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First Morphological Identification of *Eimeria* spp. and *Cryptosporidium* spp. in Different Wild Rodent Species from Central and Northwest Iran

İran'ın Orta ve Kuzeybatı Bölgeleri'ndeki Farklı Yabani Kemirgen Türlerinde Eimeria spp. ve Cryptosporidium spp.'nin İlk Morfolojik Tanımlaması

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ABSTRACT

Objective: Wild rodents act as important hosts and reservoirs for both zoonotic and non-zoonotic pathogens, playing a key role in maintaining and transmitting infectious agents in nature. Their presence can lead to contamination of food and water sources, affecting both humans and animals.

Methods: This study examined 138 dead rodents from six species (*Microtus socialis*, *Rattus norvegicus*, *Mus musculus*, *Meriones libycus*, *Apodemus witherbyi*, and *Ellobius lutescens*) collected from three regions in Iran. Fecal samples were analyzed for *Eimeria* spp. and *Cryptosporidium* spp. using potassium dichromate cultivation and sugar flotation for *Eimeria*, and modified Ziehl-Neelsen staining for *Cryptosporidium*.

Results: The infection rate for *Eimeria* spp. was 5.79%, and the identified species included *E. falciformis*, *E. papillata*, *E. miyairii*, *E. musculoidei*, and *E. hungaryensis*. For *Cryptosporidium* spp., a 4.34% infection rate was observed. While *Eimeria* infections were limited to three rodent species, *Cryptosporidium* was detected in all six.

Conclusion: This study presents the first morphological identification of *Eimeria* species in rodents in Iran, with findings consistent with host-parasite relationships reported globally. Additionally, the widespread presence of *Cryptosporidium* spp. in multiple rodent species emphasizes the epidemiological importance of these animals as potential reservoirs of zoonotic pathogens. These results contribute to a better understanding of protozoan diversity and distribution in rodent populations of Iran.

Keywords: Rodent, *Emeria, Cryptosporidium*, Iran

ÖZ

Amaç: Yabani kemirgenler, hem zoonotik hem de zoonotik olmayan patojenler için önemli konakçı ve rezervuarlar olup, doğada enfeksiyöz etkenlerin devamlılığının sağlanması ve yayılmasında kilit rol oynamaktadır. Bu canlıların varlığı, hem insan hem de hayvanlar için gıda ve su kaynaklarının kontaminasyonuna neden olabilmektedir.

Yöntemler: Bu çalışmada, İran'ın üç farklı bölgesinden toplanan altı türe ait (*Microtus socialis, Rattus norvegicus, Mus musculus, Meriones libycus, Apodemus witherbyi* ve *Ellobius lutescens*) toplam 138 ölü kemirgen incelenmiştir. Dışkı örnekleri, *Eimeria* spp. için potasyum dikromat inkübasyonu ve şeker flotasyon yöntemi, *Cryptosporidium* spp. için ise modifiye Ziehl-Neelsen boyama yöntemi kullanılarak analiz edilmiştir.

Bulgular: *Eimeria* spp. enfeksiyon oranı %5,79 olarak belirlenmiş; tanımlanan türler arasında *E. falciformis, E. papillata, E. miyairii, E. musculoidei* ve *E. hungaryensis* yer almıştır. *Cryptosporidium* spp. için saptanan enfeksiyon oranı ise %4,34'tür. *Eimeria* enfeksiyonları yalnızca üç kemirgen türünde görülürken, *Cryptosporidium* altı türün tamamında tespit edilmiştir.

Sonuç: Bu çalışma, İran'daki kemirgenlerde *Eimeria* türlerinin ilk morfolojik tanımlamasını sunmakta olup, elde edilen bulgular dünya genelinde bildirilen konak-parazit ilişkileri ile uyumludur. Ayrıca, *Cryptosporidium* spp.'nin birçok kemirgen türünde yaygın olarak bulunması, bu hayvanların zoonotik patojenler için potansiyel rezervuarlar olarak epidemiyolojik önemini vurgulamaktadır. Elde edilen veriler, İran'daki kemirgen popülasyonlarında protozoon çeşitliliği ve dağılımının daha iyi anlaşılmasına katkı sağlamaktadır.

