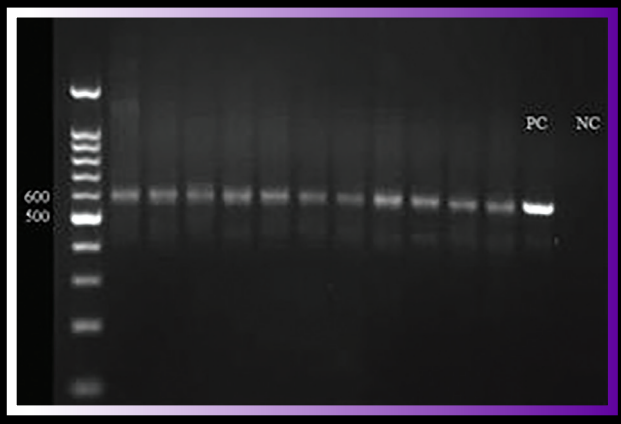
E-ISSN 2146-3077

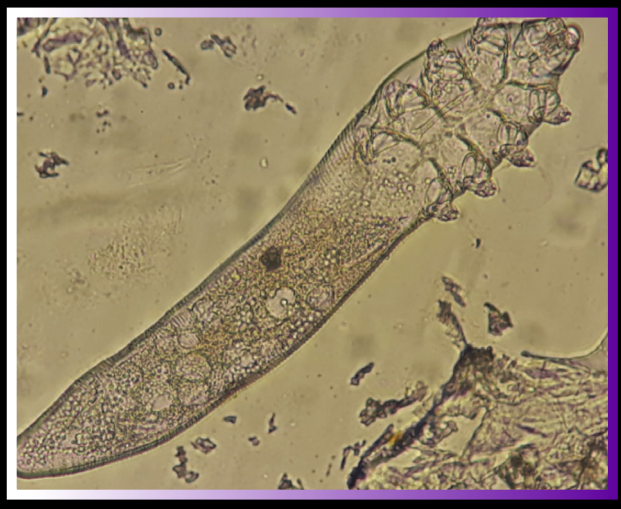
Cilt/Volume: 49
Sayı/Issue: 2
Haziran/June 2025

Türkiye PARAZİTOLOJİ Dergisi

TURKISH JOURNAL OF PARASITOLOGY

Özgün Araştırmalar / Original Investigations





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EDİTÖRDEN

2025 yılının ikinci sayısını 7 özgün araştırma makalesi ile çıkarmaktayız. Özgün araştırmalar arasında; İran'da yapılan *Leishmania major*'un genetik çeşitliliğini araştıran bir çalışma, kedilerdeki hepatozoonozun filogenetik karakterizasyonu, fascioliasis tanısını ele alan bir çalışma, Siirt ilimizdeki bağırsak parazitlerin durumunu ortaya koyan bir makale, pandemi sonrası *Demodex* enfestasyonlarındaki değişimi araştıran bir çalışma, kist hidatik ile ilgili tezlerin incelendiği bir çalışma ile *Lucilia serricata* larva salgılarının *Echinococcus granulosus* üzerine olan etkilerini irdeleyen bir makale yer almaktadır.

Dergimizin ESCI için de başvurusu yeniden yapılmış olup sonucu beklenmektedir. Bu sürece büyük katkısı olan ve gönderilen makalelere özveri ile hakemlik yapan, bu sayının sonunda da listesi yayınlanan akademisyenlerimize de teşekkür etmek ve minnetlerimi sunmak isterim.

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E-posta/E-mail: info@galenos.com.tr/ yayin@galenos.com.tr

Web: www.galenos.com.tr

Publisher Certificate Number: 14521

Online Publication Date: Temmuz / July 2025

E-ISSN: 2146-3077

Üç ayda bir yayımlanan süreli yayındır.
International scientific journal published quarterly.

Türkiye PARAZİTOLOJİ Dergisi

Türkiye PARAZİTOLOJİ Dergisi

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Journal is published online.

Owner: Yusuf Özbel on Behalf of Turkish Society of Parasitology

Responsible Manager: Yusuf Özbel

Contact

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İÇİNDEKİLER/CONTENTS

ÖZGÜN ARAŞTIRMALAR / ORIGINAL INVESTIGATIONS

Türkiye PARAZİTOLOJİ Dergisi

Original Investigation
Özgün Araştırma

Turkiye Parazitol Derg 2025;49(2):47-51
DOI: 10.4274/tpd.galenos.2025.92400

Genetic Diversity Analysis of *Cysteine Proteinase B* Gene in *Leishmania major* Isolates from Southwest Iran

İran'ın Güneybatısındaki Leishmania major İzolatlarında Sistein Proteinaz B Geninin Genetik Çeşitlilik Analizi

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Cite this article as: Rahdar M, Maghsoudi MR, Beiromvand M, Kardooni T. Genetic diversity analysis of *cysteine proteinase B* gene in *Leishmania major* isolates from southwest Iran. Turkiye Parazitol Derg. 2025;49(2):47-51.

ABSTRACT

Objective: Selecting an effective target for *Leishmania* vaccination requires identifying a protein antigen with low or no genetic diversity. The *cysteine proteinase B* (CPB) gene has emerged as a promising immunogenic target, though its diversity requires evaluation across different regions. Given Iran's status as a hyperendemic region for cutaneous leishmaniasis, this study aimed to collect and analyze 30 *Leishmania major* isolates from various areas of Khuzestan Province in Southwestern Iran.

Methods: The *CPB* gene was amplified via polymerase chain reaction, and its nucleotide sequence was determined. Sequence analysis was performed using MEGA5 software, with subsequent comparison to National Center for Biotechnology Information database entries. A phylogenetic tree was constructed to compare isolated strains with reference strains from other geographic regions and species.

Results: The phylogenetic analysis demonstrated that the *CPB* gene of the isolated strains in different regions of Khuzestan province formed a clade with certain *Leishmania major* strains from various parts of the world. Overall, the genotypic analysis of the *CPB* gene in Khuzestan province indicated genetic similarity among 29 isolates. In contrast, one sample from Dezful (north of Khuzestan Province) exhibited a significant difference from the reference strain, resulting in notable amino acid changes.

Conclusion: Based on these findings, the *CPB* gene holds promise as a potential candidate for vaccination development against cutaneous leishmaniasis in this region.

Keywords: *Leishmania major*, cysteine proteinase B, phylogeny

ÖZ

Amaç: *Leishmania* aşısı için etkili bir hedef seçimi, düşük veya sıfır genetik çeşitliliğe sahip bir protein antijenin belirlenmesini gerektirir. *Sistein proteinaz B (CPB)* geni, umut vaat eden bir immünojenik hedef olarak öne çıkmakla birlikte, çeşitliliğinin farklı bölgelerde değerlendirilmesi gerekmektedir. İran'ın kutanöz leishmaniasis için hiperendemik bir bölge olması nedeniyle, bu çalışma İran'ın Güneybatısındaki Huzistan Eyaleti'nin çeşitli bölgelerinden 30 *Leishmania major* izolatını toplamayı ve analiz etmeyi amaçlamıştır.

Yöntemler: *CPB* geni PCR yöntemiyle çoğaltıldı ve nükleotit dizisi belirlendi. Dizi analizi, MEGA5 yazılımı kullanılarak gerçekleştirildi ve sonrasında Ulusal Biyoteknoloji Bilgi Merkezi veritabanındaki dizilerle karşılaştırıldı. Coğrafi bölgeler ve türler arasındaki referans suşlarla izole edilen suşları karşılaştırmak üzere bir filogenetik ağaç oluşturuldu.

Bulgular: Filogenetik analiz, Huzistan Eyaleti'nin farklı bölgelerinden izole edilen suşların *CPB* geninin, dünyanın çeşitli bölgelerindeki *Leishmania major* suşlarıyla bir klad oluşturduğunu göstermiştir. Genotipik analizler, Huzistan Eyaleti'ndeki *CPB* geninin 29 izolat arasında genetik benzerlik sergilediğine işaret etmektedir. Buna karşılık, Dezful'dan (Huzistan Eyaleti'nin kuzeyi) elde edilen bir örnekte referans suşla belirgin farklılıklar gözlemlenmiş olup, bu durum amino asit düzeyinde kayda değer değişikliklerle sonuçlanmıştır.

Sonuç: Bu bulgulara dayanarak, *CPB* geninin bu bölgede kutanöz leishmaniasis'e karşı aşı adayı olarak potansiyel taşıdığı sonucuna varılmıştır.

Anahtar Kelimeler: *Leishmania major*, sistein proteinaz B, filogeni

Received/Geliş Tarihi: 23.10.2023 **Accepted/Kabul Tarihi:** 20.06.2025 **Epub:** 07.07.2025 **Publication Date/Yayınlanma Tarihi:** 18.07.2025

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Leishmania major, the causative agent of cutaneous leishmaniasis, places nearly 10% of the global population at risk of infection. Annually, approximately two million new leishmaniasis cases are reported worldwide (1), with Iran representing a key endemic region where the disease constitutes a significant public health burden. The World Health Organization has prioritized leishmaniasis as an emerging and uncontrolled disease, prompting extensive efforts to develop effective vaccines, including the exploration of numerous candidate proteins (2).

Among these, cysteine proteinase B (CPB) stands out due to its critical role in parasite immune evasion (3). This 36-kDa protein, encoded by a 12,939-*nucleotide* gene on chromosome 8, is expressed in both promastigote (flagellated) and amastigote (non-flagellated) life cycle stages of Leishmania. CPB facilitates disease pathogenesis and serves as a key antigenic target. Phylogenetically, three cysteine proteinase types A, B, and C have been identified in Leishmania, with CPA and CPB homologous to mammalian Cathepsin-B, and CPC resembling Cathepsin-L (4).

Notably, CPB exhibits marked interspecies variability. For instance, *Leishmania mexicana* harbors 19 tandemly repeated *CPB* gene copies, while Leishmania donovani and Leishmania infantum possess five and one copies, respectively (5). Functional studies reveal that CPB deficiency in *L. mexicana* disrupts glycoprotein 63 (GP63) expression and impairs phagolysosomal activity (6). Furthermore, Rafati et al. (6) demonstrated that natural CPB isoforms in *Leishmania major* (*L. major*) elicit immune responses in human lymphocytes, infected murine models, and cutaneous leishmaniasis patients. Genetic polymorphisms in CPB contribute to phenotypic and genotypic diversity across Leishmania strains, underscoring its evolutionary plasticity (7).

Despite these advances, no provincial-level analysis of CPB genetic diversity has been conducted in Khuzestan, a hyperendemic tropical region in Iran. This study addresses this gap by performing molecular and genotypic analyses of CPB in *L. major* isolates from diverse geographic locations within the province. Identifying structural homogeneity in CPB across isolates would support its candidacy for region-specific vaccine development, offering a targeted strategy to mitigate cutaneous leishmaniasis in this high-risk population.

METHODS

Ethical Approval and Sample Collection

This study received ethical clearance from the Medical Ethics Committee (IR.TUMS.MEDICINE.REC.1400.014). Thirty *L. major* isolates were obtained from cutaneous leishmaniasis patients across five geographic subdivisions of Khuzestan Province (north, south, east, west, and central; six samples per region). Promastigotes were cultured in biphasic NNN medium supplemented with RPMI 1640 until reaching stationary phase, followed by cryopreservation at -20 °C. Genomic DNA extraction was performed on 3×10^6 promastigotes using the Qiagen DNA Mini Kit (catalog no. 51304, South Korea), with PBS-washed parasites processed according to manufacturer protocols. DNA concentration was quantified spectrophotometrically at 260 nm.

Primary Polymerase Chain Reaction (PCR)

Primary PCR amplification targeting kinetoplast DNA minicircles (kDNA) was conducted to *differentiate L. major* from *L. tropica*, utilizing a Sinaclone PCR Kit (Shiraz, Iran). The species-specific 620-bp amplicon was generated using primers:

Forward: 5'-GGGGTTGGTGTAAAATAGGG-3'

Reverse: 5'-TTTGAACGGGATTTCTG-3'

Reactions (25 µL total volume) contained 12.5 µL master mix, 2.5 µL primer pair (10 pmol/µL), 5 µL template DNA, and 5 µL DNase-free water. Thermocycling parameters included: Initial denaturation: 95 °C for 3 min

35 cycles of:

Denaturation: 93 °C for 40 s

Annealing: 63 °C for 40 s

Extension: 72 °C for 1 min

Final extension: 72 °C for 5 min

Amplification products were resolved on 2% agarose gels stained with ethidium bromide (0.5 µg/mL) and visualized under ultraviole illumination.

CPB Proliferation

CPB gene was used to design primers amplification 1100 bp nucleotide band. Primers included:

Forward: 5'-ACGGTCTTAGCGTGCGAGTTGTG-3'

Reverse: 5'-TCGTGCAGCACATGTCGCTTG-3'

PCR reactions (25 µL) comprised 12.5 µL master mix, 1 µL each primer (10 pmol/µL), 5 µL DNA template, and 5.5 µL DNase-free water. Thermal cycling conditions were:

5 minutes at 95 °C for initial denaturation and 35 cycles including 94 °C for 1 min, 57 °C 30 seconds for annealing, 72 °C 1 minute for extension. The PCR process was ended at 72 °C 10 min for final extension. The electrophoresis of PCR products was performed using gel agarose 2% and ethidium bromide, and the bands appeared by gel documentation apparatus.

Phylogeny Analysis

Sequence alignments and phylogenetic trees were constructed using MEGA5 software. Evolutionary relationships were inferred via the Neighbor-joining algorithm with Kimura 2-parameter distance modeling. Topological robustness was evaluated through 1,000 bootstrap replicates, with clade stability thresholds set at ≥70%. Nucleotide substitution patterns were analyzed under a gamma-distributed rate variation model (shape parameter α=0.5).

Statistical Analysis

Statistical analysis was performed using SPSS software, version 22 (SPCC Inc., Chicago, IL, USA). Descriptive analysis was used to calculate the mean and standard deviation and parametric tests (ANOVA) and non-parametric tests (Mann-Whitney and Wilcoxon) were used. Significant differences were considered at a p-value of less than 0.05.

Demographic data

The study cohort comprised 30 cutaneous leishmaniasis patients, with a male-to-female ratio of 12:18 (40% vs. 60%). Age distribution revealed a predominance of cases in the 21-30-year demographic, though infections spanned ages 2 to 80 years. Cutaneous lesions localized predominantly to the hands (21/30 cases, 70%), followed by facial involvement (9/30, 30%). Four

patients exhibited lesions at multiple anatomical sites, and 50% of cases presented with more than one lesion.

Primary PCR

Primary PCR amplification targeting kDNA minicircles confirmed *L. major* as the sole causative agent across all isolates, evidenced by a species-specific 620-bp amplicon (Figure 1). Electrophoretic differentiation from *L. tropica*, which produces an 800-bp fragment under identical conditions, excluded coinfection or misclassification. Subsequent amplification of the *CPB* gene yielded a conserved ~1,170 bp product in all isolates (Figure 2), confirming target gene integrity across geographically distinct *L. major* strains from Khuzestan Province.

Sequencing Analysis

Sequencing of 10 representative isolates (GenBank accessions OM037016-OM037025) revealed minimal nucleotide divergence from the *L. major* Friedlin reference strain, with one notable exception: Isolate OM037023 exhibited 14 nonsynonymous substitutions, resulting in substantial amino acid alterations within the CPB catalytic domain (Figures 3, 4). Clinically, infections involving OM037023 correlated with atypical presentations, including larger lesion sizes (>5 cm diameter), multidermatomal spread, and reduced responsiveness to antimonial therapy. The clinical changes in this study may be due to the presence of *Leishmanis* RNA virus 2 (LRV2). Çulha et al. (8) in Hatay-Türkiye failed to show LRV2 in 20 isolates of *Leishmania* included 17 *L. tropica*, 2 *L. major* and 1 *L. infantum*. Mirabedini et al. (9) in Iran, out of four isolates of *L. major*, three samples were positive for LRV2. The changes mentioned are described in Table 1.

Using the neighbour-joining method, the phylogenetic analysis of the gene encoding CPB DNA sequence was performed in *L. major*, along with the rest strains available in Genebank. The sequence patterns of the *CPB* gene in *L. major* isolated from patients in this study were compared to those of species in GenBank, demonstrating that all isolates, except for isolate number 8, exhibit a high degree of similarity with all isolates and standard Friedlin strain (Figure 5).

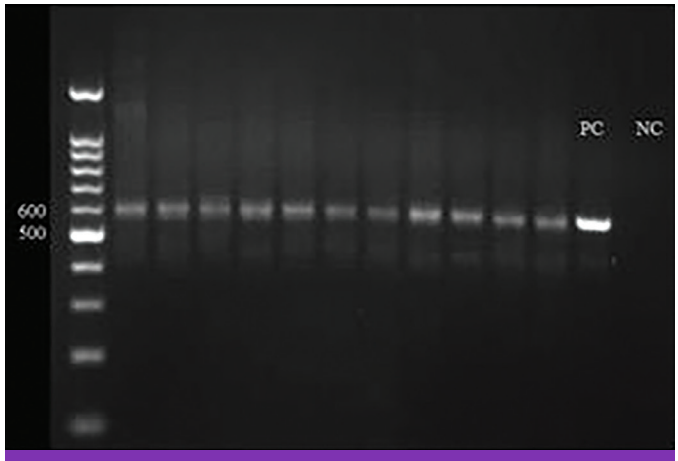

Figure 1. The electrophoresis of isolated *Leishmania major* show 620 bp nucleotides in PCR

PC: Positive control, NC: Negative control, PCR: Polymerase chain reaction

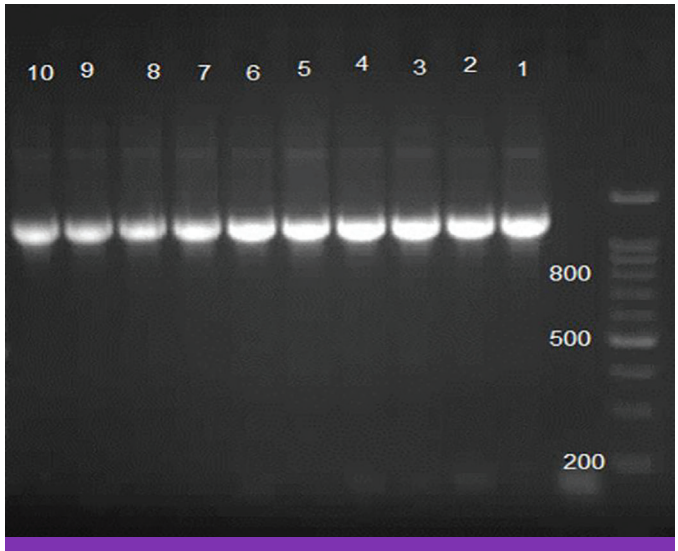

Figure 2. The electrophoresis of *cysteine proteinase B* gene PCR show a 1170 bp nucleotide in all isolated samples

PCR: Polymerase chain reaction

DISCUSSION

CPB constitutes a critical virulence factor in *Leishmania* spp., mediating parasite survival through differentiation roles in host cell invasion, immune evasion, and nutrient acquisition (10-12). Experimental validation of CPB's pathogenicity was demonstrated by Denise et al. (13), who observed attenuated cutaneous lesion

Figure 3. Nucleotide alignment of OM037023 isolate with Friedlin standard strain

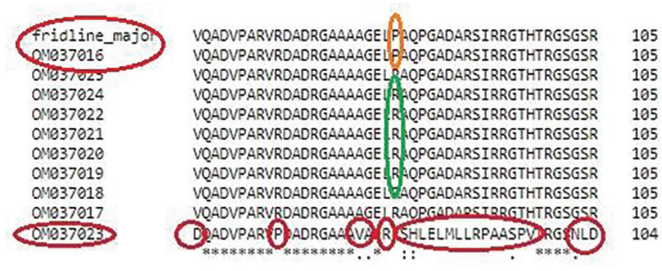

Figure 4. Amino acid alignment of OM037023 and other isolates with Friedlin standard strain

progression in BALB/c mice infected with CPB-deficient *L. mexicana* mutants. This phenotype correlated with enhanced Th1-polarized immunity (e.g., elevated IFN-γ), whereas *CPB* gene complementation restored lesion expansion and parasite proliferation via Th2 axis activation, evidenced by upregulated interleukin (IL)-4, immunoglobulin (Ig)G1, and IgE titers (13,14). Such immunological modulation underscores CPB's dual role as both a virulence effector and immunomodulator, motivating ongoing efforts to develop CPB-targeted chemotherapeutics and subunit vaccines (15,16).

Based on this, much attention has been paid to this enzyme for therapeutic purposes as well as vaccine production. Denise et al. (13) demonstrated that in Balb/C mice infected with Mexican *L. mexicana* strains with *CPB* gene disruption, disease severity was reduced, and induction of protective TH1 response led to weak expansion of cutaneous lesions. Reintroducing the *CPB* gene into the mutant strains increased lesion growth and parasite proliferation and led to TH2 axis activity, as evidenced by increased levels of IL4, IgG1, and IgE (13).

