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Original Article

The prevalence of *Cryptosporidium spp.* in sheep and goats in Urmia, Iran

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Abstract

Cryptosporidiosis, a zoonotic disease caused by *Cryptosporidium spp*., affects both domestic animals and humans. In newborn ruminants, it often manifests as outbreaks of diarrhea, leading to significant morbidity and economic losses. This study aimed to determine the prevalence of *Cryptosporidium spp*. infection in small ruminants in Urmia, Iran. A total of 560 small ruminants from seven farms located in seven villages around Urmia were examined across four seasons. *Cryptosporidium* oocysts were concentrated using formalin-ether sedimentation method, and oocysts were identified through a modified Ziehl-Neelsen staining procedure. Of the 560 animals, 67 (11.96%) tested positive for *Cryptosporidium spp*. Sheep exhibited a higher prevalence (17.86%) compared to goats (6.07%). Statistical analysis revealed significant associations between infection prevalence and factors such as fecal consistency, age, sex, and season. These findings underscore the potential zoonotic threat of *Cryptosporidium spp*. in the region, indicating the need for public health awareness and effective management strategies in small ruminant farming.

Key words: *Cryptosporidium*, Prevalence, Small ruminants, Urmia, Iran

Introduction

Cryptosporidiosis, caused by protozoan parasites of the genus *Cryptosporidium*, is a globally important zoonotic disease affecting humans and a wide range of vertebrate hosts, including mammals, birds, and reptiles (Roellig and Xiao 2020). These apicomplexan parasites are responsible for gastrointestinal illness characterized by diarrhea, which can be particularly severe in immunocompromised individuals such as those with HIV/AIDS (Gerace, Presti et al, 2019).

Molecular studies have identified nearly 40 *Cryptosporidium* species and more than 50 genotypes, with *C. hominis* and *C. parvum* being the primary etiologic agents of human infection (Kabira, et al, 2020; Roellig and Xiao, 2020). *C. parvum* is also a major pathogen in livestock, commonly associated with neonatal diarrhea in ruminants such as calves, lambs, and goat kids (Constable, Hinchcliff et al, 2017; Mammeri, et al, 2019). Infected animals shed large numbers of environmentally resistant oocysts, which contaminate water, food, and soil, facilitating transmission between animals and humans (Santin, 2020).

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Small ruminants, including sheep and goats, are increasingly recognized as important reservoirs of zoonotic *Cryptosporidium* species (Robertson, 2009; Mi, et al, 2014), particularly in rural and peri-urban areas where close contact with humans enhances the risk of cross-species transmission. In these hosts, infection is primarily associated with neonatal diarrhea, leading to dehydration, poor growth, and sometimes death, thereby causing substantial economic losses through reduced productivity and increased treatment costs (Constable, et al, 2017; Mammeri, et al, 2019).

The persistence of oocysts in the environment perpetuates the infection cycle and underscores the significance of *Cryptosporidium* as a continuing threat to both animal and public health, warranting further investigation. Despite the importance of cryptosporidiosis in small ruminants, there remains a significant gap in knowledge regarding the prevalence and distribution of *Cryptosporidium spp*. infections in sheep and goats, particularly in regions like the northwest of Iran. Urmia, the capital of West Azerbaijan province, has a large population of small ruminants, but limited studies have investigated the prevalence of *Cryptosporidium* infections in these animals in this region. Therefore, the present study was undertaken to determine the prevalence of *Cryptosporidium spp.* infections in sheep and goats in Urmia and its surrounding areas. Additionally, the study sought to identify potential risk factors associated with infection, such as the presence of diarrhea, age, sex, and seasonality.

Materials and Methods

Ethical Approval

The study protocol was reviewed and approved by the Bioethics and Animal Welfare Commission of the Faculty of Veterinary Medicine, Urmia University, Iran (IR-UU-AEC-33/75). All animal handling and sample collection procedures adhered to ethical guidelines for the humane treatment of animals.