Anahtar Kelimeler: Kemirgen, *Eimeria, Cryptosporidium*, İran

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Türkiye PARAZİTOLOJİ Dergisi

INTRODUCTION

Rodents are among the most common reservoirs of zoonotic infections, and they play an important role in the spread of infectious diseases to people and domestic animals. They comprise 42% of the world's mammalian biodiversity and are the biggest order of extant mammals. They are remarkably diverse. Rodents are thought to be the most successful and adaptable group of mammals (1,2). *Eimeria* spp. and *Cryptosporidium* spp. are protozoan genera of significant concern due to their impact on human and animal health. *Cryptosporidium* species cause cryptosporidiosis, a gastrointestinal disease that can be particularly severe in immunocompromised individuals. In contrast, the *Eimeria* species are primarily associated with coccidiosis, which affects a wide range of animal hosts and leads to substantial economic losses in the livestock industry (3,4).

There are about 2,000 species of *Eimeria* known to exist in invertebrate hosts as well as mammals, birds, reptiles, and other vertebrates. *Eimeria* species are found worldwide because their hosts are widely dispersed; over 200 species of coccidia, especially *Eimeria* spp., have been identified in rodents. Most species of *Eimeria* are homoxenous, which means they only infect one type of host. Nonetheless, certain avian *Eimeria* species have been found to have a restricted oligoxenous host range, a trait that could aid in the spread of the parasite by allowing livestock and wildlife to become infected (5,6). On the other hand, the protozoan genus *Cryptosporidium* spp. can infect a wide range of hosts, including humans, domestic animals, and wildlife. The oocysts of *Cryptosporidium* spp. are highly resistant to environmental conditions and can persist for extended periods, enabling transmission through contaminated food and water. Numerous rodent species have been identified as hosts of *Cryptosporidium* spp., making them significant reservoirs of the parasite. They may actively contribute to the spread of zoonotic *Cryptosporidium* species and play a role in cryptosporidiosis in both humans and animals (7,8).

The primary objective of this research is to identify and characterize *Eimeria* spp. and *Cryptosporidium* spp. species present in rodents from selected regions. This study aims to expand existing knowledge on the epidemiology of these parasites within rodent populations. The research is conducted in the central and northwestern regions of Iran, specifically in the cities of Arak, Saqqez, and Urmia, where rodent populations are abundant and frequently interact with human habitats.

METHODS

A total of 138 rodents were collected from central and northwestern Iran, specifically from Arak (n=45), Saqqez (n=41), and Urmia (n=52). The rodents were trapped in grain warehouses, cereal pastures, or accidentally captured in homes by rodenticide poisoning (Table 1). All rodents were found dead in the traps before being transported to the laboratory. The samples were securely transported in sealed bags following biosecurity protocols. Following the collection, the taxonomic classification of the rodents was performed and recorded. After the dissection of the gastrointestinal tract, fecal samples were collected from the distal part of the intestine, where the feces had a distinct shape and consistency. These samples were transferred to a 2.5% potassium dichromate solution. Additionally, a portion of the fecal sample was prepared for smear examination and identification of *Cryptosporidium*.

Examination of *Eimeria* Oocysts

For this purpose, after fecal sample collection, a portion was placed in a 2.5% potassium dichromate preservative solution and stored at 4 °C in a refrigerator for 3 days to allow the oocysts to complete the sporulation process. After that, one slide was prepared from each sample using the direct smear method, and another was prepared using the sugar flotation method (9).

The *Eimeria* oocysts were identified using a light microscope at magnifications of ×10 and ×40, with each oocyst containing four sporocysts. After observing the target oocysts, further imaging and morphometric measurements of all oocyst samples on each slide were performed using a Dino-Eye digital camera. This camera, which allows simultaneous connection to a light microscope and a computer, facilitated detailed imaging at ×40 and ×100 magnifications using immersion oil.

