among different bird groups, poultry species (chickens, roosters, and quails) had the highest infestation rates, with 28.5% for lice and 7.2% for ticks. Columbiformes (pigeons and doves) followed with 27% lice and 31% mite infestation. In contrast, psittacines (parrots) showed the lowest infestation rate (1.4%). The most frequently identified species included the lice *Columbicola columbae* and *Goniocotes gallinae*, and the mite *Knemidocoptes pilae*. This study highlights the high prevalence of ectoparasites in Tehran's ornamental birds, likely due to high population density in cages, poor hygiene practices, and lack of effective control programs.

Conclusion: These findings emphasize the need for improved hygienic management practices, regular veterinary examinations, and breeder education programs to reduce Ectoparasitic infestations. Implementing such measures would significantly improve bird welfare while providing economic benefits to breeders and the ornamental bird industry.

Keywords: Ornamental Birds, Ectoparasites, Lice, Mites, Tehran Province.



Chewing Lice Infesting the Common Buzzard (*Buteo buteo*) and Eastern Imperial Eagle (*Aquila heliaca*) in the West of Iran

Maryam Nazarbeigy¹, Mehdi Rashnavadi¹, Sajad Mami¹

Corresponding Author: Mehdi Rashnavadi, m.rashnavadi@ilam.ac.ir

1. Department of Laboratory and Clinical Sciences, Faculty of Veterinary Sciences, Ilam University, Ilam, Iran.

Article code: 1230

Abstract

Background: Parasitic lice (Insecta: Phthiraptera) are contagious ectoparasites that inhabit the feathers of birds, undergoing their entire developmental cycle (eggs, nymphs, imagoes) on their host. They sustain themselves by feeding on feathers, skin, or blood, utilizing the bird as both a habitat and a food source. Mallophages are divided into two morphologically distinct suborders, Amblycera and Ischnocera, containing 30% and 70% of all avian species, respectively. They are a group of economically important ectoparasitic insects infesting birds and mammals. They do not only affect the fitness, viability and productivity of their hosts, but also play a role as reservoirs and in the transmission of infectious diseases among them. The current study aimed to report the number of ectoparasites of wild birds in Ilam province.

Methods: In February 2024, two species of wildlife birds Common Buzzard (*Buteo buteo*) and Eastern Imperial Eagle (*Aquila heliaca*) were referred to the Clinic of the Faculty of Veterinary Sciences, Ilam University, due to broken wings. After undergoing treatment measures and hematology tests, external parasites were collected by visually inspecting the plumage and were subsequently preserved in 70% ethanol. Lice were cleared and slide-mounted in Canada balsam. Then they were examined and identified using a stereomicroscope and diagnostic keys.

Results: In the study of isolated samples, infestation with three species of chewing lice was identified: *Laemobothrion maximum* (n=9) in *Aquila heliaca*, and two other species, *Craspedorrhynchus platystomus* (n=3) and *Degeeriella fulva* (n=2), from *Buteo buteo*. Blood picture of the birds was normal.

Conclusion: *Craspedorrhynchus platystomus* and *Degeeriella fulva* in Buzzard (*Buteo buteo*) were reported for the first time from Hamedan province in 2024. Hence, the present record could be considered as the first record from Ilam province. These lice have not been described previously from Iran.

Keywords: Wild birds, Parasitic Infestation, Biodiversity, Ectoparasite, Ilam.



Investigation of the Anti-Leishmanial Effects of Essential Oils from *Zataria multiflora* and *Thymus vulgaris* Against *Leishmania major*: A Detailed Analysis of Phytochemical Composition and Therapeutic Efficacy

Kambiz Karimi¹, Mahmoodreza Moein², Qasem Asgari¹, Mohammad Hossein Motazedian¹

Corresponding Author: Mohammad Hossein Motazedian, motazedm@sums.ac.ir

1. Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

Article code: 1233

Abstract

Background: Leishmaniasis, a neglected tropical disease, impacts millions, especially in resource-limited areas. This study examines the chemical composition and anti-leishmanial potential of *Zataria multiflora* and *Thymus vulgaris* essential oils against *Leishmania major*, a key cause of cutaneous leishmaniasis.

Methods: Gas chromatography-mass spectrometry (GC-MS) was employed to identify the constituents of the essential oils from *Z. multiflora* and *T. vulgaris*. The anti-leishmanial effects of the essential oils (31.25-1000 µg/ml) on *L. major* promastigotes were assessed using the flow cytometry. The cytotoxic effects of the essential oils on J774 cells were evaluated using the MTT assay over a 48-hour period. The anti-amastigote effects of the essential oils were also investigated at concentrations ranging from 31.25 to 1000 µg/mL over 24 and 48 hours.

Results: The main compounds in *T. vulgaris* were thymol (49.83%), γ-terpinene (14.93%), and cymene (10.99%), while *Z. multiflora* primarily contained thymol (46.46%) and carvacrol (32.47%). The IC₅₀ values for the essential oils of *T. vulgaris* and *Z. multiflora* against promastigote forms were 300.8 and 278.7 µg/mL, respectively. Additionally, The IC₅₀ values of the essential oils of *T. vulgaris* and *Z. multiflora* against amastigote forms after 48 hours were determined to be 298 µg/mL and 264 µg/mL, respectively. The CC₅₀ values for the essential oils of *T. vulgaris* and *Z. multiflora* on J774 cells were found to be 820.4 and 799.6 µg/mL, respectively.

Conclusion: This study demonstrates the anti-leishmanial potential of *Z. multiflora* and *T. vulgaris* essential oils against *L. major*. but require further in vivo validation.

Keywords: Essential Oil, Cutaneous leishmaniasis, Phytochemical Composition, *Zataria Multiflora*, *Thymus Vulgaris*.



Recumbency and Anemia associated with Severe *Linognathus vituli* Infestation in a Calf in East Azerbaijan Province, Iran: A Case Report

Yaghoub Firouzivand¹

Corresponding Author: Yaghoub Firouzivand, yaghoub.firouzivand@iaau.ac.ir

1. Department of Pathobiology, Male.C., Islamic Azad University, Malekan, Iran.

Article code: 1234

Abstract

Background: Lice infestation in livestock is widespread throughout the world. Sucking lice posing significant health risks to livestock, including severe anemia, compromised body condition, increased disease susceptibility, and mortality. This report describes a case of severe infestation by the cattle long-nosed louse (*Linognathus vituli*) in a calf.

Methods: In May 2024, a 3-month-old Holstein female calf (body weight: 55 kg) from the Turkamanchay district, was presented with recumbency and anorexia. According to the owner, the calf had exhibited progressive weakness and reluctance to stand for 24 hours, culminating in an inability to nurse. Clinical examination revealed lateral recumbency, lateral head tilt, hypothermia (muzzle, ear tips, and extremities), and pale oral and ocular mucous membranes. Vital parameters included a body temperature of 38.6°C, heart rate of 120 bpm, and respiratory rate of 40 breaths/min. Hematological analysis demonstrated marked anemia (PCV: 23%; RBC count: $5.4 \times 10^6/\mu\text{L}$; hemoglobin: 7.4 g/dL). Gross inspection identified extensive infestation by bluish-brown lice (*Linognathus vituli*), concentrated on the neck and dorsum, alongside adherent grayish-white nits on the haircoat. To remove lice from the body, Topical 1% flumethrin (pour-on) and subcutaneous 1% ivermectin (1 mL/50 kg) and to compensate for anemia, Intramuscular administration of phosphorus, vitamin B₁₂, and iron (Cyanoferrin®; 2–3 mL/100 kg) were administered. Within 72 hours, the calf regained ambulatory capacity and appetite.

Results: Considering the clinical signs and the relative recovery of symptoms after the treatments, the possibility of recumbency and anorexia may be associated with the severe infestation with *Linognathus vituli*.

Conclusion: Infestations by *Linognathus vituli* (sucking lice) pose serious health risks to livestock, particularly in calves, causing anemia and lethargy. Veterinary professionals must consider ectoparasitosis in differential diagnoses. Integrated control—combining ectoparasitocides and nutritional support—is essential to reduce morbidity/mortality. Further research on *L. vituli*'s epidemiological impact on herd productivity is needed.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: livestock, Sucking lice, Long-nosed cattle louse, Ivermectin, Hematological Parameters.



Morphomolecular Characterization of *Babesia* spp. in Camels in Southeastern Iran

Reza Shafiei¹, Hadi Mirahmadi²

Corresponding Author: Reza Shafiei, reza_shafi@yahoo.com

1. Department of Pathobiology, North Khorasan University of Medical Sciences, Bojnurd, Iran.
2. Department of Parasitology and Mycology, Zahedan University of Medical Sciences, Zahedan, Iran.

Article code: 1235

Abstract

Background: Babesiosis, a globally significant zoonotic disease impacting veterinary health, necessitates accurate diagnostic strategies to guide control measures. This study integrates morphological and molecular approaches to characterize *Babesia* species in camels from southeastern Iran. Totally, 140 blood samples were collected from camels in Qaen, Nehbandan, Iranshahr, and Zahedan.

Methods: Blood smears were analyzed via Giemsa staining for morphological identification, while PCR targeting the ITS1 region enabled molecular detection and species differentiation.

Results: Microscopic examination revealed a 10% prevalence of *Babesia* spp., whereas PCR demonstrated significantly higher sensitivity, detecting a 19.28% infection rate ($p = 0.003$). Regional disparities were notable, with Zahedan exhibiting the highest prevalence. All PCR-confirmed cases were identified as *Babesia caballi*, underscoring its regional dominance.

Conclusion: This morphomolecular approach highlights the limitations of microscopy in subclinical or low-parasitemia infections and reinforces PCR's critical role in species-specific diagnosis. The high prevalence in Zahedan signals urgent needs for targeted surveillance and prophylactic strategies. By bridging traditional and modern diagnostic techniques, this study advances understanding of camel babesiosis epidemiology in Iran and advocates for integrated morphomolecular methods to enhance veterinary diagnostic frameworks and mitigate zoonotic risks.

Keywords: Babesiosis, Camel, Giemsa staining, Microscopic examination, Molecular technique, PCR.



Diversity and Prevalence of Parasites in Domestic Pigeons in North-west of Iran: Insights and Implications

Atousa Mohseni¹, Mansour Ebrahimi², Emad Changizi³

Corresponding Author: Atousa Mohseni, atoomohseni@gmail.com

1. Department of Pathobiology, School of Veterinary Medicine, University of Shiraz, Shiraz, Iran.
2. Doctor of Veterinary Sciences, Veterinary Parasitology
3. Department of Pathobiology, School of Veterinary Medicine, University of Semnan, Semnan, Iran.

Article code: 1236

Abstract

Background: Since ancient times, pigeons have been companions to humans, serving as a source of food, hobbies, pets, and religious symbols.

Methods: This study was conducted to assess the prevalence of parasites in domestic pigeons, examining a total of 280 birds for various parasitic infections.

Results: The results indicated that 39.2% of the examined pigeons (n=110) were infected with one or more types of parasites. Among the detected parasites, cestodes included *Raillietina* spp. at a prevalence of 7.5%. Nematodes, specifically *Ascaridia columbae*, were found in 13.9% of the pigeons. Ectoparasitic infestations were significantly represented, with *Columbicloa columbae* affecting 33.9%, *Menopon gallinae* at 28.2%, and *Lipeurus caponis* at 10.3%. Additionally, haemoparasites were identified, with *Haemoproteus columbae* present in 16.7% of the pigeons and *Leucocytozoon* spp. detected at a lower prevalence of 1.4%. Protozoan infections were also notable, particularly *Trichomonas gallinae*, which showed a prevalence of 21.7%.

Conclusion: These findings illustrate a diverse range of parasitic infections in domestic pigeons, indicating moderate to high levels of infestation. This study highlights the need for effective management strategies to control parasitic infections in domestic pigeon populations, which may have implications for both animal health and public health. Future research is necessary to further explore the impact of these parasites on pigeon health and to develop targeted interventions for prevention and control.

Keywords: Prevalence, Parasites, Domestic Pigeon, West-Azerbaijan.



The Parasitic Profile of Indigenous Poultry: Prevalence Rates and Species Diversity in West-Azerbaijan

Atousa Mohseni¹, Emad Changizi², Mansour Ebrahimi³

Corresponding Author: Atousa Mohseni, atoomohseni@gmail.com

1. Department of Pathobiology, School of Veterinary Medicine, University of Shiraz, Shiraz, Iran.
2. Department of Pathobiology, School of Veterinary Medicine, University of Semnan, Semnan, Iran.
3. Doctor of Veterinary Sciences, Veterinary Parasitology.

Article code: 1237

Abstract

Background: This study aimed to assess the prevalence of parasites in indigenous poultry in Piranshahr, West-Azerbaijan.

Methods: A total of 80 birds were necropsied. Internal organs, feather, and leg skin chips were thoroughly examined for the presence of parasites.

Results: The findings revealed a notable prevalence rate of 67.5%, with 54 out of 80 poultry testing positive for various parasites. Among the isolated parasites, two species of helminths were identified: *Ascaridia galli*, present in 18.7% of the examined birds, and *Heterakis gallinarum*, found in 25% of the cases. Additionally, one species of cestode, *Raillietina spp.*, was documented, although specific prevalence percentages were not detailed for this group. The study also highlighted the presence of three ectoparasite species, which contributed to the overall parasitic burden in the poultry population. *Menacanthus stramineus* was the most prevalent ectoparasite, affecting 48.7% of the birds, followed by *Menopon gallinae* at 41.2% and *Lipeurus caponis*, which affected 11.2% of the poultry.

Conclusion: These findings underscore the significant impact of parasitic infections on indigenous poultry in the region, indicating a need for effective management and control strategies to mitigate the health risks associated with these parasites. Moreover, the results contribute to the existing knowledge of poultry health in West Azarbaijan and can serve as a basis for future research and intervention programs aimed at improving poultry health and productivity in the region.

Keywords: Parasites, Prevalence, Native Poultry, Piranshahr.



Prevalence of *Theileria equi* Infection in Piranshahr in 2024 Using Microscopy and PCR Approaches

Atousa Mohseni¹, Mansour Ebrahimi², Emad Changizi³

Corresponding Author: Atousa Mohseni, atoomohseni@gmail.com

1. Department of Pathobiology, School of Veterinary Medicine, University of Shiraz, Shiraz, Iran
2. Doctor of Veterinary Sciences, Veterinary Parasitology.
3. Department of Pathobiology, School of Veterinary Medicine, University of Semnan, Semnan, Iran.

Article code: 1238

Abstract

Background: This study investigates the prevalence of *Theileria equi* in equines in West-Azerbaijan, employing both light microscopy and polymerase chain reaction (PCR) methods for detection.

Methods: The present study was conducted on 46 apparently-healthy horses in Piranshahr, located in West-Azerbaijan, Iran from May to September 2024. The animals were grouped based on sex (27 males and 19 females) and age (24 older: > 3 years and 22 younger: ≤3 years). Blood samples were collected from the jugular veins of the horses and placed in sterile tubes containing EDTA; these samples were either examined immediately for blood smears or stored at -20°C for subsequent analysis.

Results: The results demonstrated a prevalence of *T. equi* of 4.3% (2/46) when assessed by light microscopy and significantly higher at 23.9% (11/46) using PCR methods. Further analysis revealed that the prevalence of *T. equi* was notably higher in older animals, with infection rates of 15.2%, compared to 8.7% in younger equines. Additionally, females exhibited a higher rate of infection (13%) than males (10.9%).

Conclusion: These findings indicate a concerning level of *T. equi* prevalence in the surveyed equine population and suggest that age and sex are important factors influencing infection rates. Horses in various regions of Iran exhibit a notable tolerance to equine theileriosis, pointing to potential endemic stability that may arise from factors such as local adaptation, genetic resilience, and the presence of mild or asymptomatic infections. Environmental conditions and effective management practices may also contribute to maintaining this stability by preventing severe outbreaks.

Keywords: *Theileria equi*, Molecular detection, North-west, Iran.



Canine Anaplasmosis in a Ghahderijani Dog (Shahrekord, Iran): A Case Report

Soroush Ghasemi¹, Abbas Ghorbiani¹, Ali Maddahi Nejad¹, Mohammad Ali Mohammadi¹

Corresponding Author: Ali Maddahi Nejad, alimaddahi3177@gmail.com

1. Department of Veterinary Medicine, School of Veterinary Science, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

Article code: 1240

Abstract

Background: Canine granulocytic anaplasmosis caused by *Anaplasma phagocytophilum* is rarely reported in Iran and has not previously been documented in the indigenous Ghahderijani breed. Accurate recognition is challenging because clinical findings overlap with other tick-borne diseases.

Methods: A 5-year-old intact male Ghahderijani herding dog from a mountain village near Shahrekord presented with lethargy, anorexia, shifting-leg lameness, fever (39.8 °C) and pale mucous membranes. Physical examination disclosed splenomegaly and multiple *Rhipicephalus sanguineus* ticks. Hematology, including platelet count and packed cell volume (PCV), was performed, and Giemsa-stained peripheral smears were examined for intracellular organisms. Because polymerase-chain-reaction facilities were unavailable, empirical therapy was started with a single intramuscular dose of imidocarb dipropionate (6.6 mg kg⁻¹) to cover possible co-infections.

Results: Blood analysis revealed thrombocytopenia ($67 \times 10^9 \text{ L}^{-1}$) and mild normocytic normochromic anaemia (PCV 28 %). Morulae compatible with *A. phagocytophilum* were present in 6 % of neutrophils. After treatment, clinical signs resolved within 72 h; platelet count increased to $183 \times 10^9 \text{ L}^{-1}$ and PCV to 33 % by day 7. Repeat smears were free of morulae, and the owner reported full recovery at 30-day telephone follow-up.

Conclusion: This first clinically confirmed case of granulocytic anaplasmosis in a Ghahderijani dog highlights the need to include *A. phagocytophilum* in the differential diagnosis of febrile thrombocytopenic dogs in western Iran. Prompt microscopic diagnosis and empiric anti-piroplasm/anti-rickettsial therapy can be effective where doxycycline or molecular tests are not immediately available.

Keywords: Anaplasmosis, *Anaplasma phagocytophilum*, Dog, Ghahderijani breed, Iran.



***Trypanosoma evansi* Infection in Camels Slaughtered in Zahedan, in the Summer of 2023**

Seyed Reza Hosseini¹, Saeed Dadkhah Tehrani¹, Ali Maddahi Nejad², Mohadeseh Soltanian Dehkordi³

Corresponding Author: Ali Maddahi Nejad, alimaddahi3177@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.
2. DVM Graduate, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Iran.
3. Bachelor's Degree in Veterinary Medicine, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Iran.

Article code: 1242

Abstract

Background: *Trypanosoma evansi* is a protozoan parasite responsible for surra, a disease that causes considerable economic losses in camels due to decreased productivity, abortion, and mortality. Despite the high camel population in Sistan and Balochistan, few studies have addressed the prevalence of this parasite using multiple diagnostic methods. The aim of this study was to compare different diagnostic methods in camels slaughtered at the Zahedan slaughterhouse

Methods: In this study, 200 blood samples were collected from male camels (3-5 years old) at the Zahedan slaughterhouse during the summer of 2023. Samples were analyzed using five Diagnostic methods: Direct Blood Smear, Thymol Turbidity test, Mercuric Chloride test, Formol Gel test, and Polymerase Chain Reaction (PCR). DNA extraction was performed via the phenol-chloroform method, and specific primers targeting *T. evansi* were used for PCR amplification (ITS Gene, Product length: 1100 bp).

Results: Positive cases were identified as follows: Direct Smear (1.5%), Thymol Turbidity (23.5%), Mercuric Chloride (20.5%), Formol Gel (16%), and PCR (24%). PCR showed the highest sensitivity, detecting chronic and latent infections more effectively. Serological methods also provided notable detection rates, with Thymol Turbidity and Mercuric Chloride showing comparable results.

Conclusion: The results confirm the presence of *T. evansi* in a significant number of slaughtered camels in Zahedan Through some diagnostic tests that are rarely used in This area. The findings emphasize the importance of molecular diagnostics, especially PCR, in accurately detecting the infection and improving surveillance strategies in camel populations. Due to camels' resistance to most diseases, serological tests can be used as rapid tests in the initial diagnosis of trypanosomiasis.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Trypanosomiasis, Diagnostic Test, PCR, Surra.



Drug Resistance and Novel Therapeutic Strategies in Babesiosis: An Integrated Review of Epidemiological, Clinical, and Molecular Data

Siamak Kakekhani¹, Erfan Eftekhari², Negin Shahri²

Corresponding Author: Siamak Kakekhani, kakekhanisiyamak@iausdj.ac.ir

1. Department of Pathobiology, Sa.C., Islamic Azad University, Sanandaj, Iran.
2. Student in Veterinary Medicine, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran.

Article code: 1243

Abstract

Background: Babesiosis, a parasitic disease caused by *Babesia* species, poses a global threat to livestock and humans. This review aims to examine recent advances in babesiosis management, focusing on drug resistance and novel treatment approaches, integrating epidemiological, clinical, and molecular data.

Methods: This comprehensive review explored recent advancements in babesiosis management. Data was gathered from reputable scientific databases including PubMed, Scopus, Web of Science, and Google Scholar, using keywords like 'Babesiosis', 'Drug resistance', 'Herbal medicines', and 'Molecular targets'. Articles on *Babesia* drug resistance to traditional drugs (e.g., imidocarb dipropionate, diminazene aceturate), novel drugs (e.g., tafenoquine, clofazimine), herbal compounds (e.g., red skin extract, annual wormwood), and new molecular targets (e.g., proteasome, PI4K) were reviewed. Selected articles were evaluated for relevance and scientific credibility. Treatment efficacy, resistance mechanisms, and comparative clinical trial results were analyzed.

Results: Traditional drugs like imidocarb dipropionate and diminazene aceturate show 70-100% efficacy in sheep and goats, but drug resistance limits their effectiveness. Novel drugs such as tafenoquine and clofazimine have reduced parasitemia by up to 90% in animal models, sometimes achieving radical cures. Herbal compounds like red skin extract (IC₅₀ of 3.4 µM) and annual wormwood (IC₅₀ of 14 µM) exhibit anti-babesial activity comparable to common drugs like quinine. Molecular studies have identified new targets, including proteasome and PI4K, for selective drug development.

Conclusion: Drug resistance diminishes the effectiveness of traditional babesiosis treatments. Novel drugs and herbal compounds show promising results. Molecular studies have identified new drug targets. The atovaquone-azithromycin regimen demonstrates similar efficacy with fewer side effects (15%) compared to clindamycin-quinine (72%). Further research on toxicity, pharmacokinetics, and compound evaluation in animal and human models is necessary.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Integrating treatment, vaccination, and vector control is crucial for sustainable babesiosis management.

Keywords: Babesiosis, Drug resistance, Herbal medicines, Combination therapy, Proteasome, Molecular targets.



***Leishmania* and the Challenges of Skin Repair: The Role of the Immune Response**

Yaser Pirali¹, Moosa Javdani², Shayan Akhavan², Moein Mirzajani²

Corresponding Author: Shayan Akhavan, sh.akhavan2000@gmail.com

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.

Article code: 1244

Abstract

Background: Cutaneous leishmaniasis (CL) is a parasitic disease caused by *Leishmania* spp., transmitted by infected sandflies. Unlike ordinary wounds, CL lesions often last long after the parasite has been removed. This delay in healing is mainly due to an overactive immune response. This study aims to examine how the immune system interferes with skin repair in CL.

Methods: After infection, *Leishmania promastigotes* enter neutrophils and macrophages. The parasite survives by stopping phagolysosome fusion. IL-12 leads to Th1 cell development, which produces IFN- γ to activate macrophages. While this helps control the parasite, it also causes extra TNF- α and tissue damage. In CL, M1 macrophages dominate and keep inflammation high, while M2 macrophages that help repair are limited. CD8⁺ T cells also worsen the damage. This ongoing immune activity blocks fibroblast action, keratinocyte movement, and blood vessel formation.

Results: The normal steps of healing, inflammation, tissue growth, and remodeling, are disrupted. Neutrophils stay longer and break down the tissue matrix. High IFN- γ and IL-12 levels stop skin cells from growing and spreading. IL-10 and TGF- β can support healing but may let the parasite persist. The outcome depends on when and where these immune signals appear.

Conclusion: For CL, killing the parasite is not enough. Healing also needs control of the immune system. Restoring a balanced immune environment is crucial to allow skin cells to proliferate and regenerate. Better understanding of how immune factors influence tissue repair may lead to more effective and holistic treatment strategies, especially in chronic or non-healing cases.

Keywords: Cutaneous leishmaniasis, Immune response, IFN- γ , IL-12.



Acaricide Resistance in Biological Tests and Molecular Mechanisms

Amir Arshia Lotfi¹, Zahra Jafari-Ardakan², Amir Mahdi Amjadi³, Mohammad Alinezhad-Taheri³, Sedigheh Nabian⁴

Corresponding Author: Sedigheh Nabian, nabian@ut.ac.ir

1. Student of Doctor of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Physiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Student of Doctor of Veterinary Medicine, University of Tehran, Tehran, Iran.
4. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1245

Abstract

Background: Acaricide resistance has become a major obstacle in controlling tick populations worldwide. Traditional bioassay methods, such as the larval immersion test and adult immersion test (AIT), are limited by their reliance on live samples, lengthy procedures, and inability to identify genetic resistance markers. This has spurred research into molecular mechanisms driving resistance, particularly detoxification pathways, to develop more effective control strategies.

Methods: Recent studies have focused on identifying enzyme systems involved in acaricide resistance. Four primary mechanisms have been elucidated:

1. Esterase-mediated resistance: Carboxylesterases (e.g., CarE genes) hydrolyze pyrethroids, as seen in fenpropathrin-resistant *Tetranychus urticae*.
2. Cytochrome P450 monooxygenase (CYP) activity: The overexpression of CYP genes (e.g., CYP392A16) in resistant strains facilitates the metabolism of pyrethroids.
3. Glutathione-S-transferase (GST) mechanism: GSTs bind xenobiotics, with differential expression observed in resistant *Tetranychus urticae*.
4. ABC transporters: These proteins efflux acaricides (e.g., macrocyclic lactones), contributing to multi-drug resistance in *Rhipicephalus microplus*.

Results: Recent findings demonstrate the complexity of acaricide resistance at the molecular level. In fenpropathrin-resistant *Tetranychus urticae*, researchers observed significant overexpression of CarE18 and CarE15 genes, which are directly linked to enhanced hydrolysis of pyrethroid compounds. Similarly, in *Tetranychus cinnabarinus*, elevated levels of CYP392A28 and related P450 enzymes were associated with metabolic detoxification of acaricides. Experimental inhibition of esterases using triphenyl phosphate (TPP) successfully reversed amitraz resistance in *Rhipicephalus microplus*, confirming the functional role of these enzymes in resistance mechanisms. Additionally, combining ivermectin with cyclosporin, an



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



inhibitor of ABC transporters, markedly improved acaricide efficacy by blocking drug efflux pathways. These results highlight the potential of these strategies to overcome resistance.

Conclusion: Molecular research has advanced our understanding of acaricide resistance, revealing actionable targets like enzyme inhibitors. Future strategies should combine genetic screening for resistance markers to mitigate evolving resistance. Integrating these approaches will be critical for sustainable tick management.

Keywords: Acaricide resistance, Esterase-mediated resistance, Cytochrome P450 monooxygenase, Glutathione-S-transferase (GST) mechanism, ABC transporters.



Case report: Simultaneous Outbreak of *Microsporum canis* and *Ixodes ricinus* Infections in a Persian Chinchilla Cat in Tehran, Iran

Amir Arshia Lotfi¹, Zahra Jafari-Ardakan², Sedigheh Nabian³

Corresponding Author: Sedigheh Nabian, nabian@ut.ac.ir

1. Student of Doctor of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Physiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1246

Abstract

Background: Persian Chinchilla cats are prone to dermatological and parasitic infections due to their long fur and genetic predispositions. *Microsporum canis*, a zoonotic dermatophyte, and *Ixodes ricinus*, a hard tick species, are common pathogens in cats. Concurrent infections with these pathogens are rare but can complicate diagnosis and treatment. This case highlights the importance of recognizing such co-infections in veterinary practice.

Methods: A Persian Chinchilla cat exhibiting alopecia, pruritus, and lethargy was examined in Tehran, Iran. Skin scrapings and hair samples were collected for fungal culture on Sabouraud Dextrose Agar supplemented with chloramphenicol and cycloheximide, as well as Mycosel Agar. Ticks were manually removed and identified morphologically. Microscopic examination of fungal elements and tick specimens was performed to confirm the pathogens.

Results: The fungal culture revealed *Microsporum canis*, supported by microscopic findings of hyphae and spores. The ticks were identified as *Ixodes ricinus* based on morphological characteristics. The cat showed improvement after treatment with topical antifungals (e.g., clotrimazole) and systemic acaricides (e.g., fipronil). The concurrent infection underscored the need for dual-pathogen screening in cats with mixed clinical signs.

Conclusion: This case demonstrates the potential for co-infections with *Microsporum canis* and *Ixodes ricinus* in Persian Chinchilla cats. Comprehensive diagnostic approaches are essential for accurate identification and effective treatment. Further research is warranted to explore the epidemiological and clinical implications of such co-infections in domestic animals.

Keywords: *Ixodes ricinus*, Persian Chinchilla cat, *Microsporum canis*, Co-infections.



Role of Probiotics in the Prevention and Reduction of Coccidiosis Effects in Poultry

Hamidreza Farzin¹, Mohadese Amiri², Majid Jamshidian-Mojaver³

Corresponding Author: Majid Jamshidian-Mojaver, m.jamshidian@rvsri.ac.ir

1. Mashhad Branch, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Mashhad, Iran.
2. PhD Student of Bacteriology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
3. Mashhad Branch, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Mashhad, Iran.

Article code: 1247

Abstract

Background: Avian coccidiosis, caused by seven *Eimeria* species (notably *E. tenella*), is a major parasitic disease in poultry that damages the intestinal lining, impairs nutrient absorption, reduces growth, increases feed conversion ratio, and raises production costs. Each species targets specific intestinal regions and varies in pathogenicity. The infection disrupts protein absorption and compromises gut integrity. Probiotics support gut health by enhancing microbiota, inhibiting pathogens like *Clostridium* and *Salmonella*, boosting immunity through increased lymphocyte levels, and reducing lesion severity. They help maintain growth during infection, lessen the impact of coccidiosis, and reduce reliance on prophylactic drugs by improving the bird's resistance to infection. To investigate the role of probiotics in enhancing intestinal health, immune response, and overall resistance in poultry affected by avian coccidiosis, with the aim of reducing disease severity, improving growth performance, and minimizing the use of prophylactic drugs.

Methods: A literature review examined the role of probiotics in avian coccidiosis using databases like PubMed and Scopus (2000–2024). Around 50 studies were analyzed, focusing on *Eimeria* pathogenesis, intestinal health, immune response, and probiotic effects on lesion severity, growth, and secondary infections in poultry, especially broilers.

Results: Probiotic supplementation in poultry with coccidiosis reduces intestinal lesions, improves microbial balance, enhances immune response, supports growth and feed conversion, lowers secondary infections, and decreases reliance on prophylactic drugs.

Conclusion: Probiotics offer a promising, natural solution by enhancing gut integrity, modulating microbiota, boosting immune response, and reducing lesion severity. Their ability to prevent secondary infections and improve growth performance makes them an effective and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



sustainable alternative to conventional treatments for the prevention and control of coccidiosis in poultry farming.

Keywords: Poultry, *Eimeria tenella* , Parasitic disease, Probiotics.



Serosurvey of *Neospora caninum* and *Toxoplasma gondii* in Stray Dogs of Fars Province, Southern Iran: Implications for Epidemiology and Control

Bahador Shahriari^{1,2}, Negin Abbasi³, Niksa Adibso³, Mostafa Omidian¹, Qasem Asgari¹, Kourosh Azizi⁴

Corresponding Author: Bahador Shahriari, sarkarib@sums.ac.ir

1. Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
3. Student Research Committee, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
4. Department of Biology and Control of Disease Vectors, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran.

Article code: 1249

Abstract

Background: Dogs, as the definitive hosts of *Neospora*, play a crucial role in the epidemiology of this parasitic infection. *Toxoplasma* can lead to clinical signs such as fever, anorexia, dyspnea, and more specific symptoms affecting the nervous and respiratory systems in dogs. These parasites play a role in the epidemiology of these dogs, potentially affecting humans, livestock, and wildlife. This study aimed to assess the seroprevalence of *Neospora caninum* and *Toxoplasma gondii* infection in a dog population in Fars Province, Southern Iran, utilizing a serological method.

Methods: Serum samples were collected from 22 stray dogs in Shiraz city, capital of Fars Province in 2014, and evaluated for anti-*N. caninum* and anti-*T. gondii* antibodies using an in-house ELISA method. For the ELISA, *Neospora* antigens were prepared from tachyzoites cultured in Vero cell culture medium, while *Toxoplasma* antigens were derived from tachyzoites propagated in mice.

Results: Among the 22 stray dogs tested, anti-*Neospora* antibodies were detected in four dogs (18.2%), with all seropositive dogs being male. The dogs' ages ranged from 1 to 9 years, with an average age of 3.81 (± 1.99). Anti-*Toxoplasma* antibodies were found in five dogs (22.7%), with two female and three male dogs testing positive. No dogs exhibited dual seropositivity for both *Neospora* and *Toxoplasma*.

Conclusion: The findings of the current indicate a relatively high seroprevalence of *Neospora* and *Toxoplasma* infections in dogs in Fars province, southern Iran. Further studies are needed



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



to explore the risk factors associated with these infections and develop effective control and prevention strategies.

Keywords: Seroepidemiology, Protozoa, Dogs, Fars Province, Iran.



Evaluation of the Effect of 7- α -Acetoxyrooleanone, an Abietane Diterpenoid Derivative, on *Leishmania Major* in *In Vitro* Conditions

Kambiz Karimi¹, Asma Mousivand², Erfan Ahmadi³, Gholamreza Hatam¹

Corresponding Author: Gholamreza Hatam, hatam908@yahoo.com

1. Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.
3. Department of Microbiology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.

Article code: 1250

Abstract

Background: Cutaneous leishmaniasis (CL), caused by the protozoan parasite *Leishmania major*, is a significant public health concern in tropical and subtropical regions, including Iran. Conventional treatments such as antimonial compounds and amphotericin B face challenges including toxicity, high cost, and drug resistance. This study investigates the potential of 7- α -acetoxyrooleanone, a diterpenoid derivative from the abietane group, as a novel anti-leishmanial agent. Abietane diterpenoids, known for diverse pharmacological properties including antimicrobial and antiparasitic activities, represent promising candidates for novel therapeutics.

Methods: This in vitro study was conducted on *L. major* promastigotes (strain MRHO/IR/75/ER) and murine macrophage cells (J774). The cytotoxic effect of 7- α -acetoxyrooleanone was evaluated at concentrations of 2.5, 5, 10, 20, 40, and 80 μ g/mL. Promastigote viability was assessed using flow cytometry and propidium iodide (PI) staining, while macrophage cytotoxicity was measured via the MTT assay. Amphotericin B (20 μ g/mL) served as the positive control. Statistical analysis was performed using SPSS version 27.

Results: 7- α -acetoxyrooleanone exhibited a dose-dependent anti-leishmanial effect, with 86.5% promastigote mortality at 80 μ g/mL ($IC_{50} = 30.41$ μ g/mL). Amphotericin B (positive control) showed 98.4% efficacy. The compound demonstrated low toxicity toward J774 macrophages ($CC_{50} = 63.27$ μ g/mL), indicating selectivity against the parasite.

Conclusion: The findings suggest potent anti-leishmanial effects of 7- α -acetoxyrooleanone against *L. major* promastigotes, with limited host-cell toxicity. Further in vivo studies are recommended to evaluate its therapeutic potential as a novel treatment for CL.

Keywords: *Leishmania major*, Abietane diterpenoid, 7- α -acetoxyrooleanone, Flow cytometry, MTT assay, Cutaneous leishmaniasis.



The Effect of Climate Conditions on the Distribution Pattern of Sheep Gastrointestinal Nematodiasis in Three Different Climate Zone of Iran Based on Geographical Information System (GIS)

Mohammad Shadman¹, Behnam Meshgi²

Corresponding author: Mohammad Shadman, shademan.m@ut.ac.ir

1. Department of veterinary parasitology, university of Tehran, Tehran, Iran.
2. Department of veterinary parasitology, university of Tehran, Tehran, Iran.

Article code: 1251

Abstract

Background: Gastrointestinal nematodes can cause death in severe forms, although they occur chronically in Iran, but generally with economic losses and clinical symptoms, such as diarrhea, anorexia, weakness, weight loss and livestock production, reduced material absorption. The fact is that the gastrointestinal nematodes of ruminants are strongly influenced by geographical and climatic conditions. The lead research has two main objectives; determines the gastrointestinal nematodes of sheep and then identifies the risk factors and factors affecting the incidence of infection in three different geographical areas in the country.

Methods: This study was conducted in two stages: field-laboratory and software. In order to study the frequency and severity of infection of sheep with gastrointestinal nematodes from three provinces of East Azerbaijan, Kerman and Gilan respectively 800, 500 and 840 and a total of 2140 sheep feces samples were taken. In the second stage, the findings were recorded using Arc-GIS software and GIS by maximum entropy (MaxEnt) method and Jackknife analysis, taking into account the climatic variables under study and was tested.

Results: The results based on the intensity of infection showed that in Kerman province the intensity of infection is related to nematodes with strongl eggs (5.5%), in East Azarbaijan province it is related to *Marshallagia* (39.8%) and in Gilan province it is related to nematodes with strongle eggs (34/2%).The results of Jackknife analysis and MaxEnt model in the investigated areas show that the rainfall of the driest season of the year (Bio17) in the two provinces of East Azarbaijan and Kerman has the greatest impact, and in Gilan province the two indicators of land slope (Slope) and vegetation density (NDVI) They have the greatest climatic impact.

Conclusion: Climatic variables such as rainfall and vegetation are considered to be the most important factors affecting the nematode infections of the gastrointestinal system of sheep. Therefore, treatment and control strategies are also subject to these conditions, and in areas where the frequency of cases is more severe.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Gastrointestinal nematodes, Sheep climatic conditions, Geographical information system (GIS).



Molecular Identification of Medicinal Leeches and Assessment of Microbial Contamination in Mashhad

Mohsen Najjari¹, Parisa Eysi², Hossien Zarrinfar³, Hadi Farsiani⁴, Arash Pourdad⁵, Rahmat Solgi⁶

Corresponding Author: Mohsen Najjari, najjarimh@mums.ac.ir

1. Department of parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Department of parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Department of parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
4. Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
5. Department of parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
6. Department of parasitology and Mycology, School of Medicine, Birjand University of Medical Sciences, Birjand, Iran.

Article code: 1253

Abstract

Background: Leeches are predominantly aquatic organisms, and for centuries, specific species have been employed in the treatment of various human and animal ailments. Their therapeutic use, particularly in traditional and complementary medicine, continues to attract scientific interest. This study aimed first to determine the molecular identity of medicinal leeches used for therapeutic purposes. Subsequently, it sought to assess the extent of microbial contamination in these leeches collected from Mashhad.

Methods: This cross-sectional descriptive study was conducted at Mashhad University of Medical Sciences during 2021–2022. Twenty medicinal leeches were collected from five sales centers in Mashhad. Morphological identification was performed using standard taxonomic keys. Each leech was aseptically divided into three parts for molecular and microbiological analyses. DNA was extracted from the posterior sucker, and species identification was carried out via PCR amplification of the ITS2 gene.

Results: Morphological analysis using diagnostic keys indicated that all collected leeches belonged to the *Hirudo orientalis* species, which was subsequently confirmed by molecular identification. Microbiological testing revealed the presence of *Enterobacteriaceae*, *Klebsiella pneumoniae*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, and *Staphylococcus saprophyticus* in the leech digestive tracts. *Aeromonas hydrophila* was the most prevalent



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



(33%), while *Plesiomonas shigelloides* had the lowest frequency (3%). Fungal contamination was detected in two isolates (10%): *Aspergillus niger* (O19) and *Aspergillus nidulans* (A2).

Conclusion: This study confirmed that the medicinal leeches used in Mashhad belong to the *Hirudo orientalis* species, as determined by morphological analysis and validated through ITS2 based molecular identification. Microbial contamination both bacterial and fungal was detected in 90% of the samples, highlighting the potential health risks associated with their therapeutic use. These findings emphasize the importance of implementing strict hygiene and quality control measures in the handling of medicinal leeches.

Keywords: *Hirudo orientalis*, ITS2 gene, Medicinal leech, Microbial contamination, Mashhad.



Renal Lesions Associated with *Theileria annulata* in a Cow from Northwest Iran

Melika Hasani¹, Bijan Esmaeilnejad², Seyede Soraya Mahmoudi², Asghar Afshari³

Corresponding Author: Seyede Soraya Mahmoudi, ss.mahmoudi@urmia.ac.ir

1. Veterinary Student of Veterinary Medicine, Urmia University, Urmia, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
3. Department of Parasite Vaccine Research and Production, Razi Vaccine and Serum Research.

Article code: 1254

Abstract

Background: Tropical theileriosis, caused by *Theileria annulata*, is one of the most prevalent and economically critical fatal diseases of cattle in tropical and subtropical regions such as Iran.

Methods: A 5-year-old cross-bred cow from Urmia County, Northwest Iran, was examined by a private veterinarian due to one-week history of anorexia and listlessness. Physical examination revealed high fever (39.5–41 °C body temperature), enlargement of the superficial lymph nodes, pale mucous membranes, and severe petechial and ecchymotic hemorrhages throughout the skin, particularly in hairless regions. Specific PCR assay were conducted on the blood sample to confirm the presence of *Theileria annulata*. Before necropsy, a urine sample was collected by cystocentesis. Detailed gross pathological findings were undertaken on different organs. A full-thickness renal sample was collected from the central lobe of both kidneys in 10% neutral buffered formalin for hematoxylin-eosin staining.

Results: The diagnosis of theileriosis was confirmed by observing different intraerythrocytic forms, including mainly signet ring and rod-, comma-, and dot-shaped ones on Giemsa-stained blood smears. Definitive diagnosis was confirmed as a positive sample by PCR using *T. annulata*-specific primers (an expected 1020-bp fragment). Diagnostic laboratory tests revealed uremia, creatininemia, and hypersthenuria. Aggressive therapeutic procedures failed to improve the animal deteriorated condition, and finally, the animal died. At necropsy, the kidney was edematous with hyperemic and dark reddish-brown pigmentation on the renal surface, and also surrounding fat was yellow. In histopathology analysis Congestion, hemorrhage, and necrosis of renal tubular epithelial cells and necrotic debris formed homogeneous casts within the tubules was observed.

Conclusion: The results of this study showed that during bovine theileriosis caused by *T. annulata*, acute tubular necrosis (ATN) may develop. Considering the widespread dispersion of tropical theileriosis, it should be included routinely in the differential diagnosis of all cattle with acute renal failure. Therefore, in future studies, larger samples of animals are required to



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



elucidate the role of the *T. annulata*-induced anemia and consequently the hypoxia in the pathogenesis of nephropathy.

Keywords: Histopathology, Uremia, Necropsy, Nephropathy.



Raman Spectroscopy and Confocal Imaging for the Identification of *Parascaris* Eggs

Ehsan Rakhshandehroo¹, Ali Owliaee¹, Tina Yaghoobpour²

Corresponding Author: Tina Yaghoobpour, Tinayaghoobpour75@gmail.com

1. Departments of Pathobiology, Shiraz University, Shiraz, Iran.
2. Departments of Clinical Sciences, Shiraz University, Shiraz, Iran.

Article code: 1257

Abstract

Background: Raman spectroscopy and 3D imaging are emerging as promising for improving diagnostic precision of the chemical structures. The Raman shift obtained in this method produces a unique spectral characterization. This technique may be utilized for accurate detection of nematode eggs. Among the nematodes, *Parascaris* is a significant and prevalent worm in Iran. Also, the eggs of this nematode are constructed of different layers and substances that may be good candidates for chemical tracing. Thus, the aim of this study was to determination and analysis of Raman signatures produced by *Parascaris* eggs.

Methods: worm samples were collected from the infected foals in Shiraz city, Iran. They were first rinsed with PBS solution and the uterus was cut to release eggs. The recovered eggs were fixed in formalin solutions at concentrations of 2% and 10% and also 70% ethanol. Then, they were subjected to the Raman spectroscopy and examined using a confocal Bx-FM microscope.

Results: The intensity of the Raman scattering spectra and its Raman shift region were successfully characterized for the eggs fixed using 2% formalin. Multiple strong peaks were seen in the Raman shift range of 3000-3500 cm^{-1} . Depiction of the vibrational properties in this crucial region with the intensities of 3396 and 3415 counts helps to find out the precise spectra of the *Parascaris* species. The confocal microscopy also offered a three-dimensional visualization and represented a morphologic view of the helminth eggs.

Conclusion: There is no adequate information on the Raman peaks that reveals helminth eggs. In this study, we characterized the unique *Parascaris* Raman spectral ranges using eggs fixed in 2% formalin. This method is suggested to be utilized for the identification of other helminth eggs that cannot be easily distinguished. Additionally, this technique could assist in investigating the effects of different treatments on the parasite eggs.

Keywords: Nematode eggs, *Parascaris*, Raman spectra, Confocal microscopy.



Case Report: Identification of *Rhipicephalus sanguineus* Ticks on a Domestic Rabbit (Black and White Mini Lop)

Amir Arshia Lotfi¹, Niayesh Mohammadi¹, Sedigheh Nabiyan², Amir Rostami³

Corresponding Author: Niayesh Mohammadi, niayeshmohammady@gmail.com

1. Student of Doctor of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Department of Internal Medicine, University of Tehran, Tehran, Iran.

Article code: 1258

Abstract

Background: *Rhipicephalus sanguineus* (commonly known as the "brown dog tick") is a globally distributed hard tick of significant medical and veterinary importance. While dogs are its primary hosts, *R. sanguineus* exhibits a multi-host feeding behavior, enabling it to parasitize other mammals, including rabbits, under certain environmental conditions. This tick species is a known vector for pathogens causing Mediterranean Spotted Fever, Ehrlichiosis, and Babesiosis, highlighting its zoonotic potential. Documenting its presence on atypical hosts is essential for understanding its expanding host range and associated public health risks.

Methods: During a clinical examination of a two-year-old female domestic rabbit (Black and White Mini Lop) kept in an open area in Mohammad Shahr, Alborz Province, Iran, two adult male *R. sanguineus* ticks were found attached beneath the chin. The ticks were carefully detached using fine-tipped tweezers and immediately preserved in 70% ethanol for further analysis. For species identification, the specimens were examined under a stereomicroscope at appropriate magnifications. Morphological features, including scutum shape, palpal structure, spiracle position, and the presence of lateral eyes, were assessed using standard taxonomic keys.

Results: Microscopic examination confirmed the ticks as *Rhipicephalus sanguineus* based on key diagnostic characteristics: reddish-brown coloration, rectangular capitulum, prominent lateral eyes and distinct spiracular plates. Although *R. sanguineus* primarily infests dogs, this case demonstrates its ability to opportunistically infest rabbits, possibly due to environmental factors (e.g., host availability, microclimate conditions).

Conclusion: This report documents the occurrence of *R. sanguineus* on a rabbit, suggesting a possible extension of its host range in response to specific environmental or host-related factors. Such findings are important for tick surveillance and the prevention of tick-borne zoonotic diseases.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Rabbit, *Rhipicephalus sanguineus*, Zoonosis, Ectoparasite.



A Review of *Neospora caninum* and Current Therapeutic Approaches in Cattle

Soroush Heidari¹, Mohammad Taha Heidari², Ali Maddahi Nejad³

Corresponding Author: Ali Maddahi Nejad, alimaddahi3177@gmail.com

1. Department of Theriogenology Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran.
2. Department of Food Hygiene And Quality Control of Veterinary Medicine, University of Urmia, Urmia, Iran.
3. DVM Graduate Faculty of Veterinary Medicine, University of Islamic Azad University of Shahrekord Branch, Shahrekord, Iran.

Article code: 1260

Abstract

Background: *Neospora caninum* is a significant protozoan parasite in veterinary medicine and a leading cause of abortion in dairy and beef cattle. It transmits vertically (mother to fetus) and horizontally, mainly via dogs, which act as definitive hosts. In dogs, the parasite reproduces sexually in the GI tract, producing oocysts shed in feces. Under suitable environmental conditions, oocysts become infectious within 24–72 hours, contributing to major economic losses in the livestock industry.

Methods: This review analyzed studies from databases like PubMed, ScienceDirect, and Scopus, using keywords such as *Neospora caninum* and bovine neosporosis therapy. Only peer-reviewed English articles (2010–2025) focused on diagnosis, treatment, or prevention in cattle were included. Over 45 papers were reviewed. The reported prevalence of *N. caninum* in herds varies between 20% and 90%, and in Iran, 20–40% of aborted cows are affected. Diagnostic methods include serological tests (ELISA, IFAT), PCR, and histopathology

Results: A concise summary of the major findings of the experiment or study. Sufficient data must be provided to permit evaluation by the reviewers and public reading of the abstracts.

Conclusion: No definitive cure or vaccine currently exists for bovine neosporosis. Control programs remain essential to limit economic losses. Although vaccine research is ongoing, no effective product has reached the market. Future research should focus on improving diagnostics and treatment options.

Keywords: *Neospora caninum*, Toltrazuril, Serological diagnosis, Vertical transmission.



An Investigation on the Infection with *Hepatozoon* sp. in Stray Dogs of Shiraz City and Its Suburbs

Tina Yaghoobpour¹, Hassan Sharifiyazdi¹, Ehsan Rakhshandehroo², Mahsa Rouhana¹

Corresponding Author: Tina Yaghoobpour, Tinayaghoobpour75@gmail.com

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Article code: 1263

Abstract

Background: Stray dogs can considerably affect public and animal health due to their role in transmitting tick-borne protozoan parasites. They are often infested with ectoparasites that spread zoonotic and non-zoonotic pathogens. Among the most significant protozoa are *Hepatozoon canis* and *Hepatozoon americanum*, causative agents of canine hepatozoonosis. Clinical signs may include fever, lethargy, weight loss, anemia, and ocular discharge. As stray dogs are widespread in our country and frequently harbor ticks, infection with *Hepatozoon* spp. is likely. This study aimed to investigate the presence of *Hepatozoon* spp. in stray dogs of Shiraz and surrounding areas and to emphasize the role of these dogs as potential reservoirs for tick-borne pathogens.

Methods: One hundred stray dogs were captured in Shiraz and its suburbs in cooperation with the municipality. Blood samples were collected into EDTA tubes during autumn and winter of 2024–2025. The dogs were free-ranging, and information on sex was unavailable. Thin smears were prepared, Giemsa-stained, and examined microscopically for protozoan stages within leukocytes, particularly neutrophils.

Results: Two samples (2%) showed gamonts morphologically consistent with *H. canis*. These appeared elongated and oval with basophilic cytoplasm and a centrally positioned nucleus. Although prevalence was low, the detection of *Hepatozoon* spp. highlights the epidemiological importance of stray dogs in this region.

Conclusion: The findings stress the need for improved ectoparasite surveillance in stray dogs. Unlike most tick-borne pathogens, *Hepatozoon* spp. are transmitted through ingestion of infected ticks, mainly *Rhipicephalus sanguineus*. Therefore, integrated vector control measures are recommended, especially in urban dog populations living close to human.

Keywords: *Hepatozoon* sp., Stray dogs, Shiraz city.



Application of Artificial Intelligence in Diagnosis and Control of Parasitic Zoonoses in Iran

Reza Soltani¹, Bahareh Meftahi^{2,3}

Corresponding Author: Reza Soltani, rezasoltani.rs.rs@gmail.com

1. *Microbiology and Biology Research Group, Food Technology and Agricultural Products Research Center, Standard Research Institute, Karaj, Iran.*
2. *Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.*
3. *Innovative Medical Research Center, MMS.C, Islamic Azad University, Mashhad, Iran.*

Article code: 1268

Abstract

Background: Parasitic zoonoses, affecting both humans and animals, are a growing global health concern with increasing prevalence in Iran. These diseases, such as echinococcosis, leishmaniasis, and toxoplasmosis, impose significant public health and economic burdens. Climate change, host migration, and drug resistance have complicated traditional control efforts. This study explores the potential of artificial intelligence (AI) technologies in improving the diagnosis and control of parasitic zoonoses in Iran, with reference to international applications.

Methods: A comprehensive literature review (2012–2024) was conducted across PubMed, Scopus, and ScienceDirect to identify peer-reviewed studies using AI in parasitology. Emphasis was placed on the use of specific algorithms such as convolutional neural networks (CNN), support vector machines (SVM), and random forests in the detection of parasites. Regional relevance and feasibility for Iran's epidemiological settings were prioritized.

Results: AI tools have demonstrated significant potential in identifying parasitic forms in microscopic images, predicting outbreak risks, and enhancing surveillance accuracy. In Iran, preliminary simulations using CNN and hybrid algorithms achieved over 90% sensitivity and specificity in diagnosing key zoonotic parasites. Despite limited implementation, these technologies could transform early detection and monitoring practices in veterinary and medical parasitology in the country.

Conclusion: AI offers a transformative approach for parasitic disease control. However, effective integration requires access to localized data, interdisciplinary collaboration, and ethical oversight. We recommend national investment in AI-assisted diagnostic platforms, training for field veterinarians, and integration of these tools into the Iranian One Health framework.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Artificial intelligence, Parasitic zoonoses, Iran, Epidemiology, Surveillance.



Emerging Trends in Tick-Borne Parasitic Diseases of Iranian Livestock

Reza Soltani¹, Bahareh Meftahi^{2,3}

Corresponding Author: Reza Soltani, rezasoltani.rs.rs@gmail.com

1. *Microbiology and Biology Research Group, Food Technology and Agricultural Products Research Center, Standard Research Institute, Karaj, Iran.*
2. *Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.*
3. *Innovative Medical Research Center, MMS.C, Islamic Azad University, Mashhad, Iran.*

Article code: 1269

Abstract

Background: Tick-borne parasitic diseases represent a significant challenge for animal health and productivity in Iran. In recent years, changing climate patterns, livestock movement, and ecological shifts have influenced the distribution and incidence of these infections. This study reviews emerging trends, risk factors, and preventive strategies for tick-borne parasitic diseases in livestock across Iran.

Methods: A systematic review of literature from 2010 to 2024 was conducted using databases including PubMed and Scopus, focusing on tick-borne protozoan and helminthic infections affecting domestic animals. Key epidemiological patterns, vector ecology, and reported control measures were analyzed.

Results: There is evidence of expanded tick habitats and increased tick activity seasons in several Iranian provinces. This has led to a rise in cases of theileriosis, babesiosis, and anaplasmosis among cattle, sheep, and goats. Identified contributing factors include climate change, inadequate tick control measures, and gaps in farmer awareness.

Conclusion: Addressing emerging tick-borne parasitic diseases requires integrated vector management, improved veterinary training, and climate-adaptive surveillance systems. These steps are essential to protect the health of livestock and ensure sustainable livestock production in Iran.

Keywords: Tick-borne diseases, Livestock, Iran, Climate change, Epidemiology.



Morphological Characterization and Complete Sequence Determination of the ITS2 Region of *Ostertagia occidentalis* in Sheep and Goat Isolates in Iran

Meisam Sabour-Darbandi¹, Behnam Meshgi², Fateme Jalousian²

Corresponding Author: Meisam Sabour-Darbandi, ms.darbandi@yahoo.com

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Shiraz, Shiraz, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1272

Abstract

Background: The stomach worm, *Ostertagia occidentalis*, is the main parasite of domestic ruminants, especially sheep and goat, in Iran. In acute infections with this nematode, causing gasteritis in animals. The pathogenicity of *Ostertagia* sp. is usually high; ostertagiosis can lead to weight loss, anemia, reduced milk and wool production, with potentially maldigestion of proteins in the alimentary canal. In the present study, ITS2-rDNA and morphological characters of *O. occidentalis* from sheep and goat isolates from Iran were evaluated.

Methods: Sixty male *O. occidentalis* adult worms were collected from the abomasum of twenty sheep and goat from Tehran and mashhad slaughters Iran. Specimens were morphologically identified based on the length and shape of spicules. The ITS2 region of four individual worms' ribosomal DNA was amplified by conventional PCR and the tow products were Sanger sequenced.

Results: The spicules were dark brown, thick, and each divided into three branches. The arrow-shaped gubernaculum was diagnosed. The mean length of the spicules was 311–321.7 micrometer. Nucleotide sequences of the 946 bp ITS2 region showed 99% similarity with *Marshallagia marshalli* and 92% similarity with *O. circumcincta* species

Conclusion: Morphological and molecular characterization clearly determinate *O. occidentalis* as prevalent abomasum nematode in goat of Iran. In the other hand, morphological results is agreeament with molecular results and recurding data in Gen Bank. But the results of molecular analysis with *Marshallagia marshalli* showed 99% similarity.

Keywords: *Ostertagiosis*, Morphology, Nematode, PCR, Iran.



The Survy of Morphological Characterization and Determination Complete Sequence of ITS2 Region of *Ostertagia (Teladorsagia) circumcincta* in Goat Isolate in Iran

Meisam Sabour darbandi¹, Behnam Meshgi², Fateme Jalousian²

Corresponding Author: Meisam Sabour darbandi, ms.darbandi@yahoo.com

1. Doctora Philosophy student of Parasitology, Faculty of Veterinary Medicine, University of Shiraz, Shiraz-Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran-Iran.

Article code: 1273

Abstract

Background: The stomach worm, *Ostertagia circumcincta* is the main parasite of domestic ruminants especially goat in Iran. In acut infections with this nematode causing gasteritis in animals. The patogenecity of *ostertagia* sp. is usually high, ostertagiasis can lead to weight loss, anemia, reduced milk and wood production, with potentially maldigestion of proteins in alimentary canal. In the present study ITS2-rDNA and morphological characters of *O.circumcincta* from goat isolate from Iran were avaluated.

Methods: Sixty of *O.circumcincta* male adult worm were collected via necropsy of abomasum from goat from different localitirs in Iran. Specimens were morphologically identified based on the length and shape of spicules. The ITS2 of ribosomal DNA were amplified by PCR and sequenced from individual nematodes.

Results: Our finding showed that the spicules are pigmented brown, slender and are 382.7 μ and 388.8 μ long. Each end in a large knobbed and a small, acut process. DNA amplification of the ITS2 produced a 946bp fragment for all the cases. ITS2 sequence analysis of samples showed exactly 100% similarity with all available data of *O.circumcincta* in GenBank.

Conclusion: Morphological and molecular characterization clearly determinate *O.circumcincta* as prevalent abomasum nematode in goat of Iran. In the other hand, morphological results is agreeament with molecular results and recurding data in Gen Bank.

Keywords: *Ostertagia circumcincta*, Morphology, ITS2, Sequence, goat, Iran.



Infection of a Persian Cat with *Otodectes cynotis* Mite in Khorramabad

Amir Hossein Ghiasvand¹, Mobina Torabi Feijani¹, Mohammadhossein Abbasi¹, Shahriar Yavari¹, Hassan Nayebzadeh¹

Corresponding Author: Amir Hossein Ghiasvand, amiirghiasvand@gmail.com

1. Department of Pathobiology, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.

Article code: 1274

Abstract

Background: External parasites are important factors in causing itchy and non-itchy skin problems in pets. The ear mite (*Otodectes cynotis*), is a member of the Psoroptidae family. This study aims to describe a case of *Otodectes cynotis* infestation in a cat, including its clinical presentation, diagnosis, and treatment.

Methods: A 1-year-old female Persian cat was referred to Mehr Veterinary Clinic in Khorramabad which is located in the west of Iran with symptoms of head shaking, ear scratching, and dark brown waxy deposits with an unpleasant odor in the ear area. After examination with an otoscope and sampling with the help of a swab from the animal's ear, the waxy secretions obtained were placed in a test tube containing 10% potassium hydroxide in a 60-degrees Celsius water bath, and after centrifugation, the sediment was examined microscopically.

Results: Based on microscopic observations and morphological characteristics, the apodemes of the first and second pairs of legs of mites were connected to each other on each side of the body. Males *Otodectes* had cup-shaped suckers on short pedicels on all legs, while females only had them on the first two pairs of legs. Additionally, males had a pair of adanal suckers used for mating. Using diagnostic keys, it was determined that the cat was infected with *Otodectes cynotis*. For treatment, first, glycerin drops were used to wash and clean the ear, then ivermectin was injected S.C at a dose of 0.2 mg/kg to the cat. In addition, topical ivermectin was applied to the inside of each ear. Follow-up of the cat's disease revealed that the cat had recovered 3 weeks after treatment.

Conclusion: *Otodectes cynotis* mite is one of the common mites in the ears of cats, which can lead to otitis externa if not treated promptly.

Keywords: Cat, *Otodectes*, Khorramabad, Lorestan.



Effects In Vitro and In Vivo Antihydatid Activity of a Nano Emulsion of Zataria Multiflora Essential Oil on the Hydatid Cysts in Laboratory Mice

Meisam Sabour Darbandi¹, Hassan Borji², Mohammad Moazeni¹

Corresponding Author: Meisam Sabour Darbandi, ms.darbandi@yahoo.com

1. Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.
2. Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1276

Abstract

Background: Cystic echinococcosis (CE) is a worldwide zoonotic infection with economic and public health importance in many parts of the world. It is the result of infection of humans and domestic ruminants with the larval stage of *Echinococcus granulosus sensu lato*.

Methods: In this study, protoscoleces of the hydatid cysts were exposed to two concentrations of nano emulsion (NE) of *Zataria multiflora* essential oil (ZMEO) (1 and 2 mg/mL) for 10 and 20 min. Viability of protoscoleces was confirmed using 0.1% eosin staining. For in vivo studies, sixteen laboratory mice were infected intraperitoneally by 1500 live protoscoleces. Five months after infection, the infected mice were divided into treatment and control groups. The mice of treatment group received the NE of ZMEO (20 mg/kg) orally via their drinking water while the mice of control group received no treatment. Two months after the start of treatment, all of the mice were necropsied and the hydatid cysts were collected. Subsequently, the numbers, sizes and weights of the collected cysts were compared between the mice of two groups.

Results: The results of in vitro scolicidal assays showed that the scolicidal power of NE of a ZMEO at concentration of 1 mg/mL was 88.01%, and 100% after 10 and 20 min respectively. NE of ZMEO showed 100% scolicidal power at a concentration of 2 mg/mL after 10 min (comparing to 4.46% for the control group). The results of in vivo studies revealed that the size of the largest cysts as well as the total number of the cysts were significantly lower in the mice treated with NE of ZMEO ($P < 0.05$).

Conclusion: NE of ZMEO may be considered as a natural scolicidal agent and a potential therapeutic tool for treatment of hydatid disease.

Keywords: Protoscolec, Zoonotic, *Echinococcus granulosus*, Nano emulsion.



Study of *Leishmania* Species of Human Cutaneous Leishmaniasis in Fasa City, Southeast Iran (2022-2024)

Shamsi Noorpisheh Ghadimi¹, Shiva Kazemi²

Corresponding Author: Shamsi Noorpisheh Ghadimi, sn.noorpisheh@yahoo.com

1. Department of Parasitology and Mycology, School of Allied Medicine Sciences, Fasa University of Medical Sciences, Fasa, Iran.
2. Student Research Committee, Fasa University of Medical Sciences, Fasa, Iran.

