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Cryptosporidium infection in lambs: prevalence and potential risk factors in villages of Mashhad city, eastern Iran

Mohammad Bagher Ghorbanzadeh[1], Elahe Ebrahimzadeh[1]*, Mohammad Azizzadeh[2], GholamReza Mohammadi[2]

[1] Department of Pathobiology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
[2] Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashad, Iran

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Abstract

Cryptosporidium is one of the major causes of diarrhea in animals and humans worldwide. The purpose of this research was to estimate the prevalence of *Cryptosporidium* infection and investigate the predisposing factors of infection in lambs in the suburbs of Mashhad, eastern Iran. In this research, a total of 400 fecal specimens of lambs aged 5-90 days were taken by rectal examination and mixed with the same volume of 2.5% potassium dichromate until further examinations. In the lab, thin fecal smears were prepared from the specimens and stained using the modified Ziehl-Neelsen technique. The stained smears were examined under 40X and 100X magnification. In addition, anamnestic, epidemiological, management, and hygiene measures data, including age, sex, breed, rectal temperature, fecal consistency, herd number, keeping with other animals, type of pen, type of bedding, colostrum consumption, source of drinking water were recorded in questionnaires and analyzed. In the current research, the intensity of infection was determined. In microscopy, 11% of the examined smears scored positive. The lowest intensity of infection was recorded in 38.63%, the average intensity of infection was found in 47.7%, and the highest intensity of infection was found in 13.63% of samples. Cryptosporidium infection was significantly influenced by some analyzed factors, including younger age, absence of colostrum intake, mud wall of the pens, and non-tap water consumption. The present study showed that the infection of lambs with *Cryptosporidium* is significant in the suburbs of Mashhad, although in most cases, the intensity of contamination was moderate.

Introduction

Cryptosporidium is a protozoan that infects the gastrointestinal tract of humans, livestock, and a wide range of animals (1). The infection can be self-limiting in immunocompetent hosts, or life-threatening in immunocompromised individuals (2). Cryptosporidiosis in lamb was first reported in Australia (3). Later, infection was reported in sheep

***Corresponding author:** *eebrahimzadeh@um.ac.ir*
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from different countries. The prevalence of *Cryptosporidium* in lambs was evaluated at 27.8% worldwide (4). Ahurai et al. reported *Cryptosporidium* infection in lambs from Iran (5). Later, *Cryptosporidium* infection in sheep/lamb was reported in different areas in Iran (6-13). Prevalence of cryptosporidiosis was reported as 13.8%, 11.3%, 17.2%, and 14.2% in Kerman (7), Kurdistan (8), Lorestan (14) and Tehran (15), respectively. The average prevalence of *Cryptosporidium* in sheep was reported to be 9.1% -9.9% in the country (16-18). Various variables, including age, sex, fecal consistency, bedding type, hygiene, colostrum feeding, management practices, feed and water sources, and climate conditions, have been considered as risk factors that affect the prevalence of cryptosporidiosis in ruminants (19, 20). In sheep, *C. xiaoi*, *C. ubiquitum*, and *C. parvum* are the most prevalent, whereas *C. hominis*, *C. andersoni*, *C. bovis*, *C. scrofarum*, *C. suis*, *C. fayeri*, *C. baileyi*, and *C. muris* have also been reported (18, 21, 22). In Iran, most studies focused on the incidence of *Cryptosporidium* in calves, and limited information has been published regarding the infection in lambs (16).

There are several reasons why *Cryptosporidium* in lamb and its risk factors should be considered:

- In lambs, *Cryptosporidium* can cause diarrhea, weight loss, economic losses, or even death (2, 18, 23).
- Major zoonotic species of *Cryptosporidium*, can be transmitted from small ruminants to humans (21).
- A large number of oocysts are excreted by livestock, contaminating the environment. Therefore, livestock should be considered an important reservoir for *Cryptosporidium* (1, 2).
- Controlling *Cryptosporidium* is challenging, as its oocyst wall resists conventional disinfection and environmental conditions. *Cryptosporidium* can infect susceptible hosts with just one oocyst (24).
- Lack of effective treatments and vaccination for the disease, there is a need to identify the risk factors that influence the transmission of infection in animals and to design and establish effective control strategies to prevent the infection on farms. However, most studies focused on identifying factors that may affect the risk of *Cryptosporidium* infection in cattle (25, 26).

The present study aimed to determine the prevalence of the *Cryptosporidium* in lambs and analyze the effect of various risk factors such as age, sex, breed, rectal temperature, fecal consistency, herd number, keeping with other animals, kind of pen, type of bedding, colostrum consumption, source of drinking water on the occurrence of cryptosporidiosis in lamb in the suburbs of Mashhad.