Study Design

This cross-sectional study was conducted in Urmia and its surrounding rural areas, located in the northwest of Iran, from January 2022 to 2023. Urmia, with a population of over 600,000 people, is the capital of West Azerbaijan province and lies at an altitude of 1368 meters above sea level. The region experiences a semi-arid climate, characterized by hot, dry summers and cold winters, with an Annual humidity ranges from 52% to 64%, average annual temperature of 8.9°C and average annual precipitation of 238.2 mm (Sima, et al, 2013; Mousavi, et al, 2024). The study area is predominantly rural, with small ruminant farming being a key agricultural activity. Sheep and goats are raised in various smallholder farms, often in close contact with humans and other animals.

Sample Collection

A total of 560 small ruminants, consisting of 280 sheep and 280 goats, were included in the study. The animals were selected from seven farms in the rural outskirts of Urmia, distributed across three geographical areas: (1) Northern villages, including Nazloo, Anzal, and Somay; (2) Central (nearby) villages, including Tape-Ghalee and Barandaz; and (3) Southern villages, including Margvar and Silvane. Ten sheep and ten goats were randomly selected from each of the villages during each of the four seasons—spring, summer, autumn, and winter—resulting in a total of 560 fecal samples.

Fresh fecal samples (5–10 grams) were collected directly from the rectum of each animal. Prior to sample collection, general data were recorded, including the presence of diarrhea, the animal's age (classified as ≤1 year or >1 year), and sex (male or female). The collected fecal samples were immediately placed in disposable containers and transported to the parasitology laboratory at the Faculty of Veterinary Medicine, Urmia University, for analysis.

Laboratory Analysis

Upon arrival at the laboratory, fecal samples were processed using the Clayton-Lane flotation method to isolate and identify *Cryptosporidium* oocysts. For each sample, 3 grams of feces were suspended in 42 ml of distilled water and left to settle for approximately one hour at room temperature. Afterward, 15 ml of the strained fecal suspension was transferred to a test tube and left to stand for 30 minutes to allow for sedimentation of larger particles. The supernatant was discarded, and a saturated sugar solution was added to the precipitate. The mixture was then centrifuged at 2000 rpm for 10 minutes to separate the oocysts from the remaining debris. The oocyst-rich supernatant was examined under a microscope for the presence of *Cryptosporidium* oocysts. Positive samples were then treated with a 2.5% potassium dichromate solution (Merk, Germany) and incubated at 28°C for 48 hours, with the suspension aerated three times daily to encourage oocyst sporulation (Hendrix and Robinson, 2016).

The oocysts were examined daily for sporocyst formation under a light microscope. The morphological characteristics of the oocysts, including shape, wall structure, color, and sporocyst residue, were recorded. Additionally, micrometry (oocyst length, width, and shape index) was performed using Image Focus software to assist in species identification, following Soulsby's (1986) identification key (Soulsby, 1986).

Statistical Analysis

Data were analyzed using SPSS version 17 (SPSS Inc., Chicago, IL, USA). The Chi-square test was used to determine the association between *Cryptosporidium* infection and various factors, including fecal consistency (diarrhea or no diarrhea), age, sex, and season. A significance level of ($P<0.05$) was considered statistically significant.

Results

Prevalence of *Cryptosporidium* Infection

Of the 560 small ruminants sampled, 67 animals (11.96%) were found to be infected with *Cryptosporidium spp*. The infection rate was significantly ($P<0.05$) higher in sheep (50/280, 17.86%) compared to goats (17/280, 6.07%).

Factors Associated with Infection

The prevalence of *Cryptosporidium* infection varied significantly according to several factors, including fecal consistency, age, sex, and season. As shown in (Table 1), the prevalence of infection was significantly higher in animals with diarrhea (22.63%) compared to those without diarrhea (8.51%) ($P<0.05$).