Prophylactic strategies leveraging CPB have shown promise in preclinical models. Doroud et al. (17) reported sustained protective immunity in BALB/c mice immunized with a CPB DNA vaccine adjuvanted with nanoparticles, manifesting as reduced lesion sizes and robust Th1 responses (IFN-γ/IL-5 ratio >3.2) compared to controls.

The existence of homogeneity with low diversity in the *CPB* gene in *Leishmania* species gives hope that the production of an immunogenic vaccine against the *CPB* gene can be used against all *Leishmania* species. Still, due to the molecular diversity of the CPB enzyme in different *Leishmania* species, it is hindered for this purpose. Gomes et al. (18) showed that the supply of *CPB* gene is different in various developmental stages of the parasite and identified stage-specific CPB expression dynamics, with promastigotes producing 1.5-fold higher protein levels than amastigotes, modulated by environmental pH and temperature fluctuation.

Saadati et al. (19) were able to isolate *L. donovani* from 3477 mosquitoes. Examining the *CPB* gene in this parasite species showed the difference between the genotypes of this gene in *L. donovani* and other species of *Leishmania*; therefore, they concluded that one type of vaccine could not be used to protect this parasite from other species. Mohammadpour et al. (20) in Fars province examined the *CPB* gene in 100 clinical samples of *Leishmania* cutaneous and showed many differences in the *CPB* gene sequence in clinical samples. Their results are different from the present study, which showed that only one of the 10 sequenced samples had genotypic and clinical differences with other isolates, proving the importance of making vaccines based on local species. The Chaouch et al. (5) study showed that the sequences of *L. tropica* and *L. major* have the least diversity, and intraspecies nucleotide polymorphisms largely lead to silent mutations, especially in the case of *L. major*. However, in *L. donovani,* there are many differences as a result of the deletion of a 39 nucleotide segment in the cysteine proteinase genotype (5).

In the present study, out of the ten samples examined in the nucleotide sequence, nine isolates were similar to the Friedline standard strain, except in a few areas that led to changes in the nucleotide sequence, and no differences were observed, and these changes did not lead to amino acid changes. Therefore, there was no change in the parasite's behaviour. Only in sample no. OM037023, significant nucleotide sequence alterations were

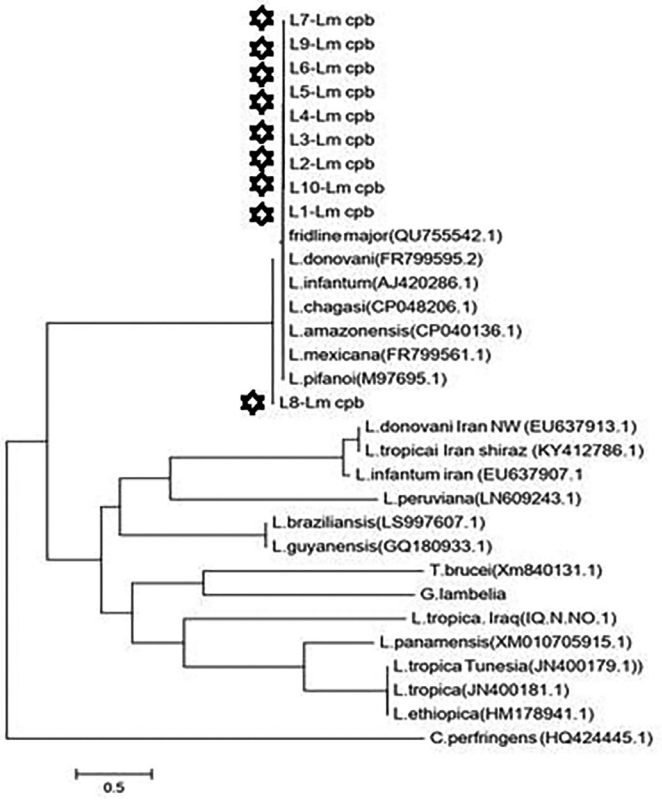

Figure 5. The phylogeny tree of studied isolates in comparison to other species of *Leishmania* in gene bank.*. Studied isolates

Table 1. Nucleotide and amino acid change in OM037023 isolate in comparison with Friedlin standard strain

Position in nucleotide sequence	Nucleotide		Amino acid	
	From	To	From	To
	G	C	Alanine	Proline
58	T	C		Stop codon
59	A	T		Stop codon
182	T	A	Valine	Asparagine
209	G	C	Arginine	Proline
236	C	T	Alanine	Valine
237	T	G	Alanine	Valine
	G	C	Glycine	Alanine
243	A	G	Without change	Without change
245	T	G	Leucine	Arginine
250	T	G	Alanine	Serine
257	C	T	Proline	Leucine
258	T	G	Proline	Leucine
260-272			Amino acids changed	
280-289			Amino acids changed	
307-318			Amino acids changed	

identified when compared to the Friedline reference strain and other samples isolates. These genetic modifications induced substantial amino acid substitutions, resulting in altered parasite pathogenicity manifested through increased lesion severity, prolonged disease duration, and reduced therapeutic efficacy. This divergence parallels reports of LRV2-associated hyperpathogenicity, though LRV2 detection protocols were not explicitly performed here. The observed genetic homologous in the *CPB* gene of *L. major* highlights its potential utility as a molecular target for region-specific vaccine development.

However, the outlier OM037023 underscores the necessity for pan-regional genomic surveillance to capture emergent virulent strains. Future studies should integrate proteomic profiling to quantify CPB expression variability across parasite life stages and assess cross-reactivity of CPB-directed antibodies against heterologous *Leishmania* species.

CONCLUSION

Vaccination in Leishmaniasis is the important and safty method for controlling the disease in endemic areas. To select of appropriated target for candidate vaccine need to identification and characterization of immunogenic and homologous gene or proteines. This study presented that *CPB* gene and protein are suitable target for designing an effective vaccine in this region. Further study is needed for evaluation LRV2 detection gene in isolated samples and increasing sample size for reaching to confidental results.

*Ethics

Ethics Committee Approval: This study received ethical clearance from the Medical Ethics Committee (IR.TUMS.MEDICINE.REC.1400.014).

Informed Consent: Informed consent was obtained from all patients to participate in this study, and this study received approval from the Research Ethics Committee.

Acknowledgments

This paper has been extracted from the M.Sc thesis of Mohammad Reza Maghsoudi and financially supported by Cellular and Molecular Research Center, Medical Basic Science Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran as grant number CMRC-0002.

Footnotes

*Authorship Contributions

Surgical and Medical Practices: M.R.M., M.B., Concept: M.R., Design: M.R., Data Collection or Processing: M.R.M., Analysis or Interpretation: T.K., Literature Search: M.R., Writing: M.R.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This manuscript is financially supported by Cellular and Molecular Research Center, Medical Basic Science Research Institute, as grant number CMRC-0002.

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Turkiye Parazitol Derg 2025;49(2):52-7
DOI: 10.4274/tpd.galenos.2025.88609

Emerging Insights into Feline Hepatozoonosis in Türkiye: Molecular Detection and Phylogenetic Characterization from the Aegean Coast

Türkiye'de Kedi Hepatozoonozuna İlişkin Ortaya Çıkan Görüşler: Ege Kıyılarından Moleküler Tespit ve Filogenetik Karakterizasyon

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Cite this article as: Hacılarlıoğlu S, Metin M. Emerging insights into feline hepatozoonosis in Türkiye: molecular detection and phylogenetic characterization from the Aegean coast. Turkiye Parazitol Derg. 2025;49(2):52-7.

ABSTRACT

Objective: The aim of this study is to investigate the presence, prevalence, and molecular characteristics of *Hepatozoon* spp. infection in domestic cats living in Aydın and İzmir provinces, located on the Aegean coast of Türkiye. The study specifically focuses on species identification and genotype distribution.

Methods: A total of 203 blood samples from domestic cats were analyzed for *Hepatozoon* spp. using polymerase chain reaction (PCR). Among the 67 PCR-positive samples, 10 (five for each province) were selected for sequencing, and bidirectional Sanger sequencing was performed. The obtained sequences were evaluated using basic local alignment search tool and phylogenetic analyses, and genotype identification was based on the *18S rRNA* gene region. Additionally, potential risk factors such as age, sex, health status and housing type were statistically analyzed for their association with *Hepatozoon* spp. infection.

Results: The overall prevalence of *Hepatozoon* spp. in cats was determined to be 33.0% (67/203). The infection rate was 43.69% in Aydın and 22% in İzmir, and this difference was found to be statistically significant (p=0.001). All 10 sequenced samples were identified as *Hepatozoon felis*, and all isolates belonged to Genotype I. A significant association was found between age and infection, with a particularly high infection rate of 44% observed in cats aged one year or younger (p=0.020).

Conclusion: This study revealed a high prevalence of *Hepatozoon* spp. infection among cats in western Türkiye, with all sequenced isolates identified as Genotype I. The notably high infection rate observed in young cats raises the possibility of transplacental transmission. These findings underscore the need for further investigations to clarify the transmission routes and risk factors associated with this protozoan parasite, the epidemiology of which remains insufficiently understood.

Keywords: Tick-borne disease, *Hepatozoon felis*, domestic cat, genotype, Türkiye

ÖZ

Amaç: Bu çalışmanın amacı, Türkiye'nin Ege kıyısında yer alan Aydın ve İzmir illerinde yaşayan evcil kedilerde *Hepatozoon* spp. enfeksiyonunun varlığını, yaygınlığını ve moleküler özelliklerini araştırmaktır. Çalışma özellikle tür tayini ve genotip dağılımına odaklanmıştır.

Yöntemler: Evcil kedilerden alınan toplam 203 kan örneği, *Hepatozoon* spp. yönünden polimeraz zincir reaksiyon (PZR) yöntemiyle analiz edilmiştir. PZR ile pozitif bulunan 67 örnekten her iki ilden beşer adet olmak üzere toplam 10 örnek dizileme için seçilmiştir ve çift yönlü Sanger dizileme yöntemi uygulanmıştır. Elde edilen diziler temel yerel hizalama arama aracı ve filogenetik analizlerle değerlendirilmiştir; genotip tayini *18S rRNA* gen bölgesine dayalı olarak gerçekleştirilmiştir. Ayrıca, yaş, cinsiyet, sağlık durumu ve barınma şekli gibi potansiyel risk faktörlerinin *Hepatozoon* spp. enfeksiyonu ile ilişkisi istatistiksel olarak analiz edilmiştir.

Bulgular: Kedilerde *Hepatozoon* spp. enfeksiyonunun genel prevalansı %33,0 (67/203) olarak belirlenmiştir. Aydın ilindeki enfeksiyon oranı %43,69, İzmir'de ise %22 olarak bulunmuştur; bu fark istatistiksel olarak anlamlı kabul edilmiştir (p=0,001). Dizilenen 10 örneğin tamamı *Hepatozoon felis* olarak tanımlanmıştır ve tüm izolatların Genotip I'e ait olduğu belirlenmiştir. Enfeksiyon ile yaş arasında anlamlı bir ilişki saptanmıştır, özellikle bir yaş ve altı kedilerde enfeksiyon oranı %44 olarak bulunmuştur (p=0,020).

Sonuç: Bu çalışma, Batı Türkiye'de kedilerde *Hepatozoon* spp. enfeksiyonunun yüksek prevalansa sahip olduğunu ve dizilenen tüm örneklerin Genotip I'e ait olduğunu ortaya koymuştur. Özellikle genç kedilerde gözlemlenen yüksek enfeksiyon oranı, transplasental bulaş ihtimalini gündeme getirmektedir. Bu bulgular, henüz tam olarak aydınlatılamamış olan bu protozoon parazitin bulaş yolları ve risk faktörlerinin daha iyi anlaşılması için ileri çalışmalara duyulan ihtiyacı vurgulamaktadır.

Anahtar Kelimeler: Kene kaynaklı hastalık, *Hepatozoon felis*, evcil kedi, genotip, Türkiye

Received/Geliş Tarihi: 03.06.2025 **Accepted/Kabul Tarihi:** 05.07.2025 **Publication Date/Yayınlanma Tarihi:** 18.07.2025

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Hepatozoonosis is a disease caused by *Hepatozoon* species which are apicomplexan protozoan parasites belonging to the family Hepatozoidae. These parasites infect mammals, birds, reptiles, and amphibians and are transmitted by various arthropod vectors. The transmission of *Hepatozoon* species occurs through ingestion of infected invertebrates, such as ticks, mites, mosquitoes, or fleas, which act as definitive hosts (1,2). Although *Hepatozoon* infection in dogs is well documented, data on feline hepatozoonosis remain limited (1). The prevalence of infection has been found to vary between 0.6% and 83.3% in various regions depending on the lifestyle of the cats, the geographical area, the diagnostic method used, and the type of samples tested (3). It has been noted that infection in cats is predominantly subclinical, with the potential for exacerbation in animals with compromised immune systems or co-infections (4,5).

Three different *Hepatozoon* species have been reported in felids so far, namely *Hepatozoon felis* (*H. felis*), *Hepatozoon canis* and *Hepatozoon silvestris* (6). Among them, *H. felis* is the earliest identified and most widespread species, affecting both wild and domestic felids worldwide (7). This species, which is the primary causative agent of feline hepatozoonosis, a disease of veterinary importance, was first detected in domestic cats in India (8). Although *H. canis* infections have only been described sporadically in domestic and wild felids, *H. felis* infections are subclinical without a significant local inflammatory response (5,7).

It has been hypothesized by some researchers that different species or genotypes may possess distinct biological characteristics and pathogenic potential (5,9). However, the most prevalent *H. felis* variant in the world is believed to be a species complex comprising multiple variants, including a genotype on a global scale. Furthermore, it has been hypothesized that multiple genotypes or species may circulate among cats in the same country or a more limited area (3,10). Phylogenetic analyses of *H. felis*, the most prevalent species infecting cats, utilizing partial *18S rRNA* gene sequences, have identified two distinct genotypes: The more common genotype I and genotype II, characterized by a lower prevalence (11). Also, (12) reported a high genetic diversity with the existence of two different genotypes of *H. felis* as a result of *18S rRNA* gene sequence analysis. Additionally, Panda et al. (12) reported high genetic diversity, identifying two distinct genotypes of *H. felis* based on *18S rRNA* gene sequence analysis.

In a recent study, *H. silvestris* was identified as a new species infecting cats. Subsequently, this species has been detected in several European wild and domestic cats, including those in Türkiye (6,9,13,14). In contrast to the typically subclinical nature of *H. felis*, *H. silvestris* has been implicated as the causative agent of severe clinical conditions such as fatal myocarditis and intussusception suggesting a potentially higher virulence to domestic cats (15,16).

The majority of research on *Hepatozoon* infections in Türkiye has focused on canine hosts and tick vectors, and comprehensive study data on cats are quite limited (14,17-27). In particular, information regarding the prevalence, species and genotype distribution, as well as potential risk factors such as age, sex, health status, and housing conditions, is notably scarce. Addressing these gaps is essential for elucidating transmission dynamics and developing effective preventive veterinary strategies. The current findings emphasize the need for further research to better understand the epidemiology of *Hepatozoon* spp. infections in cats. Accordingly, the present study was conducted to investigate the presence, prevalence, molecular characteristics, and phylogenetic structure of *Hepatozoon* species in domestic cats from Aydın and İzmir provinces, located on the Aegean coast of Türkiye, while also evaluating potential risk factors associated with infection.

METHODS

Ethically Approval

The study was performed under the instructions and approval of the Institutional Animal Ethics Committee of Aydın Adnan Menderes University (protocol no: 64583101/2024/019, date: 18/01/2024). Animal owners participating in the study were informed about the purpose and procedures of the research, and written informed consent was obtained before blood sample collection.

Blood Samples

In this study, a total of 203 blood samples were used from domestic cats as part of the MSc thesis titled *"Investigation of the Prevalence of Ehrlichia Species in Cats Living in İzmir and Aydın Provinces"*. These samples were collected from cats brought to veterinary hospitals or clinics in the Aydın and İzmir provinces, located in the Aegean coast of Türkiye, between 2024 and 2025. The age of the animals was recorded at the time of sampling and categorized into three groups: One year or younger, 1-4 years, 4 and over 4 years. Blood samples were collected in tubes containing ethylene diamine tetraacetic acid (EDTA) (BD Vacutainer®, Franklin Lakes, NJ, USA) for polymerase chain reaction (PCR) analysis and were stored at -20 °C until further processing.

Genomic DNA Extraction, PCR Amplification and Sequencing

Genomic DNA was extracted from the collected blood samples (200 µL) using the Invitrogen Genomic DNA Extraction kit (Invitrogen by Life Technologies, Thermo Fisher Scientific, USA), following the manufacturer's protocol. A total of 200 µL of each blood sample was used for DNA extraction. Amplification of an approximately 600 bp fragment of the *18S rRNA* gene of *Hepatozoon* spp. was performed using the primer pair HepF300: 5′-GTT TCT GAC CTA TCA GCT TTC GAC G-3′ and Hep900: 5′-CAA ATC TAA GAA TTC ACC TCT GAC-3′, following the protocol described by Ujvari et al. (28). PCR reactions were carried out using a Techne TC-512 thermocycler (Techne, UK), in a final volume of 50 µL, containing 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 0.25 U of VitaTaq DNA polymerase, 0.5 µM of each primer, and 2 µL of DNA template. The amplification process included an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min. A positive and a negative control were included in all PCR reactions. The positive control consisted of DNA extracted from a naturally infected dog, while double-distilled water was used as the negative control. A total of 10 µL of each PCR product was electrophoresed on a 1.5% agarose gel containing SafeView™ nucleic acid stain (10 µL/mL; ABM Inc., Richmond, Canada) in Tris-acetate-EDTA buffer. Electrophoresis was conducted using a horizontal gel system at 120 V for 1 hour. For species identification, PCR products from *Hepatozoon*-positive

samples were sequenced bidirectionally using the same primers through Sanger sequencing at Atlas Biotechnology (Ankara, Türkiye). However, due to financial constraints, only ten positive samples (five from each province) were selected for sequencing. The resulting sequences were aligned and assembled into consensus sequences using Geneious Prime 2025.1.2 software (https://www.geneious.com). These consensus sequences were subsequently compared with reference sequences in the GenBank database using the basic local alignment search tool (BLAST) algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The resulting sequence data were submitted to the GenBank database under the following accession numbers: PV765406- PV765415.

Phylogenetic Analysis

A phylogenetic tree was constructed using the online tool available at https://www.phylogeny.fr. The reference sequences retrieved from the NCBI GenBank database (https://www.ncbi.nlm.nih.gov) AY628681.1, KC138533.1, and LC179794.1 (*H. felis*); KY649446.1 and KC138531.2 (*H. canis*); and AF130361.1 (*H. catesbianae*) (11); OQ862293-OQ862310 (22); OM214003-OM214012 (14) were used in the analysis. GenBank accession number, host and country of origin were included in the phylogenetic tree.

Statistical Analysis

All statistical analyses were performed using the SPSS software package (IBM SPSS for Windows, version 25). Associations between potential risk factors and the presence of infection were assessed using the chi-square (χ^2) test. A p-value less than 0.05 was considered statistically significant.

RESULTS

Demographic Characteristics and Molecular Prevalence of *Hepatozoon* spp.

A total of 203 domestic cats were included in this study, comprising 100 from İzmir and 103 from Aydın provinces. Of these, 129 were female and 74 were male. Based on age distribution, 75 cats were younger than 1 year, 118 were between 1 and 4 years old, and 10 were older than 4 years. Regarding housing conditions, the majority were outdoor cats (n=161), followed by cats with both indoor and outdoor access (n=12), while only 30 were strictly indoor cats. Importantly, all animals included in the study had no prior history of antiparasitic treatment.

Among the total 203 blood samples screened, 67 (33.0%) were found to be positive for *Hepatozoon* spp. by PCR. Region-specific prevalence rates were 22.0% (22/100) in İzmir and 43.6% (45/103) in Aydın.

Sequence and Phylogenetic Analysis

Phylogenetic analysis of *Hepatozoon*-positive samples was conducted based on the *18S rRNA* gene sequences obtained in this study, along with reference sequences retrieved from the GenBank database. Both BLAST results and phylogenetic tree reconstruction confirmed that all positive samples belonged to *H.* felis (Figure 1). Nucleotide sequence analysis of the *H. felis 18S rRNA* gene revealed a sequence similarity ranging from 98.58% to 100%. Moreover, the *H. felis* sequences obtained in this study clustered with previously reported sequences from Türkiye (11,22), which have been classified as *H. felis* genotype I.

Risk Factor Analysis

Out of 203 cats examined, 67 (33.00%) were positive for *Hepatozoon* spp. by PCR. Statistical analysis revealed a significant association between infection and age (p=0.020), with the highest positivity observed in cats ≤1 year (44.00%) (Table 1). A significant difference was also found between provinces, with higher prevalence in Aydın (43.6%) compared to İzmir (22.00%) (p=0.001) (Table 1). No significant associations were detected for sex (p=0.288), housing type (p=0.363), or clinical status (p=0.625), although slight differences in infection rates were noted across categories (Table 1). These findings suggest that younger age and geographic location may act as potential risk factors for *Hepatozoon* spp. infection in the studied cat population, while sex, housing conditions, and clinical status showed no statistically significant associations.