Materials and methods

Study area

Mashhad is a city in northeastern Iran surrounded by the Hezar Masjid and Binaloud mountain ranges, located in the Kashfroud river basin and the Mashhad plain. The climate is variable, but generally temperate and arid, characterized by hot and dry summers and cold and wet winters. In Mashhad, the highest recorded temperature is 43°C while the lowest is -22°C. The average annual precipitation has been 250 mm in recent years.

Sampling

The sample size was calculated using the formula, with a prevalence of 15% (based on unpublished local studies), an accuracy of 0.05, and a confidence level of 95%, resulting in 202 samples. The sample size was increased to 400 samples to account for the cluster sampling method. Thirty-six epidemiologic units were randomly selected, and samples were collected from each cluster. The epidemiologic units were: Nowruzabad Targah, Ahmedabad Kazqan, Kalate Ali, Kuroj, Cheshme Aliyoun, Saad Abad, Kalate Sarhang, Chechehe (Kaltaqiyya), Qala Pakhtuk, Berg, Takrok Sofli, Najm, Kazemabad Thursday, Qeshlaq, Helali, Darghan Kameli, Soleimani, Sangbar, Khorsafli, Serghaye, Amrodak, Stork, Bazekhor, Sij, Niqab, Barroud, Farrokhd, Ashgabat, Piweh Gene Rabat Sefid, Moinabad Sefli, Chah Mullah, Qasr, Cheshme

Reza, Maqsood Abad, Mian Velayat, Khatayan. At least ten stool samples were obtained from each unit (Figure 1).

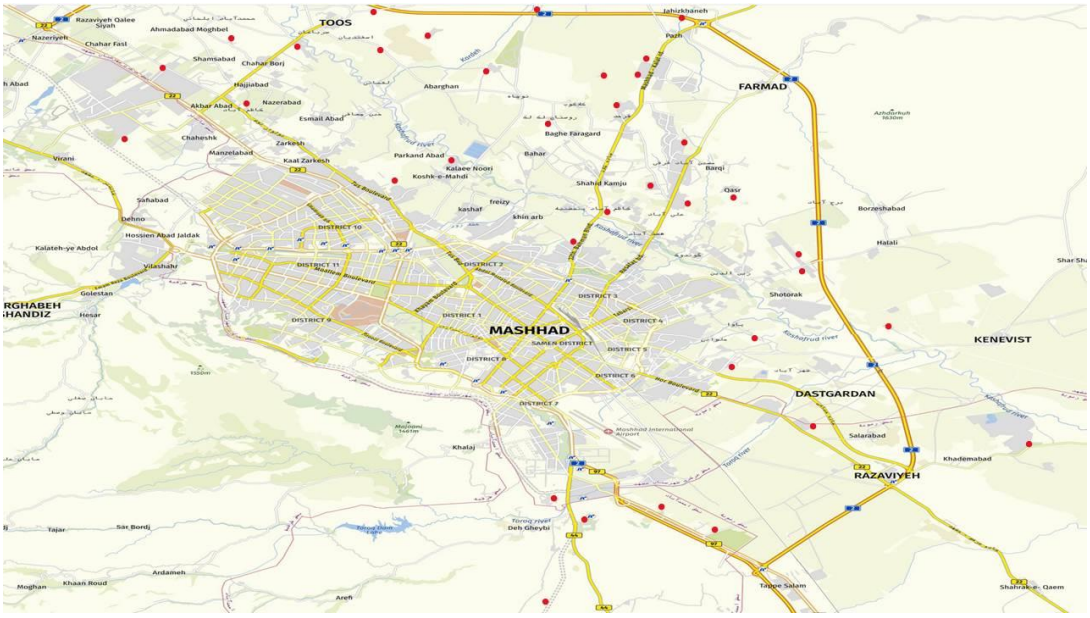

Fig. 1. Sampling location

A total of 400 fecal samples were collected directly from the rectum of lambs aged 5 - 90 days in 2021-2022. The samples were transferred to the sampling containers and mixed with an equal volume of 2.5% potassium bichromate solution to preserve specimens. A questionnaire was completed for each lamb, which included the following characteristics of the animal: age, sex, breed, rectal temperature, fecal consistency (diarrhea or normal), herd size, keeping with other animals, pen type, bedding type, colostrum intake, and drinking water source. The fecal consistency was scored based on different concentrations of feces: normal and consistent (score 1), semi-loose (score 2), loose (score 3), and diarrheal (score 4). Parameters are detailed in Table 1.