Article code: 1278

Abstract

Background: The goal of this study was to know the identity of *Leishmania* species responsible of cutaneous leishmaniasis (CL) in Fasa city, Southeast Iran.

Methods: *Leishmania* parasites were isolated from 140 patients suspected of having CL and referred to Department of Parasitology and Mycology at Fasa University of Medical Sciences, Fasa, Iran. Samples collected on skin lesions were examined both microscopically (after Giemsa staining) and molecularly (after PCR-RFLP). Primers targeting 18S rDNA, the complete internal Transcribed (ITS1), and partial 5.8S rDNA sequences were used to detect *L. Major* and *L. tropica*.

Results: On the 140 examined patients, 60 exhibit *Leishmania* sp. by microscopical examination, all confirmed by PCR. For ten patients with negative microscopical examination, PCR was positive for three of them. Among these 60 positive samples, 5 (8%) was infected by *L. tropica* and 55 (92%) by *L. major*. The most clinical feature was papule (80%), and all patients were Iranians and residents of Fasa County. The highest number of infections was noted during the autumn and winter months. Most lesions due to *L. major* were located on the feet, while *L. tropica* lesions appeared on the head and back. Studied patients had an age range between 6 to 52 years old and most abundant lesions were related to patients with 8-20 years old.

Conclusions: *Leishmania major* is the most prevalent species in n Fasa which is condidered as ZCL foci in Fars pronince. *L. tropica* also occurs in the region but is less prevalent.

Keywords: *Leishmania tropica*, *Leishmania major*, PCR-RFLP, Iran, Human.



Investigation of the Anti-Leishmanial Potential of *Lapachol* on Promastigote Forms of *Leishmania major*: An In Vitro Study

Kambiz Karimi¹, Erfan Ahmadi², Gholamreza Hatam¹

Corresponding Author: Gholamreza Hatam, hatam908@yahoo.com

1. Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Microbiology, Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran.

Article code: 1279

Abstract

Background: Leishmaniasis encompasses a spectrum of complex diseases caused by over 20 species of the genus *Leishmania*, an obligate intracellular protozoan belonging to the class *Kinetoplastida*, family *Trypanosomatidae*. Although historically first-line, pentavalent antimonials for leishmaniasis are limited by toxicity. Second-line treatments (amphotericin B, miltefosine, pentamidine) also face challenges of limited efficacy, toxicity, resistance, and long treatment times. *Lapachol*, with its diverse therapeutic properties, is investigated in this study for its *in vitro* leishmanicidal activity against *Leishmania major*, addressing the need for new leishmaniasis treatments.

Methods: *L. major* promastigotes (MRHO/IR/75/ER strain) and J774 murine macrophage cells were used in this *in vitro* study. Concentrations of *lapachol* at 5, 10, 20, 40, 80, and 160 µg/mL were used to evaluate its cytotoxic effects. Flow cytometry and propidium iodide (PI) staining were employed to determine promastigote viability, while the MTT assay was used to quantify macrophage cytotoxicity. As a positive control, Amphotericin B was used at a concentration of 20 µg/mL. SPSS version 27 was used to carry out the statistical analysis.

Results: *Lapachol* exhibited a dose-dependent anti-leishmanial effect, with 97.41% promastigote mortality at 160 µg/mL (IC₅₀ = 31.27 µg/mL). Amphotericin B (positive control) showed 98.4% efficacy. The compound demonstrated low toxicity toward J774 macrophages (CC₅₀ = 143.89 µg/mL), indicating selectivity against the parasite.

Conclusion: The findings indicate that *Lapachol* exhibits potent anti-leishmanial activity against *L. major* promastigotes, with a favorable toxicity profile towards host cells. Further *in vivo* investigations are warranted to fully ascertain its therapeutic potential as a novel treatment modality for cutaneous leishmaniasis (CL).

Keywords: *Leishmania major*, *Lapachol*, Flow cytometry, MTT assay, Cutaneous leishmaniasis, In vitro.



Molecular Identification of *Anaplasma phagocytophilum* in Sheep in East Azarbaijan Province

Ahmad Nematollahi¹, Abbas Imani¹, Behzad Ghorbanzadeh¹, Elahe Jahanifard¹

Corresponding Author: Ahmad Nematollahi, anemat@tabrizu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Article code: 1280

Abstract

Background: Anaplasmosis is one of the most important diseases transferred by Ixodidae ticks in ruminants. The disease is caused by different species of *Anaplasma*. The aim of this study was to determinate the rate of infection to *Anaplasma phagocytophilum* among sheep of East Azarbaijan province.

Methods: Two hundred blood samples were collected from sheep randomly. The extracted DNA from blood cells were amplified by two *A.phagocytophilum*-primers, which amplify an approximately 1468bp and 926bp DNA fragment from region of 16S rRNA gene.

Results: Results indicate that 7 (3.5%) out of 200 sheep blood samples were *Anaplasma* spp. positive by first cycle of nested PCR. All sheep positive samples were analyzed for the presence of *A. phagocytophilum* and 5 of sheep blood samples (2.5%) were positive for *A.phagocytophilum*. The analysis show that there is not significant difference among the infection rate to *A. phagocytophilum* with sex and age of sheep. Two positive samples were randomly sequenced, and by comparing the sequence results with the sequences registered in the Gene Bank, it was observed that both samples had 100% similarity to the *A.phagocytophilum* sequence registered in the gene bank.

Conclusion: This study revealed that the level of infection to *A.phagocytophilum* in sheep in the province of East Azarbaijan is low, and the need for testing with primers related to other *Anaplasma* species is recommended.

Keywords: *Anaplasma phagocytophilum*, PCR, Sheep, East Azabaijan.



Microscopic Investigation of Haemosporidian Parasites in Raptors from Kerman Province, Iran

Hemad Shafiei¹, Mohammadjavad Jahedi², Mohammad Zamani Ahmadmahmudi¹

Corresponding Author: Hemad Shafiei, Hemad.shafiei@uk.ac.ir

1. Department of Clinical Science, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
2. DVM student, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Article code: 1285

Abstract

Background: Avian haemosporidians are widespread blood parasites capable of infecting a diverse range of bird species across the globe. Reported infection rates vary significantly, ranging from less than 50% to nearly 100% in different avian populations. These haemoparasites can have profound effects on host physiology, behavior, and ecology, influencing factors such as health, reproductive success, population dynamics, and even sexual selection. In severe cases, persistent infections may contribute to species decline or extinction. The aim of this study was to investigate the prevalence of blood parasites in wild birds of Kerman province.

Methods: To assess the prevalence of haemosporidian infections in Iranian birds, a study was conducted on 38 individuals from 6 different raptor families (*Buteo buteo*, *Buteo rufinus*, *Accipiter brevipes*, *Falco tinnunculus*, *Aquila nipalensis*, *Bubo bubo*). Blood sampling was conducted from November 2021 to September 2023 at a protected wildlife rehabilitation center in Kerman, Iran. The presence of blood parasites was determined through microscopic examination of Giemsa-stained blood smears.

Results: one bird (Long-legged buzzard) was detected as harboring *Haemoproteus* spp. . *Haemoproteus* spp. was detected in one out of 38 birds examined (2.6%).

Conclusion: Considering that only a single sample tested positive among all the specimens examined in this study, it can be inferred that neither the age of the birds nor the specific sampling sites within Kerman Province had a statistically significant association with the occurrence of infection. The low prevalence observed in this study implies that the region may not be a highly suitable habitat for the transmission of these parasites, possibly due to environmental conditions or limited vector availability. Consequently, the findings suggest that Kerman may not be an optimal area for extensive future research on avian haemosporidians and their interactions with host species and vectors. However, continued monitoring is recommended to track potential changes in parasite distribution and prevalence over time.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Blood parasite, *Haemoproteus*, Birds of prey, Wild birds.



Scaly Face Disease in Budgerigars: Clinical and Microscopic Confirmation of *Knemidocoptes pilae* Infestation

Mohammadjavad Jahedi¹, Hemad Shafiei²

Corresponding Author: Mohammadjavad Jahedi, mjjahedi1380@gmail.com

1. DVM student, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
2. Department of Clinical Science, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Article code: 1286

Abstract

Background: Scaly face, a prevalent dermatological condition in budgerigars (*Melopsittacus undulatus*), is primarily caused by the burrowing mite *Knemidocoptes pilae* (Acari: Epidermoptidae). This ectoparasite infests the non-feathered regions of the bird, particularly the cere, beak, eyelids, and legs, leading to hyperkeratosis, crust formation, and severe deformities if left untreated. The mite's life cycle is completed entirely on the host, facilitating rapid transmission in aviary settings, especially among immunocompromised or stressed individuals. Although the condition is rarely fatal, chronic infestations can impair feeding, vision, and overall avian welfare.

Methods: Suspected cases of *Knemidocoptes pilae* infestation were diagnosed through clinical examination of characteristic lesions (hyperkeratosis, crusting, and beak deformities) and confirmed via microscopic analysis of skin scrapings.

Results: Microscopic examination of skin scrapings confirmed the presence of *Knemidocoptes pilae* mites in suspected cases, validating active infestation. Clinical assessment revealed characteristic lesions, including hyperkeratosis, crust formation, and beak deformities, consistent with scaly face disease.

Conclusion: This study confirms *Knemidocoptes pilae* as the cause of scaly face disease in budgerigars, based on typical lesions and microscopic detection of mites. While *K. pilae* primarily affects budgerigars (*Melopsittacus undulatus*), other *Knemidocoptes* species such as *K. mutans* and *K. jamaicensis* have been reported in poultry, canaries, finches, and wild birds, causing similar dermatological conditions. These mites typically infest non-feathered areas, leading to hyperkeratosis, crusting, and deformities. Since the entire life cycle occurs on the host, transmission is rapid in aviaries, especially among stressed or immunocompromised birds. Though seldom fatal, chronic infestations can impair vision, feeding, and general welfare. Preventive measures, including routine screening, isolation of affected birds, and improved husbandry, are essential. Early treatment with appropriate acaricides and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



environmental management helps control spread and improves outcomes across different avian species.

Keywords: *Knemidocoptes pilae*, Scaly face disease, Budgerigar, Avian ectoparasites.



***Theileria* Infection in Camels in Kashan, Central Iran**

Zainab Sadeghi-Dehkordi¹, Somayeh Moradi¹, Alireza Sazmand¹

Corresponding Author: Zainab Sadeghi-Dehkordi, z.sadeghidehkordi@basu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran.

Article code: 1287

Abstract

Background: Infection with tick-borne protozoan parasites of the genus *Theileria* is economically important, with various clinical outcomes depending on the parasite species and the livestock host. So far, different *Theileria* species, e.g., *T. equi*, *T. annulata*, and *T. ovis*, have been detected in the blood of camels. This study aimed to investigate the presence of *Theileria* spp. in one-humped camels (*Camelus dromedarius*) of the Kashan area, in central Iran, by microscopic examination and to identify the species by molecular tools.

Methods: From June to November 2024, blood specimens were collected from 80 randomly selected camels aged 1 to 15 year and both sexes (45 males, 55 females). Blood smears were examined microscopically for the presence of intraerythrocytic piroplasms. Genomic DNA has been extracted from the specimens and will be subjected to genus-specific PCR and Sanger sequencing. For the statistical analysis, camels were grouped into G1 (<3 years), G2 (3–5 years), and G3 (>5 years).

Results: *Theileria*-resembling inclusions were diagnosed in the blood smear of 12 camels (15%). Positivity was higher in males/females? and in the age groups G3 ($P < 0.05$). The conventional PCR assay is in progress.

Conclusion: The results of this study indicate the presence of *Theileria* species in one-humped camels from the Kashan region. These findings highlight the potential role of camels in the epidemiology of theileriosis in the area and emphasize the importance of continuous monitoring of camel health to prevent the spread of tick-borne diseases, particularly piroplasmosis, in different regions of Iran. Further molecular research and a more detailed investigation of *Theileria* species in camels from this region could contribute to improving strategies for the control and prevention of related diseases.

Keywords: *Camelus*, Tick-borne, Piroplasmosis.



A Report on the Ectopic Presence of a Hookworm Belonging to the Family Ancylostomatidae in the Urinary Bladder of a Male Domestic Rabbit (*Oryctolagus cuniculus*)

Sadegh Motamedi¹, Saeed Dadkhah Tehrani², Parinaz Heydari³

Corresponding Author: Sadegh Motamedi ,Dr.Sadegh.motamedi@gmail.com

1. Graduated Department of Veterinary Surgery, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Parasitology, Shahr-e-Kord Aranch, Islamic Azad University, Shahr-e-Kord, Iran.
3. Graduate Department of Veterinary Medicine, Faculty of Veterinary Medicine, Shahr-e-Kord Branch, Islamic Azad University, Shahr-e-Kord, Iran.

Article code: 1288

Abstract

Background: Hookworms of the Ancylostomatidae family are typically intestinal parasites of carnivores and, less commonly, humans. Their presence in non-gastrointestinal organs is considered aberrant and rare. This study reports the unusual localization of a hookworm in the urinary bladder of a domestic male rabbit (*Oryctolagus cuniculus*), a host species not commonly associated with this parasite family.

Methods: A 9-year-old domestic male rabbit was presented with acute hematuria, abdominal pain, and restlessness. Clinical examination revealed hypogastric tenderness, and ultrasonography showed an ill-defined echogenic mass in the bladder. A cystostomy was performed, during which a 10 cm long parasite was surgically extracted. The specimen was preserved in 70% ethanol, cleared with lactophenol, and examined microscopically. Morphological characteristics were compared to known descriptions of Ancylostomatidae species.

Results: Macroscopic evaluation revealed a reddish, blood-feeding worm approximately 10 cm in length. Microscopy demonstrated a distinct anterior bend and an oral capsule with prominent teeth—hallmark traits of Ancylostomatidae hookworms. The worm's presence in the bladder is highly atypical; prior experimental studies have shown that rabbit tissues can support aberrant larval migration, though no prior reports documented adult worms in the bladder. The mode of migration is uncertain, possibly involving bloodstream dissemination or ascending infection via the urethra.

Conclusion: This case highlights a rare instance of aberrant Ancylostomatidae localization in a rabbit bladder, emphasizing the need to consider parasitic infections—especially ectopic



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



ones—in the differential diagnosis of urinary tract disorders in rabbits. Future cases may benefit from molecular diagnostics to determine the specific species involved.

Keywords: Ancylostomatidae, Rabbit, Cystostomy, Bladder.



Hydatid Cyst, *Dicrocoelium dendriticum*, and *Fasciola* spp. Infections of Slaughtered Animals in Hamedan, Iran (2024)

Zainab Sadeghi Dehkordi¹, Khadigeh Rahmati¹

Corresponding Author: Zainab Sadeghi Dehkordi, z.sadeghidehkordi@basu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, University of Bu-Ali Sina, Hamedan, Iran.

Article code: 1289

Abstract

Background: Hydatid cyst, *Dicrocoelium dendriticum*, and *Fasciola* spp. are significant public health and economic challenges in livestock, leading to fundamental losses as well as the control cost programs, treatment expenses, livestock mortality, carcass condemnation, and reduced productivity. The aim of this study was to evaluate the prevalence of parasitic infection in slaughtered animals in Hamedan abattoir, during 2024.

Methods: The data were prepared by the Veterinary Organization and statistically evaluated using the Uni stat v. 21 for Excel program. The results of routine meat inspection of slaughtered animals were considered during 2024, following by 9087 calves, 11471 goats and 63176 sheep slaughtered.

Results: 10.42% and 8.71% of the slaughtered cattle were found positive for hydatid cysts in liver and lungs, respectively. Meanwhile, the prevalence of Hydatid cyst in the liver and lung, *D. dendriticum* and *Fasciola* spp. in the population of slaughtered sheep were 5.83% and 8.3%, 3.05% and 1.6%, respectively. Additionally, the prevalence of Hydatid cyst in the liver and lung, *D. dendriticum* and *Fasciola* spp. in the slaughtered goats were 3.39% and 4.09%, 2.06% and 1.089%. Parasitic infection in *sheep* (18.78%) was higher than *goats* (10.62%), but this difference was not statistically significant ($p \geq 0.05$). The highest and lowest prevalence of Hydatid cyst and *Dicrocoelium* were recorded in March and August. Comparison, the highest and lowest prevalence of *Fasciola* spp. were detected in September and April, respectively.

Conclusion: This study identified hydatid disease as a significant issue in slaughtered animals, indicating the need for preventative and control measurements. Therefore, the economic impact on the veterinary Industry and risk factors regarding to public health should be considered to target preventive measures.

Keywords: *Fasciola*, Hydatid cyst, *Dicrocoelium*, Livestock.



The First Report of *Setaria labiatopapillosa* Isolated from the Abdominal Cavity of a Goat in Ahvaz, Iran

Alireza Alborzi¹, Saleh Esmailzadeh², Afsaneh Nazarpour Kalaei¹, Boshra Khajehian³, Hossein Hosseini³, Seyedeh Zahra Bayegan³

Corresponding Author: Alireza Alborzi, a.alborzi@scu.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Pathology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
3. Department of Clinical Science, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1294

Abstract

Backgrounds: *Setaria* species are filarial nematodes that inhabit the abdominal cavity of ungulates, mainly bovines, and in very few cases from small ruminants. The goal is to report the first case of the nematode, *Setaria labiatopapillosa*, in goats from the Khuzestan region.

Methods: In November 2024, a 10-year-old native female goat was autopsied in the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz. One individual worm was observed in the animal's abdominal cavity and preserved in 70% ethanol, and transferred to the parasitology lab. The worm was clarified with lactophenol, and it was identified morphologically under a light microscope using a diagnostic key.

Results: The specimen was a female *Sataria labiatopapillosa*, measuring 76 mm in length. The anterior and posterior widths of it were 269.7 and 30.8 microns, respectively. The length and the distance between the peribuccal prominences were 19.9 and 42.9 microns, respectively. The length and width of the tail end knob were 7.54 and 12.93 microns, respectively.

Conclusion: Although reports of infections with different species of *Setaria* isolated from the abdominal cavities of cattle and buffaloes, as well as cerebrospinal nematodiasis in sheep and goats, exist in Iran, the isolation of *Setaria labiatopapillosa* in goats from Ahvaz is the first report in this region. The presence of this nematode in goats in Ahvaz indicates infection of its final hosts, and because of the presence of its intermediate host (mosquitoes) in the region, it could contribute to the potential spread of *Setaria* in southwestern Iran.

Keywords: Goat, *Setaria*, Ahvaz, Iran.



Detection and Phylogenetic Analysis of LRV2 in *Leishmania* Isolates from Qom Province, Iran

Seyedeh Tara Mosavi Zadeh^{1,2}, Elham Gholami², Negar Seyed², Shima Hadifar², Homa Hajjaran³, Sima Rafati², Tahereh Taheri²

Corresponding Author: Tahereh Taheri, tahereh_t@yahoo.com

1. Department of Basic Sciences University of Islamic Azad University, North Tehran Branch, Tehran, Iran.
2. Department of Immunotherapy and Leishmania Vaccine Research, Pasteur Institute of Iran, Tehran, Iran.
3. Department of Medical Sciences University of Tehran University, Tehran, Iran.

Article code: 1302

Abstract

Background: Leishmaniasis remains a significant public health challenge across most provinces of Iran, highlighting the urgent need for effective treatments, vaccines, and control measures. Recent studies have underscored the role of *Leishmania* RNA virus (LRV) in influencing disease severity, inflammatory responses, and treatment outcomes. LRV type 2 (LRV2) is predominantly found in *Leishmania major* (*L. major*), with occasional reports in *L. tropica* and *L. infantum* from provinces such as Golestan and Esfahan. This study aimed to detect LRV2 in *Leishmania* parasites isolates from patients in Qom city, Qom province (north-central Iran), and to investigate their genetic relationships through phylogenetic analysis, providing insights for future research and control strategies.

Methods: Clinical specimens from 16 patients were cultured, and parasites were subjected to genomic DNA and RNA extraction. Species identification was performed using the ITS1-PCR-RFLP technique. Detection of LRV2 targeted the RNA-dependent RNA polymerase (RdRp) gene via reverse transcription PCR (RT-PCR), semi-nested PCR, and Sanger sequencing of cDNA. Sequence data were analyzed with bioinformatics tools, and viral loads were quantified by quantitative real-time PCR (qRT-PCR) using RdRp-specific primers.

Results: Of the 16 isolates, 15 isolates were identified as *L. major*, and one as *L. tropica*. Among the *L. major* isolates, eight (53.3%) tested positive for LRV2 by RT-PCR. Phylogenetic analysis revealed that these eight LRV2-positive *L. major* isolates from Qom were closely related to strains previously reported in Golestan province, northeastern Iran. qRT-PCR confirmed the presence of LRV2 and quantified viral loads in these samples.

Conclusion: Given Qom's status as a major religious hub attracting numerous visitors from Iran and abroad, the detection of LRV2-infected *Leishmania* parasites highlights the need for



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



enhanced surveillance and further research into the virus's impact on transmission dynamics, clinical outcomes, and drug resistance in the region.

Keywords: Cutaneous leishmaniasis, *Leishmania major*, *Leishmania tropica*, *Leishmania* RNA virus 2.



BCG Training Effects on Mice Peritoneal Macrophages Infection Caused by *Leishmania major* and *Leishmania tropica*

Negin Elahirad¹, Zohreh Askarypour¹, Mahmoud Nateghi-Rostami¹

Corresponding Author: Mahmoud Nateghi-Rostami , m_nateghi@pasteur.ac.ir

1. Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran.

Article code: 1303

Abstract

Background: Cells of the innate immune system undergo functional reprogramming in response to BCG treatment through a process known as trained immunity which provides non-specific protection to unrelated infections. We evaluated the effects of BCG on protection of peritoneal monocytes against infection by *Leishmania major* and *L. tropica* using an infectivity assay.

Methods: 6 weeks old BALB/c female mice were injected intravenously with BCG (40µg/animal) or PBS (200 µL). After 1-week in vivo BCG trained and non-trained peritoneal monocytes (2×10^5 cells/200 µL medium) were added into chamber slides and incubated overnight at 37 °C incubator, with 5% CO₂. Then cells were infected with live stationary-phase promastigotes of *L. major* or *L. tropica* (2×10^6 /well; 10:1 ratio). Cell cultures were incubated for 6 h and 24 h. Cells were fixed, stained and examined under a light microscope to determine the infection index, the percentage of infected cells and the mean number of intracellular parasites/infected cells. Infection index = percentage of infected cells $\times$ mean number of parasites per infected cell.

Results: *L. major* infected less macrophages in trained cells after 24 h. Also, infection index was lower in trained cells at 6 h but increased after 24 h in comparison to non-trained cells, indicating killing of the parasites early at infection in presence of BCG ($p < 0.05$, 6 h vs. 24 h). *L. tropica* infected more macrophages in trained cells during the incubation time. A significant increase of parasite number/infected macrophage and infection index was seen after 24 h in trained cells showing a reduction in killing capability of trained macrophages later at infection in presence of BCG ($p < 0.05$, 6 h vs. 24 h).

Conclusion: Data show that while infection outcomes are dependent on the *leishmania* spp., the macrophage killing potential is different in early vs. late infection. Trained innate immunity induced by BCG improves the immune response towards *L. major* at early infection.

Keywords: BCG, Trained immunity, Macrophage, Leishmaniasis.



Nematode Infection of Terrestrial Slug *Drusia ibera* (Eichwald, 1841) from North of Iran

Sepideh Safarzadeh¹, Mousa Tavassoli¹, Ebrahim Ghale Kafai¹, Laya Shamsi²

Corresponding Author: Sepideh Safarzadeh, Sepiide.safarzadeh@gmail.com

1. Department of Pathobiology, Parasitology Division, Faculty of Veterinary Medicine, Urmia University, Iran.
2. Ph.D of Veterinary Parasitology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article code: 1305

Abstract

Background: Terrestrial slugs are globally distributed gastropods that may act as both intermediate and definitive hosts for various parasitic species. Nematodes in slugs can exist as commensals, parasites, or pathogens. This study aimed to investigate the presence of nematode larvae in population of the slug species *Drusia ibera* (Eichwald, 1841) in North of Iran.

Methods: A total of 119 slugs were collected from both urban and rural regions in northern Iran and maintained under laboratory conditions with controlled humidity as well. Species identification was performed using morphological keys and radula staining techniques. Moreover, to assess nematode infection, the radula was dissected and the visceral organs were examined using the Baermann technique.

Results: Radula analysis revealed a tooth formula of 1C + 40L + 18M. Parasitological examination of adult *D. ibera* specimens showed nematode eggs containing *Strongyloides*-like larvae, and adult nematodes belonging to the genera *Rhabditis* and *Panagrolaimus*.

Conclusion: The findings of this study confirm that *D. ibera* in northern Iran harbors various nematode species, including potentially pathogenic forms. This highlights the ecological and parasitological importance of slugs as possible reservoirs and vectors for nematode transmission, which could have an impact on animal and public health in endemic regions.

Keywords: *Drusia ibera*, Nematode infection, Terrestrial slug, Radula, *Rhabditis*, *Panagrolaimus*, Iran.



Case Report of *Cotugnia mohekarii* Infection in a Laughing Dove (*Spilopelia senegalensis*) from Ahvaz, Iran

Alireza Alborzi¹, Afsaneh Nazarpour Kalaei¹

Corresponding Author: Alireza Alborzi, a.alborzi@scu.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1307

Abstract

Backgrounds: Due to the vast biodiversity and the presence of various species of wild birds in Iran, studying the parasites of these birds can greatly contribute to maintaining ecological balance and public health. The laughing dove (*Spilopelia senegalensis*) not only plays a significant role in nature but also has a special value and place in various cultures, arts, and beliefs. Because these birds are exposed to various threats, preserving and protecting them means preserving an important cultural and natural symbol.

Methods: An accidentally dead dove was transferred to the Parasitology Department of Shahid Chamran University of Ahvaz. After necropsy, to isolate helminth parasites, different parts of the digestive tract (esophagus, crop, gizzard, small intestine, and cecum) were washed separately using a sieve and water. After preparation, the isolated worms were stained with carmine acid and identified based on their morphological features and diagnostic keys.

Results: No helminth parasites were observed in the examination of the bird's esophagus, crop, gizzard, and cecum. However, two types of tapeworms (Cestodes) were identified in its small intestine, including *Cotugnia* and *Raillietina*.

The genus *Cotugnia*, since it has four cup-shaped suckers and a rostellum without hooks, might be *Cotugnia mohekarii*, which needs further investigation.

Conclusion: Since these birds are not under veterinary care, they can act as a reservoir for parasites, increasing the risk of their survival and transmission to other host birds.

Keywords: Laughing dove, Tapeworm, Ahvaz, Iran.



Prevalence of Metacestode Infections in Afshari Sheep Slaughtered in Bandar Abbas

Mohammad Mandegari¹, Mohammad Mazrouei Sebdani¹, Amin Ahmadi^{2,3}, Zahra Nasr Esfahani¹, Soheila Bakhshayesh¹

Corresponding Author: Mohammad Mazrouei Sebdani, mohammadmazrouei93@gmail.com

1. Department of Veterinary Medicine, Islamic Azad University, B.C. , Baft , Iran
2. Department of basic sciences, Faculty of veterinary Medicine, Ardakan University, Ardkan, P.O.Box 184, Iran.
3. Biology and Animal Reproduction Science Research Institute , Ardekan University , Ardakan , P.O.Box 184, Iran.

Article code: 1309

Abstract

Background: Prevalence of Metacestode Infections in Afshari Sheep Slaughtered in Bandar Abbas. This study was conducted to assess the prevalence of metacestode infections in Afshari sheep slaughtered in Bandar Abbas. Metacestodes, the larval forms of certain cestode parasites, pose significant health risks to both animals and humans, making their monitoring critical for animal and public health.

Methods: The investigation took place during the winter and spring of 1403 and involved 1,100 sheep randomly selected from a slaughterhouse in Bandar Abbas. In collaboration with the local veterinary authorities, samples were examined directly on the slaughter line under stringent hygienic protocols. The heart and skeletal muscles were inspected for *Cysticercus ovis*, the peritoneal cavity for *Cysticercus tenuicollis*, and the liver and lungs for hydatid cysts.

Results: The results revealed that 85% (935 out of 1,100) of the sheep were infected with at least one type of metacestode. *Cysticercus ovis* exhibited the lowest prevalence at 2.1%, predominantly localized in the heart (91%). *Cysticercus tenuicollis* was primarily found in the peritoneal cavity (97.6%), while hydatid cysts were mostly identified in the heart (84.5%) and, to a lesser extent, in the lungs (15.5%).

Conclusion: These findings underscore a high prevalence of metacestode infections in slaughtered Afshari sheep in Bandar Abbas, highlighting the urgent need for enhanced parasite control strategies. Implementing effective control measures is essential to protect both livestock health and public safety.

Keywords: Metacestodes, Afshari sheep, Bandar Abbas city, Slaughterhouse, *Cysticercus ovis*, Hydatid cyst, *Cysticercus tenuicollis*.



Evaluation of Ivermectin Efficacy Against Gastrointestinal Nematodes in Equines

Negar Askari¹, Mohammad Rahim Haji Hajikolaei¹, Alireza Alborzi², Afsaneh Nazarpour Kalaei²

Corresponding Author: Negar Askari, negar.askari079@gmail.com

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1310

Abstract

Background: Ivermectin is a macrocyclic lactone anthelmintic drug used to manage gastrointestinal parasites in horses. Recently, there have been concerns about its effectiveness, particularly regarding early egg reappearance and the survival of immature larvae up to 21 days after treatment. As a result, it is recommended that the treatment be repeated 21 days later. To evaluate the efficacy of ivermectin, this study was conducted on free-grazing horses that were naturally infected with gastrointestinal parasites and had adult worms in their feces.

Methods: *Parascaris equorum* and *Strongylus* spp. infections in three horses were identified by fecal sampling and microscopic examination. Each horse received an oral dose of ivermectin paste at 0.2 mg/kg of body weight. Fecal samples were collected separately from each horse before treatment and on days 1, 2, 7, 14, 21, 28, and 35 after treatment. The eggs per gram (EPG) of feces for each horse were counted using the Clayton-Lane fecal egg flotation technique.

Results: The results of our study showed a decreasing trend in the number of eggs per gram of feces (EPG) after treatment. The mean EPG before treatment (day 0) was 39; on day 1, it was 29.67; and on day 2, it was 20. From day 7 to day 35, no nematode eggs were observed in the feces.

Conclusion: Our findings suggest that a single oral dose of Ivermectin could be a reliable and effective choice for treating and controlling parasitic infections; however, more studies are needed.

Keywords: Equine, Gastrointestinal parasite, Nematode, Ivermectin, Anthelmintic efficacy.



Investigation of Nematode Infection and Radula Structure in *Limacus flavus* (Linnaeus, 1758)

Ebrahim Ghale Kafai¹, Mousa Tavassoli¹, Sepideh Safarzadeh¹, Laya Shamsi²

Corresponding Author: Ebrahim Ghale Kafai, st_e.ghalekafai@urmia.ac.ir

1. Department of Pathobiology, Parasitology Division, Faculty of Veterinary Medicine, Urmia University, Iran.
2. Ph.D in Veterinary Parasitology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article code: 1312

Abstract

Background: Slugs are reported worldwide and recognized as significant agricultural pests. Nematodes exhibit diverse associations with slugs, ranging from commensalism to parasitic and pathogenic relationships. This study aimed to investigate the radula structure of the slug *Limacus flavus* (Linnaeus, 1758) and its infection with nematode larvae in northern Iran.

Methods: A total of 104 slugs were collected from three geographical regions in Talesh County, Gilan Province: urban (N 37.80484421°, E 48.90812941°; altitude 39 m above sea level), plain (rice fields) (N 37.81837209°, E 48.90879460°; altitude 31 m above sea level), and mountainous (N 37.87553619°, E 48.64855561°; altitude 1516 m above sea level). The slugs were identified using taxonomic identification keys. Their buccal masses were dissected and digested in 7% potassium hydroxide (KOH) solution to extract the radulae, which were then stained with Mallory II staining. To detect nematode infection, the visceral organs of the slugs were examined for nematode larvae using the Baermann funnel technique.

Results: The radular tooth formula of *L. flavus* (Linnaeus, 1758) was determined, with the maximum number of tooth rows being 145 ± 5 . The central tooth was rectangular with rounded edges at the radula's center. All examined *L. flavus* specimens were infected with larval nematodes of the genera *Strongyloides*, *Rhabditis*, and *Alloionema*.

Conclusion: This study revealed infection with various nematode larvae in slugs. Further research is recommended to identify parasitic nematodes and elucidate the role of *L. flavus* (Linnaeus, 1758) as an intermediate host.

Keywords: Radula, *Limacus flavus*, *Strongyloides*, *Rhabditis*, *Alloionema*, Nematode, Iran.



Allergenic Effects of *Echinococcus granulosus*

Hadi Ebrahimnejad¹, Zahra Moayyed²

Corresponding Author: Zahra Moayyed, Zahra.moayed5931@gmail.com

1. Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
2. Faculty of Veterinary Medicine; Shahid Bahonar University of Kerman, Kerman, Iran.

Article code: 1319

Abstract

Background: Cystic echinococcosis (CE), caused by the parasite *Echinococcus granulosus*, not only leads to tissue damage but also stirs potent allergic responses. Surprisingly, several of its proteins behave like classic allergens, structurally conserved and biologically active, capable of triggering IgE-mediated hypersensitivity.

Methods: This study was performed through a comprehensive search across multiple scientific databases such as PubMed to ensure broad coverage of peer-reviewed research.

Results: Key allergens in *E. granulosus* include well-characterized diagnostic proteins AgB and Ag5. AgB, a potent Th2-skewing protease inhibitor, induces IL-4 and IL-13 without promoting IL-12. Ag5, with serine protease activity, also provokes IgE responses. A newly identified allergen from protoscoleces of *E. granulosus*, EA 21, shows strong homology with allergens from *Malassezia*, and *Schistosoma*, and exhibits significant IgE-binding variation in allergic versus non-allergic individuals. Another critical allergen presents in protoscoleces and hydatid fluid is Eg EF-1 β/δ , an elongation factor that binds IgE in 90% of cystic echinococcosis patients with allergic symptoms, promoting a Th2-skewed immune response. Epitope mapping shows striking similarity between this and *Strongyloides stercoralis* elongation factors. These antigens, especially paramyosins and elongation factors, display cross-reactivity across species, not just among parasites but even with environmental allergens like birch pollen (Bet v 7) or enzymes used pharmaceutically (e.g. Thiomucase®), implicating a broader clinical impact.

Conclusion: *Echinococcus granulosus* allergens blur boundaries between infection and allergy. These discoveries on these allergens not only open new paths for diagnostics and therapeutics but also emphasize the importance of monitoring food sources. Ingesting hydatid-infested meat/offal may expose individuals to hidden parasite allergens, triggering severe allergic reactions. This highlights the critical need for accurate veterinary screening in slaughterhouses and ensuring food safety.

Keywords: *Echinococcus granulosus*, Allergens, Hypersensitivity, Food safety.



Immunoenhancing Effects of Vitamin D₃ on *Leishmania tropica*-Infected Macrophages

Razie Zeinali¹, Ali Afgar², Iraj Sharifi³, Zahra Babaei³, Amirhosein abbasi⁴

Corresponding Author: Zahra Babaei, bzahra580@gmail.com

1. Department of Parasitology and Mycology, Afzalipour Faculty, Kerman University of Medical Sciences, Kerman, Iran.
2. Research Center for Hydatid Disease in Iran, Kerman University of Medical Sciences, Kerman, Iran.
3. Leishmaniasis Research Center, Kerman University of Medical Sciences, Kerman, Iran.
4. Laboratory Department, Asa Health Test Company, Tehran, Iran.

Article code: 1323

Abstract

Background: Cutaneous leishmaniasis caused by *Leishmania tropica* remains a persistent global health concern, particularly due to emerging drug resistance. The World Health Organization has highlighted the urgent need for novel or adjunctive therapies to improve treatment outcomes.

Methods: This study evaluated the effects of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], both alone and in combination with meglumine antimoniate (Glucantime®), on macrophages infected with two *L. tropica* isolates—one Glucantime®-responsive and one resistant. Macrophages were treated with 10–100 nM concentrations, and outcomes assessed included parasite burden, cytokine gene expression, antioxidant enzyme activity (catalase and superoxide dismutase), and lipid peroxidation (MDA levels).

Results: Vitamin D₃, alone or with Glucantime®, significantly reduced both promastigote and amastigote counts, especially in infections caused by the drug-responsive isolate. Treatment enhanced pro-inflammatory cytokines (IFN-γ, TNF-α, IL-12p40) while suppressing anti-inflammatory markers (IL-4, IL-10, TGF-β). Antioxidant activity improved, accompanied by reduced oxidative damage as indicated by decreased MDA levels.

Conclusion: These results support the potential of 1,25(OH)₂D₃ as a promising adjunct therapy to Glucantime®, capable of boosting host immune responses, mitigating oxidative stress, and improving efficacy against *L. tropica* infection, particularly in sensitive strains.

Keywords: Vitamin D, *Leishmania tropica*, Cutaneous leishmaniasis, Drug resistance, Macrophage, Combination therapy.



Evaluation of Golden Eagle Lice Infestation in Northern Iran

Seyedeh Mohanna Zarei¹, Behrad Asghari¹, Sara Sadat Pourmirafzali Firouzabadi¹, Maryam Hosseinpour Mohammadabadi¹, Seyed Javad Nourbakhsh Lamouki¹

Corresponding Author: Seyedeh Mohanna Zarei, mohannazarei73@gmail.com

1. Department of Parasitology, Bab.C., Islamic Azad University, Babol, Iran.

Article code: 1324

Abstract

Background: Golden eagles (*Aquila chrysaetos*) are native birds of prey that inhabit highlands and cold regions and can host many parasites, including ectoparasites. Golden eagles are known to harbor several genera of chewing lice such as *Degeeriella*, *Falcolipeurus*, and *Laemobothrion*, which are considered host-specific ectoparasites of large raptors. Previous researches in Europe and Asia have consistently reported these lice on *Aquila chrysaetos*, confirming their natural association with this species. This study aims to evaluate of lice infestation in golden eagles in northern Iran.

Methods: In this study, a total of 8 golden eagles, including 5 males and 3 females, were seized by environmental agents from illegal hunters in Mazandaran province. All samples were sent to the Parasitology Laboratory of the Faculty of Veterinary Medicine, Islamic Azad University, Babol Branch, in order to examine their ectoparasites.

Results: Three species of parasites were identified, including *Degeeriella aquilarum*, *Falcolipeurus suturalis*, and *Laemobothrion maximum*. Parasitic infection was found in all eagles. Five male eagles (100%) and two females (66.66%) were infected with *Degeeriella aquilarum*. Two male eagles (40%) and one female eagle (33.33%) were infected with *Falcolipeurus suturalis*. *Laemobothrion maximum* infection was found in three male eagles (60%) and one female eagle (33.33%).

Conclusion: Given the results of similar studies, the conservation of this valuable species requires the involvement of expert specialists in areas threatening this bird. The survival and conservation of this bird's population depend on identifying threatened and endangered species and helping to protect its population. Further research on this species, as well as reviewing relevant studies on the conservation of other exotic birds, should provide appropriate strategies for controlling factors leading to population decline.

Keywords: Ectoparasite, Golden Eagle, *Degeeriella aquilarum*, *Falcolipeurus suturalis*, *Laemobothrion maximum*.



Identification of Gastrointestinal Parasites of Native Poultry in Mazandaran Province

Nasrollah Vahedi Noori¹, Javad Abbasi Harazi², Farnaz Seilanian Toosi³, Mahmood Reza Rezaie Erami⁴

Corresponding Author: Nasrollah Vahedi Noori, nsvahedii@gmail.com

1. *Animal Parasitic Disease Research Department, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran*
2. *Doctor of Veterinary Medicine, Working in the Private Sector.*
3. *Doctor of Veterinary Medicine Student, Faculty of Veterinary Medicine , Islamic Azad University, Babol Branch, Babol, Iran.*
4. *Animal science, Mazandaran Agricultural and Resources Research Center, Mazandaran, Iran.*

Article code: 1325

Abstract

Background: Native poultry plays an important role in rural families, especially in developing countries, by providing cheap protein. More than 75% of native chickens in developing countries are raised traditionally and with minimal facilities in rural and nomadic homes. Therefore, they are susceptible to most microbial infections, especially parasites. Considering the role of parasitic infections in native poultry, this study was conducted on native poultry of Mazandaran province.

Methods: For this purpose, 100 native chickens were randomly selected from different regions of Mazandaran province. Gastrointestinal helminth parasites were identified by necropsy and microscopic examination.

Results: A total of 100 native birds examined, all of them had gastrointestinal parasitic infections. The present study shows that four genera of digestive parasites have been identified in native poultry of Mazandaran province, including: *Ascaridia* (75%), *Cheilosporura* at a rate of 90%, *Tetrameres* (20%) and *Hypoderaeum* (15%).

Conclusion: The results of this study showed that the infection with gastrointestinal parasites is high in native poultry of Mazandaran province, and this issue can be related to the type of keeping and free feeding in their environment. Although parasitic diseases in native poultry do not cause death, they have a significant impact on slowing down their growth rate.

Keywords: Gastrointestinal, Parasites, Native Poultry, Mazandaran.



Evaluation of an Herbal Anticoccidial Compound in Experimental Coccidiosis in Broiler Chickens

Negar Najafifard¹, Fatemeh Arabkhazaeli², Seyed Ahmad Madani³, Sarvenaz Azadnia¹, Fatemeh Babaei⁴, Negin Mahdavi Jafari⁵, Mohammad Saberi⁶

Corresponding Author: Fatemeh Arabkhazaeli, farab@ut.ac.ir

1. DVM student, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
4. Master student, Department of Biology, Faculty of Basic Sciences, University of Shahed.
5. Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
6. PhD student, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1326

Abstract

Background: The Poultry industry accounts for supplying a major part of animal protein worldwide. Coccidiosis is a parasitic disease affecting the poultry industry. Drug resistance, concerns about chemical residues in poultry products, and global demand for healthy meat production have led to research to produce herbal compounds. In this study, an herbal compound for the control of coccidiosis was investigated.

Methods: Broiler chickens were randomly divided into eight groups supplied with feed and anticoccidial agents accordingly. The experiment started when the chickens were 14 days old, challenge groups received oocysts while the treatment groups were supplied with in-feed salinomycin and the herbal anticoccidial compound in 2 different doses (300 and 500 ppm). Oocyst per gram of feces, weight gain, and macroscopic and microscopic lesion scores were recorded and evaluated to assess the anticoccidial effect of the herbal compound.

Results: In general, the groups with anticoccidial additives in the diet had a significantly higher final weight than the untreated group ($p < 0.05$). The salinomycin-treated group showed slightly higher weight gain than the others, and the challenged-untreated control group had the lowest weight gain. The herbal compound in both concentrations was able to reduce the rate of oocyst excretion with a slight difference from the salinomycin group, and all three groups showed a significant difference compared to the control group in reducing the oocysts per gram (OPG).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



In general, the groups including different additives in the diet had a significant effect in preventing macroscopic intestinal lesions. In microscopic assessment, the salinomycin group was able to significantly reduce the parasite load, followed by 300 ppm concentration of the herbal anticoccidial, more than the other two groups.

Conclusion: It can be concluded that the type of treatment has a significant effect on the level of parasitic infection, and herbal anticoccidials may be effective in reducing the parasite infection severity comparable to salinomycin.

Keywords: Anticoccidial, Herbal, Coccidiosis, Poultry, Salinomycin.



A Report on Parasitic Contamination of Raw Edible Vegetables in Mazandaran Province

Nasrollah Vahedi Noori¹, Farnaz Seilanian Toosi²

Corresponding Author: Nasrollah Vahedi Noori, nsvahedii@gmail.com

1. Parasitic Diseases Research Department, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.
2. Doctor of Veterinary Medicine Student, Faculty of Veterinary Medicine, Islamic Azad University, Babol Branch, Babol, Iran.

Article code: 1327

Abstract

Background: Parasitic infections are one of the major health problems in most parts of the world, especially in developing countries such as Iran. The prevalence of these diseases is high in tropical or subtropical regions, reaching up to 90% in some areas. Lack of familiarity with hygiene principles is one of the important factors in the spread of parasitic infections. Many parasites spend part of their development in the external environment as eggs or cysts and are transmitted to humans through water, soil, food, or direct contact, harming their health. This article reports on the observation of parasites and parasite eggs in a raw edible vegetables sample including (leek, basil, mint, chives, parsley, watercress, tarragon, and coriander).

Methods: The sample was examined for parasitic and protozoan contamination according to the FAO method after washing, centrifugation, sediment preparation, and staining.

Results: The results of the examination showed the eggs of the parasite *Enterobius vermicularis* and the adult parasite *Echinococcus sp.*

Conclusion: It is recommended that necessary measures be taken to increase public awareness about proper disinfection of vegetables. Care should be taken in hygienic washing of vegetables that are consumed raw, and by educating people about hygiene principles, the prevalence of parasitic diseases can be reduced.

Keywords: Parasitic, Contamination, Vegetables, Mazandaran.



The Importance of Hard Ticks in Contamination and Transmission of Protozoan Pathogens in Domestic Livestock in Mazandaran Province

Nasrollah Vahedi Noori¹, Sayed Hamid Aghajanzadeh¹, Farnaz Seilanian Toosi²

Corresponding Author: Nasrollah Vahedi Noori, nsvahedii@gmail.com

1. Parasitic Diseases Research Department, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.
2. Doctor of Veterinary Medicine Student, Faculty of Veterinary Medicine, Islamic Azad University, Babol Branch, Babol, Iran.

Article code: 1328

Abstract

Background: Ticks (Acari: *Ixodidae*) transmit a wide variety of pathogens to vertebrates including viruses, bacteria, protozoa and helminths. Ticks are considered to be second worldwide to mosquitoes as vectors of human diseases, but they are the most important vectors of disease-causing pathogens in domestic and wild animals. Due to its geographical and climatic conditions, Mazandaran Province is one of the most susceptible areas to the spread of hard ticks. Therefore, the occurrence of tick-borne diseases in humans and livestock is very likely in Mazandaran Province. So far, much research has been conducted on hard ticks in Mazandaran Province.

Methods: Between 2005 and 2011, research was conducted in Mazandaran Province to identify hard ticks in ruminants (cattle, sheep, and goats) as well as contamination with pathogenic protozoa such as *Babesia*, *Anaplasma*, etc. using PCR.

Results: The results of these studies in Mazandaran Province show that 13 species of hard ticks from 6 genera are actively present in the province. These genera include: *Ixodes*, *Rhipicephalus*, *Hyalomma*, *Boophilus*, *Haemaphysalis*, and *Dermacentor*. On the other hand, the results of various studies in Mazandaran Province show that pathogenic protozoa such as *Babesia*, *Anaplasma*, and *Theileria* are highly prevalent in domestic livestock in the province.

Conclusion: The species diversity of hard ticks in Mazandaran province is high. Therefore, ticks, in addition to their direct harmful effects on livestock and humans, also have the potential to transmit many parasitic agents such as protozoa between livestock and humans. This article provides a comprehensive analysis of tick infestation in domestic livestock in Mazandaran province and their role in the transmission of protozoan pathogens.

Keywords: Hard Ticks, Protozoan Pathogens, Domestic Livestock, Mazandaran.



Occurrence of Cutaneous Leishmaniasis in an 8-Year-Old Male Poodle Dog: A Case Report

Behrad Asghari¹, Seyed Mohammad Hosseini¹, Amir Hossein Moshrefi¹, Samin Katuli¹

Corresponding Author: Seyed Mohammad Hosseini, dr_hosseini2323@yahoo.com

1. Department of Pathology, Bab.C., Islamic Azad University, Babol, Iran.

Article code: 1330

Abstract

Background: Leishmaniasis is a parasitic disease caused by protozoa of the genus *Leishmania*, and it manifests in three main clinical forms: cutaneous, mucocutaneous, and visceral. Cutaneous leishmaniasis is the most prevalent form in tropical and subtropical regions. Leishmaniasis can produce skin lesions that may appear as dry, persistent ulcers, or as moist lesions that tend to bleed easily and heal more rapidly. Over time, both types of lesions can heal, leaving behind depressed, depigmented scars. The disease is transmitted by female sandflies of the genus *Phlebotomus*. This study aimed to characterize the histopathological and cytological features of cutaneous leishmaniasis in a canine case, emphasizing the diagnostic value of Giemsa-stained smears and tissue evaluation.

Methods: An 8-year-old male Poodle dog was brought to a diagnostic laboratory on December 11, 2024, with multiple ulcerative nodules and dry skin lesions on the nasal area. According to the owner, the lesions had progressively increased during the week before examination. The sample was collected from the ulcerative lesions on the nasal area, and a skin smear was prepared and stained using Giemsa stain for microscopic evaluation.

Results: Light microscopic examination of the Giemsa-stained skin smear demonstrated the presence of *Leishmania* amastigotes located extracellularly (outside macrophages), along with epidermal hyperkeratosis. Microscopic observations also revealed the presence of Leishman bodies within macrophages and neutrophils. These findings confirm the parasitic infection in the cutaneous lesions of the dog.

Conclusion: Based on the clinical presentation, microscopic evidence of both intracellular and extracellular *Leishmania* amastigotes, and histopathological features such as lymphocytic infiltration, inflammation, histiocytes, plasma cells, and hyperemia, a definitive diagnosis of cutaneous leishmaniasis in the dog was confirmed. This case highlights the importance of considering leishmaniasis in dogs presenting with ulcerative skin lesions in endemic regions and emphasizes the value and utility of Giemsa-stained skin smear cytology and histopathology for accurate diagnosis.

Keywords: Cutaneous Leishmaniasis, Poodle Dog, Giemsa Stain, Amastigotes.



An Outbreak of Acute Theileriosis in a Sheep Flock in Kerman, Iran

Mohammad Mazrouei Sebdani¹, Soheila Bakhshayesh¹, Zahra Nasre Esfahani¹

Corresponding Author: Mohammad Mazrouei Sebdani, m.mazrouei@iau.ir

1. Department of Veterinary Medicine, Islamic Azad University, B.C., Baft, Iran.

Article code: 1331

Abstract

Background: Theileriosis is a tick-borne protozoan disease of ruminants caused by *Theileria* spp., transmitted primarily by *Hyalomma* ticks. The disease is endemic in many parts of Iran and responsible for considerable economic losses due to high morbidity and mortality.

Methods: An acute outbreak occurred in a flock of 200 adult sheep in Kerman Province, Iran. The flock was presented to the university's mobile veterinary clinic with sudden deaths and progressive illness. Clinical signs included anorexia, marked weakness, persistent high fever, anemia, icteric mucous membranes, and generalized lymphadenopathy, with pronounced enlargement of the prescapular nodes. Approximately 30 animals died shortly after cessation of feed intake. Due to logistical limitations in the field, laboratory confirmation using blood smear or molecular techniques was not feasible. Nevertheless, the consistency and severity of clinical manifestations were considered sufficient for a definitive clinical diagnosis of ovine theileriosis. Affected animals received Buparvaquone (Butalex®, 1 mL/25 kg body weight [BW] every 48 h), Oxyflunex® (1 mL/10 kg BW for four days), and Phosphorus–Vitamin B12 (Cobafos®, standard dosing). Supportive care included Ivermectin 1% (1 mL/25 kg BW, repeated after two weeks), Dexamethasone (2.5 mL/head for three days), GLU-Ca (100 mL/head for three days), and oral Vitamin AD3E (for three days). Housing disinfection and acaricide application were also implemented.

Results: Within one week, mortality markedly declined, and surviving sheep exhibited significant improvement, including restored appetite, reduced fever, normalization of mucous membranes, and regression of lymph node swelling.

Conclusion: This outbreak underscores the importance of timely clinical recognition of ovine theileriosis in field conditions where diagnostic resources are limited. Prompt treatment with buparvaquone and supportive therapy, combined with vector control, proved effective in reducing mortality. Preventive measures such as vaccination, farmer education, and regular veterinary supervision remain essential for sustainable control.

Keywords: *Hyalomma* Tick, Theileriosis, *Theileria*, Animal Husbandry.



Epigenetic Orchestration by MicroRNAs in *Toxocara canis*: Implications for Parasite Development, Host Immune Evasion, and Novel Diagnostic and Vaccination Strategies

Fatemeh Ramzi¹, Sima RoshanFar², Mohadeseh Ramzi³, Golnaz Mobayen⁴, Masoud Lahouty⁵

Corresponding Author: Fatemeh Ramzi , Ramziifatemeh637@gmail.com

1. Department of Medical Parasitology and Mycology. School of Public Health Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Medical Parasitology & Mycology. School of Medicine Urmia University of Medical Sciences, Urmia, Iran.
3. Department of Medical Parasitology and Mycology . School of Public Health Tehran University of Medical Sciences, Tehran, Iran.
4. STD Disease Pediatric Health Research Center. Tabriz University of Medical Sciences. Tabriz, Iran.
5. STD Disease Pediatric Health Research Center. Tabriz University of Medical Sciences. Tabriz, Iran.

Article code: 1333

Abstract

Background: *Toxocara canis* is a parasitic nematode capable of evading host immune responses and persisting in both definitive (dogs, cats) and paratenic (humans) hosts. Emerging studies have highlighted the regulatory role of microRNAs (miRNAs) in controlling parasite development, immune adaptation, and latent infection. These non-coding RNAs modulate critical genes like foxo, TGF- β , and SEMA7A, as well as signaling pathways such as MAPK, Wnt, and PI3K-Akt during larval migration and adult stages.

Methods: This Narrative review included studies from 2013 to 2025, retrieved from PubMed, Scopus, ScienceDirect, Web of Science, and Google Scholar. Search terms included *T. canis*, microRNA, extracellular vesicles, host immunity, drug resistance, larval development, and diagnostic biomarkers. In vivo, in vitro, and bioinformatics-based studies involving RNA-seq and qRT-PCR were analyzed.

Results: Identified miRNAs such as Tc-miR-2305, Tc-let-7-5p, Tc-miR-2881, and Tc-miR-5126 were differentially expressed across parasite tissues including intestine, gonads, and body wall. These miRNAs target host-relevant genes such as ABC transporters, CD22, TGFB1, and CD96, influencing immune evasion. Parasite-derived extracellular vesicle (EV)-associated miRNAs were shown to suppress host TH1 responses while promoting regulatory T cell (Treg) pathways. Additionally, some miRNAs detectable in host blood or bone marrow have potential as non-invasive diagnostic markers. miRNA-mediated regulation also contributes to larval



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



developmental arrest in paratenic hosts and is implicated in benzimidazole resistance mechanisms.

Conclusion: *T. canis* miRNAs act as essential epigenetic regulators that enable parasite survival, migration, immune suppression, and apoptosis avoidance in host environments. These findings provide a basis for developing miRNA-based diagnostic kits, RNAi therapeutics, and novel vaccines targeting parasite persistence and transmission in human and veterinary contexts.

Keywords: *Toxocara canis*, MicroRNA, Extracellular Vesicles, Host Immune Modulation, RNA-Based Vaccine.



NETosis-Mediated Immune Escape in Fascioliasis: A Narrative Review on Diagnostic Advances and Immunotherapeutic Prospects

Mohadeseh Ramzi¹, Sima RoshanFar², Golnaz Mobayen³, Fatemeh Ramzi⁴, Masoud Lahouty⁵

Corresponding Author: Mohadeseh Ramzi, mramzi9731@gmail.com

1. Department of Medical parasitology and Mycology. School of Public Health Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Medical Parasitology & Mycology. School of Medicine Urmia University of Medical Sciences, Urmia, Iran.
3. STD Disease Pediatric Health Research Center. Tabriz University of Medical Sciences. Tabriz Iran.
4. Department of Medical parasitology and Mycology. School of Public Health Tehran University of Medical Sciences, Tehran, Iran.
5. STD Disease Pediatric Health Research Center. Tabriz University of Medical Sciences. Tabriz Iran.

Article code: 1334

Abstract

Background: Fascioliasis, caused by the trematodes *Fasciola hepatica* and *Fasciola gigantica*, remains a significant challenge in human and veterinary medicine due to its complex immunopathology and chronic nature. Neutrophils, key effectors of the innate immune system, respond to parasitic invasion by releasing neutrophil extracellular traps (NETs), consisting of DNA decorated with antimicrobial proteins such as neutrophil elastase, myeloperoxidase, and histones. These NETs immobilize and neutralize parasites; however, *Fasciola* spp. have developed mechanisms to evade this defense. This narrative review synthesizes current knowledge regarding NETosis in fascioliasis, with emphasis on diagnostic advances and potential immunotherapeutic strategies.

Methods: This review explores NETosis involvement in fascioliasis and its implications for diagnosis and immunotherapy. A total of 23 peer-reviewed articles published between 2015 and 2025 were reviewed. Literature was sourced from PubMed, Scopus, and Google Scholar using keywords such as “*Fasciola hepatica*,” “*Fasciola gigantica*,” “NETosis,” “immune evasion,” and “immunomodulation.” Selected studies focused on species-specific NETosis induction, immunomodulatory effects of parasite excretory-secretory products, and methods such as fluorescence microscopy, immunofluorescence staining, and extracellular DNA quantification. Emphasis was also placed on studies utilizing inhibitors like diphenyleneiodonium (DPI) and Sivelestat to differentiate ROS-dependent and independent NETosis pathways.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Results: Both *F. hepatica* and *F. gigantica* triggered suicidal and vital NETosis in neutrophils, depending on host-parasite interactions and exposure duration. *F. gigantica* induced stronger NET responses in bovine than ovine neutrophils. ESPs from both species, especially *F. hepatica*, induced vital NETosis without apoptosis, suggesting immunoregulatory potential. Inhibitors like DPI and Sivelestat helped distinguish ROS-dependent from independent pathways. Both species employed proteolytic enzymes and ROS suppression to degrade NETs, enabling immune evasion and chronic liver pathology.

Conclusion: Understanding how *Fasciola* spp. manipulate NETosis offers opportunities for improved diagnostics and immunotherapeutics. Further research is needed to translate these insights into effective clinical applications.

Keywords: Fascioliasis, NETosis, Immune Evasion, Immunotherapy.



Coincidence of *Babesia canis* and *Candida* spp. Infections in a German Shepherd Dog

Dorin Motamedi¹, Somayeh Bahrami¹, Sajjad Alishiri¹, Reza Naghi ha Najaf Abadi², Bahman Mosallanejad³

Corresponding Author: Dorin Motamedi, doriindmo@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Mycology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
3. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1336

Abstract

Background: In a survey assessing the probable presence of systemic *Candida* infections in dogs, we encountered a notable case coinfecting with *Babesia canis*. This association aligns with the hypothesis that *Babesia*-induced immunosuppression - particularly through T-cell dysfunction and cytokine imbalance - may predispose dogs to opportunistic fungal invasions. Co-infections are rarely reported but carry significant clinical implications. This case report documents a rare coinfection of *Babesia canis* and *Candida* spp. in a young German Shepherd dog.

Methods: A 4-month-old male German Shepherd dog presented to the veterinary hospital exhibiting severe lethargy, anorexia, and pale membranes. A blood sample was taken from the cephalic vein of the dog in EDTA-coated tubes for blood smear preparation and DNA extraction. The hematological parameters were assessed. The conventional PCRs targeting the partial 18S rRNA gene of *Babesia* spp. and the ITS for *Candida* spp. were used. For species identification, the PCR product of the *Babesia* sample was Sanger sequenced.

Results: Blood tests revealed that RBC, HCT, and HGB levels were below the normal range, indicating the dog was suffering from anemia. Blood smears exhibited intraerythrocytic *Babesia* merozoites (piroplasms). Biochemical analysis indicated mild hyperbilirubinemia and elevated liver enzymes, suggestive of hemolysis and secondary hepatic involvement. Molecular confirmation was achieved, followed by BLAST analysis of the sequenced product, which identified the isolate as *B. canis*. Concurrently, fungal ITS amplification confirmed systemic *Candida* spp. infection.

Conclusion: This case demonstrates that co-infection with *Babesia* and *Candida* can occur, likely due to the immunosuppressive effects of *Babesia* infestation. The weakened immune



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



system caused by *Babesia* infection may predispose the host to opportunistic fungal infections such as *Candida*. This highlights the importance of considering secondary infections in cases of babesiosis, especially when immune suppression is evident, and underscores the need for comprehensive diagnostic approaches to ensure effective treatment.

Keywords: *Babesia*, *Candida*, Dog, Coinfection.



Study of the Prevalence of Gastrointestinal Parasitic Worms in Domestic Dogs in Tabriz City

Elyar Azimi Zang Abad¹, Saba Sabbaghkar², Mahdi Farshbaf Nobarian³, Salar Sartipi⁴

Corresponding Author: Elyar Azimi Zang Abad, elyar.azimi@gmail.com

1. Department of Basic Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.
2. Department of Veterinary Medicine, University of Shabestar, Shabestar, Iran.
3. Department of Medical Sciences, Tabriz, Iran.
4. Department of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Article code: 1337

Abstract

Background: With the increasing trend in pet dog ownership, strict adherence to health protocols has become essential to prevent the transmission of zoonotic diseases between dogs and humans. Neglecting such protocols poses serious risks to both canine health and human well-being.

Methods: Fecal samples were collected from dogs of both age groups: under 6 months and 6 months or older. All animals had a documented history of routine vaccination and antiparasitic treatment. Samples were obtained directly from the rectum using sterile gloves to minimize contamination. The flotation technique was employed for the detection of helminth eggs, utilizing a saturated zinc sulfate (ZnSO_4) solution (specific gravity: ~ 1.18) as the flotation medium.

Results: This study aimed to assess the prevalence of gastrointestinal helminth infections in domestic dogs in Tabriz, Iran. A total of 176 dogs were examined at the veterinary clinic, of which 34 cases (19.31%) were found to be infected with at least one species of gastrointestinal helminth. Among the infected dogs, 21 were under 6 months of age, including 11 cases of *Toxocara* spp. Infection, 3 cases of hookworm infection, and 7 cases with co-infection of *Toxocara* spp. And hookworms. The remaining 13 infected dogs were over 6 months old, comprising 6 cases of *Toxocara* spp. Infection, 3 cases of hookworm infection, and 4 cases of co-infection with both parasites. No cestode infections were detected in any of the examined dogs.

Conclusion: The findings indicate a relatively high prevalence of gastrointestinal helminths among domestic dogs (19.31%). In dogs over 6 months of age, key contributing factors included exposure to contaminated environments (e.g., parks), use of counterfeit anthelmintics available on the market, purchase of dogs from unhygienic breeding centers, and inadequate implementation of routine deworming protocols in some veterinary clinics in Tabriz. These



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



findings emphasize the necessity for public education, regulatory control over antiparasitic medications, and standardized deworming programs.

Keywords: Gastrointestinal Helminths, Domestic Dogs, Deworming, Zoonoses, Parasitology, Tabriz.



Epidemiology and Prevalence of *Neospora caninum* Infection and Its Impact on Abortion in Cattle in Hormozgan Province, Iran

Seyyed Amirali Foroughi¹, Fateme Azizipour²

Corresponding Author: Seyyed Amirali Foroughi, Ali1379foroughi@gmail.com

1. Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Islamic Azad, Karaj Branch.
2. Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Islamic Azad, Karaj Branch.