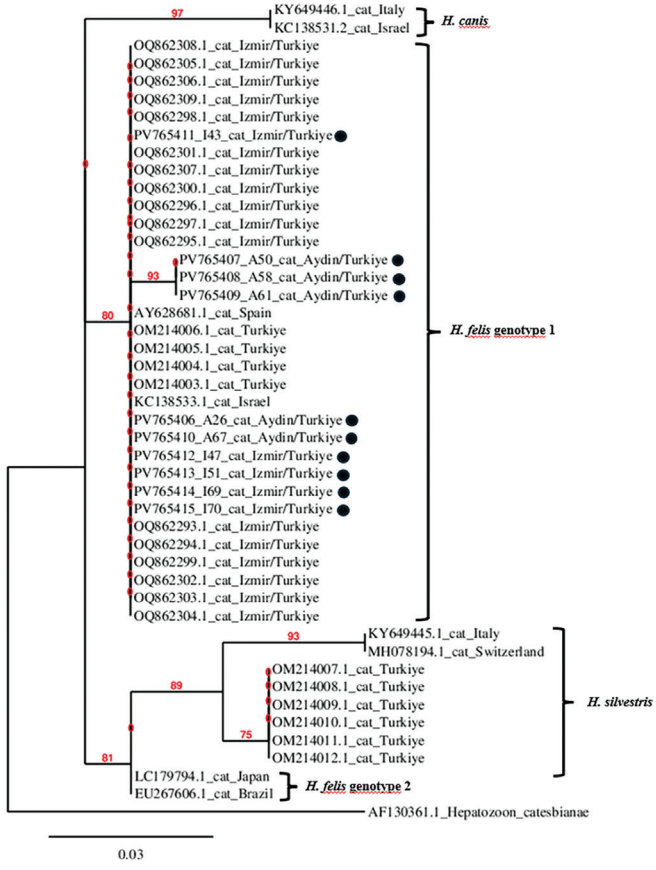

Figure 1. Phylogenetic tree based on the *18S rRNA* gene sequences of *Hepatozoon felis* detected in cats from Türkiye, along with representative sequences of *H. felis*, *H. canis*, and *H. silvestris* obtained from felids. Additionally, all *H. felis* and *H. silvestris* isolates previously reported from Türkiye and available in GenBank were included in the analysis. *H. catesbianae* was used as the outgroup. Molecular evolutionary genetic analysis was performed using the online platform available at https://www.phylogeny.fr. The phylogenetic tree includes information on the host species, country of origin, and GenBank accession number for each sequence. Black circles indicates the *H. felis* sequences obtained in the present study from cats in İzmir (coded with "i") and Aydın (coded with "A"), Türkiye

Table 1. Association between *Hepatozoon* spp. infection and potential risk factors in cats

Variable		Total (n)	PCR positive (%)	p-value
Age	≤1 year	75	33 (44.00)	0.020*
	1-4 years	118	33 (27.97)	
	>4 years	10	1 (10.00)	
Sex	Female	129	46 (35.66)	0.288
	Male	74	21 (28.38)	
Housing type	Indoor	30	11 (36.67)	0.363
	Indoor-outdoor	12	6 (50.00)	
	Outdoor	161	50 (31.06)	
Clinical status**	Viral, bacterial, or parasitic infections	34	10 (29.41)	0.625
	Unknown	169	57 (33.73)	
Province	İzmir	100	22 (22.00)	0.001*
	Aydın	103	45 (43.69)	

*: Indicates p-values considered as statistically significant (<0.05) based on the chi-square test, **: Indicates clinical status refers to 34 of the 203 cats examined that were diagnosed with other infectious diseases, including 30 viral infections (Feline Leukemia Virus, Feline Herpesvirus Type 1, Feline Calicivirus, Feline Infectious Peritonitis, and Feline Immunodeficiency Virus); one parasitic infection (*Toxocara cati*); and three bacterial infections of unknown etiology, PCR: Polymerase chain reaction

DISCUSSION

This study primarily aimed to investigate the presence and molecular characterization of *Hepatozoon* spp. in domestic cats from two provinces in the Aegean coast of Türkiye, with a particular focus on species identification and genotype distribution. Of the 203 cats tested, 33.0% (67/203) were found to be positive for *Hepatozoon* spp. by PCR analysis. This notably high prevalence highlights the widespread presence of this protozoan parasite in feline populations in the western part of Türkiye. Among the 67 *Hepatozoon*-positive cats, 10 samples were successfully sequenced. Subsequent BLAST and phylogenetic analyses confirmed that all sequenced samples belonged to *H. felis* (Figure 1).

Previous studies have demonstrated the presence of *Hepatozoon* spp. infection in cats in Türkiye, with varying prevalence rates reported across different regions. The prevalence observed in this study (33.0%) is considerably higher than those reported in previous molecular studies conducted in other regions of Türkiye, which documented infection rates of 2.37%, 10.8%, and 18.64% (14,22,23). In a study conducted on 1012 stray cats from İzmir province, which is located in the same region as the current study (23%), reported a much lower prevalence of *H. felis* (2.37%) compared to our findings. Notably, the samples used in that study were collected approximately a decade earlier than those analyzed in the present investigation. It is possible that ecological changes in recent years such as global warming, rapid urban population growth, and the increased transcontinental movement of companion animals have contributed to a rise in the transmission rates of vector-borne pathogens worldwide (29), potentially influencing the current distribution of *Hepatozoon* spp. infections.

The prevalence of *Hepatozoon* spp. detected in Aydın (43.69%) was significantly higher than that observed in İzmir (22%) (p=0.001). A review of the existing literature reveals that the prevalence of *Hepatozoon* spp. infection in cats shows considerable variation across different geographical regions worldwide, with reported rates ranging wide 0.6-83.3% (3,5,10,11,30-33). The findings of the present study are in agreement with this broad global variability in prevalence rates. Although the samples in both provinces were selected in equal numbers and from cats with similar characteristics, specifically stray cats without a history of antiparasitic treatment, the results varied. This discrepancy may be attributed to the fact that some of the sampling areas in Aydın shifted from urban to rural settings.

Although biological and epidemiological knowledge about *H. felis* remains limited, *Rhipicephalus sanguineus* is considered one of its main potential vectors (34). The ingestion of ticks during grooming behavior in cats may also contribute to the transmission of *Hepatozoon* species (2). In the present study, the previously reported presence of tick species belonging to the genus *Rhipicephalus* in the study area (35) supports the infection rates observed.

In the current study, all sequenced samples were identified as *H. felis*. These findings are consistent with previous molecular investigations conducted in Türkiye, particularly in İzmir and Tekirdağ, where *H. felis* was the only *Hepatozoon* species detected in cats, with prevalence rates of 2.37% and 10.8%, respectively (22,23). However, a recently published study from the northern part of Türkiye reported the presence of both *H. felis* and *H. silvestris* in feline hosts (14). Additionally, a case report from Aydın documented the detection of *H. canis* in a cat by both microscopic examination and PCR analysis; however, sequence analysis was not performed in the study (26).

The taxonomic status of *Hepatozoon* spp. infecting felids is not fully established (10). *H. felis* has been identified as the predominant species infecting cat populations worldwide (7). Due to the high genetic variability observed across various studies, *H. felis* has been proposed to represent a species complex (36). To date, phylogenetic analysis based on 18S rRNA sequences revealed that two distinct genotypes of *H. felis* genotypes I and II have been reported in domestic cats (11). Among these, genotype I is recognized as the most common genotype infecting domestic cats. Pereira et al. (11) reported that *H. felis* genotype I has been detected in cats from Europe, Asia, Africa, and South America. Phylogenetic analysis in this study revealed that all sequenced isolates clustered within *H. felis* Genotype I. Similarly, studies conducted in Türkiye have also identified *H. felis* genotype I

(14,22), supporting the notion that this genotype is the most prevalent one in the country.

In a study conducted by Attipa et al. (31), non-healthy cats were found to have a threefold higher risk of *Hepatozoon* spp. infection compared to healthy individuals (p=0.010); however, the authors noted that the observed health problems might not be directly caused by hepatozoonosis and could instead result from coinfections with other pathogens. In the present study, no statistically significant association was found between *Hepatozoon* spp. infection and the presence of viral, bacterial, or other parasitic agents, possibly due to the limited number of infected animals (34 out of 203). Additionally, in line with previous findings (2,14,31), no significant association with sex was observed.

To date, studies have not identified a significant association between age and *Hepatozoon* infection in cats (2,7,14,31). However, in the present study, a statistically significant difference was observed in cats aged one year or younger (p=0.020). Similarly, Attipa et al. (31) reported that 21% of kittens under six months of age tested positive for *Hepatozoon* spp. In line with these findings, the present study found a *Hepatozoon* spp. positivity rate of 44% among cats aged one year or younger, which was statistically significant. When comparing overall age distributions, it appears that differences in age group classifications among studies may lead to inconsistent results. Nonetheless, the notably high infection rates detected in young cats in this study are of particular importance. These findings raise the possibility of vertical (transplacental) transmission from queen to offspring. Indeed, studies on *H. canis* in dogs have demonstrated that transplacental transmission is possible (37,38). Vertical transmission is an important adaptation in many parasites that enables the persistence of infection within a population.

Previous studies have reported a significant association between infection and outdoor access (7,14,31). In the present study, a statistically significant difference was initially detected in cats reported to live indoors. However, interviews conducted with cat owners revealed that these animals had frequent contact with other animals that had access to outdoor environments. Upon reevaluation of the living conditions, it became evident that these cats were indirectly exposed to external sources. These findings support the vector–host transmission route and indicate that animals living outdoors or in contact with the outdoor environment are at increased risk of infection.

The present study provides valuable insights into the current epidemiological status of *Hepatozoon* spp. infections in domestic cats from two provinces located in the Aegean cost of Türkiye. Molecular analyses confirmed that *H. felis* is the predominant species circulating in these areas, particularly among free-roaming cats.

Hepatozoon spp. infections in cats appear to be largely asymptomatic or present with occasional subclinical manifestations, which may contribute to underdiagnosis in routine clinical practice (7). Given the relatively high prevalence of *Hepatozoon* spp. observed in present study and its widespread distribution among free-roaming feline populations (22,33), this parasitic infection warrants further attention.

CONCLUSION

In conclusion, this study provides up-to-date epidemiological data on feline hepatozoonosis in western Türkiye and confirms *H. felis* genotype I as the predominant species. Raising awareness among veterinarians and incorporating *Hepatozoon* spp. into routine diagnostic panels is essential, particularly in areas with high stray cat populations. Further molecular surveillance and larger-scale studies are needed to better understand transmission dynamics and potential risk factors.

*Ethics

Ethics Committee Approval: The study was performed under the instructions and approval of the Institutional Animal Ethics Committee of Aydın Adnan Menderes University (protocol no: 64583101/2024/019, date: 18/01/2024).

Informed Consent: Animal owners participating in the study were informed about the purpose and procedures of the research, and written informed consent was obtained before blood sample collection.

Acknowledgments

The authors would like to thank Yusuf Darçın for their valuable contributions to the study.

Footnotes

*Authorship Contributions

Concept: S.H., M.P., Design: S.H., M.P., Data Collection or Processing: S.H., M.P., Analysis or Interpretation: S.H., M.P., Literature Search: S.H., M.P., Writing: S.H., M.P.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This research was partially supported by Aydın Adnan Menderes University Scientific Research Projects Coordination Unit with the code ADU-BAP VTF-24002.

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Turkiye Parazitol Derg 2025;49(2):58-62
DOI: 10.4274/tpd.galenos.2025.42650

Fasciola hepatica Diagnosis: Clinical and Laboratory Clues from A University Hospital Experience

Fasciola hepatica Tanısında Klinik ve Laboratuvar İpuçları: Bir Üniversite Hastanesi Deneyimi

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Cite this article as: Sağmak Tartar A, Aşan MA, Bozdağ A, Bahçecioğlu İH, Akbulut A, Demirdağ K. *Fasciola hepatica* diagnosis: clinical and laboratory clues from a university hospital experience. Turkiye Parazitol Derg. 2025;49(2):58-62.

ABSTRACT

Objective: Fascioliasis is a trematode infection caused by *Fasciola hepatica* or *Fasciola gigantica*. Diagnosis of fascioliasis is often delayed. This study aims to contribute to reducing the incidence of the disease by determining regional epidemiology and guiding public health measures, as well as increasing awareness among physicians through the examination of clinical, laboratory, and imaging findings.

Methods: Patients diagnosed with fascioliasis at the Infectious Diseases Clinic and Gastroenterology Clinic of Fırat University Hospital between 2011 and 2022 were included in the study. Demographic information, clinical findings, complete blood count, biochemical parameters, radiological imaging reports, treatment, and prognosis were examined. Patient data were obtained from the hospital automation system, files, and epicrisis.

Results: Of the 19 patients followed, 15 (78.9%) were female. The mean age was 62.36±12.30 years. Fifteen patients (78.9%) lived in rural areas. Seven patients (36.8%) were involved in animal husbandry. Twelve patients (63.2%) had a history of consuming watercress. The most commonly observed symptoms were loss of appetite, right upper quadrant pain, nausea-vomiting, and night sweats. All patients were treated with triclabendazole without any side effects. Statistically significant differences were found in the levels of eosinophilia, alanine transaminase, gamma-glutamyl transferase, alkaline phosphatase, total bilirubin, and direct bilirubin between the beginning of treatment and 1 month in our patients ($p<0.05$). One patient was lost due to intervening cardiac problems.

Conclusion: Fascioliasis is also present in our region, and a significant history of watercress consumption is noteworthy epidemiologically. *Fasciola hepatica* should be considered in patients presenting with abdominal pain accompanied by peripheral eosinophilia. Increasing awareness among physicians will contribute to preventing delays in diagnosis and treatment.

Keywords: Fascioliasis, *Fasciola hepatica*, eosinophilia, watercress

ÖZ

Amaç: Fascioliasis, *Fasciola hepatica* veya *Fasciola gigantica*'nın neden olduğu trematod enfeksiyonudur. Fasiyoliyazis tanısında sıklıkla gecikme olur. Bu çalışmada bölgesel olarak epidemiyolojinin belirlenmesi ile, alınacak halk sağlığı önlemlerine yön vererek hastalık insidansının azaltılmasına katkı sağlanması, klinik, laboratuvar ve görüntüleme bulgularının irdelenmesiyle ise hekimler arasında farkındalığın artırılması hedeflenmektedir.

Yöntemler: Çalışmaya 2011-2022 tarihleri arasında Fırat Üniversitesi Hastanesi Enfeksiyon Hastalıkları ve Gastroenteroloji Kliniği'nde fascioliyazis tanısı ile takip edilen hastalar dahil edildi. Demografik bilgileri, klinik bulguları, tam kan sayımı, biyokimyasal parametreler, radyolojik görüntüleme raporları, tedavi ve prognozları incelendi. Hasta bilgilerine hastane otomasyon sistemi, dosya ve epikrizlerden ulaşıldı.

Bulgular: Takip edilen 19 hastanın 15'i (%78,9) kadındı. Yaş ortalaması 62,36±12,30 olarak bulundu. Hastaların 15'i (%78,9) kırsal bölgede yaşamaktaydı. Yedi (%36,8) hasta hayvancılıkla uğraşmaktaydı. On iki (%63,2) hastanın su teresi yeme öyküsü

Received/Geliş Tarihi: 02.04.2024 **Accepted/Kabul Tarihi:** 20.06.2025 **Epub:** 03.07.2025 **Publication Date/Yayınlanma Tarihi:** 18.07.2025

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Türkiye PARAZİTOLOJİ Dergisi

vardı. En sık saptanan semptom ve bulgu iştahsızlık, sağ üst kadranda ağrı, bulantı-kusma ve gece terlemesiydi. Hastaların hepsine triklobendazol başlandı. Yan etki görülmedi. Hastalarımızın tedavi başlangıcı ve 1 ayda bakılan eozinofili, alanin transaminaz, gama-glutamil transferaz, alkalen fosfataz, toplam bilirubin ve direkt bilirubin değerlerinde istatistiksel anlamlı fark saptandı (p<0,05). Bir hasta araya giren kardiyak sorunları sebebiyle kaybedildi.

Sonuç: Fascioliasis bölgemizde de görülmektedir ve epidemiyolojik olarak önemli oranda su teresi tüketme öyküsü dikkat çekmektedir. Periferik eozinofilinin eşlik ettiği karın ağrısı ile başvuran hastalarda *Fasciola hepatica* düşünülmelidir. Hekimlerde farkındalığın artması tanı ve tedavideki gecikmeleri önlemeye katkı sağlayacaktır.

Anahtar Kelimeler: Fascioliasis, *Fasciola hepatica*, eozinofili, su teresi

INTRODUCTION

Fascioliasis is a trematode infection caused by *Fasciola hepatica* (*F. hepatica*) or *Fasciola gigantica* (*F. gigantica*) (1). *F. hepatica* has a global distribution, while *F. gigantica* is predominantly seen in tropical regions.

F. hepatica is globally distributed, especially in sheep farming areas with temperate climates. Humans are accidental hosts and mainly acquire infection by consuming watercress or aquatic plants. Infection can also occur through other freshwater plants such as lettuce, mint, clover, and parsley. Outbreaks resulting from the consumption of contaminated water containing live metacercariae (2) have been described (3). Most infected individuals have mild clinical manifestations; morbidity increases with the fluke burden (4). Abdominal pain accompanied by peripheral eosinophilia should raise suspicion of fascioliasis. A careful dietary history, including consumption of watercress or potentially contaminated water-washed raw vegetables, should be obtained. Diagnosis of fascioliasis is often delayed. In a study of patients with fascioliasis in developed countries, acute infection-related symptoms occurred more than 1 month before diagnosis in 73% of cases (5). Another study involving patients with biliary phase fascioliasis found even longer delays (symptoms occurred 1 to 208 weeks; mean 64 weeks) (6). In this study, clinical symptoms and findings, laboratory and radiological imaging findings, treatment, and prognosis of patients followed with fascioliasis diagnosis at the infectious diseases and gastroenterology clinic between 2011 and 2021 were retrospectively examined. Determining regional epidemiology will contribute to reducing the incidence of the disease by guiding public health measures, while examining clinical, laboratory, and imaging findings aims to increase awareness among physicians.

METHODS

This study was conducted in accordance with the principles of the Helsinki Declaration. Ethical approval for the study was obtained from the Non-Interventional Ethics Committee of Fırat University Faculty of Medicine (date: 21.01.2022, number: 6284). Patients diagnosed with fascioliasis at the Infectious Diseases Clinic and Gastroenterology Clinic of Fırat University Hospital between 2011 and 2022 were included in the study. Patient consent was not obtained because the study was retrospective. Demographic information, clinical findings, complete blood count, biochemical parameters, radiological imaging reports, treatment, and prognosis were examined. Patient data were obtained from the hospital automation system, files, and discharge summaries.

Statistical Analysis

For statistical analysis, the IBM SPSS Statistics 22 version package program (SPSS Inc., Chicago, IL, USA) was utilized. The normality of quantitative data was examined using the Shapiro-Wilk test. Mean ± standard deviation was employed for continuous variables exhibiting normal distribution, while median (+25th-75th) was used for non-normally distributed data. Classified data were analyzed in terms of frequency and percentage. Due to the non-normal distribution of the data, the Wilcoxon signed-rank test, a non-parametric test, was used for comparisons between dependent groups. A p-value <0.05 was considered statistically significant.

RESULTS

Nineteen patients diagnosed with fascioliasis were followed between 2011 and 2022. Of these patients, 15 (78.9%) were female, and 4 (21.1%) were male. The mean age was determined to be 62.36±12.30 years. Fifteen patients (78.9%) resided in rural areas. Seven patients (36.8%) were involved in animal husbandry. Twelve patients (63.2%) had a history of consuming watercress. In one patient (5.3%), there was a history of contact with lake-stream water. Extrahepatic involvement was not detected in any of the patients. Hepatic involvement was present in 11 patients (57.9%), while biliary involvement was present in 8 patients (42.1%). The number of flukes was unknown in 11 patients (57.9%), while single fluke was detected in 5 patients (26.3%), 2 flukes in 2 patients (10.5%), and 7 flukes in 1 patient (5.3%).

The symptoms and findings of the patients are provided in Table 1. Various laboratory parameters at the time of admission and at the 1st month of treatment are presented in Table 2.