Table 1: The prevalence of *Cryptosporidium spp*. infection in sheep and goats in Urmia according to feces status, sex, and age

Animals	Feces status		Age (year)		Sex	
	Normal	Diarrhea	≤1	>1	Female	Male
No. of examined sheep	193	87	132	148	134	146
No. of infected sheep (%)	26(13.47)[a]	24(27.59)[b]	36(27.27)[a]	14(9.46)[b]	11(8.21)[a]	39(26.71)[b]
No. of examined goats	230	50	144	136	113	167
No. of infected goats (%)	10(4.35)[a]	7(14.00)[b]	14(9.72)[a]	3(2.21)[b]	3(2.65)[a]	14(8.38)[b]
No. of total examined	423	137	276	284	247	313
No. of total infected (%)	36(8.51)[a]	31(22.63)[b]	50(18.12)[a]	17(5.99)[b]	14(5.67)[a]	53(16.93)[b]

[a] and [b] Statistically significant ($P<0.05$)

Age was another significant factor influencing the prevalence of *Cryptosporidium* infection. Infection was more common in animals ≤1 year old (18.12%) than in older animals (>1 year) (5.99%) (P<0.05).

Sex also played a role in infection rates. Male animals had a significantly higher prevalence of *Cryptosporidium* infection (16.93%) than females (5.67%) (P<0.05).

Seasonality was another important factor influencing infection rates. According to (Table 2) the highest prevalence of *Cryptosporidium* was observed in the spring (23.57%) and summer (15.00%), while the lowest prevalence occurred in autumn (6.43%) and winter (2.86%) (P<0.05).

Table 2: The prevalence of *Cryptosporidium spp.* **infection in sheep and goats in Urmia according to season**

Animals	Season			
	spring	summer	autumn	winter
No. of examined sheep	70	70	70	70
No. of infected sheep (%)	23(32.86)^{a}	16(22.86)^{a}	7(10.00)^{b}	4(5.71)^{b}
No. of examined goats	70	70	70	70
No. of infected goats (%)	10(14.29)^{a}	5(7.14)^{b}	2(2.86)^{c}	0(0.00)^{c}
Total examined	140	140	140	140
Total infected (%)	33(23.57)^{a}	21(15.00)^{b}	9(6.43)^{c}	4(2.86)^{c}

$^{a, b}$ and ^{c} Statistically significant (P<0.05)

Discussion

Cryptosporidium is a significant parasitic protozoan responsible for cryptosporidiosis in both humans and a wide range of animal species. This protozoan, has demonstrated remarkable adaptability and resilience in various environmental conditions. Over the past few years, numerous studies have been conducted on *Cryptosporidium* across different regions globally (Young, et al, 2014; Ali Ahmed, et al, 2025). However, information regarding the prevalence of *Cryptosporidium* infection in small ruminants, as compared to cattle, remains scarce in Iran (Firoozi, et al, 2019; Haghi, et al, 2020). This represents the first reported evidence of *Cryptosporidium spp.* infection in small ruminants in Urmia, Iran.

The results of this study reveal that cryptosporidiosis is prevalent in sheep and goats in Urmia city, with infection rates of 11.42% and 2.50%, respectively. This demonstrates the widespread presence of *Cryptosporidium* in the region and underscores the potential public health risk associated with animal-to-human transmission. To accurately identify the strain of *Cryptosporidium* involved, molecular characterization using polymerase chain reaction (PCR) is required. Differences in *Cryptosporidium* prevalence between sheep and goats are quite common in epidemiological studies. Greater host susceptibility, flock management practices, and grazing behaviors can be the resions (Hatam-Nahavandi, et al, 2019; Chen, et al, 2022).

The prevalence of *Cryptosporidium* infection in sheep has been reported to vary considerably in both Iran and other regions worldwide, with rates ranging from 1.7% to 85% (Gharekhani, et al, 2014; Sadeghi Dehkordi, et al, 2016; Dalimi and Tahvildar, 2017; Yang, et al, 2021; Chen, et al, 2022; Papanikolopoulou, et al, 2022; Cheng, et al, 2024). In comparison to the previous studies, the prevalence of *Cryptosporidium* infection in sheep reported in this study is lower than that observed in Hamadan (43.04%) and Esfahan (19%), but higher than in Kerman (13.8%), Yazd (12.5%), Tehran (1.7%), Kurdestan (1.6%), Lorestan (5.8%), Mazandaran (4.1%), Fars (6.3%), Mashad (11%), and Bushehr (3.7%) (Fasihi Harandi and Fotouhi, 2008; Vahedi, et al, 2009; Heidari, 2011; Gharekhani, et al, 2014;

Sadeghi Dehkordi, et al, 2016; Shafieyan, et al, 2016; Dalimi and Tahvildar, 2017; Ghorbanzadeh, et al, 2024). A summary of the prevalence of *Cryptosporidium* infection in sheep from other countries is provided in Table 3.

