



Microscopic and Molecular Prevalence of *Cryptosporidium* spp. in Lambs in Siirt, Turkey

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CRYPTOSPORIDIOSIS is a zoonotic disease that occurs acutely or chronically in young or immune-compromised animals and humans, caused by *Cryptosporidium* species. *Cryptosporidium* is recognized as one of the major enteropathogens associated with neonatal diarrhea in ruminants. The aim of this study is to determine the prevalence of *Cryptosporidium* spp. in lambs in Siirt province using microscopic and molecular methods. The fecal materials of the study collected from 194 randomly selected lambs of different sexes, up to 4 weeks of age, in various farms. As a result of microscopic examination, *Cryptosporidium* spp. oocysts were found in 54 (27.84%) of 194 samples, while specific bands were obtained in 63 (32.47%) samples as a result of Nested PCR analysis. A statistically significant relationship was found between lambs with and without diarrhea ($P < 0.001$) while no statistically significant relationship was found between age groups, gender, and locations ($P > 0.05$). In conclusion, the data obtained from this study revealed that *Cryptosporidium* infection is present in Siirt province, and *Cryptosporidium* spp. should be considered as one of the agents in the etiology of neonatal diarrhea in lambs.

Keywords: *Cryptosporidium* spp., Lamb, Molecular, PCR, Siirt, Turkey.

Introduction

Cryptosporidiosis is a zoonotic disease in the phylum Apicomplexa that occurs in young or

immunocompromised animals and humans are caused by *Cryptosporidium* species and is one of the most important diseases of the last century [1-4]. *Cryptosporidium* is a worldwide coccidial

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protozoan [5] and is recognized as one of the major enteropathogens associated with neonatal diarrhea in ruminants [6, 7]. It is considered to be one of the main causes of morbidity and mortality in young farm animals and causes significant economic losses worldwide [6, 8]. From an economic point of view, it is considered a serious disease, particularly for lambs and calves [1-3, 9]. It is also considered to cause diarrhea cases in various animal species and humans, either by itself or in combination with other agents [5]. Contaminated water is the most important factor in the prevalence of *Cryptosporidium* oocysts, followed by contaminated food and animal-human contamination [10, 11].

The most prominent clinical symptom of cryptosporidiosis is diarrhea, which lasts 2-12 days. Typically, animals 1-5 weeks old age are exposed to infection. Lambs aged 5-12 days are most susceptible to the disease, which causes mild or severe diarrhea accompanied by infection, depression, dehydration, decreased milk intake, growth retardation, stiffness, hyperpnea, slow gait, anorexia [6, 12, 13].

This study aimed to determine *Cryptosporidium* spp. prevalence in lambs microscopically and molecularly in the Siirt province of Turkey.

Material and Methods

The Study Area and Animal Material

This study was carried out in the Şirvan, Kurtalan, Baykan, and Aydınlar districts of Siirt province located in the South eastern Anatolia Region of Turkey (37° 55' 48" N, 41° 56' 23" E). The fecal material of the study collected from 194 randomly selected lambs of different sexes, up to 4 weeks old in various farms.

Sample Collection and Preparation

Fecal samples were taken from the rectum of each lamb using disposable latex gloves. The samples were placed in fecal containers and labeled. The sex, age, and location information of the animals were recorded.

Microscopic examination

After the collected samples are brought to the laboratory, they were stained with Kinyoun's acid-fast method and examined under a microscope (Leica DM500, Switzerland) at 100x magnification [14].

DNA extraction

DNA extraction was performed in all fecal samples using the Gene Matrix Stool DNA Purification Kit (Poland, Cat E3575-01) according to the manufacturer's protocol. The obtained DNAs were stored at -20°C.

PCR Amplification

The primers described by Xiao *et al.* [15] were used to amplify the SSU rRNA gene region. 5'-TTCTAGAGCTAATACATGCG-3' and 5'-CCCATTTCCTTCGAAACAGGA-3' Primers were used to amplify the 1325 bp gene region in the first step of nested PCR. 5'-GGAAGGGTTGTATTTTAGATAAAG-3' and 5'-AAGGAGTAAGGAACAACCTCCA-3' Primers were used to amplify the 826-864 bp gene region in the second step of Nested PCR. Protocol for both reactions was performed according to Ayan and Orunç Kılınç [16]. The reaction was performed in an automatic thermal cycler (Eppendorf Mastercycler® pro) device. Subsequently, 1.5% agarose gel was prepared and stained with RedSafe™ Nucleic Acid Staining Solution. The PCR products were run on an agarose gel afterward, and images were obtained on the gel imaging device (Syngene bioimaging system).

Statistical Analysis

The results between the groups were evaluated by the chi-square test. A value of $P < 0.05$ was considered statistically significant.

Ethical Approval

Ethical approval for this study was obtained from the Siirt University Local Ethics Committee for Animal Experiments (Decision No: 2023/01/07).