To identify *Eimeria* species, images measured with Dino-Eye software were analysed based on key morphological features, including oocyst and sporocyst size, oocyst layers, and the presence of structures like micropyle, polar granules, and Stieda body and large or small refractile globules (6).

Examination and Identification of *Cryptosporidium*

To identify *Cryptosporidium* spp. in fecal samples, the modified Ziehl-Neelsen acid-fast staining method was employed. Two fecal smears were prepared for each sample following standard protocols. Under ×100 magnification, *Cryptosporidium* oocysts were observed as minute red granules within a green-blue background, indicative of the characteristic acid-fast staining properties of the parasite (10).

Table 1. Distribution of rodent species by city and capture location

Rodent species	Arak (n=45)	Saqqez (n=41)	Urmia (n=52)	Total (n=138)	Capture location
Mus musculus	18	15	16	49	Homes, Grain Warehouses
Rattus norvegicus	12	10	10	32	Homes, Grain Warehouses
Microtus socialis	7	8	15	30	Cereal Pastures
Apodemus witherbyi	3	4	3	10	Cereal Pastures, Grain Warehouses
Ellobius lutescens	3	2	5	10	Cereal Pastures
Meriones libycus	2	2	3	7	Cereal Pastures, Occasionally Grain Warehouses
Total	45	41	52	138	

Statistical Analysis

Descriptive statistical analyses were performed in the study. All data were entered into Microsoft Excel, and descriptive measures, including frequencies and percentages, were calculated using the software's built-in functions.

RESULTS

A total of 138 rodents were examined, and six species were diagnosed: *Microtus socialis, Rattus norvegicus, Mus musculus, Meriones libycus, Apodemus witherbyi,* and *Ellobius lutescens* (11). These species are presented in Table 2.

Out of 138 rodents, 8 (5.79%) were infected with *Eimeria* spp. Among these, 5 were male and 3 were female. The infected rodents included *M. musculus* (n=4), *R. norvegicus* (n=2), and *A. witherbyi* (n=2). One case of simultaneous infection with *Eimeria* spp. and *Cryptosporidium* spp. was observed in *A. witherbyi*. In the 8 positive samples for *Eimeria*, 5 species were diagnosed: *E. musculoidei* (Figure 1) from *M. musculus* (n=1), *E. falciformis* (Figure 2) from *M. musculus* (n=2), *E. papillata* (Figure 3) from *M. musculus* (n=1), *E. miyairii* (Figure 4) from *R. norvegicus* (n=2), and *E. hungaryensis* (Figure 5) from *A. witherbyi* (n=2).

Additionally, 6 (4.34%) out of 138 rodents were infected with *Cryptosporidium* spp. (Figure 6). Among these, 3 were male and 3 were female. The infected rodent species included *M. musculus, R. norvegicus, M. socialis, E. lutescens, and A. witherbyi* (Table 3).

DISCUSSION

This study examined the occurrence of *Emeria* spp. and *Cryptosporidium* spp. in wild rodents from western, central, and northern Iran, identifying a diverse range of coccidian parasites, including *Eimeria* species *E. musculoidei, E. falciformis, E. papillata, E. miyairii, and E. hungaryensis*.

Rodents serve as hosts for a wide range of *Eimeria* species, most of which exhibit strict host specificity, even among different rodent species. Most *in vivo* studies on *Eimeria* spp. have been conducted in rodents, such as the house mouse, due to economic considerations (3).

E. falciformis is reported as the most prevalent *Eimeria* species in *M. musculus*, infecting the epithelial cells of the cecum and upper colon. This species can cause acute coccidiosis in rodents. *E. papillata* is commonly used in laboratory studies on anticoccidial agents, as it specifically infects *M. musculus*. *Eimeria hungaryensis* is a host-specific coccidian that primarily infects the genus *Apodemus*, consistent with our study findings. This species cannot be transmitted to *M. musculus, Microtus arvalis, Clethrionomys glareolus,* or *Cricetus cricetus*. *Eimeria miyairii* is an uncommon species that infects the epithelial cells of the villi and, occasionally, the glands of Lieberkühn in the small intestine of the Norway rat (*R. norvegicus*), its primary host. This species is host-specific and cannot infect *M. musculus, Sylvilagus floridanus, Cavia porcellus,* or *Spermophilus tridecemlineatus. E. musculoidei* has been identified in the upper ileum of *Mus (Leggada) musculoides* (its primary host) in Africa and is also suspected to infect the house mouse (*M. musculus*) in Asia (6,12,13).