Table 1. Patients' symptoms and findings

Symptom	n	(%)
Night sweats	7	36.8
Nausea-vomiting	13	68.4
Anorexia	19	100
Diarrhea	1	5.3
Weight loss	4	21.1
Myalgia	1	5.3
Bloating	1	5.3
Cough	1	5.3
Right upper quadrant pain	17	89.5
Jaundice	5	26.3
Hepatomegaly	4	21.1
Cholelithiasis	5	26.3
Urticaria	1	5.3

Table 2. Various laboratory parameters detected at the time of admission and at 1st month of

Parameter	Median (interquartile range) at admission	Median (interquartile range) at 1st month	p-value
WBC (mm^3)	9.51 (6.61-13.23)	6.27 (4.66-8.26)	0.117
Eosinophil (mm^3)	9.6 (2.3-44)	5.3 (2.4-9.4)	0.01*
Hemoglobin (g/dL)	13 (11.4-14.1)	12.1 (11.6-13.1)	0.610
Platelet (mm^3)	275,000 (223,000-339,000)	265 (239-321)	0.530
Alanine aminotransferase (U/L)	34 (27-160)	25 (17.5-37)	0.019*
Aspartate aminotransferase (U/L)	28 (23-91)	23 (18-28.5)	0.066
Alkaline phosphatase (U/L)	165 (96-232)	95 (75-103)	0.028*
Gamma-glutamyl transferase (U/L)	79 (47-194)	26 (20.5-41.5)	0.011*
Total bilirubin	0.7 (0.4-2.3)	0.4 (0.35-0.65)	0.017*
Direct bilirubin	0.2 (0.1-1.5)	0.1 (0.1-0.15)	0.011*

*: $p<0.05$ was considered statistically significant, WBC: White blood cell

No parasite eggs were detected in the stool of any patient. Imaging findings were obtained form 12 patients. Magnetic resonance imaging (MRI) was used for 7 patients (58.3%), computed tomography (CT) for 4 patients (21.1%), and no significant findings were found in one patient (8.3%). Eight patients (42.1%) were diagnosed with endoscopic retrograde cholangiopancreatography (ERCP), and 2 patients (10.5%) were diagnosed with biopsy. One patient underwent serological testing, which yielded a positive result.

All patients were initiated on triclabendazole. No side effects were observed. One patient was lost due to intervening cardiac problems. Eighteen patients were discharged with recovery.

DISCUSSION

F. hepatica is endemic in Central and South America (especially Bolivia and Peru), Europe (especially Portugal, France, Spain, and Türkiye), Asia (especially China, Vietnam, Taiwan, Korea, and Thailand), Africa, and the Middle East. Globally, 180 million people are at risk (6,7). In Türkiye, the incidence rates of cases vary according to regional distribution. The actual number of fascioliasis cases in our country is likely higher than the detected case numbers due to the asymptomatic course of cases, difficulties in considering the disease, especially in rural areas, and delays in diagnosis. In previous studies published in our province, the prevalence of fasciola hepatica immunoglobulin G positivity was found to be 2.78% (8).

Sheep and cattle are the most important definitive hosts of *F. hepatica*; goats, buffaloes, horses, donkeys, camels, pigs, deer, and rabbits can also be infected. Snails are intermediate hosts. Humans are accidental hosts and usually acquire infection by ingesting watercress or water chestnut grown in sheep farming areas. Infection can also be transmitted by other freshwater plants such as lettuce, mint, clover, and parsley. Humans can acquire infection by ingesting contaminated water containing live metacercariae. Outbreaks have been described to develop in this way (9,10). The incidence of animal and human infection increases in rainy years due to the increase in snail numbers and the longer survival of encysted cercariae (11). In our patients, a history of watercress consumption was present in 12 (63.2%) patients, and a history of animal husbandry was present in 7 patients. It can be said that watercress is the most significant item in the epidemiological history in our region. A careful dietary history, including the consumption of raw vegetables washed in contaminated water, should be obtained.

In endemic areas, the likelihood of infection in very young children and women is higher. Studies conducted in our country also emphasize female dominance (12). Sheep, cattle, or humans acquire infection by ingesting vegetation containing metacercariae. Metacercariae exit the duodenum and migrate to the bile ducts through the intestinal wall, peritoneal cavity, and liver parenchyma, where they mature into adults. In humans, this takes three to four months. Adult flukes are found in the larger bile ducts of mammalian hosts. Adult flukes can partially obstruct the bile ducts and cause thickening, dilation, and fibrosis of the proximal bile duct (13).

Liver damage is associated with the parasite burden. The number of adult flukes reaching the biliary tree is usually low. The estimated lifespan of adult *F. hepatica* flukes in humans is 9 to 13 years. Many infections are mild; morbidity increases with fluke burden (14). Among the forms of infection are the acute (hepatic) phase, chronic (biliary) phase, ectopic fascioliasis, and pharyngeal fascioliasis. In this study, while the number of flukes was unknown in 11 (57.9%) patients, single fluke was detected in 5 (26.3%) patients, 2 flukes in 2 (10.5%) patients, and 7 flukes in 1 (5.3%) patient. Extrahepatic involvement was not detected in any of the patients. Hepatic involvement was present in 11 (57.9%) patients, while biliary involvement was present in 8 (42.1%) patients.

Symptoms in the acute (hepatic) phase generally begin 6 to 12 weeks after ingesting metacercariae. The early phase of hepatic migration is usually associated with fever, right upper quadrant pain, and hepatomegaly. Jaundice is occasionally observed. Other symptoms include anorexia, nausea, vomiting, weight loss, myalgia, cough, and urticaria. Prominent peripheral eosinophilia is almost always present. The acute phase can be complicated by hemobilia or subcapsular hepatic hematomas (9). In most cases, acute symptoms typically resolve about six weeks later, but widespread hepatic parenchymal necrosis can occur in very severe infections.

Extrahepatic symptoms can also occur in the acute phase, possibly due to an immunological or allergic mechanism (11). A Loeffler-like syndrome or right-sided pleural effusion

containing eosinophils may be observed (6,15). Urticaria may be associated with itching and dermatographia (16). Pericarditis, cardiac conduction abnormalities, meningeal symptoms, focal neurological changes, or seizures have also been described but are rare.

The chronic phase (biliary phase) typically begins approximately six months after the primary infection and may persist for around ten years or even longer. Although often asymptomatic, patients may present with epigastric and right upper quadrant pain, diarrhea, nausea, vomiting, weight loss, and hepatomegaly. It may cause biliary obstruction, leading to complications such as gallstone formation, cholangitis, obstructive jaundice, and, through obstruction of the pancreatic duct, recurrent episodes of pancreatitis. Prolonged and/or severe infection may also result in sclerosing cholangitis and biliary cirrhosis. Peripheral eosinophilia may or may not be present during the biliary phase (5).

Ectopic fascioliasis remains unknown whether extrahepatic involvement in ectopic fascioliasis results from hematogenous dissemination of the parasites or their migration through soft tissues. Ectopic fascioliasis leads to eosinophilic and mononuclear infiltration, accompanied by secondary tissue damage. The most frequently affected site is the subcutaneous tissue of the abdominal wall. Other organs that may also be involved include the lungs, heart, brain, muscles, genitourinary system, skin, and eyes.

Pharyngeal fascioliasis is a form of fascioliasis observed in the Middle East, typically transmitted through the consumption of undercooked viscera—particularly liver—from infected animals. The live parasite may attach to the upper respiratory tract or proximal segments of the gastrointestinal tract, leading to allergic pharyngitis characterized by edema and obstruction. In severe cases, airway compromise and asphyxiation may occur.

In this study, the most commonly detected symptoms were anorexia, right upper quadrant pain, and nausea-vomiting. Five of the cases were evaluated as being in the chronic phase, presenting with cholangitic findings. The symptoms and clinical features of the remaining patients were consistent with the hepatic phase. Similar to our study, abdominal pain and anorexia were the most frequent symptoms in the study by Binici.

Fascioliasis should be considered in patients presenting with abdominal pain and hepatomegaly accompanied by peripheral eosinophilia.

Diagnosis can be established by identifying eggs in stool, duodenal aspirates, or bile samples. Eggs may not be detected in feces during the acute phase of infection or in ectopic fascioliasis. Since egg shedding may be intermittent, examination of multiple specimens may be required; negative stool examinations do not exclude the diagnosis. In this study, no parasite eggs were detected in the stool of any patient.

The diagnosis may be made during surgery or endoscopy for biliary obstruction when adult flukes are found in the biliary tree. On ERCP, adult flukes may appear as small, radiolucent linear, elliptical, or crescent-like shadows, with jagged, irregular margins in the gallbladder or dilated bile ducts. Laparoscopy may also demonstrate nodules in the liver capsule.

Serology generally becomes positive early in the hepatic migration phase; therefore, it is useful for diagnosis before eggs appear in the stool. It is also useful in ectopic diseases when eggs are not detected in the stool. Serological tests include indirect hemagglutination, complement fixation, counterimmunoelectrophoresis, immunofluorescent tests, and enzyme-linked immunosorbent assay. The sensitivity of these tests is good, but their specificity is not optimal. Cross-reactions occur with other parasitic infections. In this study, serological testing was performed in one patient, and it was positive.

Real-time polymerase chain reaction on stool is emerging as an alternative diagnostic technique with high sensitivity, but is not yet commercially available.

Useful radiographic tools for fascioliasis include CT, ultrasonography, cholangiography, ERCP, and MRI (17). Ultrasonography, cholangiography, and ERCP are useful in the biliary phase of infection (18).

The treatment of fascioliasis consists of antihelminthic therapy. Additional intervention may be required depending on the nature of the clinical picture. The preferred treatment is triclabendazole; bithionol and nitazoxanide are alternative options (19,20). Triclabendazole is an imidazole derivative. It is effective against all stages of fascioliasis with a cure rate of over 90% (21). The dosage consists of orally administering 10 mg/kg for one or two days. The drug is relatively well tolerated; absorption is preferably increased by postprandial administration following a fatty meal.

The mechanism of resistance of triclabendazole against Fasciola is not known (22). Treatment failures have been reported in humans and may be due to resistance; however, some studies have shown improvement after repeated triclabendazole doses (23-25).

Triclabendazole prolongs the QTc interval. The primary complication of treatment is biliary obstruction due to dead parasites, which may occur three to seven days after treatment and may require removal by ERCP. In this study, no side effects or complications related to triclabendazole were observed in any of the patients.

Post-treatment follow-up should include resolution of eosinophilia, clearance of eggs in stool, and a decrease in serology titers. However, difficulties in detecting eggs in stool and accessing serological tests limit the role of these parameters in follow-up. Resolution of biliary tract findings on ultrasound after treatment may also be beneficial (26).

CONCLUSION

In conclusion, Fascioliasis is the most widely distributed vector-borne parasitic disease known, with the widest latitude, longitude, and altitude distribution. It is also seen in our region, and a significant history of watercress consumption epidemiologically stands out. *F. hepatica* should be considered in patients presenting with abdominal pain accompanied by peripheral eosinophilia. Increasing awareness among physicians will contribute to preventing delays in diagnosis and treatment.

*Ethics

Ethics Committee Approval: This study was conducted in accordance with the principles of the Helsinki Declaration. Ethical approval for the study was obtained from the Non-Interventional Ethics Committee of Fırat University Faculty of Medicine (date: 21.01.2022, number: 6284).

Informed Consent: Patient consent was not obtained because the study was retrospective.

Footnotes

*Authorship Contributions

Surgical and Medical Practices: A.S.T., M.A.A., A.B., İ.H.B., A.A., K.D., Concept: A.S.T., İ.H.B., K.D., Design: A.S.T., A.A., Data Collection or Processing: A.S.T., M.A.A., Analysis or Interpretation: A.S.T., M.A.A., A.B., İ.H.B., A.A., K.D., Literature Search: A.S.T., M.A.A., A.B., Writing: A.S.T., M.A.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Turkiye Parazitol Derg 2025;49(2):63-7
DOI: 10.4274/tpd.galenos.2025.97659

Frequency of Intestinal Parasites in Patients Admitted to the Microbiology Laboratory of Siirt Training and Research Hospital

Siirt Eğitim ve Araştırma Hastanesi Mikrobiyoloji Laboratuvarına Başvuran Hastalarda Bağırsak Parazitlerinin Sıklığı

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Cite this article as: Gündüz L, Aydemir S, Taş Cengiz Z, Yılmaz H. Frequency of intestinal parasites in patients admitted to the microbiology laboratory of Siirt Training and Research Hospital. Turkiye Parazitol Derg. 2025;49(2):63-7.

ABSTRACT

Objective: The aim of this study was to determine the frequency of intestinal parasites in patients admitted to the Microbiology Laboratory of Siirt Training and Research Hospital.
Methods: The study was conducted between 19.04.2021 and 30.11.2021 with a total of 300 patients (150 children and 150 adults) between the ages of 1-90 years who were referred to the Microbiology Laboratory with a request for stool sample analysis and who admitted to Siirt Training and Research Hospital with different complaints. The samples were evaluated by nativ-Lugol, formol-ethyl acetate concentration and modified acid-fast staining methods. The samples in which *Entamoeba* spp. eggs were detected were evaluated for *Entamoeba histolytica/Entamoeba dispar* antigen using *Entamoeba* antigen cassette test.
Results: In this study, one or more than one type of intestinal parasite was found in 21.3% of 150 pediatric patients, 24% of 150 adult patients and 22.7% of 300 patients. The highest rate was *Blastocystis* (18%) and the lowest rate was *Ascaris lumbricoides* (0.7%). Although intestinal parasites were found at a higher rate in adults (24%) compared to pediatric age group (21.3%), there was no significant difference between the age groups in terms of parasite frequency in the statistical evaluation. There was a statistically significant difference between diarrhea (p=0.022) and anorexia (p=0.014) and intestinal parasite positivity.
Conclusion: It was concluded that it would be appropriate to evaluate patients admitted to hospitals with complaints such as diarrhea and loss of appetite in terms of intestinal parasites. Although this study gives an idea about the prevalence of intestinal parasites in Siirt Region, there is a need for larger scale studies in the region including more people.
Keywords: Intestinal parasites, prevalence, Siirt

ÖZ

Amaç: Bu çalışmanın amacı, Siirt Eğitim ve Araştırma Hastanesi Mikrobiyoloji Laboratuvarına başvuran hastalarda bağırsak parazitlerinin sıklığını belirlemektir.
Yöntemler: Çalışmaya, 19.04.2021-30.11.2021 tarihleri arasında, Siirt Eğitim ve Araştırma Hastanesi'ne farklı şikayetlerle başvurup dışkı istemiyle mikrobiyoloji laboratuvarına yönlendirilen ve 1-90 yaş aralığında olan toplam 300 hasta (150 çocuk ve 150 erişkin) dahil edildi. Örnekler nativ-Lugol, formol-etil asetat konsantrasyon ve modifiye asit-fast boyama yöntemi ile değerlendirildi. *Entamoeba* spp. yumurtası saptanan örnekler *Entamoeba* antijen kaset testi kullanılarak *Entamoeba histolytica/Entamoeba dispar* antijeni yönünden değerlendirildi.
Bulgular: Bu çalışmada dışkı örnekleri incelenen 150 çocuk hastanın %21,3'ünde, 150 erişkin hastanın %24'ünde, toplam 300 hastanın %22,7'sinde bir ya da birden fazla türde bağırsak parazitine rastlandı. En yüksek oranda *Blastocystis* (%18), en düşük oranda *Ascaris lumbricoides*'e (%0,7) rastlandı. Erişkinlerde (%24), çocuk yaş grubuna (%21,3) göre daha yüksek oranda intestinal parazitlere rastlansa da yapılan istatistiksel değerlendirmede parazit sıklığı bakımından yaş grupları arasında anlamlı bir fark belirlenmedi. Hastalarda görülen semptomlardan ishal (p=0,022) ve iştahsızlık (p=0,014) ile intestinal parazit pozitifliği arasında istatistiksel olarak anlamlı fark belirlendi.
Sonuç: Özellikle ishal ve iştahsızlık gibi şikayetler ile hastanelere başvuran hastaların intestinal parazitler yönünden de değerlendirilmesinin uygun olacağı kanaatine varılmıştır. Bu çalışma Siirt Bölgesi'nde intestinal parazit sıklığı ile ilgili olarak bir fikir vermekle beraber, yörede çok daha fazla kişinin dahil edileceği, daha büyük çapta çalışmalara ihtiyaç vardır.
Anahtar Kelimeler: İntestinal parazitler, prevalans, Siirt

Received/Geliş Tarihi: 08.03.2024 **Accepted/Kabul Tarihi:** 30.05.2025 **Epub:** 25.06.2025 **Publication Date/Yayınlanma Tarihi:** 18.07.2025

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Intestinal parasites are broadly classified into protozoa and helminths. Among the pathogenic intestinal protozoan infections, *Cryptosporidium* spp., *Giardia intestinalis* and *Entamoeba histolytica* are of major public health importance (1). *Blastocystis* is the most common intestinal protozoan parasite in humans (2). Among intestinal helminths, especially soil-transmitted helminths such as *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale* and *Necator americanus* are in the category of neglected tropical diseases. (3). *Enterobius vermicularis* is the most common intestinal helminth in middle- and high-income countries, especially in school-age children (4).

Intestinal parasite infections can be asymptomatic or cause clinical findings such as malnutrition, malabsorption, anemia, growth retardation, learning disabilities and diarrhea (5). According to the global health estimates of the World Health Organization, diarrheal diseases, including intestinal parasitic diseases, rank eighth among the causes of mortality (3). The problems caused by these infections depend on factors such as the type of parasite, the severity of the infection, and the socio-economic characteristics of societies (5).

Intestinal parasite infections affect more than two billion people worldwide, mainly in low- and middle-income countries (6). There are differences in the prevalence, species distribution and spread dynamics of intestinal parasites between countries and regions (3). The prevalence of intestinal parasites in a community is associated with factors such as inadequate infrastructure and clean water supply, malnutrition, lack of hygiene, high population density, illiteracy, political instability, civil unrest and climate changes (7). It is also predicted that there may be an increase in the prevalence of parasitic diseases in developed countries due to many reasons such as climate change and mass migration (1).

The aim of this study was to determine the frequency of intestinal parasites in patients admitted to the Microbiology Laboratory of Siirt Training and Research Hospital.

METHODS

The study was conducted between 19.04.2021 and 30.11.2021 with a total of 300 patients (150 children and 150 adults) between the ages of 1-90 years who applied to Siirt Training and Research Hospital with different complaints and were referred to the microbiology laboratory with a request for stool sample analysis. For the study, the leftover stool samples collected from the patients were used. The samples were analyzed in the microbiology laboratory of the hospital where they were collected and in the Parasitology Research Laboratory of Van Yüzüncü Yıl University Faculty of Medicine.

Non-Interventional Clinical Research Ethics Committee of Van Yüzüncü Yıl University approved the study protocol (decision number: 2021/05-20, date: 16.04.2021).

Stool samples were examined within one hour after collection. After macroscopic examination, the samples were evaluated by nativ-Lugol, formol-ethyl acetate concentration and modified acid-fast staining methods. The preparations prepared by nativ-Lugol method were examined at X100 and X400 magnification and the stained preparations were examined at X1000 magnification under a light microscope (8). If the number of *Blastocystis* forms in each microscope field was 5 or more, "abundant *Blastocystis*" was considered (9). The samples in which *Entamoeba* spp. eggs were detected were evaluated for *E. histolytica/Entamoeba dispar* antigen using *Entamoeba* antigen cassette test (True Line, China). The collected stool samples were stored in the refrigerator at +4 °C.

Statistical Analysis

Z (t) test and Fisher's exact test were used to compare the rates for categorical variables. In addition, chi-square test was performed to determine the relationship between categorical variables. Statistical significance level was taken as 5% and SPSS (ver:26) statistical package program was used for calculations.

Table 1. Parasite positivity according to age groups

Parasite type	Child (1-18 age) (N=150)	Adult (19-90 age) (N=150)	Total (N=300)	p
	Positive n (%)	Positive n (%)	Positive n (%)	
Blastocystis (≤4)	19 (12.7)	12 (8.0)	31 (10.3)	0.183
Abundant Blastocystis (≥5)	6 (4.0)	17 (11.3)	23 (7.7)	0.016
Blastocystis (all positive)	25 (16.7)	29 (19.3)	54 (18)	0.548
*E. histolytica/E. dispar**	3 (2.6)	3 (9.4)	6 (4.1)	0.99
E. coli	2 (1.3)	7 (4.7)	9 (3.0)	0.089
G. intestinalis	2 (1.3)	4 (2.7)	6 (2.0)	0.409
H. nana	2 (1.3)	2 (1.3)	4 (1.3)	0.99
A. lumbricoides	-	2 (1.3)	2 (0.7)	0.498
Total	32 (21.3)	36 (24)	68 (22.7)	0.581

N: Total number of patients, n: Number of positive patients, p: Significance value, *: by *Entamoeba* antigen cassette test, N: 146 (114 children, 32 adults)

RESULTS

In this study, one or more than one type of intestinal parasite was found in 32 (21.3%) of 150 pediatric patients, 36 (24%) of 150 adult patients and 68 (22.7%) of a total of 300 patients. Six different parasite species were detected in 68 positive patients. In our study, *Blastocystis* (18%) and *A. lumbricoides* (0.7%) were detected at the highest and lowest rates, respectively, and *E. histolytica/E. dispar* (4.1%), *G. intestinalis* (2%), *E. coli* (3%) and *Hymenolepis nana* (1.3%) were also found (Table 1).