Results

As a result of microscopic examination, *Cryptosporidium* spp. oocysts were found in 54 (27.84%) samples (Fig. 1), while in Nested PCR results, 63 (32.47%) samples showed *Cryptosporidium* spp. specific bands of 826-864 bp in size (Fig. 2). The highest positivity among age groups was found in the 0-15 days group, while the highest positivity among locations was Şirvan district. The highest positivity between genders was found in females (Table 1). A statistically significant relationship was found between lambs with and without diarrhea ($P < 0.001$), while no statistically significant relationship was found between age groups, gender, or locations ($P > 0.05$).

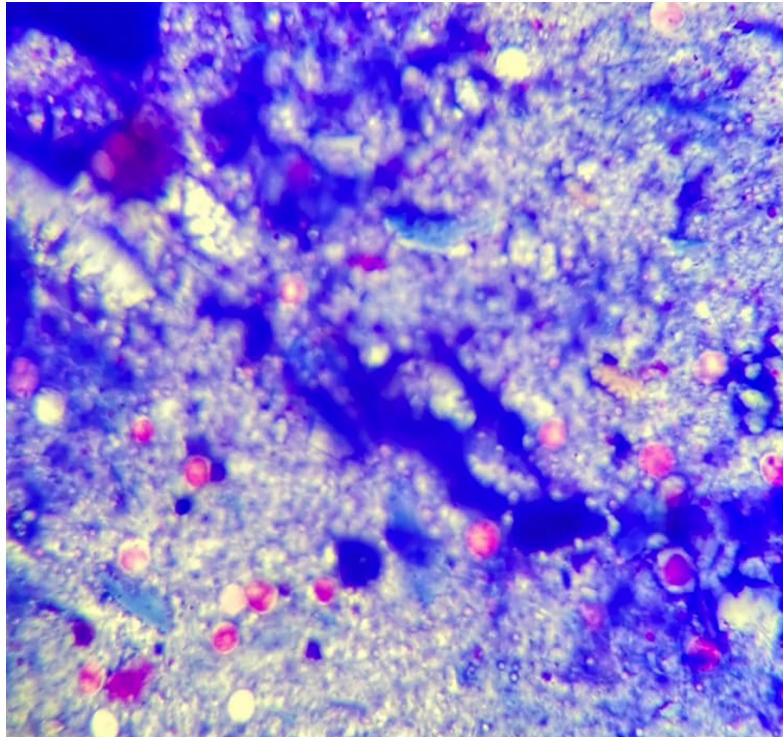


Fig. 1. *Cryptosporidium* oocysts stained with Kinyoun's acid-fast method (x100 magnification).

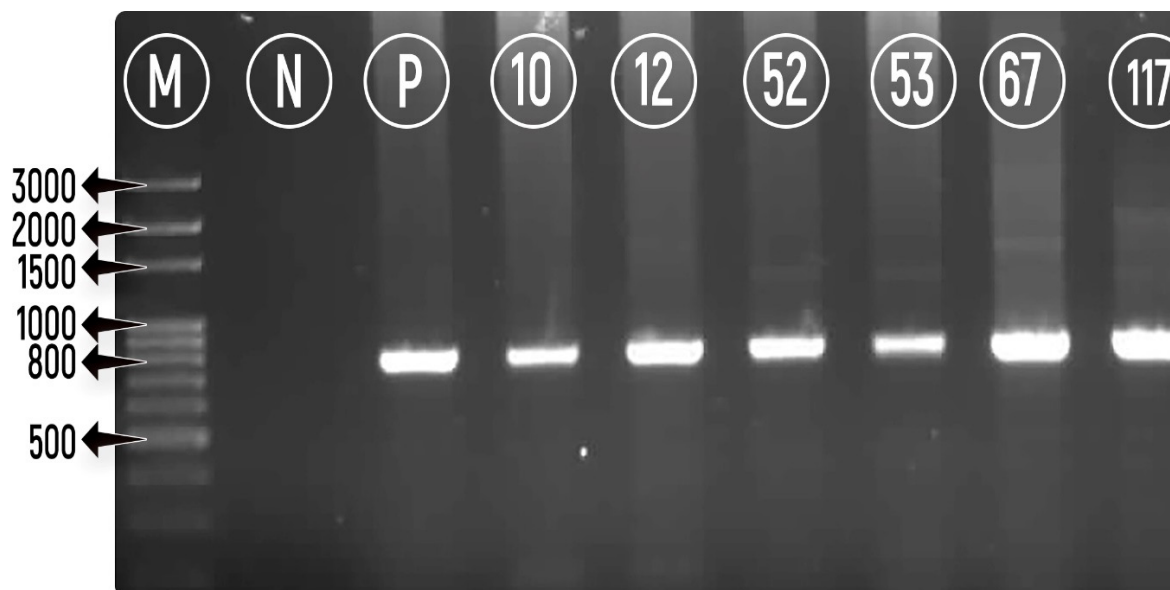


Fig. 2. Amplification of *Cryptosporidium* spp. using nested-PCR. Lanes M: Marker, N: Negative control, P: positive control, 10, 12, 52, 53, 67, and 117 represent *Cryptosporidium* sp. (826-864 bp).