There are few reports on *Eimeria* spp. infection in rodents in Iran, and most studies have been limited to genus-level identification.

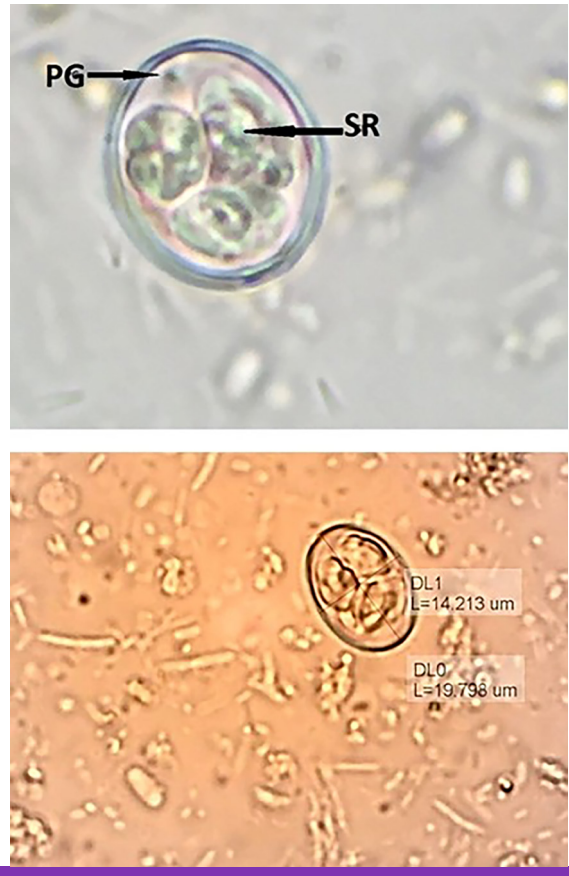

Figure 1. Oocysts of *E. musculoidiei* are spherical to ellipsoidal (16.9×19.7 µm; length-to-width ratio 1.16, average of 30 oocysts), with smooth, single-layered, pale yellow to brown walls (~1 µm thick), no micropyle, and 1-several polar granules (PG). Sporocysts are lemon-shaped (10.3×6.9 µm; length-to-width ratio 1.49), lack Stieda bodies, and contain coarse residual granules (SR). Host: *Mus musculus*

Table 2. Distribution of rodent species, including the number and percentage of males and females in the sample population (n=138)

No	Rodent species	N/%		Male		Female	
1	*Mus musculus*	49	35.50%	37	26.81%	12	8.70%
2	*Rattus norvegicus*	32	23.20%	20	14.49%	12	8.71%
3	*Microtus socialis*	30	21.73%	19	13.77%	11	7.97%
4	*Apodermus witherbyi*	10	7.25%	9	6.53%	1	0.73%
5	*Ellobius lutescens*	10	7.25%	5	3.63%	5	3.63%
6	*Meriones libycus*	7	5.07%	4	2.91%	3	2.17%
Total		138	100%	94	68%	44	32%

A study conducted in 2014 reported an *Eimeria* spp. infection rate of 22.5% in rodents from Meshkin Shahr, located in northwestern Iran (14). Additionally, a study conducted in Iraq in 2022 reported a 28% infection rate in *M. musculus* (15). Another study in Egypt reports 19.9% *Emeria* spp. infection in *Psammomys obesus* (16). However, Reports on *Eimeria* infections in rodents from Iran and neighboring countries remain limited. This study provides the first detailed morphological identification of *Eimeria* species in rodents from Iran.

The first detection of *Cryptosporidium* spp. in mice dates back to 1907. Since then, more than 40 species and over 120 genotypes of *Cryptosporidium* spp. have been identified (17).