Article code: 1339

Abstract

Background: *Neospora caninum* is an obligate intracellular protozoan parasite and a major cause of abortion in cattle worldwide. The parasite has a complex life cycle. dogs serving as definitive hosts and cattle as intermediate hosts. Vertical transmission plays a critical role in the maintenance of infection within herds, posing a serious threat to reproductive performance and herd productivity. Despite its economic significance, data on the prevalence and impact of *N. caninum* in southern regions of Iran, particularly Hormozgan province, remain scarce. This study was conducted to evaluate the seroprevalence of *N. caninum* and its association with abortion in both indigenous and imported cattle in Bandar-Abbas region.

Methods: A total of 120 serum samples were collected from dairy (60 samples) and beef cattle (60 samples) across several counties in Hormozgan province in a cross-sectional design. The presence of anti-*N. caninum* antibodies was determined using a commercial enzyme-linked immunosorbent assay (ELISA). Additionally, placental and fetal liver tissues from aborted cases were subjected to polymerase chain reaction (PCR) analysis for direct detection of *N. caninum* DNA. Relevant data including abortion history, cattle origin (local or imported), and herd management practices were also recorded.

Results: Serological analysis revealed that 15% of cattle were seropositive for *N. caninum*. PCR confirmed the parasite's DNA in eight tissue samples. The prevalence was significantly higher in imported cattle (20%) compared to indigenous cattle (10%) ($P < 0.05$). A statistically significant correlation was also observed between seropositivity and a history of abortion.

Conclusion: This study provides evidence of active *N. caninum* infection in cattle in Bandar-Abbas region, with a clear association with abortion. The findings highlight the need for improved surveillance, preventive strategies, and farmer education to mitigate the reproductive and economic losses associated with neosporosis.

Keywords: *Neospora caninum*, Abortion, Cattle, Hormozgan Province.



Seroprevalence of Cystic Echinococcosis among the Population with Substance Use Disorders in Fars Province, Southern Iran: A Cross-sectional Study

Sara Shahriarirad¹, Seyed Abolfazl Soltani¹, Mina Zarrabi¹, Sahel Rohampour¹, Ghazal Shayan¹, Mostafa Omidian¹, Jamal Sarvari², Bahador Shahriari^{3, 4}

Corresponding Author: Bahador Shahriari, Sarkarib@sums.ac.ir

1. Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran.
2. Department of Microbiology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
3. Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
4. Basic Sciences in Infectious Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

Article code: 1340

Abstract

Background: Individuals with substance use disorders (SUDs) may have reduced access to preventive care or delayed diagnosis, which could theoretically increase vulnerability to neglected tropical diseases like *cystic echinococcosis* (CE). This study aimed to determine the seroepidemiological status of human CE among people with substance use disorder (drug addiction) in Fars Province.

Methods: From 2022 to 2024, a total of 1,400 individuals were enrolled in a cross-sectional study. After obtaining informed consent, participants completed a pre-designed questionnaire capturing basic epidemiological and demographic information. Venous blood was collected from each participant. Serological testing for anti-hydatid cyst antibodies was performed using the AgB-ELISA method. Data were analyzed using SPSS software.

Results: Of the 1,400 participants, 1,299 (92.8%) were male and 125 (8.9%) were female. The majority (52.7%) were between 40 and 60 years of age. A total of 233 individuals (16.6%) resided in rural areas, and 1,176 (83.4%) in urban areas. Among the participants, 1,221 (87.2%) were non-injecting drug users, and 179 (12.8%) reported injecting drug use. Anti-hydatid cyst antibodies were detected in 125 individuals, yielding a seroprevalence rate of 8.9%. The highest prevalence (8.9%) occurred in the 40–60 age group. Seroprevalence was higher in males (9.3%) compared to females (4%), and was more common among urban residents and non-



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



injecting drug users. A statistically significant association was observed between seropositivity and age, gender, residence area, and type of drug use ($P > 0.05$).

Conclusion: The findings indicate a considerable rate of hydatid cyst infection among individuals with SUD in Fars Province. Given the specific risk behaviors associated with this population, including limited access to healthcare, poor sanitation, and lower socioeconomic status, it is essential for health authorities to implement targeted preventive strategies to control the spread of CE in the region.

Keywords: Hydatid cyst, Seroepidemiology, Substance Use Disorder, Fars.



The Role of Artificial Intelligence in Revolutionizing Parasitology Education in Veterinary Medicine

Kimia Taheri Kartiji¹, Maedeh sadat Moghaddasi sani¹, Mahsa Shahbakhsh¹

Corresponding Author: Mahsa Shahbakhsh, M1.shahbakhsh@gmail.com

1. *Department of pathobiology, Faculty of Veterinary Medicine, Islamic Azad University, Garmsar Branch, Iran.*

Article code: 1342

Abstract

Background: Parasitology is a fundamental and vital discipline within veterinary sciences, playing a crucial role in the diagnosis, prevention, and control of parasitic diseases in animals. Mastery of the morphology, life cycle, and morphological distinctions of parasites poses significant challenges for students due to the complexity and diversity of parasitic organisms. Given the extensive content volume, variability among parasite species, and limited educational resources, there is an urgent need for innovative and efficient instructional methodologies.

Methods: Incorporating AI not only improves the accuracy and precision of learning outcomes but also reduces reliance on physical resources such as microscopes and specimen collections. Since precise knowledge and practical skills in parasitology directly impact clinicians' ability to diagnose and treat parasitic diseases, leveraging technologies like AI can substantially elevate students' academic performance, reduce diagnostic errors, and better prepare them for clinical practice. This paper explores the various applications of AI in parasitology education, discussing its advantages, potential challenges, and future prospects within the field of veterinary medicine.

Results: Given the conceptual and practical complexities involved in teaching parasitology particularly in disciplines like veterinary medicine AI could serve as a transformative educational aid. Technologies such as machine learning, image recognition, and augmented reality facilitate a shift away from traditional, passive learning towards more personalized, interactive, and precise instructional approaches. However, effective implementation of AI necessitates appropriate data infrastructure, user training, and adherence to ethical considerations. Challenges such as limited domain-specific data in veterinary parasitology and high technical costs must be addressed through strategic planning and long-term investments.

Conclusion: In summation, AI should not be viewed as a replacement but rather as a complementary tool that enhances conceptual understanding and practical skills, ultimately preparing students more effectively for real-world clinical situations.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Artificial intelligence, Medical parasitology, Virtual learning, Veterinary medicine.



Molecular Detection of *Babesia* and *Theileria* Species in Sheep and Goats with a Recent Abortion History in East Azerbaijan Province, Northwest Iran

Parisa Shahbazi¹, Reza Ayoubi¹, Ahmad Nematollahi¹, Monireh Khordadmehr¹, Soheila Aghaalizadeh¹, Maedeh Nazadi¹, Alireza Zandna¹

Corresponding Author: Parisa Shahbazi, p.shahbazi56@gmail.com

1. Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Article code: 1344

Abstract

Background: *Theileria* and *Babesia* belong to a group of protozoan parasites known as *Apicomplexa* within the *Piroplasmida* order. Both *Theileria* and *Babesia* disrupt normal hematological functions in their respective hosts, causing hypoxia, anemia and systemic disease. Immunosuppressive effects associated with such infections may act to increase the susceptibility of pregnant animals to secondary infections and abortion. Thus, the current study demonstrated the prevalence of *Theileria* and *Babesia* infections in small ruminants with a new abortion evidence in East Azerbaijan province, Iran.

Methods: In this study, 200 blood samples from 43 flocks were collected from goat and sheep in nine cities of East-Azerbaijan province, in calving seasons (during fall and winter seasons of 2023). The conventional PCR method using the specific primers was employed for the detection of the *Theileria* and *Babesia* genomes after extracting DNA from the whole blood samples.

Results: Molecular findings revealed the infection rates of 70% and 8% for *Theileria* and *Babesia*, respectively. *Theileria ovis* (64%), *Theileria lestoquardi* (6%), and co-infection with *Babesia* species (8%, *B. ovis* and *B. motasi*) were detected in the positive samples. All *Babesia* positive samples were *Theileria* positive too, so 8% co-infection with *Babesia* and *Theileria* spp. were observed in this study.

Conclusion: Taken together, the detection of *Theileria* and *Babesia* infections with a much higher rate, particularly in the autumn and winter seasons which can result in hypoxia and anemia, can play an indirectly remarkable role in the abortion of animals in this area. Therefore, the importance of efficient management practices to prevent and control of these infections aims at protecting the health and productivity of the small ruminants in this area.

Keywords: Sheep, Goat, *Theileria*, *Babesia*, Abortion.



Application of Artificial Intelligence in Diagnosis and Epidemiological Surveillance of *Toxoplasma gondii*: A Narrative Review

Farnaz Seilanian Toosi¹, Nasrollah Vahedi Noori²

Corresponding Author: Nasrollah Vahedi Noori, Nsvahedii@gmail.com

1. DVM Student, Bab.C. Islamic Azad University, Babol, Iran.
2. Parasitic Diseases Research Department, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

Article code: 1345

Abstract

Background: *Toxoplasma gondii* is an obligate intracellular protozoan parasite with a complex life cycle involving felines as definitive hosts and a wide range of intermediate hosts, including humans and domestic animals. Human toxoplasmosis can range from asymptomatic infection to severe neurologic and ocular disease, particularly in immunocompromised individuals and during congenital transmission. Conventional diagnosis methods such as serology (ELISA, IFA), PCR, and neuroimaging often require expert interpretation and can lack accuracy in early or atypical presentations. Recently, artificial intelligence (AI) has shown promising capabilities in the medical field, including the detection, classification, and prediction of parasitic diseases.

Methods: This narrative review summarizes current literature on the applications of AI in the diagnosis and epidemiological surveillance of *Toxoplasma gondii*. Data were collected from PubMed, Scopus, and Google Scholar, focusing on machine learning (ML), deep learning (DL), and image recognition technologies applied to microscopy, radiology, and serological data.

Results: Several AI-based models have demonstrated high performance in diagnosing *Toxoplasma gondii*. For instance, an image-based fuzzy clustering model using CycleGAN architecture achieved over 93% accuracy in detecting tachyzoites from microscopy images. Deep learning approaches such as MobileNetV2 integrated with U-Net have been used to segment ocular toxoplasmosis lesions with high precision. Moreover, predictive models using climatic and spatial data have been proposed to forecast regional outbreaks in both animal and human populations.

Conclusion: Artificial intelligence offers a valuable enhancement to traditional diagnostic approaches for *Toxoplasma gondii*, improving speed, sensitivity, and consistency. Its integration into veterinary and human parasitology can facilitate early detection, reduce diagnostic errors, and support large-scale epidemiological control. Further development and validation across diverse settings are warranted.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Artificial Intelligence, Diagnosis, Epidemiological, *Toxoplasma gondii*.



A Comprehensive Review of YOLOv9 and Deep Learning Approaches for Automated Malaria Parasite Detection in Blood Smears with Rouleaux Formation

Ali Motamedi¹

Corresponding Author: Ali Motamedi, nariman.motamedi.1999@gmail.com

1. DVM Student, Ka.C., Islamic Azad University, Karaj, Iran.

Article code: 1346

Abstract

Background: Malaria is a life-threatening disease spread to humans by some types of mosquitoes. It is mostly found in tropical countries. Usually, female Anopheles mosquitoes carrying the Plasmodium parasite. Two dangerous Plasmodium parasite types cause malaria in humans are *P. falciparum* and *P. vivax*. For diagnosing the traditional methods relies on microscopic examination of stained blood smears. While considered the gold standard, this method suffers from observer subjectivity, inter reader variability, and the limited availability of skilled microscopists. These factors hinder timely and reliable diagnosis, especially in resource limited regions.

Methods: Artificial Intelligence (AI), specifically deep learning and object detection models like YOLO (You Only Look Once), has revolutionized automated malaria diagnosis. Earlier YOLO versions (v5 to v8) showed high accuracy in detecting *Plasmodium* parasites. These models are usually trained using transfer learning on annotated malaria datasets, supported by data augmentation such as flipping, rotation, and noise addition. However, complex smear morphologies like Rouleaux formations where red blood cells stack and overlap remain difficult to detect accurately.

Results: YOLOv9 introduces notable upgrades, including Programmable Gradient Information (PGI) and the Generalized Efficient Layer Aggregation Network (GELAN), which enhance gradient flow and feature reuse without increasing computational cost. Early results show YOLOv9 performs better than its predecessors in detection accuracy and inference speed, especially in crowded blood smear fields. However, a lack of diverse annotated datasets containing Rouleaux formations reduces its generalizability and limits benchmark comparisons.

Conclusion: YOLOv9 presents a major advancement in AI-powered malaria detection, particularly for challenging smear structures. Wider clinical application requires improved dataset diversity, real world validation, and low resource deployment strategies. Strengthening



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



interdisciplinary collaboration and investing in local diagnostic infrastructure are essential to fully harness the potential of AI for equitable and scalable malaria diagnosis worldwide.

Keywords: YOLOv9, Malaria, Rouleaux Formation, Deep Learning.



The Impact of Latent Toxoplasmosis on Human Mental Health and Neurological Disorders: A Review

Maedeh Bakhshandeh¹, Simin Jalili Abjazi¹, Mahsa Khademi Kalantari¹, Somayeh Bahrami¹

Corresponding Author: Maedeh Bakhshandeh, maedehbakhshandeh1998@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1348

Abstract

Background: *Toxoplasma gondii* infects approximately one-third of the global population. While typically asymptomatic in immunocompetent individuals, growing evidence suggests a link between latent infection and various neuropsychiatric disorders, including schizophrenia, bipolar disorder, autism spectrum disorder (ASD), obsessive-compulsive disorder (OCD), attention-deficit/hyperactivity disorder (ADHD), and generalized anxiety. Proposed mechanisms involve chronic neuroinflammation, modulation of dopaminergic and GABAergic pathways, and structural brain alterations in areas such as the frontal cortex, thalamus, and cingulate gyrus. This review aimed to assess recent epidemiological and neurobiological evidence to clarify the potential causal relationship between *T. gondii* infection and mental disorders and to highlight research priorities.

Methods: We reviewed seven studies published between 2010 and 2024, retrieved from PubMed, Scopus, Web of Science, and Google Scholar, including meta-analyses and large cohort studies. Data were evaluated based on seroprevalence, odds ratios, and neurobiological findings. Six articles met the eligibility criteria and were included.

Results: Meta-analyses reported a pooled *T. gondii* IgG seroprevalence of 38% in individuals with neuropsychiatric disorders, compared to 25% in healthy controls. The strongest associations were found with schizophrenia (OR: 3.3–4.8) and ASD (OR: 4.78), followed by ADHD (OR: 2.50), OCD (OR: 1.86), and anxiety (OR: 1.48). Seropositive individuals often show more severe symptoms and a chronic course. Neuroimaging studies revealed gray matter reduction in key brain regions. Experimental research supports a role for parasite-induced neuroimmune activation and neurotransmitter dysregulation in behavioral and cognitive impairments.

Conclusion: Latent toxoplasmosis is significantly associated with several major psychiatric and neurological disorders. Although causality is not yet established, current findings underscore the need for screening at-risk groups and further interdisciplinary research into mechanisms and interventions.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Autism, Human, Mental Health, Schizophrenia, Toxoplasmosis.



Comparison of Diagnostic Accuracy between Wet Mount and PCR Methods for Trichomoniasis Detection in Pigeons from Tehran

Ali Naser¹, Zahra Davanloo¹, Seyedeh Ommolbanin Ghasemian², Leyla Derakhshan³, Behrad Asghari¹

Corresponding Author: Ali Naser, dr.alinaser@yahoo.com

1. Department of Parasitology, Bab.C., Islamic Azad University, Babol, Iran.
2. Department of Parasitology, Beh.C., Islamic Azad University, Behbahan, Iran.
3. Department of Parasitology, Shu.C., Islamic Azad University, Shushtar, Iran.

Article code: 1349

Abstract

Background: Trichomoniasis is a prevalent parasitic disease in birds caused by the flagellated protozoan *Trichomonas gallinae*. It predominantly affects pigeons and birds of prey. Clinical manifestations include anorexia, diarrhea, dysphagia, caseous plaques in the oral and pharyngeal cavities, and foul-smelling breath in the mouth. The parasite has a direct life cycle and does not require an intermediate host. Transmission occurs through contaminated water and shared food sources or via regurgitated feeding from infected parents to their offspring. Diagnosis typically relies on clinical signs and microscopic examination of wet mount slides prepared from the oral-pharyngeal region. Accurate sampling and thorough microscopic analysis are essential for identifying the flagellated protozoan. Given the possibility of false-negative results in wet mount tests due to sampling or processing errors, this study aimed to evaluate the effectiveness of PCR and compare its diagnostic accuracy with that of the wet mount method.

Methods: Samples were collected from 60 pigeons presented to a veterinary clinic. Using sterile swabs, specimens were aseptically obtained from both the crop and oral cavity of each bird. For each bird, one sample was prepared and examined using wet mount microscopy, while the other was stored in PBS microtubes for PCR analysis. Microscopic examination was performed on fresh samples, and PCR was conducted on the preserved specimens.

Results: Among the 60 pigeons tested, wet mount microscopy identified *T. gallinae* in 24 cases (40%), while PCR detected the parasite in 51 cases (85%).

Conclusion: Wet mount microscopy evaluation showed high specificity and positive predictive value but demonstrated lower sensitivity and overall accuracy compared to PCR. The discrepancy between the two diagnostic methods suggests that wet mount microscopy may miss a substantial number of infections that PCR can detect. A statistically significant difference was observed between the two methods at the 95% confidence level.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Trichomonas gallinae*, Flagellated Protozoan, Wet Mount, PCR.



Assessment of *Cytauxzoon felis* Prevalence and Its Impact on Oxidative Stress and Antioxidant Defense in Feline Populations

Ali Naser¹, Behrad Asghari¹, Sedighe Sharifpour¹, Seyedeh Ommolbanin Ghasemian²

Corresponding Author: Ali Naser, dr.alinaser@yahoo.com

1. Department of Parasitology, Bab.C., Islamic Azad University, Babol, Iran.

2. Department of Parasitology, Beh.C., Islamic Azad University, Behbahan, Iran.

Article code: 1350

Abstract

Background: Cytauxzoonosis is one of the most important blood parasites affecting both wild and domestic cats. The present study aimed to investigate the prevalence of *Cytauxzoon felis* in domestic cats and to evaluate the impact of the disease on oxidative stress parameters, specifically free radical levels and the balance between oxidant and antioxidant mechanisms in the body.

Methods: Blood samples were collected from 100 cats. One portion of each sample was used for PCR analysis; concurrently, the remaining samples were analyzed to assess antioxidant indices and oxidative stress parameters, including catalase activity and lipid peroxidation levels in both healthy and infected cats. Statistical analyses were conducted using Microsoft Excel and SPSS version 24.

Results: The findings revealed that 3 out of 100 cats were infected with *C. felis*, indicating a prevalence rate of 3%. Infected cats exhibited a statistically significant 9% reduction in catalase enzyme activity compared to uninfected cats ($P = 0.004$). Furthermore, lipid peroxidation levels increased by 19.3% in infected cats relative to the parasite-free group, also showing statistical significance ($P = 0.021$).

Conclusion: Stray cats, due to their unrestricted movement and potential to serve as reservoirs, play a crucial role in the transmission of *C. felis*. Therefore, assessing the prevalence of this parasite in cat populations is essential for developing effective preventive strategies.

Keywords: *Cytauxzoon felis*, Blood parasite, Cat, Oxidative stress, Catalase, Lipid peroxidation.



Prevalence of Hemoparasites and Ectoparasites of Horses in Gonbad-e Kavus, Iran: A Cross-Sectional Study

Ali Naser¹, Sedighe Sharifpour¹, Seyedeh Ommolbanin Ghasemian², Leyla Derakhshan³

Corresponding Author: Ali Naser, dr.alinaser@yahoo.com

1. Department of Parasitology, Bab.C., Islamic Azad University, Babol, Iran.
2. Department of Parasitology, Beh.C., Islamic Azad University, Behbahan, Iran.
3. Department of Parasitology, Shu.C., Islamic Azad University, Shushtar, Iran.

Article code: 1351

Abstract

Background: Horse breeding has long been practiced in Iran, with provinces like Golestan, especially Gonbad-e Kavus, serving as major centers. Despite the economic and cultural importance of equine health, parasitic infections particularly hemoparasites and ectoparasites remain a significant challenge. This study aimed to determine the prevalence of hemoparasites and ectoparasites, along with associated risk factors, in horses from Gonbad-e Kavus.

Methods: A cross-sectional survey was conducted over six months (during autumn and winter 2022/23), involving 128 horses showing clinical signs of parasitic infection. Macroscopic examination for ectoparasites was performed over the entire body, and microscopic evaluation was conducted on Giemsa-stained blood smears. Data on age, breed, and sex were also recorded and analyzed.

Results: Out of 128 blood samples, 100 (78.1%) tested positive for Piroplasmosis, with no other hemoparasites detection. The prevalence of infection across age groups was as follows: 18 horses under 2 years, 20 aged 2–4 years, 16 aged 4–6 years, 20 aged 6–8 years, 14 aged 8–10 years, and 12 over 10 years. No statistically significant differences were found among the age groups. ($P = 0.980$). Regarding sex, 52 males and 48 females were infected. In terms of breed, 38 Crossbred, 34 Turkmen, and 28 Thoroughbred horses were infected, with no statistically significant differences detected ($P = 0.589$). The prevalence of ectoparasitic infection was reported in 18 horses, equivalent to 14.06%.

Conclusion: These findings indicate a high prevalence of hemoparasitic infection in the region, raising significant concern. The low rate of ectoparasitic infestation reflects the effective external parasite control measures in the local equine management. However, enhanced surveillance and prevention strategies for blood-borne parasites are recommended to protect animal health and the regional equine industry.

Keywords: Horses, Hemoparasites, Ectoparasites, Piroplasmosis, Gonbad-e Kavus.



Recombinant expression of a *Wolbachia* WSP from *Phlebotomus sergenti* (Diptera: Psychodidae)

Fateme Bamshad^{1,2}, Naseh Maleki-Ravasan¹, Jalal Babaie¹, Fateh Karimian¹, Asal Katebi³, Soheila Ajdary³

Corresponding author: Soheila Ajdary, sohary@yahoo.com

1. Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran.
2. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
3. Department of Immunology, Pasteur Institute of Iran, Tehran, IR Iran.

Article code: 1356

Abstract

Background: *Leishmania tropica* -induced cutaneous leishmaniasis is primarily transmitted directly between humans, necessitating accurate diagnosis and management of both symptomatic and asymptomatic cases—particularly in urban settings where outbreaks are more likely. *Wolbachia*, a non-pathogenic endosymbiont of the sand fly vector, has immunostimulatory properties but remains challenging to study due to its intracellular nature. This study aimed to produce a recombinant *Wolbachia* WSP (rWSP) protein.

Methods: *Phlebotomus sergenti* sand flies were collected from Bam, Kerman Province—an endemic area for anthroponotic cutaneous leishmaniasis (ACL) in Iran—during their active season in June and July 2023. *Wolbachia* endobacteria were detected through molecular identification based on amplification and sequencing of a ~650-bp fragment of the *wsp* gene using universal primers. Specific primers containing *Bam*HI and *Xho*I restriction sites were designed to clone the *wsp* gene into pTG TA and subsequently pET21a vectors. Key expression parameters were optimized to maximize recombinant protein yield.

Results: Colony PCR and sequence analysis confirmed the successful insertion of a 630-bp fragment of the *wsp* gene into the pET21a vector. Optimization of expression conditions showed that the highest rWSP yield was achieved at 37 °C using 0.5 mM IPTG after 4 h of induction. The rWSP protein (~25 kDa) was successfully expressed at both small and large scales. Protein expression was confirmed by SDS-PAGE and Western blotting in both formats.

Conclusion: *Wolbachia* and its associated molecules have emerged as promising targets in leishmaniasis research due to their potential role in modulating host-vector-pathogen interactions. The successful production and purification of rWSP provides a foundation for studying its immunogenicity, developing antibody-detection assays to monitor *Wolbachia* exposure, evaluating the impact of anti-*Wolbachia* immune responses on disease severity, exploring its utility in vaccine or immunotherapy development, and understanding its possible



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



involvement in treatment resistance—ultimately contributing to improved control of cutaneous leishmaniasis in endemic areas.

Keywords: Endosymbiont, Cutaneous leishmaniasis, *Leishmania tropica*, Immunogenicity, Antibody detection.



Application of Artificial Intelligence in Diagnosis of Protozoan Parasites in Veterinary Medicine: A Review of Innovative Technologies and Global Studies

Mohammad Mahdi Darvishi¹, Parinaz Hanafi², Parnia Shams², Mohammad Yazdan Ashouri², Behrad Asghari³

Corresponding Author: Parinaz Hanafi, parinaz82hanafi@gmail.com

1. Department of Veterinary Pathobiology Sciences, GA.C., Islamic Azad University, Garmsar, Iran.
2. Department of Parasitology, Doctor Of Veterinary Medicine Student, Gar.C., Islamic Azad University, Garmsar, Iran.
3. Department of Parasitology, Doctor Of Veterinary Medicine Student, Bab.C., Islamic Azad University, Babol, Iran.

Article code: 1358

Abstract

Background: Recent advancements in artificial intelligence (AI) and machine learning have led to a paradigm shift in the diagnosis of protozoan parasites. This abstract presents an overview of seven specialized AI-based platforms designed to identify parasites such as *Babesia*, *Coccidia*, *Trypanosoma*, *Theileria*, *Leishmania*, *Giardia* and *Entamoeba* in livestock.

Methods: Platforms like Parasight, AI-Parasite Scanner, HemoparAI, ProtozoVision, VetScopeAI, Techcyte, and YOLOv4 have been implemented in countries including India, Spain, Italy, China, Brazil, USA and Turkey. These technologies employ deep learning algorithms to analyze blood smears, fecal images, or lesion photographs, achieving diagnostic accuracy between 81% and 97%, with detection times under 10 minutes.

Results: Parasight in India uses EfficientNet and SimCLR models to detect *Babesia*; HIPRA in Spain developed YOLOv7-based tools to identify *Coccidia* in poultry; HemoparAI in Italy targets *Trypanosoma* with YOLO-TrypPA; ProtozoVision in China identifies drug-resistant *Theileria* strains; VetScopeAI in Brazil detects *Leishmania* through smartphone images of lesions; Techcyte in the USA diagnoses intestinal protozoa including *Giardia* and *Entamoeba* from stained slides; and YOLOv4 in Turkey identifies freshwater protozoa for aquaculture monitoring. These platforms significantly reduce human error and improve diagnostic speed.

Conclusion: AI-driven platforms have revolutionized the diagnosis of protozoan parasite in veterinary practice by enhancing accuracy, efficiency, and accessibility. Despite challenges such as data dependency and the need for algorithm localization, these tools serve as adjunct



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



aids in preventive and control programs for parasitic diseases in both livestock and companion animals.

Keywords: Artificial Intelligence, Parasite Diagnosis, Digital Veterinary Medicine, Deep Learning, Smart Systems.



Molecular Investigation of *Cytauxzoon* Infection in Owned and Stray Cats in Tehran City

Hadis Boujarzadeh¹, Hamidreza Shokrani², Alireza Rocky³, Ali Forouharmehr⁴

Corresponding Author: Hamidreza Shokrani, shokrani.hm@lu.ac.ir

1. Graduate Student in Veterinary Medicine, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
3. Department of Clinical Science, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
4. Department of Animal Science, Faculty of Agriculture, Lorestan University, Khorramabad, Iran.

Article code: 1361

Abstract

Background: Cytauxzoonosis, an emerging tick-borne disease of domestic cats and wild felids, is caused by the protozoa belonging to the genus *Cytauxzoon*. *Cytauxzoon felis* causes a rapidly progressive, fatal disease in domestic cats. Infected domestic cats show clinical signs such as pyrexia, anorexia, dehydration, depression, icterus, and hepatosplenomegaly. The present study was conducted to determine the frequency of *Cytauxzoon* infection in owned and stray cats in Tehran, Iran.

Methods: From January to May 2020, a total of 90 blood samples were collected from domestic cats (50 stray and 40 owned) referred to the Central Veterinary Hospital of Tehran. Microscopic evaluation was performed on the Giemsa-stained blood smears. DNA extraction from blood samples was done using the MBST kit. DNA specimens were tested for the DNA of *C. felis* using a polymerase chain reaction assay targeting the *18S rRNA* gene.

Results: In microscopy 9 specimens were suspected to have piroplasm-like organisms, but no blood sample was PCR-positive for *C. felis*.

Conclusion: It is likely that *Cytauxzoon felis* is absent among cats in Tehran or the infection rate is too low. However, due to the limited number of cats, these results cannot be attributed to the cat population of Tehran city. This findings suggest that just specific methods, such as PCR, is suitable to detect the infection in the areas with low parasitemia.

Keywords: Cytauxzoonosis, *18S rRNA*, PCR, Cat.



The Prevalence of Avian Trichomoniasis in Domestic Pigeons (*Columba livia domestica*) in Khorramabad

Mazyar Rashidi¹, Hamidreza Shokrani²

Corresponding Author: Hamidreza Shokrani, shokrani.hm@lu.ac.ir

1. Graduate Student in Veterinary Medicine, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.

Article code: 1362

Abstract

Background: *Trichomonas gallinae* is a common flagellated protozoan of birds that can cause the avian trichomoniasis. The Columbidae species are the main hosts of *T. gallinae*. The present study was designed to investigate the frequency of *Trichomonas* infection in domestic pigeons (*Columba livia domestica*) from Khorramabad and compare the light- microscopic results obtained from wet smear and Giemsa stain methods.

Methods: In total, 130 domestic pigeons were randomly selected from various locations of Khorramabad city. Two slides were prepared by taking a swab from the oropharynx of each bird. The first slide was immediately investigated by a light microscope for observation of the movement of the parasites. The second one was dried at room temperature, fixed in methanol and stained with Giemsa (Merck, Germany) for microscopic detection of *T. gallinae*.

Results: Out of the total of 130 pigeons, 41 (31. 5%) were infected with *T. gallinae* using the wet smear method. The detection rate of the *Trichomonas* infection was significantly higher in wet smear compared to Giemsa stain method ($p < 0.0001$). All the Giemsa stain positive samples were positive in wet smear. The prevalence of *Trichomonas* infection was significantly higher in nestling birds than in adults ($p < 0.02$). However, no significant difference was found between genders.

Conclusion: The wet smear method is a sensitive technique for the detection of avian trichomoniasis. According to the results, avian trichomoniasis is common in the pigeons of Khorramabad city. The asymptomatic nestling pigeons must be considered as an important source of infection and the identification and treatment of these birds could be effective in reducing the incidence of disease.

Keywords: *Trichomonas gallinae*, Wet smear, Prevalence, Pigeon.



Neosporosis in Dogs from Kermanshah, Iran

Mohammad Hossein Abbasi¹, Hamidreza Shokrani², Saman Salmani³, Bahman Mosallanejad⁴

Corresponding Author: Hamidreza Shokrani, shokrani.hm@lu.ac.ir

1. Graduate Student in Veterinary Medicine, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
3. Department of Clinical Science, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
4. Department of Clinical Science, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1363

Abstract

Background: *Neospora caninum* is known to cause abortion in cattle and hind limb paralysis in dogs. Diagnosis of infection is possible through serological and molecular methods. This study aimed to investigate the seroprevalence of *N. caninum* in dogs from Kermanshah city.

Methods: In January and February 2023, a total of 60 whole blood samples were obtained from domestic dogs of various breeds in Kermanshah city. Blood samples were obtained from the cephalic vein and were centrifuged at 1,500g for 10 min. All sera were diluted at 1:25, followed by serial dilution up to 1:400 in 96 round-bottom-well microplates. The modified direct agglutination test (MAT) was used to diagnose the infection. The method is based on the presence of the *Neospora* tachyzoites as antigen and reveals the IgG antibodies in serum.

Results: Out of the total 60 cases, 16.66% (10/60) had positive serum titers. There was no statistically significant difference in the seroprevalence between males and females ($P = 0.30$).

Conclusion: The modified direct agglutination test (MAT) is a suitable method for screening animals for *Neospora* antibodies. Our findings highlight the need for focus on preventative efforts in dogs of these areas. Preventative efforts such as reducing the number of stray dogs and improving the sanitary conditions must be considered.

Keywords: *Neospora caninum*, Seroprevalence, Dog, Modified direct agglutination.



The Prevalence Rate of Infection to *Toxoplasma gondii* in Domestic and Industrial Breeding Poultry in Isfahan City, Iran, 2020 by Modified Agglutination Test

Nader Pestehchian^{1,2}, Mahnaz Sami¹, Hossain Yousofi-Darani¹, Hosseinali Yousefi¹, Reza Kalantari¹

Corresponding Author: pestechian@med.mui.ac.ir

1. Department of Medical Parasitology and Mycology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

2. Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

Article code: 1367

Abstract

Background: *Toxoplasma gondii* (T. gondii) is a pathogenic and zoonotic parasite, which felines implicate as definitive hosts; intermediate hosts are warm-blooded vertebrates. The protozoa can cause serious symptoms in humans, while in poultry is usually asymptomatic. The prevalence of *Toxoplasma gondii* in poultry due to the way poultry are fed is an important indicator of the distribution of oocysts in the environment; in addition, consumption of raw or under cooked meat of chickens can cause infection in human and other animals. Therefore, in this study, the prevalence rate of infection to *toxoplasma gondii* in domestic and industrial breeding poultry in Isfahan City, Iran, was assessed.

Methods: From three groups of domestic breeding, broiler, and laying eggs poultry, 60 serum samples blood samples were collected. On collected serums, serological modified agglutination test (MAT) was performed. *Toxoplasma gondii*-specific antibodies were assayed by this test. Then, the obtained results were analyzed.

Results: By performing the MAT serological test, 20, 15, and 30 samples were positive in domestic breeding, industrial broiler, and in laying hen samples, respectively. Therefore, relative frequency was 33.3, 25.0, and 50.0 percent in domestic breeding, industrial broiler, and industrial laying eggs, respectively, which by using the chi-square test ($P < 0.050$) between the three groups, a significant difference was observed serologically.

Conclusion: A considerable percent of domestic and industrial poultry was infected with *Toxoplasma gondii*. Therefore, preventive measures should be conducted to provide safe foods for vertebrate animals and human.

Keywords: Agglutination, Poultry, Prevalence, Serology, Iran, *Toxoplasma gondii*.



A Review of Drug Resistance Mechanisms in *Acanthamoeba*: Challenges and Opportunities for Veterinary Therapeutics

Maedeh Bakhshandeh¹, Simin Jalili Abjazi¹, Mahsa Khademi Kalantari¹, Somayeh Bahrami¹

Corresponding Author: Somayeh Bahrami, s.bahrami@scu.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1370

Abstract

Background: *Acanthamoeba* spp. Are widespread free-living protists that cause serious infections such as keratitis and granulomatous amoebic encephalitis (GAE) in humans and animals. In veterinary medicine, treatment failure is common due to the resilience of cysts, rare drug-resistant genotypes (e.g., T5), and adaptive drug tolerance. Delayed diagnoses and lack of standardized treatment protocols further complicate management. This review synthesizes current knowledge on resistance mechanisms and their therapeutic implications in animal hosts. The aim is to identify key resistance factors and highlight gaps in current veterinary strategies requiring future research and innovation.

Methods: Evidence was integrated from genomic, clinical, and pharmacological studies published between 2006 and 2025. Data were sourced from Google Scholar, Scopus, and PubMed, focusing on resistance mechanisms, drug efficacy (in vitro/in vivo), and translational veterinary applications. Ten peer-reviewed articles were critically reviewed.

Results: Agents like hexamidine, clotrimazole, and voriconazole show strong trophozoiticidal activity but limited or uncharacterized cysticidal effects. Amphotericin B resistance appears strain-wide, likely mediated by mitochondrial pathways and multidrug resistance (MDR) transporters. Genomic studies reveal horizontal gene transfer (e.g., *adeF*, *amrA*, *amrB*) from bacterial endosymbionts, particularly in ocular isolates. Co-incubation experiments suggest *Acanthamoeba* can enhance antibiotic resistance in intracellular bacteria such as *Pseudomonas putida*. T5 (*A. lenticulata*) is linked to aggressive, refractory infections. Although PHMB–hexamidine combinations show synergistic effects in animals, treatment protocols remain non-standardized. Manual cysticidal assays limit drug screening. Infections often go undiagnosed in veterinary settings.

Conclusion: Resistance in *Acanthamoeba* stems from cyst resilience, gene transfer, and limited drug efficacy. Urgent needs include rapid diagnostics, high-throughput screening, and clinical trials for combination therapies. Future research should target molecular resistance mechanisms and develop standardized treatment protocols for veterinary use.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Acanthamoeba*, Drug Resistance, Cysticidal Agents, Horizontal Gene Transfer, Veterinary Medicine, Keratitis.



Pigeon Fly (*Pseudolynchia canariensis*) Infestation in Urban Pigeons(*Columba livia*): A Systemic Review

Ali Safari¹, Saman Moradian²

Corresponding Author: saman.moradian@iau.ir

1. Graduated Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, Bab.C., Islamic Azad University, Babol, Iran.
2. Graduated Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, Bab.C., Islamic Azad University, Babol, Iran.

Article code: 1371

Abstract

Background: *Pseudolynchia canariensis*, a hematophagous ectoparasite of pigeons (*Columba livia*), significantly impacts avian health and productivity. Beyond causing anemia, irritation, and reduced vitality in pigeons, it also transmits pathogens such as *Haemoproteus columbae* and poses zoonotic risks through occasional human bites; however, there is limited research on its zoonotic potential. Given its high prevalence and the close contact between pigeons and humans, understanding its biology and effective control measures is essential for both animal and public health.

Methods: This systematic review synthesizes findings from published literature, laboratory research, and field reports on *P. canariensis*. Data were gathered on parasite morphology, life cycle, epidemiology, hematological impacts, and current strategies for prevention and treatment. Both conventional insecticidal approaches and traditional methods were evaluated.

Results: *P. canariensis* was found to infest up to 63.3% of feral pigeons, with an average of 1.81 flies per host. Infestation caused significant hematological disturbances, including reduced RBC, hemoglobin, and zinc levels, along with increased WBC, lymphocytes, MDA, and inflammatory cytokines (TNF- α , IL-1 β). Effective control measures included deltamethrin, permethrin, ivermectin, and silver-chitosan nanocomposites, while traditional practices such as apple cider vinegar and salt baths showed supplementary benefits. Proper loft hygiene and pigeon management significantly reduced infestation rates.

Conclusion: *P. canariensis* is a prevalent and harmful ectoparasite in pigeons. Its control requires an integrated approach combining chemical treatment, environmental hygiene, and biosecurity practices. Continued surveillance and research into resistance patterns and alternative eco-friendly treatments are vital for sustainable management and reducing public health risks.

Keywords: Ectoparasite, Pigeon, *Pseudolynchia canariensis*, Pigeon fly.



Prevalence of *Cryptosporidium* in Free-range Cattle in the Eastern Part of Golestan Province

Mohadese Chitband¹, Elahe Ebrahimzadeh¹

Corresponding Author: Elahe Ebrahimzadeh, eebrahimzade@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1373

Abstract

Background: *Cryptosporidium* is a protozoan parasite that causes gastrointestinal disease in cattle and presents zoonotic risks. Free-grazing cattle may be at an increased risk of exposure to infection; however, data from the eastern Golestan Province are limited. This study investigates the prevalence of *Cryptosporidium* in this region.

Methods: A cross-sectional study was carried out during the spring on 348 free-grazing cattle in eastern Golestan Province. Fresh fecal samples were collected from the ground immediately after defecation and examined for the presence of *Cryptosporidium* oocysts using the modified Ziehl–Neelsen staining technique.

Results: In this study, 348 fecal samples were collected from free-grazing cattle and calves in villages across eastern Golestan Province, including Kalaleh, Maraveh Tappeh, Galikesh, and Minoodasht counties. The samples consisted of 289 adult cattle and 59 calves. Samples were examined microscopically using the modified Ziehl–Neelsen staining technique. Overall, 82 samples (23.5%) were found to be infected with *Cryptosporidium*. Results indicated that 69 samples (19.8%) from adult cattle and 13 samples (3.7%) from calves tested positive for *Cryptosporidium*. Considering the potential role of livestock in spreading *Cryptosporidium* oocysts and associated public health risks, maintaining hygiene and controlling risk factors in traditional, free-grazing livestock farming are essential.

Conclusion: This study shows that *Cryptosporidium* infection is present in free-roaming cattle and calves in eastern Golestan Province, with a considerable prevalence. Because *Cryptosporidium* is zoonotic and affects both animal and public health, it is crucial to adopt effective hygiene practices and manage risk factors in traditional livestock farming to lower infection rates and safeguard both animals and humans.

Keywords: *Cryptosporidium*, Free-grazing cattle, Golestan Province, Zoonosis.



Molecular Detection of Protozoan Parasites in the Semen of Dogs in Iran

Pouria Rafiei¹, Baharak Akhtardanesh², Seyed Morteza Aghamiri², Seyede-Mahshid Mostafavi², Alireza Sazmand¹

Corresponding Author: Alireza Sazmand; alireza.sazmand@basu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Article code: 1374

Abstract

Background: Semen-borne parasites of dogs and their epidemiological importance have not received adequate attention compared to those of livestock. This study aimed to investigate the possible presence of several blood and tissue parasites in the semen of donor dogs using molecular methods.

Methods: From March to May 2022, semen specimens were collected by digital manipulation from 33 adult male purebred dogs of various breeds, all with AKC-certified pedigrees, residing in a breeding kennel in Kerman. The average age of the dogs was 3.58 ± 1.96 years (range: 8 months to 8 years). Genomic DNA was extracted from the specimens using the Exgene DNA extraction kit, following the manufacturer's instructions. The presence of DNA of *Trypanosoma* spp., *Babesia* spp., *Hepatozoon* spp., *Leishmania* spp., and *Neospora caninum* was examined by group-, genus-, and species-specific primers using conventional polymerase chain reaction (cPCR), and confirmed by Sanger sequencing.

Results: cPCR revealed the presence of *Trypanozoon* DNA in 3 out of 33 samples (9%). Obtained nucleotide sequences were identical to *Trypanosoma evansi* type A. DNA of other tested pathogens was not detected.

Conclusion: The detection of *T. evansi* DNA in dogs' semen suggests the potential role of sexual transmission of this parasite in dogs in Iran. This finding warrants further investigation into the reproductive and epidemiological implications of venereal transmission of vector-borne diseases.

Keywords: *Trypanosoma evansi*, PCR, Sexual transmission, Vector-borne pathogens, Venereal transmission.



Recent Advances in Multivalent and Targeted Vaccine Design Against Babesiosis: From Antigen Selection to Immunological Efficacy

Zahra gahvechi amiri¹, Mohadeseh Ramzi², Fatemeh ramzi²

Corresponding Author: Zahra gahvechi amiri, Zahraamiri672@gmail.com

1. Department of Parasitology and Mycology . School of Medicine . Shahid Beheshti University of Medical Sciences , Tehran , Iran.
2. Department of Medical parasitology and Mycology . School of Public Health Tehran University of Medical Sciences , Tehran , Iran.

Article code: 1376

Abstract

Background: Babesiosis is a significant hemoprotezoan disease in domestic animals, primarily caused by *Babesia bovis* in cattle and *Babesia gibsoni* in dogs. Effective disease control remains challenging due to limited immunogenicity of conventional vaccines, the complexity of producing live-attenuated strains, and the incomplete efficacy of current therapeutic regimens. Over the past decade, research has increasingly focused on the development of recombinant subunit vaccines and antigen-specific platforms, resulting in notable advances in immunoprophylaxis against *Babesia* spp.

Methods: This narrative review examines progress in subunit and recombinant vaccines against *Babesia bovis* and *Babesia gibsoni*. Twenty-three articles published between 2014 and 2025 were analyzed, focusing on key antigens, expression systems such as yeast, viral vectors, and DNA, as well as preclinical evaluations and immune outcomes, particularly the role of adjuvants and Th1 responses.

Results: Merozoite surface antigens such as MSA-2a₁, MSA-2b, and MSA-2c in *Babesia bovis*, and adhesive proteins like BgTRAP and BgP50 in *Babesia gibsoni*, have shown strong immunogenicity in recombinant platforms. When formulated with Montanide ISA 720, multivalent vaccines elicited robust IgG responses, elevated IFN- γ and TNF- α levels, and significantly inhibited merozoite invasion. These responses indicate the induction of a protective Th1-type immunity. In addition to surface antigens, ribosomal proteins such as P0 and metabolic enzymes including DHFR-TS and IMPDH represent promising cross-protective targets. Combining multiple antigens enhanced both humoral and cellular immunity, offering broader protection against diverse *Babesia* strains. Optimizing antigen combinations and adjuvant formulations may further improve vaccine efficacy and support the development of next-generation multivalent vaccines against bovine and canine babesiosis.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: The findings highlight that advanced vaccine strategies utilizing functional antigen combinations, optimized recombinant expression systems, and Th1-biased adjuvants can lay the foundation for the next generation of safe and effective vaccines against babesiosis in animals of all ages. Nevertheless, transitioning these findings to field-based veterinary trials and long-term efficacy assessments remains a critical next step in translational vaccine development.

Keywords: *Babesia* spp., Recombinant subunit vaccine, Merozoite surface antigens, Th1 immune response.



Biological Control of Nematodes: A Review of *Bacillus thuringiensis* and Its Nematicidal Compounds

Azar Motamedi Boroojeni¹, Kazem Hajati Bardmili¹

Corresponding Author: Kazem Hajati Bardmili, Kazem82hb@gmail.com

1. Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Article code: 1377

Abstract

Background: Nematode infections are a threat to agriculture, animal welfare, and public health, leading to substantial economic losses and increased healthcare burdens. Many chemical nematicides are currently less effective due to the rising prevalence of drug-resistant nematodes. Furthermore, many are restricted because of their toxicity and environmental impact. Consequently, scientists have become more interested in biological control, a vital alternative to chemical methods. A variety of helpful microbes have been discovered that show great potential for nematode control. However, most of them are only effective against plant-parasitic nematodes. This review aims to present *Bacillus thuringiensis* as a promising approach for the biological control of nematodes. *Bacillus thuringiensis* (*Bt*) is a gram-positive bacterium that produces powerful compounds that kill nematodes—not just the ones that target plants, but those that affect animals too.

Methods: The findings from studies on the nematicidal activity of *Bt*, with a focus on its bioactive compounds and their mechanisms of action, were summarized. In addition, some studies addressing the application of *Bt* in controlling nematode infections in animals were reviewed to highlight its potential in veterinary medicine.

Results: *Bt* produces crystal proteins, a significant group of toxins with potent activity against nematodes, which are effective against a wide range of insect orders, nematodes, mites, protozoa, and even human cancer cells. These compounds exhibit strong efficacy against both animal and plant-parasitic nematodes, highlighting their broad-spectrum potential in biological control.

Conclusion: The remarkable potential of *Bt* paves the way for the development of indigenous and recombinant products, as well as *Bt*-derived nanoparticles.

Keywords: Nematode, *Bacillus thuringiensis*, Biological control, Crystal proteins.



Molecular Detection of Protozoan Parasites in Bottled Drinking Water from Different Cities of Iran

Paniz Khodakarami¹, Zahra Qeysari¹, Zahra Kharaghani¹, Pouria Rafiei¹, Somayeh Bahrami², Alireza Sazmand¹

Corresponding Author: Alireza Sazmand, alireza.sazmand@basu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Bu-Ali Sina University, Hamedan, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran.

Article code: 1378

Abstract

Background: Bottled water is widely considered a safe alternative to tap water. However, contamination with protozoan parasites such as *Cryptosporidium*, *Acanthamoeba*, and *Giardia* remains a public health concern, particularly due to their resistance to conventional disinfection methods. This study aimed to investigate the presence of these protozoa in commercially available bottled water brands across various cities in Iran.

Methods: From March to April 2024, a total of 42 bottled drinking water from different brands were purchased from stores in Hamedan, Tehran, Amol, and Razan. From each brand, 250 mL of water was centrifuged, and the genomic DNA was extracted from the resulting sediments. Specimens were tested for the presence of DNA of *Cryptosporidium*, *Acanthamoeba*, and *Giardia* by conventional PCR assay.

Results: DNA of the examined parasites was not detected.

Conclusion: While a negative result for protozoan DNA in the examined bottled water is reassuring and generally a positive sign for the effective water sourcing, filtration, and packaging processes, it doesn't guarantee the water is completely free of all microorganisms; therefore, the results should be interpreted with caution. Indeed, the results suggest that the concentration of the parasites or their DNA, if present, falls below the detection limit of the method used. Further examinations with more sensitive methods, such as real-time PCR, targeting different genes of the parasites, and more advanced water concentration techniques, may be necessary to confirm their absence or to identify any potentially present at low levels.

Keywords: Bottled water, *Cryptosporidium*, *Giardia*, *Acanthamoeba*.



Chewing Lice in a Common Myna (*Acridotheres tristis*): A Case Report

Shirin Aliakbari¹, Fatemeh Arabkhazaeli^{2, 3}, Mohammad Bagher Ahoo⁴, Mohammad Reza Haddadmarandi⁵

Corresponding Author: Fatemeh Arabkhazaeli, farab@ut.ac.ir

1. DVM student, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Iran.
3. Iranian Research Center for Ticks and Tick-borne Diseases, Faculty of Veterinary Medicine, University of Tehran, Iran.
4. Iranian Museum of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Iran.
5. Central Veterinary Laboratory, Tehran, Iran.

Article code: 1379

Abstract

Background: Lice are common ectoparasites that affect a variety of animal species. While avian lice species are limited to chewing lice, the discomfort, damage, and pathogen transmission caused by them is noteworthy. Despite international reports, data on lice infestations in Common Myna within Iran remain limited.

Methods: An ectoparasite removed from a common myna was referred to the parasitology laboratory for species diagnosis. The referred louse was stored in ethanol 70%. After being cleared with 10% KOH for 18 hours, the collected louse was temporarily mounted in lactophenol for morphological examination.

Results: Based on morphological characteristics, including oral spinous process, the recovered louse was identified as *Menacanthus eurysternus*. This species is a member of Amblycera based on the presence of maxillary palps and concealed four-segmented antennae.

Conclusion: *Brueelia chayan* (Phloptoridae), *Myrsidea invadens*, and *Menacanthus eurysternus* (Menoponidae) have been reported so far in Iran. This study aims to contribute to the documentation of lice species associated with the Common Myna in Iran. The identified ectoparasite was *Menacanthus eurysternus*, belonging to the family Menoponidae. The prevalence and species composition of lice infesting the Common Myna are important for understanding the epidemiology and control of avian ectoparasites.

Keywords: Lice, Common Myna, *Acridotheres tristis*, *Menacanthus*, Phthiraptera.



Helminthic infection in a Rhesus Macaque (*Macaca mulatta*): A Case Report

Mohammad Hossein Asadi¹, Tannaz Eghdami¹, Seyed Kian Mohseni Mofidi², Alireza Ghorbani²

Corresponding author: asadi.mmd@yahoo.com

1. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article code: 1380

Abstract

Background: Helminthic infections in non-human primates (NHPs) represent a pivotal concern in veterinary and biomedical research paradigms, particularly due to their zoonotic implications. Notably, *Ascaris lumbricoides* (Nematoda: Ascarididae) exhibits prevalence across humans, Suids, and NHPs, posing risks to animal welfare and facilitating cross-species transmission in captive environments. In this report, helminthic infections in a male, adult rhesus macaque (*Macaca mulatta*) at Urmia Zoo, located in Urmia, West Azerbaijan Province, Iran, in May 2025 are discussed. It should be noted that the monkey was kept under veterinary supervision.

Method: Three grams of monkey feces were examined by Clayton-Lane flotation technique using Sheather's sugar solution with specific gravity of 1.12, which is high enough to float nematode eggs, cestode eggs, and protozoan cysts.

Results: Three eggs were found following examination, with average dimensions of $65 \times 40 \mu\text{m}$ (length $\times$ width), all of them had same characteristics, like pitted egg shell, oval rounded form and thick outer walls. Mentioned characteristics are most related to *A. lumbricoides* fertilized egg. Mitosis cell division had begun in one of them, although the other two were also fertilized and zygotic mass was present, but no division could be seen.

Conclusion: The detection of fertilized *A. lumbricoides* eggs in rhesus monkey feces highlights the need for routine parasitological screening in captive monkey populations in laboratories and zoos, where most human contact with this species occurs. The zoonotic propensity of *A. lumbricoides* mandates stringent biosecurity protocols to prevent cross-species transmission. The diagnostic efficacy of Clayton-Lane flotation technique reaffirms its strength in helminth eggs recovery. Therefore, sustained surveillance by appropriate diagnostic tests combined with targeted anthelmintic treatments is essential to protect human and animal populations to prevent the transmission of such zoonotic diseases. In order to eliminate the ever-important *Ascaris* species, molecular confirmation for species identification is recommended.

Keywords: *Ascaris lumbricoides*, Flotation Technique, Rhesus Monkey, Zoonoses.



Helminth Infections in a Deer: A Case Report

Tannaz Eghdami¹, Mohammad Hossein Asadi¹, Seyed Kian Mohseni Mofidi², Alireza Ghorbani²

Corresponding author: Tannaz.eghdamio@gmail.com

1. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article code: 1381

Abstract

Background: Helminth infections in wild ruminants (e.g., cervids) constitute a critical nexus in veterinary and ecological health paradigms. Notably, *Moniezia expansa* (Cestoda: Anoplocephalidae) exhibits high prevalence in small ruminants and concurrently, *Neoascaris vitulorum* (Nematoda: Toxocaridae) predominantly causes severe infection in juvenile ruminant hosts. In this report, helminth infections in a male 3-5-year-old red deer (*Cervus elaphus*) at Urmia Zoo, located in Urmia, West Azerbaijan Province, Iran in May 2025 are discussed. It should be noted that the deer was kept under veterinary supervision .

Methods: Three grams of this sample were analyzed using the Clayton-Lane flotation technique, which employed Sheather's sugar solution with a specific gravity of 1.12. This solution is effective in floating nematode eggs, cestode eggs, and protozoan cysts, thereby facilitating their detection.

Results: During the examination of the sample, a *M. expansa* egg was observed, which was recognizable due to its triangular shape, pyriform apparatus, thick bent walls, and lemon-shaped larva. Also, an egg with a thick and uneven outer wall (pitted eggshell) and a dividing zygotic mass was observed, which according to its morphological characteristics, can be said to be a species of the Toxocaridae family and since deer are ruminants, it could be an egg of *N. vitulorum*.

Conclusion: The identification of *M. expansa* and *N. vitulorum* eggs in deer feces underscores the urgent need to monitor wildlife reservoirs for epizootic risks. The Clayton-Lane flotation technique's proven efficacy in isolating helminth eggs confirms its value in parasitological diagnosis. Wild deer likely serve as reservoirs, posing a threat to domestic ruminants on common pastures. Restricting access to these areas offers a practical strategy to reduce helminth infections in livestock. Therefore, sustained surveillance of helminths in wild populations is essential for elucidating epidemiological patterns and developing robust control measures to safeguard ruminant health.

Keywords: Deer, Epizootic Diseases, Flotation Technique, *Moniezia expansa*, *Neoascaris vitulorum*.



Prevalence of Canine Heartworm (*Dirofilaria immitis*) in Golestan Province Shelters: A Zoonotic Risk

Arvin Azadeh¹, Ali Safari², Saman Moradian³

Corresponding Author: Saman Moradian, saman.moradian@iau.ir

1. Graduated Master's Degree of Veterinary Parasitology, Faculty of Veterinary Medicine, Go.C., Islamic Azad University, Gorgan, Iran.
2. Graduated Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, Bab.C., Islamic Azad University, Babol, Iran.
3. Graduated Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, Bab.C., Islamic Azad University, Babol, Iran.

Article code: 1383

Abstract

Background: *Dirofilaria immitis*, the causative agent of canine heartworm disease, is a parasitic nematode with a global distribution. The organism infects the cardiovascular and pulmonary systems of dogs and is passed to the dog via the mosquito vector. Zoonotically competent, the organism is capable of infecting humans and has a considerable public health importance, most notably in regions of frequent human–canine contact. This study aimed to determine the prevalence of *Dirofilaria immitis* infection in shelter dogs in Golestan Province, and to assess potential epidemiological factors such as age and sex associated with infection.

Methods: To assess the prevalence of *Dirofilaria immitis* infection in dogs, 150 blood samples were collected from dogs residing in shelters in Golestan Province, Iran. The samples were processed under standard conditions and transferred to the laboratory for analysis. Three methods of diagnosis were employed: direct smear, modified Knott's test, and rapid tests (commercial kits).

Results: The findings showed that female dogs were more infected (15.9%) than male dogs (14.5%). The infection was more prevalent in dogs with ages ranging from 2 to 3 years (23.3%).

Based on the type of diagnostic method, all three tests were positive in most dogs (47.8%). However, in a smaller number of cases, only Knott's test was positive (4.3%). Additionally, in some dogs, both Knott's test and direct smear were positive (39.1%), as well as Knott's test and rapid test (8.6%).

Conclusion: This study confirms the presence of *Dirofilaria immitis* infection among shelter dogs in Golestan Province, with a relatively higher prevalence in female dogs and those aged 2–3 years. The modified Knott's test demonstrated high diagnostic value, often correlating with other diagnostic methods. Given the zoonotic potential of *Dirofilaria immitis*, these findings



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



also underscore the importance of public health surveillance and vector control measures to reduce the risk of transmission to humans.

Keywords: *Dirofilaria immitis*, Dog, Golestan, Shelter.



Application of Artificial Intelligence in Leishmaniasis Diagnosis

Mohammadreza Motezarre¹, Yasaman Hosseini¹, Anahita Gharagozlou¹, Armina Jalali¹, Sedigheh Nabian²

Corresponding Author: Sedigheh Nabian, nabian@ut.ac.ir

1. DVM Student, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1384

Abstract

Background: Diagnosis of Leishmaniasis, a parasitic disease with significant global health impact, traditionally relies on diagnostic methods like microscopy that are often time-consuming and prone to errors. The integration of artificial intelligence (AI) and machine learning (ML) offers promising avenues for automated and enhanced diagnosis of this neglected tropical disease (NTD). This review summarizes current research on the application of AI in Leishmaniasis diagnosis, to improve diagnostic accuracy and efficiency.

Methods: This abstract draws upon multiple studies that employ diverse methodologies of Leishmaniasis diagnosis, including the development of deep learning models such as Convolutional Neural Networks (CNNs) like AlexNet and specialized frameworks like LeishFuNet. The data analyzed ranges from microscopic images of Leishmania parasites in blood smears or tissue samples, to macroscopic images of cutaneous leishmaniasis (CL) lesions. Skin scraping samples were obtained from the lesions of 292 patients and were subsequently stained with Giemsa. Additionally, the potential of ChatGPT to assist in differential diagnosis based on clinical vignettes has been explored.

Results: Research indicates that AI models can achieve high accuracy in detecting Leishmania parasites and identifying Leishmaniasis. LeishFuNet demonstrated promising capabilities in patient-level Leishmania detection. AlexNet achieved an average accuracy of 95.04% in identifying pictures of 292 CL lesions. Studies using CNNs on microscopic images have also reported high sensitivity and specificity in parasite detection. Studies on ChatGPT suggest its potential in providing accurate differential diagnoses for visceral leishmaniasis (VL) in a significant percentage of cases.

Conclusion: AI-based tools can offer economical, rapid, and accurate diagnostic solutions, especially valuable in endemic regions with limited resources and expertise. Future research should focus on validating these findings across larger and more diverse datasets, addressing challenges related to image quality and differential diagnosis, and developing user-friendly AI-



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



powered tools to further harness the potential of AI in controlling and potentially eradicating Leishmaniasis.

Keywords: Leishmaniasis, Artificial Intelligence, Machine Learning, Neglected Tropical Disease.



Visceral leishmaniosis in dogs from Hamedan, Iran

Alireza Sazmand¹, Georgiana Deak², Ali Kalantari-Hessari³, Pouria Rafiei¹, Navid Etemadi⁴, Hessam Hajilou⁴, Mina Malekmohammadi⁵, Farhad Mahra⁶, Leili Moradi¹, Monireh Khordadmehr⁷, Seyedmousa Motavallihaghi⁸, Ali Fattahi-Bafghi⁹, Mariaelisa Carbonara¹⁰, Stephanie Oren¹¹, Domenico Otranto^{10,12}

Corresponding Author: Alireza Sazmand, alireza.sazmand@basu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Bu-Ali Sina University, 6517658978, Hamedan, Iran.
2. Department of Parasitology and Parasitic Diseases, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 400372, Cluj-Napoca, Romania.
3. Department of Basic Sciences, Faculty of Veterinary Medicine, Bu-Ali Sina University, 6517658978, Hamedan, Iran.
4. Dr. Hajilou's Veterinary Clinic, Hamedan, Iran.
5. Opal Veterinary Clinic, Hamedan, Iran.
6. Dr. Mahra's Veterinary Clinic, Hamedan, Iran.
7. Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, 516665-1647, Tabriz, Iran.
8. Department of Medical Parasitology and Mycology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.
9. Department of Parasitology and Mycology, Infectious Diseases Research Center, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
10. Department of Veterinary Medicine, University of Bari, 70010, Bari, Italy.
11. Department of Anatomic Pathology, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 400372 Cluj-Napoca, Romania.
12. Department of Veterinary Clinical Sciences, City University of Hong Kong, Kowloon Tong, Hong Kong, China.

Article code: 1386

Abstract

Background: *Leishmania infantum* is a sandfly-borne parasite, responsible for diseases in many animal species, including humans. Dogs are the main reservoir hosts and play a crucial role in the epidemiology of the disease. This study aims to report for the first time the presence of *L. infantum* in a western province of Iran, which was previously considered not endemic for this parasite, by confirming the infection in severely infected dogs by parasitological, histopathological, and molecular methods.

Methods: From April 2024 to June 2025, six mongrel dogs with poor body condition and skin ulcers were presented to veterinary clinics in Hamedan. Impression smears and scrapes of the



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



wounds were examined microscopically for *Leishmania* amastigotes. In addition, spleen, skin, and lymph node samples from two dead dogs were collected in a 10% buffered formalin solution, processed, sectioned, stained using hematoxylin and eosin, and examined microscopically. Genomic DNA was extracted from skin wounds' scrapes and tested for the presence of *Leishmania* spp. by conventional PCR targeting a fragment of the parasite's ITS-2 region. PCR products were Sanger sequenced.

Results: Clinical signs included cachexia, severe muscle wasting, muco-/cutaneous lesions multifocally distributed and variably sized, enlargement of lymph nodes, rough and dry hair coat, with alopecic regions characterized by hyperkeratosis, crusts and scales, desquamation, seborrheic dermatitis, and thick dandruff with bright reflections. Amastigotes were observed in scrapes, skin, spleen, and lymph nodes. DNA sequences were identical to *L. infantum*.

Conclusion: These cases should serve as a warning for the need for integrated control measures, including stray animal population control through spaying and neutering campaigns, molecular and serological surveillance, and accessible prophylaxis and treatments. Public awareness about the potential spread of this zoonotic disease in the region and methods of prevention to reduce the risk of infection in both humans and pets are recommended.

Keywords: emerging disease, *Leishmania infantum*, zoonotic, public health.



A Cross-sectional Study of Helminth Parasites in Stray Dogs in Iran Using a One Health Approach

Mohammad Bagher Ahoo^{1,2}, Fateme Jalousian¹, Seyed Hossein Hosseini^{1,2}

Corresponding author: Seyed Hossein Hosseini, hhoseini@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. The parasitology museum, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1388

Abstract

Background: Stray dogs are known reservoirs of zoonotic parasites, the One Health concept is critical in addressing such parasitic threats. This study was conducted to determine the prevalence and diversity of helminth parasites in stray dogs from the southwestern and southern Caspian Sea regions of Iran using a One Health framework.