Of the 68 positive patients, eight (11.8%) had two species and two (2.9%) had three species of parasites. Although multiple intestinal parasites were found at a higher rate in adults (5.3%) than in the paediatric age group (1.3%), there was no significant difference in the frequency of multiple parasites between the age groups on statistical analysis (Table 2).

Although intestinal parasites were found at a higher rate in adults (24%) compared to pediatric age group (21.3%), there was no significant difference between the age groups in terms of parasite frequency in the statistical evaluation. When the age groups were compared in terms of parasite positivity, there was a statistically significant difference only in the frequency of abundant *Blastocystis* (p=0.016; Table 1).

In our study, parasites were found in 20.7% of females and 24.5% of males and no statistically significant relationship was found between gender and parasite frequency (Table 3). There was a statistically significant difference between diarrhea (p=0.022) and anorexia (p=0.014) among the symptoms observed in our patients and intestinal parasite positivity (Table 4).

DISCUSSION

Siirt province, located in the Southeast of Türkiye, is one of the provinces with the lowest socio-economic development level in Türkiye (10). In 2022, in the socio-economic development ranking survey of the districts conducted by the Ministry of Industry and Technology, it was determined that five of the seven districts of Siirt were among the least developed districts (11). Since socio-economic development affects the prevalence of intestinal parasites, a high prevalence of intestinal parasites in Siirt is an expected result. In this study, 22.7% intestinal parasites were detected. This rate is not representative of the prevalence in Siirt province. However, since the sample of the studies on the prevalence of parasites in provinces in Türkiye generally consists of patients admitted to hospitals, the 22.7% rate found in this study helps to compare the prevalence of parasites in Siirt province with other provinces. In studies investigating the prevalence of parasites in patients admitted to hospitals in different provinces in Türkiye, it was determined that the rate was 3.6% in Ankara (5); 2.96% in İstanbul (12), 2020), 6% in İzmir (13); 12.3% in Mardin (14), 16.8% in Niğde, 10.8% in Sivas, 11.43% in Van [pre Coronavirus disease-2019 (COVID-19) + COVID-19 process] (7). When the studies are compared, it is seen that the prevalence of parasites in Siirt province is higher than in other provinces.

G. intestinalis and *E. histolytica* are the most common protozoa causing gastroenteritis worldwide. *Blastocystis*, another intestinal protozoan, is currently the most common parasite in the gastrointestinal tract of humans and its pathogenicity is controversial, as reported in numerous epidemiologic studies (2). In this study, *Blastocystis* was found to be the most common intestinal parasite in Siirt Region.

Table 2. Distribution of multiple parasitism by age groups

Parasite types	Child (1-18 age) (n=150)	Adult (19-90 age) (n=150)
E. histolytica/E. dispar + *Blastocystis*	-	2
G. intestinalis + *Blastocystis*	1	1
E. coli + *Blastocystis*	1	2
A. lumbricoides + *Blastocystis*		1
G. intestinalis + *H. nana* + *E. coli*		1
G. intestinalis + *Blastocystis* + *E. coli*		1
Total*	2 (1.3%)	8 (5.3%)

*: According to Fisher's exact test, the difference between the two groups was not significant (p=0.130)

Table 3. Parasite positivity according to gender

Parasite type	Female (N=145) n (%)	Male (N=155) n (%)	Total (N=300) n (%)	p
Blastocystis (≤4)	10 (6.9)	21 (13.5)	31 (10.3)	0.055
Abundant Blastocystis (≥5)	14 (9.7)	9 (5.8)	23 (7.7)	0.213
Blastocystis (all positive)	24 (16.6)	30 (19.4)	54 (18.0)	0.527
*E. histolytica/E. dispar**	3 (4.2)	3 (4.1)	6 (4.1)	0.934
E. coli	5 (3.4)	4 (2.6)	9 (3.0)	0.661
G. intestinalis	3 (2.1)	3 (1.9)	6 (2.0)	0.934
H. nana	3 (2.1)	1 (0.6)	4 (1.3)	0.290
A. lumbricoides	2 (1.4)	-	2 (0.7)	0.233
Total	30 (20.7)	38 (24.5)	68 (22.7)	0.428

N: Total number of patients, n: Number of positive patients, p: Significance value, *: by *Entamoeba* antigen cassette test, N: 146 (72 females, 74 males)

Table 4. Parasite positivity according to patients' symptoms

Symptoms	Features	Intestinal parasite		p
		Positive number (%) n=68	Negative number (%) n=232	
Abdominal pain	Yes (n=152)	33 (21.7)	119 (78.3)	0.689
	No (n=148)	35 (23.6)	113 (76.4)	
Diarrhea	Yes (n=94)	29 (30.9)	65 (69.1)	0.022
	No (n=206)	39 (18.9)	167 (81.1)	
Constipation	Yes (n=5)	1 (20.0)	4 (80.0)	0.886
	No (n=295)	67 (22.7)	228 (77.3)	
Nausea	Yes (n=37)	6 (16.2)	31 (83.8)	0.317
	No (n=263)	62 (23.6)	201 (76.4)	
Vomiting	Yes (n=7)	1 (14.3)	6 (85.7)	0.592
	No (n=293)	67 (22.9)	226 (77.1)	
Loss of appetite	Yes (n=27)	1 (3.7)	26 (96.3)	0.014
	No (n=273)	67 (24.5)	206 (75.5)	
Weight loss	Yes (n=4)	2 (50.0)	2 (50.0)	0.222
	No (n=296)	66 (22.3)	230 (77.7)	
Developmental delay	Yes (n=9)	2 (22.2)	7 (77.8)	0.974
	No (n=291)	66 (22.7)	225 (77.3)	
Fever	Yes (n=14)	3 (21.4)	11 (78.6)	0.910
	No (n=286)	65 (22.7)	221 (77.3)	

With advancing age, the frequency of intestinal parasites generally decreases due to factors such as increased hygiene awareness and the development of a certain degree of immunity against some parasites. In the studies (15-17) in which this criterion was considered, it was found that the prevalence of intestinal parasites generally decreased with increasing age. However, the prevalence of some parasites such as *Blastocystis* was reported to be higher in adults than in children (18,19). In this study, parasite positivity was higher in people older than 18 years (24%) than in those younger than 18 years (21.3%), but there was no statistically significant relationship between the age groups in terms of the frequency of parasite detection.

The most common symptoms in intestinal parasitosis are gastrointestinal symptoms such as diarrhea, nausea/vomiting and abdominal pain. In a study, it was reported that 63.2% of patients infected with intestinal parasites were symptomatic and a significant association was found between intestinal parasites and diarrhea, abdominal pain and fever (20). In another study, a statistically significant difference was found between abdominal pain and nausea and the incidence of intestinal parasites (21). In this study, a statistically significant difference was found between diarrhea and anorexia and the incidence of intestinal parasites.

CONCLUSION

In this study, intestinal parasites were found at a rate of 22.7% and pathogenic parasites other than *Blastocystis* were found at low rates. The reason for this may be that both physicians in primary health care institutions provide symptomatic treatment to patients and people pay more attention to hygiene rules due to the COVID-19 pandemic process. As a result, it was concluded that it would be appropriate to evaluate patients admitted to hospitals with complaints such as diarrhea and loss of appetite in terms of intestinal parasites. Although this study gives an idea about the prevalence of intestinal parasites in Siirt Region, there is a need for larger-scale studies in the region, including many more people.

The protozoan and helminth parasites detected in this study are usually transmitted via fecal-oral route. Therefore, it is of great importance to comply with hygiene rules sufficiently. Considering that both the drinking water and the foodstuffs consumed may contain infective forms of parasites, the use of safe water sources and taking care to wash foodstuffs thoroughly or cooking them if risky will be effective in reducing the parasitosis rate in Siirt Region.

***Information:** The results of the present study are summarized from L.G.'s master's thesis.

*Ethics

Ethics Committee Approval: Non-Interventional Clinical Research Ethics Committee of Van Yüzüncü Yıl University approved the study protocol (decision number: 2021/05-20, date: 16.04.2021).

Informed Consent: Written informed consent was obtained from the patient who participated in this study.

Footnotes

*Authorship Contributions

Concept: L.G., Z.T.C., Design: L.G., Z.T.C., Data Collection or Processing: L.G., Z.T.C., S.A., H.Y., Analysis or Interpretation: L.G., Z.T.C., S.A., H.Y., Literature Search: L.G., S.A., Writing: L.G., S.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Turkiye Parazitol Derg 2025;49(2):68-73
DOI: 10.4274/tpd.galenos.2025.43265

Investigation of *Demodex* Prevalence Due to Mask Use After the COVID-19 Pandemic with Cellophane Tape Method

COVID-19 Pandemisi Sonrası Maske Kullanımına Bağlı Demodex Yaygınlığının Selofan Bant Yöntemi ile Araştırılması

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Cite this article as: Ataş AD, Baysal Bakay B. Investigation of *Demodex* prevalence due to mask use after the COVID-19 pandemic with cellophane tape method. Turkiye Parazitol Derg. 2025;49(2):68-73.

ABSTRACT

Objective: *Demodex* species in the family Demodicidae are hair follicle scabies agents. *Demodex* species are transmitted from person to person through close contact, shared towels, make-up materials, etc. This study was conducted to obtain data on the relationship between mandatory mask use and demodicosis during the Coronavirus disease-2019 (COVID-19) period.
Methods: The study included 510 students who used masks in necessary environments since the beginning of the pandemic and participated in the study voluntarily. Cellophane tapes were applied to the relevant areas three times. In addition, both eye lashes were pulled from both eyes and adhered to the cellophane tapes. The samples were examined under light microscope at different magnifications (x10, x40).
Results: *Demodex folliculorum* and *Demodex* brevis were detected in 38 (7.5%) of 510 students. *D. folliculorum* was detected in 33 of the positive students and both *D. folliculorum* and *D. brevis* were detected in 5 students, 2 of whom were male and 3 of whom were female. *Demodex* spp. was found in 12 (4.9%) of 245 students who answered "no" to the question "Do you have acne or skin complaints on your face?" and in 26 (9.8%) of 265 students who answered "yes". While this parameter was statistically significant, the other parameters were not statistically significant.
Conclusion: The COVID-19 period has changed people's lifestyles and habits in many ways. It has made the use of masks obligatory. During mandatory mask use, factors that may increase the presence of *Demodex* spp. should not be ignored.
Keywords: COVID-19, *Demodex folliculorum*, *Demodex brevis*, face mask, university students

ÖZ

Amaç: Demodicidae familyasında yer alan *Demodex* türleri kıl folikülü uyuzu etkenleridir. *Demodex* türleri insandan insana yakın temas, ortak kullanılan havlular, makyaj malzemeleri vb. yollarla bulaşır. Bu çalışma, Koronavirüs hastalığı-2019 (COVID-19) sürecinde zorunlu maske kullanımı ile demodikozis arasındaki ilişki hakkında veri elde etmek amacıyla yapılmıştır.
Yöntemler: Çalışmaya pandeminin başlangıcından itibaren zorunlu ortamlarda maske kullanan ve çalışmaya gönüllü olarak katılan 510 öğrenci dahil edilmiştir. Selofan bantlar ilgili bölgelere üç kez uygulanmıştır. Ayrıca her iki göz kirpikleri her iki gözden çekilerek selofan bantlara yapıştırılmıştır. Örnekler ışık mikroskobu altında farklı büyütmelerde (x10, x40) incelenmiştir.
Bulgular: *Demodex folliculorum* ve *Demodex brevis* 510 öğrencinin 38'inde (%7,5) tespit edilmiştir. Pozitif öğrencilerin 33'ünde *D. folliculorum*, 2'si erkek 3'ü kız olmak üzere 5'inde hem *D. folliculorum* hem de *D.brevis* tespit edilmiştir. "Yüzünüzde sivilce veya cilt şikayetiniz var mı?" sorusuna "hayır" cevabı veren 245 öğrencinin 12'sinde (%4,9), "evet" cevabı veren 265 öğrencinin 26'sında (%9,8) *Demodex* spp. bulunmuştur. Bu parametre istatistiksel olarak anlamlı bulunurken, diğer parametreler istatistiksel olarak anlamlı bulunmamıştır.
Sonuç: COVID-19 dönemi insanların yaşam tarzlarını ve alışkanlıklarını birçok yönden değiştirmiştir. Maske kullanımını zorunlu hale getirmiştir. Zorunlu maske kullanımı sırasında *Demodex* spp. varlığını artırabilecek faktörler göz ardı edilmemelidir.
Anahtar Kelimeler: COVID-19, *Demodex folliculorum*, *Demodex* brevis, yüz maskesi, üniversite öğrencileri

Received/Geliş Tarihi: 02.09.2024 **Accepted/Kabul Tarihi:** 25.04.2025 **Epub:** 29.04.2025 **Publication Date/Yayınlanma Tarihi:** 18.07.2025

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Demodex species in the family Demodicidae are "hair follicle scabies agents" that usually settle in facial hair and sebaceous follicles in humans and present with various symptoms. *Demodex folliculorum* is reported to be the most common ectoparasite in humans. *Demodex* species show cosmopolitan distribution and are widely observed all over the world and in our country (1).

Demodex spp. are transmitted from person to person through close contact, shared towels, make-up materials, etc. (1-3). Although it is claimed to be apathogenic by some researchers, it is also known to be pathogenic in cases such as perioral dermatitis, immune system weakness, and the presence of acne vulgaris (1,2).

The diagnosis of demodicosis can be made with a standard superficial skin biopsy (SSSB) sample including the follicle contents; by direct slide-to-lamel examination of epilated eyelashes; and by microscopic examination of the material obtained by sticking and pulling the cellophane tape (CT), which is widely used especially in field applications, to the suspicious area (1).

It is recommended that personal care and cleaning products and hand and face towels should not be shared for individual and social protection against infestation. It has been reported that inadequate skin cleansing or keeping the skin moist (such as the use of masks) may create a suitable environment for the parasite to settle (1). With the Coronavirus disease-2019 (COVID-19) pandemic, public health authorities are advising people to wear face masks to reduce respiratory transmission. Wearing a face mask can cause changes in the microenvironment, skin barrier function, and microbiome of human skin. Protective equipment such as masks and gloves used for protection can cause skin diseases such as acne, folliculitis, seborrheic dermatitis, and eczema as a result of inappropriate use (4-6).

This study was planned considering the possibility that prolonged moist skin conditions due to mask use may create a suitable environment for the settlement of parasites of the genus *Demodex* as well as skin disorders. This study was conducted to obtain data on the relationship between the mandatory use of masks and demodicosis. We believe that our study results will contribute to the precautions that can be taken in similar situations that we may encounter in the future.

METHODS

Working Group

This study was conducted with the permission of Sivas Cumhuriyet University Non-Interventional Clinical Research Ethics Committee with the decision of 2021-11/45 dated 17.11.2021. This study was conducted between December 2021 and June 2022. The population of the study consisted of students studying at Sivas Cumhuriyet University Faculty of Medicine, Faculty of Dentistry, Faculty of Pharmacy, Faculty of Health Sciences, Vocational School of Health Services, Faculty of Veterinary Medicine. The age range of the students in the study was 18-23 years old. The study included 510 students who used masks in necessary environments since the beginning of the pandemic and participated in the study voluntarily.

Demodex spp. Examination

After short seminars about *Demodex* spp. and Demodicosis, volunteer students filled out the questionnaire and consent forms. Afterwards, CT samples were taken from the nose, forehead, cheeks, and lower lip. Cellophane tapes were applied to the relevant areas three times each and withdrawn by pressing on them. In addition, both eye lashes were pulled from both eyes and adhered to the CTs. *Demodex* species were identified using literature (1,2,5,7). The samples were examined under light microscope at different magnifications (x10, x40) in the laboratory of Sivas Cumhuriyet University Faculty of Medicine, Department of Parasitology.

Statistical Analysis

For the analysis of the data, descriptive statistical methods, means, standard deviations, medians, frequencies and percentages were used by loading the data into the IBM SPSS 22.0 program. Normality assumption was checked according to Kolmogorov-Smirnov or Shapiro-Wilk test. Parametric tests were used for variables that met the parametric assumption; non-parametric tests were used for variables that did not meet the parametric test assumption. $P<0.05$ was considered statistically significant.

RESULTS

D. folliculorum and/or *D. brevis* were detected in 38 (7.5%) of 510 students (Figures 1, 2). *D. folliculorum* was detected in 33 of the positive students and both *D. folliculorum* and *D. brevis* were detected in 5 students, of which 2 were male and 3 were female.

The distribution of the presence of *Demodex* spp. in the students according to the various questions asked is shown in Table 1. *Demodex* spp. was found in 12 (4.9%) of 245 students who answered "no" to the question "Do you have acne or skin complaints on your face?" and in 26 (9.8%) of 265 students who answered "yes". It was statistically significant ($p=0.035$, $p<0.05$). Other parameters were not statistically significant (Table 1).

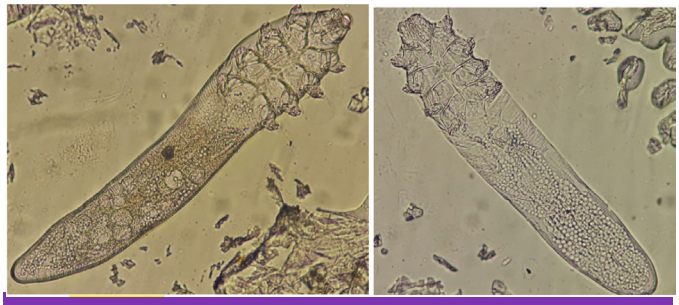

Figure 1. *D. folliculorum* adult (x400)

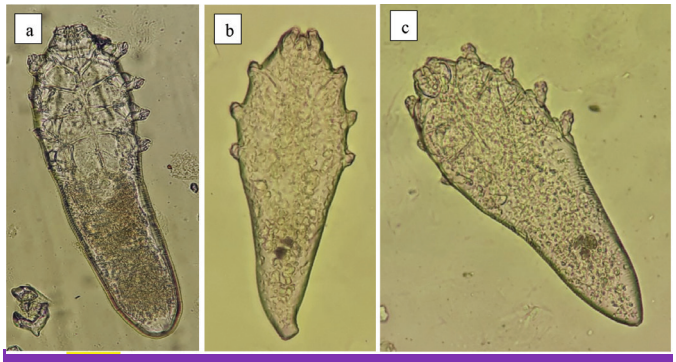

Figure 2. *D. brevis*, a-adult, b-larva, c-nymph (x400)

DISCUSSION

Demodex species can generally be found on the forehead, eyes, nose and around the mouth where sebaceous glands are high; they can also be found on the scalp, outer ear, chest and genital area (2). It can be found all over the world and in all races. The total infestation rate in different study groups generally varies between 17% and 72% in healthy people and can reach up to 100% in people over 96 years of age (1).

It has been reported that *Demodex* spp. can be transmitted more easily through close contact in crowded living spaces such as dormitories, kindergartens, etc., and in the common use of cleaning equipment (7). Some molecular analyses show that frequent and close physical contact leads to mite transmission and haplotypes are likely to be common within families (8).

In university students; Çetinkaya et al. (7), 35.9% in 92 students; Miman et al. (9), 11.0% in 100 students; Kaplan et al. (10), 10.07% in 258 students; Özdemir et al. (11), 47.4% in 270 students; Zeytun et al. (12), 50.1% in 385 students; Sevgen and Mor (13), 42.7% in 375 students; Yilmaz and Akkas (14), 34.5% in 171 students; Ding and Huang (15), 11.5% in 613 students; Isa et al. (16), 17.2% in 390 students were found to have *Demodex* spp.

There is no standard method for *Demodex* examinations; CT method, squeezing method or skin scraping can be used. Liwtin et al. (1) found a 91% positivity rate with the CT method and 34% with the compression method. They recommend the SSSB method as the most commonly used method to compare mite densities between patients with dermatosis and healthy controls (1). However, it has been reported in the literature that the SSSB technique has some limitations (17). Çetinkaya et al. (7) and Miman et al. (9) and, on the other hand, stated that they found the SSSB method more successful and the CT method more unsuccessful. In our study, *Demodex* spp. was detected in 38 (7.5%) of 510 university students who had CT taken from different parts of their faces and answered the survey questions. We believe that the use of only the CT method may be the reason for the low detection rate of *Demodex* spp.

Studies investigating the presence of *Demodex* spp. especially in patients with different dermatologic symptoms on the face have also been conducted. In these studies, Karabay and Çerman (3), 52.0%; Yazısız et al. (18), 69.9%; Maldonado-Gómeza et al. (19), 29.7% *Demodex* spp. were detected and *Demodex* infestation was associated with acne vulgaris, rosacea, and seborrheic dermatitis. Of the people in our study, 163 had COVID-19 and *Demodex* spp. was detected in 14 of them. However, no statistically significant result was found between COVID-19 and *Demodex* spp.