Many of these species pose a significant threat to human health and economically important animals, with rodents serving as crucial reservoirs for their transmission. Rodents can act as reservoirs for highly prevalent *Cryptosporidium* species such as *C. parvum*, which infects both humans and animals, and *C. meleagridis*, which primarily infects birds (18,19). Additionally, rodents can facilitate the adaptation of certain rare *Cryptosporidium* species to humans, including *C. ditrichi, C. mortiferum, C. tyzzeri,* and *C. viatorum* (20).

Most studies on rodents focus on their role as reservoirs of zoonotic agents, with *Cryptosporidium* being one of the most significant. Rodents can serve as reservoirs for *Cryptosporidium*, easily spreading thick-walled oocysts into food and water sources (8). Reports indicate that approximately 17-20% of rodents worldwide are infected with *Cryptosporidium* spp., with *C. parvum* being the most commonly detected species. Among rodent hosts, muskrats are considered the most relevant reservoir (4,21). Based on the results, all rodent species involved in the study can act as reservoirs for *Cryptosporidium* spp.

Several studies have reported *Cryptosporidium* spp. infections in rodents in Iran. A study conducted in 2016 in the Torkaman Sahra Region identified *Cryptosporidium* spp. in 6.6% of *R. norvegicus* samples (22). In the same year, another study reported that only 3% of rodent samples tested positive for *C. parvum* in Ahvaz City (22), while *Cryptosporidium* spp. was detected in just 1% of *R. norvegicus* specimens in Tehran (23,24). However, meta-analyses suggest that the overall prevalence of *Cryptosporidium* in rodents across Iran ranges from 18% to 20%. These findings indicate that *Cryptosporidium* spp. infection rates in Iranian rodent populations are consistent with those reported in other regions worldwide, highlighting the potential role of rodents as reservoirs for this parasite (25,26).

Figure 2. Oocysts of *E. falciformis* are spherical to slightly ellipsoidal (18.3×28.2 µm; length-to-width ratio ~1.7, average of 30 oocytes), with a single-layered wall, no micropyle, and a Stieda body (SB). Sporocyst residuum (SR) are present, with sporocysts measuring 12.2×7.2 µm. Oocysts are capable of enlarging to a size range of 13-24×15-26 µm. Host: *Mus musculus*