TABLE 1. Distribution of parameters according to the positive results.

Parameters	(n)	Positive				P
		Diarrheic		Non-diarrheic		
		(n)	(%)	(n)	(%)	
Sex						
Female	117	27	23.08	9	7.69	NS
Male	77	20	25.97	7	9.09	
Age (Days)						
0-15	116	31	26.72	9	7.76	NS
16-30	78	16	20.51	7	8.97	
Diarrhea						
Yes	103	47	45.63	-	-	***
No	91	-	-	16	17.58	
Location						
Şirvan	60	19	31.67	6	10.00	NS
Kurtalan	52	13	25.00	3	5.77	
Aydınlı	45	9	20.00	4	8.89	
Baykan	37	6	16.22	3	8.11	
Method						
Microscopy	194	43	22.16	11	5.67	NS
Nested PCR	194	47	24.23	16	8.25	
Total	194	47	24.23	16	8.25	

***: $P < 0.001$, ^{NS}: Non-significant

Discussion

Cryptosporidium spp. is one of the most common intestinal pathogens in both diarrheal and non-diarrheal ruminants and is seen as one of the most important causes of early lamb diarrhea [1, 6, 8]. Common in many parts of the world, Cryptosporidiosis is also one of the most important zoonotic diseases. The disease is more common in livestock workers, young people, and people with weakened immune systems [17].

Cryptosporidium prevalence in lambs varies according to countries and regions. Many studies were performed on lambs with or without diarrhea in many parts of the world, for which the prevalence of the disease was reported as follows: 3.7% in Brazil [18], 44.8% in Spain [19], 10.1% in Poland [20], 42.1% in Serbia [21], 13.1% in Belgium [22], 23% in Canada [23], 77% in America [24], 20% in Trinidad and Tobago [5], and 11.2% in Tunisia [25]. In studies carried out on lambs in Turkey, a prevalence rate of 1%-79.1% has been reported [1, 3, 6, 8, 12, 26-31]. Studies show that *cryptosporidium* infections are quite common in animals in Turkey and around the world.

As a result of microscopic examination and PCR analyses of this study, 27.84% and 32.47% prevalence were determined respectively. The prevalence obtained as a result of this study was found to be lower than the findings of some researchers [8, 19, 21, 24]; similar to the findings of certain researchers [3, 23, 28, 29], and higher than the findings of other researchers [1, 5, 6, 8, 12, 18, 20, 22, 25]. The potential reasons for the differences between the studies can be include as geographical location, type of farm, number of animals in the farm, sample size, animal species, age of the animal, diarrhea status, nursing status, water resources, and methods used. Clinically sick and latently infected animals contaminate feed and water, transferring the disease to other animals. The wide host range of *Cryptosporidium*, combined with the massive oocyst output from infected hosts, results in a high level of contamination of the environment with this parasite [5]. This situation may be among the reasons for the prevalence obtained as a result of this study.

Al-Zubaidi [4] reported that PCR method was more sensitive than microscopic method.

In this study, the higher prevalence detected by PCR method coincides with the findings of the researcher.

Some studies [4, 5, 8, 10] have reported a higher prevalence in animals with diarrhoea. In this study, a higher prevalence was also determined in the diarrheal groups compared to those without diarrhea, and a statistically significant difference was observed ($p < 0.001$).

It has been reported that Cryptosporidiosis causes serious problems in small ruminants such as calves, lambs, and kids, and age is the most important risk factor for its spread [3]. Previous studies [6, 8, 10, 17, 32, 33] show that lambs aged 1-15 days are generally affected by this disease more than other age groups. In this study, similar to the findings of the researchers, a higher prevalence was found in the 1-15 days age group. This could be due to the underdeveloped immune system in young animals and their inability to mount an effective immune response to eliminate the infection.

Some researchers [4, 34-36] reported that they found a higher prevalence in males than females in their studies. Similarly, in this study, higher prevalence was found in males than females, which supports the researchers. In a study carried out on goat kids, it was revealed that sex had no effect on the elimination of *Cryptosporidium* oocysts [37].

Conclusion

In conclusion, the data obtained from this study revealed that Cryptosporidial infection is present in Siirt province, and *Cryptosporidium* spp. should be considered as one of the agents in the etiology of neonatal diarrhea in lambs. Therefore, in addition to keeping cryptosporidiosis cases in mind during diarrhea treatment protocol in clinical practice, more studies should be performed to evaluate its impact on the human population and livestock industry from a public health perspective.

Conflicts of interest

The authors declare that there is no conflict of interest.

Funding statement

The authors declared that this study received no financial support.

Authors contributions

ÖYÇ, BAÇ and AA designed the research plan, organized the study, KE, MAS and VB collected the samples. AA, ÖOK and ÖOA made the laboratory analysis, ÖYÇ and BAÇ wrote the manuscript.

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