While there is limited data on the prevalence of *Cryptosporidium* infection in goats across different regions of Iran, global reports indicate infection rates ranging from 0.00% to 72.5% (Romero-Salas, et al, 2016; Papanikolopoulou, et al, 2022; Cheng, et al, 2024). In this study, the prevalence of *Cryptosporidium* infection in goats was lower than that reported in Kerman (17.6%) (Fasihi Harandi and Fotouhi, 2008) and Kurdestan (18.86%) (Khezri and Khezri, 2013), but higher than that in Yazd (2%) (Firoozi, et al, 2019). A summary of the prevalence of *Cryptosporidium* infection in goats from other countries is presented in Table 3.

Table 3: The prevalence of *Cryptosporidium* infection in sheep and goats

Country	Sheep	N	Goats	N	Reference
Poland	10.1%	159	0.00%	91	(Majewska, et al, 2000)
Egypt	2.5%	120	-	-	(Mahfouz, et al, 2014)
Iraq	13.3%	45	17.7%	45	(Mahdi and Ali, 2002)
Tunisia	11.2%	89	0.00%	184	(Soltane, et al, 2007)
India	1.8%	55	-	-	(Maurya, et al, 2013)
New Guinea	2.2%	276	4.4%	228	(Koinari, et al, 2014)
Poland	19.2%	234	37.1%	105	(Kaupke, et al, 2017)
Algeria	14.5%	62	8.7%	92	(Baroudi, et al, 2018)
Turkey	-	-	67.9%	112	(Sayin ipek, 2017)
China	-	-	11.4%	604	(Mi, et al, 2014)
Mexico	67.5%	80	72.5%	80	(Romero-Salas, et al, 2016)
Spain	30%	673	30%	184	(Castro-Hermida, et al, 2007)

The variability in the prevalence of cryptosporidiosis across different studies can be attributed to a range of factors, including regional climate conditions, breed differences, the immune status of the animals, environmental factors (e.g., humidity, temperature, rainfall), seasonal variations, laboratory methods and the sanitary conditions of livestock management (Constable, et al, 2017; Ghorbanzadeh, et al, 2024). Therefore, comparisons of prevalence rates of *Cryptosporidium* infection across different regions should be interpreted with caution, as aligning animal characteristics and management conditions across studies is inherently challenging.

To assess the relationship between animal’s risk factors and *Cryptosporidium* infection, the prevalence of infection was evaluated in relation to fecal status, age, sex, and season.

In this study, a history of diarrhea in the animals was significantly associated with *Cryptosporidium* infection. This suggests that diarrhea is a common clinical manifestation of *Cryptosporidium* infection in small ruminants and is likely a key indicator for diagnosis. This finding is consistent with the previous studies conducted on sheep and goats by Fasihi Harandi and Fotouhi (2008), Sayin Ipek (2017), Firoozi et al. (2019), Papanikolopoulou et al. (2022), and Ghorbanzadeh et al. (2024). *Cryptosporidium* is a well-established cause of neonatal diarrhea in small ruminants (Constable, et al, 2017). The higher the concentration of *Cryptosporidium* oocysts in the environment, the greater the risk of infection to animals. It is likely that the persistence of diarrhea in newborns, which can last up to two weeks, combined with the large number of oocysts shed into the environment by infected animals, contributes to the spread of *Cryptosporidium*. This infection can rapidly disseminate through direct contact between animals or through environmental contamination. Preventative measures such as avoiding overcrowding, isolating sick animals during diarrheal outbreaks, disinfecting facilities, and administering anti-protozoal treatments such as Halofuginone lactate and Paromomycin are essential for controlling the spread of infection and minimizing both morbidity and mortality rates in affected herds (Constable, et al, 2017; Thomson, et al, 2017).