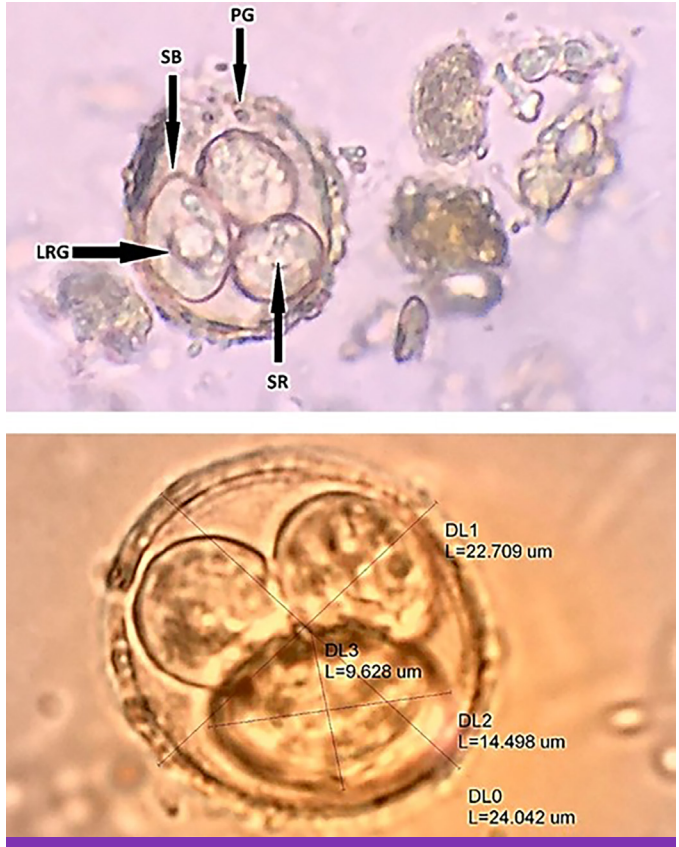

Figure 3. Oocysts of *E. papilata* are spherical to ellipsoidal, particularly at the poles, with a single-layered wall (~1.2 µm thick) and a yellowish-brown color. The wall may appear rough or coarse, sometimes exhibiting striations. Oocysts measure 19.2×22.4 µm (average of 30 oocysts), with a length-to-width ratio of ~1.16. They lack a micropyle, contain 1-3 oval-shaped polar granules (PG) and a large refractile globule (LRG), and possess Stieda bodies (SB) and sporocyst residuum (SR). Host: *Mus musculus*

Study Limitations

The results of this study were based on morphometric measurements and taxonomic identification keys. However, molecular methods could not be applied, which represents a limitation since such approaches could have provided more accurate confirmation and deeper insights. Furthermore, although the presence of *Eimeria* and *Cryptosporidium* species in rodents was identified, their geographical and regional distribution patterns were not evaluated. Future studies should incorporate molecular techniques and spatial analyses to provide a more comprehensive understanding of the epidemiology of these parasites.

CONCLUSION

In conclusion, the findings of this study provide the first detailed morphological descriptions of *Eimeria* species infecting rodents in Iran. The rodent host species identified in this study align with the results of similar studies conducted worldwide, further supporting the consistency of rodent species as hosts for various *Eimeria* species across different geographic locations. The results of *Cryptosporidium* spp. infection in terms of species were consistent with previous studies conducted in Iran and

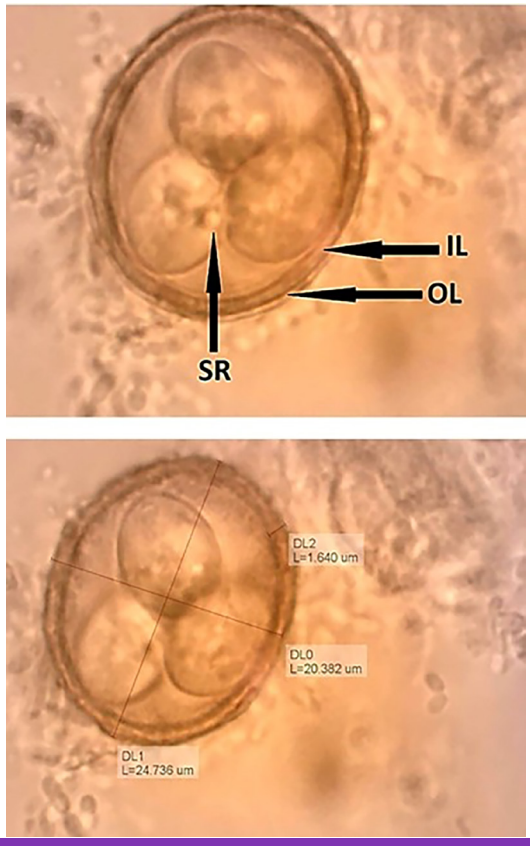

Figure 4. Oocysts of *E. miyairii* are spherical with a thick, rough, yellowish-brown wall, measuring 24.73 × 20.38 µm, an average of 30 oocysts. Sporocysts measure 11.23×8.6 µm. The wall is ~1.6 µm thick, radial, and striated, with two layers (the inner layer, IL, thicker than the outer layer, OL, and sometimes striated). Oocysts lack micropyle, polar granules, and oocystic remnants but contain sporocystic remnants (SR). Host: Rattus norvegicus