Methods: The carcasses of 85 stray dogs from northern Provinces of Iran were submitted to the parasitology museum at the faculty of Veterinary Medicine, University of Tehran. Full necropsy examinations were conducted, including the gastrointestinal tract, lungs, heart, kidneys, and sinuses. They were examined macroscopically and microscopically for helminths. Parasites were preserved in 70% ethanol or 30% formalin, stained with lactophenol or azocarmine, and identified morphologically using standard taxonomic keys and confirmed at the Iranian Parasitology Museum.

Results: a total of 16 species of helminths were identified: 7 cestodes, 7 nematodes, 1 acanthocephalan, and 1 pentastomid. Overall, 83.5% of the dogs were infected with at least one parasite, and 62.3% were co-infected with multiple species. The most prevalent helminths were *Dipylidium caninum* (45.9%), *Ancylostoma caninum* (35.3%), *Dirofilaria immitis* (30.6%), and *Echinococcus granulosus* (21.2%). Zoonotic roundworms were also present: *Toxocara canis* in 21.1% and *Toxascaris leonina* in 7.1% of dogs.

Conclusion: These findings emphasize the role of stray dogs as a significant source of environmental contamination and zoonotic risk. The One Health approach is vital to understand these complex transmission cycles and to guide prevention strategies. This study highlights a high burden of helminthic infections in stray dogs in northern Iran. Given the zoonotic potential of several identified parasites, it is essential to implement integrated control strategies including regular deworming, stray dog population management, public education, and environmental sanitation. Adopting a One Health approach is a practical framework for sustainable parasitic disease control in endemic regions.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Echinococcus granulosus*, One Health approach, *Toxocara canis*, Zoonotic helminths.



Comparative Analysis of Native and Denaturing Conditions for the Extraction and Purification of Recombinant Proteins: Focus on *Toxocara canis* Recombinant C-Type Lectin

Narges Amininia¹, Parviz Shayan¹, Seyed Hossein Hosseini^{1,2}, Fateme Jalousian¹

Correspondence Author email: Fateme Jalousian, jalousian_f@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. The Iranian Museum of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1389

Abstract

Background: Different methods for solubilization and purification of recombinant proteins play a crucial role in optimizing protein yield and preserving its biological activity. *Toxocara canis* C-type lectin (*T.canis*-CTL), a key protein secreted by the infective larvae of *Toxocara canis* (*T.canis*). This study aimed the purification of recombinant *T. canis*-CTL under native and denaturing conditions to enhance protein recovery while maintaining its functional integrity.

Methods: the recombinant protein was expressed in *Escherichia coli* BL21 (DE3) using the pET32a vector, with IPTG induction. Various lysis buffers, including those containing urea and lysozyme, were tested for their effectiveness in solubilizing the protein. Purification was performed using Ni-NTA affinity chromatography under both native and denaturing conditions.

Results: indicated that the urea-containing lysis buffer (8 M, pH 8) along with sonication provided the highest yield of soluble protein. Both denatured and native recombinant proteins were successfully purified using Ni-NTA agarose and sepharose resin, with denaturation not affecting the biological activity of the protein. The immunogenicity of the recombinant protein was confirmed by immunizing a New Zealand male rabbit, which developed a specific IgG response.

Conclusion: these findings suggest that denaturation conditions are effective in maximizing recombinant protein yield without compromising its immunological activity. The results highlight the importance of optimizing solubilization and purification conditions to improve recombinant protein production. The preservation of *T. canis*-CTL's immunological activity under Denaturing conditions supports its potential for therapeutic and vaccine development.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: C-type lectin, Immunological activity, Protein purification, Recombinant protein, *Toxocara canis*.



Antitrematodal Effects of *Allium sativum* (Garlic) Extract Against *Fasciola* spp.

Yasaman Hosseini¹, Mohammadreza Motezarre¹, Anahita Gharagozlou¹, Armina Jalali¹, Behnam Meshgi²

Corresponding Author: Behnam Meshgi, bmeshgi@ut.ac.ir

1. DVM Student, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1390

Abstract

Background: Fascioliasis is a parasitic disease caused by *Fasciola hepatica* and *Fasciola gigantica*, affecting both livestock and humans. Current anthelmintic treatments are increasingly limited by the emergence of drug resistance. *Allium sativum* (Garlic), a medicinal plant with well-documented antiparasitic properties, contains bioactive compounds such as allicin that may offer alternative therapeutic potential. This review summarizes findings from both in vitro and in vivo studies evaluating the effects of *A. sativum* extracts on various life stages of *Fasciola* species.

Methods: This review summarizes in-vitro methodologies reported in studies on *Fasciola* species. Reviewed articles included protocols such as miracidial incubation and exposure to alcoholic or hydroalcoholic *Allium sativum* extracts for *F. hepatica*, and treatment of *F. gigantica* flukes or tissue strips with essential oils. Efficacy assessments were based on survival time, motility (microscopy/software), and spontaneous muscular activity (SMA) parameters, including amplitude, frequency, and baseline tension.

Results: The hydroalcoholic extract of *A. sativum* significantly reduced the survival time and motility of *F. hepatica* miracidia in a dose-dependent manner. At a concentration of 10 µg/mL, miracidia were rapidly destroyed, and egg development was markedly inhibited. The essential oil of *A. sativum* significantly decreased the frequency and amplitude of SMA in adult *F. gigantica*, inducing complete and irreversible flaccid paralysis at a concentration of 3 mg/mL in both whole organisms and tissue strip preparations.

Conclusions: Findings from both in vitro and in vivo studies indicate that *A. sativum* extracts exhibit substantial anthelmintic activity, including inhibition of *F. hepatica* egg development, reduced miracidial viability, and paralysis of adult *F. gigantica*. These results support further investigation into garlic-based formulations as a natural alternative or adjunctive treatment strategy for controlling fascioliasis in livestock, and potentially in humans.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Allium sativum*, Fluke, *Fasciola*, Plant.



Human Dirofilariasis Due to *Dirofilaria* spp. in Iran

Armina Jalali¹, Yasaman Hosseini¹, Mohammadreza Motezarre¹, Anahita Gharagozlou¹, Behnam Meshgi²

Corresponding Author: Behnam Meshgi, Bmeshgi@ut.ac.ir

1. DVM Student, Faculty of Veterinary Medicine, University of Tehran, Tehran-Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran-Iran.

Article code: 1391

Abstract

Background: Human dirofilariasis is a zoonotic infection caused by the filarial nematodes *Dirofilaria immitis* and *Dirofilaria repens*, which are primarily transmitted by mosquitoes. These parasites usually infect canids, but human cases have been reported in several parts of the world, including Iran. Although human dirofilariasis is rare, cases have been documented in various provinces of Iran, especially in regions with abundant mosquito populations, such as Bandar Abbas in Hormozgan Province.

Methods: This study reviews documented cases of human dirofilariasis in Iran, with a focus on ocular and subcutaneous manifestations. Diagnostic approaches included retrospective analysis of case reports, histopathological examination, serological testing, and polymerase chain reaction (PCR) for species confirmation. Particular attention was given to a case from Bandar Abbas involving a 20-year-old woman diagnosed with a subcutaneous forehead nodule caused by *D. repens*.

Results: A total of 13 human cases of dirofilariasis have been reported in Iran, most of which involved *D. repens*, particularly in ocular areas. The highest number of cases occurred in Khuzestan and Hormozgan provinces. Clinical symptoms included subcutaneous nodules, ocular infections, and rare cases of *D. immitis* found in the eye and in association with testicular hydrocele. Molecular diagnostic methods have demonstrated high accuracy in identifying *Dirofilaria* species, even in cases where microfilariae were absent.

Conclusion: This study emphasizes the growing concern regarding human dirofilariasis in endemic regions of Iran and underscores the importance of accurate and early diagnosis to avoid misdiagnosis. Given the increasing number of reported cases and the zoonotic potential of these parasites, healthcare professionals should consider dirofilariasis as a possible diagnosis, especially in patients presenting with unexplained subcutaneous or ocular nodules. Improved surveillance, greater public awareness, and enhanced diagnostic methods are essential for effective management and control of this emerging public health issue.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Human dirofilariasis, Zoonotic disease, Ocular infection, Iran.



Emerging Parasitic Zoonoses in Iran: An Overlooked One Health Challenge

Fatemeh Sayareh¹, Hesameddin Akbarein²

Corresponding Author: Fatemeh Sayareh, dr.sayareh@gmail.com

1. *Graduated from Faculty of Veterinary Medicine, Shahid Bahonar University, Kerman, Iran.*
2. *Division of Epidemiology & Zoonoses, Department of Food Hygiene & Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*

Article code: 1392

Abstract

Background: The One Health concept highlights the interconnectedness of human, animal, and environmental health. Understanding this interdependence is crucial for controlling zoonotic diseases. Parasitic zoonoses transmit between humans and animals via direct contact, contaminated food or water, and vectors. Implementing the One Health strategy is key in combating these diseases, which demand collaboration among physicians, veterinarians, researchers, public health professionals, and policymakers. This paper reviews zoonotic parasitic diseases in Iran, with updated insights on parasites affecting wildlife and domestic animals.

Methods: This research is a review study in which articles published in authentic databases such as Scopus, ScienceDirect, Google Scholar and PubMed and also library search were used. The terms “Emerging Parasitic Disease”, “Parasitic Zoonoses”, “Iran” and “One Health” were used simultaneously to search in English sources.

Results: Iran’s diverse climate and socio-economic challenges, along with limited public health infrastructure, contribute to the persistence and spread of zoonotic parasitic diseases. These infections remain a significant public health issue. This review identifies major parasitic zoonoses reported in Iran, focusing on transmission routes, affected hosts, and public health risks. Effective control requires coordinated research and targeted actions. National surveillance systems should monitor zoonotic parasites and risk factors such as human activities (e.g., illegal wildlife trade), which may introduce pathogens into new areas.

Conclusion: Emerging parasitic zoonoses in Iran pose increasing threats to humans and animals. Tackling this issue demands a One Health approach promoting interdisciplinary collaboration and surveillance. Strengthening research, improving monitoring, and raising awareness of risk factors are crucial for controlling these diseases. Integrated strategies will help Iran reduce health risks and support global efforts against emerging zoonotic threats.

Keywords: Emerging Parasitic Disease, Parasitic Zoonoses, Iran, One Health.



Molecular Detection of Tick-Borne Parasites in Ardabil Province

Zahra Heidari¹, Faraz Malek Bagali²

Corresponding Author: Zahra Heidari, zahra61.h@gmail.com

1. Department of Medical Microbiology, Parasitology and Immunology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
2. Students Research Committee, Ardabil University of Medical Sciences, Ardabil, Iran.

Article code: 1395

Abstract

Background: *Theileria* and *Babesia* are among the most important causes of parasitic zoonotic diseases in the world and transmitted by ticks. Due to the appropriate geographical conditions and the high abundance of the livestock industry in Ardabil province, the aim of this study was the molecular identification of tick-borne parasites, *Babesia* spp. and *Theileria* spp. in northwestern Iran, 2024.

Methods: This study employed molecular techniques to hunt down two dangerous parasites, *Babesia* spp. and *Theileria* spp., in 841 ticks collected from cattle, goats, and sheep. In total, 26 pooled samples were subjected to DNA extraction using the Qiagen kit according to the manufacturer's protocol. In this study utilizing PCR that targets the 18S rRNA gene followed by sequencing. Sequence analysis was conducted utilizing data available in GenBank for *Babesia* spp. and *Theileria* spp. isolates, employing bioinformatics tools including the standard nucleotide BLAST.

Results: Using PCR and 18S rRNA gene sequencing, the study uncovered their genetic footprints in 23.07% (*Babesia*) and 15.38% (*Theileria*) of the tested samples. Eleven tick species belonging to six genera were identified. The most abundant hard tick species was *Rhipicephalus bursa* (42.26%), while the most abundant soft tick species was *Argas persicus* (98.93%). PCR method analyses showed that out of 26 pooled samples, 6 (23.07%) pools were positive for 18S rRNA *Babesia* spp, 4 (15.38%) pools were positive for *Theileria* spp..

Conclusion: This is one of the comprehensive reports on Tick-borne parasites in ticks of livestock in Ardabil province. The results of this study demonstrate the presence of *Theileria* and *Babesia* parasites in ticks collected from various regions of Ardabil Province, northwestern Iran. The high prevalence of tick-borne parasites (*Babesia* and *Theileria*) in the area indicates that these parasites are actively circulating in the region, posing a potential threat to both livestock and human health.

Keywords: *Babesia*, Iran, PCR, *Theileria*, Tick.



Logistic Regression Modeling for the Prediction of *Leishmania major* PTR1 Inhibition

Yasamin Raziee^{1,2}, Sima Rafati², Negar Seyed²

Corresponding Author: Negar Seyed, negarse@gmail.com

1. DVM student, Azad Islamic University, Alborz, Iran.
2. Department of Immunotherapy and Leishmania Vaccine Research, Pasteur Institute of Iran, Tehran, Iran.

Article code: 1396

Abstract

Background: Leishmaniasis, a zoonotic neglected tropical disease, suffers from limited and inadequate treatment options. Pteridine reductase 1 (PTR1) is an NADPH-dependent short-chain dehydrogenase reductase that catalyzes the reduction of pterins and folates, serving as a metabolic bypass for dihydrofolate reductase (DHFR) in *Leishmania spp.* which has been identified as a new drug target. In this study, we applied a logistic regression model to rank the top 10 positive and negative features that can help predict the efficiency of another library on the PTR1 receptor based on molecular descriptors.

Methods: 271 compounds examined in vitro studies, which had evident experiments and were labeled as active or inactive, were exported from the PubChem database and analyzed using RDKit and Mordred. This analysis calculated molecular descriptors, resulting in 1229 features of data. StandardScaler normalized the features and VariableDrop discarded highly correlated variables ($|p| > 0.7$). After earning the 334 features logistic regression with class balancing was executed on an 80:20 train-test split. The model was evaluated using Receiver Operating Characteristic-Area Under Curve, Precision-Recall Area Under Curve, Matthews Correlation Coefficient, accuracy, and log-loss. Feature coefficients were obtained and visualized for interpretability.

Results: The logistic regression model gave a Receiver Operating Characteristic - Area Under Curve of 0.939, Precision-Recall Area Under Curve of 0.953, Matthews Correlation Coefficient of 0.78, and log-loss of 0.3319, reflecting superb predictive performance and calibration. Analysis of coefficients identified significant predictive features, including IC1, EState_VSA11, fr_Ar_OH, fr_azo, and fr_thiophene. These descriptors reflect the influence of molecular topology, aromaticity, and electronic distribution on PTR1 inhibitory activity.

Conclusion: Our results indicate that logistic regression is able to precisely classify features of PTR1 inhibitors. The discovered features provide insights into structure-activity relationships and can potentially be used to guide the rational design of anti-leishmanial



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



compounds. This research supports the use of interpretable machine learning for early-stage virtual screening pipelines.

Keywords: Leishmaniasis, Logistic Regression, Machine Learning, PTR1.



Investigation of Drug Resistance in Gastrointestinal Parasites of Ruminant Livestock

Setayesh Sadat Naseri¹, Mobina Sadat Naseri²

Corresponding Author: Setayesh Sadat Naseri, setayesh.n359@gmail.com

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Zabol, Zabol, Iran.

Article code: 1399

Abstract

Background: Drug resistance in gastrointestinal parasites of ruminants, particularly nematodes, stands as a major and increasing issue in veterinary medicine worldwide. This challenge stems primarily from the overuse of anti-parasitic drugs, misapplication of treatment regimens, and genetic changes in the parasites.

Methods: Various studies underscore the severity of the problem in different parts of the country.

Results: In Saqqez area, resistance against albendazole has exceeded to 71% and the suspected resistance against fenbendazole was 90.66%. Nevertheless, in Gilan no resistance has been reported, and Khuzestan has a rate of resistance of 35% in ivermectin. There are multiple biological processes responsible for resistance formation, including mutations in the β -tubulin gene (particularly mutations at codons F200Y, F167Y and E198A), activation of drug efflux systems, and the number of drug-degrading enzymes. Various detection methods, including the Fecal Egg Count Reduction Test (FECRT), Larval Development Test (LDT), and molecular techniques, PCR- ARMS or NGS, have been developed. In recent times, artificial intelligence (AI) has been advocated as the latest approach to forecasting resistance patterns more reliably. Integrated control measures are being adopted to meet this challenge.

Conclusion: These include rotation of drugs, combination therapies, better pasture and quarantine controls, and new technologies such as nanomedicines and subunit vaccines. In the end, in the fight against drug resistance to parasites, the target may not be to do away with anthelmintics, but to instead use them smarter—more strategically and efficiently: one that balances efficacy with future sustainability in parasite control.

Keywords: Ruminant Parasites, β -Tubulin Gene Mutation, Anthelmintic Resistance, Gastrointestinal Parasites.



Evaluating Serum Carotenoid in Poultry Experimental Coccidiosis

Ali Naseri¹, Fatemeh Arabkhazaeli^{2,3}, Seyed Ahmad Madani⁴, Negar Najafifard¹, Sarvenaz Azadnia¹

Corresponding Author: Fatemeh Arabkhazaeli, farab@ut.ac.ir

1. DVM student, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Society of Animal Parasitic Diseases and zoonoses, Iran, Tehran, Iran.
4. Department of Animal and Poultry Health and nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1401

Abstract

Background: Coccidiosis is an intestinal parasitic disease caused predominantly by *Eimeria* species that can cause severe effects on the performance of poultries and the well-being of free-living birds. Pathogenesis involves inhibiting the absorption of the essential dietary components from the alimentary tube and growth impairment. Carotenoids are essential biomolecules that enhance immune status, inhibit oxidative stress, and increase intestinal integrity.

Methods: Broiler chickens randomly divided into challenged and non-challenged groups orally received oocyst on 14 day-old. Carotenoid concentration was measured 7 days post inoculation via spectrophotometry.

Results: The mean carotenoid level in non-challenge birds was 2.27% higher than the challenge group. Non-Parametric Kruskal-Wallis test showed difference in carotenoid level between the groups though the significance does not cross the threshold ($p = 0.055$). While the data suggest a potential decrease in carotenoid levels in challenge birds, the evidence is not conclusive. The higher mean carotenoid level in non-challenge birds, suggests a biologically meaningful difference despite marginal statistical significance.

Conclusion: The marginal difference of carotenoid absorption in this study highlights the need to conduct studies on more diverse groups of broiler breeds available in Iran to draw a baseline data and in field studies to assess the reproducibility and practical value of this biomarker in assessing poultry intestinal health. Though coccidiosis is associated with the reduced blood carotenoid level, many other factors including the diet and genetic factors may contribute as well. Serum carotenoid levels in poultry are not a consistent indicator of coccidiosis severity.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



While intestinal damage from *Eimeria* can reduce carotenoid absorption, birds may maintain circulating levels by mobilizing reserves from the liver and fat. Carotenoids therefore play more of a supportive role in immunity and health rather than serving as a reliable diagnostic marker for coccidial infection.

Keywords: Coccidiosis, Poultry, Carotenoid, *Eimeria*, intestinal health



Bovine Theileriosis as a Model for Studying Human Leukemia

Sina Nazifi¹, Mohammadreza Aslani¹, Nazanin Gharib¹

Corresponding Author: Sina Nazifi, nazifisinavet@gmail.com

1. Department of Clinical Sciences School of Veterinary Medicine University of Shahrekord, Shahrekord, Iran.

Article code: 1403

Abstract

Background: Bovine theileriosis is a tick-borne hemoparasitic disease caused by protozoan species of the genus *Theileria*, primarily *Theileria parva* and *Theileria annulata*. These parasites infect bovine lymphocytes, inducing the uncontrolled proliferation of infected cells, which resembles a cancerous state. *Theileria* parasites exhibit a complex life cycle that involves a mammalian host (cattle) and tick vectors, specifically *Hyalomma* species for *T. annulata* and *Rhipicephalus appendiculatus* for *T. parva*.

Methods: This review pinpoints the existing knowledge gaps in published literature which needs attention on bovine theileriosis as a model for human leukosis.

Results: The schizont stage of the parasite infects host lymphocytes, transforming them into a proliferative and immortalized state, akin to lymphoid neoplasms in humans, such as acute lymphoblastic leukemia. *Theileria* schizonts transform bovine lymphocytes into a cancer-like state, inducing continuous proliferation without the need for external growth factors, resembling the autonomous growth seen in human leukemias, particularly T-cell and B-cell leukemias. The parasite manipulates signaling pathways, such as NF- κ B, which are also dysregulated in human lymphoid neoplasms. For instance, *T. parva* activates the NF- κ B pathway to enhance cell survival, mirroring mechanisms observed in human acute lymphoblastic leukemia. A unique feature of theileriosis is the reversibility of cellular transformation (Treatment with buparvaquone). This reversibility provides a controlled system for studying tumor oncogenesis, which is highly valuable for human leukemia research. *Theileria*-infected cells evade immune surveillance by downregulating MHC class I expression and altering cytokine profiles, similar to immune evasion strategies in human leukemias.

Conclusion: Bovine theileriosis is a robust model for human leukemia due to its induction of cancer-like transformation in bovine lymphocytes, the reversible nature of this transformation, and shared molecular pathways, such as NF- κ B and c-Myc. Despite limitations, such as species-specific differences, ongoing research into the molecular mechanisms of *Theileria* offers promising avenues for understanding and treating human leukemias.

Keywords: Bovine, Theileriosis, Human Leukemia, Model.



Emerging Biotherapeutic Strategies for Wound Management in Companion Animals: A Review of Efficacy and Practical Considerations

Sogand Fallahfar¹, Mahdi Shojaee¹, Mahsa Shahbakhsh²

Corresponding Author: m1.shahbakhsh@gmail.com

1. Department of parasitology science school of veterinary science university of Islamic azad: Garmsar branch, Garmsar, Iran.
2. Department of pathobiology science School of veterinary science University of Islamic azad: Garmsar branch, Garmsar, Iran.

Article code: 1406

Abstract

Background: Wound care in companion animals (cats and dogs) remains a major clinical challenge due to the multifactorial nature of healing and infection control. Rising antibiotic resistance and limitations of synthetic treatments have increased interest in biotherapeutic alternatives such as larval (maggot) therapy and natural products. This review summarizes contemporary veterinary research and compares biotherapeutics with conventional allopathic approaches.

Methods: A literature review examined 41 peer-reviewed articles published after 2000, including original research, reviews, and clinical case studies, primarily sourced from Google Scholar. Studies focused on experimental larval therapy, wound infection management, and comparisons of natural biotherapeutic agents with traditional chemical treatments (e.g., fipronil) in *Canis familiaris*.

Results: Evidence indicates larval therapy can aid debridement of chronic and infected wounds by removing necrotic tissue and reducing bacterial burden, including resistant strains such as MRSA. Natural biotherapeutic products demonstrated promising efficacy against parasites compared with fipronil. Reported benefits include reduced reliance on systemic antibiotics and acceptable safety when protocols are followed. Practical barriers to implementation include owner reluctance toward maggot therapy and limited availability of appropriate dressing materials.

Conclusion: Although studies on large animals are limited, emerging larval technologies and other biotherapeutic approaches appear to be safe and effective alternatives for managing infected wounds in companion animals. Adoption could help mitigate antimicrobial resistance and improve outcomes, but additional experimental and clinical research is needed to establish optimal protocols, address logistical and regulatory issues, and promote clinician training and owner acceptance.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Veterinary wound care, maggot therapy, Biotherapeutics, Companion animals.



Molecular Investigation and Genotyping of *Cryptosporidium* in Fecal Samples of Parrots in Tehran City

Alireza Rastegar Moghaddam¹, Saloomesh Shirali^{1,2}, Kaveh Parvandar¹, Melika Mohammadi¹

Corresponding Author: Saloomesh Shirali, Saloomesh Shirali@iau.ac.ir

1. Department of Veterinary Pathobiology, SR.C., Islamic Azad University, Tehran, Iran.
2. Department of Biotechnology, Ahv. C., Islamic Azad University, Ahvaz, Iran.

Article code: 1409

Abstract

Background: Cryptosporidiosis is one of the important diseases in livestock, birds, and humans, which is of great importance both in terms of health and economy. This disease is caused by *Cryptosporidium* and causes damage through settling on the surface of the mucous cells of the digestive and respiratory systems. The current research aimed to perform molecular and genotypic analysis of *Cryptosporidium* in fecal samples collected from parrots in Tehran.

Methods: From April to November of 2022, 36 samples were collected from the fresh feces of clinically parrots by referring to bird sales centers in Tehran. Stool samples were concentrated by the formol-ether method. The samples were stained using the Ziehl–Nelson method. To detect and observe *Cryptosporidium* oocysts, all the spreads were examined with an optical microscope. DNA extraction from the stool samples was performed using the AccuPrep® Stool DNA Extraction Kit manufactured by Bioneer, Korea. Polymerase chain reaction was utilized to identify *Cryptosporidium* avian genotype III, employing a specific primer pair targeting the 18s ribosomal RNA gene, resulting in a 141 bp fragment.

Results: Of the 36 stool samples obtained, 0 samples (0%) of the total samples were found to be positive for *Cryptosporidium* infection using the Ziehl-Nelson method. Out of 36 parrot feces samples examined, only one sample (2.77%) was found positive for *Cryptosporidium* avian genotype III.

Conclusion: Compared to reports from other regions of Iran and worldwide, the prevalence of *Cryptosporidium* avian genotype III in this study is comparatively lower. The reason for the low prevalence may be the density of parrots per unit area in the maintenance and sales centers. Thus, it is necessary to conduct more studies in this field and consider appropriate strategies for the prevention and control of this disease.

Keywords: *Cryptosporidium*, parrot, Ziehl-Nelson, PCR.



Investigating the Prevalence of *Hepatozoon canis* in Dogs of Gilan Province by PCR

Parvin Mojerloo¹, Saloomesh Shirali^{1,2}, Hooman Rounaghi¹, Melika Mohammadi¹

Corresponding Author: Saloomesh Shirali, Shirali@iau.ac.ir

1. Department of veterinary pathobiology, SR.C., Islamic Azad University, Tehran, Iran.
2. Department of Biotechnology, Ahv. C., Islamic Azad University, Ahvaz, Iran.

Article code: 1410

Abstract

Background: *Hepatozoon canis* is a protozoan parasite that infects dogs and cats. This parasite has a global distribution but is most common in tropical and subtropical regions. It is mainly transmitted by the brown dog tick (*Rhipicephalus sanguineus*). Given that no study has been conducted on the prevalence of this protozoan in Gilan Province, this study aimed to investigate *Hepatozoon* kinesis in blood samples from dogs in Gilan Province using molecular PCR.

Methods: In this research, a number of 60 dog collars were randomly selected from among the stray and owned dogs in different regions of Gilan province, according to the geographical climatic conditions, without considering the age of the breed. After the complete clinical examination, the blood samples were sent to the laboratory. Blood smears were examined microscopically after Giemsa staining, and polymerase chain reaction was performed to examine the genes of *Hepatozoon canis*.

Results: Examination of blood samples taken from dogs and examination of the specific 18SrRNA gene with a size of 660 base pairs after electrophoresis showed that out of a total of 60 blood samples taken from dogs in Gilan province, 8 samples (13.3%) carried the *Hepatozoon canis* gene.

Conclusion: This study is the first report of infection with *Hepatozoon canis* in dogs in Gilan province. An important point in accurately diagnosing the prevalence of this protozoan was the correct use of diagnostic methods, as *Hepatozoon canis* can cause the spread of infection and disease in dogs in the region. Also, given that in this study, infection was seen more in stray and tick-infected dogs, therefore, tick control programs by relevant organizations should be considered in the prevention and control of disease in dogs.

Keywords: Dog, Gilan province, *Hepatozoon canis*, PCR.



Survey of Gastrointestinal Protozoa in Dogs in the Capital of Mazandaran Province

Fatemeh Zahra Gharib¹, Shayan Shafaei², Mohammadreza Youssefi³

Corresponding Author: Fatemeh Zahra Gharib, fzgharib@iau.ac.ir

1. Department of Clinical Sciences, Bab.C., Islamic Azad University, Babol, Iran.
2. Graduate of DVM, Bab.C., Islamic Azad University, Babol, Iran.
3. Department of Parasitology, Bab.C., Islamic Azad University, Babol, Iran.

Article code: 1411

Abstract

Background: Gastrointestinal protozoan infections caused by *Giardia*, *Cryptosporidium*, and *Entamoeba* represent important causes of diarrhea and malnutrition in canine populations. These parasites demonstrate particular pathogenicity in animals. The zoonotic potential of these organisms necessitates consideration of both veterinary and public health implications. Therefore, the purpose of the present study was to investigate the level of contamination with gastrointestinal protozoa in dogs in the center of Mazandaran province.

Methods: From December 2021 to February 2022, fecal samples were collected from 120 dogs in urban and rural areas of Babol, Sari, and Babolsar. Techniques included sodium formalin-ether sedimentation, Ziehl-Neelsen staining for *Cryptosporidium*, and Trichrome staining for *Giardia* and *Entamoeba*. Data were analyzed using SPSS v26 with the one-way ANOVA and Duncan's post hoc test.

Results: Overall, 6.67% of dogs were positive for protozoan infections. Among males (n=74), 93.2% were uninfected, while 4.1% had *Giardia* and 2.7% had *Entamoeba*. Among females (n=46), 93.5% were uninfected, 4.3% had *Giardia*, and 2.2% had *Cryptosporidium*. No significant gender-based differences were observed ($P>0.05$). Prevalence was higher in dogs under 1 year (6.8% *Giardia*, 2.3% *Entamoeba*, 2.3% *Cryptosporidium*) but not statistically significant ($P=0.359$). Diarrheic dogs had significantly higher protozoan infections (11.8% *Giardia*, 5.9% *Entamoeba*, 2.9% *Cryptosporidium*; $P=0.016$). No association was found between fecal mucus/blood and protozoan presence.

Conclusion: This study found a 6.67% prevalence of protozoan infections in dogs from Mazandaran, with diarrhea being a key clinical indicator. The lack of demographic associations suggests broad risk across all dogs. The zoonotic potential of these pathogens highlights the need for routine screening, hygiene practices, and public awareness. These findings support targeted control measures to safeguard both animal and human health in the region.

Keywords: Protozoa, *Giardia*, *Entamoeba*, *Cryptosporidium*, Dog, Mazandaran.



Case Report of Infestation of a Steppe Eagle (*Aquila sp.*) by Three Genera of Chewing Lice in Ahvaz, Iran

Maryam Abbaszadeh¹, Somayeh Bahrami¹, Mansour Mayahi²

Corresponding Author: Maryam Abbaszadeh, maryamabbaszadeh241375@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Livestock, Poultry and Aquatic Animal Health, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1412

Abstract

Background: Chewing lice (*Phthiraptera*), as obligate ectoparasites, are commonly found on birds. These parasites primarily feed on feathers, skin debris, and in some cases, the host's blood. Severe infestations can lead to persistent itching and skin lesions, threatening the host's health. The aim of this study was to report the infestation of three genera of chewing lice on a Steppe Eagle (*Aquila sp.*) from Ahvaz, Iran.

Methods: A Steppe Eagle (*Aquila sp.*) with a wing injury was transferred from the Ahvaz Department of Environment to the Poultry Section of the Veterinary Hospital at Shahid Chamran University of Ahvaz. For ectoparasite examination, the bird was referred to the Parasitology Department of the Faculty of Veterinary Medicine. The eagle was safely restrained without causing harm during sampling, and its head, breast, and wings were carefully examined. Lice were gently removed using forceps and preserved in 70% ethanol. The specimens were then cleared in 10% KOH solution for 24 hours, rinsed with distilled water, dehydrated through an ascending ethanol concentration series, mounted on slides with Canada balsam, and examined under a light microscope. Identification of the specimens was based on the study by Gustafsson et al., (2018).

Results: Examination of the head, breast, and wings revealed a significant infestation of the Steppe Eagle (*Aquila sp.*) with chewing lice from the genera *Laemobothrion* (25), *Degeeriella* (13), and *Colpocephalum* (33), totaling 71 lice.

Conclusion: Parasitic infections, particularly ectoparasite infestations in raptors, play an important role in ecosystems. Although treatment is generally deprioritized due to the ecological importance of parasites, monitoring and management are essential to reduce the risk of zoonotic disease transmission. Severe infestations threaten host health and increase the risk of parasite transmission to humans and domestic animals. Therefore, continuous surveillance and adherence to biosecurity principles are vital for protecting wildlife and public health.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Chewing Lice, Steppe Eagle (*Aquila sp.*), Birds, Ahvaz.



Sensitive Detection of Specific Cell-free DNA in Serum Samples of Sheep Infected with Hydatid Cyst

Mahboubeh Hadipour¹, Hossein Yousofi Darani¹

Corresponding Author: Hossein Yousofi Darani, yousofidarani@gmail.com

1. Department of Pathobiology, Faculty of Veterinary Medicine Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

Article code: 1413

Abstract

Background: Laboratory diagnosis of hydatid cyst usually performed by detection of anti-parasite antibodies in patient serum. However, this method is not useful for follow-up of patients after surgery. One way to solve this problem is detection of specific cell-free DNA (cfDNA) of *Echinococcus granulosus sensu lato* in the sera. So in this work detecting (cfDNA) in sera of naturally infected sheep was investigated.

Methods: Modified phenol-chloroform method was used for extract of DNA from 35 infected sheep. Two mitochondrial genes, i.e. NADH dehydrogenase subunit I (nad1) and Cytochrome C oxidase subunit I (cox1), were considered the optimal targets for PCR amplification, and four sets of primer pairs were designed for PCR and semi-nested PCR. From each extracted sample, two DNA volumes (5 and 10 µl) were amplified using both standard PCR and semi-nested PCR.

Results: Standard and semi-nested PCR on 0.5 ml of serum samples detected *Echinococcus* DNA in 8 and 12 out of 35 sheep, respectively; however, using 2 ml of serum samples, they detected 24 and 27 samples. By increasing the volume of template DNA, the PCRs detected 29 and 33 out of 35 samples. The results were confirmed by sequencing

Conclusions: Using Larger volumes of serum for extraction of DNA, and by using greater volumes of DNA for PCR resulted in increasing the sensitivity of test to 95% with semi-nested PCR protocol. This method can be investigated further to use for the diagnosis of echinococcosis in humans.

Keywords: Hydatid cyst, Cell free DNA, Sheep, PCR.



Investigation of Bacterial and Parasitic Agents Associated with Neonatal Calf Diarrhea in Fars Province

Atefeh Shirazi¹, Yahya Tahamtan¹, Masoumeh Hayati¹, Hajar Molaee¹, Sona Amiri¹

Corresponding Author: Yahya Tahamtan, yahyatahamtan@yahoo.com

1. Microbiology Department, Razi Vaccine and Serum Research, Shiraz-Branch Education and Extension Organization (AREEO), Shiraz, Iran.

Article code: 1414

Abstract

Background: Neonatal calf diarrhea (NCD), or scours, is a major issue in dairy farming, causing significant economic losses due to high mortality and morbidity. NCD results from infectious agents (parasites, bacteria, viruses) and non-infectious factors like poor colostrum intake, gut dysbiosis, and inadequate hygiene or nutrition. This study examined the prevalence of several parasitic and bacterial agents in NCD in Fars Province.

Methods: From January to July 2025, Fecal specimens were collected from 60 diarrheic calves (aged <10 days) and 60 healthy calves from dairy farms in Fars Province. Specimens were transported to the lab, and DNA was extracted using the CedExtra purification kit. PCRs using specific primers were used to detect *Cryptosporidium parvum*, *Giardia* spp. and bacterial (*E. coli* K99, *E. coli* O157, *Salmonella typhimurium*, *S. enteritidis*, *Clostridium perfringens*).

Results: In the diarrheic group, four samples tested positive for *C. parvum*, and five samples tested positive for *E. coli* O157. *C. perfringens* type A was found in all samples (100%) from both groups. Other tested pathogens were not detected.

Conclusion: The limited detection of targeted pathogens, except for *E. coli* O157 and *C. parvum*, indicates that viruses (e.g., rotavirus, coronavirus), other parasites (e.g., *Eimeria* spp.), or non-infectious factors like poor hygiene or inadequate colostrum intake may drive NCD in Fars Province. According to the result, *C. perfringens* type A was found in all samples suggesting it is a normal intestinal flora component. Its role in NCD needs further investigation, possibly via alpha toxin detection using ELISA. Broader diagnostic approaches are needed to identify additional causes and mitigate NCDs' economic impact.

Keyword: Neonatal calf diarrhea, *Cryptosporidium parvum*, *Giardia* spp., *E. coli* O157.



Case Report of Parasitic Infection in a Stray Cat from Ahvaz, Iran

Alireza Alborzi¹, Saleh Esmailzadeh², Sara Larki¹, Afsaneh Nazarpour Kalaei¹, Maryam Abbaszadeh¹, Fatemeh Madankan¹

Corresponding Author: Alireza Alborzi, a.alborzi@scu.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Pathology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1415

Abstract

Background: Parasites are disease-causing agents in cats that pose a risk to their health. Some of them, as zoonotic parasites, can be transmitted from cats to humans. Stray cats are more exposed to parasitic infections and play a significant role in their survival and transmission to other hosts. Identifying these infections in any area aids in their diagnosis, control, prevention, and treatment.

Methods: A stray cat that had died due to an accident was sent to the pathology section of the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz. To detect external parasites, the surface of the animal's body was examined. Internal organs (heart and lungs) and various parts of the gastrointestinal tract (esophagus, stomach, and intestines) were examined for internal parasites. The collected parasites were preserved in 70% ethanol and transferred to the parasitology lab.

Results: The external parasite examination revealed two fleas, *Ctenocephalides felis*. No helminth parasites were observed in the heart and lungs. Internal parasitic infection included nematodes from the stomach, *Physaloptera praeputialis* (three males and three females). Additionally, 35 cestodes, *Joyeuxiella* spp. were identified in the intestines.

Conclusion: The results indicate the presence of various internal and external parasites in the animal. Since these animals are not under veterinary care, they act as reservoirs for parasitic infections, increasing the risk of their survival and transmission to humans and other animals.

Keywords: Cat, Parasitic Infection, Ahvaz, Iran.



Evaluation of Published Primer Sets for Identification of *Cryptosporidium* and *Giardia* spp. by Nested PCR

Masoumeh Hayai¹, Yahya Tahamtan ¹, Atefeh Shirazi¹, Ali Shirazinezhad¹

Corresponding Author: Masoumeh Hayati, hayatimax@yahoo.co.uk

1. Department of research, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Shiraz, Iran.

Article code: 1416

Abstract

Background: A nested PCR protocol has been introduced in some published articles for detection of *Cryptosporidium* and *Giardia* spp. based on the small subunit of rRNA region. In this study, an attempt was made to evaluate these provided primers, which were published by Xiao et al. 1999, Park et al. 2006, Adamaska et al. 2012 and Wei et al. 2020.

Methods: These primers were examined using the NCBI BLAST program (<https://www.ncbi.nlm.nih.gov/Blast.cgi>). The sequences of these outer primers are as follows: F1:TTCTAGAGCTAATACATGCG and R1: CCCTAACCTTCGAAACAGGA for detection of *Cryptosporidium* and F1:AAGTGTGGTGCAGACGGACTC and R1:CTGCTGCCGTCCTTGGATGT for detection of *Giardia*.

Results: The results showed that the first-stage nested reverse primer for *Cryptosporidium* identification is defective and does not have complete similarity to any of the *Cryptosporidium* strains in the GenBank. Its highest similarity (query coverage = 70%) was found for only 14 bases at its 5' end identical to about 150 *Cryptosporidium* strains in the GenBank. Also, the forward primer for the first stage of nested PCR for *Giardia* detection is defective and does not have complete similarity with any of the *Giardia* strains in the gene bank. The highest sequence similarity of this primer for 18 bp at its 5' end was found in only one of the *Giardia lamblia* strains (U09492.1) in the gene bank. For the other strains, this similarity becomes less.

Conclusion: It can be concluded that the use of these outer primers is not appropriate. It can also be suggested to use other primers or the internal or second stage primers in a single PCR. If a nested PCR test is needed, the second-stage reverse primer can be used for the first stage of *Cryptosporidium* identification and the second-stage forward primer for the first stage of *Giardia* identification.

Keywords: *Cryptosporidium*, *Giardia*, PCR, Primer.



Species Distribution and Seasonal Dynamics of Tick Infestation in Dromedary Camels (*Camelus Dromedarius*) in Marand Suburbs

Farnaz Malekifard¹, Mousa Tavassoli¹, Adel Jafari¹

Corresponding Author: Farnaz Malekifard, f.malekifard@urmia.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article code: 1420

Abstract

Background: Ticks are important ectoparasites that cause economic losses in animal husbandry in Iran and worldwide. This study aimed to determine the frequency and species diversity of hard ticks during four seasons in dromedary camels (*Camelus dromedarius*) in Marand, East Azerbaijan province.

Methods: From January 2022 to January 2023, a total of 138 camels (59 male and 79 female) were randomly selected and examined for tick (Acari) infestation. Among the examined camels, 63 were less than 3 years old, while 75 were more than 3 years old. The ticks collected from the body surface of examined camels were transferred to the laboratory and identified according to the keys.

Results: 54 camels (39.13%) were infested with a total number of 386 Ixodid (hard) ticks. Mean intensity for each animal was 7.14. The highest prevalence of hard ticks was found in <3-year-old camels ($P<0.05$). However, no significant differences were found based on the sex of the animal ($P>0.05$). Ticks were of two genera and four species. *i.e.* *Hyalomma dromedarii* (58.80%), *Hyalomma anatolicum* (21.76%), *Hyalomma marginatum* (5.95%), and *Rhipicephalus sanguineus sensu lato* (13.47%). Ixodid ticks' infestations occurred throughout the year, with the highest prevalence in spring ($P<0.05$).

Conclusion: This study presents the first data on tick infestation of camels in Marand suburbs. Further studies to investigate the potential tick vectors involved in the transmission of hemoparasites in camels in the region are recommended.

Keywords: Hard ticks, Camels, Marand, Ectoparasite



Major Symbiotic Bacteria Associated with Nematodes

Mohadese Amiri¹, Majid Jamshidian-Mojaver², Hamidreza Farzin³

Corresponding Author: Hamidreza Farzin, hrfarzin@yahoo.com

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Iran.
2. Mashhad Branch, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Mashhad, Iran.
3. Mashhad Branch, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Mashhad, Iran.

Article code: 1422

Abstract

Background: *Entomopathogenic* nematodes of the genera *Steinernema* and *Heterorhabditis* play a key role in biological pest control. A distinct feature of these nematodes is their specific symbiotic association with bacteria that reside in their intestines and contribute to the infection and killing of insect hosts. The most important of these bacteria are *Xenorhabdus* spp. and *Photorhabdus* spp. *Xenorhabdus* bacteria are symbionts of *Steinernema* nematodes. These are Gram-negative, facultative anaerobic bacteria that are released from the nematode's gut into the insect hemocoel upon infection. They produce toxins, hydrolytic enzymes, and antimicrobial compounds that rapidly kill the insect host and prevent decomposition by other microbes. Notably, *Xenorhabdus nematophila* is widely studied for its application in agriculture. *Photorhabdus*, on the other hand, is symbiotic with *Heterorhabditis* nematodes. Like *Xenorhabdus*, it is also Gram-negative but distinguished by its ability to produce bioluminescence. Species such as *Photorhabdus luminescens* not only contribute to insect mortality but also generate a wide variety of secondary metabolites with antibacterial, antifungal, and even pharmaceutical properties. This unique nematode–bacteria symbiosis forms the basis for environmentally friendly approaches to pest control, serving as an effective alternative to chemical pesticides. Moreover, the bioactive metabolites produced by these bacteria have strong potential for development in pharmaceutical industries. In conclusion, the symbiotic bacteria *Xenorhabdus* and *Photorhabdus* represent a powerful natural system for pest management and biotechnological innovation, combining ecological safety with high efficacy.

Methods: This review systematically examines symbiotic bacteria associated with entomopathogenic nematodes, focusing on *Xenorhabdus* and *Photorhabdus*. Using scientific databases, key studies on their taxonomy, symbiosis mechanisms, insect pathogenesis, and biotechnological applications were analyzed to highlight their role in biological pest control and production of bioactive compounds.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Results: Nematode symbiotic bacteria enable eco-friendly pest control and yield bioactive compounds for agriculture and medicine.

Conclusion: Symbiotic nematode bacteria enable sustainable pest control and yield bioactive compounds for agriculture and medicine.

Keywords: Symbiotic bacteria, *Xenorhabdus*, Biological pest control, Insect pathogenesis.



Canine Diectophymosis: A Case of Renal Parasitism in a Pitbull Dog

Javad Jafari¹, Mostafa Rahimaf¹, and Mohadeseh Hosseinzadeh¹

Corresponding Author: Javad Jafari, javaadjafari1993@gmail.com

1. Private Veterinary Practitioner, Darkoob Veterinary Clinic, Rasht, Iran.

Article code: 1425

Abstract

Background: *Diectophyma renale* infection affects numerous mammal species, primarily in temperate regions. We report the first documented case of *D. renale* infection in a domestic dog from Rasht, Iran.

Methods: The patient was a 20-month-old female dog (30 kg) that presented with anorexia. The owner reported hematuria and purulent genital discharge. The diagnosis was confirmed through complete blood count (CBC), serum biochemistry, and abdominal ultrasound.

Results: Complete blood count and serum biochemistry revealed renal injury. Consequently, abdominal ultrasound was also carried out to rule out other possible pathologies in the differential diagnoses and to confirm the absence of concurrent abdominal pathology. Ultrasound revealed a 35 × 30 mm hypoechoic oval lesion in the mid-cortex of the right kidney. Urinalysis demonstrated hematuria, and microscopic examination revealed *D. renale* eggs. Nephrectomy revealed a live female worm in the right kidney.

Conclusion: While *D. renale* infection is typically a postmortem finding in dogs, this case demonstrates that both ultrasound and urinalysis are essential diagnostic tools. Early diagnosis was achieved through combined imaging and laboratory analysis, enabling successful treatment before end-stage renal damage occurred.

Keywords: Dog, *Diectophyma renale*, Kidney, Pit Bull.



The Transformative Impact of Artificial Intelligence on Diagnostic and Management Strategies in Veterinary Parasitology

Mahdi Shojaei¹, Sogand fallahfar¹, Mahsa Shahbakhsh²

Corresponding Author: Mahdi Shojaei, Mahdish29m@gmail.com

1. Department of Parasitology science School of Veterinary science University of Islamic azad: Garmsar branch, Garmsar, Iran.
2. Department of Pathobiology science School of Veterinary science University of Islamic azad: Garmsar branch, Garmsar, Iran.

Article code: 1426

Abstract

Background: Intestinal parasitic infections remain a substantial public and animal health burden worldwide, with zoonotic species posing significant risks, particularly in regions with inadequate sanitation and high human–animal contact. To review the current advancements and challenges in applying artificial intelligence (AI)–based systems for the diagnosis of parasitic diseases in veterinary practice.

Methods: Peer-reviewed publications indexed in PubMed, Scopus, and Google Scholar were searched using combinations of terms such as artificial intelligence, machine learning, deep learning, parasitic disease, and veterinary parasitology. Studies included were English-language reports that evaluated AI-assisted diagnostic systems or compared them with conventional methods. Seminal original research and recent review articles were prioritised; reference lists were also hand-searched.

Results: Identified AI-augmented systems, such as Automated Diagnosis of Intestinal Parasites (DAPI) and VETSCAN IMAGYST, demonstrated sensitivities up to 94% and high concordance with expert microscopy. Deep learning methods, particularly convolutional neural networks, improved automation of parasite recognition from microscopic images. Hyperspectral imaging coupled with neural networks enhanced parasite detection in aquaculture, enabling real-time, non-invasive assessments. Challenges remain, including limited availability of annotated datasets, risk of model overfitting, data governance concerns, and absence of standardised protocols.

Conclusion: AI technologies have shown strong potential to improve diagnostic accuracy, reduce observer bias, and streamline laboratory workflows in veterinary parasitology, but further standardisation and validation are required.

Keywords: Artificial intelligence, Veterinary parasitology, Deep learning, Diagnostic methods, Hyperspectral imaging.



The Role of Parasites in Modulating Intestinal Bacterial Populations

Anahita Gharagozlou¹, Armin Gharagozlou², Yasaman Hosseini¹, Armina Jalali¹, Mohammadreza Mottezarre³, Fatemeh Moradalian¹, Behnam Meshgi⁴

Corresponding Author: Behnam Meshgi, bmeshgi@ut.ac.ir

1. DVM. Student, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Student of Medicine, Gilan University of Medical Sciences(GUMS), Rasht, Iran.
3. DVM. Student, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
4. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1427

Abstract

Background: Extensive research has elucidated the presence of intracellular bacteria within parasitic organisms. Notably, in the 1970s, intracellular bacteria were identified in filarial nematodes such as *Brugia malayi* and *Dirofilaria immitis*. These bacteria are transmitted transovarially (from female worms to their offspring) and influence both immunological and pathological outcomes. However, it remains unclear whether the interaction between parasites and bacteria is limited to this transmission method, or if more complex organisms, including mammals, particularly humans and birds, and other higher life forms, become susceptible to bacterial infections following parasitic infections. Furthermore, it is yet to be determined whether colonization by specific bacterial species could reduce susceptibility to parasitic infections.

Methods: A comprehensive review of existing literature was conducted. *Blastocystis* is an intestinal protozoan parasite commonly found in humans and animals. Despite its high genetic diversity and evidence suggesting the pathogenic potential of certain genotypes, the pathogenic impact of this parasite remains incompletely understood, necessitating further research to identify both its pathogenic and non-pathogenic strains.

Results :Several studies indicate that *Blastocystis* colonization is generally associated with higher gut microbial diversity, including increased *Clostridia* and decreased Enterobacteriaceae. Unlike typical dysbiosis in gut disorders, *Blastocystis* may reflect a balanced microbiota. Though findings on *Entamoeba* spp. are less consistent, some studies suggest possible interactions with other microbes, pointing to a commensal or even beneficial role for certain protozoa in healthy individuals.

Conclusion: Current evidence indicates that protozoa like *Blastocystis* may be linked to a healthier and more diverse gut microbiota, rather than disease. However, due to its genetic diversity and varying effects, further standardized, longitudinal, and mechanistic studies are



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



needed to determine whether it influences gut microbiota or simply reflects the host's gut health.

Keywords: *Blastocystis*, Gut microbiota, Microbial diversity, Commensal protozoa, Dysbiosis.



Study of the Prevalence of *Cryptosporidium* in Dogs in Southeastern Iran Using the Concentration Method

Mahdi Khoshsima Shahraki¹, Saeid Reza NourollehiFard², Mansour Dabirzadeh¹

Corresponding Author: Mansour Dabirzadeh, mdabirzadeh20002000@gmail.com

1. Department of Parasitology and Mycology, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

Article code: 1429

Abstract

Background: *Cryptosporidium*, a zoonotic parasite, infects humans and dogs via contaminated food or water, causing diarrhea, weight loss, and lethargy. Stray dogs are particularly susceptible, posing a public health risk. This study investigates the epidemiology of *Cryptosporidium* in dogs in southeastern Iran to inform control strategies.

Methods: In a six-month cross-sectional study (spring-summer), 260 fecal samples from dogs (stray, herd, shelter) in Sistan and Kerman regions were randomly collected. Samples were analyzed using the formalin-ether concentration method with safety precautions, and slides were stained with the Ziehl-Neelsen method to detect *Cryptosporidium* oocysts under microscopy. Data on dog age, water source, dog type, diet (raw/cooked), and region were analyzed using SPSS v26, employing chi-square, t-tests, mean, and standard deviation.

Results: Of 260 samples, 2 (0.8%) tested positive for *Cryptosporidium*. Statistical analysis revealed a significant association ($P < 0.05$) between infection and water source (contaminated vs. clean) and dog type (stray, herd, shelter), with stray dogs showing higher prevalence. No significant correlations were found with age, diet, or region.

Conclusion: Access to stagnant water significantly increases *Cryptosporidium* infection risk in dogs, particularly strays, due to reliance on contaminated public water sources. This aligns with studies in similar ecological settings, where standing water facilitates human-livestock transmission. Control measures should include improved water quality, stray dog management, and public health education. Future research should explore seasonal variations and zoonotic transmission pathways to enhance intervention efficacy. This study highlights the need for integrated zoonotic disease control in southeastern Iran.

Keywords: *Cryptosporidium*, Epidemiology, Dogs, Kerman, Zoonotic Disease.



A Study on the Prevalence of Theileriosis Among Ruminants in Three Selected Regions of South Khorasan

Afsane Hoseini¹, Maryam Arbabi Dastgerd¹, Amir Nasiraei Tabas¹, Atiye Etebari¹

Corresponding Author: Afsane Hosseini, hoseinivetpharmacy@gmail.com

1. Doctorate of Veterinary Medicine, Birjand, Iran.

Article code: 1431

Abstract

Background: Theileriosis is a protozoal disease caused by *Theileria* spp. affecting mostly warm-blooded vertebrates worldwide. It is one of the common tick-borne diseases among domestic animals in tropical and subtropical regions, which has various adverse effects on health, economy and animal welfare. The purpose of this study was to determine the prevalence of theileriosis among small and large ruminants in South Khorasan during the warm season.

Methods: This study was conducted from March to July 2025 on 1100 ruminants suspected of having theileriosis in Birjand, Khusf, and Sarbisheh. After the clinical examination and observation of clinical signs, samples from peripheral blood were collected for parasite detection. The blood smear was stained with Giemsa stain and examined under a light microscope ($\times 100$) in the veterinary laboratory.

Results: The results showed that 24.5% of the studied ruminants were infected with *Theileria* (155 large ruminants and 115 small ruminants). Among the positive cases, 18% showed exclusively anorexia and pale mucous membranes, with no signs of icterus.

Conclusion: Arthropods have a crucial role in the transmission of theileriosis, and effective control strategies such as vaccination and environmental sanitation are essential. Therefore, combating these vectors is the first and most important step in controlling the spread of the disease.

Keywords: Theileriosis, Warm season, Tick, South Khorasan.



Artificial Intelligence in the Diagnosis of *Babesia*: A Step Toward Smarter Veterinary Hemoparasitology

Amir Yosef Momenzadeh¹, Shayeste Noshadian¹, Saber Rahmati²

Corresponding Author: Amir Yosef Momenzadeh, Amiryosefmomenzzadeh@gmail.com

1. Veterinary Medicine Student, University of Urmia, Urmia, Iran.

2. Doctor of Veterinary Medicine, Neyshabur, Iran.

Article code: 1432

Abstract

Background: Early and accurate diagnosis of blood parasites in animals is critical for the control and management of hemotropic diseases. However, microscopic evaluation of blood smears requires high expertise from parasitologists and experienced veterinarians. Emerging technologies, such as automated digital scanning and image analysis using artificial intelligence (AI) algorithms, are gaining traction as tools to support diagnostic decision-making.

Can AI replace veterinary parasitologists in the future or at least play a significant role in facilitating diagnosis?

Methods: This study used 20 blood smear images of *Babesia* spp. from veterinary textbooks, along with two confirmed clinical samples *Babesia canis* (PCR-confirmed dog case) and *Babesia bigemina* (microscopy-confirmed cow case). The Grok-3 AI model, built on a convolutional neural network (CNN) and adapted via transfer learning, analyzed images to identify host species, staining methods, and diagnose *Babesia* infection. The model's performance was evaluated using accuracy, precision, recall, and F1-score, with and without supplementary inputs (host species, staining method, clinical data).

Results: Initial model performance was 20% accuracy in host identification, 100% in staining method recognition, and 80% in final diagnosis. Supplementary data improved species identification to 75%, and clinical data elevated diagnostic accuracy to 100%. These results emphasize the importance of contextual input for reliable AI interpretation.

Conclusion: AI showed strong potential in diagnosing *Babesia* infections, especially when supported by clinical metadata. While it cannot replace parasitologists, it offers promise in education, telemedicine, and remote diagnostics. However, real-world testing on broader datasets remains essential for validation. AI can effectively assist in hemoparasite diagnosis, but its reliability heavily depends on access to clinical and contextual data. With further refinement, such tools could become integral to veterinary diagnostics.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Artificial Intelligence, CNN, Grok, Image Analysis, Hemoparasite.



Prevalence of Tick-Borne Haemoparasites in Clinically Suspected Cattle in Northern Iran

Saman Moradian¹, Ali Safari²

Corresponding Author: Saman Moradian, saman.moradian@iau.ir

1. Graduated Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, Bab.C., Islamic Azad University, Babol, Iran.
2. Graduated Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, Bab.C., Islamic Azad University, Babol, Iran.

Article code: 1433

Abstract

Background: Haemoparasitic infections are among the most significant health challenges affecting cattle. These infections, caused by protozoa such as *Theileria*, *Babesia*, and *Anaplasma* species, are primarily transmitted by ticks and lead to considerable economic losses in the livestock industry due to decreased productivity, increased treatment costs, and mortality. Although these pathogens primarily affect animals, some species—particularly *Babesia* and *Anaplasma*—have zoonotic potential and can be transmitted to humans. Haemoparasites have generally been shown to cause destruction of RBCs resulting in anaemia, jaundice, anorexia, weight loss and infertility. The aim of this study was to determine the prevalence and diversity of haemoparasitic infections in jaundiced cattle in the cities of Sari and Neka, and to highlight their clinical, economic, and potential zoonotic importance.

Methods: This study was conducted during the spring and summer of 2024 on 200 cattle with jaundice. After clinical examination and observation of clinical signs such as mucous membrane jaundice, fever, enlargement of superficial lymph nodes, or pallor, the animals were enrolled in the study. To investigate the presence of haemoparasites, blood smears were prepared from the ear vein. The blood smears were then fixed with 96% methanol, stained with Giemsa, and examined under a light microscope.

Results: The results of this investigation showed that 36% of the examined cattle were infected with theileriosis, 6% with babesiosis, and 10% with anaplasmosis. Additionally, 8% of the cases showed co-infection with *Theileria* spp. and *Anaplasma* spp.

Conclusion: The findings of this study highlight that a significant proportion of cattle suspected of jaundice were infected with haemoparasites, with *Theileria* spp. being the most prevalent. The presence of co-infections, particularly with *Theileria* and *Anaplasma* species, underscores the need for comprehensive diagnostic approaches to ensure accurate detection



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



and appropriate treatment. These results emphasize the importance of regular surveillance, tick control programs, and prompt therapeutic interventions to reduce the burden of haemoparasitic diseases in cattle populations. Performing PCR is recommended in future studies for a more accurate assessment of the prevalence of haemoparasites.

Keywords: *Anaplasma*, *Babesia*, Haemoparasites, *Theileria*.



Cerebral Hydatid Cyst in Humans from an Endemic Region in Northeastern Iran (Mashhad): A Case Report

Farnaz Seilanian Toosi¹, Sara Kazemi¹, Nasrollah Vahedi Noori², Seyyed Mohammad Aghapour¹

Corresponding Author: Nasrollah Vahedi Noori, Nsvahedii@gmail.com

1. DVM Student, Bab.C., Islamic Azad University, Babol, Iran.
2. Parasitic Diseases Research Department, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

Article code: 1434

Abstract

Background: Cystic echinococcosis (CE) is a chronic zoonotic disease caused by the larval stage of *Echinococcus granulosus*. Although the liver and lungs are the most commonly affected organs, cerebral hydatidosis is a rare manifestation.

Methods: A 62-year-old female presented with headache, seizures, and unilateral motor weakness. Brain MRI with contrast was performed, revealing a multilocular cystic lesion in the right frontal lobe. The patient was referred to neurosurgery with a presumptive diagnosis of cerebral hydatid cyst

Results: MRI demonstrated a well-defined, multilocular cystic lesion measuring 66×50 mm in the right frontal lobe. The lesion appeared hypointense on T1-weighted images and hyperintense on T2-weighted images, with no enhancement after contrast injection. The cyst was surgically removed without rupture, and postoperative recovery was clinically satisfactory.

Conclusion: Given that Khorasan Razavi province is a highly endemic area for *Echinococcus granulosus* in carnivores, the presence of hydatid cysts in both livestock and the human population is not unexpected. This has important public health implications. Therefore, implementing public health strategies such as hygienic slaughter of livestock in abattoirs and routine deworming of stray dogs plays a vital role in preventing and controlling the infection.

Keywords: Cerebral Hydatid Cyst, Humans, Mashhad.



Epidemiology of Toxocariasis by Concentration Method in Dogs of Southeast Iran

Mansour Dabirzadeh¹, Saeidreza NourollahiFard², Mansour Aminzadeh Gowhar³, Mahdi Khoshshima shahraki⁴

Corresponding Author: Mahdi Khoshshima Shahraki, khoshshima6144@vet.uk.ac.ir

1. *Professor of Medical Parasitology, Department of Parasitology and Mycology, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran.*
2. *Professor of Veterinary Parasitology, Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.*
3. *Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.*
4. *Department of Parasitology and Mycology, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran.*

Article code: 1435

Abstract

Background: The epidemiology of toxocariasis in dogs is of vital importance due to its high prevalence (especially in puppies up to 80%), serious zoonosis potential, and public health threat. It is easily spread in the environment through eggs excreted in the feces of infected dogs, and humans (especially children) become infected by accidentally ingesting contaminated soil or water containing contaminated eggs and vegetables. In the present study, feces from 260 dogs were examined for the epidemiology of toxocariasis.

Methods: In this descriptive study, 260 dog feces were examined for 6 months in the spring and summer seasons in a cross-sectional and completely random manner in the two regions of Sistan and Kerman. In this study, dog feces samples (stray, herd, and shelter) were examined using the concentration method (formalin-ether) with safety precautions, and all parasite slides were examined under a microscope with 10 and 40 lenses to examine *Toxocara* eggs.

Results: The results of this study show that out of a total of 260 dog feces samples examined, 24 (9.2%) were infected with *Toxocara*. The age distribution of the dogs, temperature at the time of sample collection, type of water consumed, type of infected dog, type of food (raw or cooked), and region of residence (Sistan or Kerman) were examined. A significant relationship was observed between the type of water consumed and the type of infected dog (stray-herd-shelter) ($P < 0.05$).

Conclusion: Among demographic variables, dog ownership status emerged as an important determinant of risk. Stray dogs had a significantly higher prevalence rate of *Toxocara* spp. (4.6%), underscoring their role as unregulated reservoirs in the transmission of common parasites between humans and livestock. Lack of veterinary care, access to contaminated



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



environments, and scavenging behaviors increase susceptibility to infection and facilitate environmental contamination.

Keywords: Toxocara, Epidemiology, Dog, kerman.



Morphological Identification of Ticks on Afshari Sheep in Kerman City

Zanko Rahmani¹, Mohammad Mazrouei Sebdani¹, Amin Ahmadi^{2,3}, Hossein Iravani¹,
Amirhosein Shekari¹

Corresponding Author: mohammadmazrouei93@gmail.com

1. Department of Veterinary Medicine, Islamic Azad University, B.C. , Baft , Iran.
2. Department of basic sciences, Faculty of veterinary Medicine, Ardakan University, Ardkan,P.O.Box 184, Iran.
3. Biology and Animal Reproduction Science Research Institute , Ardekan University , Ardakan , P.O.Box 184, Iran.