When the relationship between the duration of daily mask wearing, frequency of mask replacement and the presence of *Demodex* spp. was examined, no statistically significant result was found. Some other studies have emphasized that long-term mask use increases the risk of adverse skin reactions, and that those who do not change their face masks every day are at greater risk of adverse skin reactions than the group that uses masks (19). It has been reported that wearing a mask for several hours

Table 1. Distribution of *Demodex* spp. according to different characteristics in the survey questions

Survey questions		*Demodex* spp.						
		Positive		Negative				
		n	%	n	%	Total	χ^2	p
Gender	Male	17	8.2	191	91.8	208	0.266	0.606
	Female	21	7.0	281	93.0	302		
Have you had a COVID-19 infection?	Yes	14	8.6	149	91.4	163	0.450	0.502
	No	24	6.9	323	93.1	347		
How often did you change masks daily?	1 per day	24	8.3	264	91.7	288	1.831	0.608
	2 per day	5	4.6	103	95.4	108		
	1 per week and >	7	8.6	74	91.4	81		
	Other/irregular	2	6.1	31	93.9	33		
How many hours did you wear your mask per day?	1-3 hours	11	7.0	146	93.0	157	3.277	0.351
	3-6 hours	18	7.8	214	92.2	232		
	6-9 hours	9	9.9	82	90.1	91		
	9 hours and >	0	0.0	30	100.0	30		
What is your daily face washing habit-frequency?	1 per day	11	11.1	88	88.9	99	2.387	0.127
	1 per day >	27	6.6	384	93.4	411		
Do you have acne, skin complaints on your face?	Yes	26	9.8	239	90.2	265	4.437	0.035
	No	12	4.9	233	95.1	245		
Did you use medication for your complaint?	Yes	11	10.0	99	90.0	110	1.321	0.250
	No	27	6.8	373	93.3	400		
Do you use cosmetics (aftershave, moisturizing cream, foundation cream, powder, etc.)?	Yes	30	8.5	322	91.5	352	1.893	0.166
	No	8	5.1	150	94.9	158		
General total		38	7.5	472	92.5	510		

COVID-19: Coronavirus disease-2019

daily may cause changes in skin microbiota, and skin barrier functions, and normal skin flora may become pathogenic. It has been stated that demodicosis should be considered in the differential diagnosis in patients with mask-related rash during the COVID-19 pandemic (20,21). It is stated that masks cause microenvironmental changes in the skin through dehydration, sebum and pH increase; *D. folliculorum*, which is considered to be a trigger in rosacea, increases inflammation by taking advantage of sebum overproduction (20,22,23). Avşar et al. (24) investigated *Demodex* spp. frequency, mask use, and personal hygiene habits among medical school students during the COVID-19 pandemic. The researchers found *Demodex* spp. presence at a rate of 47.5% in samples taken from the cheek area and 13.9% in samples taken from the forehead area of those using masks. They commented that the more frequent occurrence in the cheek area may be due to prolonged mask use during the COVID-19 pandemic (24). In a study investigating adverse skin reactions due to the use of protective equipment by healthcare workers during the pandemic process, Turan and Nacar (25) found itching in 23%, scarring on the bridge of the nose in 18%, and pimple/acne formation in the area where the mask was worn in 13% due to mask use.

In studies examining whether the prevalence of *Demodex* spp. infection varies according to gender difference, no statistically significant result was found (2,7,9,11-14,18). In our study, there was no statistically significant result between genders. The fact that it is found slightly higher in men is interpreted as androgen-induced higher sebum production in men and exogenous lipid applications in cosmetics may affect the growth of *Demodex* mites (2). However, Isa et al. (16) found statistically significant results in men compared to women in their study. The researchers attributed this to the fact that men are generally more active throughout the day, which may result in more sebum and sweat secretion (16).

When the relevant literature is examined; results and interpretations differ in terms of the presence of *Demodex* spp. in issues involving personal hygiene. For example, Karaman et al. (26) reported that frequent face washing may cause dryness in the skin, as a result of which the rate of mites will be less on clean skin; Zeytun et al. (12) found that daily face washing frequency and shared towel use affected the frequency of *Demodex* spp. and *Demodex* infestation decreased with increasing number of daily face washes; Forton et al. (27) reported that *Demodex* mites were less common in those who washed their face with soap, and that there was a significant decrease in the frequency of parasites in those who washed their face three times or more daily and had regular skin care. There are also studies in the literature reporting that *Demodex* infestation increases as the frequency of face washing and bathing increases. However, it was also reported that there was no statistically significant result between personal hygiene, self-care and the presence of *Demodex* spp. in these studies (10,13,18,28,29).

In our study, the presence of *Demodex* was found to be 8.5% in those who used cosmetic materials and make-up, and 5.1% in those who did not use it. However, this result was not statistically significant (Table 1). In some other studies, although not statistically significant, it was reported that the presence of *Demodex* was found more frequently in those who did not wear make-up and did not use cosmetics (13,18,24). On the other hand, Okyay et al. (29) reported that living conditions in crowded groups, frequency of daily face washing and hygienic and cosmetic practices such as lotion use did not affect the prevalence of *Demodex* spp. Çetinkaya et al. (7) reported a statistically significant relationship between the use of facial cleansing products only and parasite positivity. They emphasized that skin care and hygiene decreased the frequency of parasites. The researchers interpreted that cosmetics contribute to the destruction of *Demodex* mites by mechanically occluding the follicle, preventing the migration and respiration of the parasite, with a toxic effect because they contain antiseptics such as alcohol (7). In some other studies, Elston and Elston (2) reported that the application of exogenous lipids in cosmetics may affect the growth of *Demodex* mites; Guner et al. (30) reported that changes in skin pH due to cosmetic products lead to disruption of skin barrier function and moisture imbalance, and that the prevalence of sensitive skin increases as the frequency of cosmetic use increases. It should also be taken into account that *Demodex* spp. can survive for long hours in cosmetic materials and this may contribute to its transmission through shared cosmetics (8).

It has been reported that the pathogenicity of *Demodex* mites increases with factors such as neglect and improper skin cleansing, intensive use of cosmetic products, and increased sebum production with sweating (30). It is also reported that *Demodex* spp. may be the cause of chronic inflammatory eruptions of the skin resembling bacterial folliculitis, rosacea, perioral dermatitis and otitis externa (2). Free fatty acids and triglycerides contribute to skin acidity. But although this acidic environment is protective against microorganisms, it is claimed that it has no effect on parasites and even facilitates the presence of parasites (30). Litwin et al. (1) emphasize that higher mite prevalence is observed in rosacea, seborrheic dermatitis, perioral dermatitis, blepharitis and chalazion; the situation is exacerbated when hormonal abnormalities or chronic diseases in the host organism coexist. Nobeyama et al. (31) found *Demodex* in 88.2% of rosacea patients; Avşar et al. (24) found *Demodex* in 52.8% of those with acne and 40.0% of those with other skin problems. In our study, statistically significant presence of *Demodex* spp. was found in people with dermatologic complaints on the face. In some previous similar studies, there were no statistically significant results related to *Demodex* in patients with various skin complaints (3,7,10,11,13,14,16). This is explained by the fact that the cause of facial problems may be multifactorial such as environmental, hormonal, and personal hygiene. However, no significant result was found between the use or non-use of medication for skin complaints.

CONCLUSION

The COVID-19 period has changed people's lifestyles and habits in many ways. It has made the use of masks obligatory. It is also possible that the presence of *Demodex* spp. may be influenced in different directions by the following factors; due to staying at home for a long time during quarantine and not going out, skin cleansing may have been neglected. When coming from outside, extreme care may be taken to clean, due to fear of contracting the COVID-19 virus. In the COVID-19 process, a lot of antiseptic materials were used. Due to the face mask, there has been a decrease in makeup applications and the use of cosmetics. There was excessive sweating due to the mask. During quarantine, sebum production increased due to excessive consumption of

fatty foods such as nuts. Due to staying at home for long periods of time, close contact has increased. However, the relationship between mandatory mask use and infestation by *Demodex* mites has not been studied in detail.

We think that the continuation of many habits acquired during the COVID-19 period and the use of only the CT method during our study may have caused the presence of *Demodex* spp. to be lower compared to previous studies. During mandatory mask use, factors that may increase the presence of *Demodex* spp. should not be ignored.

*Ethics

Ethics Committee Approval: This study was conducted with the permission of Sivas Cumhuriyet University Non-Interventional Clinical Research Ethics Committee with the decision of 2021-11/45 dated 17.11.2021.

Informed Consent: In order to conduct the study, "approved information form" was obtained from the students who agreed to participate in the study in accordance with the letter of Sivas Cumhuriyet University Faculty of Medicine Dean's Office dated 22.02.2022 and numbered 135575; Sivas Cumhuriyet University Rectorate dated 22.03.2022 and numbered 137740.

Footnotes

*Authorship Contributions

Surgical and Medical Practices: A.D.A., B.B.B., Concept: A.D.A., B.B.B., Design: A.D.A., B.B.B., Data Collection or Processing: A.D.A., B.B.B., Analysis or Interpretation: A.D.A., B.B.B., Literature Search: A.D.A., B.B.B., Writing: A.D.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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Turkiye Parazitol Derg 2025;49(2):74-81
DOI: 10.4274/tpd.galenos.2025.09226

Bibliometric Analysis of All Theses on Echinococcosis in Türkiye

Türkiye'deki Ekinokokkozis Üzerine Yapılmış Tüm Tezlerin Bibliyometrik Analizi

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Cite this article as: Davarcı İ, Güdücüoğlu H. Bibliometric analysis of all theses on echinococcosis in Türkiye. Turkiye Parazitol Derg. 2025;49(2):74-81.

ABSTRACT

Objective: Türkiye is the country with the highest number of published articles on echinococcosis worldwide, it is expected that medical specialization, PhD and master's students in Türkiye would conduct theses on this topic. These theses can provide insights for future studies. Therefore, this study aims to evaluate the medical specialization, PhD, and master's theses related to echinococcosis conducted in Türkiye.

Methods: The relevant theses were accessed individually from the "detailed search" section of the National Thesis Center webpage of the Council of Higher Education by using the search terms "Ekinokok", "*Echinococcus*", or "Hidatik" without selecting a year range.

Results: A total of 202 theses (113 medical specialization theses, 42 PhD theses, and 47 master's theses) were included in the study. When examined on a provincial basis, it was found that the highest number of theses were conducted in Ankara (n=36). At the university level, İstanbul University had the most theses. Medical specialization theses were mostly conducted in the fields of general surgery and radiology, while PhD and master's theses were primarily conducted in the fields of parasitology and veterinary medicine. Most of the theses conducted in the internal and surgical sciences were retrospective in design, while those in the basic sciences were mainly diagnostic in nature. A moderate positive correlation was found between the number of theses and the years for medical theses (r=0.341, p=0.027), and a strong positive correlation was found for PhD theses (r=0.505, p=0.001), master's theses (r=0.619, p=0.000), and all theses combined (r=0.761, p=0.000).

Conclusion: It was observed that at least one thesis related to echinococcosis is conducted each year, with an increase in the number of PhD and master's theses in recent years.

Keywords: Academic dissertation, bibliometric analysis, echinococcosis

ÖZ

Amaç: Türkiye, dünyada ekinokokkozis üzerine en fazla makale yayımlanan ülke olup, Türkiye'deki tıpta uzmanlık, doktora ve yüksek lisans öğrencilerinin bu konuda tez çalışmaları yapması beklenmektedir. Bu tezler, gelecekteki çalışmalar için önemli bilgiler sunabilir. Bu nedenle, bu çalışmanın amacı, Türkiye'de ekinokokkozis ile ilgili olarak yürütülen tıpta uzmanlık, doktora ve yüksek lisans tezlerini değerlendirmektir.

Yöntemler: İlgili tezlere, Yükseköğretim Kurulu Ulusal Tez Merkezi web sayfasındaki "detaylı arama" bölümünden "Ekinokok", "*Echinococcus*" veya "Hidatik" anahtar kelimeleri kullanılarak herhangi bir yıl aralığı seçilmeksizin tek tek erişilmiştir.

Bulgular: Çalışmaya toplamda 202 tez (113 tıpta uzmanlık tezi, 42 doktora tezi ve 47 yüksek lisans tezi) dahil edilmiştir. İller bazında incelendiğinde, en fazla tezin Ankara'da (n=36) yapıldığı belirlenmiştir. Üniversite düzeyinde ise İstanbul Üniversitesi en çok tez yapılan kurum olarak öne çıkmıştır. Tıpta uzmanlık tezleri en çok genel cerrahi ve radyoloji alanlarında yapılırken, doktora ve yüksek lisans tezleri genellikle parazitoloji ve veterinerlik alanlarında gerçekleştirilmiştir. Dahili ve cerrahi bilimlerde yapılan tezlerin çoğu retrospektif tasarıma sahipken, temel bilimlerde yapılan tezler genellikle tanı odaklıdır. Tıbbi tezler ile yıllar arasında orta düzeyde pozitif bir korelasyon (r=0,341, p=0,027), doktora tezleri ile güçlü bir pozitif korelasyon (r=0,505, p=0,001), yüksek lisans tezleri ile (r=0,619, p=0,000) ve tüm tezler bir arada değerlendirildiğinde (r=0,761, p=0,000) güçlü bir pozitif korelasyon bulunmuştur.

Sonuç: Her yıl ekinokokkozis ile ilgili en az bir tez çalışması yapıldığı ve son yıllarda doktora ve yüksek lisans tezlerinin sayısında artış olduğu gözlenmiştir.

Anahtar Kelimeler: Akademik tez, bibliyometrik analiz, ekinokokkozis

Received/Geliş Tarihi: 20.01.2025 **Accepted/Kabul Tarihi:** 21.04.2025 **Epub:** 21.05.2025 **Publication Date/Yayınlanma Tarihi:** 18.07.2025

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***This study was presented as an oral presentation at the "11th National and 4th International Congress of Hydatidology, 2-4 October 2024, Edirne, Türkiye".**

Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Echinococcosis, a chronic and neglected zoonosis characterized by the formation of cysts, is caused by the larval stages of parasites belonging to the genus *Echinococcus* and affects both humans and animals. Over the past two decades, despite the concerted efforts of scientists worldwide to minimize *Echinococcus* helminth infections, the disease continues to result in a significant number of human cases globally. Increased tourism and travel mobility have contributed to the spread of echinococcosis even in developed countries. The disease are classified as alveolar echinococcosis caused by *E. multilocularis* and cystic echinococcosis caused by *E. granulosus*. Notably, cystic echinococcosis accounts for over 95% of all echinococcosis cases, the most common larval form is the hydatid cyst, and cases are most commonly localized in the liver (1). However, cases of infection have also been reported in the spleen, kidneys, peritoneal cavity, skin, muscles, and, rarely, the heart, spine, brain, and ovaries (2). In Türkiye, the prevalence of *E. granulosus* in stray dogs exceeds 10%, particularly in the Central Anatolia and Eastern Anatolia Regions. Moreover, the number of human cases has increased steadily each year from 2008 to 2019 (3). Therefore, alongside proper washing of fresh fruits and vegetables, reducing the number of stray dogs is of critical importance in preventive strategies (4).

These parasites cause significant health problems and economic losses for both humans and animals. The World Health Organization reports that over 1 million individuals are affected by echinococcosis annually worldwide, with the disease being highly prevalent in endemic regions such as Central Asia, Mediterranean countries, East Africa, South America, and Western China (2). Echinococcosis is estimated to cause approximately 19,300 deaths and result in the loss of 871,000 disability-adjusted life years globally each year, with hepatic echinococcosis accounting for 60-75% of these cases (5,6). Türkiye has emerged as the leading country in echinococcosis research globally, contributing 531 articles, which account for 20.3% of the total 2,605 publications worldwide (7). Another study highlights that between 2000 and 2019, the top five countries with the most publications on echinococcosis were Türkiye, China, Iran, Germany, and the United States (2). Although Turkish researchers have received the highest total number of citations in this field, their average citation values are not particularly high (2). Original research articles and bibliometric analyses like this article will contribute to increasing the number of citations. Bibliometric analysis, a method that quantitatively evaluates studies and provides in-depth knowledge about a specific research area, systematically examines a large body of work using mathematical and statistical approaches. This method also evaluates the co-citations, authors, journals, institutions, countries, and keyword trends of articles across different research domains, offering researchers a comprehensive literature review and helping measure and assess productivity in the field. As a result, it aids in identifying potential future research areas (1,8,9).

In the literature, we did not encounter any bibliometric analysis of theses on echinococcosis conducted in Türkiye. Given that Türkiye is the country with the highest number of publications on echinococcosis worldwide, it is expected that medical specialization, PhD, and master's students in Türkiye would conduct theses on this topic. These thesis studies can provide valuable insights for future research. Therefore, this study aims to evaluate the Medical Specialization, PhD, and Master's theses on echinococcosis conducted in Türkiye.

METHODS

Data Collection

This descriptive study was conducted between June 1, 2024, and June 31, 2024. The relevant theses were accessed individually through the "detailed search" section of the National Thesis Center website of the Council of Higher Education, using the search terms "Ekinokok" or "*Echinococcus*" or "Hidatik" without restricting the search to a specific time period (10). Theses in which echinococcosis was not the primary focus, or in which echinococcosis was studied alongside multiple other factors, were excluded from the study. Theses specifically addressing the diagnosis, treatment, or prevalence of echinococcosis, as well as those involving patients diagnosed with hydatid cyst, were included in the analysis. The titles, page numbers, topics, authors' names and sexes; supervisors' names, titles, and sexes; university names, and the characteristics of the provinces where the studies were conducted were recorded. The study did not require ethical committee approval as the data used were publicly available.

Statistical Analysis

The data were analyzed using IBM SPSS 21 software. Descriptive statistics were presented as counts, percentages, medians, and 25^{th}-75^{th} percentile values. Continuous variables with skewness and kurtosis levels between ±2 were assumed to follow a normal distribution. Data were analyzed using descriptive statistics, the chi-square test for categorical variables, and Kruskal-Wallis, ANOVA, and Pearson correlation analysis for continuous variables. A p-value of <0.05 was considered statistically significant in the results.

RESULTS

According to the research results, 293 theses were retrieved. Theses that contained the search terms in the abstract but did not focus on echinococcosis as their primary topic were excluded, resulting in a total of 202 theses being analyzed (113 medical specialization theses, 42 PhD theses, and 47 master's theses).

According to available records, the earliest thesis on echinococcosis dates back to 1976 and was prepared by the department of general surgery as a medical specialization study. The distribution of theses by year is presented in Figure 1.

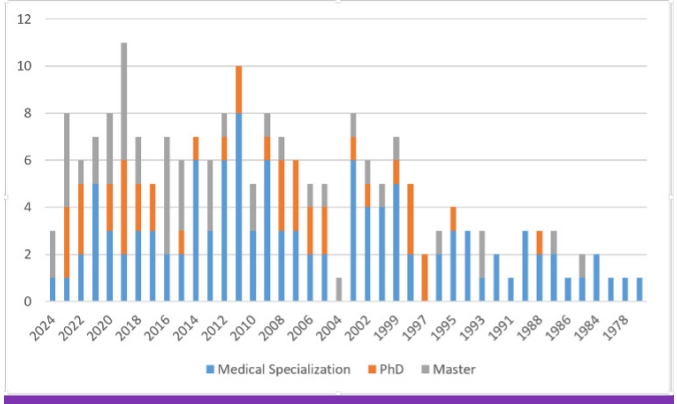

Figure 1. Distribution of theses by year

Table 1. Characteristics of theses

	Medical specialization	PhD	Master	p	Cramer's V	X^2
Title of the consultant						
Professor	54 (47.8%)*	32 (76.2%)	27 (57.4%)	**0.005**	**0.201**	**15.077**
Associate professor	20 (17.7%)*	7 (16.7%)	15 (31.9%)			
Assistant professor/medical specialist	25 (34.5%)*	2 (7.1%)	4 (10.6%)			
City						
Metropolitan	103 (91.2%)*	32 (76.2%)	36 (76.6%)	**0.015**	**0.203**	**8.336**
Small city	10 (8.8%)	10 (23.8%)	11 (23.4%)			
Experimental/hospital/field						
Experimental	21 (18.6%)	10 (23.8%)	3 (6.4%)	**0.000**	**0.363**	**53.368**
Hospital	85 (75.2%)	13 (31.0%)	20 (42.6%)			
Field	7 (6.2%)	19 (45.2%)	24 (51.1%)			
Who the research is conducted on						
Experimental animal/animal	11 (9.7%)	18 (42.9%)	19 (40.4%)	**0.000**	**0.372**	**27.931**
Human/strain/animal and human together	102 (90.3%)	24 (57.1%)	28 (59.6%)			

*: Kruskal-Wallis

Figure 2. Distribution of all theses by province*
** It was prepared using the website https://paintmaps.com.*

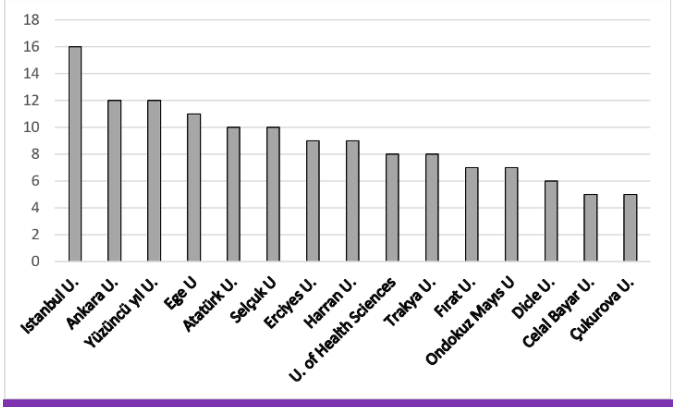

Figure 3. Distribution of theses according to the centers where they were conducted
U: University

A total of 113 supervisors (60.8%) were professors, 42 (22.6%) were associate professors, 19 (10.2%) were assistant professors, and 12 (6.4%) were specialist doctors. The characteristics of the theses are presented in Table 1.