Parasitology analysis

Following filtration through a 100-mesh sieve, the samples were centrifuged for 2 minutes at 2000 rpm, and the supernatant was removed. A thin smear was made from the sediment and stained with the modified Ziehl-Neelsen staining technique. Briefly, the smears were fixed with methanol for 3 minutes, stained with basic fuschin for 30 minutes, and stained with 2.5% acid alcohol. Then, they were counterstained with malachite green for 5 minutes (27). After washing and drying, the smears were observed under a light microscope at 40X and 100X magnification using oil immersion to detect Cryptosporidium oocysts. Cryptosporidium oocysts appeared as bright red spherical oocysts on a green background. A fecal sample was considered positive if at least one Cryptosporidium oocyst was clearly recognizable. The number of oocysts observed in the smear was used to determine the infection intensity of the positive samples, following the method of Irshad Ahamed (28). Three categories of infection intensity were defined based on the oocyst count at different magnifications: low (+1) for less than five oocysts at 100X, medium (+2) for 1-10 oocysts at 40X, and high (+3) for more than 11 oocysts at 40X.

Statistical analysis

Statistical Product and Service Solutions (SPSS) version 20.0 was used to analyze the data collected. The prevalence was estimated overall and separately for different factors (age groups, sex, diarrhea status, and stool consistency). The chi-square test (χ^2) was applied to determine statistical differences in the prevalence and other variables. Fisher exact test was used when the expected frequency was below 5. A significance level of $p < 0.05$ (CI; 95%) was considered for all tests. Mann-Whitney U test was used to compare fecal consistency scores between "fecal positive" and "fecal negative" lambs.

Results

Oocysts of *Cryptosporidium* were detected in 44 stool samples out of the 400 samples examined, representing a rate of 11% with a 95% confidence interval (7.95%-14.05%). The parasite was identified as red spherical structures with a diameter ranging from 4 to 6 microns on a green background. Out of the 44 positive samples, 17 (38.6%) had low intensity, 21 (47.7%) had medium intensity, and 6 (13.63%) had high intensity. The majority of the cases exhibited medium contamination intensity (2+).

The association between the occurrence of *Cryptosporidium* infection and the variables of age, sex, breed, rectal temperature, fecal consistency (diarrhea or normal), herd size, keeping with other animals, pen type, bedding type, colostrum intake, and drinking water source was investigated.

The median score of fecal consistency in "fecal positive" lambs was significantly higher than that of "fecal negative" (Tables 1 and 2).

The age of lambs and the prevalence of *Cryptosporidium* infection were significantly associated, such that lambs under one month had a significantly higher risk of infection than lambs aged two months and above ($p<0.05$). The type of pen wall also influenced the prevalence of *Cryptosporidium* infection, with lambs in mud pens having a higher infection rate than lambs in fence pens ($p<0.05$). The source of drinking water was another factor affecting the prevalence of *Cryptosporidium* infection, with lambs drinking untaped water having a higher infection rate than lambs drinking tap water ($p<0.05$). Colostrum intake was inversely related to the prevalence of *Cryptosporidium* infection, with lambs that consumed colostrum having a lower infection rate than lambs that did not consume colostrum ($p<0.05$; Table 1). However, other studies in Iran reported higher prevalence rates than this study. For example, the rates were 26.86% in Tehran and 31% in Kurdistan.

Discussion

This study revealed that 11% (44/400) of lambs were infected with *Cryptosporidium* spp. The results of the study are in line with previous studies carried out in Kurdistan (10.24 and 11.3%), Saveh (10.5%), and Kerman (13.8%) (7-9, 29). The prevalence of *Cryptosporidium* infection in lambs in this study was higher than the reports from some previous studies in Iran, which showed prevalence rates below 10%. These studies were conducted in Tehran (6.75%), Amol (4.09%), Hamadan (2.5%), and Yazd (7.5%) (5, 12, 30, 31). The prevalence rates reported in some other studies in Iran, however, were higher than this study, such as studies conducted in Tehran (26.86%) and Kurdistan (31%) (6,32). In neighboring countries such as Turkey and Iraq, cryptosporidiosis among sheep/lambs has been reported at different levels (33-35). The variation in the prevalence of cryptosporidiosis in different research can be explained by the influence of factors such as study design, sample size, diagnostic methods, weather conditions of the region, breed variation, immune status of the animal, environmental conditions (humidity, temperature, rainfall), the season of the year and sanitary conditions of livestock (36).