Article code: 1437

Abstract

Background: Tick borne parasitic diseases pose a major threat to livestock health and productivity worldwide. As primary vectors of numerous pathogens, ticks contribute to substantial economic losses through reduced yields, anemia, paralysis, and zoonotic disease transmission. This study aimed to identify ixodid tick species infesting Afshari sheep in Kerman Province, Iran, using morphological criteria.

Methods: The total Afshari sheep were randomly selected from three flocks of sheep. Ticks were collected from common predilection sites (auricular, axillary, and inguinal regions) and identified to the species level in the Ardekan School of Paraveterinary Medicine using standard taxonomic keys.

Results: A total of 94 ticks (59 males, 35 females) were collected, representing four species: *Hyalomma anatolicum anatolicum* (n=75), *Dermacentor marginatus* (n=10), *Hyalomma asiaticum* (n=6), and *Rhipicephalus bursa* (n=3). *H. anatolicum anatolicum* was the dominant species.

Conclusion: Afshari sheep in Kerman Province harbor multiple ixodid tick species, particularly *H. Anatolicum anatolicum*, a known vector of *Theileria* spp. and Crimean-Congo Hemorrhagic Fever virus. These findings underscore the need for targeted tick control measures and provide essential baseline data for developing integrated parasite management programs, including acaricide use, improved biosecurity, and vaccination strategies.

Keywords: Ticks, Afshari Sheep, Kerman, *Theileria*.



Detection of *Theileria* spp. and *Babesia ovis* in Sheep Using Molecular Methods in Taybad, Iran

Erfan Sepahvand-Mohammadi¹, Seyed Omid Naghibi Torbat², Zahra Davoodian³, Seyed Mohamadreza Bolandi⁴

Corresponding author: Mohamadreza Bolandi, Mreza.Bolandi@Gmail.com

1. Department of Clinical Sciences, Ka.C., Islamic Azad University, Karaj, Iran.
2. Veterinary Aquatic Animal Health Expert, Mashhad Veterinary Network, Iran Veterinary Organization, Mashhad, Iran.
3. Department of Clinical Sciences, Ka.C., Islamic Azad University, Garmsar, Iran.
4. Department of Pharmacology, Ka.C., Islamic Azad University, Karaj, Iran.

Article code: 1438

Abstract

Background: This study aimed to examine infections caused by *Theileria* and *Babesia* species in sheep using polymerase chain reaction (PCR) techniques in Taybad, Iran. *Theileria* and *Babesia* are apicomplexan protozoa with complex life cycles involving both vertebrate and invertebrate hosts. These parasites, transmitted via tick vectors, are recognized as major agents of parasitic diseases in Iran. Traditional diagnostic methods include microscopic examination and serological tests, but molecular techniques like PCR have recently become more widely used for detection.

Methods: In this research, 56 blood samples were collected from sheep and tested using species-specific primers targeting *Theileria annulata*, *T. ovis*, *T. lestoquardi*, and *Babesia ovis*. Two PCR approaches—semi-nested and competitive PCR—were employed.

Results: The PCR analysis revealed the presence of *B. ovis*, *T. ovis*, and *T. annulata* in 34 (86.4%), 29 (42.4%), and 25 (24%) samples, respectively, while *T. lestoquardi* was not detected in any sample.

Conclusion: The detection of subclinical infections with protozoa that exhibit high morbidity suggests that these animals may serve as asymptomatic carriers, contributing to the persistence and transmission of these parasites in the area. Taybad's location as a border city further emphasizes the importance of monitoring, as similar hemoprotozoan infections have been reported in adjacent regions such as Afghanistan and neighboring Iranian provinces. Consequently, livestock in Taybad face an elevated risk of infection with *Babesia* and *Theileria* species. The findings of this study contribute valuable insights into the epidemiology of theileriosis and babesiosis and may inform strategies for disease prevention and control in the region.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Theileria* spp, *Babesia ovis*, Taybad, Sheep.



Nasopharyngeal Myiasis in a Motor Vehicle Accident Victim: A Case Report from Shushtar, Khuzestan Province, Iran

Seyede Manizhe Heidar Nejadi¹, Pegah Golafshan²

Corresponding Author: Seyede Manizhe Heidar Nejadi, reyhane_zn@yahoo.com

1. Shoushtar Faculty of Medical Sciences, Shoushtar, Iran.
2. Student Research Committee, School of Public Health and Safety, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Article code: 1440

Abstract

Background: Rhino-myiasis is a rare yet dangerous form of parasitic infection in humans in which the larvae of various flies, particularly those of the families Oestridae, Calliphoridae, and Sarcophagidae, develop within the nasal cavity, causing tissue destruction, severe inflammation, and, in advanced cases, necrosis with extension into adjacent structures. This report aims to present myiasis of case of a 55-year-old man, an accidental driver, with expelling larvae from the nasal cavities caused to died due to the severity of his initial traumatic injuries and progressive respiratory failure in Khatam (S) Hospital in Shushtar, Khuzestan Province, Iran.

Methods: Larvae were removed mechanically and endoscopically via the nasal and oral routes. The infected areas were irrigated with 0.2% chlorhexidine and normal saline, and preventive measures against secondary infection were implemented. For species identification, the collected larvae were placed in a container with fresh liver tissue and incubated at 26 ± 2 °C and 65% humidity. After one week they pupated, and by days 28–30 they emerged as adult flies. (Fly larvae were cultured to complete their development and facilitate improved identification).

Results: Based on larval morphology—tegument bearing distinct transverse grooves, plate-shaped posterior stigmas, and prominent oral hooks—a provisional diagnosis of myiasis was *Sarcophaga africa*.

Conclusion: Given its classification as a neglected disease, addressing nasal myiasis requires enhanced clinical awareness, the development of standardized diagnostic protocols, and expanded epidemiological studies, particularly in tropical and subtropical regions. To achieve this, it is recommended to strengthen suspected-case reporting systems, promote interdisciplinary collaboration between otolaryngology, medical microbiology, and parasitology, and educate local communities on preventative measures against contaminated



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



sources. Implementing these measures is essential to reduce diagnostic delays, improve treatment outcomes, and prevent life-threatening complications.

Keywords: Myiasis, Nasal Cavity, *Sarcophaga africa*, Iran.



Emerging Recombinant Antigen Strategies and Immune Modulation for Effective Control of *Neospora caninum*: A Narrative Review

Maryam Hassan Nejad¹, Amirhossein Ramzi², Sima RoshanFar¹, Mohadeseh Ramzi³

Corresponding Author: Maryam Hassan Nejad, mhasannejhad07@gmail.com

1. Department of Medical Parasitology & Mycology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran.
2. Department of Microbiology, Ardabil Branch, Islamic Azad University, Ardabil, Iran.
3. Department of Medical parasitology and Mycology . School of Public Health Tehran University of Medical Sciences , Tehran , Iran.

Article code: 1441

Abstract

Background: *Neospora caninum* is a globally prevalent intracellular protozoan parasite recognized as a primary causative agent of bovine neosporosis, leading to abortion and reproductive failure in dairy and beef cattle. Despite extensive research, effective therapeutic agents and universally protective vaccines remain unavailable. Consequently, recent investigations have focused on recombinant antigen development and DNA-based vaccination strategies aimed at enhancing serological diagnosis and eliciting robust protective immunity.

Methods: This narrative review critically synthesizes data from 26 peer-reviewed articles published between 2012 and 2025, retrieved from PubMed, Scopus, and Google Scholar databases. Search terms included “*Neospora caninum*,” “recombinant antigens,” “neosporosis diagnosis,” “DNA vaccines,” and “vertical transmission.” Selected studies addressed recombinant protein production, diagnostic assay performance, immunogenicity profiling (Th1/Th2 balance), and protective efficacy in murine and bovine models. Particular emphasis was placed on prime-boost vaccination regimens, cytokine responses (notably IFN- γ), and outcomes such as reduction in cerebral infection and prevention of vertical transmission.

Results: Among recombinant antigens, *NeSAG1* demonstrated the highest diagnostic reliability, exhibiting superior sensitivity and specificity in serological assays. *NcMIC4* and *NePrx2* showed moderate to low diagnostic efficacy. Protein-based vaccines predominantly induced Th2-biased immune responses, insufficient for effective protection. In contrast, prime-boost strategies combining DNA vaccines with recombinant proteins, especially involving *NeSAG1* and *NeSRS2*, elicited dominant Th1 responses characterized by elevated IFN- γ secretion and IgG2a production, conferring significant protection against cerebral neosporosis in murine models. Additionally, *MIC10* and *p24B* proteins showed promise in reducing vertical transmission, meriting further evaluation under field conditions.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: Effective control of *N. caninum* requires strong Th1-mediated immunity. DNA and DNA/protein vaccines provide better protection than protein-only vaccines. Challenges include epitope mismatches, Th2-biased responses, and ineffective adjuvants. Future progress depends on identifying immunogenic antigens, improving delivery systems, and directing immune responses toward protection. Simultaneously, developing accurate serological markers is essential for early diagnosis and integration into prevention programs.

Keywords: *Neospora caninum*, Recombinant antigens, DNA vaccines, Vertical transmission.



Increasing Drug Resistance among Zoonotic Protozoan Parasites: A Worldwide Review

Amir Hessam Khani Sarhoozaki¹ Mahyar Yarahmadi Khorasani², Ali Fatahi², Mehran Arian Shokooh³

Corresponding Author: Amir Hessam Khani Sarhoozaki, hessam.kh79@gmail.com

1. DVM Student, Faculty of Veterinary Medicine, Islamic Azad University, Baft Branch, Baft, Iran.
2. Veterinary Practitioner, Iran.
3. DVM Student, Faculty of Veterinary Medicine, Islamic Azad University, Garmsar Branch, Garmsar, Iran.

Article code: 1445

Abstract

Background: Zoonotic protozoans, including *Leishmania*, *Trypanosoma*, *Toxoplasma gondii*, and *Cryptosporidium* spp., are major causes of human and animal diseases globally. The emergence of drug resistance in these parasites threatens effective treatment and control, particularly in regions with limited healthcare resources.

Methods: This narrative review was conducted using articles retrieved from PubMed, Scopus, and open-access sources up to 2024. Studies were selected based on their relevance to drug resistance patterns, molecular mechanisms, and control strategies for zoonotic protozoa. Data were synthesized to provide a global perspective on emerging resistance trends.

Results: Drug resistance in *Leishmania* spp. is widespread, especially against pentavalent antimonials in South Asia, with increasing resistance to miltefosine and amphotericin B. These resistances are linked to mutations in drug transporters and thiol metabolism pathways. *Trypanosoma* spp. show resistance through altered drug uptake carriers and efflux mechanisms, complicating treatment of sleeping sickness and Chagas disease. Although clinical resistance in *Toxoplasma gondii* is rare, in vitro studies reveal mutations in dihydrofolate reductase-thymidylate synthase, suggesting potential future treatment failures. For *Cryptosporidium* spp., limited data indicate reduced nitazoxanide efficacy, particularly in immunocompromised individuals.

Conclusion: The growing drug resistance among zoonotic protozoan parasites poses a serious global health challenge. Strategies such as developing new drugs, implementing combination therapies, and strengthening surveillance systems are essential to sustain treatment efficacy and control zoonotic infections.

Keywords: Drug resistance, *Cryptosporidium*, Global health, *Leishmania*, *Toxoplasma*, *Trypanosoma*, zoonotic parasites.



Retrospective Review of *Toxocara* spp. Prevalence in Domestic Dogs and Cats: A Global and Local Perspective

Amir Hessam Khani Sarhoozaki¹, Ali Fatahi², Mahyar Yarahmadi Khorasani², Mehran Arian Shokooh³

Corresponding Author: Amir Hessam Khani Sarhoozaki, hessam.kh79@gmail.com

1. DVM Student, Faculty of Veterinary Medicine, Islamic Azad University, Baft Branch, Baft, Iran.
2. Veterinary Practitioner, Iran.
3. DVM Student, Faculty of Veterinary Medicine, Islamic Azad University, Garmsar Branch, Garmsar, Iran.

Article code: 1446

Abstract

Background: *Toxocara canis* and *Toxocara cati* are major intestinal nematodes of dogs and cats with significant veterinary and public health importance. They are found worldwide, with higher prevalence in areas lacking sanitation, routine deworming, and in pets with close human contact. Infected animals may present gastrointestinal signs, malnutrition, impaired growth, or, in severe cases, systemic illness. From a One Health perspective, controlling *Toxocara* infections in companion animals is essential to reduce environmental contamination with infective eggs.

Methods: This retrospective review focused on published data regarding the prevalence of *T. canis* in dogs and *T. cati* in cats. Literature searches in PubMed, Scopus, and Web of Science covered studies up to 2024 using keywords “*Toxocara canis*,” “*Toxocara cati*,” “dogs,” “cats,” “prevalence,” and “epidemiology.” Eligible studies were peer-reviewed reports on domestic dogs and cats based on coprological, necropsy, or molecular methods. Studies solely on human seroprevalence or non-domestic animals were excluded.

Results: Prevalence of *T. canis* in dogs ranges from 5% to over 20%, with higher rates in puppies under six months due to vertical and lactogenic transmission. *T. cati* in cats shows prevalence of 3% to 17%, higher in stray or shelter cats than in owned cats. Risk factors include outdoor access, absence of routine anthelmintic treatment, and hunting or raw meat diets. Iranian data follow global trends, with rural and stray populations most affected.

Conclusion: *Toxocara* spp. infections remain common in dogs and cats. Effective control relies on regular deworming, public awareness, and environmental hygiene. Improving companion animal health will reduce contamination and potential zoonotic transmission.

Keywords: Cats, Dogs, *Toxocara canis*, *Toxocara cati*, Prevalence, Review, Zoonosis.



Microbial Symbiosis Between *Trichomonas vaginalis* and HPV in Cervical Carcinogenesis: A Narrative Review of Pathological Interactions and Clinical Implications

Maryam Hassan Nejad¹, Sima RoshanFar², Golnaz mobayen³

Corresponding Author: Maryam Hassan Nejad, mhasannejhad07@gmail.com

1. Department of Medical Parasitology & Mycology, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran.
2. Department of Medical Parasitology & Mycology, School of Medicine Urmia University of Medical Sciences, Urmia, Iran
3. STD Disease Pediatric Health Research Center . Tabriz University of Medical Sciences, Tabriz Iran.

Article code: 1447

Abstract

Background: Human papillomavirus (HPV) and *Trichomonas vaginalis* are two highly prevalent sexually transmitted pathogens, each independently associated with reproductive tract disorders, including cervical neoplasia. Emerging evidence suggests that these microorganisms may engage in a pathological microbial symbiosis that facilitates viral persistence and progression of precancerous cervical lesions. This narrative review aims to explore the biological mechanisms underlying their interaction and to assess its clinical relevance in the context of cervical carcinogenesis.

Methods: This review examined the topics of HPV, cervical cancer, sexually transmitted infections, *T. vaginalis*, and microbial symbiosis. A literature search was conducted across PubMed, Scopus, Web of Science, and Google Scholar databases for the period spanning 2012 to 2025. Articles that specifically addressed the pathogenic interplay between HPV and *T. vaginalis* were selected. In total, 24 key studies were critically reviewed and synthesized.

Results: The literature indicates that the coexistence of *T. vaginalis* and high-risk HPV types—particularly HPV16 and HPV18—is associated with an increased risk of high-grade cervical intraepithelial neoplasia (CIN2/3). *T. vaginalis* promotes a conducive microenvironment for viral persistence by altering vaginal pH, disrupting epithelial barriers, modulating the vaginal microbiota, inducing chronic inflammation, and secreting proteolytic enzymes such as cysteine proteinases. Furthermore, the expression of parasite-derived adhesion proteins (e.g., TvAP33, TvAP65) appears to enhance viral entry into host cells by upregulating epithelial viral receptors.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: The microbial symbiosis between *T. vaginalis* and HPV represents a pathophysiological model that may contribute significantly to cervical carcinogenesis through immune evasion, epithelial disruption, and enhanced viral retention. Simultaneous screening for both pathogens, expansion of HPV vaccination coverage, and modulation of the vaginal microbiome may serve as essential strategies for cervical cancer prevention and control. Future research should focus on molecular interventions targeting this synergistic interaction to mitigate oncogenic progression.

Keywords: HPV, *Trichomonas vaginalis*, Cervical Cancer, Microbial Symbiosis.



Overview of Parasitic Diseases in Ostriches

Mohammadjavad Aghasizadeh¹, Hamideh Zeynali¹, Mostafa Sadeghidokht¹

Corresponding Author: Mohammadjavad Aghasizadeh, vet.daniyal@gmail.com

1. DVM, Department of Research and Development of Behinparvar company, Mashhad, Iran.

Article code: 1448

Abstract

Background: Ostrich (*Struthio camelus*) farming has expanded significantly in recent years due to the high commercial value of its meat, leather, and feathers. However, parasitic diseases pose a major threat to bird health and farm profitability. This review outlines the most prevalent parasitic infections in ostriches, caused by protozoa, nematodes (roundworms), cestodes (tapeworms), and ectoparasites such as lice and mites, each of which imposes distinct pathological and economic burdens.

Methods: In this research, credible and diverse sources were used to gather information and advance the study's objectives. Among the most important sources utilized are Veterinary Problems, The Ostrich: Biology, Production and Health, Huchzermeyer FW, Veterinary Parasitology, William J. Foreyt, The Ostrich Biology, Production and Health, D.C. Deeming, and several other articles. Additionally, by using data and citing these references, an effort was made to provide more comprehensive analyses.

Results: Gastrointestinal parasites can impair nutrient absorption, induce intestinal inflammation, and lead to reduced growth performance and productivity. These conditions may result in decreased egg output, lower leather and feather quality, and increased veterinary expenses. Ectoparasites damage the skin through blood and tissue feeding, causing irritation, pruritus, reduced skin quality, and serving as vectors for secondary infections. Raising awareness of these diseases and promoting timely intervention through diagnostic sampling (e.g., fecal or skin analysis) are essential to mitigate losses.

Conclusion: Controlling parasitic diseases in ostriches requires a comprehensive approach that includes strict hygiene practices, disinfection of equipment, proper litter management, and effective control of insect vectors. Key preventive measures involve balanced nutrition, reduced stocking density, quarantine of infected birds, and prompt veterinary care. Farmer education and proactive disease management are vital to ensuring the sustainability and profitability of ostrich farming operations.

Keywords: Ostrich, Parasitic diseases, Protozoa, Nematodes, Ectoparasites, Disease management.



Applications of Artificial Intelligence in the Diagnosis of Canine Babesiosis: A Mini Review

Sara Kazemi¹, Seyyed Mohammad Aghapour¹, Farnaz Seilanian Toosi¹, AmirMohammad Malekpour¹

Corresponding Author: Sara Kazemi, sarakazemi.dvm@gmail.com

1. DVM Student, Bab.C., Islamic Azad University, Babol, Iran.

Article code: 1450

Abstract

Background: Canine Babesiosis is a severe tick-borne disease mainly caused by *Babesia canis* and *Babesia gibsoni*. These parasites destroy red blood cells, causing organ failure and death without proper intervention. Perhaps the key concern of babesiosis is its potential to spread between species. Although *B. microti* is primarily zoonotic, infecting animals and rarely infecting humans through tick bites, it may cause severe infections especially in immunocompromised individuals. Transmission may also occur through blood or organ transplants, elevating it from a zoonotic threat to a significant global health concern. Veterinary diagnosis still relies on slow, error-prone blood smear microscopy. This study aims to explore the applications of artificial intelligence in diagnosing canine babesiosis, evaluating various machine and deep learning models for early and accurate detection of this disease.

Methods: This review synthesizes findings from AI-driven diagnostic tools, focusing on machine and deep learning models. Data were gathered from PubMed, ScienceDirect, and Google Scholar. The included studies, published between 2015 and 2024, examined AI applications in diagnosing *Babesia* infections in dogs. Only peer-reviewed research reporting diagnostic accuracy was considered, while studies without AI methods, lacking relevant data, or of low methodological quality were excluded.

Results: Emerging AI technologies improve precision and productivity in diagnosing babesiosis. Deep ensemble learning achieved 97.75% accuracy; another using SimCLR and EfficientNet_b2 reached 97.07%. Hybrid models with DenseNet121 and Support Vector Machine (SVM) achieved 95.50%, improved to 96.62% with ensemble approaches. Machine learning models using hematology data achieved 100% sensitivity and 95.7% specificity, identifying indicators like platelet count and mean platelet volume.

Conclusion: AI technologies can provide timely, cost-effective, and accurate detection of canine *Babesia* infections in under-resourced areas. Further work is needed to enhance diagnostic systems for other veterinary applications by increasing datasets and tailoring algorithms for various *Babesia* species.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Canine Babesiosis, Tick-borne, Zoonotic, Artificial Intelligence.



Reengineering Serodiagnosis of Strongyloidiasis: A Narrative Review of Recombinant and Chimeric Antigen Innovation

Fatemeh Ramzi¹, Amirhossein Ramzi², Zahra gahvechi amiri³, Sima RoshanFar⁴, maryam Hassan Nejad⁴, Mohadeseh Ramzi¹

Corresponding Author: Fatemeh Ramzi, Ramziifatemeh637@gmail.com

1. Department of Medical parasitology and Mycology . School of Public Health Tehran University of Medical Sciences, Tehran, Iran.
2. Department of Microbiology, Ardabil Branch, Islamic Azad University, Ardabil, Iran.
3. Department of Parasitology and Mycology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Department of Medical Parasitology & Mycology, School of Medicine Urmia University of Medical Sciences, Urmia, Iran.

Article code: 1453

Abstract

Background: Strongyloidiasis, caused by the nematode *Strongyloides stercoralis*, remains a diagnostic challenge due to the low sensitivity of parasitological methods and frequent cross-reactivity in serological assays. Recent advancements in recombinant protein technology have led to the development of novel biomarkers that enhance diagnostic accuracy and feasibility. This narrative review synthesizes progress in recombinant antigen design and their application as high-performance serodiagnostic tools for *S. stercoralis* infection, highlighting potential impacts on clinical and epidemiological practices.

Methods: A narrative literature review was performed through systematic searches of PubMed, Scopus, and Google Scholar, using keywords including “*Strongyloides stercoralis*,” “recombinant protein,” “diagnostic biomarker,” “immunochromatographic assay,” and “serodiagnosis.” Thirty-five peer-reviewed publications dated from 2010 to 2025 were critically appraised. Selection focused on studies detailing recombinant antigen identification, expression platforms, assay.

Results: Multiple studies demonstrate that recombinant antigens, notably NIE and SsIR, outperform crude preparations by delivering enhanced sensitivity and specificity in serological assays. Chimeric proteins, such as SsIR-Ss1a, exhibit superior solubility, immunogenicity, and performance, with ELISA sensitivities surpassing 90% and specificities up to 97%. Immunochromatographic rapid diagnostic tests (RDTs) using combinations of these antigens achieve sensitivities around 95% and specificities near 90%, with excellent reproducibility and inter-reader agreement. Peptide-tagged recombinant proteins (e.g., Ss3a7K) show promising potential, with specificities of 100% and sensitivities near 80%. Notably, whole-blood RDTs



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



enable point-of-care diagnosis without serum separation, supporting use in field settings. Despite these advances, large-scale validation and standardized production remain essential for clinical implementation.

Conclusion: Recombinant protein biomarkers address limitations of classical diagnostic methods. Multiplex strategies improve diagnostic precision, vital for detection and surveillance. Point-of-care RDTs offer rapid, accessible tools in endemic, resource-limited areas. Future priorities include scalable antigen production, assay harmonization, and broader validation. Integrating recombinant diagnostics with treatment monitoring may further enhance control efforts.

Keywords: *Strongyloides stercoralis*, Recombinant Antigen Diagnostics, Serological Cross-reactivity, Point-of-Care Testing (POCT).



A Study on Gastrointestinal Parasites in Captive Reptiles from Tehran and Alborz Provinces

Mehrasa SheikhEsmaili¹, Fateme Jalousian^{1, 2}, Amir Rostami¹

Corresponding Author: Fateme Jalousian, jalousian_f@ut.ac.ir

1. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1454

Abstract

Background: Reptiles can serve as definitive, intermediate, or mechanical hosts for various helminths and protozoan parasites. This study aimed to investigate the gastrointestinal parasites present in captive reptiles housed in zoological centers in Tehran and Alborz Provinces, Iran.

Methods: A total of 138 fecal samples were collected from reptiles across five major zoological centers known for maintaining reptile collections. The samples represented 13 genera/species of snakes, 9 of lizards, 4 of turtles. All samples were examined using saturated Zinc Chloride flotation, and staining with the Trichrome and Ziehl- Neelsen.

Results: parasitic infection was observed in 18 cases of lizards, included 13 cases of oxyurid eggs, 2 cases of combined *Cryptosporidium* spp. and oxyurid infection in iguanas, 1 case of ciliated protozoan cysts in an Argentine black and white tegu, 1 case of *Isospora* spp. infection in a Caspian lizard, and 1 case of *Balantidium coli* (*B. coli*) infection in a black-throated monitor. Snakes parasitic infections were reported in 5 cases, including 2 infections with *Cryptosporidium* spp. and 3 with ascarid nematodes. Among turtles, 12 cases were found to be infected: 4 with oxyurid eggs, 3 with Heterakidae eggs, 1 with mixed oxyurid and *B. coli*, 2 with *B. coli* alone, and 2 with amoebic cysts.

Conclusion: The results of this study highlight a clear correlation between management practices and parasitic burden in captive reptiles. The highest rate of infection was observed in lizards, particularly with oxyurid nematodes, which are commonly associated with herbivorous and omnivorous reptiles in captive environments. The relatively low infection rate in snakes may be attributed to differences in diet, husbandry practices, and possibly natural resistance. gastrointestinal parasitism remains a significant health issue in captive reptiles, particularly in facilities with limited resources and inconsistent treatment protocols.

Keywords: Captive reptiles, Fecal examination, Parasitic infection, Reptile health management.



Medicinal Plants as a Source of Antiparasitics: A Review

Fatemeh Forouzanfar¹

Corresponding author: Fatemeh Forouzanfar, forouzanfarff@gmail.com

1. Department of Neuroscience, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Article code: 1456

Abstract

Background: Parasites are organisms that obtain their food by eating other organisms or their products in nature. Antiparasitic drugs available for the treatment of parasitic infections often lead to *toxicity* and clinical *resistance* to *drugs*. Medicinal plants have several components with different chemical and pharmacological characteristics. *Many medicinal plants* are a valuable source for treating various diseases and possess antiparasitic properties. This study reviews medicinal plants with antiparasitic properties.

Methods: A review of published articles was conducted using databases such as PubMed, Scopus, and Google Scholar. Keywords included medicinal plants, antimalarial, antiparasitic, anthelmintic, and antiprotozoal effects.

Results: Many medicinal plants demonstrated potent antiparasitic activity, such as *Azadirachta indica*, *Allium sativum*, *Moringa oleifera*, *Alstonia scholaris*, *Artemisia annua*, *Centella asiatica*, *Justicia adhatoda*, and *Curcuma longa*. A wide variety of phytochemicals, such as alkaloids, flavonoids, and volatile essential oils obtained from herbs, exhibit antiparasitic properties. Disrupted membrane permeability, neurotoxic activity, and antioxidant activity are suggested for the mode of action of antiparasitic plants. Some studies showed that plant combinations added therapeutic efficacy. Besides, studies showed plant essential oils are secondary metabolites with pharmaceutical importance, particularly anthelmintic activities.

Conclusion: Medicinal plants offer a rich source of antiparasitic agents with diverse mechanisms of action. While promising in preclinical studies, additional pharmacological, toxicological, and clinical evaluations are needed to validate their efficacy and safety.

Keywords: Antiparasitic agents, medicinal plants, *Curcuma longa*, *Artemisia annua*.



Ectoparasite Prevalence on Dogs in Western Iran: Epidemiological Patterns in Shepherd and Stray Populations

Alireza Ghorbani¹, Maryam Hataminejad^{2,3}, Amirreza Ghorbani⁴, Erfan Darmanloo⁵

Corresponding Author: Maryam Hataminejad, hatamiavin168@gmail.com

1. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
2. Toxoplasmosis Research Center, Communicable Diseases Institute, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
3. Department of Parasitology and Mycology, Faculty of Medicine, Mazandaran University of Medical Science, Sari, Iran.
4. Department of Microbiology, College of Science, Agriculture and Modern Technology, Shiraz Branch, Islamic Azad University, Shiraz, Iran.
5. Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran.

Article code: 1457

Abstract

Background: Ectoparasitic infestations in dogs represent a substantial concern for public health, primarily because these parasites can serve as vectors for the transmission of various zoonotic pathogens, thereby facilitating the spread of infectious diseases from animals to humans.

Methods: This cross-sectional study investigated the prevalence, species diversity, and demographic factors associated with ectoparasites (ticks, mites, fleas, lice) infesting shepherd (n=283) and stray dogs (n=185) across four western Iranian provinces (Kurdistan, Hamadan, Markazi, Zanjan) from February to July 2023. Dogs (N=468) were randomly sampled from 22 locations. Ectoparasites were collected via full-body examination, preserved in 70% ethanol, and morphologically identified using taxonomic keys.

Results: Infestation prevalence was 20.1% (57/283) in shepherd dogs and 24.9% (46/185) in stray dogs ($p>0.05$). Eleven ectoparasite species were identified. Ticks were predominant (14.3% of dogs), followed by mites (5.6%), fleas (3.8%), and lice (1.2%). The most prevalent species were *Rhipicephalus sanguineus* (ticks), *Sarcoptes scabiei* var. *canis* (mites), *Ctenocephalides canis* (fleas), and *Trichodectes canis* (lice). Juvenile dogs (<1 year) had significantly higher infestation rates (28.0%) compared to adults ($p<0.05$, χ^2 test), while no sex-based differences were observed ($p>0.05$). Significant spatial variation occurred, with the highest prevalence in Kurdistan province (31.4%), attributed to favorable climatic and ecological conditions for arthropods.

Conclusion: particularly strays, serve as substantial reservoirs for medically and veterinarily important ectoparasites in western Iran, notably *R. sanguineus*, a key vector for pathogens like



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Rickettsia spp. and *Coxiella burnetii*. These findings underscore the need for evidence-based surveillance and control programs targeting arthropod-borne diseases, with a focus on stray dog populations in peri-urban/urban interfaces.

Keywords: Ectoparasites, *Ctenocephalides canis*, Dog, Iran, *Rhipicephalus sanguineus*, *Trichodectes canis*.



Morphological Identification of *Rhipicephalus* spp. in Small Asian Mongooses (*Herpestes javanicus*) in Sistan and Baluchestan Province, Southeastern Iran

Seyede Mahshid Mostafavi¹, Mahdiye Mollashahi², Seyede Mahnoosh Mostafavi³, Kian Mosaali¹

Corresponding Author: Seyede Mahshid Mostafavi, seyedemahshidmostafavi@gmail.com

1. Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Bahonar of Kerman, Iran.
2. Doctorate of Veterinary Medicine (DVM) graduated.
3. Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Chamran of Ahvaz, Iran.

Article code: 1458

Abstract

Background: Mongooses (*Herpestes* spp.) are often overlooked in studies of ectoparasites, despite their potential role as reservoirs for medically and veterinary important tick species. Their ecological habits—frequenting areas shared by wildlife, livestock, and humans—make them a potential link in the maintenance and spread of immature ticks, particularly *Rhipicephalus* spp. In Iran, the tick fauna of mongooses remains largely uncharacterized. This study set out to identify and describe the morphological features of *Rhipicephalus* ticks infesting wild mongooses in southeastern Iran.

Methods: In 2024, 30 Small Asian Mongooses (*Herpestes javanicus*) (18 males and 12 females) were collected from roadside habitats in northern Sistan and Baluchestan Province (Nimruz, Zahak, Zabol, Hirmand). All mongooses were died because of accident. Following anesthesia, the animals were externally examined for ectoparasites. Retrieved ticks were preserved in 70% ethanol and identified under a stereomicroscope using standard morphological keys, focusing on key structures such as the scutum, leg segmentation, anal groove shape, and hypostome dentition.

Results: 22 Ticks were found on 7 of the 30 mongooses (23.33%). All ticks belonged to the species *Rhipicephalus sanguineus*, and were adults (4 males, 3 females). No immature stages were observed.

Conclusion: The detection of *Rhipicephalus sanguineus* in Small Asian Mongooses (*Herpestes javanicus*) indicates a potential role for these animals in supporting adult tick populations. These findings underscore the need for continued surveillance of mongoose-associated ticks in



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Iran, particularly given the zoonotic risk posed by *Rhipicephalus* spp. Future molecular studies will be essential to clarify their role in vector-borne disease cycles

Keywords: *Rhipicephalus*, ticks, Herpestes, tick-host interaction



Evaluation of the Diversity of Freshwater Snail Species in Borujen City

Yaser Pirali¹, Hamidreza Yoonesi Borujeni¹, Behnam Meshgi², Fatemeh Nasiri Dashtaki¹,
Seyed Reza Hosseini¹

Corresponding Author: Fatemeh Nasiri Dashtaki, fatemeh.nasiridashtaki@stu.sku.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1460

Abstract

Background: Many freshwater snails in the evolutionary process of *Trematodes* are intermediate hosts, which are vectors of various parasitic diseases. Snails have specific biological needs and geographical distribution. Purpose of this study is to identify species diversity of freshwater snails in Borujen city.

Methods: In this regard, in summer and autumn of 2018 and summer of 2019, at 9 stations located in this city (*Imam Qais spring, Gandman wetland, Borujen aqueduct, Siasard spring, Ordobar spring, Sefid Spring, Dehno Zaghi spring, Choghakhor wetland* And Gelooger spring), search for snail fishing was carried out which was successful at 7 stations. The available snails were sampled (754 from Imam Qais Spring, 259 from Gandoman Wetland, 131 from Siasard Spring, 78 from Ordobar Spring, 312 from Zaghi Dehno Spring, 520 from Choghakhor Wetland, and 546 from Golugard Spring) and after the transfer to the laboratory, the family and genus of the caught samples were identified with help of snail identification keys (appearance characteristics, length, width, type of spire, apex, aperture, and number of shells) and reliable sources in this regard.

Results: It was determined that the following snails live in this city: *Physa acuta*, *Planorbis planorbis*, *Lymnaea truncatula*, *Lymnaea gedrosiana*, and *Lymnaea stagnalis*. *Lymnaea truncatula* and *Lymnaea gedrosiana*, known hosts of *Fasciola hepatica*, *Fasciola gigantica*, and *Schistosoma turkestanicum*, were identified in the city, suggesting the area is favorable for the emergence of these diseases. Previous research in Iran had mentioned two species, *T. doriae* and *Pisidiu sp.*, in Borujen city, but these species were not found in this study. Instead, two new species, *Planorbis planorbis* and *Physa acuta*, were identified.

Conclusion: The findings indicate that Borujen city is a suitable environment for the emergence of diseases caused by trematode infections, such as fascioliasis and schistosomiasis, due to the presence of the snail species *Lymnaea truncatula* and *Lymnaea gedrosiana*. Given the identification of two new species, *Planorbis planorbis* and *Physa acuta*, this study revises



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



previous reports regarding the species found in the area. Preventive measures should be planned to address the potential for disease emergence in the region.

Keywords: Freshwater Snails, *Planorbis planorbis*, *Physella acuta*, *Lymnaea* spp.



Diphyllobothriasis: A Zoonotic Infection Affecting Humans and Livestock and Its Role in Causing Anemia

Seyed Reza Hosseini¹

Corresponding Author: Seyed Reza Hosseini, rezahosseini2912@gmail.com

1. Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Shahrekord, Shahrekord, Iran.

Article code: 1461

Abstract

Background: Diphyllobothriasis is a parasitic infection caused by the tapeworm *Diphyllobothrium latum*, also known as the fish or broad tapeworm. This zoonotic disease affects humans and fish-eating mammals, including domestic animals like dogs and cats. It is common in regions where raw or undercooked freshwater fish is consumed, such as Scandinavia, Siberia, parts of Asia, and South America. The parasite's life cycle involves freshwater or marine fish as intermediate hosts, and humans become infected by eating fish containing plerocercoid larvae. These larvae develop into adult tapeworms in the small intestine and can live for years. The aim of this article is to review the zoonotic infection diphyllobothriasis in humans and livestock and its role in causing anemia.

Methods: In this review article, relevant sources were retrieved from reputable scientific databases such as PubMed, Science Direct, and Google Scholar. The literature search was conducted using keywords related to the topic of the study. The search covered publications from the year 2020 to 2025.

Results: Studies have shown that infection with *Diphyllobothrium latum* is often asymptomatic, but 20–30% of patients may experience gastrointestinal symptoms such as abdominal pain, diarrhea or constipation, fatigue, and weight loss. A key complication is vitamin B12 deficiency due to the parasite's absorption of the nutrient in the small intestine. Around 35–40% of infected individuals show low B12 levels, though only 2–3% develop clinical megaloblastic anemia. Diagnosis is based on detecting eggs or worm segments in stool, supported by blood tests showing macrocytic anemia. Treatment with praziquantel or niclosamide, along with vitamin B12 supplementation, is generally effective.

Conclusion: Diphyllobothriasis emphasizes the need for public education and better food hygiene in endemic areas. Early diagnosis and treatment are essential to prevent vitamin B12 deficiency anemia.

Keywords: *Diphyllobothrium latum*, zoonotic disease, vitamin B12, anemia, praziquantel



Tick Infestation in Free-Ranging Tortoises (*Testudo graeca*) from Sirch and Lalehzar Regions of Kerman Province, Southeastern Iran

Seyede Mahshid Mostafavi¹, Kian Mosaali¹, Mahdiye Mollashahi², Seyede Mahnoosh Mostafavi³

Corresponding Author: Seyede Mahshid Mostafavi, seyedemahshidmostafavi@gmail.com

1. *Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Bahonar of Kerman, Kerman, Iran.*
2. *Doctorate of Veterinary Medicine (DVM) graduated.*
3. *Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Chamran of Ahvaz, Ahvaz, Iran.*

Article code: 1462

Abstract

Background: Chelonians serve as hosts for various ectoparasites, including ixodid ticks, some of which are vectors of tick-borne pathogens. *Hyalomma aegyptium* is a reptile-associated tick species widely distributed across arid regions of the Middle East. This study aimed to assess the prevalence and species composition of ticks parasitizing free-ranging tortoises (*Testudo graeca*) in the Sirch and Lalehzar districts of Kerman Province, areas notable for their high elevation and distinctive microclimatic conditions.

Methods: A cross-sectional field survey was conducted from May to September 2024. A total of 10 mature *T. graeca* individuals (6 females and 4 males) were manually captured through active searching in natural habitats across Sirch and Lalehzar. Each tortoise was thoroughly examined for ectoparasites, focusing on axillary, inguinal, and cervical skin folds. Ticks were carefully removed using forceps, preserved in 70% ethanol, and morphologically identified to species level under a stereomicroscope employing standard taxonomic keys.

Results: Tick infestation was detected in 5 out of 10 tortoises (50% prevalence), comprising 2 males and 3 females. A total of 17 adult ticks were collected (distributed as 3 ticks on male 1, 1 tick on male 2, 4 ticks on female 1, 3 ticks on female 2, and 6 ticks on female 3), all morphologically identified as *Hyalomma aegyptium*. No larval or nymphal stages were observed.

Conclusion: This study provides updated data on the ectoparasitic burden of free-ranging tortoises in two highland regions of Kerman, demonstrating a moderate prevalence of *Hyalomma aegyptium*. Considering the known vector competence of this tick species and its potential role in zoonotic pathogen maintenance, these findings highlight the importance of



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



long-term ecological surveillance of chelonian-associated ticks in Iran's arid and semi-arid zones.

Keywords: *Testudo graeca*, *Hyalomma aegyptium*, Ectoparasites, Chelonians, Kerman.



Investigation of Parasitic Diseases in Koi Fish (*Cyprinus carpio koi*) from an Aquaculture Farm in Rasht City, Gilan Province

Fatemeh Madankan¹, Mohammad Hossein Razi Jalali^{1,3}, Mehrzad Mesbah^{2,3}, Hossein HamidiNejat¹, Mohammad Vali Mohammadpour Choshali²

Corresponding Author: Fatemeh Madankan, F_madankan@stu.scu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Livestock, poultry and aquatic animal health, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
3. Member of Excellence center of warm water fish health, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1465

Abstract

Background: Aquatic animals that are today known under the name ‘koi fish’ are descendants of the common carp, which, over the course of thousands of years, have been established in Central Asia and from there have spread to other parts of the world. Aquatic animals, in addition to providing a portion of the food for human society, can also be cultivated as ornamental. The health and safety status of koi fish under various environmental conditions can make a significant contribution to reproductive efficiency, egg quality, and protection of offspring. However, despite the importance of this fish, relatively few studies have been conducted on its diseases and parasites.

Methods: On 17 November 2024 a total of 25 koi fish suffering from chronic mortality were referred from two breeding farms in Rasht, Gilan Province—both using river water—to the Aquatic Animal Health and Diseases Department at the Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz. All organs of the fish, including the skin, swim bladder, muscles, digestive system, and others, were examined. Subsequently, necessary procedures were undertaken for the morphological identification of the parasitic species detected.

Results: According to examinations conducted, unicellular protozoans *Trichodina sp.* and *Ichthyobodo necator* were isolated from the body surface of the fish, and metacercariae of the family *Cyathocotylidae*, *Falcatus* metacercaria, and *Stellantchasmus* were isolated and identified from the peritoneal cavity and muscles. Additionally, cestodes diagnosed as *Schyzocotyle acheilognathi* were identified from the gastrointestinal tract of the fish.

Conclusion: Early detection of parasitic diseases in an aquaculture center particularly in facilities using river water is of high priority. Following diagnosis, an appropriate therapeutic



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



or managerial response is essential to prevent the rapid loss of fish stocks. Parasitic infections in humans and animals can be transmitted by freshwater fish that act as second intermediate hosts. The results obtained from this study will provide preliminary information for further management to prevent parasite outbreaks.

Keywords: *Cyprinus rubrofuscus*, Parasitic diseases, Ornamental fish, Aquatic animal diseases.



Emerging Role of Artificial Intelligence in Advancing Parasitology: Current Applications and Future Perspectives

Mahsa Shahbakhsh¹, Maryam Shahbakhsh²

Corresponding Author: Mahsa Shahbakhsh, m1.shahbakhsh@gmail.com

1. Graduate of Department of parasitology. School of veterinary medicine. University of Tehran.
2. Department of Electrical Engineering Electronics and Telecommunications. School of Electrical and Computer Engineering. University of Sistan and Baluchestan.

Article code: 1466

Abstract

Background: Parasitology is the scientific study of parasites, including their life cycles, modes of transmission, diagnosis, prevention, and treatment of parasitic diseases. In this context, Artificial Intelligence (AI) has emerged as a transformative tool in parasitology, offering advanced diagnostic capabilities, precise drug discovery, predictive modeling, personalized treatment, educational opportunities for students, vector control, epidemic detection, understanding disease transmission patterns, and insights into drug resistance mechanisms.

Methods: The present study aims to delineate the current and potential applications of AI in parasitology and to explore possible future directions in this rapidly evolving field. All studies published since 2010 related to this field have been examined.

Results: Predictive AI algorithms analyze large volumes of epidemiological, environmental, and demographic data, aiding in understanding parasite transmission dynamics and disease prevalence. This enhances public health interventions, resource allocation, and preparedness strategies, thereby facilitating preventive measures to reduce disease outbreak impacts. In diagnostic applications, AI-based image analysis enables rapid and accurate identification of parasites, which is especially valuable in remote areas with limited diagnostic resources. Computational AI methods also assist in drug discovery by identifying new therapeutic targets and predicting the efficacy and safety of potential drug candidates, thereby streamlining the development process and leading to more effective and targeted treatments. Furthermore, AI can contribute to educational advancements by improving student learning outcomes, reducing diagnostic errors, and enhancing preparedness for entering the workforce.

Conclusion: However, the potential of AI in parasitology is limited by the complex life cycles of parasites, heterogeneity among different species, and the specialized knowledge required. Additionally, data shortages and ethical considerations constrain widespread implementation. To realize AI's full potential in real-world applications, these barriers must be addressed.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Through further research and collaboration, innovative techniques can be developed to combat parasitic infections effectively.

Keywords: Artificial intelligence, Health care, Machine learning, Parasitic diseases.



Mesenchymal Stem Cells and CAR-T Therapy for *Toxoplasma gondii*-Associated Miscarriage: A Cell-Based Approach for Maternal–Fetal Protection

AmirReza Kanani^{1,4}, Hossein Javid^{1,2,3}

Corresponding author: Hossein Javid, Javidh@varastegan.ac.ir

1. Department of Medical Laboratory Sciences, Varastegan Institute for Medical Sciences, Mashhad, Iran.
2. Department of Clinical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Surgical Oncology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
4. Student Research Committee, Varastegan Institute for Medical Sciences, Mashhad, Iran.

Article code: 1468

Abstract

Background: *Toxoplasma gondii* is a common protozoan parasite that causes vertical transmission, leading to abortion and fetal death. Available antiparasitic therapies are few and are frequently contraindicated during pregnancy, thus there is a pressing need for new treatment strategies. Early study have shown that stem cell therapy, in particular mesenchymal stem cells (MSCs) and immunotherapy by methods of chimeric antigen receptor T-cell (CAR-T) therapy can improve immune response and limit adverse consequences in maternal-fetal settings.

Methods: Data were reviewed from January 2022 to July 2025 using Google Scholar, PubMed and Web of Science. Search terms included “*Toxoplasma gondii*,” “Mesenchymal Stem Cells,” “Miscarriage,” “CAR-T Therapy,” and “Zoonotic Parasites.” Studies were selected based on relevance, clinical validation, and citation impact. The inclusion criteria were included English, human subjects or animal models, and pregnancy. The exclusion criteria involved immunotherapy interventions other than MSC or CAR-T and non-pregnant subjects.

Results: Umbilical cord derived mesenchymal stem cells (UC-MSCs) and bone marrow derived MSCs (BM-MSCs) have been shown to modulate immune responses and inflammatory reactions in animal models of infection with *Toxoplasma gondii*. In animal experiments the rate of vertical transmission was markedly reduced, and fetal survival was improved following MSC treatment. Notably, CAR-T cells targeting *T. gondii* have only been tested in animal models, but similar therapies in cancer patients have been safe. Transplantation of MSCs combined with CAR-T cells may promote immunity and reduce the occurrence of miscarriage caused by *T. gondii* infection.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: Cell-based therapies, such as mesenchymal stem cells (MSCs) and CAR-T immunotherapy, become the candidate approaches for controlling *T. gondii*-induced miscarriage. For development of their clinical application, standardized trials are essential to examine their safety, efficacy, and long-term effects on maternal–fetal outcome. Future study of the optimal dosing, combination schedules, and integration of these therapies should also occur in the context of standard prevention strategies in populations at risk.

Keywords: *Toxoplasma gondii*, Mesenchymal Stem Cells, Miscarriage, CAR-T Therapy, Zoonotic Parasites.



The Link Between Parasitic Infections and Inflammatory Bowel Disease (IBD)

Elahe Mousavi¹, Sanaz Dehbashi^{2,3}, AmirReza Kanani^{2,3}

Corresponding author: Sanaz Dehbashi, DehbashiS@varastegan.ac.ir

1. Student Research Committee, Varastegan Institute for Medical Sciences, Mashhad, Iran.
2. Department of Medical Laboratory Sciences, Varastegan Institute for Medical Sciences, Mashhad, Iran.
3. Department of Clinical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Article code: 1469

Abstract

Background: Deficiencies in vital micronutrients, such as vitamins A, D, and B12, are frequently observed in individuals with inflammatory diseases such as inflammatory bowel disease (IBD). Infectious parasitic diseases (IPDs) and malnutrition remain prevalent in low-income countries, significantly contributing to the global burden of gastrointestinal disorders. In this article, we evaluate the relationship between Parasitic Infections and Inflammatory Bowel Disease.

Methods: A systematic review was conducted using academic databases including Google Scholar, PubMed, ResearchGate, and Web of Science. Publications up to July 2025 were screened using keywords such as parasitic infections, intestinal infections, malnutrition, and micronutrients. Out of 18 initially relevant articles, 6 were selected based on predefined inclusion criteria and language relevance.

Results: Findings from the reviewed literature indicate IBD patients had a significantly higher prevalence of intestinal parasitic infections (IPIs). Moreover, there is a notable correlation between chronic parasitic infections and the development of IBD, particularly among malnourished populations in sub-Saharan Africa, South Asia, and Southeast Asia. The most frequently identified parasites in these regions include *Giardia lamblia*, *Cryptosporidium*, *Ascaris lumbricoides*, and *Entamoeba histolytica*.

These infections are associated with typical IBD symptoms such as abdominal pain, diarrhea, and intestinal distension, as well as diminished immune function. Also, IPD has been linked to low serum concentrations of ferritin, zinc, vitamin C, which are critical for maintaining mucosal integrity and immune defense. Additionally, different studies reported that 30% of IBS patients (a condition often overlapping symptomatically with IBD) were infected with at least one intestinal parasite, with *Blastocystis hominis* and *Giardia lamblia* being the most common.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: The intersection of parasitic infections, micronutrient deficiencies, and malnutrition forms a critical axis in the pathogenesis of IBD. The severity of these effects depends on factors such as parasite type, infection burden, age, and environmental conditions. Future research should focus on elucidating the gut–immune–nutrition relationship.

Keywords: Parasitic infections, Intestinal infections, Malnutrition, Micronutrients.



The Role of Artificial Intelligence and Novel Techniques in Overcoming Zoonotic Parasite Detection and Outbreak Management in Wildlife

Zahra Nabizadeh¹, Leyli Shabani Vieh², Fatemeh Karimi Kesheh³, Mehdi Farjoud⁴, Edris Motamedi⁴, Sahar AriaeeNasab⁵, Mohamad Arad Zandieh⁶

Corresponding Author: Mohamad Arad Zandieh, M.aradzandieh@ut.ac.ir

1. DVM Student, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
2. DVM Student, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.
3. DVM Student, Faculty of Specialized Veterinary Sciences, Islamic Azad University.
4. DVM Student, Faculty of Veterinary Sciences, Islamic Azad University.
5. DVM Student, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran.
6. Department of food Hygiene and Quality Control, Division of Epidemiology, Faculty of Veterinary Medicine, University of Tehran, Iran.

Article code: 1470

Abstract

Background: Wildlife is a natural reservoir of many zoonotic parasites and the source of over 70% of emerging diseases. Habitat destruction, deforestation, and exposure to wild animals have increased the risk of zoonotic diseases spillover, such as toxoplasmosis and ehrlichiosis. However, parasite identification remains difficult due to limited sampling, insufficient data, and unidentified infection sources. Recently, diagnostic accuracy has improved through modern diagnostic methods, especially artificial intelligence-based tools and extensive databases.

Methods: A comprehensive review of articles from 2013 to 2025 in the PubMed, MDPI, and Google Scholar databases was conducted using the keywords "Wildlife", "Zoonotic parasites", "Artificial intelligence", and "Diagnostic method".

Results: Research has focused less on eukaryotic parasites like protozoa and helminths due to genetic complexity and variable life cycles. However, detection methods have advanced; For example Mini-FLOTAC combined with Fill-FLOTAC, is used to detect zoonotic trematodes in rodents. Artificial intelligence tools (e.g., Techcyte) and machine learning models such as Random Forest and XGBoost have been effective in parasite detection and outbreak prediction. Combining surveillance systems with AI can increase the efficiency of disease detection and prediction; providing a platform for early warning systems and helping reduce diseases spread. Molecular methods and genetic techniques such as PCR and gene sequencing have enabled differentiation wildlife origins of parasites. Additionally, field kits and ecological modeling using GPS and genetic data have proven useful in analyzing wild hosts.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: Wildlife is an important reservoir of many zoonotic parasites, and their identification has been challenging due to sampling limitations and a lack of epidemiological data. The use of comprehensive data and a One Health approach is crucial for the development of adaptive surveillance systems and sustainable control. New methods based on new technologies and AI can improve the accuracy of parasite detection and screening. Therefore, investment in this area is highly recommended.

Keywords: Artificial intelligence, Diagnostic method, Wildlife, Zoonotic parasites.



The Impact of Parasites on Neurobiology and Mental Health (Review)

Shiva Hafezi Ahmadi^{1,2}, Sanaz Dehbashi³

Corresponding Author: Sanaz Dehbashi, dehbashis@varastegan.ac.ir

1. Department of Medical Laboratory Sciences, Varastegan Institute for Medical Sciences, Mashhad, Iran.
2. Student Research Committee, Varastegan Institute for Medical Sciences, Mashhad, Iran.
3. Assistant Professor, Department of Medical Laboratory Sciences, Varastegan Institute for Medical Sciences, Mashhad, Iran.

Article code: 1471

Abstract

Background: Parasites such as *Toxoplasma gondii*, *Plasmodium falciparum*, *Taenia solium*, and *Trypanosoma brucei* are pervasive in the environment and significantly impact health, particularly mental health. The purpose of this review is to analyze the available research on neurobiological mechanisms that show how parasites influence brain function and behavior.

Methods: A literature search was conducted via searching PubMed, Scopus, and Google Scholar for articles published between 2013 and 2025. Some of the search terms that were used were parasites, neurobiology, neurotransmission, behavioral changes, and neuroinflammation. An overall total of 22 studies had been decided on based on relevance, methodology, and translational capacity.

Results: Recent studies revealed that parasites can invade the CNS or influence it indirectly by triggering neuroinflammation. These processes may affect brain homeostasis, influence neurotransmission, and lead to significant behavioral or cognitive changes. They use three broadly defined mechanisms: (1) psychoneuroimmunological, (2) neuropharmacological, and (3) genomic- and proteomic-based mechanisms. The two main ways that parasite infections affect the nervous system are through blood-brain barrier crossing and inflammatory responses. Parasites affect biogenic amines, including dopamine, serotonin, and octopamine. According to recent studies, *T. gondii* affects the levels of arginine vasopressin and testosterone. Parasitic infections have been implicated in the development of mental illnesses like schizophrenia, depression, epilepsy, and sleep disturbances. Neurocysticercosis affects cognitive function, leading to problems with concentration and memory.

Conclusion: Studying how parasites influence host behavior will also help us integrate genomic, proteomic, and neurophysiological perspectives on behavior. Recent papers show that parasites tend to use complex, multifaceted mechanisms to alter the behavior of their hosts. Altered neurotransmission, microglial response, brain regions, and desynchronization of diurnal rhythms seem to be shared mechanisms by which parasites influence mental health.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Utilization of novel brain-imaging technology such as fMRI and positron emission tomography will be important in examining these relationships further.

Keywords: Zoonotic Parasites, Neuroinflammation, Neurotransmission, Mental Health, *Toxoplasma gondii*.



Muelleriosis in a Traditional Sheep Flock: A Histopathological Case Report from Northwestern Iran

Rastin Bakhtiari Lafmejani¹, Donya Pourbagher¹, Seyede Soraya Mahmoudi²

Corresponding Author: Seyede Soraya Mahmoudi, s.sorayamahmoudi@gmail.com

1. Doctor of veterinary medicine student, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article code: 1472

Abstract

Background: *Muellerius capillaris* is a metastrongyloid nematode of the family Protostrongylidae and a frequent etiologic agent of chronic verminous pneumonia in small ruminants, in special sheep. It can cause substantial economic loss by means of decreased productivity and being prone to secondary infections.

Methods: The present investigation was conducted in a traditional sheep farm in rural area located in vicinity of Urmia, the West Azerbaijan Province, northwestern Iran where multiple animals were observed to suffer from unceasing cough, gasping, and poor growth. The flock was formed of 50 sheep and was maintained in the non-industrial pasture-based extensive farming system. At necropsy of recently dead and selectively removed animals, we found that firm and grayish nodules were present in the subpleural area of the lungs in 22 animals (44%). H&E staining was performed on the tissue specimens.

Results: On microscopic examination, larvae and adult worms of *Muellerius capillaris* were found within granulomas, infiltrating the lung parenchyma. There was prominent interstitial inflammation, increased alveolar septal thickness, lymphocytic inflammatory infiltrate and formation of multinucleate giant cells. No bacterial pathogenic growth was identified in culture, validating the parasitic cause. On the basis of these findings, the diagnosis was muelleriosis.

Conclusion: This paper highlights the critical need for early clinical diagnosis and histopathologic proof of infection with *M. capillaris* in extensively farmed sheep flocks. A diagnosis provided the opportunity for prompt treatment with anthelmintics such as levamisole and ivermectin, as well as better rotational grazing to minimize recontamination. Monitoring and targeted deworming are necessary to control parasitic lung disease in extensive rural conditions.

Keywords: *Muellerius capillaris*, Sheep, Lungworm, Urmia.



Application of Artificial Intelligence in Diagnosis and Epidemiological Control of *Neospora caninum*: A Narrative Review

Farnaz Seilanian Toosi¹, Nasrollah Vahedi Noori², Sara Kazemi¹, Seyyed Mohammad Aghapour¹

Corresponding Author: Nasrollah Vahedi Noori, Nsvahedii@gmail.com

1. DVM Student, Bab.C. Islamic Azad University, Babol, Iran.
2. Parasitic Diseases Research Department, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran.

Article code: 1473

Abstract

Background: *Neospora caninum* is an intracellular protozoan parasite that causes major reproductive losses in cattle through abortion and infertility, and can also result in neuromuscular disease in dogs. Despite the widespread use of diagnostic tools like ELISA and PCR, challenges remain in early detection, especially at the herd level. In recent years, artificial intelligence (AI) has emerged as a transformative tool in veterinary medicine, offering capabilities such as pattern recognition, decision support, and epidemiological forecasting.

Methods: This narrative review explores the scientific literature from 2020 to 2024 that discusses the use of AI in diagnosing and monitoring *Neospora caninum* infections. Relevant studies were identified using keywords such as “*Neospora caninum*,” “machine learning,” “artificial intelligence,” “bovine abortion,” and “veterinary diagnostics.” Both diagnostic applications and herd-level predictive models were considered.

Results: AI-based approaches have shown considerable promise in the management of neosporosis. Machine learning models have been used to predict the risk of infection based on clinical and serological data, with high accuracy. Additionally, tools developed for parasitic detection in companion animals have demonstrated the feasibility of AI integration in routine veterinary diagnostics, highlighting its practical potential.

Conclusion: Artificial intelligence offers a promising tool to support the diagnosis and control of *Neospora caninum*. It can improve herd surveillance, risk prediction, and early detection in both farm and clinical settings. While AI holds great potential in veterinary medicine, it also presents several challenges. High setup costs, lack of transparency in decision-making, and ethical concerns around reducing human oversight must be considered. In addition, questions remain about system reliability, accuracy, over-reliance on automation, and data privacy. Addressing these limitations is essential for safe and effective integration of AI into veterinary practice.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Artificial Intelligence, Diagnosis, Epidemiological Control, *Neospora caninum*.



Revolutionizing Parasitic Disease Diagnostics: The Rise of AI and Molecular Technologies in Human and Veterinary Health

Mostafa Kami^{1,2,3}, Ali abbasi¹, Najva Zivdari⁴, Mahdi Fakhar^{5,6,7}

Corresponding Author: Mahdi Fakhar, mahdifakhar53@gmail.com

1. Doctor of Veterinary Medicine, Bab.C., Islamic Azad University, Babol, Iran.
2. Young Researchers and Elite Club, Bab.C., Islamic Azad University, Babol, Iran.
3. The Health of Plant and Livestock Products Research Center, Mazandaran University of Medical Sciences, Sari, Iran.
4. DVM Student, Department of Microbiology and Food Hygiene, Faculty Veterinary Medicine, Lorestan University, Khorramabad, Iran.
5. Toxoplasmosis Research Center, Communicable Diseases Institute, Mazandaran University of Medical Sciences, Sari, Iran.
6. Iranian National Registry Center for Lophomoniasis (INRCL), Imam Khomeini Hospital, Mazandaran University of Medical Sciences, Sari, Iran.
7. Department of Medical Microbiology and Immunology, School of Medicine, Qom University of Medical Sciences, Qom, Iran.

Article code: 1475

Abstract

Background: Parasitic infections remain a significant burden on public and veterinary health systems, especially in endemic and low-resource regions. Conventional diagnostic tools, such as manual microscopy, are often limited by low sensitivity, subjectivity, and labor-intensive procedures. Recent breakthroughs in artificial intelligence (AI), deep learning, and molecular diagnostics are rapidly transforming the detection and management of parasitic diseases across both human and animal populations.

Methods: This review synthesizes recent studies (2024–2025) on the application of AI-based diagnostic platforms, smart microscopy, and molecular tools in parasitology. Peer-reviewed literature was analyzed to evaluate diagnostic performance, field applicability, and cross-species relevance of emerging technologies.

Results: AI-enhanced microscopy has shown superior accuracy in detecting soil-transmitted helminths compared to the traditional Kato-Katz method. Tools such as the AiDx Assist platform enable automated detection of *Schistosoma* eggs in stool and urine samples, with proven reliability in field settings. Deep learning models—including YOLO-based object detection—have achieved high precision in classifying helminth ova. Moreover, molecular assays like multiplex PCR and CRISPR-Cas platforms offer increased specificity in identifying co-infections and asymptomatic carriers. These innovations also support epidemiological



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



surveillance and predictive modeling under the One Health paradigm, strengthening early warning systems and zoonotic disease control strategies.

Conclusion: AI and next-generation molecular diagnostics represent a transformative leap in parasitic disease management. By offering rapid, scalable, and accurate tools, they bridge critical gaps in both human and veterinary diagnostics. To ensure sustainable impact, interdisciplinary collaboration and ethical implementation across diverse health systems remain essential.

Keywords: AI-Parasitology, Smart Diagnostics, One Health, CRISPR-Tech.



Regulatory Immune Pathways Underlying the Persistence of *Echinococcus granulosus*: Mechanistic Insights from Experimental Models

Hannaneh Mirzaei Rad¹, Amirhossein Gohari², Maryam Mirzaei³

Corresponding Author: Hannaneh Mirzaei Rad, hannamirzaei@gmail.com

1. Doctor of veterinary Medicine candidate, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Doctor of veterinary Medicine, Islamic Azad University, Babol Branch, Babol, Iran.
3. Medical Doctor, Tehran, Iran.

Article code: 1478

Abstract

Background: Cystic echinococcosis, a chronic parasitic disease caused by the larval form of *Echinococcus granulosus*, is sustained by a remarkably adaptive host-parasite interaction. Among the most striking features of this infection is the parasite's ability to shape the host immune system toward a regulatory and anti-inflammatory state. Recent studies suggest that hydatid cyst fluid induces epigenetic reprogramming in host macrophages, particularly through the upregulation of histone demethylase KDM5B. This process leads to TRAF6 inhibition and subsequent suppression of pro-inflammatory cytokines such as IL-1 β . In addition, parasite-mediated induction of arginase-1 in myeloid cells suppresses T cell activation and contributing to disease chronicity.

Methods: We employed a murine model using 6-8-week-old female BALB/c mice housed under standard specific-pathogen-free conditions. Immune profiling was carried out through multiparametric flow cytometry, ELISA, and co-culture assays. The experimental design included analysis of regulatory T cell (T_{reg}) populations, arginase-1-expressing macrophages, and expression of co-stimulatory molecules on antigen-presenting cells. Additionally, the effect of IL-10 and arginase blockade were evaluated in vitro to assess functional immune recovery.