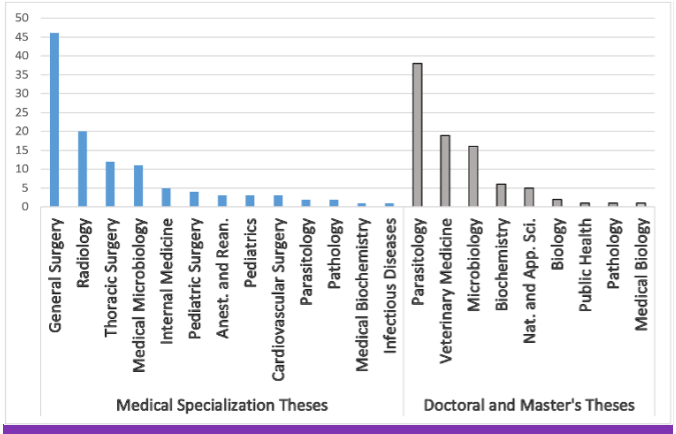

Figure 4. Distribution of theses by department
Anest. and Rean.: Anesthesia and Reanimation, Nat. and App. Sci.: Natural and Applied Science

On a provincial basis, the highest number of theses was conducted in Ankara (n=36) (Figure 2), followed by İstanbul (n=20), İzmir (n=17), and Konya (n=16). The most master's theses were conducted in Van (n=6), the most PhD theses in İzmir (n=6) and Ankara (n=6), and the most medical specialization theses in Ankara (n=28) (Supplement 1).

In studies on echinococcosis, İstanbul University ranks first with 16 theses, followed by Ankara University and Van Yüzüncü Yıl University with 12 theses each, and Ege University with 11 theses (Figure 3). The highest number of medical specialization theses were conducted at İstanbul University (n=10); the most PhD theses were conducted at Ege University (n=5) and Ankara University (n=5); and the most master's theses were conducted at Van Yüzüncü Yıl University (n=6) and Erciyes University (n=5) (Supplement 2).

It is observed that the highest number of medical specialization theses were conducted in general surgery and radiology, while the majority of PhD and master's theses were completed in the

Table 2. Distribution of medical specialization theses according to internal, surgical and basic sciences

	Internal medicine sciences	Surgical medical sciences	Basic medical sciences
Title of the consultant			
Professor	13 (56.5%)	30 (47.6%)	11 (84.6%)
Associate professor	4 (17.45)	14 (22.2%)	2 (15.4%)
Assistant professor/ medical specialist	6 (26.1%)	19 (30.2%)	0 (0.0%)
Council of higher education/ministry of health			
Council of higher education	24 (82.8%)	59 (84.3%)	14 (100.0%)
Ministry of health	5 (17.2%)	11 (15.7%)	0 (0.0%)
Experimental/hospital/field			
Experimental	1 (3.4%)	16 (22.9%)	4 (28.6%)
Hospital	27 (93.1%)	53 (75.7%)	5 (35.7%)
Field	1 (3.4%)	1 (1.4%)	5 (35.7%)
Who the research is conducted on			
Experimental animal	0 (0.0%)	8 (11.4%)	0 (0.0%)
Animal	0 (0.0%)	2 (2.9%)	1 (7.1%)
Human	28 (96.6%)	54 (77.1%)	9 (64.3%)
Strain	1 (3.4%)	6 (8.6%)	2 (14.3%)
Human and animal	0 (0.0%)	0 (0.0%)	2 (14.3%)
Aim			
Risk factor	2 (6.9%)	3 (4.3%)	2 (14.3%)
Treatment	7 (24.1%)	22 (31.4%)	1 (7.1%)
Diagnosis	9 (31.0%)	6 (8.6%)	10 (71.4%)
Method comparison	1 (3.4%)	0 (0.0%)	1 (7.1%)
Retrospective	10 (34.5%)	39 (55.7%)	0 (0.0%)
Method used			
Biochemical	2 (6.9%)	4 (6.0%)	1 (7.1%)
Serological	2 (6.9%)	0 (0.0%)	9 (64.3%)
Radiological	8 (27.6%)	3 (4.5%)	0 (0.0%)
Microscopic	0 (0.0%)	2 (3.0%)	0 (0.0%)
Molecular	0 (0.0%)	1 (1.5%)	3 (21.4%)
Method research	7 (24.1%)	17 (25.4%)	1 (7.1%)
Macroscopic	0 (0.0%)	1 (1.5%)	0 (0.0%)
Retrospective	10 (34.5%)	39 (35.5%)	0 (0.0%)
City			
Metropolitan	27 (93.1%)	65 (92.9%)	11 (78.6%)
Small city	2 (1.8%)	5 (4.4%)	3 (21.4%)
Region			
Marmara	6 (20.7%)	13 (18.6%)	3 (21.4%)
Aegean	3 (10.3%)	4 (5.7%)	3 (21.4%)
Central Anatolia	14 (48.3%)	24 (34.3%)	6 (42.9%)
Eastern Anatolia	1 (3.4%)	9 (12.9%)	1 (7.1%)
Black Sea	1 (3.4%)	2 (1.8%)	0 (0.0%)
Southeastern Anatolia	3 (10.3%)	13 (18.6%)	1 (7.1%)
Mediterranean	1 (3.4%)	5 (7.1%)	0 (0.0%)

departments of parasitology and Veterinary Medicine (Figure 4).

Most of the theses in internal medicine and surgical sciences are retrospectively designed, while in basic sciences, the majority of theses focus on diagnostics (Table 2).

A moderate positive correlation was found between the number of theses over the years and medical theses (r=0.341, p=0.027), a strong positive correlation with PhD theses (r=0.505, p=0.001), a strong positive correlation with master's theses (r=0.619, p=0.000), and a strong positive correlation with the total number of theses (r=0.761, p=0.000).

DISCUSSION

Türkiye is the leading country in the world in terms of the number of publications on echinococcosis, followed by China, Germany, and the United States (7). In a country where extensive research is conducted on echinococcosis, it is expected that a high number of theses would also be produced on the same topic. In this study, a bibliometric analysis was conducted using data from the National Thesis Center to examine theses on echinococcosis. Since the first thesis on echinococcosis in 1976, it has been observed that at least one thesis has been completed on this subject each year. Particularly in recent years, there has been a noticeable increase in the number of PhD and master's theses.

When examining the sex of the researchers conducting the theses, a balanced distribution is observed in PhD and master's theses, while male dominance is more pronounced in medical specialization theses. This sex disparity in medical specialization theses seems to be primarily due to the surgical disciplines. Additionally, when considering the sex of thesis advisors, male dominance is evident in medical specialization, PhD, and master's theses. This may be related to the higher number of professors compared to associate professors in Türkiye, or it could be due to professors perceiving themselves as more competent in this field. Medical specialization theses are mostly designed in hospitals, whereas approximately half of the PhD and master's theses are conducted in the field.

Although the highest number of theses were completed at İstanbul University, the majority of theses on echinococcosis have been conducted in Ankara. The higher number of theses conducted in Ankara compared to other provinces can be attributed to several key factors. First, Ankara is home to long-established higher education institutions such as Hacettepe University, Ankara University, and Gazi University, which host numerous academic departments. These universities actively conduct research in fields directly related to echinococcosis, including medicine, veterinary sciences, and basic sciences. Additionally, as the capital city, Ankara's proximity to major public health institutions such as the Ministry of Health and the Ministry of Agriculture and Forestry provides strong research infrastructure and increased opportunities for institutional collaboration. Moreover, the presence and activity of multiple veterinary faculties and departments of parasitology in these universities contribute to the concentration of theses on zoonotic diseases. The combination of these factors may have positioned Ankara as the leading province in the production of theses on echinococcosis. The highest number of medical specialization theses were completed in Ankara, PhD theses in Ankara and İzmir, and master's theses in Van. The locations where theses or research are conducted may not always correlate with disease

prevalence. This simply indicates that there are more researchers interested in echinococcosis at these centers.

The primary treatment approach for the clinical diagnosis of hepatic echinococcosis is a combination of surgical intervention and the use of albendazole; however, the rapid progression of the disease limits the number of patients who can benefit from surgery. Therefore, it is crucial to focus on developing effective drugs and vaccines to control echinococcosis and improve early diagnosis. Such advancements will significantly impact future treatment methods for echinococcosis, which remains a global health concern (7).

Imaging techniques play a critical role in the diagnosis, community screening, and follow-up processes of echinococcosis in humans. Ultrasound and X-rays are widely used for diagnosing liver and lung lesions associated with cystic echinococcosis and alveolar echinococcosis, respectively. To confirm the diagnosis, serological tests with varying levels of sensitivity and specificity can assist in detecting antigens or antibodies in both definitive and intermediate hosts. Current approaches to echinococcosis treatment involve a combination of surgical intervention and pharmacotherapy, along with long-term follow-up of cases (2). When analyzing medical specialization theses by department, the majority of these theses were conducted in general surgery. Given that echinococcosis most commonly affects the liver and can be treated both medically and surgically, it falls within the scope of general surgery. On the other hand, radiology is the department most frequently consulted by general surgery doctors for both the diagnosis and treatment of echinococcosis in their patients. Therefore, it is natural that a large number of theses on echinococcosis have been conducted in both departments. Furthermore, in PhD and master's theses, the department with the most theses is parasitology, which is expected given that echinococcosis is caused by a parasitic agent.

We believe that academic theses conducted on echinococcosis, a zoonotic disease of public health importance, may have indirect impacts in various areas such as early diagnosis, preventive measures, treatment options, and raising public awareness. The fact that these theses are predominantly carried out within disciplines such as surgery and parasitology supports both clinical applications and laboratory-based diagnostic processes, while those conducted in the field of veterinary medicine contribute to the public health perspective within the One Health framework. Moreover, the concentration of theses in major metropolitan areas and well-established universities suggests that the academic expertise in these centers may influence the development of health policies. These data can provide an academic foundation for planning regional or national strategies in the fight against echinococcosis in Türkiye.

It is observed that theses in internal medicine and surgical disciplines are predominantly designed retrospectively, whereas those in basic sciences are more focused on diagnostics. When retrospective studies are excluded, internal medicine disciplines tend to use radiological methods, surgical disciplines primarily focus on methodological research, and basic sciences predominantly employ serological methods. The predominance of retrospective study designs in theses conducted within internal medicine and surgical departments may be explained by the ease of access to patient data stored in hospital information management systems, as well as the challenges researchers face in conducting prospective studies due to heavy clinical workloads. Additionally, one of the reasons why clinical disciplines produce more theses can be attributed to the high number of cystic echinococcosis cases. In contrast, the emphasis on diagnostic approaches in theses from basic science disciplines can be associated with the widespread availability of laboratory infrastructure, a strong culture of experimental research, and greater opportunities for fieldwork in these departments. When retrospective studies are excluded, the prevalence of radiological methods in internal medicine theses, methodological research in surgical theses, and serological techniques in basic science theses reflects the variations shaped by each discipline's research traditions, methodological frameworks, and available technical resources.

This study includes only theses published in the National Thesis Center database. Since the electronic submission of theses was not mandatory prior to 2018, some theses prepared before this date may not be included in the database, which may have limited the scope of the study. Additionally, as the research is limited to theses prepared in Türkiye, the possibility of international comparison is restricted. Future studies are recommended to analyze additional dimensions such as sample size, findings, and contributions to the literature. Comparative studies involving international databases (e.g., ProQuest, Open Access Theses and Dissertations) could help assess Türkiye's scientific contribution on a global scale. Examining the changes over time in the methods used in echinococcosis theses (e.g., laboratory techniques, epidemiological models, experimental studies) may reveal scientific development trends. Analyzing the relationship between theses and health policies may be useful to measure the practical impact of the academic knowledge produced. Moreover, the distribution of thesis advisors by academic titles and affiliated institutions could be investigated to better understand the relationship between academic productivity and fields of specialization.

CONCLUSION

In conclusion, it has been determined that the number of theses increases each year, field studies are more frequently included in PhD and master's theses, the highest number of theses are conducted in Ankara, clinical disciplines produce more theses, and retrospective studies are the most commonly conducted in medical specialization theses.

*Ethics

Ethics Committee Approval: The study did not require ethical committee approval as the data used were publicly available.

Informed Consent: N/A.

Footnotes

*Authorship Contributions

Concept: İ.D., Design: İ.D., H.G., Data Collection or Processing: İ.D., H.G., Analysis or Interpretation: İ.D., H.G., Literature Search: İ.D., H.G., Writing: İ.D., H.G.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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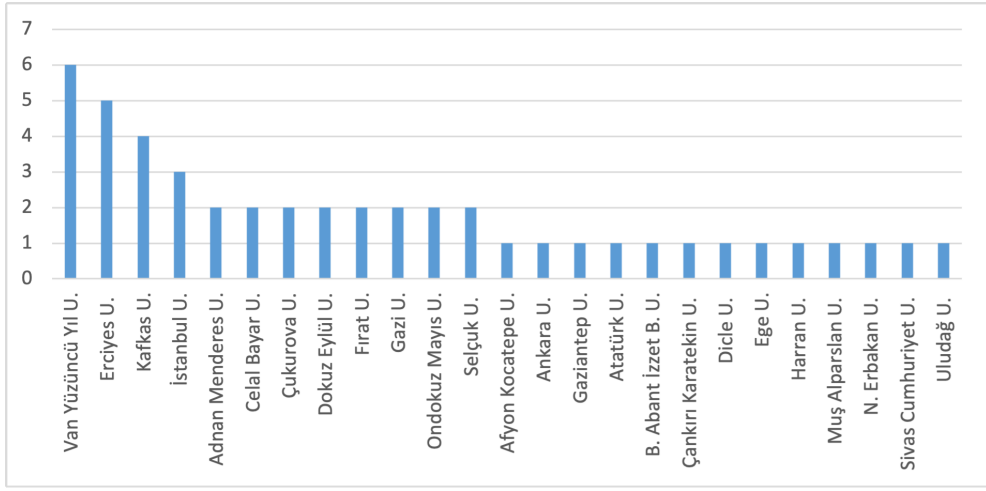
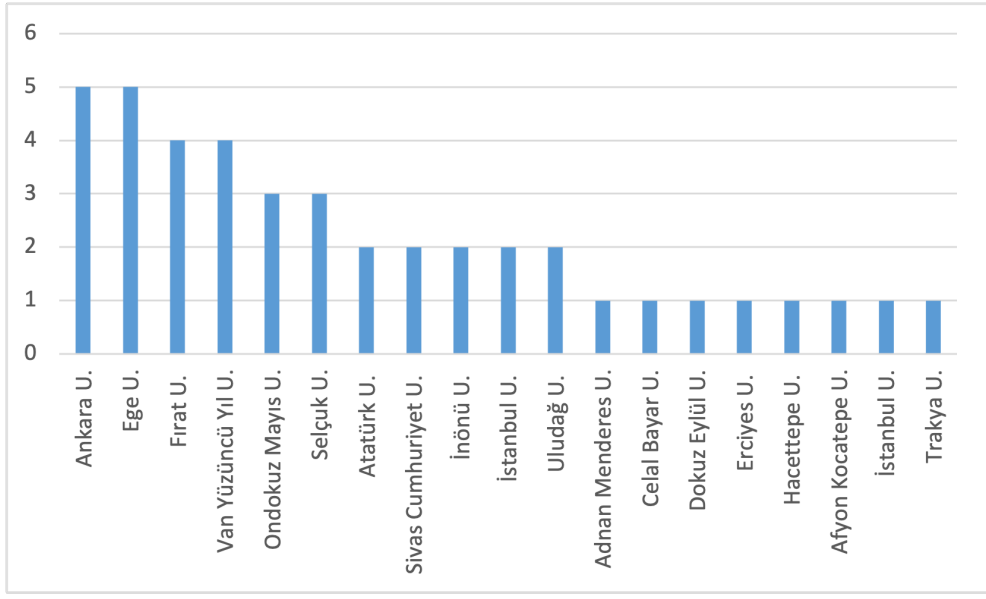
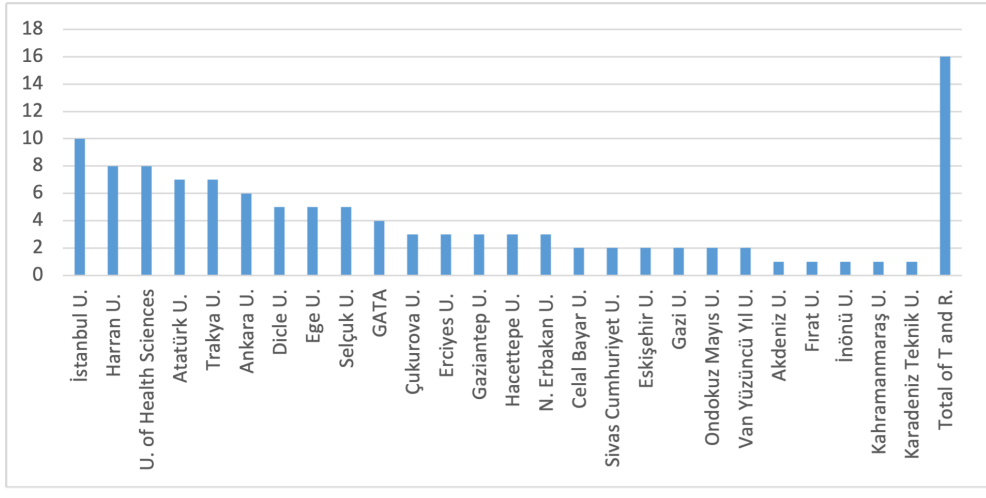
Supplement 1. Distribution of master's theses by province

Supplement 1. Distribution of PhD theses by province

Supplement 1. Distribution of medical specialization theses by province







Supplement 2. Distribution of master's theses by universities

Distribution of PhD theses by universities.

Distribution of medical specialization theses by universities.

B. Abant İzzet B. U.: Bolu Abant İzzet Baysal University, N. Erbakan U.: Necmettin Erbakan University, GATA: Gülhane Military Medical Academy, N. Erbakan U.: Necmettin Erbakan University, Kahramanmaraş U.: Kahramanmaraş Sütçü İmam University, Total of T and R.: Total of Training and Research Hospitals

Turkiye Parazitol Derg 2025;49(2):82-7
DOI: 10.4274/tpd.galenos.2025.36854

Effects of *Lucilia sericata* Larval Secretion on *Echinococcus granulosus*

Lucilia sericata Larva Sıvısının Echinococcus granulosus Üzerine Etkileri

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Cite this article as: Aldı Fİ, Tabakçıoglu K, Polat E, Şakru N. Effects of *Lucilia sericata* larval secretion on *Echinococcus granulosus*. Turkiye Parazitol Derg. 2025;49(2):82-7.