Age was another significant factor in this study. Statistical analysis revealed that *Cryptosporidium* infection was more prevalent in animals under 1 year of age. This finding is consistent with the previous research (Fotouhi Ardakani, et al, 2008; Papanikolopoulou, et al, 2022; Ghorbanzadeh, et al, 2024). Younger animals are more susceptible to infection, while older animals develop immunity through repeated exposure, resulting in increased resistance to *Cryptosporidium* (Thomson, et al, 2017).

The higher number of positive cases among male animals may be attributed to their younger age, as males are typically kept for slaughter at a younger age.

In this study, the prevalence of infection in sheep and goats was higher during the spring and summer months. This finding aligns with that of Fasihi Harandi and Fotouhi (2008) and is likely linked to the lambing season, when newborn animals with cryptosporidial diarrhea are show a higher prevalence of cryptosporidial diarrhea, further contributing to the environmental contamination and transmission of *Cryptosporidium* (Fasihi Harandi and Fotouhi, 2008). Additionally, warmer temperatures and higher humidity during the spring and summer months in this region favor the survival and transmission of *Cryptosporidium* oocysts in the environment (King and Monis, 2007; Yang, et al, 2022).

In conclusion, this study provides important insights into the prevalence of *Cryptosporidium* infection in small ruminants in Urmia, Iran. The findings highlight the need for improved control measures to mitigate the spread of cryptosporidiosis, particularly in regions where small ruminant farming is prevalent. Management practices such as improved sanitation, and isolation and treatment of infected animals could help reduce the environmental load of oocysts and limit the transmission of *Cryptosporidium* to both animals and humans. Further studies are needed to explore the molecular epidemiology of *Cryptosporidium* in small ruminants and to identify potential interventions for controlling the disease.

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Competing Interests

The authors declare that there is no conflict of interest.

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بررسی میزان آلودگی گوسفندان و بزهای ارومیه به انواع *کریپتوسپوریدیوم*

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چکیده

کریپتوسپوریدیوزیس، یک بیماری مشترک بین انسان و دام است که توسط گونه‌های *کریپتوسپوریدیوم* در انواع حیوانات و انسان ایجاد می‌شود. در نشخوارکنندگان تازه متولد شده، اغلب به صورت اسهال رخ داده و با ضررهای اقتصادی قابل توجهی همراه است. مطالعه حاضر با هدف تعیین میزان آلودگی نشخوارکنندگان کوچک در شهرستان ارومیه به انواع *کریپتوسپوریدیوم* انجام شد. در مجموع ۵۶۰ نشخوارکننده کوچک (۷۰ رأس گوسفند و ۷۰ رأس بز در هر فصل) از گله‌های اطراف ارومیه در چهار فصل سال مورد بررسی قرار گرفتند. به منظور جستجوی اووسیست‌های *کریپتوسپوریدیوم*، نمونه‌ها به روش فرمالین اتر تغلیظ و پس از تهیه گسترش روی لام به روش زیل نلسسون اصلاح شده رنگ‌آمیزی شدند. از ۵۶۰ حیوان مورد بررسی، ۶۷ مورد (۱۱/۹۶ درصد) به انواع گونه‌های *کریپتوسپوریدیوم* آلوده بودند. میزان آلودگی در گوسفندان (۱۸/۸۶ درصد) بیش‌تر از بزهای مورد بررسی (۶/۰۷ درصد) بود. تجزیه و تحلیل آماری داده‌ها همچنین وجود یک ارتباط معنی‌دار بین میزان آلودگی و عواملی مانند قوام مدفوع، سن، جنس و فصل را نشان داد. نتایج این مطالعه خطر زئونوتیک بالقوه انواع گونه‌های *کریپتوسپوریدیوم* را در منطقه تأکید کرده و نشان دهنده لزوم آگاهی بهداشت عمومی و استراتژی‌های مدیریت مؤثر در گله‌های نشخوارکننده کوچک منطقه است.

کلمات کلیدی: *کریپتوسپوریدیوم*، شیوع، نشخوارکنندگان کوچک، ارومیه، ایران

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