Results: In infected mice, a consistent expansion of CD4⁺FOXP3⁺ T_{regs} was noted in both splenic and pericystic compartments, averaging a 65% increase relative to uninfected controls. Pericystic fluid analysis revealed elevated levels of IL-10 (about 285 pg/ml) and TGF- β (about 145 pg/ml). Antigen-presenting cells showed downregulation of CD80/CD86 and MHC II, coinciding with a fourfold increase in ARG1⁺ macrophage populations. Functionally, CD4⁺ T cells displayed reduced proliferative responses when co-cultured with antigen-loaded APCs from infected mice, a defect partially reversed upon neutralization of IL-10 or inhibition of arginase activity.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: Our findings reinforce the concept that *E.granulosus* employs a multi-layered strategy to manipulate host immunity,favoring persistence.The expansion of regulatory cell subsets,suppression of APC functionality,and metabolic inhibition via arginase-1 collectively shape a microenvironment that dampens effector T cell responses.These results underscore that potential of adjunctive immunotherapies such as targeting IL-10 signaling or arginase pathways to enhance the efficacy of conventional antiparasitic regimens in chronic CE.

Keywords: *Echinococcus granulosus*, Tregs,IL-10, Cystic echinococcosis, Hydatid cyst, Immunoregulation, Immunosuppression, TGF- β , APCs, Arginase-1.



Addressing Therapeutic Challenges in Giardiasis Management in a Household Samoyed

Tina Daneshrad¹, Siamak Mashhadi Rafiee²

Corresponding Author: Tina Daneshrad, tina.daneshrad3@gmail.com

1. Department of Clinical Sciences, Faculty of Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Article code: 1480

Abstract

Background: *Giardia duodenalis* is a flagellated protozoan parasite that affects a wide range of mammalian hosts, including humans and dogs. It is considered one of the most important zoonotic gastrointestinal parasites worldwide, with transmission routes that include ingestion of contaminated water, food, or direct contact with infected feces. In household settings, close physical interactions between dogs and humans heighten the risk of cross-species transmission. Beyond its enteric manifestations, *Giardia* can induce intestinal dysbiosis, which may worsen clinical outcomes and complicate treatment response. Metronidazole is commonly used to treat giardiasis in dogs, but adverse reactions—particularly gastrointestinal—can limit its utility.

Methods: A one-year-old intact male Samoyed was presented with marked weight loss (initial body weight: 9 kg) despite having a normal appetite. Feces were dark and malodorous. Hematologic evaluation revealed a left shift, and fecal analysis identified *Giardia* cysts and significant intestinal dysbiosis. Oral metronidazole was initiated but caused severe vomiting, including post-water intake emesis. Even after switching to injectable metronidazole and combining it with antiemetics (ondansetron and metoclopramide), vomiting persisted. Due to suspected metronidazole intolerance, the drug was discontinued, and supportive care with fluid therapy, ampicillin, and enrofloxacin was continued. Anti-*Giardia* therapy was replaced with oral fenbendazole.

Results: Vomiting resolved shortly after stopping metronidazole. Over seven days, the dog's clinical signs improved significantly. Fecal color and odor normalized. A follow-up fecal test showed no evidence of *Giardia* cysts or dysbiosis. The dog gained 2 kg, reaching 11 kg in body weight.

Conclusion: This case illustrates the dual challenge of managing giardiasis as both a clinical and public health concern. The zoonotic potential of *Giardia duodenalis* requires increased awareness among clinicians and pet owners. Furthermore, it underscores the importance of



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



being alert to adverse drug reactions—especially metronidazole-induced vomiting—and the value of alternative therapies such as fenbendazole. Timely diagnosis, flexible therapeutic strategies, and strong communication with pet owners are essential for successful management and prevention of zoonotic spread.

Keywords: Giardiasis, Zoonosis, *Giardia duodenalis*, Dysbiosis, Veterinary Parasitology



Parasitic Infections in Companion Animals: Implications for Lameness and Neuromuscular Disorders

Leyli Shabani Vieh¹, Zahra Nabizadeh², Edris Motamedi³, Niknaz Rajaei⁴, Parsa Amoozadeh¹, Parham Rahmanian⁵

Corresponding Author: Parham Rahmanian, Parham.rahmanian@gmail.com

1. DVM Student, Faculty of Veterinary Medicine, Semnan University
2. DVM Student, Faculty of Veterinary Medicine, Urmia University
3. DVM Student, Faculty of Veterinary Sciences, Islamic Azad University
4. Graduated DVM, Private Clinician
5. Doctor of Veterinary Medicine, Faculty of Specialized Veterinary Sciences, Islamic Azad University.

Article code: 1481

Abstract

Background: Lameness is a significant welfare concern in companion animals, affecting comfort, quality of life, and mobility. Parasitic infections may cause lameness directly by damaging the musculoskeletal or nervous systems or by migrating through body tissues. Indirect mechanisms include blood-borne parasites, transmission of infectious diseases, or ectopic localization in tissues such as the CNS and blood vessels. Some parasites also release neurotoxins, leading to motor dysfunction and paralysis.

Methods: A comprehensive review of articles from 2016 to 2025 was conducted in the PubMed, ScienceDirect, and Google Scholar databases using the English keywords parasitic diseases, lameness, companion animals, and neuromuscular disorders.

Results: The findings of this review indicate that parasites induce neuromuscular disorders in dogs and cats through various mechanisms. Ticks, as vectors of the pathogens *Borrelia burgdorferi* and *Anaplasma phagocytophilum*, play a role in the development of lameness. Hepatozoonosis (*Hepatozoon americanum*) can cause severe lameness by inducing myositis, muscle atrophy, and periosteal proliferation, particularly in dogs. Additionally, in infections such as toxoplasmosis and neosporosis, lameness may be accompanied by myopathy, hind limb paralysis, diffuse myalgia and muscular atrophy. In felines, lameness associated with *Gurltia paralyans*, *Toxoplasma gondii*, and uncommon parasites such as *Trichinella* has been reported, leading to necrotizing pyogranulomatous myositis. Tick paralysis, caused by the neurotoxic venom of *Ixodes holocyclus*, is characterized by ascending flaccid paralysis and can be life-threatening. Unlike other vector-borne diseases, its pathogenesis is directly related to the tick's unique toxin-mediated mechanism. *Gurltia paralyans* cause paralysis of the hind



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



limbs, progressive ataxia, and occasionally tetraplegia by damaging the meningeal or spinal blood vessels. Ataxia is also reported in toxoplasmosis, gurltiosis, and neosporosis.

Conclusion: Parasitic infections may cause lameness; clinical signs can range from mild limping to progressive paralysis. Early, targeted antiparasitic therapy is essential for symptom control and prevention.

Keywords: Parasitic infection, Companion animals, Lameness, Neuromuscular disorders.



Faunistic Study of Ectoparasitic Arthropods in Dogs of Sanandaj County, Iran

Zahra Ghashghaei¹, Ghazaaleh Adhami², Pouria Rafiei³

Corresponding Author: Zahra Ghashghaei, ghashghaei.zahra@yahoo.com

1. Veterinary graduate, Sa.c., Islamic Azad University, Sanandaj, Iran.
2. Department of Pathobiology, Sa.c., Islamic Azad University, Sanandaj, Iran.
3. Veterinary graduate, Sa.c., Islamic Azad University, Sanandaj, Iran.

Article code: 1484

Abstract

Background: Ectoparasitic arthropods are common in domestic and stray dogs, potentially affecting animal health and playing a role in transmitting zoonotic pathogens. Given their veterinary and public health relevance, this study aimed to assess the ectoparasite fauna in dogs from Sanandaj County, western Iran.

Methods: A cross-sectional survey was conducted on 200 dogs, including 100 strays and 100 guard dogs. Ectoparasites were collected following standard parasitological procedures. For each dog, variables such as sex, breed, hair coat type, presence of pruritus, alopecia, and anatomical location of parasites were recorded. Identification was performed using morphological keys, and chi-square tests were applied to assess potential associations.

Results: Ectoparasites were detected in 47% of stray dogs and 22% of guard dogs. Among stray dogs, the most frequent species were *Boophilus annulatus* (68.08%) and *Rhipicephalus sanguineus* (23.40%), while *Sarcoptes scabiei*, *Demodex canis*, and *Otodectes cynotis* had lower prevalences. Guard dogs showed a different distribution, with *Rhipicephalus sanguineus* (40.91%) and *Boophilus annulatus* (36.37%) being dominant. *Ctenocephalides canis* (4.54%), *Demodex canis* (13.64%), and *Otodectes cynotis* (4.54%) were also observed. Statistically significant associations were found between parasite presence and hair coat type in stray dogs ($P < 0.05$).

Conclusion: The relatively high ectoparasite burden observed, particularly among stray dogs, underscores the need for regular monitoring and targeted control strategies. Preventive measures are essential to mitigate the risk of zoonotic transmission and improve canine health in the region.

Keywords: Ectoparasites, Dog, Prevalence, Sanandaj, Arthropod fauna.



The Role of CO₂ in Attracting Soft Ticks (Argasidae) and Its Practical Use in Poultry Management

Shirin Aliakbari¹, Parviz Shayan²

Corresponding Author: Shirin Aliakbari, Shirin.aliakbari@ut.ac.ir

1. DVM student, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1485

Abstract

Background: Soft ticks, especially *Argas persicus*, are nocturnal blood-feeding creatures that target avian hosts. They hide in crevices of roosts, making them difficult to detect. CO₂, released during exhalation, acts as a chemical signal that guides ticks to their hosts. Regarding contamination of poultry housing, understanding the biological response of ticks to CO₂ can assist in managing and monitoring both commercial and backyard poultry flocks.

Methods: Articles published between 1980 and 2025 were investigated using the *Argas persicus* soft ticks CO₂ trap and poultry search keywords. Documented and reliable relevant data were reviewed and extracted, and subsequently utilized to produce this article.

Results: Soft ticks detect host cues through chemosensory organs, such as the Haller's organ on the foreleg, which likely includes CO₂-sensitive sensilla. Using CO₂ traps reduces the need for widespread chemical treatments. Dry ice-baited devices, compared to unbaited probe traps, collected significantly more ticks.

Conclusion: *Argas persicus* has been reported in multiple poultry flocks across Iran. Their infestation can cause pathogen transmission and economic losses. CO₂-based traps provide a reliable and effective method for detecting and managing soft ticks in poultry.

Keywords: CO₂ Trap, Soft Tick, *Argas persicus*, Poultry.



Enhancing Anti-Acanthamoeba Potential of *Satureja khuzistanica* Essential Oil through Ultrasonic-Nanoemulsification Approach

Somayeh Bahrami¹, Fatemeh Navidi¹, Maryam Ghaderi-Ghahfarokhi², Mehdi Zarei²

Corresponding Author: Maryam Ghaderi-Ghahfarokhi, m.ghaderi@scu.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1489

Abstract

Background: Acanthamoeba is a genus of microscopic, single-celled organisms commonly referred to as amoebae. These amoebae are found widely in the environment and infrequently cause disease in humans. It can infect multiple organs throughout the body, causing widespread infections and potentially spreading to the central nervous system. Acanthamoeba causes keratitis, resulting in corneal ulcers, vision loss, and, in some cases, blindness. This study aimed to assess the antiamoebic effect of *Satureja khuzistanica* essential oil (SEO) and its nanoemulsion (SEO-NE).

Methods: In this study, SEO-NE was prepared using the ultrasonic emulsification method. The amoebicidal effects of various concentrations of SEO (500-2000 µg/ml) and SEO-NE (50-200 µg/ml) were evaluated on both trophozoites and cysts of the reference Acanthamoeba strain (ATCC 30010) for durations of 1-3 hours in nutrient (PYG) and non-nutrient (PAS) medium. At each time point, the survival rates of cysts and trophozoites were assessed by staining with trypan blue and counting on a hemocytometer slide.

Results: SEO at a concentration of 1000 µg/L effectively eliminated all trophozoites of the standard Acanthamoeba strain within 2 hours in both PAS and PYG media. Cysts were eliminated in PYG after 2 hours at this concentration, but in PAS, complete eradication required 2000 µg/L for the same duration of SEO. The effects of SEO-NE on trophozoites of the standard strain indicated that, in PAS medium, 100 µg/L, and in PYG medium, only 50 µg/L, were enough to completely eradicate the trophozoites. Additionally, 50 µg/L of SEO could eliminate all amoeba cysts within the first hour in PYG medium, whereas 100 µg/L was needed in PAS medium to achieve the same effect within the same time.

Conclusion: Our findings demonstrate that SEO and SEO-NE can effectively combat Acanthamoeba infections. The effectiveness of SEO improved with the nanoemulsification technique, which reduces the overall dose and side effects.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Acanthamoeba, essential oil, Nanoemulsion, *Satureja khuzistanica*.



Systematic Review of the Effect of Prolonged In Vitro Passage on the Immunopathogenicity of *Theileria* spp.

Mohaddeseh Karimi Kesheh¹, Parviz Shayan², Fatemeh Karimi Kesheh³

Corresponding Author: Mohaddeseh Karimi Kesheh, Mohaddesehkarimik78@gmail.com

1. DVM student, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. DVM student, Faculty of Veterinary Medicine, Science and Research Branch, Islamic Azad University, Tehran, Iran.

Article code: 1490

Abstract

Background: Theileriosis, caused by *Theileria* species, poses a serious threat to livestock, particularly in tropical and subtropical regions. *T. lestoquardi* and *T. annulata* belong to the most causative agent of theileriosis in Iran. Infection induces cellular changes, promoting cancer-like properties in host cells, including persistent proliferation and resistance to apoptosis, presenting challenges for livestock health. Prolonged *in vitro* passage results in the loss of invasive properties, while maintaining immune responses supporting the development of an attenuated live vaccine.

Methods: A systematic literature review was performed using PubMed, ScienceDirect, and Google Scholar with keywords. The search covered peer-reviewed articles published from 2006 to 2025. Articles were screened based on title and abstract relevance before a detailed review of full texts for potential inclusion.

Results: The review found that, in *T. lestoquardi*, increased passage numbers led to significant decreases in MMP-9 and MMP-2 activity, demonstrating reduced invasive potential. Concurrently, TNF- α mRNA expression decreased at high passages. Lymphoblastoid cells infected with *T. lestoquardi* exhibited a loss of virulence after 105 passages, resulting in only mild febrile responses and low parasitemia. *T. parva*-infected cells similarly showed down-regulation of NF- κ B and MMP9 pathways, yet retained expression of genes that activate CD8⁺ T cell responses. Notably, *T. annulata* showed limited invasiveness and was less pathogenic in rabbits, while still eliciting strong immune responses. The experimental induction of long-term immunity via ITM against *T. parva* was also noted.

Conclusion: The study highlights that reducing *Theileria* pathogenicity through methods like prolonged passages and abnormal host replication effectively lessens invasion while preserving



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



immunogenicity. This balance supports the potential development of safe, live attenuated vaccines, as transformation-related pathways are suppressed, ensuring the presence of vital CD8+ T cell epitopes essential for vaccine efficacy. The findings underline the significance of these approaches in controlling *Theileria*-induced diseases in livestock.

Keywords: *Theileria*, *T. annulata*, *T. lestoquardi*, Immunogenicity, Vaccination.



Survey of Gastrointestinal Helminth Fauna in Native Dogs in Eastern Gilan Province

Elahe Noorollahi¹, Fatemeh zahra Gharib², Shohreh Alian³

Corresponding Author: Fatemeh zahra Gharib, fzgharib@iau.ac.ir

1. Resident of Department of Surgery, K.C., Islamic Azad University, Karaj, Iran
2. Department of Clinical Sciences, Bab.C., Islamic Azad University, Babol, Iran
3. Department of food hygiene, Faculty of veterinary medicine, Amol University of Special Modern Technologies, Amol, Iran.

Article code: 1492

Abstract

Background: Common parasitic worms in dogs can be transmitted to humans, posing significant health risks. In some cases, the larvae of these parasites migrate to vital human organs, potentially leading to serious health complications and even death.

Methods: This study was conducted on 100 dogs from various regions of eastern Gilan Province to investigate the prevalence of gastrointestinal helminthic infections. Stool samples were examined using the Clayton-Lane method.

Results: According to the findings, 61% of the examined dogs were infected with helminths. Among these, *Toxocara canis* was the most prevalent parasite (30%), followed by *Ancylostoma caninum* (9%), *Trichuris vulpis* (4%), and *Dipylidium caninum* (2%). There was no statistically significant relationship between parasitic infection and the sex of the dogs. However, a statistically significant association was found between infection and both the dogs' living conditions, stray dogs had a higher infection rate, and age, with older dogs showing a higher prevalence of infection. Overall, more than half of the dogs were infected with gastrointestinal helminths.

Conclusion: The high prevalence of gastrointestinal helminths among dogs in eastern Gilan may be attributed to inadequate environmental health monitoring. It is essential to identify the most common endemic parasites through epidemiological studies so that relevant authorities can develop targeted public health education and intervention programs.

Keywords: Dog, Helminth, Gastrointestinal, Eastern Gilan.



Microsatellite Analysis as a Tool for Revealing the Genetic Diversity and Origin of *Leishmania tropica*

Mobina Mohammadi¹, Parviz Shayan¹

Corresponding Author: Parviz Shayan, pshayan@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1493

Abstract

Background: *Leishmania tropica* is a vector-borne parasite responsible for cutaneous leishmaniasis. The Spread of cutaneous leishmaniasis necessitates an investigation into the epidemiology and evolution of this parasite. The genetic exchange between *Leishmania* populations may change population structure and complicate molecular epidemiological studies. However, Microsatellite analysis can be a beneficial method for phylogenetic and epidemiological studies. Microsatellite or short tandem repeats (STRs) are the neutral consecutive repeats of nucleotides ranging from 1 to 6 bases, mostly in the non-coding regions of the genome. In this study, we review the use of Multi Locus Microsatellite Typing (MLMT) as a tool for revealing *L. tropica* origin and genetic diversity.

Methods: Various studies have utilized Microsatellite markers and compared them among the different strains of *L. tropica*. Literature searches of electronic databases from 2004 to 2015 were conducted in July 2025. Databases used include PubMed, Google Scholar, ScienceDirect, and PLOS. There were no language restrictions in the searches. This search used the following keywords: “Microsatellite,” “Leishmania tropica,” “MLMT,” and “Microsatellite analysis”.

Results: various studies proved MLMT to be a beneficial tool for epidemiological and phylogenetic studies of *L. tropica*. The pioneering study done by Schwenkenbecher showed that *L. tropica* likely came from Africa and mostly reproduces clonally, but its genetic patterns vary based on geography and how it's transmitted, which highlights the ability of the MLMT method in epidemiological studies. Another study employed the MLMT method to compare the population structure of *Leishmania killicki* and *L. tropica*, supporting the hypothesis that *L. killicki* belongs to the *L. tropica* complex, despite the strong genetic differentiation between them.

Conclusion: MLMT method is a promising method for epidemiological and phylogenetic studies. The microsatellite analysis of *L. tropica* can provide valuable information about the origin and subpopulations of the parasite, which can help inform prophylactic actions.

Keywords: Microsatellite, MLMT, *Leishmania tropica*, Zoonotic parasites, Epidemiology.



Investigation on *Toxocara* spp. Eggs in Breeding Dogs in Tehran Province, Iran

Ali Ahmadi¹, Fateme Jalousian¹

Corresponding Author: Ali Ahmadi, ahmadiali26@ut.ac.ir and, Fateme Jalousian, jalousian_f@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1494

Abstract

Background: Toxocariasis is a significant zoonotic infection caused by the larval stages of *Toxocara canis* and *Toxascaris leonina*, primarily transmitted through ingestion of embryonated eggs present in contaminated environments. Dogs, particularly those in breeding facilities, can serve as important reservoirs for these parasites, contributing to soil contamination and increased human exposure risk. This study aimed to assess the frequency of *Toxocara* spp. eggs in the feces of breeding dogs in Tehran Province, Iran.

Methods: A total of 100 fecal samples were collected from breeding dogs aged between 1 to 7 years, maintained in breeding kennels in Tehran Province. The samples included 56 females and 44 males. The samples were examined using the Willis flotation method with saturated sodium chloride (NaCl) solution at a density of 1.2. Microscopic evaluation was conducted under X10 and X400 magnification.

Results: The results revealed that 6% of the samples contained *Toxocara canis* eggs and 1 % were positive for *Toxascaris leonina* eggs. Notably, all infected samples were from dogs aged 1–3 years. A positive correlation was observed between age and susceptibility to Ascaridiidae infection ($r= 6.22$), indicating that younger dogs are at higher risk. No mixed infections were identified.

Conclusion: These findings suggest that even in regulated breeding environments, parasitic contamination remains a concern. The environment in which the dogs are kept plays a critical role in transmission, underscoring the need for rigorous sanitation, routine deworming programs, and regular parasitological monitoring. Given the zoonotic potential of these helminths, increasing awareness among breeders and implementing standardized veterinary protocols are essential steps in reducing the public health burden.

Keywords: *Toxocara canis*, *Toxascaris leonina*, Willis flotation, Zoonotic parasites, Kennel hygiene.



Acute Bilateral Hyphema and Sudden Blindness Associated with Ehrlichiosis in a German Shepherd Dog: A Case Report

Reza Amjadi¹, Alireza Ghaffari Cherati², Parviz Noushivani³

Corresponding Author: Reza Amjadi, rezaamjadii@yahoo.com

1. School of veterinary medicine, Islamic Azad University, Babol branch, Babol, Iran.
2. Department of small animal internal medicine, Ara Pet Hospital, Sari, Iran.
3. Department of veterinary clinical pathology, Ara Pet Hospital, Sari, Iran.

Article code: 1497

Abstract

Background: Ehrlichiosis is a tick-borne disease caused by *Ehrlichia* species that can lead to systemic and ocular manifestations in dogs. Sudden blindness with hyphema is an uncommon but critical presentation that requires prompt diagnosis and intervention.

Methods: A 5-year-old female german shepherd dog was referred to Ara Veterinary Hospital on March 18, 2026, due to acute blindness. Clinical ophthalmic evaluation revealed bilateral hyphema and a positive menace response in the left eye. Hematologic and biochemical profiles were obtained. Further evaluations, including corneal staining, canine distemper virus (CDV) testing, and polymerase chain reaction (PCR) for *Ehrlichia* and *Leishmania*, were performed.

Results: Complete blood count (CBC) showed a reversed neutrophil-to-lymphocyte ratio with a normal hemogram. Biochemistry indicated hypoalbuminemia, hyperglobulinemia, and elevated liver enzymes like Aspartate Aminotransferase (AST), Alkaline Aminotransferase (ALT) and Alkaline Phosphatase (ALP), suggestive of systemic inflammation or hepatic involvement. Canine distemper virus (CDV) test was negative. Corneal staining was negative. On April 8, Polymerase chain reaction (PCR) testing confirmed a positive result for *Ehrlichia* and negative for *Leishmania*. The dog was diagnosed with ehrlichiosis, and the initial therapy was induced. The case received empirical treatment with antibiotics (cap doxycycline100, 2.5 cap) and corticosteroids (Tab Prednisolone5, 4 tab), alongside topical ophthalmic therapy (opht drop Atropine1% 1drop, opht drop chloramphenicol 1drop and opht drop prednisolone 1drop). Clinical improvement in ocular signs and general condition was observed over the following days. Case is much better now and her vision is back.

Conclusion: This case illustrates that *Ehrlichia* infection should be considered a differential diagnosis in dogs with sudden blindness and bilateral hyphema, particularly in endemic regions. Early diagnosis and appropriate antibiotic and supportive therapy can lead to favorable outcomes.

Keywords: Ehrlichiosis, Dog, Hyphema, PCR, Ophthalmology



Study of the Prevalence of *Taenia* spp. Isolated from Dogs in Southeastern Iran Using the Concentration Method

Maziar Jajarmi¹, Saeidreza NourollahiFard¹, Mansour Dabirzadeh², Mahdi Khoshsimashahraki^{1,2}

Corresponding Author: Mahdi Khoshsimashahraki, khoshsimashahraki6144@vet.uk.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman Iran
2. Department of Parasitology and Mycology, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran.

Article code: 1498

Abstract

Background: *Taenia* spp. is one of the significant zoonotic parasites causing taeniasis in humans and animals, recognized as a public health and economic concern in many regions worldwide. *Taenia* spp. are among the most critical cestodes that lead to the formation of cysts or infections in humans and animals. Control programs, including regular deworming of dogs, slaughtering animals in equipped abattoirs, and proper disposal of infected viscera, have effectively reduced the prevalence of this parasite. This study aims to investigate the epidemiology of *Taenia* spp. in dogs in southeastern Iran.

Methods: In this descriptive cross-sectional study, 260 dog fecal samples were collected over six months during the spring and summer seasons in two regions of southeastern Iran, Sistan and Kerman, using a completely randomized sampling method. Fecal samples from stray, herd, and shelter dogs were examined with strict safety precautions using the formalin-ether concentration method to detect contamination with *Taenia* spp. eggs.

Results: The study revealed that out of 260 dog fecal samples examined, 13 cases (5%) were infected with *Taenia* spp. eggs. Statistical analyses were conducted using chi-square and t-tests to evaluate the association between the presence of *Taenia* spp. and factors including dogs' age, type of water consumed, dog type (stray, herd, or shelter), type of food (raw or cooked), and region of residence (Sistan or Kerman). The results indicated that temperature, type of water consumed, and dog type were significantly associated with the presence of *Taenia* spp. in fecal samples ($P < 0.05$).

Conclusion: This study underscores a significant public health challenge due to low awareness and inadequate routine veterinary care among dog owners in southeastern Iran. The findings highlight the need for improved control measures and public education. Future research should



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



prioritize molecular diagnostic techniques, such as PCR, to identify *Taenia* strains and their transmission sources.

Keywords: Epidemiology- Dog- *Taenia* spp-Sistan.



A Study on the Frequency and Diversity of Ectoparasites in Stray Dogs in Shiraz

Shadi Shahvand¹, Zahra Mohammadi¹, Mohammad-salar Tasdighi¹, Seyed Mostafa Razavi¹

Corresponding Author: Shadi Shahvand, shahvandshadi99@gmail.com

1. Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Article code: 1499

Abstract

Background: Stray dogs are important reservoirs for various ectoparasites, some of which can act as vectors for zoonotic pathogens. In Shiraz, the growing population of stray dogs increases the risk of transmitting parasitic diseases to both animals and humans. This study was conducted to determine the frequency and diversity of ectoparasites in stray dogs in Shiraz, with the aim of providing baseline data to support prevention and control programs for zoonotic diseases.

Methods: Between April and June 2025, 35 consecutive stray dogs captured by the Shiraz municipal animal control unit were examined (convenience sampling; each dog evaluated once; no restriction on age or sex). A standardized clinical inspection and body-surface combing were performed. Ectoparasites were removed with fine forceps from the skin and hair coat, preserved in glycerin–alcohol, and analyzed at the Parasitology Laboratory, Shiraz University. Specimens were identified morphologically using standard taxonomic keys.

Results: Of 35 dogs, 15 (42.9%) were infested with at least one ectoparasite species. Identified taxa among infested dogs included: *Rhipicephalus sanguineus* (7 specimens; 46.7% of positive dogs), *Hippobosca* spp. (5; 33.3%), *Rhipicephalus bursa* (2; 13.3%), and *Ctenocephalides canis* (1; 6.7%).

Conclusion: Stray dogs in Shiraz carry multiple ectoparasite taxa, including hematophagous groups such as certain ticks, fleas, and louse flies; although some ectoparasites feed on blood, others may not depending on species or life stage. These infestations may contribute to skin lesions, irritation, and anemia in dogs, and can facilitate transmission of vector-borne pathogens. Regular monitoring and targeted control are recommended. Given the small sample size, these findings should be considered preliminary and warrant larger studies.

Keywords: Zoonotic diseases, Vector-borne pathogens, *Rhipicephalus sanguineus*, *Hippobosca* spp, *Ctenocephalides canis*.



Global Research Trends on Re-emerging Parasitic Diseases: A Bibliometric Analysis (2000-2025)

Masoud Imani¹, Sina Etminan²

Corresponding Author: Masoud Imani, masoudimani7@gmail.com

1. *Department of Biostatistics, School of Public Health, Iran University of Medical Sciences, Tehran, Iran.*
2. *Faculty of Veterinary Medicine, University of Garmsar, Semnan, Iran.*

Article code: 1502

Abstract

Background: Re-emerging parasitic diseases continue to pose significant threats to global public health, necessitating ongoing research to understand evolving epidemiological patterns. This study aimed to systematically examine global research trends, collaborative networks, and prominent research themes on re-emerging parasitic diseases from 2000 to 2025 through a comprehensive bibliometric analysis.

Methods: We conducted a bibliometric analysis using peer-reviewed articles indexed exclusively in the Web of Science (ISI) database, spanning publications from January 2000 to July 2025. Relevant articles were retrieved using search terms such as re-emerging parasitic diseases, parasitic infections, and re-emerging parasites. Data analysis was performed using bibliometric software, specifically VOSviewer and the Bibliometrix package in R. These tools enabled us to visualize collaboration networks, citation analysis, thematic mapping, and co-occurrence of keywords, providing insights into the evolution of research trends over the defined period.

Results: A total of 1,785 articles met the inclusion criteria, showing a significant upward trend in publications after 2010, indicating increasing scholarly interest. The United States, Brazil, and China were identified as leading countries in research output and international collaboration. Major research hotspots included malaria resurgence, Chagas disease epidemiology, schistosomiasis control strategies, and the impact of climate change on parasite transmission dynamics. Network analyses highlighted strong collaborative links among research institutions globally, although regional collaboration clusters were also evident. Keyword analysis revealed emerging themes, including climate-related factors, parasite drug resistance, and vector ecology.

Conclusion: This bibliometric analysis highlights critical trends and research priorities in the domain of re-emerging parasitic diseases. The findings underscore the importance of international collaboration and emphasize the need to address research gaps related to parasite



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



ecology, climate impacts, and innovative control measures. These insights can inform policymakers, researchers, and healthcare providers about future directions and priorities in global parasitology research.

Keywords: Re-emerging Parasitic Diseases, Bibliometric Analysis, VOSviewer, Global Health.



Revolutionizing Parasitic Disease Control: The Role of Artificial Intelligence in Diagnosis and Public Health

Fatemeh Valikhani¹, Yaser Habibi¹, Mohammed Reza Anani¹, Mobina Rajabzadeh¹, Tohid Javaheri²

Corresponding Author: Fatemeh Valikhani, fvalikhani162@gmail.com

1. Department of Biology, Faculty of Sciences, Mashhad Branch, Islamic Azad University, Mashhad, Iran.
2. Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran.

Article code: 1504

Abstract

Background: Parasitic infections including malaria, leishmaniasis and schistosomiasis are diseases of public health concern, especially in resource-poor countries. Challenges like late diagnosis, lack of qualified health care professionals and limited access to diagnostic facilities hinder the control of the disease. In recent years, the use of artificial intelligence (AI) for improving the accuracy and efficiency of parasite detection, disease surveillance and public health strategies has been explored.

Methods: This narrative review is based on a systematic search of peer-reviewed papers published during the period 2015-2025 in the databases PubMed, Scopus, Web of Science. The search terms used included Artificial Intelligence and Parasitic Diseases, Machine Learning and Digital Diagnosis. Publications included were experimental research, systematic reviews and AI-based approaches in the detection, diagnosis, and monitoring of parasitic infestations.

Results: For the parasite detection from the microscopic images, machine learning approaches, specially the Convolutional Neural Network (CNN), have achieved high-performance accuracy over 95% in the recognition of parasites like Plasmodium, Leishmania, and Schistosoma. Beyond image processing, AI models have been utilized to forecast the spread disease in epidemiology and to prioritize the allocation of resource and data-driven decision on public health campaigns. A few AI-based mobile applications have also been demonstrated to possibly enable field diagnostics in low-resource settings.

Conclusions: Artificial intelligence has great potential in revolutionizing the control of parasitic diseases through accuracy and precision of diagnosis, promoting active surveillance of diseases. The introduction of AI technologies in the existing healthcare systems may lead to an improved performance of the intervention, particularly in resource-constrained regions. In the future, progress will rely on multidisciplinary teamwork, continued investment in research, and creation of available and context-specific AI tools suited to endemic areas.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Artificial Intelligence, Parasitic Diseases, Machine Learning, Digital Diagnosis.



A Case Report of Severe *Hepatozoon canis* Infection in a Mixed-breed Puppy in Sari

Reza Amjadi¹, Siavash Faramarz², Parviz Noushervani³

Corresponding Author: Reza Amjadi, rezaamjadii@yahoo.com

1. School of veterinary medicine, Islamic Azad University, Babol branch, Babol, Iran.
2. Department of small animal internal medicine, Ara pet hospital, Sari, Iran.
3. Department of veterinary clinical pathology, Ara pet hospital, Sari, Iran.

Article code: 1505

Abstract

Background: Vector-borne parasitic diseases such as *Hepatozoon canis* are of increasing importance in veterinary medicine, particularly during the warmer months when tick activity intensifies. This case highlights the correlation between seasonal tick prevalence and the emergence of hemoprotozoan infections in dogs.

Methods: In June 2023 (the onset of summer), a 3-month-old female mixed-breed puppy named Fandogh presented to Ara Veterinary Hospital with signs of vomiting, though she remained alert and maintained appetite. Canine parvovirus (CPV) rapid kit was negative. Complete blood count (CBC) was performed, and blood samples were collected periodically for follow-up during the study period.

Results: On June 14, 2023, leukocytosis and mild non-regenerative anemia were identified, and severe *H. canis* infection was confirmed in leukocytes. Treatment was initiated on June 15 using subcutaneous injections of imidocarb dipropionate (0.2–0.55 cc) every two weeks, combined with supportive therapy including Ringer, dextrose 50%, famotidine, metoclopramide, B-complex, and cefazolin. Therapy continued regularly through November 5, 2023. Serial blood tests showed gradual improvement, with normalization of leukogram and hemogram by September 23. The dog currently remains clinically healthy and parasite-free. Practical recommendations for prevention and control include regular tick control measures, especially during warmer months, environmental tick management, and routine health monitoring in endemic areas.

Conclusion: This case underscores the role of seasonal tick proliferation in the transmission of *H. canis*. Early detection and regular treatment using imidocarb dipropionate can lead to favorable outcomes, even in severe infections. Proactive tick control measures are essential during the warmer seasons to prevent such vector-borne diseases.

Keywords: *Hepatozoon canis*, Tick-borne disease, Hemoprotozoan, Seasonal parasitic infection.



A Comparative Review of Amphotericin B, Meglumine Antimoniate (Glucantime), and Curcumin as Treatment Agents for Cutaneous Leishmaniasis Caused by *Leishmania major*

Mobina Mohammadi¹, Parviz Shayan¹

Corresponding Author: Parviz Shayan, pshayan@gmail.ut.ac.ir

1. Research Center for Ticks and Tick-borne Diseases, Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1508

Abstract

Background: *Leishmania major*, a protozoan parasite belonging to the genus *Leishmania*, is the causative agent of Old World cutaneous leishmaniasis. Since the 1940s, pentavalent antimonials, such as meglumine antimoniate, have been considered the first-line treatment for leishmaniasis. Administration of pentavalent antimonials comes with side effects, including but not limited to hepatotoxicity, cardiotoxicity, pancreatitis, and injection site pain. Amphotericin B deoxycholate has been used as a second-line treatment for cutaneous leishmaniasis. On the other hand, a recent study has evaluated the effect of nanoliposomal Curcumin on CL skin lesions caused by *L. major*. Curcumin is an extract of the rhizome turmeric (diferuloylmethane), which has antioxidant, anti-inflammatory, apoptotic, and anti-proliferative activity.

Methods: Literature searches of electronic databases from 2007 to 2024 were conducted in July 2025. Databases used include PubMed, Google Scholar, ScienceDirect, and PLOS. There were no language restrictions in the searches. This search used the following keywords: cutaneous leishmaniasis, Glucantime, Curcumin, and Amphotericin B.

Results: For decades, pentavalent antimonials have been the drug of choice; however, they are accompanied by many side effects. Due to the studies reviewed, the most frequently reported clinical adverse effects of these drugs were musculoskeletal pain, gastrointestinal disturbances, and headaches. Amphotericin B, as another treatment option, comes with downsides such as systemic and renal toxicity, cost, and difficulty of intravenous administration. The introduction of lipid-associated amphotericin B products with less renal toxicity has enabled more widespread use; however, a high percentage of liposomal amphotericin B is bound to the liposome and is not biologically effective. A recent study showed the potential of Curcumin as a new anti-leishmanial drug.

Conclusion: Nanoliposomal curcumin could be considered as a potential drug against cutaneous leishmaniasis caused by *L. major* with no side effects compared to Glucantime or Amphotericin B.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Cutaneous leishmaniasis, Glucantime, Amphotericin B, Curcumin.



A Survey of Gastrointestinal Parasite Infection in Horses and Donkeys in the Dargaz Area, Khorsan Razavi Province

Mohammad Hosein Gholinezhadnokhandan¹, Abbas Jadidoleslami², Zahra Poorejbari³, Ali Parsafar⁴, Gholamreza Razmi⁵

Corresponding Author: Gholamreza Razmi, razmi@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.

Article code: 1514

Abstract

Background: Gastrointestinal parasitic infections can lead to progressive weight loss, anemia, and, in severe cases, death in horses. As donkeys and horses often graze alongside sheep in mixed herding systems, the risk of cross-species parasite transmission increases. However, limited information is available regarding gastrointestinal parasitic infections in equids in the Dargaz area. This study aimed to investigate the prevalence of gastrointestinal parasites in equids in this area.

Methods: A cross-sectional study was conducted during the spring season on 87 equids in the Dargaz area. Individual fecal samples were collected and transferred to the parasitology laboratory for examination. The presence of parasite eggs and oocysts was assessed using the Clayton-Lane flotation technique with zinc chloride.

Results: A total of 87 fecal samples were examined, including 58 from donkeys and 29 from horses. The frequency of gastrointestinal parasitic infections was as follows:

- *Eimeria leuckarti* oocysts: 7 donkeys (12.06%) and 5 horses (17.24%)
- *Parascaris equorum* eggs: 2 donkeys (3.44%) and 4 horses (13.79%)
- *Dicrocoelium* eggs: 7 donkeys (12.06%) and 1 horse (3.44%)
- *Trichuris* eggs: 1 donkey (1.72%)
- *Strongyle* spp. eggs: 22 donkeys (37.93%) and 11 horses (37.93%)

Conclusion: This study revealed a moderate frequency of gastrointestinal parasitic infections among equids in the Dargaz region. Given the potential health impacts of these infections, regular antiparasitic treatment and monitoring are recommended to improve equid health.

Keywords: Equids, Farm Animals, Gastrointestinal Parasites, Dargaz County.



Diversity and Prevalence of Cestode Infections in Three Lizard Species from the Arid Regions of Sistan and Nehbandan, Southeastern Iran

Kian Mosaali¹, Mahdiye Mollashahi², Seyede Mahnoosh Mostafavi³, Seyede Mahshid Mostafavi¹

Corresponding Author: Kian Mosaali, moosaalikian@gmail.com

1. Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Bahonar of Kerman, Kerman, Iran.
2. Doctorate of Veterinary Medicine (DVM) graduated
3. Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Chamran of Ahvaz, Ahvaz, Iran.

Article code: 1516

Abstract

Background: Reptilian hosts play a significant ecological role in arid environments and can harbor a variety of helminths, including cestodes, which may affect both individual health and ecosystem dynamics. Despite the ecological importance of lizards in the Iranian plateau, parasitological surveys in remote desert areas remain scarce. This study aimed to assess the prevalence and diversity of cestode parasites in three lizard species inhabiting the understudied regions of Sistan and Nehbandan, southeastern Iran.

Methods: During spring and summer field expeditions in 2024, a total of 48 dead lizards were collected from diverse habitats within Sistan and Nehbandan. The specimens included 32 individuals of *Trapelus agilis* (locally known as Agama Chabok), 10 *Varanus griseus* (Desert Monitor, or Bozmache Biabani), and 6 *Phrynocephalus maculatus* (Agama Sarvazaghi). After dissection, gastrointestinal tracts were examined under a stereomicroscope. Recovered cestodes were fixed in 70% ethanol.

Results: Cestode infections were detected in all three species, with varying prevalence rates. According to the results of the contamination observed in these lizards, it is absolutely necessary that more detailed studies should be carried out to accurately examine the cestode species and other digestive parasites of lizards.

Conclusion: This study provides novel data on the helminth fauna of reptiles in southeastern Iran and highlights the role of Sistan and Nehbandan as endemic areas for cestode transmission among desert-dwelling lizards. Further investigations employing molecular techniques are recommended to clarify species identities and assess potential public health or ecological implications.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Cestode, *Trapelus agilis*, *Varanus griseus*, *Phrynocephalus maculatus*, Helminths, Sistan, Nehbandan, Iran.



Tick Diversity and Distribution in Iranian Reptiles: A Narrative Review (1922–2025)

Mehrasa SheikhEsmaili¹, Parviz Shayan²

Corresponding Author: Parviz Shayan, pshayan@ut.ac.ir

1. Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1518

Abstract

Background: Reptiles in Iran are traditionally considered hosts for hard ticks (Ixodidae) and occasionally soft ticks (Argasidae). Recent surveys, particularly in Iran's border and western provinces, confirm widespread reptilian infestation, mainly by *Hyalomma aegyptium*. This review compiles data from historical literature, recent fieldwork, and global distribution models (up to 2025), including reports from Nemrut Mountain on the Iran–Turkey border.

Methods: Data from 1922 to 2025 were obtained from peer-reviewed sources. Ticks were identified using morphological and molecular methods, and records on host species, geography, and infestation prevalence were compiled.

Results: Numerous tick species parasitize Iranian reptiles, particularly near borders. *Hyalomma aegyptium* is the dominant species in the northwest, infesting *Testudo graeca* at 60–100% prevalence and *Trachylepis spp.* at ~60%. *Rhipicephalus sanguineus* (~14%) and *Haemaphysalis spp.* (~17%) are also reported on skinks in Nemrut. *H. marginatum* has been isolated from *Macrovipera lebetina* in Kermanshah and Lorestan (18–22%). Occasional findings of soft ticks (*Argas spp.*) have been noted on *Varanus griseus* in Sistan and Baluchestan, though rarely and without quantified prevalence. Global data confirm that *H. aegyptium*'s highest density occurs in Iran and Turkey, corresponding to its reptilian hosts' range.

Conclusion: *Hyalomma aegyptium* is the most prevalent reptilian tick in Iran, especially on tortoises and skinks in the northwest. Other ixodid species and rare soft ticks are present, but hard ticks dominate and hold potential veterinary and zoonotic significance. Future research should focus on standardized field studies, pathogen screening, and GIS mapping to better assess risks to wildlife and public health.

Keywords: Ticks, Reptiles, Iran, *Hyalomma aegyptium*, Ixodidae.



Possible Contribution of Migratory Birds to Dengue Virus Spread: A Narrative Review

Mehrasa SheikhEsmaili¹, Parviz Shayan²

Corresponding Author: Parviz Shayan, pshayan@ut.ac.ir

1. Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1519

Abstract

Background: Dengue fever, caused by the Dengue virus (DENV), is a significant mosquito-borne disease transmitted primarily by *Aedes aegypti* and *Aedes albopictus*. While humans are the principal amplifying hosts, increasing attention has been given to the role of vertebrates—particularly migratory birds—in virus persistence and transmission. Iran's location on the Afro-Eurasian bird flyways and the rise in imported dengue cases highlight the importance of evaluating birds' ecological contribution to DENV spread.

Methods: This review evaluated studies published from 2000 to 2024 via PubMed, ScienceDirect, Scopus, SID, and Magiran. A total of 58 articles were screened, and 27 relevant studies focusing on sero-surveillance, ecological modeling, and fieldwork in birds and mosquitoes were critically analyzed. Keywords included Dengue, Migratory birds, Reservoirs, Vector-borne diseases, and Iran.

Results: Serological evidence from Portugal and Brazil revealed anti-Flavivirus antibodies in wild birds such as *Columba livia*, *Anas platyrhynchos*, and *Ardea cinerea*, indicating prior exposure. Although viral isolation from birds in Iran is lacking, the overlap between bird migration routes and DENV-endemic provinces (Sistan and Baluchestan, Hormozgan, Bushehr, Khuzestan) suggests possible involvement. Birds may serve as mechanical carriers of infected mosquitoes or short-term hosts in sylvatic cycles. However, experimental infections have shown minimal DENV replication in avian tissues, implying an indirect epidemiological role.

Conclusion: While birds are unlikely to serve as biological reservoirs for DENV, they may still influence viral ecology by transporting mosquitoes, providing blood meals, or acting as ecological sentinels in surveillance systems. Their role in bridging sylvatic and urban transmission—especially in tropical migratory hubs—warrants further multidisciplinary research in Iran, particularly on avian seroprevalence, host-vector interactions, and detection of viral RNA in bird populations.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Migratory birds, Dengue virus, Vector-borne diseases, *Aedes* mosquitoes, Iran.



Survey of Filariasis in Shelter Dogs from Selected Cities of Khorasan Razavi Province, Iran

Amin Bakhshani¹, Safa Moghadam², Neda Sasan Nejad³, Alireza Ghodrati⁴, Hassan Borji⁵

Corresponding Author: Amin Bakhshani, aminbakhshani@gmail.com

1. Graduated of Veterinary Parasitology (Ph.D degree), Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.
2. Graduated of Doctor of Veterinary Medicine, Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.
3. Veterinarian at Aria Veterinary Hospital, Mashhad, Khorasan Razavi Province, Iran.
4. Veterinary Head Office of Torghabeh and Shandiz, Khorasan Razavi Province, Iran.
5. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.

Article code: 1520

Abstract

Background: Filariasis in dogs, caused by species like *Dirofilaria immitis*, *Dirofilaria repens*, and *Acanthocheilonema reconditum*, is reported in Iran. *D. immitis* is especially important due to its heart localization and potential to infect humans. While *D. repens* and *A. reconditum* are less clinically significant, all filarial species are a public health concern. Many filarial worms carry *Wolbachia* bacteria, which have a symbiotic relationship with the worms. The aim of this study was to investigate the presence of filarial infections and the presence of *Wolbachia* in shelter dogs from some regions of Khorasan Razavi province, Iran.

Methods: From July 2021 to March 2024, blood samples were collected from 420 dogs across four cities in Iran: Mashhad (236), Neyshabour (122), Daregaz (41), and Kalat (21). All dogs were over 1 year old and of both sexes. Microfilariae presence was assessed using the modified Knott's test, with identification based on morphometric and morphological features. PCR was used for molecular detection of filarial DNA (*cox1* gene) and *Wolbachia* (*ftsZ* gene). Morphometric analysis of microfilariae was also performed.

Results: Of 420 samples, 31 (7.4%) were positive for microfilariae, all identified as *A. reconditum* based on morphometric and morphological features. Molecular methods confirmed these results. No *Wolbachia* was detected in infected dogs. Infection was found in all cities, with the highest prevalence in Mashhad (8.5%), followed by Neyshabour (6.6%), Daregaz (4.9%), and Kalat (4.8%). The average microfilariae length was $238.27 \pm 9.93 \mu\text{m}$. No significant differences in infection rates were found by gender or age ($P > 0.05$).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: No *D. immitis* infection was found in shelter dogs from the study areas, suggesting the absence of the definitive host of this parasite in these regions. Further research is needed in other regions to assess the presence of filarial nematodes, especially *D. immitis*.

Keywords: Dog, Khorasan Razavi, *Acanthocheilonema reconditum*, PCR.



Dermatitis Caused by *Ornithonyssus bacoti* in Laboratory Rats

Mohammad Farshad Golestanifar¹, Niknaz Rajaei², Mobina Ghorbaninasab³, Seddighe Nabian⁴

Corresponding Author: Mohammad Farshad Golestanifar, mfgolestanifar@gmail.com

1. DVM graduated, Islamic Azad University, Yazd province, Iran.
2. DVM graduated, Islamic Azad University, Olom Tahghighat branch, Tehran province, Iran.
3. Faculty of Veterinary Medicine, University of Tehran, Tehran province, Iran.
4. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1522

Abstract

Background: The tropical rat mite, *Ornithonyssus bacoti*, is an ectoparasite mite primarily associated with rats (*Rattus rattus*) but also infests other small mammals, such as mice (*Mus musculus*) and gerbils (*Meriones unguiculatus*). This mite belongs to the phylum Arthropoda, class Arachnida, and order Acari and is found in all continents except the Arctic and Antarctic regions. The mite exclusively consumes blood at all developmental stages. The presence of *O. bacoti* can lead to nonspecific dermatitis due to the action of their chelicerae and saliva, making diagnosis challenging unless the parasites are identified in the vicinity of the host's sleeping area or environmental dust. The purpose of this study is to recommend hygiene principals to prevent the transmission of parasites to mice and laboratory staff.

Methods: In laboratory setting, three rats presented with a history of severe itching, erythema and local ulcers on neck, back and around eyes. Affected skin areas were scraped with a scalpel blade and slide samples were stained with KOH.

Results: Upon microscopic examination, the existence of *O. bacoti* was confirmed. A sharp caudal apex of the Scutum, an oval genital shield, and a cranially positioned anus are among the microscopic diagnostic signs of this parasite. Treatment included the application of appropriate acaricides such as Permethrin and, additionally, local administration of Ivermectin(15 drops) once weekly for three weeks to ensure that elimination of mites from rats and their surroundings was effective. From the second week, the lesions showed significant improvement.

Conclusion: Close body contact between laboratory personnel and infested animals can facilitate the transition of dermatitis caused by *O. bacti*. This report highlights the critical need for stringent biosecurity protocols in laboratory animal husbandry to mitigate potential



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



zoonotic risks to human health and emphasizes on effective biosecurity measures for safeguarding both animal welfare and human health.

Keywords: *Ornithonyssus bacoti*, Arthropoda, Biosecurity, Zoonosis.



Explainable AI in Zoonotic Parasite Diagnosis: Enhancing Real-Time Clinical Trust and Transparency across One Health Systems

AmirReza Kanani^{1,4}, Sanaz Dehbashi^{1,2,3}

Corresponding author: Sanaz Dehbashi, DehbashiS@varastegan.ac.ir

1. Department of Medical Laboratory Sciences, Varastegan Institute for Medical Sciences, Mashhad, Iran.
2. Department of Clinical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Surgical Oncology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
4. Student Research Committee, Varastegan Institute for Medical Sciences, Mashhad, Iran.

Article code: 1524

Abstract

Background: Parasitic zoonotic diseases are still posing threats to global health, particularly in low-income countries because of limited capacity of health-care setting and delayed diagnosis. Although artificial intelligence has the potential to significantly aid the clinical diagnosis process, the non-transparent nature of AI models makes this solution unreliable for real-time medical and veterinary decision-making. Explainable AI resolves this issue by outputting explicit and user-interpretable results on which clinicians and veterinarians can rely. This review aims to evaluate the One Health perspective for cross-disciplinary interpretability and collaboration.

Methods: Data were reviewed from January 2022 to July 2025 using PubMed, Web of Science, Scopus, and Google Scholar. Search terms included Explainable AI, zoonotic parasites, interpretable machine learning, clinical decision support, and One Health. Studies were selected based on relevance, clinical validation, and citation impact. The exclusion criteria were included bacterial infections, viral infection, and non-zoonotic diseases.

Results: Explainable models such as Shapley Additive explanations (SHAP), Local Interpretable Model-agnostic Explanations (LIME), Random Forest classifier, and Extreme Gradient Boosting (XGBoost) have proven effective in diagnosing of *Toxoplasma gondii*, *Echinococcus multilocularis*, *Trypanosoma cruzi*, and *Cryptosporidium parvum*. Some studies conducted in rural areas demonstrated that POCT-integrated XAI models improved diagnostic precision and enabled earlier intervention. These tools are also proved themselves in point-of-care testing (POCT), especially in underserved communities.

Conclusion: Explainable AI fills the gap between algorithmic complexity and clinical applicability. Amid zoonotic disease outbreaks, transparent models enable effective triage and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



risk classification, even under uncertain conditions Future research should focus on developing cross-platform, mobile-integrated XAI systems to make fair in real-time parasite diagnostics, especially in resource-limited One Health environments.

Keywords: Explainable Artificial Intelligence (XAI), Zoonotic Parasites, Point-of-Care Testing (POCT), Diagnostic Equity, One Health Framework.



The Study of the Effect of Ethanolic *Citrullus colocynthis* Extract on *Leishmania major* In Vitro

Nadia Taiefi Nasrabadi¹

Corresponding Author: Nadia Taiefi Nasrabadi, drtaiefi@gmail.com

1. Department of parasitology, Karaj Branch, Islamic Azad University, Karaj, Iran.

Article code: 1525

Abstract

Background: Leishmaniasis is one of the zoonotic diseases, that is caused by *Leishmania*, it is from order kinetoplastida and in vertebrate lives in mononuclear cells and there are transmitted by sandflies. Today it is treated by Antimoan compounds like Glucantime that has side effect and cardiac and hepatic toxicity. In this study the antileishmania effect of etanolic extract of *Citrullus colocynthis* was evaluated in culture.

Methods: After *Leishmania major* MRHO/IR/75/ER was cultured in RPMI and determined logarithmic phase in 72 times, various concentrations of *Citrullus colocynthis* extract (0.1%, 0.01%, 0.001%) was added to tubes containing parasites.

Results: The results showed that in various concentrations of *Citrullus colocynthis* extract, with the passage of time survival percent reduced, so that in 300 minute in three concentrations it reaches zero and in 75, 90, 105, 120, 180 minutes, had significant differentiation in various concentrations and whatever it's concentration is more.

Conclusions: The survival rate of *Leishmania major* was inversely proportional to the concentration of *Citrullus colocynthis* extract. The most effective concentration was found to be 0.1% of the extract in pure distilled water.

Keywords: *Leishmania major*, *Citrullus colocynthis* extract, survival rate, in vitro.



***Hepatozoon felis* Infection in a Cat Undergoing FIP Treatment: A Case Report**

Reza Amjadi¹, Parviz Noushirvani², Reza Javadzadeh³, Alireza Ghaffari Cherati⁴

Corresponding Author: Reza Amjadi, rezaamjadi@yahoo.com

1. School of veterinary medicine, Islamic Azad University, Babol branch, Babol, Iran.
2. Department of veterinary clinical pathology, Ara pet hospital, Sari, Iran.
3. Department of small animal internal medicine, Ara pet hospital, Sari, Iran.
4. Department of small animal internal medicine, Ara pet hospital, Sari, Iran.

Article code: 1526

Abstract

Background: Feline infectious peritonitis (FIP) is a fatal viral disease with a wide spectrum of clinical presentations. Hepatozoonosis, on the other hand, is a rare protozoal infection in cats, caused by *Hepatozoon felis*. Coexistence of these two infections is extremely rare and scarcely reported. Here, we describe a case of a young female cat with effusive FIP that, following successful treatment, was incidentally diagnosed with *H. felis*.

Methods: On 2 May 2025, a one-year-old female domestic cat was presented to Ara Veterinary Hospital with anorexia, lethargy, abdominal distension, muscle wasting, cachexia, and pale mucous membranes. Diagnostic workup included complete blood count (CBC), serum biochemistry, and abdominal ultrasonography. Blood smears play major role in diagnosis of blood parasites such as *H. felis*.

Results: Laboratory findings revealed lymphopenia, toxic neutrophil changes, giant platelets, left shift (2+), a markedly decreased albumin-to-globulin ratio ($A/G = 0.35$), and non-regenerative anemia. The biochemical panel showed increased AST, hypoalbuminemia, hypocalcemia and hyponatremia. Ultrasonography findings were consistent with FIP. The cat underwent an 85-day antiviral treatment regimen for FIP and achieved full clinical recovery. However, on 28 July 2025, a follow-up CBC smear revealed the incidental presence of *H. felis* gamonts within neutrophils. Specific antiprotozoal therapy was initiated with Doxycycline (100 mg capsule, for 4 weeks). After 3 weeks of treatment, follow-up CBC showed no detectable gamonts, confirming a favorable therapeutic response.

Conclusion: This case highlights the importance of thorough hematological evaluation even in clinically recovered cats. Co-infection of *H. felis* and FIP, although rare, may occur and requires awareness for timely diagnosis and treatment. The accidental detection of *H. felis* in a cat recovering from FIP underscores the complexity of feline infectious diseases. Detection of



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



H. felis may occur without obvious clinical signs and should not be overlooked, especially in areas with potential vector exposure.

Keywords: *Hepatozoon felis*, FIP, Co-infection, Vector-borne infection.



Detection of *Moniliformis moniliformis* in Long-eared Hedgehogs (*Hemiechinus auritus*) in Nehbandan in South Khorasan Iran

Kian Mosaali¹, Mahdiye Mollashahi², Seyede Mahnoosh Mostafavi³, Seyede Mahshid Mostafavi¹

Corresponding Author: Kian Mosaali, moosaalikian@gmail.com

1. Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Bahonar of Kerman, Kerman, Iran.
2. Doctorate of Veterinary Medicine (DVM) graduated
3. Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Chamran of Ahvaz, Ahvaz, Iran.

Article code: 1528

Abstract

Background: *Moniliformis moniliformis* is a zoonotic acanthocephalan with a broad host range, including small mammals and insectivores. Despite its global distribution, data on its occurrence in Longe-eared hedgehogs (*Hemiechinus auritus*) in Iran are scarce. Considering the increasing ecological interaction between synanthropic wildlife and domestic animals, monitoring intestinal helminths in hedgehogs may provide insight into the local epidemiology of neglected zoonotic parasites. This study aimed to identify and describe *M. moniliformis* infection in hedgehogs from northeastern Iran.

Methods: During a parasitological survey conducted in 1403, a total of 11 Longe-eared hedgehogs (6 male and 5 female) were collected from roadside habitats of Nehbandan in South Khorasan. Following euthanasia in compliance with ethical standards, the gastrointestinal tracts were dissected and examined for helminths. Acanthocephalans were isolated from the small intestine and preserved in 70% ethanol. Specimens were cleared in lactophenol and analyzed morphologically under light microscopy, focusing on proboscis armature, body segmentation and reproductive structures.

Results: *M. moniliformis* was detected in 2 female (18.18 %) of the hedgehogs that examined. Adult worms were creamy-white and cylindrical. Histopathologic evaluation of intestinal mucosa showed localized inflammatory infiltration and mild villous atrophy at attachment sites.

Conclusion: This study documents the occurrence of *M. moniliformis* in Longe-eared hedgehogs from Iran, providing the first detailed morphological report from the region. The zoonotic potential of this parasite, combined with its presence in synanthropic hosts, highlights



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



the need for integrated wildlife–livestock–human surveillance programs. Further molecular characterization is recommended to trace local transmission pathways.

Keywords: Longe-eared hedgehogs, Acanthocephalan, Zoonosis, Wildlife.



Prevalence and Genetic Diversity of *Blastocystis* sp. in Human and Animal Fecal Samples Identified by High-Resolution Melting Analysis

Ghodratollah Salehi Sangani¹, Ahmad Hosseini-Safa², Bibi Razieh Hosseini Farash¹, Pooria Salehi Sangani¹, Mehdi Zarean¹, Elham Moghaddas¹, Saeid Zoghdar Moghadam¹, Lida Jarahi³, Ali Gholizadeh⁴, Fariba Berenji¹

Corresponding Author: Fariba Berenji, berenjif@mums.ac.ir and, Ali Gholizadeh, aligholizadeh1997patho@gmail.com

1. Department of Parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Department of Medical Laboratory Sciences, Faculty of Allied Medicine, Iran University of Medical Sciences, Tehran, Iran.
3. Department of Community Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
4. Department of Medical Laboratory Sciences, Faculty of Allied Medicine, Gonabad University of Medical Sciences, Gonabad, Iran.

Article code: 1529

Abstract

Background: *Blastocystis* sp. is an intestinal protozoan parasite widely distributed in humans and animals, transmitted through the fecal–oral route. Its prevalence ranges from around 20% in industrialized countries to over 60% in developing regions. Although the clinical significance of this organism remains controversial, it has been found in both symptomatic and asymptomatic carriers. Microscopical examination is commonly used for detection but lacks sensitivity and subtype resolution. Molecular techniques have become essential for accurate diagnosis. The present study aimed to determine the prevalence and subtype distribution of *Blastocystis* sp. in human and animal stool samples using High-Resolution Melting (HRM) curve analysis as a novel molecular approach.

Methods: A total of 730 fecal specimens were collected from humans and domestic animals. Initial screening was performed by direct microscopy. Samples negative for *Blastocystis* were



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



inoculated into a biphasic culture medium and re-examined after 2–3 days. Subtyping was carried out through HRM analysis of real-time PCR amplicons using specific primers.

Results: Six distinct subtypes were identified. ST7 (30%) was the most prevalent overall, followed by ST3 (28%), ST2 (16%), ST1 (14%), ST5 (6%), and ST14 (6%). In humans, ST3 was the dominant subtype, also detected in poultry and sheep, while ST7 was mainly restricted to animal hosts. The presence of ST1–ST3 in both humans and animals suggests possible zoonotic transmission pathways.

Conclusion: Our findings demonstrate that ST3 and ST7 are the most common subtypes in this region, with differing distributions between humans and animals. HRM analysis proved to be a rapid, cost-effective, and reliable tool for subtype characterization. This method can strengthen epidemiological surveillance, reduce reliance on sequencing, and improve understanding of cross-species transmission dynamics.

Keywords: *Blastocystis*; Zoonosis; Subtype; Khorasan province; HRM.



A Survey of *Acarapis woodi* in Festaion in Apiaries in Iran

Golnush Bolvardi¹, Sedigheh Nabian^{1,2}, Alireza Arab³, Kamyar Ahmadi³, Abbas gerami Sadeghian¹, Ali Ahmadi¹

Corresponding Author: Sedigheh Nabian, nabian@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Center of Tick and Tick-borne Disease, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Veterinary Organization, Tehran, Iran.

Article code: 1536

Abstract

Background: Beekeeping is crucial for global food needs due to its role in pollination. However, Iran is not fully utilizing its beekeeping potential, despite its share in honey trade and production per colony. One of the most important reasons for this is bee diseases such as acarapisosis. Acarapisosis is a disease of the adult honey bees caused by the tarsonemid mite, known as tracheal mite *Acarapis woodi*. This mite is an internal parasite of respiratory system, living and reproducing mainly in large prothoracic trachea of the honey bees. *Acarapis woodi* can cause bothe mechanical injuries and physiological disorders consequent to the obstruction of air ducts, lesion in the tracheal walls, and the depletion of haemolymph.

Methods: Microscopic methods used in this study and for each sample 50 adult honey bees collected from different apiaries. In total 9350 honeybees from 180 aiparies in summer 1403, and 9250 honey bee samples from 200 aipiaries in spring 1404 were collected from different apiaries of Iran. For microscopic detection thoracic discs were prepared from adult bees then muscle cleared from the discs by heating at 60⁰ C in a 5-10% solution of potassium hydroxide for 2 hours then thracheas separated from discs and examine at 200-400 magnification.

Results: As a result of this study , it was determined that *A.woodi* is not present in bee colonies in Iran. None of the the 380 apiaries thart surveyed , were infected with *A.woodi*.

Conclusion: Acording to past research in the north of country , a large number of apiaries were infected with *A.woodi*. but no contamination was found in this study. One of the reasons for this result can be attributed to the use of new antiparasitic drugs.

Keywords: *Apis mellifera*, Honeybee, Prevalence, *Acarapis woodi*, Iran.