ABSTRACT

Objective: *Echinococcus granulosus* causes echinococcosis when its larvae settle in various organs, especially the liver and lung, of humans and herbivorous animals such as sheep and cattle. Echinococcosis are endemic in Mediterranean countries including Türkiye, the Middle East and South Africa. Echinococcosis treatments are generally surgical excision or benzimidazoles. Maggot debridement therapy is a wound treatment with *Lucilia sericata* larvae. While the larvae consume the old-damaged tissues and bacteria in the wound site, the maggots secrete an antimicrobial fluid. In this study, we aimed to determine the effect of *Lucilia sericata* larval secretions on *Echinococcus granulosus* protoscoleces *in vitro*.
Methods: Various DNA damage markers were used to analyze the therapeutic potential of the larval secretions. For this purpose, protoscoleces were cultured and treated with different concentrations of larval secretions. Comet test was performed to determine DNA damage. Expression of *EgATM, EgRad9* and *EgTopo2a* genes was analyzed by quantitative real time polymerase chain reaction.
Results: The viability of the control group was 94% and the viability of the protoscoleces treated with larval secretions was 73%. Comet test showed that larval secretions caused DNA damage in protoscoleces. According to quantitative real time polymerase chain reaction results; 1:1 larval secretions increased *ATM* and *Rad9* gene expression 3.2-fold and *Topo2a* gene expression 2.2-fold compared to control groups ($p<0.05$).
Conclusion: These data showed that *in vitro* larval secretion induced DNA damage in *Echinococcus granulosus* protoscoleces and increased the expression of *EgATM, EgRad9* and *EgTopo2a* genes. However, further *in vitro* and *in vivo* studies are needed.
Keywords: *Echinococcus granulosus*, *Lucilia sericata*, DNA damage, Comet assay, gene expression

ÖZ

Amaç: *Echinococcus granulosus*'un larvaları, insanlarda ve koyun, sığır gibi otçul hayvanlarda özellikle karaciğer ve akciğer gibi çeşitli organlara yerleşerek ekinokokkozise neden olur. Ekinokokkozis, Türkiye dahil olmak üzere Akdeniz ülkeleri, Orta Doğu ve Güney Afrika'da endemiktir. Ekinokokkozis tedavileri genellikle cerrahi eksizyon veya benzimidazoller ile yapılmaktadır. *Lucilia sericata* larvaları ile uygulanan Maggot debridman tedavisi, bir yara tedavi yöntemidir. Larvalar, yara bölgesindeki eski ve hasar görmüş dokuları ve bakterileri elimine ederken, antimikrobiyal bir sıvı salgılar. Bu çalışmada, *Lucilia sericata* larval sekresyonlarının *Echinococcus granulosus* protoskoleksleri üzerindeki etkisini *in vitro* olarak belirlemeyi amaçladık.
Yöntemler: Larval sekresyonların tedavi potansiyelini analiz etmek için çeşitli DNA hasarı belirteçleri kullanıldı. Bu amaçla, protoskoleksler kültüre alındı ve farklı konsantrasyonlarda larval sekresyonlar ile muamele edildi.
Bulgular: DNA hasarını belirlemek için Comet testi uygulandı. *EgATM*, *EgRad9* ve *EgTopo2a* genlerinin ekspresyonu, kantitatif gerçek zamanlı polimeraz zincir reaksiyonu ile analiz edildi. Kontrol grubunda canlılık oranı %94 iken, larval sekresyonlar ile muamele edilen protoskolekslerde canlılık oranı %73 olarak belirlendi. Comet testi, larval sekresyonların protoskolekslerde DNA hasarına yol açtığını gösterdi. Kantitatif gerçek zamanlı polimeraz zincir reaksiyonu sonuçlarına göre; 1:1 oranında larval sekresyon, *ATM* ve *Rad9* gen ekspresyonunu kontrol gruplarına kıyasla 3,2 kat, *Topo2a* gen ekspresyonunu ise 2,2 kat artırdı ($p<0,05$).
Sonuç: Bu veriler, *in vitro* olarak larval sekresyonların *Echinococcus granulosus* protoskolekslerinde DNA hasarını indüklediğini ve *EgATM*, *EgRad9* ve *EgTopo2a* genlerinin ekspresyonunu artırdığını göstermiştir. Ancak, bu sonuçların desteklenmesi için daha fazla *in vitro* ve *in vivo* çalışmalara ihtiyaç vardır.
Anahtar Kelimeler: *Echinococcus granulosus*, *Lucilia sericata*, DNA hasarı, Comet testi, gen ekspresyonu

Received/Geliş Tarihi: 11.12.2024 **Accepted/Kabul Tarihi:** 22.04.2025 **Publication Date/Yayınlanma Tarihi:** 18.07.2025

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***One of the authors of this article (N.Ş.) is a member of the Editorial Board of this journal. She was completely blinded to the peer review process of the article**

Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Echinococcus granulosus is a parasite that causes echinococcosis when its larvae settle in various organs and tissues, especially the liver and lungs, of humans and herbivorous animals such as sheep and cattle (1, 2). Echinococcosis is found on every continent except Antarctica. It is endemic in tropical and subtropical regions, especially where livestock farming is widespread, such as Mediterranean countries, Türkiye, the Middle East, South America, New Zealand, and South Africa. In endemic areas, incidence rates can be 50/100.000 or more (3).

The disease is treated by surgery, punction-aspiration-injection-reaspiration method or chemotherapy. In chemotherapy, benzimidazoles are used, particularly albendazole (ABZ). ABZ is a benzimidazole derivative, and the active metabolite responsible for its anthelmintic effects is ABZ sulfoxide. Systemic absorption of the drug is low; therefore, high doses are required to treat the disease, which makes it difficult for the patient to tolerate the drug (4). Additionally, teratogenic and embryotoxic effects of the drug have been reported in experimental animal studies (5). ABZ is also used as a protoscolocidal agent, they are used during surgical and percutaneous treatment of echinococcosis against the risk of secondary cysts or recurrence (6). For this purpose, various chemical compounds and plant extracts have been frequently tested in recent years (3,7).

The use of *Lucilia sericata* larvae in wound treatment is known as maggot therapy. They provide wound healing by debridement and disinfection and by inducing the secretion of wound-healing stimulants (8). In addition, excretions/secretions (ES) secreted by larvae also show antimicrobial activity. Studies have proven that larval ES contain various proteinases, DNases and antimicrobial substances such as lusifensin and lusimycin (9-12). The effect on many Gram-positive and some Gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus,* various fungi and parasites of the genus *Leishmania* has been studied (8,13). Studies on cancer cell lines have shown that larval ES limits tumor growth and metastasis and causes increased expression of apoptotic genes, consequently affecting eukaryotic cells at the molecular level (14,15).

Cellular DNA undergoes physical and/or chemical changes due to various factors such as free oxygen radicals, intracellular stresses, ultraviolet radiation and ionizing radiation (X-ray), resulting in DNA damages (single or double-strand breaks, base changes, base dimers and/or nucleotide losses or gains). DNA damage can be detected by various methods, such as Comet and TUNEL (16). In addition, gene expression is considered to be the best means of identifying DNA damage, and quantitative real time-polymerase chain reaction (qRT-PCR) has been the most widely used method for this. Gene regions such as *ATM, ATR, Rad9, p53* and *Topo2* have been used in various studies for this purpose (17-19).

In this study, we aimed to determine the effect of Lucilia sericata larval secretions on Echinococcus granulosus protoscoleces *in vitro* and whether the larval secretions can be used as treatment option. Various DNA damage markers were used to analyze the therapeutic potential of the larval secretions.

METHODS

Collection of Cyst Fluid and Determination of Viability

This study was conducted with the approval of Trakya University Faculty of Medicine Scientific Research Ethics Committee (no: TÜTF-BAEK-2021/368, date: 20.09.2021). The study does not require informed consent.

One hydatid cyst material used in our study was obtained fresh from newly slaughtered sheep at Edirne Meat and Meat Products Integrated Facility, Industry and Commerce Company Slaughterhouse. Cyst fluid was obtained based on the method of Smyth and Davies (20). The cyst contents were transferred to sterile falcon tubes and kept at room temperature for 30 min to allow the protoscoleces to settle. At the end of the time, the upper liquid was discarded, and the cyst material at the bottom of the tube was used for viability determination with 0.1% eosin.

This study was conducted with the approval of Trakya University Faculty of Medicine Scientific Research Ethics Committee (no: TÜTF-BAEK-2021/368, date: 20.09.2021). The study does not require informed consent.

Protoscolex Culture

The culture of *E. granulosus* protoscoleces was performed by modifying the method of Smyth and Davies (20). Briefly, the cyst material was washed 4 times with Hank's Balanced Salt Solution, and after each washing, protoscoleces were allowed to settle at room temperature for 30 min. In the last step, after waiting for 30 minutes, the upper liquid was discarded, replaced with RPMI medium (maintenance medium; RPMI 1640 with 20% FBS, 1% penicillin-streptomycin solution), and washed for 10 minutes; the medium was renewed at the end of the time. This mixture was distributed into 5 flasks, with the number of protoscolices per mL being equal to each flask (approximately 1000 protoscolices per mL). RPMI medium was added to each flask to a final volume of 5 mL, and the flasks were incubated in a 37 °C CO_2 incubator for 24-48 hours.

Preparation and Application of Larval Secretion

Lucilia sericata larvae were obtained from the insectarium of İstanbul University-Cerrahpaşa Traditional and Complementary Medicine Research and Development Center. Larval secretion was prepared according to the method of Akbas et al. (21). Briefly, second and third-stage approximately 2000 sterile larvae were placed in a sterile beaker, and 5 mL of distilled water was added to them. 5 mL of distilled water was added at one-hour intervals for 5 hours, and the larvae were allowed to release their secretions into the water. After all, 30 mL of larval ES collected. After the process, the liquid was centrifuged at 1.500 rpm for 10 min, and the supernatant was separated for use. After this liquid was sterilized by filtration, various dilutions were prepared, and 5 mL each of 1:1, 1:2, and 1:4 dilutions were added to the flasks. ABZ solution at a concentration of 20 µg/mL was added to the fourth flask as a positive control. Nothing was added to the fifth flask and used as a negative control. After treatment, the flasks were incubated at 37 °C for 5 hours for Comet test and microscopic examination and 24-48 hours for qRT-PCR.

Comet Test

Comet test was performed by modifying the method of Singh et al. (22). Briefly, 5 hours after the application, the samples

taken from the flasks were mixed with 0.75% low melting point agarose and spread on slides previously coated with 1.5% normal melting point agarose. The slides were first kept in a lysis solution for 2 hours and then in an alkaline electrophoresis buffer for 1 hour. At the end of the time, electrophoresis was started by setting the current to 300 mA, voltage to 24 V and time to 1 hour. After electrophoresis, the slides were stained with ethidium bromide and visualized under a fluorescence microscope (Olympus BX51, 40x magnification). The results of the Comet test were evaluated from Grade 0 to +4 with a visual scoring diagram from Noroozi et al. (23); Grade 0 no damage (<5%), Grade 1 low damage (5-25%), Grade 2 medium damage (25-45%), Grade 3 high damage (45-70%) and Grade 4 very high damage (>70%). Collins method was used to calculate the total score (24): 50 comets are scored each flask (total score was between 0-200), and each comet assigned a value of 0 to 4 according to its class and total score calculated.

qRT-PCR

For the detection of *EgATM* (*EGR_01160*), *EgRad9 (EGR_01432)* and *EgTopo2a* (*EGR_04559*) gene expressions of *E. granulosus* in samples taken from treated flasks, qRT-PCR was performed based on the method of Higuchi et al. (25). Each experiment was carried out in triplicate. RNA isolation was performed according to the recommendations of the kit manufacturer (QuickEx Total RNA Extraction Kit, Nucleogene, Türkiye). Then, the cDNA of each group was synthesized using the isolated RNAs according to the instructions of the kit manufacturer (cDNA Synthesis Kit with RNase Inh.-High Capacity, A.B.T., Türkiye). Gene-specific primer sequences were designed (Table 1) and these were synthesized commercially (Genesuz, Türkiye). Applied Biosystems Quantistudio 6 Flex real-time PCR instrument (Thermo Fisher Scientific, USA) was set up according to kit instructions (qPCR Probe Master Mix-2X kit, Nucleogene, Türkiye) and qRT-PCR was performed. As a result of the process, gene expressions of EgATM, EgRad9 and EgTopo2a of *E. granulosus* were calculated by the ΔΔCt method, based on Ct values (26).

Statistical Analysis

GraphPad Prism 9 statistical software was used to perform statistical analyses. Comparisons between two groups were analyzed by using t-test, and the comparison between multiple groups was analyzed by using One-Way ANOVA. $P<0.05$ indicated that the difference was statistically significant.

RESULTS

Implementation Results

The viability rate of the hydatid cyst material, in which protoscolex was detected on direct microscopic examination and included in the study, was 95%.

When the culture flasks were applied and microscopically examined 5 hours later, the viability rates were 73% for larval ES 1:1, 80% for larval ES 1:2, 86% for larval ES 1:4, 70% for ABZ and 94% for control.

Comet Test

According to the results of the Comet test performed 5 hours after the application, total scores were 2 for control, 120 for larval ES 1:1, 90 for larval ES 1:2, 63 for larval ES 1:4 and 127 for ABZ. Grade 4 damage was observed mainly in Larval ES 1:1 application (36%), Grade 0 damage was observed mainly in Larval ES 1:2 and Larval ES 1:4 applications (31.3% and 37.3%, respectively), and Grade 3 damage was observed mainly in ABZ application (36.9%). In the control group, the majority was Grade 0 damage (96.8%), and no Grade 2, Grade 3 and Grade 4 damage was observed. The larval ES 1:1 group had the most Grade 4 damage (36%).

qRT-PCR

EgATM gene expression increased 3.2, 2.6, 1.8 fold in Larval ES dilutions 1:1, 1:2, 1:4 respectively and 4.5-fold in ABZ treated protoscoleces. Gene expression in protoscoleces from all treatment groups was statistically significant compared to the gene expression in control group protoscoleces ($p<0.05$).

EgRad9 gene expression increased 3.2, 1.4, 1.8 fold in Larval ES dilutions 1:1, 1:2, 1:4 respectively and 13.5-fold in ABZ treated protoscoleces. Gene expression in protoscoleces from all treatment groups was statistically significant compared to gene expression in control group protoscoleces ($p<0.05$).

EgTopo2a gene expression increased 2.2, 1.3, 1.6 fold in Larval ES dilutions 1:1, 1:2, 1:4 respectively and 12.9-fold in ABZ treated protoscoleces. Gene expression in protoscoleces from all treatment groups was statistically significant compared to the gene expression in control group protoscoleces ($p<0.05$). All gene expression increases are given in the Table 2.

DISCUSSION

E. granulosus, which causes hydatid cyst disease, is a prevalent parasite worldwide, threatening public health and causing economic losses by causing diseases in humans and animals

Table 1. Gene-specific primer sequences

Gene/gene ID	Primer sequences
EgATM 36336875	Forward primer: 5'- GTT CCT ACA GTC CAT CCT AAT- 3' Reverse primer: 5'- CTC CAT CAA GCC AGC ATT- 3';
EgRad9 36337147	Forward primer: 5'- ACC TGT CAA ATC GTG ATG TCG T- 3' Reverse primer: 5'- TCA CTT CAG TTT GAC TTG GCC T- 3';
EgTopo2a 36340274	Forward primer: 5'-CTT ACC ACT GCG GCC AAG TC- 3' Reverse primer: 5'- GCC ACC AGG GGT ATC AGA GT- 3'
Egβ-actin 36344784	Forward primer: 5'-GCG ATG TAT GTA GCT ATC CAG GCA GTG CTC TCG CT- 3' Reverse primer: 5'-CAA TCC TCC AGA CAG AGT ATT TGC GTT CCG GAG GA- 3'

Table 2. Gene expression increases

Gene/gene expression increase (fold)	Larval ES 1/1	Larval ES 1/2	Larval ES 1/4	ABZ
EgATM	3.2	2.6	1.8	4.5
EgRad9	3.2	1.4	1.8	13.5
EgTopo2a	2.2	1.3	1.6	12.9

ABZ: Albendazole

(6). Hydatid cysts are an important health problem in Türkiye, and cases are reported from all over the country. According to Ministry of Health data, the number of cases has increased in recent years; while the number of cases was 408 in 2008, the number of cases was reported as 1.867 at the end of 2019 (27). The estimated incidence rate of hydatid cyst disease in Türkiye is 0,8-2,0/100.000 (28). The disease is usually asymptomatic for years, and when it reaches a specific size, symptoms appear due to pressure on adjacent organs. The disease is most commonly recognized when radiologic tests are performed for other purposes (2,3).

During surgical and percutaneous treatment, protoscolicidal agents are used by many clinicians against secondary cyst formation or recurrence due to cyst rupture. Especially in the last 50 years, a wide range of protoscolicidal agents such as chemical compounds, plant extracts or natural compounds have been studied, and the search for effective alternative treatments for *E. granulosus* has increased. ABZ is frequently used as a protoscolicidal agent during surgical and percutaneous procedures (7). Apart from ABZ, harmin (19), thymol (29) and carvacrol (30) were the most studied chemical compounds as protoscolicidal agents *in vitro*. Besides, extracts of various plants such as *Cinnamomum zeylanicum* (31), *Curcuma longa* (32) and *Nigella sativa* (33), which were studied as protoscolicidal agents, showed a 100% killing effect on protoscoleces depending on dose and time. As a result of our study, the viability rate of protoscoleces 5 hours after treatment was 73% for larval ES 1:1 and 70% for ABZ. Since ABZ is frequently used as a protoscolicidal agent, we used ABZ as a positive control in our study. However, we attribute the lower lethality of the application compared to the literature to its short incubation period.

Lucilia sericata larvae are used in maggot therapy, which is therapy for various ulcers such as diabetic foot, ischemic skin, and decubitus ulcers (8,34). Studies have revealed the antimicrobial activity of secretions obtained from larvae (11,12,35-39). According to the results of studies in the literature, Larval ES showed bactericidal and/or bacteriostatic effects against various Gram-positive and Gram-negative bacteria (37-39). Cerovský et al. (11) isolated lucifensin from larval ES and identified it as a key component of the antibacterial effect. Although there are fewer studies on the effect of larval ES on fungi, Pöppel et al. (12) succeeded in isolating lucimycin, the compound responsible for the antifungal effect. As a result of studies on fungi, the antifungal activity of larval ES against various opportunistic pathogenic fungi has also been proven (35,36).

Besides of these studies, the antiparasitic effect of larval ES on protozoan parasites of the genus *Leishmania* has been also proven (13,40,41). As a result of the studies first conducted by Polat et al. (13), many researchers have focused on the anti-leishmanial effects of larval ES. However, no other parasite other than *Leishmania* spp. has been studied in the literature.

In our study, *in vitro* protoscolicidal effects of larval ES were examined for the first time, and viability was found to be significantly reduced compared to the control. In addition, the protoscolicidal activity of larval ES was also determined by showing DNA damage by the Comet test and changes in gene expression levels by qRT-PCR.

Lu et al. (19) and Gong et al. (18) used the Comet test in their study in which they evaluated the antiparasitic activity and DNA damage mechanisms of harmin in *Peganum harmala* seeds against *E. granulosus*. The results of these studies showed that the nuclei of the protoscoleces in the control group were compact without tails, while the nuclei of the protoscoleces in the harmin-treated group formed tails. As a result of our study in which the *in vitro* activity of larval ES on *E. granulosus* protoscoleces was examined, it was found that larval ES caused damage in protoscolex DNA, similar to the data in the literature. In terms of total DNA damage score, ABZ (127/200) and larval ES 1:1 (120/200) caused the most damage. Ramírez et al. (42) and Oztas et al. (43) have shown the DNA damaging effect of ABZ, in our study, ABZ caused DNA damage in the protoscoleces, and the results of our study overlap with these studies. Average damage grade of larval ES 1:1 was 4 (36%), while the average damage grade of the control group was 0 (96.8%). Moreover, the group with the highest damage grade 4 was the larval ES 1:1 group, with 36%, followed by the larval ES 1:2 group, with 22.9% and the ABZ group, with 16%. In this context, larval ES causes more severe DNA damage depending on the concentration.

In studies with *E. granulosus*, genes encoding various proteins have been used to determine the gene expression of a chemical substance in the parasite (17-19,44). The *ATM, Rad9*, and *Topo2a* genes that we used in our study are genes that are involved in the identification of DNA damage, especially at cell cycle checkpoints. The results of our study showed that 1:1 dilution of larval ES increased the expression of *EgATM* and *EgRad9* genes 3.2-fold and *EgTopo2a* gene expression 2.2-fold compared to the control. Cabrera et al. (17) showed that the expression of *Rad9* increased in the oxidative damage of *E. granulosus* protoscolex DNAs. Gong et al. (18) and Lu et al. (19) investigated the effects of harmin on *E. granulosus* and reported that the expression levels of *EgATM, EgP53, EgRad54* and *EgTopo2a* genes increased in harmin-treated protoscoleces. These results are consistent with the results of our study and indicate that larval ES induces *ATM-Rad9-Topo2a* signaling pathways by causing DNA damage.

In the present study, the effects of *L. sericata* larval ES on *E. granulosus* protoscoleces *in vitro* were reported. As a result of the study, larval ES caused DNA damage by showing a protoscolicidal effect. It increased the expression of *ATM-Rad9-Topo2a* genes involved in the cell cycle. In conclusion, the efficacy of larval ES against *E. granulosus* has been determined, and further *in vitro* and *in vivo* studies are needed.

*Ethics

Ethics Committee Approval: This study was conducted with the approval of Trakya University Faculty of Medicine Scientific Research Ethics Committee (no: TÜTF-BAEK-2021/368, date: 20.09.2021).

Informed Consent: The study does not require informed consent.

Footnotes

*Authorship Contributions

Surgical and Medical Practices: F.İ.A., Concept: E.P., N.Ş., Design: E.P., N.Ş., Data Collection or Processing: F.İ.A., K.T., Analysis or Interpretation: K.T., E.P., N.Ş., Literature Search: F.İ.A., N.Ş., Writing: F.İ.A., N.Ş.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This work was supported by Trakya University Scientific Research Projects Commission (grant numbers: TUBAP-2022/26).

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