Table 1. Predisposing factors affecting the prevalence of cryptosporidiosis in lambs

Risk factors / Cryptosporidium infection	Oocyst Negative (N/%)	Oocyst Positive (N/%)	P-value
Age			
5-30 d	225(86.54)	35(13.46)	
30-60 d	96(91.43)	9(8.57)	0.009
60-90 d	35(100)	0	
Gender			
Male	181(87.4)	26(12.6)	0.3
Female	175(90.07)	18(9.3)	
Breed			
Afshari	251(89.3)	30(10.7)	0.3
Bouri	46(84.6)	9(16.4)	
Afshari-Kordi	59(92.2)	5(7.8)	
Rectal temperature			
Fever +	23(82.4)	5(17.6)	0.3
Fever -	333(89.5)	39(10.5)	
Fecal consistency			
Normal (score 1)	275(77.2)	8(18.2)	
Semi-loose (score 2)	27(10.4)	11(25)	0.001
Loose (score 3)	26(7.3)	9(20.5)	
Diarrheal (score 4)	18(5.1)	16(34.6)	
Herd size			
<100	209(52.25)	30(7.5)	0.4
101-200	115(28.75)	10(2.5)	
>200	32(8)	4(1)	
Keeping with other animals			
+	330(88.7)	42(11.3)	0.7
-	26(99.2)	2(7.1)	
Type of barn wall			
Brick	186(91.2)	18 (8.8)	
Cementum	141(86)	141(23)	0.03
Mud	7(70)	7(3)	
Fence	22(100)	22(0)	
Type of bedding			
Cementum	119(89.5)	14(10.5)	0.001
Soil	237(88.8)	30(11.2)	
Source of drinking water			
Tap water	247(92.2)	21(7.8)	0.003
Non-tap water	109(82.6)	23(17.4)	
Colostrum consumption			
Colostrum intake +	271(97.9)	24(8.1)	0.001
Colostrum intake -	61(79.2)	16(20.8)	

Table 2. Description of stool consistency score in “fecal positive” and “fecal negative” lambs

Cryptosporidium infection	Number	Median	25% (Q1)	75%(Q3)	Minimum	Maximum
Negative	356	1	1	1	1	4
Positive	44	3	2	4	1	4

Out of the positive samples, 17 (38.6%) had low contamination (+1), 21 (46.7%) had moderate contamination (+2), and 6 (13.63%) had high contamination (+3). The majority of the cases showed a moderate level of contamination (2+). The results of the current study differ from those reported by Ahamed (28), who reported that 36.84% of samples had low contamination (+1), 24.59% had moderate contamination (+2), and 38.57% had high contamination (+3). The variations in Jammu and Kashmir may be due to humid climatic conditions (28). There was a significant association between the occurrence of cryptosporidiosis and the fecal consistency in lambs. The lambs with diarrheal feces had the highest prevalence of the infection.

The relationship between cryptosporidiosis prevalence and fecal consistency has been confirmed by several studies across different regions and populations (7, 9, 28, 34, 37-39). Therefore, the prevalence of cryptosporidiosis is strongly associated with diarrhea (2, 40). There has been no association found between diarrhea and *Cryptosporidium* outbreaks in some studies (11, 13). However, it should be noted that in diarrheal feces, oocysts are dispersed in a larger volume of feces, and fecal concentration before smear preparation can increase the probability of detecting oocysts.

The age of the lamb was a relevant factor in this study, as the statistical analysis revealed that the *Cryptosporidium* infection was most common among lambs aged 5 to 30 days. This finding is consistent with previous studies (7, 28, 34, 39, 41). It may be due to the immaturity of young animals' immune systems (20, 28, 41). Furthermore, lambs that received colostrum had a lower infection rate compared to those that did not. It has been demonstrated that hyperimmune colostrum can protect newborn calves against cryptosporidiosis (42). Lambs drinking non-tap water had a higher infection rate than lambs drinking tap water. Some studies have verified the influence of this factor on the transmission of cryptosporidiosis in ruminants (35, 43, 44).

A significant association was found between the occurrence of Cryptosporidium and the type of pen wall in this study. In pens with mud walls, *Cryptosporidium* was more prevalent than in pens with brick, cement, or fence walls. *Cryptosporidium* oocysts are resistant to harsh environmental conditions, so effective disinfectants such as ammonia with a flamethrower are needed to eliminate them. In mud pens, hygiene and disinfection practices were less conducive, which led to the survival of *Cryptosporidium* oocysts in the environment and contamination of water and food. Previous studies have demonstrated that animal housing conditions affect the incidence of cryptosporidiosis in different hosts (20, 45).

Identifying other etiological agents of diarrhea in lambs, such as bacterial and viral pathogens, can enhance our comprehension of the contribution of *Cryptosporidium* to diarrheal disease in lambs.

Conclusions

This study figures out that *Cryptosporidium* infection is considerable in lambs in the suburbs of Mashhad. *Cryptosporidium* infection was significantly increased by factors such as younger age, absence of colostrum intake, mud wall of the pens, and non-tap water consumption. When devising a policy to control the infection, it is important to take into account these predisposing factors. Consequently, the community should be educated on cryptosporidiosis's public health and economic implications.

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Ethical approval

The project was conducted according to the ethical principles and the national norms and standards for conducting Medical Research in Iran (IR.UM.REC.1401.107).

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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