The First Report of Infestation of Small Asian Mongoose (*Herpestes javanicus auropunctatus*) with *Ctenocephalides canis* Flea in Iran

Saeed Dadkhah Tehrani¹, Seyed Reza Hosseini¹, Mehran Riahi²

Corresponding Author: dr.s.reza@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.
2. Graduated Department of Veterinary Poultry Hygiene and Disease, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.

Article code: 1538

Abstract

Background: Known external parasites, due to their blood-feeding nature, are capable of transmitting various types of pathogens from animal to animal and from animal to human, and therefore are of zoonotic importance. Today, the mongoose (*Herpestes javanicus auropunctatus*) is kept as a pet alongside humans, so it is necessary to study the identification of fleas observed on this animal.

Methods: A one-year-old male mongoose (*Herpestes javanicus auropunctatus*) presented to the veterinary clinic with symptoms of weakness, lethargy, poor appetite, and mild anemia on examination of the mucous membranes. After a thorough examination and examination of the external body covering, external parasites were diagnosed in the initial examination. In the laboratory, the samples were clarified with KOH solution and then identified using a valid diagnostic key.

Results: Laboratory studies and observation of the number of spines and rows of pectoral and chin ridges, the forward slope of the head, and the size of the third pair of legs confirmed the presence of the flea *Ctenoscephalides canis* in this species, which was consistent with the diagnostic key.

Conclusion: This is the first report of mongoose infestation with the *Ctenocephalides canis* flea in Iran. Given the zoonosis of this parasitic agent and the keeping of mongooses as pets in human societies, and the issue of disease transmission, it should be noted that periodic veterinary care is a priority and that necessary awareness should be given to pet owners, especially those who keep mongooses.

Keywords: Flea, Disease transmission, Pet animal, Zoonoses.



Equine Leishmaniasis: Overview of Etiology, Clinical Manifestations, Diagnosis, and Management

Mohammad Hekmatmanseh¹, Mehdi Hekmatmanesh², Elahe Ghazi Shehni Zad³, Somayeh Bahrami^{1,5}

Corresponding author: Mohammad Hekmatmanesh, hekmatmanesh45@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
3. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
4. Society of Animal Parasitic Diseases and Zoonoses, Iran.

Article code: 1540

Abstract

Background: This review provides an overview of equine leishmaniasis, addressing etiology, clinical manifestations, diagnostic approaches, treatment options, and management challenges in equine populations.

Methods: An extensive search was performed across prominent scientific databases, including PubMed, ScienceDirect, Google Scholar, and Web of Science, to ensure comprehensive coverage. The search utilized keywords Equine leishmaniasis, Parasitology, *Leishmania*, and *Leishmania infantum* to identify pertinent publications. The inclusion criteria focused on articles published from 2010 to 2025, emphasizing methodological quality, relevance to research objectives, and contribution to understanding equine leishmaniasis. A total of 32 studies were selected following screening, providing a robust foundation for the review.

Results: Equine leishmaniasis is mainly caused by *Leishmania infantum*, a protozoan transmitted by infected phlebotomine sandflies. Clinical manifestations range from cutaneous lesions and lymphadenopathy to systemic signs such as fever and weight loss; some cases progress to visceral disease. Reports come from regions where sandfly vectors are prevalent. Documented countries include Portugal, Spain, Italy, France, Greece, Bulgaria, Turkey, Brazil, Argentina, Tunisia, Algeria, and Morocco. These areas report endemic *L. infantum* infection affecting equines and canines. Prevalence varies with environmental conditions, vector density, and horse management. Diagnosis combines clinical assessment with serology and PCR. Treatment options include antileishmanial agents such as amphotericin B or miltefosine, but therapeutic responses vary and long-term management may be required. Managing



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



leishmaniasis in horses is challenging due to diagnostic nuances, limited evidence-based treatments, and complex epidemiology.

Conclusion: Evidence from 2010–2025 indicates that while diagnosis is best supported by combining clinical evaluation with serology and PCR, variability in test performance and low parasite burdens complicate case confirmation. Therapeutic options yield inconsistent outcomes and often require prolonged follow-up. Given the sparse, fragmented literature and the disease's complex eco-epidemiology at the human–canine–equine interface, management should prioritize accurate diagnosis, judicious therapy, and integrated vector control.

Keywords: Equine leishmaniasis, *Leishmania infantum*, Diagnosis, Vector control, Treatment.



Scabies in Pet Rabbits in 2025: A Case Report

Abbas Jadidoleslami¹, Fatemeh Jadidoleslami², Mostafa Imani³, Ali Moshaverinia¹

Corresponding Author: Ali Moshaverinia, moshaverinia@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Independent Researcher, Muscat, Oman.
3. BSc graduated, department of veterinary science, Shahid Hasheminejad Educational Complex, University of Applied Science and Technology, Mashhad, Iran.

Article code: 1541

Abstract

Background: *Sarcoptes scabiei*, the causative agent of scabies, is a burrowing mite affecting humans and various mammals, including rabbits. This study reports severe infestation of scabies in pet rabbits, emphasizing the zoonotic potential and the need for preventive measures to control transmission from domestic animals to humans. This report aims to describe the infection of domestic rabbits with *S. scabiei*, highlight its zoonotic potential, and underscore the need for accurate diagnostic and historical data to enhance monitoring and management.

Methods: Two pet rabbits (*Oryctolagus cuniculus*), sourced from a breeding center in Mashhad, Iran, were examined post-mortem in January 2025. Parasitological analyses were conducted, including skin scrapings clarified in KOH 10% to identify potential agents such as burrowing mites. The isolated mites were identified based on standard keys.

Results: Both rabbits exhibited severe scabies symptoms, including crusting and peeling skin around the paws, sores near the nails caused by scratching, hyperkeratosis of the paws, the skin of the ears and nose. Microscopic analysis confirmed *S. scabiei* and its eggs in skin samples. The second rabbit died shortly after the first, showing identical symptoms and rapid disease progression. No signs of scabies were observed in family members or the breeder in contact with the infected rabbits.

Conclusion: This study highlights the presence of scabies in pet rabbits in Mashhad and its zoonotic implications. Factors such as handling stress, housing changes, and a potentially contaminated environment likely worsened the infection. The zoonotic nature of *S. scabiei* underscores risks to human health. Effective management requires sourcing animals from reputable centers, implementing quarantine protocols, disinfecting environments, and regular skin health monitoring. These findings emphasize the critical need for improved parasite control and management of comorbidities in laboratory and pet settings to prevent outbreaks and protect public health.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Oryctolagus cuniculus*, *Sarcoptes scabiei*, Sarcoptic mange, Zoonotic disease, Iran.



A Review on the Role of Climate Change on the Occurrence of Parasitic Diseases

Ali Naseri¹, Mirabi.S¹, Akbarein.H²

Corresponding Author: Ali Naseri, naseri.ali@ut.ac.ir

1. Undergraduate student, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Division of Epidemiology & Zoonoses, Department of Food Hygiene & Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1542

Abstract

Background: Climate change has a significant influence on the distribution, transmission, and disease occurrence of parasitic diseases worldwide. Temperature, precipitation, and humidity govern the parasite and vector lifecycle, often their geographic range and seasonal activity. Increasing temperatures have the potential to allow vector habitats to spread to new locations, and enhanced precipitation and humidity construct optimum breeding pools for vectors like mosquitoes and freshwater snails. Natural disasters, including droughts and floods, also alter transmission patterns, which can be the cause of emerging or re-emerging parasitic diseases. With growing climate change, understanding these climatic effects is important in order to forecast disease trends and improve control strategies, ultimately minimizing the burden of parasitic diseases in vulnerable groups.

Methods: This research is a review-type study in which authentic articles published in databases such as Scopus, Google Scholar, ResearchGate and other scientific references were used. To search in English sources from the words “Climate change and Parasitic disease” was used.

Results: Climatic change has a significant influence on the transmission dynamics of parasitic diseases by affecting the habitat, lifecycle, and population dynamics of both parasites and vectors. Increased temperature accelerates the development of parasites and expands the geographical range of vectors such as mosquitoes and snails, which leads to increased transmission of the disease and the expansion of parasitic diseases to new locations. Precipitation and humidity are conducive to vector breeding and survival, while extreme conditions in the form of floods and droughts can potentially disrupt or enhance disease transmission cycles. The alterations in the patterns, seasonality, and intensity of parasitic diseases such as malaria, schistosomiasis, lymphatic filariasis, and soil-transmitted helminths are due to these environmental changes. There should be continued vigilance and climate-



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



sensitive public health action to prevent the evolving menace of climate variability to parasitic disease control measures.

Keywords: Climate Change, Parasitic Disease, increasing temperatures, Forecast disease.



The Application of Artificial Intelligence in the Prediction, Diagnosis and Treatment of Parasitic Diseases

Mohammadsaeed Azamian¹, Hesameddin Akbarein²

Corresponding Author: Mohammadsaeed Azamian, saeedazamian7798@gmail.com

1. DVM, Graduated from Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.
2. DVM, DVSc, Assistant professor of Epidemiology, Division of Epidemiology & Zoonoses, Department of Food Hygiene & Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1543

Abstract

Background: Parasitic diseases such as malaria, leishmaniasis, etc., continue to pose major global health challenges, particularly in low-resource regions. Traditional diagnostic methods, including manual microscopy and surveillance systems, are often labor-intensive, error-prone, and difficult to scale, limiting timely and effective disease control. Recent advances in artificial intelligence (AI), especially machine learning (ML) and deep learning (DL), offer promising solutions for improving prediction, diagnosis, and treatment. This review systematically examines AI applications in parasitology, highlighting their role in enhancing diagnostic accuracy, outbreak forecasting, and treatment optimization.

Methods: This review utilized original articles published between 2020 and 2024 across Scopus, ScienceDirect, Google Scholar, ResearchGate, PubMed, SID, and library sources. Studies addressing AI applications in parasitic disease detection, prediction, and treatment were included. Data extraction focused on AI methodologies (e.g., convolutional neural networks [CNNs]), dataset types (clinical records, environmental variables), and performance metrics. A qualitative synthesis was performed to assess model performance and applicability.

Results: AI-based techniques, particularly CNNs, demonstrated higher sensitivity and specificity than manual microscopy in detecting intestinal protozoa and blood parasites. Predictive models integrating environmental, sociodemographic, and clinical data effectively identified transmission dynamics and provided early outbreak warnings. Moreover, AI supported drug resistance monitoring, novel drug target identification, and demonstrated robustness across diverse populations. Beyond diagnostics, automated image analysis contributed to parasitology education and training, facilitating broader accessibility of expertise.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: AI is revolutionizing parasitic disease management by delivering rapid, accurate, and scalable solutions for prediction, diagnosis, and treatment. CNNs represent a powerful diagnostic tool, while predictive models enable early intervention and outbreak mitigation, particularly in endemic regions. Despite these advances, challenges remain, including limited dataset diversity, computational resource constraints, and barriers to implementation in low-resource settings. Future research should prioritize expanding dataset inclusivity and addressing infrastructural limitations to fully realize AI's potential impact on global health.

Keywords: Artificial Intelligence, Parasitic Infections, Predictive Modeling, Deep Learning.



The Necessity of Rational Prescribing of Anthelmintic and Antiprotozoal Drugs to Prevent Antimicrobial Resistance

Mohammadsaeed Azamian¹, Hesameddin Akbarein²

Corresponding Author: Mohammadsaeed Azamian, saeedazamian7798@gmail.com

1. DVM, Graduated from Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.
2. DVM, DVSc, Assistant professor of Epidemiology, Division of Epidemiology & Zoonoses, Department of Food Hygiene & Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1544

Abstract

Background: This study hypothesizes that irrational use of veterinary drugs, particularly antiprotozoals and anthelmintics, in food-producing animals significantly contributes to antimicrobial resistance (AMR), threatening animal, human, and environmental health under the One Health approach.

Methods: This review utilized original articles published between 2020 and 2024 across ScienceDirect, Google Scholar, ResearchGate, PubMed, and SID. Library researches were also conducted. English keywords such as AMR, Anthelmintic drug, Antiprotozoal drug and Rational prescription as well as their Persian equivalents were employed for literature search.

Results: Widespread irrational use of antiprotozoals and anthelmintics in livestock, characterized by excessive prescriptions, inappropriate dosages, and non-compliance with withdrawal periods, was found to exacerbate antimicrobial resistance (AMR), anthelmintic and antiprotozoal resistance. Misuse and abuse of these drugs rendered treatments ineffective against key pathogens, including bacteria, helminthes and protozoans, due to genetic mutations and resistance gene transfer. High drug residues in animal-derived products, such as meat, milk, and eggs, were identified, posing significant risks to consumer health by contributing to AMR dissemination. These practices also threatened environmental health through residual contamination, undermining the One Health approach and increasing the risk of global economic impacts, including food trade disruptions.

Conclusion: Irrational veterinary drug use drives AMR and anthelmintic resistance, compromising treatment efficacy and posing significant public health concerns. Rational drug administration, adherence to withdrawal periods, and regular resistance monitoring are essential to mitigate these challenges. Strengthened global surveillance and national and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



international programs are critical to promote sustainable veterinary practices and ensure food safety within the One Health approach.

Keywords: AMR, Anthelmintic drug, Antiprotozoal drug, Rational prescription.



Association Between Bovine Leukemia Virus Infection and Parasitic Susceptibility in Cattle: A Review

Nikta Amiri¹, Mohammadreza Rezaeigolestani²

Correspondence: Mohammadreza Rezaeigolestani, mr.rezaee@um.ac.ir

1. Master student, Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Assistant professor, Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1548

Abstract

Background: Bovine leukemia virus (BLV), the causative agent of enzootic bovine leukosis (EBL), causes persistent infection and immune dysfunction in cattle. BLV-infected animals often exhibit decreased CD4⁺ T-cell counts, impaired neutrophil and macrophage functions, and elevated anti-inflammatory cytokines such as IL-10. These immunological changes may increase susceptibility to secondary infections, including parasitic diseases, potentially leading to reduced animal health and productivity. This study aimed to review existing literature addressing the potential association between BLV infection and parasitic infections in cattle.

Methods: A structured search was conducted using PubMed, Google Scholar, and SID for publications addressing BLV, immune function, and parasitic diseases in cattle. Both epidemiological and experimental studies were included when relevant.

Results: Direct epidemiological studies linking BLV infection with increased prevalence of specific parasitic infections are scarce. However, reports consistently describe immunosuppression in BLV-positive cattle, including reduced humoral and cellular immune responses. Such immune dysfunction is known to facilitate opportunistic infections and has been associated with increased incidence of infectious diseases at the herd level. These findings suggest that BLV-positive cattle may be at higher risk of parasitic infections, although targeted field studies are lacking.

Conclusion: Evidence from immune function studies and herd-level disease associations supports the hypothesis that BLV infection could predispose cattle to parasitic infections. Further research specifically designed to evaluate BLV–parasite interactions is required to confirm this association and guide control strategies to improve cattle health and productivity.

Keywords: Enzootic Bovine Leukosis (EBL); Immunosuppression; Parasitic infections; Cattle.



Host Spectrum and Impact of *Trypanosoma evansi* Infections Across Animal Species

Elahe Ghazi Shehni Zad¹, Mohammad Yarmahmoudi¹, Mehdi Hekmatmanesh², Mohammad Hekmatmanseh³, Somayeh Bahrami^{3,5}

Corresponding author: Elahe Ghazi Shehni zad, eli1999gh@gmail.com

1. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
3. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
4. Society of Animal Parasitic Diseases and Zoonoses, Iran.

Article code: 1550

Abstract

Background: While horses and camels typically exhibit severe clinical signs of *Trypanosoma evansi*, the impact on other species can vary. This review discusses a more detailed look at its presence and impact across various species.

Methods: Comprehensive searches were performed in PubMed, Scopus, Web of Science (Core Collection), Google Scholar, and CAB Abstracts using database-tailored combinations of keywords and controlled terms (e.g., *Trypanosoma evansi*, surra, host range, reservoir, camelid, equine, bovine, wildlife, morbidity, mortality, productivity, economic impact). Eligible records were English-language articles, reviews, confer, and theses that reported primary data or substantive syntheses on *T. evansi* occurrence, transmission, clinical/pathological effects, productivity or economic outcomes, diagnostics, or control in any animal host. A total of 50 studies were selected after rigorous screening, providing a robust foundation for the review.

Results: Goats and sheep can be infected by *T. evansi*, it's less common than other species. Infected animals may exhibit weight loss, lethargy, anemia, infertility, and abortion in Buffalos and cattle. Severe infections can lead to significant morbidity. There are reports of *T. evansi* infections in pigs. Various wild animals, such as antelopes, capybaras, ocelots, wallabies, tigers, jaguars, bears, rhinoceros, and other ruminants, can harbor *T. evansi*. These species may serve as reservoirs for the parasite, contributing to maintenance of the parasite in ecosystem. While *T. evansi* infection in dogs has been documented in multiple regions, feline cases are sporadically reported. Some studies have indicated that deer, coatis, bats, and armadillos can be infected with *T. evansi* as well. The impact on deer populations is less studied, and the



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



clinical significance can vary. Some clinical signs in certain animals include anorexia, ataxia, and leukopenia.

Conclusion: *T. evansi* threatens many animal species, so knowing its host prevalence is essential for effective control.

Keywords: *Trypanosoma*, Clinical signs, Transmission, Animal species, Reservoirs.



Parasitology and Histopathology Investigation of *Cryptosporidium* in Industrial Poultry of Mashhad City

Nima komeili¹, Elahe Ebrahimzadeh¹, Zahra Moosavi¹

Corresponding Author: Elahe Ebrahimzadeh, eebrahimzade@um.ac.ir

1. Department of pathobiology, faculty of veterinary medicine, Ferdowsi university of Mashhad, Mashhad, Iran.

Article code: 1554

Abstract

Background: Cryptosporidiosis is an important zoonotic protozoan disease caused by *Cryptosporidium* species. In poultry, various species such as *Cryptosporidium baileyi*, *C. galli*, *C. meleagridis*, and *C. avium* have been reported. These organisms are capable of infecting the gastrointestinal and respiratory tracts, and transmission occurs via environmentally resistant oocysts. Infected birds may exhibit clinical signs such as diarrhea, lethargy, and respiratory disturbances. This study aimed to investigate the presence of *Cryptosporidium* spp. in broiler chickens in Mashhad County using parasitological and histopathological methods.

Methods: A total of 300 broiler chicken carcasses were examined. Impression smears were prepared from the bursa of Fabricius, cloaca, trachea, and intestine, and stained using the modified Ziehl–Neelsen technique to detect oocysts. For histopathological evaluation, tissue samples were fixed in 10% neutral buffered formalin, processed routinely, and stained with hematoxylin and eosin (H&E).

Results: Among the examined samples, only one was definitively identified as positive for *Cryptosporidium* oocysts, while two were considered suspected cases; however, these lacked the definitive morphological features required for confirmation. Histopathological examination of the studied tissues revealed no evidence of *Cryptosporidium* infection or characteristic lesions such as epithelial hyperplasia or inflammatory infiltration.

Conclusion: The findings indicate a low prevalence of *Cryptosporidium* spp. in broiler chickens in Mashhad. This underscores the importance of maintaining proper hygienic and management practices in poultry farms to minimize the risk of cryptosporidiosis.

Keywords: *Cryptosporidium*, Hematoxylin and eosin, Chickens, Oocysts.



Study of Helminth Parasites in the Digestive Tract of Donkeys in Farahan Villages of Markazi Province

Abdolhossein Dalimi¹, Fatemeh Rezaee¹, Faezeh Kafi Mousavi¹, Majid Pirestani¹

Corresponding Author: Abdolhossein Dalimi, dalimi_a@modares.ac.ir

1. Department of Medical Parasitology, Faculty of Medical Sciences, Tarbiat Modares University-Tehran, Iran.

Article code: 1556

Abstract

Background: One of the most common factors reducing the health and productivity of donkeys is infection with gastrointestinal parasites. The main aim of our study was to detect digestive tract helminth parasites of donkeys in Farahan villages of Markazi Province.

Method: In this study, parasitic infection of 19 donkeys from Farahan villages, Markazi Province in 2024 was investigated. Fecal samples were collected directly from the animal and examined microscopically using the sediment & Baermann methods.

Results: The examination of the fecal samples revealed the presence of several helminth species. The most common parasite identified was *Strongylus* spp., found in 5 donkeys, accounting for 26.31% of the samples. *Oxyuris equi* was detected in 2 donkeys (10.52%), while *Strongyloides westeri* was also present in 2 donkeys (10.52%). Additionally, *Dictyocaulus* larvae were found in 1 donkey (5.26%).

Conclusion: This study showed a relatively low prevalence of gastrointestinal parasites in donkeys in Farahan villages. According to the findings of this study, *Strongylus* spp. is the most common gastrointestinal helminth parasite in donkeys in this region.

Keywords: Donkey, Helminth, Gastrointestinal parasite, Iran.



Histopathologic Investigation of the Caffeic Acid Effects on the Liver and Kidney of Laboratory Mice Infected by Hydatid Cyst

Mohammad Sajjad Ghorbani Parsa¹, Zahra Moosavi¹, Elahe Ebrahimzadeh

Corresponding Author: Zahra Moosavi, zmoosavi@um.ac.ir

1. Department of Pathobiology Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1559

Abstract

Background: Hydatidosis is a zoonotic parasitic disease caused by the larval stage of *Echinococcus granulosus*, leading to the formation of hydatid cysts in various organs. Common treatments include surgery and albendazole administration, which may cause significant side effects. Therefore, researchers are seeking alternative therapies or combinations with safer drugs to reduce the required albendazole dose. Caffeic acid, a natural plant-derived compound, has shown promising therapeutic potential. This study aimed to investigate the histopathological effects of caffeic acid on the liver and kidneys of mice infected with hydatid cysts.

Methods: Fixed liver and kidney tissues from 30 male and female laboratory mice were divided into the following groups: control, Caffeic acid 25 mg/kg/bw, Caffeic acid 50 mg/kg/bw, albendazole 200 mg/kg/bw, Caffeic acid 25 mg/kg/bw and albendazole 100 mg/kg/bw, Caffeic acid 50 mg/kg/bw and albendazole 100 mg/kg/bw. The samples were processed routinely for histopathological examinations. The lesions classified based on severity.

Results: Histopathological analysis showed that Ca50 and Al100+Ca50 had the greatest effects in reducing liver congestion and inflammation. The Ca50, Al200, and Al100+Ca50 most effectively decreased degeneration and necrotic lesions compared with the control and Ca25 groups. In the kidney, Ca50, Al200, and Al100+Ca50 markedly reduced necrosis, with no significant differences among them, indicating similar efficacy. Ca50 showed the greatest anti-inflammatory effect. Ca50 and Al100+Ca50 had the highest overall therapeutic impact on liver and kidney injuries caused by hydatid cysts. The combination of caffeic acid and albendazole showed a synergistic effect, allowing for possible reduction of albendazole dosage to minimize side effects. The results also indicate a dose-dependent effect, as Ca25 had minimal impact compared to Ca50.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: The caffeic acid has a significant reduction in liver and kidney lesions in mice infected with hydatid cysts compared to the control group, showing its anti-parasitic properties. There was no significant difference between the treatment groups.

Keywords: Caffeic acid, Hydatid cyst, Histopathology, Mice.



Morphological Identification of *Rhipicephalus sanguineus* Infestation on Wild Canids (*Vulpes vulpes* and *Canis aureus*) in Northeastern Iran Including Golestan Forests

Seyede Mahshid Mostafavi¹, Kian Mosaali¹, Shahab Shirzahi², Leila Saravani³

Corresponding Author: Seyede Mahshid Mostafavi, seyedemahshidmostafavi@gmail.com

1. Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Bahonar of Kerman, Kerman, Iran.
2. Department of Medical Faculty, Zahedan Azad University, Zahedan, Iran.
3. Department of Poultry, Apiculture, and Sericulture Health and Disease Management, General Directorate of Veterinary Services, Sistan and Baluchestan Province, Iran.

Article code: 1560

Abstract

Background: *Rhipicephalus sanguineus* is a widely distributed tick species known for transmitting various pathogens affecting both domestic and wild animals. Northeastern Iran, including the ecologically rich Golestan forests, hosts wild canids such as the red fox (*Vulpes vulpes*) and golden jackal (*Canis aureus*), which can serve as reservoirs for these ectoparasites. However, data on tick infestations in these wild hosts within this region remain limited. This study aimed to morphologically identify *R. sanguineus* ticks infesting wild canids and characterize their infestation patterns.

Methods: During June and July 2025 (Tir 1404), five wild canids—two red foxes (*Vulpes vulpes*) and three golden jackals (*Canis aureus*)—were sampled from Khorasan Razavi, North Khorasan, and the Golestan Forest regions. All samples were selected from road fatalities. Each animal underwent thorough physical examination, and all ticks were carefully collected. Ticks were identified morphologically using stereomicroscopy, with species, sex, and developmental stage recorded.

Results: A total of 18 adult ticks were collected, all identified as male *R. sanguineus*. Hosts included one male and one female red fox, and two males and one female golden jackal. Notably, no female ticks were found among the samples, suggesting possible behavioral or ecological factors influencing tick-host dynamics in this area.

Conclusion: This study documents the morphological identification of *R. sanguineus* infestation on wild canids in northeastern Iran, including the biologically diverse Golestan forests. The predominance of male ticks highlights the need for further ecological studies to better understand tick behavior and population dynamics. Ongoing surveillance of tick



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



infestations in wildlife is crucial for assessing their role in the transmission of tick-borne diseases affecting both animals and humans.

Keywords: *Rhipicephalus sanguineus*, *Vulpes vulpes*, *Canis aureus*, Tick infestation, Golestan Forests, Northeastern Iran.



Molecular Detection of *Encephalitozoon* Species in Dogs and Cats in Tabriz City, Iran

Parisa Shahbazi¹, Ali Seyed Zadeh¹, Ahmad Nematollahi¹

Corresponding Author: Parisa Shahbazi, p.shahbazi56@gmail.com

1. Department of Pathobiology, Faculty of Veterinary medicine, University of Tabriz, Tabriz, Iran.

Article code: 1563

Abstract

Background: *Encephalitozoon* is a genus of microsporidia that includes three recognized species: *E. cuniculi*, *E. hellem*, and *E. intestinalis*. These obligate intracellular parasites are capable of infecting a wide range of vertebrate hosts and are of significant zoonotic concern. Infections caused by *Encephalitozoon* spp. can lead to clinical manifestations such as chronic diarrhea, weight loss, and fever, particularly in immunocompromised individuals and susceptible animal hosts. Understanding the presence and distribution of these pathogens in domestic and stray animal populations is essential for both veterinary and public health monitoring.

Methods: A total of 100 fecal samples were collected from domestic and stray dogs and cats across Tabriz, Iran, with breed, sex, age, and sampling location recorded for each animal. Genomic DNA was manually extracted from the samples, and *Encephalitozoon* species were detected using a multiplex-nested PCR assay targeting species-specific regions of the small subunit ribosomal RNA (SSU rRNA) gene. Selected PCR-positive amplicons were sequenced by PISHGAM Biotechnology Company, registered in GenBank, and a phylogenetic tree was constructed using MEGA-6 software.

Results: PCR analysis revealed *Encephalitozoon* DNA in 11% of the fecal samples (7 dogs, 4 cats out of 100). A ~300 base pair amplicon specific to the *Encephalitozoon* genus was amplified in all positive samples. Sanger sequencing of three selected amplicons showed the highest nucleotide similarity to *E. hellem*, confirming it as the most frequently identified species. The non-parametric Mann-Whitney test indicated a significantly higher infection rate in females compared to males ($p < 0.05$). However, the Kruskal-Wallis test showed no significant difference in infection rates based on age and breeding conditions across the four groups of dogs and cats ($p > 0.05$).

Conclusion: The findings of this study demonstrate that *E. hellem* is the predominant species of microsporidia present in fecal samples of both domestic and stray dogs and cats in Tabriz, Iran.

Keywords: Microsporidia, *Encephalitozoon*, Dog, Cat, Tabriz, PCR.



Application of Artificial Intelligence in Predicting *Plasmodium* spp. Transmission under Climate Change Scenarios: A National and Global Perspective

Azin Salehe¹

Corresponding Author: Azin Salehe, Azin.salehe@gmail.com

1. Department of Veterinary, School of Veterinary Medicine, Islamic Azad University, Shahrekurd, Iran.

Article code: 1568

Abstract

Background: Malaria remains a major vector-borne disease worldwide, and *Plasmodium* spp. poses significant public health challenges in Iran's Southeastern Provinces. Climate change is expected to expand malaria transmission zones and prolong transmission seasons. Artificial intelligence (AI) offers advanced tools for integrating climatic and epidemiological data to predict disease dynamics and optimize intervention strategies. This review aims to evaluate the role of AI in predicting malaria transmission under climate change scenarios in both national and global contexts.

Methods: A systematic review was conducted using PubMed, ScienceDirect, and WHO malaria surveillance reports (2018–2024). Studies employing AI-based predictive models, including Convolutional Neural Networks (CNNs) and Maximum Entropy (MaxEnt), were analyzed. Evaluation criteria included accuracy, sensitivity, and area under the curve (AUC). Climate change projections were based on IPCC scenarios, particularly RCP 8.5 (a high-emission scenario).

Results: WHO data indicate endemic *Plasmodium vivax* foci in Sistan-Baluchestan, Hormozgan, and Kerman provinces. AI-driven models predict northward expansion of malaria risk to Fars, Bushehr, and Khuzestan by 2050, with transmission seasons potentially extending to 7–9 months annually. Globally, similar expansions are projected in South Asia, East Africa, and southern Europe. These findings are synthesized from multiple studies reviewed in this work.

Conclusion: Integrating AI-driven climate and epidemiological models can enhance early warning systems and malaria control strategies. National health programs should incorporate predictive analytics to mitigate climate-driven transmission risks.

Keywords: Artificial Intelligence, *Plasmodium* spp., Climate Change, Disease Prediction, Iran.



Leishmaniasis in Equids and Its Potential Role in the Epidemiology of the Disease in Iran: A Review

Zahra Salehzadeh Kazerouni¹, Maedeh Beiki Zare², Mohammad Reza Norouzi³, Mahmoud Nateghi-Rostami⁴

Corresponding Author: Mahmoud Nateghi-Rostami, m_nateghi@pasteur.ac.ir

1. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Iran.
2. Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Iran.
3. Doctor of Veterinary Medicine, Faculty of Veterinary Medicine. Islamic Azad University, Shooshtar Branch, Tehran, Iran.
4. Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran.

Article code: 1569

Abstract

Background: *Leishmania* parasites infect various mammals, including domestic and wild animals. Equine leishmaniasis is uncommon and often appears as mild or subclinical cutaneous forms, contributing to underdiagnosis. Thus, Equids may serve as potential reservoirs, influencing disease epidemiology and transmission.

Methods: This review was based on searches in PubMed, Scopus, Web of Science, and other data bases using the terms: leishmaniasis, equid leishmaniasis, equine leishmaniasis, epidemiology, and *Leishmania infantum* in different combinations using Boolean operators (AND, OR, NOT). Systematic reviews, cross-sectional studies and case reports were analysed. Publications from 1936 to 2025 were included to determine the potential role of equids in the epidemiology of leishmaniasis.

Results: Approximately 110 relevant studies were identified worldwide, but only one from Iran. Equine leishmaniasis is more prevalent in the New World ($\approx 20\text{-}36\%$ in Brazil, mainly due to *L. braziliensis*), whereas Old World reports are sporadic and usually involve *L. infantum*. A study in Eastern Spain found 28.5% seroprevalence in apparently healthy horses, suggesting possible underestimation of the infection in Europe. Evidence suggests infection risk is influenced by breed, morphotype, outdoor living and season. Clinical information is scarce, with most cases being subclinical or limited to cutaneous papules and ulcerated nodules around the eyes, muzzle, pinnae, neck, scrotum and legs. Diagnostic approaches include PCR-based molecular techniques, serological tests (DAT, ELISA, IFAT), and microscopy. In Iran, *L. infantum* DNA was recently identified in a donkey in Fars Province, making the first molecular evidence in equids.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: Although usually nonfatal, equine leishmaniasis is epidemiologically important due to proximity to humans and reservoir hosts. Considering the endemicity of *L. infantum* in Iran, screening of local equids is recommended and may support effective disease control.

Keywords: Equids, Equine leishmaniasis, *Leishmania infantum*, Epidemiology, Iran.



Systematic Review of *Encephalitozoon cuniculi* Within a One Health Framework: Epidemiology, Diagnosis, and Prevention

Amir Rostami¹, Reza Kalantari^{2,3}

Corresponding Author: Reza Kalantari, reza.kalantari75@yahoo.com

1. Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Internal Medicine, Faculty of Specialized Veterinary Sciences, The Science and Research Branch of Islamic Azad University, Tehran, Iran.
3. Research Institute for Herbal Medicines and Metabolic Disorders, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

Article code: 1570

Abstract

Background: *Encephalitozoon cuniculi*, a microsporidian parasite, lives inside cells and can infect humans and animals. Rabbits, livestock, and people are affected. Resistant spores spread through eating, inhalation, contact, and vertical transfer. Immunocompromised humans can develop severe systemic disease, whereas rabbits develop neurological, renal, and ocular disorders. Despite global reports, the study is spread among veterinary, pharmacological, and environmental sectors, making it challenging to gain a complete picture. We reviewed studies from 2000 to June 2025 to fill this gap by combining epidemiology, diagnostic issues, and illness prevention in a One Health paradigm.

Methods: PubMed, Scopus, Web of Science, Google Scholar, and two national databases (SID, IranMedex) were searched for regional evidence. MeSH terms and free-text keywords like *Encephalitozoon cuniculi*, zoonosis, rabbits, livestock, diagnostics, preventive, and One Health were combined using Boolean operators. We evaluated articles published since 2000, as they demonstrate sophisticated diagnostic procedures and credible prevalence data. In two steps, we removed duplicates and poor studies from the records. All articles were quality-checked. Thematically synthesized prevalence, risk factors, diagnosis, and preventive interventions.

Results: Sanitation, population density, and diagnostic methods affected rabbit prevalence from 17% to 97%. Human infections were rare but severe in immunocompromised people, while sheep and goats were asymptomatic reservoirs. Direct or indirect contact, vertical transmission, and pollution spread it. ELISA, IFAT, PCR for confirmation and genotyping, and histopathology for lesions were used to diagnose. Sanitation, carrier screening, education, and interdisciplinary collaboration were reported as preventive methods.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: *E. cuniculi* presents a continual zoonotic threat owing to its extensive host diversity and diagnostic constraints. This systematic review brings together scattered research and stresses how important it is for One Health to work together to improve surveillance, facilitate diagnosis, and put in place long-lasting preventive measures.

Keywords: *Encephalitozoon cuniculi*, Zoonosis, Microsporidia, One Health, Rabbits, Prevention.



Literature Review of Promising Antiparasitic Effects of Phytotherapy Against *Ctenocephalides felis*

Mohammad Amrollahi-Sharifabadi¹, Mobina Torabi Feijani², Amirhossein Ghiasvand², Hamidreza Shokrani³

Corresponding Author: Mobina Torabi Feijani, Torabi.mo@fv.lu.ac.ir

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
3. Department of Pathobiology, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.

Article code: 1571

Abstract

Background: Ectoparasites, such as fleas, are among the most common parasitic agents that infest companion animals worldwide. These parasites can function as vectors for vector-borne diseases, transferring several zoonotic pathogens between pets and humans. The cat flea, *Ctenocephalides felis*, is one of the most significant ectoparasites in domestic cats worldwide. On the other hand, several limitations exist for current medications in managing this parasitic infestation, and thus, introducing novel therapies is an unmet research need. In this regard, we supposed that phytoterapy with potential insecticidal effects on the cat fleas, and at the same time with negligible toxicity for non-target pets, are a promising therapy. This study aimed to investigate the potential benefits of phytoterapy for the biological control of the mentioned parasitic infestation.

Methods: This study is an analytical-narrative review on the relevant literature. It retrieved and critically evaluated the data on the beneficial properties of phytoterapy against *Ctenocephalides felis*. It used MeSH terms including cat flea, *Ctenocephalides felis*, antiparasitic agent, herbal medicine, and phytoterapy.

Results: Our results revealed that certain herbal medicines, including *Artemisia vulgaris*, *Citrus limon*, *Juniperus communis*, *Lavandula officinalis*, *Melissa officinalis*, and *Thuja plicata*, can introduce promising benefits for flea control in domestic cats. The species *Syzygium aromaticum* from the Myrtaceae family, commonly known as clove, contains the compound Eugenol. Studies showed that Eugenol has ectoparasiticidal as well as ovicidal activities for flea elimination, reducing the flea population effectively. Additionally, neem seed extract contains a powerful insecticidal and ectoparasiticidal compound known as



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Azadirachtin. Also, the volatile oils of *Illicium verum* and *Pelargonium graveolens* showed insecticidal activities against cat fleas.

Conclusion: Presented phytotherapy agents with insecticidal properties of known active ingredients can be promising options for ectoparasitic control in domestic cats.

Keywords: Ectoparasite, Flea control, Companion animal, Phytotherapy, Infestation.



A Review of New Toxoplasmosis Control Approach in Urban and Domestic Cats: Public Health Impact and Zoonotic Implication

Arian Esfandiary¹, Golnaz Azizimotlagh², Parva Abiar³, Fatemeh Ghaderi⁴, Mohammad Bahmanzadegan Jahromi⁵, Parham Rahmanian³

Corresponding Author: Parham Rahmanian, parhamrahmanian1373@gmail.com

1. Faculty of Veterinary Medicine, Razi University, Kermanshah, Iran.
2. Faculty of Veterinary Medicine, Zabol University, Zabol, Iran.
3. Faculty of Veterinary Medicine, Islamic Azad University, Science and Research branch, Tehran, Iran.
4. Department of Clinical Science, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran.
5. Faculty of Veterinary Medicine, Islamic Azad University, Kazerun branch, Kazerun, Iran.

Article code: 1574

Abstract

Background: Toxoplasmosis, a widespread zoonotic disease, is caused by *Toxoplasma gondii*. Cats, both domestic and urban, serve as the main hosts, facilitating transmission to humans through contaminated soil, water, or food, leading to notable public health implications. The purpose of this review is to review recent developments in toxoplasmosis control with particular emphasis on the role of cats and pet owners.

Methods: This narrative review analyzes published English and open-access articles via databases such as ScienceDirect, PubMed, and Google Scholar from 2015 to 2025. Keywords such as “Cat or Feline,” “Public Health,” “Toxoplasmosis,” and “*Toxoplasma gondii*” were searched in databases. The included articles were synthesized, and the analysis was carefully reviewed.

Results: In this study we discovered that traditional methods of preventing *T. gondii* include keeping cats indoors exclusively, cleaning the litter box every day, and cooking food products at the appropriate temperature. While limitations exist, several innovative approaches show promise. Live-attenuated feline vaccines (e.g., T-263 strain) have demonstrated reductions in oocyst shedding in experimental settings. Although no FDA-approved treatments specifically target feline toxoplasmosis, veterinarians successfully use drugs to mitigate acute-stage. Advances in cutting edges technologies such as -artificial intelligence assisted epidemiological surveillance and predictive modeling- can identify risk factors and forecast high-risk periods or locations. Crucially, pet owners' knowledge gaps and communication barriers between public health staffs, vet practitioners and official veterinary organization staffs highlight the need for interprofessional collaboration.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: Effective toxoplasmosis control requires a multi-faceted approach, integrating traditional preventive measures with novel technologies and robust interprofessional collaboration. Addressing knowledge gaps, improving communication, and refining feral cat management can collectively reduce oocyst shedding and mitigate the public health burden of *T. gondii*.

Keywords: Cats, Public Health, Toxoplasmosis, *Toxoplasma gondii*, Zoonotic Disease.



Microscopical and Molecular Study of *Nosema* Infection in Some Apiaries in Iran

Ali Ahmadi¹, Sedigheh Nabian¹, Ali Nikpay², Mahsa shahbakhsh³, Alireza Arab⁴, Kamyar Ahmadi⁵, Abbas Gerami Sadeghian¹

Corresponding Author: Ali Ahmadi, ahmadiali26@ut.ac.ir

1. Department of Parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran and Center of Tick and Tick-borne Disease, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran.
3. Ph.D in parasitology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
4. Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.
5. Veterinary Organization, Tehran, Iran.

Article code: 1575

Abstract

Background: Nosemosis is a parasitic disease of adult honey bees (*Apis mellifera*) caused by two different microsporidian parasites, *Nosema apis* and *Nosema ceranae*. These species are distributed globally and can cause economic losses in different agricultural sectors in the world. The present study aimed to investigate the rate of *Nosema* infection in different seasons of the year in Iranian apiaries.

Methods: In this study, 1043 adult honeybee samples were collected from 239 apiaries in summer 1403 and 1037 samples from 219 apiaries in spring 1404 from different apiaries in Iran. The abdomen of 25 honeybees was separated and ground up with 5 mL of water. Then 20 ml of water was added to it. A drop of the suspension was observed with a light microscope, and the number of spores was counted. Positive samples were examined using the Multiplex PCR method.

Results: The results of this study were analyzed using the Chi-square test. The prevalence of *Nosema* infection in different sampled apiaries in the spring and summer seasons was 10.5% and 13.81%, respectively. The study showed that the difference in the rate of *Nosema* infection in the two seasons was not statistically significant ($P\text{-value} = 0.23 > 0.05$).

Conclusion: The results of this study did not show a significant difference between the infection rates in the summer and spring seasons. Molecular analysis of *Nosema* species indicates that *N. ceranae* is currently the only agent of Nosemosis in the country, which is



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



consistent with numerous reports from many countries around the world regarding the replacement of *N. apis* by *N. ceranae* and changes in the epidemiological characteristics of the disease. It is necessary to complete the results of this study in future studies with a larger number of samples, and also to examine the level of contamination in other seasons.

Keywords: *Apis mellifera*, honeybee, prevalence, seasonal pattern, Iran.



First Molecular Detection and Phylogenetic Analysis of *Menacanthus stramineus* and *Argas persicus* in Ornamental Birds from Iran

Soroor Edrisnia¹, Saloomesh Shirali^{1,2}, Bahar Shemshadi¹

Corresponding author: Saloomesh Shirali, Saloomesh.shirali@iau.ac.ir

1. Department of Veterinary Pathobiology, SR.C, Islamic Azad University, Tehran, Iran.

2. Department of Biotechnology, Ahv. C, Islamic Azad University, Ahvaz, Iran.

Article code: 1580

Abstract

Background: Ectoparasites such as lice and ticks are common in ornamental birds worldwide. Despite the large ornamental bird population in Tehran Province, little is known about their molecular identity and genetic diversity. This study aimed to determine the prevalence, and phylogenetic relationships of ectoparasites in ornamental birds.

Methods: From August 2022 to April 2023, 2,000 ornamental birds from 200 shops in Tehran city and surrounding areas (*Parand*, *Robat Karim*, *Shahriar*), representing 4 orders, 6 families, 7 genera, and 7 species, were examined using manual inspection. Cardboard and sticky traps were used in cages and shops to collect off-host parasites such as *Dermanyssus gallinae* and *Argas persicus*. Specimens were preserved in 96% ethanol and morphologically identified using standard keys. DNA was extracted from selected lice and tick samples. PCR targeted *COX1* and *16S* rRNA (ticks) and *18S* rRNA (lice). PCR products were Sanger sequenced, followed by BLAST analysis and phylogenetic tree construction using the *Neighbor-Joining* method in MEGA7.

Results: Among 2,000 birds, 505 ectoparasites were collected (25.25%). Two main species were identified: *Argas persicus* (13%, 260 NO.) in *Columba livia domestica* and *Menacanthus stramineus* (12.25%, 245 NO.) in *Chinese Shanghai fowl*. BLAST analysis showed genetic similarities with *Argasidae* and *Menoponidae* species in GenBank. Phylogenetic analysis revealed Iranian *A. persicus* closely related to Spanish strains, while *M. stramineus* showed marked divergence. This is possibly a novel species. Sequences were submitted under OP522004, OP532983, and OP452933.

Conclusion: This first molecular characterization of ectoparasites in Tehran ornamental birds confirmed species identity, revealed genetic variation, and highlighted the need for surveillance, improved biosecurity, and awareness of zoonotic risks.

Keywords: Ornamental birds, *Argas persicus*, *Menacanthus stramineus*, Molecular identification.



Phenotypic and Molecular Detection of *Babesia* spp. in Domestic Dogs from Guilan Province, Northern Iran

Ali asbaghi Jamalabad¹, Soroor Edrisnia¹, Saloomesh Shirali^{1,2}, Kiumarth Amini³

Corresponding author: Saloomesh Shirali, Saloomesh.shirali@iau.ac.ir

1. Department of Veterinary Pathobiology, SR.C, Islamic Azad University, Tehran, Iran.

2. Department of Biotechnology, Ahv. C, Islamic Azad University, Ahvaz, Iran.

3. Department of Veterinary Basic Science, SR.C, Islamic Azad University, Tehran, Iran.

Article code: 1584

Abstract

Background: Canine babesiosis is an important tick-borne disease affecting dogs and with zoonotic potential. *Babesia* spp. invade erythrocytes, causing signs from mild anemia to severe illness. In *Guilan* province, northern Iran where conditions favor tick vectors the risk of transmission is high, yet regional data are scarce. This study investigated phenotypic and molecular features of *Babesia* spp. in dogs from *Guilan* and compared diagnostic sensitivity and specificity.

Method: In this cross-sectional study, 50 blood samples were randomly collected from dogs in various areas of *Guilan*. Blood smears were prepared, stained using Giemsa, and examined microscopically under oil immersion (1000×) across at least 100 fields per slide. Morphological features of suspected parasites were recorded. Additionally, DNA was extracted from each sample, and PCR targeting the conserved 18S rRNA gene of *Babesia* spp. was performed. Amplification products were analyzed via agarose gel electrophoresis and compared with known *Babesia* controls.

Results: Microscopic evaluation identified intraerythrocytic *Babesia* spp. in 2 out of 50 samples (4%), with parasites exhibiting round to pear-shaped morphology (~4.54 µm). PCR analysis detected *Babesia* DNA in 4 samples (8%), confirming higher diagnostic sensitivity compared to microscopy.

Conclusion: This study highlights the limitations of microscopic methods and supports the use of PCR as a more sensitive diagnostic tool for detecting *Babesia* spp. Incorporating molecular techniques into routine diagnostics is essential for early and accurate detection, timely treatment, and effective disease control in endemic regions such as *Guilan*.

Keywords: *Babesia*, Molecular diagnosis, Tick-borne disease, 18S rRNA.



Frequency of Trichomoniasis in Domestic Pigeons in Aligudarz Region and Assessment of Its Sensitivity to Anti-protozoal Drugs

Homayun Rajabipour¹, Majid Taati²

Corresponding Author: Homayun Rajabipour, homayun.rjp@gmail.com

1. Department of Basic Sciences, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
2. Doctor of Veterinary Medicine, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.

Article code: 1585

Abstract

Background: Trichomoniasis, caused by *Trichomonas gallinae*, is a protozoal disease affecting domestic and wild pigeons, resulting in oral and upper digestive tract lesions, morbidity, and potential mortality. Although antiprotozoal drugs such as metronidazole, ronidazole, and carnidazole are commonly used, concerns about reduced efficacy exist. This study aimed to determine the frequency of trichomoniasis in domestic pigeons in Aligudarz, Iran, and to evaluate the in vitro inhibitory effects of commonly used antiprotozoal agents.

Methods: A cross-sectional study was conducted on 100 domestic pigeons randomly selected from households in Aligudarz. Oral swabs were collected sterily and smears were prepared, and the smears were examined microscopically. Positive samples were cultured in modified Diamond medium and approximately 1×10^5 trophozoites were used per well for each test. The inhibitory effects of metronidazole, ronidazole and carnidazole were determined using the broth microdilution method. Data were analyzed descriptively and the chi-square test was used to compare the inhibitory effects between drugs.

Results: Out of 100 examined pigeons, 35 were positive for *T. gallinae*, resulting in a frequency of 35%. In vitro assays showed inhibition in 90% of isolates by ronidazole, 85% by metronidazole, and 80% by carnidazole. Although ronidazole showed the highest inhibitory effect, statistical analysis indicated no significant difference among the three drugs ($\chi^2=1.87$, $df=2$, $p=0.39$).

Conclusion: Trichomoniasis is relatively frequent in domestic pigeons in Aligudarz. All tested drugs demonstrated comparable inhibitory effects. These findings emphasize the importance of rational drug use, ongoing monitoring of treatment efficacy, and consideration of alternative strategies to manage trichomoniasis effectively.

Keywords: Trichomoniasis, *Trichomonas gallinae*, Domestic pigeons, Drug inhibitory effect.



Comparison of Simple and Nested PCR Methods for the Detection of *Neospora caninum* in the Brain of Aborted Bovine Fetuses

Asma Keyvanlou Shahrestanaki¹, Gholamreza Razmi²

Corresponding Author: Gholamreza Razmi, razmi@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1588

Abstract

Background: *Neospora caninum* is a significant cause of bovine abortion, leading to considerable economic losses in both dairy and beef industries. Early detection of this parasite in aborted fetuses is critical for managing its spread in herds. While conventional diagnostic methods like histopathology provide useful information, they lack the sensitivity needed for early detection. PCR-based techniques, particularly nested-PCR, have gained popularity due to their high sensitivity and specificity for detecting *N. caninum* DNA. This study compares the effectiveness of simple and nested PCR methods for detecting *N. caninum* in the brain tissue of aborted bovine fetuses.

Methods: Brain samples were collected from 105 aborted bovine fetuses at the Center of Excellence in Ruminant Abortion, Ferdowsi University of Mashhad, during 2022-2023. Two PCR methods were employed for detection: a simple PCR targeting the *pNc5* gene and internal transcribed spacer 1 (ITS1), and a nested-PCR technique using the same targets. The PCR products were analyzed via gel electrophoresis, and detection was based on the appearance of specific bands corresponding to *N. caninum* DNA.

Results: Out of the 105 samples, 26 were positive for *N. caninum* using the nested-PCR method (24.8%), while 19 were positive using the simple PCR assay (18%). Both PCR methods showed good agreement, but nested-PCR exhibited higher sensitivity, detecting more positive samples. These findings suggest that while both techniques are reliable for detecting *N. caninum*, nested-PCR offers a slight advantage in terms of detection rate.

Conclusion: This study highlights that nested-PCR is a more sensitive method compared to simple PCR for detecting *Neospora caninum* in the brain tissue of aborted bovine fetuses. Both PCR techniques are suitable for molecular diagnosis, but nested-PCR may be preferable when higher sensitivity is required. Further research is needed to validate these methods in different field settings and enhance diagnostic accuracy.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Neospora caninum*, Nested-PCR, Simple PCR, Bovine Abortion, Molecular diagnosis.



A Review of CRISPR-Cas9-Driven Insights into the Molecular Basis of Antimalarial Drug Resistance in *Plasmodium falciparum*

Mohammed Reza Anani¹, Yaser Habibi¹, Fatemeh Valikhani¹, Mobina Rajabzadeh¹, Tohid Javaheri²

Corresponding Author: Mohammad Reza Anani, reza1380top@gmail.com

1. Department of Biology, faculty of Sciences , Mashhad Branch, Islamic Azad University, Mashhad ,Iran.
2. Department of Biology ,Faculty of Sciences, University of Guilan , Rasht, Iran.

Article code: 1589

Abstract

Background: The emergence of drug-resistant *Plasmodium falciparum* strains poses a growing challenge to global malaria elimination efforts. Resistance to front-line antimalarials such as artemisinin and chloroquine has been linked to specific gene mutations. However, the functional roles of these mutations are still being explored. The development of CRISPR-Cas9 genome editing has provided a precise tool for investigating the genetic mechanisms underlying drug resistance in *Plasmodium*.

Methods: This narrative review analyzed studies published between 2015 and 2025, retrieved from PubMed, Scopus, and Web of Science using search terms like CRISPR-Cas9, *Plasmodium*, and drug resistance. Selected studies used CRISPR-Cas9 to target genes including Pfk13 and pfprt, followed by drug susceptibility assays and whole-genome sequencing to assess gene function and detect off-target effects.

Results: Editing of Pfk13 confirmed its contribution to artemisinin resistance, while modification of pfprt resulted in phenotypes consistent with chloroquine resistance. Several studies also uncovered novel resistance-related loci by disrupting uncharacterized genes. Reported editing efficiencies were typically above 80%, demonstrating CRISPR's reliability in *Plasmodium* research. Furthermore, CRISPR enabled systematic, high-throughput screening of genes potentially linked to drug response.

Conclusion: CRISPR-Cas9 has transformed malaria research by enabling precise, targeted analysis of drug resistance genes. It not only confirms known resistance mechanisms but also facilitates the discovery of new genetic factors. Integrating CRISPR with genomic surveillance and drug efficacy monitoring could significantly advance strategies for controlling resistance in endemic regions

Keywords: CRISPR-Cas9, *Plasmodium falciparum*, Drug Resistance, Genome Editing, Pfk13.



Neospora caninum and Bovine Reproductive Failure: A Review of Epidemiology, Diagnostics, and Control Strategies

Fazel Sadeghian¹, Arash Kheradmand², Roohollah Dehghani Tafti²

Corresponding Author: Fazel Sadeghian, Sadeghianfazel@gmail.com

1. Graduated from the faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.
2. Department of Clinical Sciences, Faculty of Veterinary Medicine, Lorestan University, Khorramabad, Iran.

Article code: 1590

Abstract

Background: *Neospora caninum* is a protozoan parasite and a major cause of reproductive failure in cattle, especially mid-gestation abortion. It is maintained mainly through vertical transmission, often without clinical signs, which makes detection and control difficult. Neosporosis causes major economic losses through abortions, reduced conception, culling, and lower productivity. In endemic regions, herd seroprevalence may exceed 50%, highlighting the need for better surveillance. This review summarizes current knowledge on the reproductive impact of *N. caninum* and key challenges in diagnosis and control.

Methods: This study is a narrative review. Literature in English from 2010 to 2025 was searched in PubMed, Scopus, and CAB Abstracts using keywords related to *N. caninum*, reproductive failure, vertical transmission, and diagnosis. Articles were included if they reported clinical, epidemiological, or experimental findings on bovine neosporosis with reproductive outcomes.

Results: Vertical transmission is the main infection route, leading to abortion, stillbirth, or weak calves. Histopathological lesions include nonsuppurative encephalitis and placentitis. Diagnostic methods such as ELISA, IFAT, and PCR show variable sensitivity and specificity depending on infection stage and sample type. Detecting subclinical infections remains difficult. Control measures include culling infected dams, managing canine hosts, and applying biosecurity. Vaccine efficacy is inconsistent and under study. Recent reports (2023–2025) confirm high seroprevalence in both beef and dairy herds, underscoring the parasite's global significance.

Conclusion: *N. caninum* remains an important yet underrecognized cause of reproductive loss in cattle. Better diagnostics and effective vaccines are priorities. Future research should emphasize detection of subclinical infections, evaluation of economic burdens, and integrated herd-level and One Health strategies to reduce its long-term impact on cattle production.

Keywords: *Neospora caninum*, Reproductive Failure, Vertical transmission, Abortion.



Organophosphate Poisoning Following Misuse of Pesticide for Pediculosis Treatment in a Child: A Case Report

Tarane Farhangi¹, Anahita Alizadeh²

Corresponding Author: Anahita Alizadeh, tar.korra@gmail.com

1. Department of parasitology, faculty of veterinary medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Pediatric toxicology, School of medicine, Mashhad University of Medical Science, Mashhad, Iran.

Article code: 1591

Abstract

Background: Pediculosis (lice infestation), caused by *Pediculus humanus capitis*, is a widespread parasitic condition affecting children globally, including in Iran. This infection can cause discomfort and lead to secondary infections from scratching. The case report focuses on a 3-year-old girl who suffered acute organophosphate poisoning due to the misuse of an organophosphate pesticide for treating pediculosis.

Methods: The child's mother applied an organophosphate spray (Diazinon) to treat the lice infestation on the child's scalp. Within minutes of application, the child began showing symptoms of poisoning, leading to her hospitalization in the emergency department. Upon arrival, the child was evaluated for vital signs and clinical symptoms, including excessive sweating, shortness of breath, nausea, and increased salivation. The treatment regimen included the administration of atropine, pralidoxime, and supportive care.

Results: On clinical examination, the child's vital signs were stable. However, laboratory tests revealed significantly low cholinesterase levels. The serum cholinesterase level was 1200 IU/lit (normal range > 4000 IU/lit), and the RBC cholinesterase level was 0.7 IU/lit (normal range > 4 IU/lit). These findings confirmed the diagnosis of organophosphate poisoning. The child was treated with atropine, pralidoxime, and supportive management, and after 48 hours, her condition improved, and she was discharged in stable health.

Conclusion: This case report highlights the serious consequences of improper use of chemical pesticides, especially in households without adequate knowledge or access to proper medical facilities. The report emphasizes the importance of safe treatment options for pediculosis, such as topical permethrin, and calls for greater awareness and education on the risks associated with misusing harmful chemicals. Effective management of pediculosis should involve both safe, accessible treatment alternatives and targeted educational efforts to prevent the misuse of dangerous substances in the home.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Organophosphate, Poisoning, Children, Cholinesterase.



The Role of One Health in the Sustainable Management and Surveillance of Parasitic Diseases in the Era of Climate Change

Mohammad Arad Zandieh¹, Hesamedin Akbarein¹

Corresponding Author: Hesamedin Akbarein, vet.epidemiologist.ut@gmail.com

1. Division of Epidemiology & Zoonoses, Department of Food Hygiene & Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Article code: 1592

Abstract

Background: Parasitic diseases pose a significant global health burden. Climate change has exacerbated the effects of this disease by causing it to emerge in new regions or re-emerge in old ones. Parasite ecology is altered by climate change, resulting in profound changes in epidemiology, distribution, and transmission dynamics. This review addresses how the One Health approach provides a sustainable framework for the surveillance and management of these complex diseases amidst a changing climate.

Methods: This article reviews recent advancement in disease surveillance based on One Health approach from 2016 to 2025. The review was conducted across databases, including PubMed/MEDLINE, Web of Science, Google Scholar, and EMBASE, using the following keywords: “One Health”, “Surveillance”, “Parasitic disease”, and “Disease control”.

Results: Climate change significantly alters parasitic disease dynamics, expanding vector habitats and accelerating parasite life cycles, leading to disease emergence in new geographic areas. Current control measures face limitations, particularly the growing challenge of drug resistance, driven by overuse of antiparasitic drugs in human and veterinary medicine. The One Health approach offers a sustainable framework through interdisciplinary collaboration and integrated strategies, including genetic resistance, vaccines, biological control, and grazing management. Advanced technologies like molecular diagnostics, GIS, and AI/ML enhance surveillance, enabling proactive detection via climate-based early warning systems and community-based initiatives. Successful One Health case studies, such as echinococcosis management in China, demonstrate the efficacy of multi-sectoral interventions in reducing disease burden.

Conclusion: A comprehensive and multifaceted One Health approach is needed to implement sustainable management practices and integrated surveillance systems. The goal of this interdisciplinary collaboration approach is to mitigate and control climate change. Globally and nationally, One Health initiatives offer significant opportunities for safeguarding health security despite challenges in resources and policies.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: One Health, Parasitic Diseases, Climate Change, Surveillance, Epidemiology.



The Role of Artificial Intelligence in the Diagnosis of Equine Parasitic Diseases: A Systematic Review

Mohammad Taghi Vajed Ebrahimi¹, Asma Keyvanlou Shahrestanaki²

Corresponding Author: Mohammad Taghi Vajed Ebrahimi, m.vajed@yahoo.com

1. Department of Physiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1594

Abstract

Background: Strongyles (particularly cyathostomins) and *Parascaris* spp. are among the most common and clinically significant gastrointestinal parasites in horses worldwide. The growing concern of anthelmintic resistance has challenged the effectiveness of routine deworming protocols, encouraging a shift toward evidence-based targeted anthelmintic treatment (TAT). However, traditional fecal egg count (FEC) methods like the McMaster technique are limited by operator dependency, low reproducibility, and inability to detect larval stages such as encysted cyathostomins. To overcome these limitations, artificial intelligence (AI)-driven diagnostic tools based on deep learning have recently been introduced, offering standardized and objective analysis.

Methods: A systematic literature review was conducted across PubMed, Scopus, and Google Scholar to identify relevant studies published between 2014 and 2024. The search included combinations of keywords: (Artificial Intelligence OR Deep Learning) AND (Equine OR Horse) AND (Parasite OR Fecal Egg Count). Only original peer-reviewed studies evaluating AI systems for detecting equine parasite eggs and reporting diagnostic performance metrics (e.g., sensitivity, specificity) were included. Data on parasite type, sample preparation, and diagnostic outcomes were extracted.

Results: Several AI-based platforms, including Vetscan Imagyst and OvaCyte™, demonstrated excellent diagnostic accuracy. Sensitivity for strongyle detection ranged from 99.2% to 100%, and specificity from 91.4% to 99.9%. For *Parascaris* spp., sensitivity was 88.9%–99.9% and specificity 93.6%–99.9%. AI systems also showed strong concordance with expert counts (CCC > 0.92), lower variation, and faster processing (10–15 minutes).

Conclusion: AI-powered diagnostic tools offer precise, consistent, and efficient alternatives to manual FEC methods. They enhance clinical decision-making for TAT programs and help



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



combat anthelmintic resistance. However, since they cannot detect prepatent infections, future approaches may benefit from combining AI with molecular or serological techniques.

Keywords: Artificial Intelligence, Equine Parasitology, Diagnostic Accuracy, Deep Learning, Fecal Egg Count.



An Elderly Patient with a Hydatid Cyst in the Liver, Suspected of *Echinococcus multilocularis*

Setareh Alizadeh¹, Elahe Ebrahimzadeh¹

Corresponding Author: Elahe Ebrahimzadeh, ebrahimzade@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1595

Abstract

Background: Hydatid cyst, caused by *Echinococcus granulosus* and *E. multilocularis*, is a zoonotic infection that is becoming more common. It mainly affects the liver and spreads through food and water contaminated with dog feces, including the eggs of *Echinococcus*.

Methods: A 71-year-old male presented with abdominal pain and unexplained weight loss. The patient underwent abdominal ultrasound and CT scan, which were used to assess liver pathology. Additional evaluation included endoscopic ultrasound (EUS) and endoscopic retrograde cholangiopancreatography (ERCP) to visualize the biliary tree and assess for ductal involvement. A surgical consultation was conducted to evaluate the possibility of liver lesion resection. The patient was later referred to a specialized hepatobiliary center for further management.

Results: Imaging revealed a heterogeneous, multilocular cystic lesion with peripheral calcifications in the right hepatic lobe. The lesion extended toward the common bile duct (CBD), causing partial biliary obstruction. EUS and ERCP showed moderate to severe narrowing of the biliary ducts. Signs of diaphragmatic involvement and compression of surrounding structures were also noted. These findings were highly suggestive of an infiltrative parasitic infection consistent with *E. multilocularis*.

Conclusion: This case highlights the uncommon clinical and radiological features of *E. multilocularis* infection. The lesion's malignant-like behavior underscores the importance of prompt diagnosis and multidisciplinary management, including advanced imaging, endoscopic procedures, and potential surgical resection.

Keywords: Hydatid cyst, *Echinococcus multilocularis*, *Echinococcus granulosus*, Zoonosis.