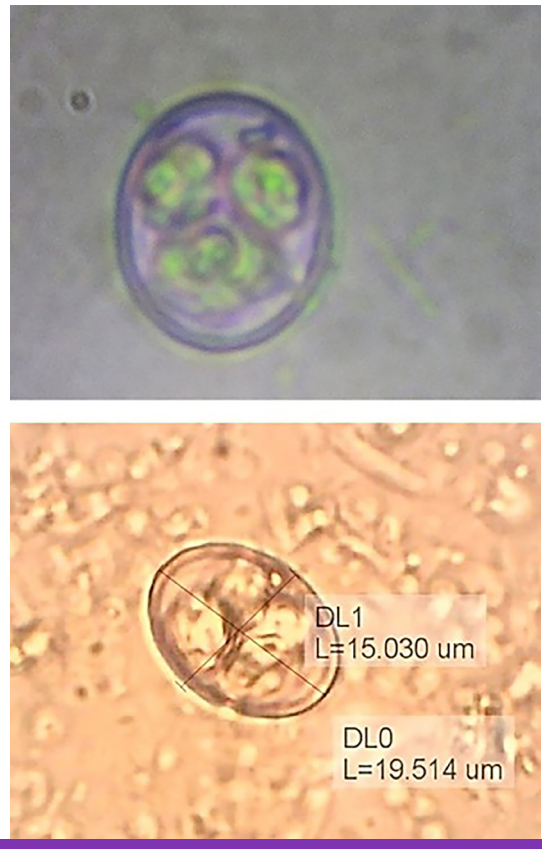

Figure 5. Oocysts of *E. hungaryensis* are spherical, with an average size of 18×20 µm. The wall is single-layered, thick, and yellowish-brown, occasionally appearing rough or coarse. They lack micropyle, oocystic remnants, and polar granules. In unsporulated oocysts, the internal space is filled with sporont granules. Host: *Apodemus witherbyi*

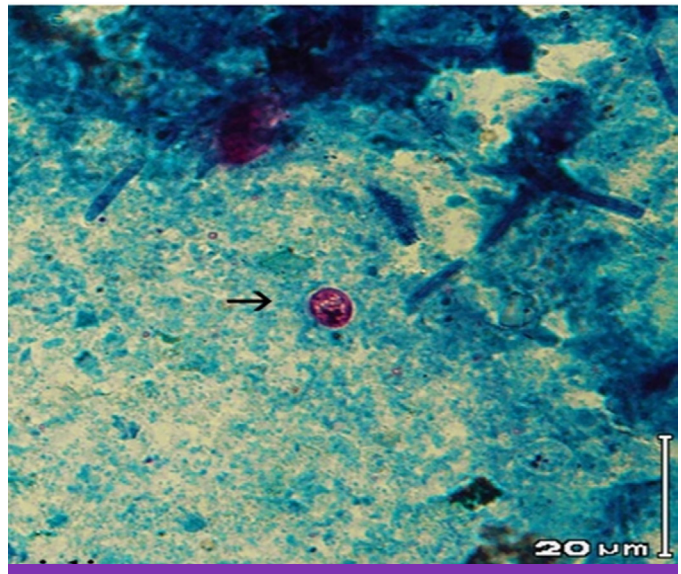

Figure 6. Oocyst of *Cryptosporidium* spp. in modified Ziehl-Neelsen staining: Oocyst is egg-shaped or spherical (4.35 µm), with a smooth wall, lacking a micropyle and sporocysts. They possess oocystic remnants and contain long, crescent-shaped sporozoites that are somewhat banana-shaped. Host: *Mus musculus*

Table 3. Infection rates of *Eimeria* spp. and *Cryptosporidium* spp. in different rodent species

Rodent species	*Eimeria* spp. (n=)	*Eimeria* spp. (%)	*Cryptosporidium* spp. (n=)	*Cryptosporidium* spp. (%)
Mus musculus	4	2.90%	1	0.73%
Rattus norvegicus	2	1.45%	1	0.73%
Apodemus witherbyi	2	1.45%	1	0.73%
Microtus socialis	0	0%	1	0.73%
Ellobius lutescens	0	0%	1	0.73%
Total	8	5.79%	6	4.34%

worldwide. However, the infection rate observed in this study was lower than the average rates reported in Iran, which could be attributed to factors such as the number of cases examined or the lack of molecular diagnostic techniques in this study.

*Ethics

Ethics Committee Approval: This study did not require ethical approval, as no live animals were harmed or directly handled during the research process.

Informed Consent: Not necessary.

Footnotes

*Authorship Contributions

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

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

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