Prevalence of *Eimeria* Infection in Free-Grazing Cattle and Calves in Eastern Golestan Province

Mohadese Chitband¹, Elahe Ebrahimzadeh¹

Corresponding Author: , Elahe Ebrahimzadeh, ebrahimzade@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1597

Abstract

Background: *Coccidiosis*, caused by protozoan parasites of the genus *Eimeria*, is a common intestinal disease in cattle and calves that leads to digestive problems, reduced growth, and economic losses in livestock production. Despite its importance, little information exists on the prevalence of *Eimeria* infection among free-grazing cattle in eastern Golestan Province, Iran. This study aimed to estimate the prevalence of *Eimeria* infection in this region.

Methods: A cross-sectional study was carried out during spring and summer on 348 free-grazing cattle from rural areas around Kalaleh, Maraveh Tappeh, Galikesh, Gonbad Kavus, and Minoodasht counties. Fresh fecal samples were collected immediately after defecation, including 289 adults and 59 calves. Detection of *Eimeria* oocysts was done using the Clayton-Lane flotation technique.

Results: Overall, *Eimeria* oocysts were found in 68 samples (19.5%). Among adults, 60 of 289 (20.8%) were infected, while 8 of 59 (13.6%) calves tested positive. These results show a moderate prevalence of infection, with a higher rate in adults than calves. The prevalence rates in the study areas were as follows: Galikash (44.11%), Minoodasht (20.58%), Gonbad Kavus (14.73%), Kalaleh (10.29%), and Maraveh Tappeh (10.29%). The intensity of contamination was “1+”, and the OPG count under a 10x lens ranged from 1 to 10 oocysts.

Conclusion: This study indicates that *Eimeria* infection in free-grazing cattle and calves in eastern Golestan Province is notable. It highlights the need for improved hygiene and parasite control in traditional farming. Regular monitoring, farmer education, and improved husbandry are essential to reduce infection, enhance animal welfare and productivity, and support sustainable livestock production.

Keywords: *Eimeria*, *Coccidiosis*, Free-grazing cattle, Golestan Province.



Cestode Infection in Eurasian Collared Dove (*Streptopelia decaocto*)

Hannaneh Moghaddam¹, Setareh Alizadeh¹, Javad Khedri¹, Elahe Ebrahimzadeh¹

Corresponding Author: Elahe Ebrahimzadeh, ebrahimzadeh@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1601

Abstract

Background: Parasitic infections in synanthropic avian species are of growing ecological and epidemiological interest, particularly due to their close interaction with human-modified environments. Eurasian Collared Dove (*Streptopelia decaocto*) is a widely distributed urban-adapted bird species that may serve as a potential reservoir for intestinal helminths, including cestodes. Monitoring such infections is crucial for understanding parasite diversity and potential zoonotic risks.

Methods: A total of 21 deceased wild Eurasian Collared Doves (*S. decaocto*) were necropsied, and their intestinal contents were specifically examined for cestode parasites. Specimens were processed using standard parasitological techniques, including lactophenol clearing and carmine staining. The preparation steps included relaxation, fixation, staining, destaining, dehydration, clearing, and permanent mounting. Microscopic examinations were conducted using both light microscopy and stereomicroscopy, with emphasis on the morphological characteristics of the scolex and proglottids for species identification.

Results: Cestode infection was confirmed in all 21 examined birds. Five different cestode species were identified: *Cotugnia digonopora* was the most prevalent, detected in 10 birds (47.6%), followed by *Choanotaenia infundibulum* in 5 birds (23.8%), *Raillietina echinobothrida* and *R. tetragona* each in 4 birds (19.0%), and *R. cesticillus* in 1 bird (4.8%). Mixed infections involving two distinct cestode species were observed in 3 out of the 21 birds (14.3%). These findings highlight a moderate level of cestode diversity in wild collared doves and suggest the potential for concurrent parasitism by multiple species within individual hosts.

Conclusion: The detection of multiple cestode species in Eurasian Collared Doves highlights their potential role as reservoir hosts for helminth parasites. These findings emphasize the importance of continuous surveillance of avian parasitic communities, not only for ecological insights but also for their public and veterinary health implications.

Keywords: Cestode, *Streptopelia decaocto*, Helminthology, Parasitology.



Geographic Distribution and Fauna of *Ixodidae* (Hard) Ticks in Northwest of Iran, in 2023-2024

Faraz Malek Bagali^{1,2}, Eslam Moradi-Asl³

Corresponding Author: Eslam Moradi-Asl, moradiasl83@yahoo.com

1. Department of Microbiology, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
2. Student Research Committee, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
3. Arthropod-Borne Diseases Research Center, Ardabil University of Medical Sciences, Ardabil, Iran.

Article code: 1603

Abstract

Background: Ticks are hematophagous ectoparasites belonging to three main families: Argasidae, Ixodidae, and Nuttalliellidae, that play a major role in transmitting zoonotic pathogens. This study was conducted to determine the species composition and geographical distribution of hard ticks infesting livestock in Ardabil, northwestern Iran, during 2023–2024.

Methods: In this cross-sectional survey, 700 tick samples were collected from cattle, sheep, and goats across 12 counties. The sample size was estimated using a single population proportion formula with an assumed prevalence of 10%, 95% confidence interval, and 3.5% precision, resulting in 700 required samples. Collected ticks were identified morphologically using standard taxonomic keys. Data analysis was performed using SPSS v25 to evaluate associations between tick species, host animals, and geographic locations.

Results: A total of 700 hard ticks were identified. *Rhipicephalus bursa* was the most prevalent species (41.7%), found in all surveyed counties. Other common species included *Rhipicephalus turanicus* (17.8%), *Hyalomma marginatum* (14.3%), and *Dermacentor marginatus* (11.1%). *Rhipicephalus sanguineus* accounted for 9.4% of the samples, while *Hyalomma scupense*, *H. sulcata*, and *H. rufipes* were rarely detected. *Ixodes ricinus* was identified in only five specimens (0.7%). Geographically, the highest tick abundance was reported in Bilehsavar (13.8%), Khalkhal (13.2%), Nir (12.8%), and Meshkinshahr (12%), while the lowest occurred in Angut (2.2%). Most counties harbored multiple species simultaneously, with *R. bursa*, *R. turanicus*, and *H. marginatum* being dominant in Bilehsavar and Khalkhal, while *H. marginatum* predominated in Kowsar. However, statistical analysis revealed no significant difference in species distribution among counties ($P > 0.05$).



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: This investigation represents one of the most comprehensive assessments of hard tick fauna in Ardabil. The findings underscore the considerable infestation burden in livestock and highlight the necessity of integrated tick control measures. Education of livestock owners and region-specific management strategies are crucial to minimize the risk of tick-borne diseases.

Keywords: Hard ticks, Ixodidae, livestock, Ardabil, Iran.



Prevalence of Tick-Borne Bacteria in Livestock, in northwestern Iran (2024)

Faraz Malek Bagali^{1,2}, Farzad Khademi³

Corresponding Author: Farzad Khademi, k_farzad@yahoo.com

1. Department of Microbiology, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
2. Student Research Committee, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
3. Arthropod-Borne Diseases Research Center, Ardabil University of Medical Sciences, Ardabil, Iran.

Article code: 1604

Abstract

Background: Ticks are blood-feeding obligate parasitic arthropods, which serve as vectors for numerous bacterial, viral, and parasitic zoonotic diseases. This descriptive cross-sectional study aimed to identify the predominant tick-borne pathogens in soft-bodied ticks (*Argasidae*) and hard-bodied ticks (*Ixodidae*) affecting livestock in northwestern Iran.

Methods: In the spring and summer of 2024, tick samples were collected from various regions of Ardabil and transported to the entomology laboratory for identification using established keys. DNA was extracted from 26 tick pools and stored at -20 °C. The study focused on detecting tick-borne pathogens, including *Coxiella burnetii*, *Anaplasma spp.*, *Borrelia spp.*, *Brucella spp.*, and *Francisella spp.*, using standard PCR and nested PCR techniques. Sequencing was performed to confirm the findings, and the distribution of tick-borne pathogens and tick species was mapped using ArcMap 10.8.1 software.

Results: A total of 841 ticks were collected from 211 herds across 12 counties in Ardabil province, representing 11 species: 2 soft-bodied ticks (*Argas persicus* and *Argas reflexus*) and 9 hard-bodied ticks (*Dermacentor marginatus*, *Rhipicephalus bursa*, *Rhipicephalus turanicus*, *Rhipicephalus sanguineus*, *Hyalomma marginatum*, *Ixodes ricinus*, *Hyalomma scupense*, *Haemaphysalis sulcata*, and *Hyalomma rufipes*). Among 26 analyzed tick pools, 6 tested positive for *Anaplasma spp.*, 11 for *Brucella spp.*, and 5 for *C. burnetii*, with no positives for *Borrelia spp.* or *Francisella spp.*.

Conclusion: This study highlighted the diversity and distribution of tick species and their pathogens in Ardabil province, emphasizing the significant risk of tick-borne diseases in the region. The detection of *Anaplasma spp.*, *Brucella spp.*, and *C. burnetii* underscores the need for proactive monitoring and control measures to protect both livestock and human health. The findings stress the importance of continued research, targeted prevention strategies, and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



effective management practices to reduce the impact of tick-borne diseases and improve public health outcomes.

Keyword: Ticks, Tick-borne pathogens, Arthropod vectors, Zoonosis.



Molecular Detection and Identification of *Crimean-Congo Hemorrhagic Fever Virus (CCHFV)* in Ixodid and Argasid Ticks from Northwestern Iran, 2024

Faraz Malek Bagali^{1,2}, Chiman Karami¹

Corresponding Author: Chiman Karami, farazmalek36@gmail.com

1. Department of Microbiology, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
2. Student Research Committee, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.

Article code: 1607

Abstract

Background: *Crimean-Congo Hemorrhagic Fever virus (CCHFV)* is a fatal tick-borne virus. Due to the absence of effective antiviral treatments and a licensed vaccine, early detection of the virus in carriers and vectors is crucial for disease control and prevention. Ardabil Province is considered an endemic region for *CCHFV* due to its extensive livestock industry and favorable ecological conditions for tick proliferation. Therefore, the aim of this study was to molecular detection and identification of *CCHFV* in Ixodid and Argasid ticks from northwestern Iran, in 2024.

Methods: 841 ticks were collected using standard methods from livestock across 12 counties in northwest of Iran and identified using valid tick identification keys. The Real-time RT-PCR method was used to detect the *CCHFV* (S) segmented genome in 26 pooled ticks. SPSS version 25 was used for statistical analysis.

Results: A total of 841 ticks from 211 herds (138 sheep, 30 goats, and 113 cattle) were collected. Eleven tick species were identified in this study. Samples were classified as *Argasidae* (22.35%) and *Ixodidae* (77.64%). They were also divided into males (49.36%) and females (50.61%). The most abundant species were *R. bursa* (39.20%) for *Ixodidae* and *A. persicus* (98.72%) for *Argasidae*, respectively. Totally 4 pools (15.3%) out of 26 were positive for *CCHFV*. Infected pools were belong to *I. ricinus* (3.8%) in goat, *H. marginatum* (3.8%) and *rhhipicefaluc.spp* (3.8%) in cattle and *H. scupense* (3.8%) in sheep. There was no significant relationship between virus contamination and tick species, nor between species caught and sampling location, $P < 0.05$.

Conclusion: This study confirms the endemic presence of *CCHFV* in Ardabil Province, highlighting the key role of *Rhipicephalus* and *Hyalomma* (hard) ticks in virus transmission.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Continued surveillance of these vectors is essential for effective disease control in the endemic region.

Keywords: *CCHFV*, Ticks, Ardabil, Real-Time RT-PCR.



Seasonal Variation of *Toxocara* Egg Contamination in Park Soils of Garmsar County, Iran

Narges Safari¹

Corresponding Author: Narges Safari, Narges.safari@iau.ir

1. Department of Veterinary Medicine, University of Garmsar branch, Islamic Azad University, Garmsar, Iran.

Article code: 1608

Abstract

Background: *Toxocara* is the etiological agent of zoonotic toxocarosis in humans and has a global distribution. Human infection occurs through direct contact with infected dogs or cats, or via contact with contaminated sources containing parasite eggs, such as soil from public spaces like parks. Eggs excreted by definitive hosts have the potential to infect a wide range of hosts, including humans. The most common route of transmission is ingestion of eggs present in soil or vegetables. The present cross-sectional study aimed to assess the presence of *Toxocara* eggs in soil from parks in Garmsar.

Methods: During the summer and autumn seasons of 1403 (Iranian calendar), 80 soil samples were collected from four public parks in Garmsar County. Each sample comprised 200 g of soil, collected at a depth of 3–5 cm. Samples were examined using a sugar-solution flotation method.

Results: All parks examined were reported as contaminated. Of the 40 samples collected in summer, 6 samples (15%) were found positive for contamination. Of the 40 samples collected in autumn, 11 samples (27.5%) were contaminated. In this region, the contamination rate with *Toxocara* eggs in autumn is significantly higher than in summer. This difference may be attributed to extreme heat and low humidity during the summer.

Conclusion: A key finding of this study is the pronounced seasonal fluctuation in the prevalence of *Toxocara* eggs in soil, which is detrimental to the survival of parasite eggs. Cooler temperatures and increased rainfall in autumn create a more favorable environment for the eggs to survive and develop. These findings illustrate how local climatic conditions act as a critical determinant in the parasite's life cycle and its environmental distribution. The contaminated soil can serve as a strong source of human infection.

Keywords: *Toxocara*, Soil, Garmsar, Seasonal distribution.



The Report of Acanthocephalan Eggs in a Long-eared Hedgehog in Isfahan Province, Iran

Mohammadreza Ghorani¹, Mahdi Salimi², Nasser Hajipour³

Corresponding Author: Nasser Hajipour, n.hajipour@tabrizu.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.
2. Department of Environmental, Wildlife Diseases Expert, Isfahan, Iran.
3. Department of Food Hygiene and Aquatic, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

Article code: 1611

Abstract

Background: Acanthocephalans are intestinal parasites with a complex life cycle involving arthropods as intermediate hosts and vertebrates, particularly rodents and insectivores, as definitive hosts. Although it has been reported from different parts of Iran, information on its occurrence in central provinces such as Isfahan is scarce. This study aimed to investigate the presence of acanthocephalan parasites in long-eared hedgehogs (*Hemiechinus auritus*) from Najaf Abad, Isfahan province.

Methods: In August 2023, an adult female long-eared hedgehog (302 g) with a history of reduced appetite was live-trapped from farmland and peri-domestic areas of Najaf Abad. Fresh fecal samples were collected directly from the animal and transferred to the laboratory in clean containers. Samples were examined by standard parasitological methods, including direct wet mount and flotation techniques, under light microscopy to identify parasite eggs. Morphological characteristics such as size, shape, and shell structure of eggs were used for identification.

Results: Microscopic examination revealed the presence of acanthocephalan eggs in the feces of the examined hedgehog. The eggs were oval, thick-shelled, and morphologically consistent with members of the family Moniliformidae. However, precise identification of parasite genus and species based solely on egg morphology was not possible.

Conclusion: This study provides the first evidence of acanthocephalan eggs in a long-eared hedgehog from Najaf Abad, Isfahan Province. Further epidemiological and ecological investigations, including recovery and identification of adult worms and studies on potential intermediate hosts, are necessary to confirm the species and to clarify its significance for public and veterinary health. Control measures targeting rodent populations could reduce the risk of transmission in agricultural environments.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Acanthocephalan, Flotation, Long-eared hedgehog, Isfahan.



In Vitro Evaluation of *Rhipicephalus sanguineus* Resistance to Commercial Formulations of Cypermethrin and Deltamethrin

Seyed Amirreza Boskabadi¹, Ali Moshaverinia²

Corresponding Author: Ali Moshaverinia, moshaverinia@um.ac.ir

1. Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1612

Abstract

Background: Ticks are obligate hematophagous ectoparasites of vertebrates and important vectors of pathogenic agents, posing threats to both human and animal health. The repeated use of chemical acaricides for tick control has led to significant problems, including the widespread development of resistance. This study aimed to assess the susceptibility of *R. sanguineus* ticks collected from infested dogs in Mashhad, Iran, to commercial formulations of cypermethrin and deltamethrin.

Methods: Five dog populations originated from different locations around Mashhad city were visually examined and collected adult female ticks identified and cultured in the parasitology laboratory. Larvae with 12-14 days old were exposed to concentrations of 0.05, 0.125, and 0.25 g/L of each compound using the Larval Packet Test (LPT) methodology. In each test, 100 larvae were placed into a packet treated with the desired concentration of acaricide, and mortality was recorded after 24 h. Lethal concentration for 99% mortality (LC₉₉) was determined using Probit analysis.

Results: Comparison of LC₉₉ values with the recommended label dosages revealed decrease in susceptibility to both examined acaricides in all five tested tick populations. Resistance ratios for cypermethrin ranged from 19 to 42, and for deltamethrin from 8 to 23.

Conclusion: This study demonstrates an *in vitro* reduction in *R. sanguineus* susceptibility to cypermethrin and deltamethrin. Continuous monitoring of acaricide resistance and the adoption of integrated tick management strategies are essential to maintain effective control.

Keywords: Tick, *Rhipicephalus sanguineus*, Acaricide resistance, Larval Packet Test, Dog.



Meat and Corn By-Products as Feedstock for Enhanced Black Soldier Fly Larvae (*Hermetia illucens*) Growth

Maryam Ghaderi-Ghahfarokhi¹, Gholam Hussein Bagheri², Somayeh Bahrami², Alireza Alborzi²

Corresponding Author: Maryam Ghaderi-Ghahfarokhi, m.ghaderi@scu.ac.ir

1. Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Parasitology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1613

Abstract

Background: Black Soldier Fly larvae (BSFL), *Hermetia illucens*, are an efficient, eco-friendly alternative to traditional livestock feed. Rich in protein and fat, they can be reared on various organic wastes such as food scraps, manure, and agricultural residues. They mature rapidly in about 21 days, making them a faster protein source than crickets or mealworms. Their ability to grow on diverse organic materials offers a more sustainable protein option compared to conventional livestock, which require substantial land and water resources.

Methods: BSFL were reared in the laboratory on three substrates—corn gluten, meat powder, and a control diet (broiler feed ration)—to evaluate growth performance. Each group was housed under controlled conditions and fed exclusively on their respective substrates. Larval growth was assessed by measuring final larval weight (g) and calculating daily growth rate (g/day) at the experiment's conclusion. Data were expressed as mean $\pm$ standard deviation.

Results: One-way ANOVA revealed a significant effect of substrate on final larval weight ($p < 0.001$). Holm-corrected pairwise comparisons indicated that larvae fed meat powder achieved significantly lower final weight (8.1 ± 0.4 g) compared to those on corn gluten (28.2 ± 0.28 g) and the control diet (28.15 ± 0.21 g) ($p < 0.001$), while no difference was found between corn gluten and control ($p \approx 1.00$). Similarly, growth rate differed significantly by substrate: larvae on meat powder grew slower (0.575 ± 0.007 g/day) than those on corn gluten (2.015 ± 0.2 g/day) and control (2.01 ± 0.01 g/day) ($p < 0.001$), with no significant difference between corn gluten and control ($p \approx 1.00$).

Conclusion : Black soldier fly larvae exhibit rapid growth when fed optimal substrates such as corn gluten, supporting their use as a sustainable alternative protein source. Substrate choice significantly influences growth outcomes.

Keywords: Black Soldier Fly Larvae, Corn By-Products, Growth rate, Meat Powder.



Anemia Caused by Heavy *Ctenocephalides felis* Infestation in Young Holstein Calves under Poor Hygienic Conditions: A Case Series from Sirch, Kerman, Iran (2024)

Seyede Mahnoosh Mostafavi¹, Seyede Mahshid Mostafavi², Kian Mosaali², Shahab Shirzahi³, Leila Saravani⁴

Corresponding Author: Seyede Mahnoosh Mostafavi, mahnooshmostafavi613@gmail.com

1. Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Chamran of Ahvaz, Ahvaz, Iran.
2. Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Bahonar of Kerman, Kerman, Iran.
3. Department of Medical Faculty, Zahedan Azad University, Zahedan, Iran.
4. Department of Poultry, Apiculture, and Sericulture Health and Disease Management, General Directorate of Veterinary Services, Sistan and Baluchestan Province, Iran.

Article code: 1615

Abstract

Background: *Ctenocephalides felis* is a hematophagous ectoparasite primarily infesting dogs, but can occasionally infest ruminants under poor hygienic conditions. While mild infestations may be subclinical, heavy flea burdens can cause clinically significant anemia, especially in young animals. This study aimed to describe clinical, hematological, and environmental findings in three Holstein calves with severe *C. felis* infestation, highlighting the role of inadequate hygiene in disease severity.

Methods: Three Holstein calves, approximately three months old (two males, one female), from a poorly managed farm in Sirch, Kerman Province, Iran, were examined after showing lethargy, anorexia, and pale mucous membranes. Complete blood count (CBC) and peripheral blood smears were performed. Flea counts were determined by manual combing and identified morphologically as *C. felis*. Blood smears were examined for hemoparasites. Environmental hygiene conditions were assessed.

Results: A total of 26 fleas were isolated from 3 calves (9 fleas from calf number 1, 7 fleas from calf number 2, and 10 fleas from calf number 3). All calves had moderate to severe anemia: hematocrit ranged from 18–22% (normal: 30–36%), hemoglobin from 6.2–7.5 g/dL (normal: 12–15 g/dL), and RBC counts from $3.8\text{--}4.5 \times 10^6/\mu\text{L}$ (normal: $7\text{--}9 \times 10^6/\mu\text{L}$). Flea counts ranged from 150–200 per calf. The farm showed poor hygiene with overcrowding, soiled bedding, and infrequent cleaning, which facilitated flea proliferation. Treatment included topical fipronil, oral iron supplementation, and environmental sanitation. All calves recovered clinically and hematologically within two weeks.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: This case series demonstrates that *C. felis* can cause significant anemia in young Holstein calves under poor hygienic conditions. Preventive measures, including regular ectoparasite control and improved farm sanitation, are essential to protect animal health and minimize economic losses.

Keywords: *Ctenocephalides felis*, Anemia, Flea infestation, Holstein calves, Farm hygiene.



A Case Report of *Hyalomma* spp. Tick Infestation in 3 Free-Roaming Dogs in Southeastern Iran (Zabol), 2024

Seyede Mahnoosh Mostafavi¹, Seyede Mahshid Mostafavi², Shahab Shirzahi³, Leila Saravani⁴

Corresponding Author: Seyede Mahnoosh Mostafavi, mahnooshmostafavi613@gmail.com

1. Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Chamran of Ahvaz, Ahvaz, Iran.
2. Department of Clinical Science, Faculty of Veterinary medicine, University of Shahid Bahonar of Kerman, Kerman, Iran.
3. Department of Medical Faculty, Zahedan Azad University, Zahedan, Iran.
4. Department of Poultry, Apiculture, and Sericulture Health and Disease Management, General Directorate of Veterinary Services, Sistan and Baluchestan Province, Iran.

Article code: 1616

Abstract

Background: Ticks of the genus *Hyalomma* are important hematophagous ectoparasites of domestic and wild animals and play a critical role in the transmission of several zoonotic pathogens, including *Theileria*, *Babesia*, and Crimean-Congo hemorrhagic fever virus. Among these, *Hyalomma* spp. is a widely distributed species in arid and semi-arid regions, including southeastern Iran. Despite its recognized veterinary and public health importance, there is a paucity of information on its occurrence in free-roaming dog populations in the Zabol region. Dogs, particularly free-roaming or stray individuals, may serve as important hosts for *Hyalomma* spp., thereby facilitating its maintenance and spread. The present study was conducted to document the occurrence and sex distribution of *Hyalomma* spp. ticks in free-roaming dogs from the suburban areas of Zabol.

Methods: In 2024, three adult free-roaming dogs (two males and one female) from suburban Zabol were clinically examined for ectoparasite infestation. Ticks were manually removed using forceps, placed in labeled containers, and preserved for identification. Morphological identification was performed under a stereomicroscope according to established taxonomic keys, and the sex of each specimen was recorded.

Results: All examined dogs were infested with *Hyalomma* spp. A total of 13 adult ticks were recovered, consisting of 10 males and 3 females. Tick burdens were as follows: Dog A—4 ticks (3 males, 1 female), Dog B—3 ticks (all males), Dog C—6 ticks (4 males, 2 females). Male ticks were predominant in all cases.

Conclusion: This study confirms the active infestation of free-roaming dogs with *Hyalomma* spp. in suburban Zabol. Given the vectorial role of this species, implementing effective tick



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



control and public awareness programs is essential to reduce the risk of tick-borne diseases in the region.

Keywords: *Hyalomma spp.*, Tick infestation, Free-roaming dogs, Epidemiology, Zabol.



Prevalence of *Rickettsia* Species in Hard Ticks In northwest Iran (2025)

Faraz Malek Bagali^{1,2}, Mahdi Rezaverdinejad³, Ahmad Enferadi³

Corresponding Author: Ahmad Enferadi, ahmad.tanyaie@gmail.com

1. Department of Microbiology, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
2. D Student Research Committee, Faculty of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
3. Department of Microbiology, Faculty of Veterinary Medicine, Urmia University, Iran.

Article code: 1617

Abstract

Background: Vector-borne zoonotic infections, including those caused by *Rickettsia* spp., are prevalent in tropical and subtropical climates. These Gram-negative, obligate intracellular bacteria are predominantly transmitted through tick vectors. The most virulent strains affecting humans are members of the spotted fever group (SFG), such as *Rickettsia rickettsii*. The present investigation was undertaken to perform molecular characterization of *Rickettsia* spp. in ticks collected from northwestern Iran during 2024–2025.

Methods: From January to September 2024, field sampling was carried out in Ardabil Province, Iran. A total of 841 ticks were recovered from livestock in 12 counties and subjected to morphological identification to the species level. Genomic DNA was extracted using the kit in accordance with the manufacturer's guidelines. The presence of *Rickettsia* spp. was assessed via PCR amplification of partial fragments of the *16S rRNA* and *gltA* genes. Amplicons were resolved by agarose gel electrophoresis, and positive samples were further confirmed through Sanger sequencing.

Results: In total, 841 ticks representing nine species across six genera—including both hard (*Ixodidae*) and soft (*Argasidae*) ticks—were collected from livestock in 12 counties of Ardabil Province during 2024. Molecular analysis revealed the presence of *Rickettsia* DNA in *Rhipicephalus turanicus* and *Rhipicephalus* spp. obtained from sheep. The detected sequences demonstrated similarity to pathogenic *R. conorii* and *R. rickettsii*, indicating that local livestock-associated tick populations may serve as reservoirs for these agents in northwestern Iran.

Conclusion: This investigation provides the first molecular evidence of *Rickettsia* spp. infection in ticks parasitizing livestock in Ardabil Province, Iran. The detection of DNA sequences closely related to *R. conorii* and *R. rickettsii* in *Rhipicephalus* ticks suggests possible silent circulation of pathogenic *Rickettsia* species in the region. Given the zoonotic potential, ongoing molecular surveillance is warranted to inform targeted public health interventions.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Rickettsia*, Ticks, Livestock, PCR.



Ticking Threat: Prevalence of *Hyalomma marginatum* in Cattle Farms of Hasan-Abad Fashafuyeh, Tehran Province

Erfan Darmanloo¹, Alireza Ghorbani², Mehrdad Ghaledari¹, Negar Shaverdi¹, Amirreza Ghorbani³

Corresponding Author: Erfan Darmanloo, erfandarmanlo@gmail.com

1. Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran
2. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
3. Department of Microbiology, College of Science, Agriculture and Modern Technology, Shiraz Branch, Islamic Azad University, Shiraz, Iran.

Article code: 1618

Abstract

Background: Ticks, particularly *Hyalomma marginatum*, are key vectors of zoonotic pathogens, posing significant threats to both livestock productivity and public health. These parasites not only reduce milk and meat yields but also increase veterinary costs for farmers. In major livestock-rearing areas such as Hasanabad-e Fashafuyeh, Tehran Province, understanding the prevalence and spatial distribution of these ticks is crucial for implementing effective control strategies and preventing disease outbreaks.

Methods: This cross-sectional field study was conducted in 14 semi-industrial and industrial dairy farms across Hasanabad-e Fashafuyeh, encompassing herds of varying sizes. Over 5,800 dairy cattle were systematically examined by carefully inspecting their entire bodies for attached ticks, with individual counts recorded on site. Each specimen was gently removed, preserved, and morphologically identified, confirming the presence of *H. marginatum* under light microscopy. Infestation rates were then calculated for each farm.

Results: Among the 14 surveyed dairy farms, only three showed evidences of *H. marginatum* infestation, highlighting its presence in the region. A total of 243 cattle were found infested with 279 ticks, with infestation rates in the affected farms ranging from 6.77% to 9.30%. The overall prevalence across all herds averaged 4.19%. The remaining 11 farms were free of infestation, indicating a patchy but noteworthy distribution pattern of the parasite.

Conclusion: The detection of *H. marginatum* in distinct hotspots signals a silent yet tangible threat to the livestock industry of Hasanabad-e Fashafuyeh. Although most farms remained tick-free, the clustered infestation pattern suggests that, without timely intervention, these pockets could become epicenters for wider outbreaks. Strengthening local surveillance and implementing integrated tick-control strategies are essential to protect animal health, sustain productivity, and safeguard the economic stability of the region's dairy sector.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: *Hyalomma marginatum*, Tick infestation, Dairy cattle, Zoonotic diseases, Tehran province.



Diagnosis of Ovine Aborted Fetuses Associated with *Toxoplasma gondii* in Mazandaran Province

Afsaneh Amouei¹, Fatemeh Daraei¹, Fatemeh Parandin², Samira Dodangeh³, Elham Kia Lashaki⁴, Saeide Mir Yaghoobzadeh¹, Azadeh Mizani⁵

Corresponding Author: Azadeh Mizani, azadeh.mizani@gmail.com

1. Mazandaran Central Veterinary Laboratory, Medical Sciences, Veterinary Administration of Mazandaran Province, Sari, Iran.
2. Department of Parasitology, School of Allied Medical Sciences, Ilam University of Medical Sciences, Ilam, Iran.
3. Technology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Department of Parasitology and Mycology, To.C., Islamic Azad University, Tonekabon, Iran.
5. Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran.

Article code: 1620

Abstract

Background: *Toxoplasma gondii* (*T. gondii*) as an obligate intracellular protozoan parasite is one of the main causes of infectious reproductive failure in small ruminants particularly sheep. This study aimed to investigate the role of *T. gondii* infection in sheep aborted fetuses using bioassay and PCR methods in Mazandaran Province, northern Iran.

Methods: A total of 48 sheep aborted fetuses were collected at various stages of gestation during lambing seasons in different parts of Mazandaran Province. The brains of all 48 aborted fetuses were bioassayed in female BALB/c mice. DNA was extracted from all brain samples following phenol-chloroform-isoamyl alcohol instructions. The RE gene, along with TOX4 and TOX5 primers, was used to detect *T. gondii* DNA using a conventional PCR assay.

Results: The mean age of aborted fetuses in sheep was 3.3 ± 0.99 . The results of the bioassay samples were negative, as no tachyzoites or cysts were observed in the peritoneal and brain specimens of the mice. The presence of *T. gondii* DNA was confirmed by the observation of a 529 bp band in 8 out of 48 fetuses (16.66%). The highest prevalence of the infection was observed in the East area (20%), followed by the Center (16.66) and the West area (12.50%) of Mazandaran Province.

Conclusion: In northern Iran, sheep and likely other livestock are commonly exposed to *T. gondii* infection. Toxoplasmosis may contribute, to abortion outbreaks and subsequent economic losses in the regions livestock industry, such as Mazandaran Province. To address this, further research including molecular genotyping of *T. gondii* strains and the development



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



of targeted control measures are essential to improve herd health management and reduce disease impact.

Keywords: *Toxoplasma gondii*, Aborted fetuses, Sheep, Iran.



Detection of *Toxocara cati* in a Deceased Stray Cat Using Morphological and Molecular Approaches: A Case Study from Shiraz, Iran

Erfan Darmanloo¹, Amirreza Ghorbani², Alireza Ghorbani³

Corresponding Author: Alireza Ghorbani, alireza.ghorbani7899@gmail.com

1. Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran.
2. Department of Microbiology, College of Science, Agriculture and Modern Technology, Shiraz Branch, Islamic Azad University, Shiraz, Iran.
3. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Article code: 1621

Abstract

Background: *Toxocara cati* is a prevalent intestinal nematode infecting domestic and feral cats, with significant zoonotic potential impacting both human and animal health. Transmission predominantly occurs via ingestion of embryonated eggs present in contaminated environments, especially in urban and peri-urban areas. Despite its widespread distribution, *T. cati* remains under-investigated compared to *Toxocara canis*, particularly in stray feline populations. This case highlights the importance of molecular diagnostics for accurate identification of *T. cati*, enhancing epidemiological insights and guiding control measures.

Methods: A deceased 2-year-old male stray cat from Shiraz was necropsied, and the intestinal contents were morphologically examined, revealing the presence of adult nematodes consistent with *T. cati*. For definitive identification, molecular analysis was conducted using PCR with species-specific primers FM1 (5'-TTGAGGGGAAATGGGTGAC-3') and FM2 (5'-TGCTGGAGGCCATATCGT-3'). PCR products were subjected to gel electrophoresis and confirmed by comparison to reference sequences, ensuring accurate diagnosis through combined morphological and molecular approaches.

Results: Necropsy revealed adult nematodes in the intestines, morphologically identified as *Toxocara cati*. PCR using primers FM1 and FM2 confirmed the parasite's identity by producing expected gene fragments. These results verify *T. cati* infection in the stray cat.

Conclusion: This case report highlights the presence of *T. cati* infection in a stray cat in Shiraz, confirmed through combined morphological and molecular methods. The findings emphasize the importance of monitoring stray animal populations as potential reservoirs of zoonotic parasites. Molecular diagnostics, alongside traditional techniques, provide accurate



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



identification crucial for effective control and prevention strategies to reduce public health risks.

Keywords: *Toxocara cati*, Stray cats, Molecular diagnosis, Zoonosis, Intestinal nematode.



Frequency and Associated Factors of *Sarcoptes scabiei* Infestation Among Suspected Patients Referred to Selected Medical Laboratories in Mashhad, Iran

Amin Bakhshani¹, Ali Moshaverinia², Bahareh Meftahi^{2,3}, Mohammad Reza Khakzad^{3,4}

Corresponding Author: Amin Bakhshani, aminbakhshani@gmail.com

1. *Graduated of Veterinary Parasitology (Ph.D degree), Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran.*
2. *Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*
3. *Innovative Medical Research Center, MMS. C., Islamic Azad University, Mashhad, Iran.*
4. *Department of Immunology, MMS. C., Islamic Azad University, Mashhad, Iran.*

Article code: 1622

Abstract

Background: Scabies is a widespread, contagious skin disease caused by the burrowing mite *Sarcoptes scabiei*, affecting humans and over 150 mammal species. Transmission primarily occurs through direct, prolonged, human-to-human contact, though zoonotic scabies can result from infested animal exposure. This study aimed to determine the frequency of scabies infestation among suspected patients in Mashhad, Iran, and to evaluate its association with age, gender, season, occupation, residence, and animal contact.

Methods: A cross-sectional study was conducted from March 2019 to March 2020. A total of 201 patients (104 females, 97 males) with pruritus and clinically suspected scabies were referred by dermatologists to selected medical laboratories, including those of the Islamic Azad University of Medical Sciences. A structured questionnaire recorded demographic information, residence (urban/rural), occupation, direct animal contact and time of symptom onset. Skin scrapings were collected from four sites—interdigital spaces of the hands, chest, abdomen, and feet—digested in 10% potassium hydroxide (KOH), and examined under light microscopy for *S. scabiei* mites or eggs. Negative specimens were re-examined to confirm results.

Results: Of 201 patients, 75 (37.31%) were positive: 39 males (52%) and 36 females (48%). The highest frequency occurred in spring and among patients under one year old (range: 6 months–61 years). The family infestation was identified in four families, three in rural and one in urban areas. Rural cases accounted for 78.7% of positives, with higher frequencies in both rural women and men compared to urban residents. Twenty-three positive cases reported animal contact via livestock farming or pet ownership. The interdigital spaces were the most frequent site of detection, followed by the abdomen, chest, and feet.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Conclusion: Although human-to-human transmission is dominant, the observed proportion of patients with animal contact suggests the need for further epidemiological and molecular studies to investigate potential cross-species transmission pathways.

Keywords: *Sarcoptes scabiei*, Frequency, Human, Animal Contact, Mashhad.



Case Report: Internal and External Parasites in Crested and Eurasian Skylarks Collected from Environmental Mortalities in Khorasan Province and the Persian Gulf Bird Market in Tehran

Mohammad Mahdi Darvishi¹, Jalil Pourhaji Motab²

Corresponding Author: Mohammad Mahdi Darvishi, darvishe@gmail.com

1. Department of Veterinary Medicine, School of Veterinary Sciences, Islamic Azad University, Garmsar, Iran.
2. Department of Veterinary Medicine, School of Veterinary Sciences, Islamic Azad University, Garmsar, Iran.

Article code: 1626

Abstract

Background: Parasitic infections are major health concerns in wild and ornamental birds. Lark species, native to Iran, are vulnerable to a variety of internal and external parasites. This study aimed to identify parasitic agents in two lark species—*Crested Lark (Galerida cristata)* and *Eurasian Skylark (Alauda arvensis)*—collected from environmental mortalities and bird markets.

Methods: Ten Crested Larks were collected from natural mortalities in Khorasan Province, and ten Eurasian Skylarks were obtained from the Persian Gulf bird market in Tehran. External parasites were removed from the body surface. Anatomical dissection was performed to examine the abdominal and pulmonary cavities. Parasites were identified using standard parasitological keys.

Results: In Crested Larks, lice of the genus *Menopon* and the nematode *Diplotrriaena sokolowi* were identified. In *Eurasian Skylarks*, lice of the genus *Menacanthus* and the same nematode were observed. *Diplotrriaena sokolowi* is an oviparous nematode with an indirect life cycle that inhabits the air sacs of birds.

Conclusion: The simultaneous presence of *Menopon*, *Menacanthus*, and *Diplotrriaena sokolowi* in two lark species from different regions of Iran highlights the importance of parasitic surveillance in native and ornamental birds. Health monitoring in bird markets is essential to prevent cross-species transmission

Keywords: Lark, External Parasite, Nematode, *Menopon*, *Menacanthus*, *Diplotrriaena sokolowi*, Iran.



Prevalence of Toxoplasmosis in Wild Cattle as Human Zoonotic Meat-borne Pathogens: A Systematic Review and Meta-analysis

Azadeh Mizani¹, Afsaneh Amouei², Elham Kia Lashaki³, Samira Dodangeh⁴, Fatemeh Parandin⁵

Corresponding Author: Fatemeh Parandin, f.parandin@yahoo.com

1. Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran.
2. Mazandaran Central Veterinary Laboratory, Medical Sciences, Veterinary Administration of Mazandaran Province, Sari, Iran.
3. Department of Parasitology and Mycology, To.C., Islamic Azad University, Tonekabon, Iran.
4. Technology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
5. Department of Parasitology, School of Allied Medical Sciences, Ilam University of Medical Sciences, Ilam, Iran.

Article code: 1627

Abstract

Background: The increasing consumer interest in game meat, along with the potential transmission of *Toxoplasma gondii* from game meat to humans, poses significant challenges in assessing the related public health risks. The diverse transmission pathways of *T. gondii* and its ability to infiltrate host organisms offer an excellent opportunity for enhancing our comprehension of this pathogen's transmission dynamics. *Toxoplasma* has traditionally been a valuable model for studying host-pathogen interactions and investigating population, community, and environmental or ecological aspects. Therefore, a meta-analysis was conducted to estimate the worldwide prevalence and related ecological factors of parasites in wild cattle.

Methods: Six databases (Google Scholar, ScienceDirect, PubMed, Scopus, Web of Science, and ProQuest) were searched for studies published up to November, 2024. Apparent pooled prevalence was analyzed using a random-effect model with a 95% confidence interval. Furthermore, a univariate and multivariate meta-regression analyses were conducted to investigate associations of species, socio-demographic, geographical, and meteorological data with the prevalence of parasites.

Results: The apparent prevalence of *T. gondii* in wild cattle was estimated at 14.4 % (CI 95%: 10.1-18.7, I²: 96.59%). According to this analysis, the prevalence estimates of the subfamily were 36.2% in Wildebeest, 9.90% in Yak, 2.10% in Muskox, 9.10% in Bison, and 52.50% in African buffalo. Meta-regression analyses showed a significant relationship among the



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



variables of wild cattle subspecies, age, gender, samples, methods, continent, temperature, rainfall, humidity, and income level with the prevalence of parasites.

Conclusion: The findings indicate the circulation of *T. gondii* but not a homogeneous exposure in wild ungulates (wild cattle), which could have significant implications for animal health, conservation, and public health.

Keywords: Ungulate, Wild cattle, Meta-analysis, Seroprevalence.



Seroprevalence of *Neospora caninum* Infection in Cows with Abortion in Khorasan Razavi, Iran

Asma Keyvanlou Shahrestanaki¹, Bahareh Meftahi¹, Narges Khaleghnia², Ali Barati¹, Gholamreza Razmi¹

Corresponding Author: Gholamreza Razmi, razmi@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Centre of Excellence in Ruminant Abortion and Neonatal Mortality, faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1628

Abstract

Background: *Neospora caninum* is a protozoan parasite responsible for abortions in cattle, causing major economic losses in the livestock industry. Early detection is essential for controlling its spread. This study aimed to determine the prevalence of *N. caninum* infection in cows with abortion in Khorasan Razavi province using ELISA.

Methods: In 2015, blood samples were collected from 94 cows that had experienced abortion between 5.5 and 7 months of pregnancy, all in their first or second gestation. Serum samples were tested for antibodies against *N. caninum* using a commercial ELISA kit (Bio K 192/2 - Bio K 192/5). Antibody titers were analyzed to determine infection prevalence.

Results: Of the 94 samples, 42 cows (44.7%) tested positive, showing significant antibody titers against *N. caninum*. Five cows (5.3%) were classified as suspected cases, while 47 (50%) tested negative. The high prevalence indicates that *N. caninum* may be a major contributing factor to abortions in this region.

Conclusion: This study confirms a notable prevalence of *N. caninum* in cows with abortion in Khorasan Razavi. The findings highlight the need for routine screening and better herd management to reduce infection rates. Considering the significant economic losses linked to abortion in cattle, preventive strategies and further research on the association between antibody titers and abortion rates are recommended.

Keywords: Abortion, Cattle, ELISA, Livestock diseases, Khorasan Razavi, *Neospora caninum*.



Study of the Practices of Rural Livestock Farmers in Saveh County Regarding Cystic Echinococcosis

Amirhossein Kakaei¹, Elahe Ebrahimzadeh², Ehsanollah Afshari Safavi³, Arman Latifi⁴

Corresponding Author: Elahe Ebrahimzadeh, ebrahimzade@um.ac.ir

1. *Doctoral Student of Veterinary Medicine, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*
2. *Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*
3. *Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*
4. *Department of Public Health, Research Center for Evidence-Based Health Management, Maragheh University of Medical Sciences, Maragheh, Iran.*

Article code: 1629

Abstract

Background: One of the most important zoonotic and parasitic diseases is hydatid cyst, which causes significant human and financial losses. Livestock farmers are among the main groups at risk of contracting this disease, and the level of their high-risk or preventive practices is effective in controlling this disease. Considering the role of livestock farmers in Saveh County in controlling this disease and the lack of study of their practice so far, we examined their performance in this study.

Methods: In this study, 460 rural livestock farmers over 15 years of age from Saveh County who had contact with dogs in their work environment completed a questionnaire including three sections: demographics, performance, and history of infection. The data were analyzed using SPSS software in terms of their high-risk and preventive practices and the relationship between demographic indicators and the performance of the farmers.

Results: The majority of the livestock farmers, 353 people (76.74%), had more than 20 years of livestock farming experience, which indicated a high level of experience in the study population. (69.8%) of the livestock farmers had herds of less than 200 heads, and (10.4%) had herds of more than 500 heads. The maximum score for the performance section was 7, which was achieved by 3 people (0.7%), and the lowest score was 1, which was achieved by 2 livestock farmers (0.4%). (84.13%) of the livestock farmers exhibited preventive practice, and (15.87%) exhibited high-risk practice.

Conclusion: Overall, the practices of livestock farmers was more preventive; however, the study of various indicators showed that there is no direct relationship between herd size and



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



livestock farming experience with the level of practice, and this indicates the need to create a platform to accelerate the transfer of experience and training among all livestock farmers.

Keywords: Cystic Echinococcosis, Saveh, Practice, Livestock.



Knowledge of Rural Livestock Farmers in Saveh County about Cystic Echinococcosis

Amirhossein Kakaei¹, Elahe Ebrahimzadeh², Ehsanollah Afshari Safavi³, Arman Latifi⁴

Corresponding Author: Elahe Ebrahimzadeh, eebrahimzade@um.ac.ir

1. *Doctoral Student of Veterinary Medicine, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*
2. *Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*
3. *Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.*
4. *Department of Public Health, Research Center for Evidence-Based Health Management, Maragheh University of Medical Sciences, Maragheh, Iran.*

Article code: 1630

Abstract

Background: Hydatid cyst is one of the most important parasitic and zoonotic diseases, and because it can be transmitted from canines and ruminants to humans, it is more prevalent in rural areas and among livestock farmers. Studies show that livestock farmers' knowledge plays a special role in controlling this disease. Given that Saveh County is one of the country's livestock farming hubs and the level of knowledge of its livestock farmers about hydatid cyst disease has not been measured so far, we investigated the level of knowledge of livestock farmers in this study.

Methods: A questionnaire that included demographic questions, history of infection, and 18 questions about knowledge of the disease was completed by rural livestock farmers over 15 years of age who were in contact with dogs in the environment. The level of knowledge of the farmers and the relationship between demographic indicators and knowledge were examined using SPSS software.

Results: In this study, out of 460 participants, 29 (6.30%) were female and 431 (93.70%) were male. 82 (17.83%) had university education and more than half of the participants, i.e. 260 (56.52%) were only literate. The maximum knowledge score was 18 and its mean was 9.47, with the lowest score being 1 and the highest score being 17. Almost half of the participants (47.17%) scored between 9 and 12, which is an average score. Only 4 (0.87%) scored the highest score, but 58 (11.7%) had a knowledge score below 7, indicating a poor level of knowledge.

Conclusion: In this study, it was found that the level of knowledge is not directly related to gender and literacy level. Overall, livestock farmers did not have an acceptable level of



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



knowledge, and these cases clearly indicate the need for specialized training for livestock farmers.

Keywords: Cystic Echinococcosis, Saveh, Knowledge, Livestock farmers.



In Vivo Evaluation of Acaricidal Effect of Seed Oil of *Melia azedarach* on *Dermanyssus gallinae*

Mohammad Reza Khoshvaght¹, Ali Moshaverinia², Ehsan Khoshvaght³

Corresponding Author: Ali Moshaverinia, moshaverinia@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran. Email: khoshvaght77@gmail.com
2. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
3. Department of Biology, Payame Noor University of Gonabad, Gonabad, Iran.

Article code: 1631

Abstract

Background: *Dermanyssus gallinae*, a blood-feeding ectoparasite of laying hens and wild birds, causes anemia, weakness, reduced egg production, and mortality in severe cases. It transmits pathogens including chicken pox virus, Newcastle virus, avian spirochaetosis, pullorum, and typhoid. Prolonged chemical acaricide use causes resistance, food residues, strict regulations, and increased demand for organic products. This study evaluated the *in vivo* acaricidal effect of *Melia azedarach* seed oil on *D. gallinae* under farm conditions.

Methods: *Melia azedarach* fruits were shade-dried, ground, and extracted with hexane. In a commercial layer house in Mashhad, the first row was sprayed with 100 liters of solution containing 5 liters seed oil, 1 liter Tween80, and 94 liters water, repeated after 7 days. The fourth row (control) was sprayed with 1 liter Tween80 and 99 liters water. The second row was treated with 1% cypermethrin. Mite populations were monitored at days 2, 7, 9, 14, 21, and 28 post-treatment using semi-transparent plastic traps.

Results: Mite counts were significantly lower in row one compared to the control at days 2, 9, 14, and 21 ($P \leq 0.05$). No significant difference was found between cypermethrin and control ($P \geq 0.05$). *Melia azedarach* seed oil efficacy on days 2, 7, 9, 14, 21, and 28 was 77.7%, 77.3%, 79.3%, 69.38%, 56.08%, and 38.67%, respectively. Cypermethrin showed 56.12% efficacy, primarily on day 2. Packed cell volume (PCV) significantly increased in the *Melia azedarach* group ($P \leq 0.05$), while control showed no significant change.

Conclusion: considerable *in vivo* acaricidal effect against *Dermanyssus gallinae* under farm conditions. The efficacy of the seed oil was higher and more persistent than cypermethrin, suggesting that it can be considered as a promising botanical alternative to chemical acaricides. Moreover, the improvement in PCV in the treated group highlights its potential in reducing the adverse health impacts of poultry red mite infestations. Further studies on dosage optimization,



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



formulation stability, and safety assessments are recommended to validate its practical application in poultry production.

Keywords: Poultry red mite 1, *Melia azedarach* 2, Cypermethrin 3, PCV 4.



Nano-Phytosomal Delivery of Phytochemicals: An Emerging Strategy for Controlling Zoonotic and Veterinary Parasitic Infections

Seyed Mobin Aghilian¹, Mohammad Mohsenzadeh², Soodeh Alidadi³

Corresponding Author: Mohammad Mohsenzadeh, mohsenzadeh@um.ac.ir

1. Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
3. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1632

Abstract

Background: Parasitic infections remain a major challenge in human and animal health due to zoonotic transmission and increasing drug resistance. Medicinal herbs contain bioactive phytochemicals with antiparasitic properties, but their clinical use is limited by poor solubility and low bioavailability. Phytosomes, phospholipid complexes of plant compounds, offer a promising nano-delivery system to enhance therapeutic efficacy.

Methods: This review summarizes recent *in vitro* and *in vivo* studies on phytosome-based formulations for parasitic diseases in animals, focusing on anti-leishmanial, anticoccidial, and scabidicidal effects. Data were extracted from peer-reviewed publications, emphasizing mechanisms, comparative efficacy, and veterinary applications.

Results: Phytosomal encapsulation improved intracellular delivery, stability, and bioavailability of key compounds such as quercetin, naringenin, andrographolide, and epigallocatechin-3-gallate (EGCG). In anti-leishmanial studies, quercetin-loaded phytosomes showed *in vitro* activity comparable to glucantime at 400 µg/ml and achieved complete lesion resolution *in vivo*, likely due to enhanced uptake, oxidative stress, enzyme inhibition, and immune modulation. Andrographolide phytosomes exhibited superior activity against *Leishmania donovani* via phospholipid-mediated membrane integration. For anticoccidial effects, green tea extract-loaded phytosomes in *Eimeria*-infected broilers reduced intestinal lesions and improved growth more effectively than unformulated extracts. This was linked to enhanced EGCG absorption, reduced oxidative stress, and suppressed inflammation through nuclear factor modulation. In scabidicidal applications, *Pistacia integerrima* gall extracts rich in naringenin and quercetin, when encapsulated in phytosomes, showed greater efficacy against



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Sarcoptes scabiei in pigs. Improved lipophilicity and membrane penetration likely disrupted cuticular respiration and physiological processes.

Conclusion: Phytosomes represent a novel strategy to overcome limitations of conventional antiparasitic drugs, offering enhanced efficacy, safety, and cost-effectiveness. Despite promising preclinical results, further studies are needed to optimize dosing, evaluate long-term safety, and confirm field usability. Addressing these gaps could strengthen integrated parasite control and improve animal and human health, as well as food safety.

Keywords: Phytosomes, Phytochemicals, Antiparasitic agents, Veterinary parasitology, Nanoencapsulation.



Assessment of *Cryptosporidium parvum* Infection and Associated Risk Factors in Calves from Dairy Farms in Southern and Southwestern Iran

Faezeh Falahatnezhad¹, Aria Rasooli¹, Mehdi Mohebbi Fani¹, Seyyed Mostafa Razavi Dinani², Saeed Hosseinzadeh³

Corresponding Author: Aria Rasooli, rasooliaria2000@yahoo.com

1. Department of Animal Health Management, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.
2. Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.
3. Department of Hygiene and Food Quality Control, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.

Article code: 1633

Abstract

Background: *Cryptosporidium* is a protozoan parasite belonging to the phylum Apicomplexa and is considered one of the most important causes of intestinal infections in humans and animals. This study aimed to investigate the prevalence of *Cryptosporidium* infection in suckling calves and to identify the associated risk factors in the southern and southwestern regions of Iran.

Methods: A total of 368 fecal samples were collected from calves on 20 dairy farms located in Fars, Khuzestan, and Kerman provinces. Fecal smears were prepared following flotation, stained using the modified Ziehl–Neelsen method, and examined microscopically for *Cryptosporidium* oocysts. Positive samples were further confirmed by PCR.

Results: Of the 368 fecal samples, 62 (16.8%) were microscopically positive for *Cryptosporidium* oocysts, of which 56 (90%) were identified as *C. parvum* by PCR. Logistic regression analysis was used to assess the prevalence and associated risk factors, calculating odds ratios (OR) with 95% confidence intervals. A significant association was found between infection and factors such as age, diarrhea, and washing practices for milk utensils.

Conclusion: *Cryptosporidium parvum* shows a high prevalence among dairy farms in southern and southwestern Iran. Given that oocyst shedding is linked to high rates of diarrhea and increased mortality in young calves, control measures aimed at reducing shedding are essential to limit transmission.

Keywords: *Cryptosporidium parvum*, Calves, Risk factors, PCR detection.



A Case of Acute Toxoplasmosis in a Veterinarian: Potential Zoonotic Transmission from a Domestic Cat in Iran

Heidarzadeh Saeid¹, Ghorbani Parsa Mohammad Sajjad¹, Ghorbani Parsa Mobina¹

Corresponding Author: Saeid Heidarzadeh, saeed.heidarzadeh2025@gmail.com

1. Doctor of Veterinary Medicine, Mashhad, Iran.

Article code: 1636

Abstract

Background: *Toxoplasma gondii* is a protozoan parasite spread worldwide. Humans acquire it primarily through ingestion of undercooked meat or contaminated food/water with oocysts shed by cats. Though generally asymptomatic in healthy individuals, it is dangerous for immunocompromised patients and pregnant women. Veterinarians and pet owners have increased exposure risk due to regular contact with cats. We present a rare case of acute toxoplasmosis in a veterinarian, linked to exposure from his household cat.

Methods: A 32-year-old veterinarian from Iran, with daily contact with a domestic cat, was diagnosed with acute toxoplasmosis during a routine student screening program. The patient had no significant symptoms, and serological tests revealed markedly elevated IgM titers (>380 IU/mL), consistent with recent infection. IgG levels were not available.

Results: The patient remained asymptomatic at the time of testing, and no common risk factors like undercooked meat consumption were identified. The domestic cat was suspected as the source of infection.

Conclusion: This case highlights the potential risk of acute toxoplasmosis in veterinarians from domestic cats, despite their professional knowledge. Although often subclinical, high IgM titers suggest recent infection. Preventive measures such as hand hygiene and avoiding raw meat for cats are essential. This case underscores the importance of educating veterinarians and pet owners about the risks of *T. gondii* transmission and the need for regular screening and strict hygiene practices. Further studies are necessary to evaluate the occupational risk of toxoplasmosis globally.

Keywords: Toxoplasmosis, *Toxoplasma gondii*, Domestic cat, Zoonosis.



The Study of the Prevalence of Hemoparasites in Camels of Northeastern Iran

Fatemeh Rezazadeh¹, Hassan Borji¹, Gholamreza Razmi¹, Javad Khedri¹

Corresponding Author: Hassan Borji, Hborji@um.ac.ir

1. Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1636

Abstract

Background: *Dipetalonema evansi* is a filarial nematode of camels, causing testicular filariasis and reduced productivity, with potential zoonotic risk due to similarity to human filariae. *Trypanosoma evansi*, a protozoan causing surra, is zoonotic and transmitted by biting flies (*Tabanus*, *Stomoxys*). Although these parasites are globally distributed, epidemiological data from northeastern Iran are limited.

Methods: A cross-sectional study was conducted in northeastern Iran on 162 camel blood samples to detect *Dipetalonema evansi* and *Trypanosoma evansi*. For *D. evansi*, Knott's method was used, and sediments were examined microscopically at 10× and 40× magnifications. *T. evansi* was identified using Giemsa-stained thin smears and buffy coat examination under a 100× oil immersion lens.

Results: Of the 162 camel blood samples analyzed, 7 (4.32%) tested positive for *Dipetalonema evansi* microfilariae, indicating moderate prevalence of infection in the studied population. No samples tested positive for *Trypanosoma evansi*.

Conclusion: This study reports a moderate prevalence of *Dipetalonema evansi* microfilariae in camels from northeastern Iran, with no detection of *Trypanosoma evansi*. Given the zoonotic potential and impact of *D. evansi* on camel health, effective control measures are essential. Although *T. evansi* was not found, its recognized pathogenicity warrants continued surveillance. Further epidemiological studies are recommended to clarify transmission dynamics and risk factors to improve prevention and control strategies, protecting animal and public health.

Keywords: *Dipetalonema evansi*, *Trypanosoma evansi*, Prevalence, Camels.



Investigation of Blood Parasites of Columbidae in Mashhad City in the Summer of 1404

Mohammad Hossein Ghafourian Abadi¹

Corresponding Author: Mohammad Hossein Ghafourian Abadi, dvm.ghafourian@gmail.com

1. Graduate of the Faculty of Veterinary Medicine, Islamic Azad University, Garmsar Branch, Garmsar, Iran.

Article code: 1643

Abstract

Background: Infection with blood parasites in Columbidae is very common and of great importance. This infection does not cause death of the bird, but it weakens the bird. Infection of Columbidae with blood parasites is important in the field of disease transmission to industrial poultry and even in common diseases. Therefore, the aim of this study was to investigate the prevalence of blood parasites in Columbidae in order to prevent irreparable economic losses to the country's poultry industry.

Methods: In this study, in order to determine the level of blood contamination, 100 Columbidae were randomly selected from different parts of Mashhad city, especially in the Tabarsi Boulevard and Panjtan areas in the summer season. After that, blood was taken from their underwing vein and blood smears were prepared from these blood samples. After fixing the smears with methanol, they were stained with Giemsa method and then examined at 100x magnification.

Results: In this study, infection with the blood parasite *Haemoproteus columbae* was observed in 17%, *Leucocytozoon* in 6%, and *Plasmodium* in 8%.

Conclusion: Since sampling was conducted in the summer, and due to the active population of insects that carry these organisms, and the domestication of these birds, infection with blood parasites was observed, therefore, by controlling these infections, increasing awareness among people, and more careful health monitoring by veterinarians, this infection can be greatly reduced.

Keywords: *Haemoproteus columbae*, *Leucocytozoon*, *Plasmodium*, Mashhad.



Phylogenetic Relationships of *Giardia duodenalis* Isolates from Human and Animal Sources Based on *tpi* and *gdh* Genes

Zeynab Khajegiri¹, Maryam Naderi Soorki¹

Corresponding author: Maryam Naderi Soorki, m.naderi.soorki@scu.ac.ir

1. Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

Article code: 1645

Abstract

Background: *Giardia duodenalis* is a globally distributed protozoan parasite that causes gastrointestinal infections in both humans and animals. This study aimed to analyze the phylogenetic relationships of *G. duodenalis* isolates from Iran and other countries using the *tpi* and *gdh* genetic loci.

Methods: Sequences of *G. duodenalis* isolates were retrieved from GenBank, including samples from humans and animals in Iran, China, India, Thailand, and Japan. DNA was extracted from fecal samples, and the *tpi* and *gdh* genes were amplified using PCR in the original studies. A Neighbor-Joining phylogenetic tree was constructed using the p-distance model, with sequences from Beaver (AY178738) and Koala (AY178737) used as outgroups.

Results: The phylogenetic tree revealed two primary clusters. Iranian human isolates grouped closely with short branch lengths, indicating high genetic similarity. Chinese and Indian isolates formed distinct clusters, reflecting geographical divergence. Iranian dog and cattle isolates were located near human isolates, suggesting a potential for zoonotic transmission.

Conclusion: The clustering of Iranian isolates indicates genetic relatedness, highlighting the potential zoonotic transmission of *G. duodenalis*. The study suggests the need for further research with larger datasets to explore these genetic relationships and zoonotic risks in more detail.

Keywords: *Giardia duodenalis*, Phylogenetic tree, *tpi*, *gdh*, Zoonosis.



Epidemiological Analysis of Cutaneous Leishmaniasis in the Catchment Area of Mashhad University of Medical Sciences, Iran (2014-2024)

Zahra Nehbandani¹, Amir sadra Keyvani²

Corresponding Author: Zahra Nehbandani, Nehbandaniz2@mums.ac.ir

1. Head of CDC Mashhad University of Medical science, Mashhad, Iran.
2. Doctor of Veterinary Medicine Student, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.

Article code: 1648

Abstract

Background: Cutaneous leishmaniasis (CL) remains a significant public health challenge in Iran. Northeastern Iran, covered by Mashhad University of Medical Sciences (MUMS), is a known endemic focus. this study aimed to conduct a longterm detailed epidemiological analysis of all reported CL cases in the MUMS catchment area over a ten-year period (2014-2024) to inform control strategies. The specific objectives of this study were to describe the demographic and socio-economic profile of patients, identify temporal trends and high-risk spatial clusters, evaluate the role of travel history as a key risk factor, determine the distribution of disease types and the rate of household transmission, and assess the treatment outcomes for the most commonly administered therapy.

Methods: A retrospective analysis was conducted on all CL cases (n=12,442) recorded in the MUMS surveillance system from 2014 to 2024. Data on demographics, travel history, disease type, treatment, and household transmission were extracted and analyzed using descriptive statistics.

Results: The mean age of patients was 29.5 years, with the highest incidence in children aged 2-9 years. Incidence decreased with age and education. A significant majority (77%) had zoonotic (rural) form. Travel history was a major risk factor, with 74% of respondents having traveled in the past year, and 34% of them to rural areas. Household infections occurred in 18% of cases. Intralesional Meglumine Antimoniate (Glucantime) was the common treatment, with a 97% success rate. Spatial analysis identified toghabeh-shandiz as the highest-risk county, with an incidence rate of 1829 per 100,000 population.

Conclusion: The study highlights the high burden of zoonotic CL in the MUMS, strongly linked to travel to rural endemic foci. The high rate of household clustering suggests active peri-domestic transmission. These findings underscore the urgent need for targeted interventions in high-incidence areas like toghabeh-shandiz and public health awareness campaigns for travelers to rural regions.



3rd National Congress Of Animal Parasitic Diseases and Zoonoses

17-18 September 2025, Ferdowsi University of Mashhad



Keywords: Cutaneous Leishmaniasis, Zoonotic Leishmaniasis, Epidemiological Analysis, Household Transmission.



سومین کنگره ملی بیماری‌های انگلی دام⁹ بیماری‌های انگلی مشترک انسان و حیوان⁹



شرکت داروسازی نصر فریمان
NASR Pharmaceutical Co.



دانشگاه فردوسی مشهد
دانشکده دامپزشکی
شهریور ماه ۱۴۰۴



National Congress of
Animal Parasitic Diseases and Zoonoses

Ferdowsi University of Mashhad - September 2025