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Occurrence and Molecular Characterization of *Cryptosporidium spp.* and *Giardia duodenalis* in Lambs and Calves in Southeastern Anatolia, Turkey

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Cryptosporidium spp. and *Giardia duodenalis* are parasitic protozoa that can infect various hosts, including humans, domestic animals, and wild animals, making them significant from medical and veterinary perspectives. These protozoa are prevalent in cattle and sheep globally, particularly affecting newborn lambs and calves. This study assessed the occurrence and genetic characteristics of *Cryptosporidium spp.* and *G. duodenalis* in asymptomatic lambs and calves under three months in Southeast Türkiye. Fecal samples were collected from 124 animals (69 lambs and 55 calves). A direct immunofluorescence test was used to confirm the presence of *G. duodenalis* cysts and *Cryptosporium spp*. oocysts. Molecular confirmation was done with Polymerase chain reaction (PCR) using *G. duodenalis SSUrRNA* and *Cryptosporidium spp. SSUrRNA* gene. Microscopic analysis of fecal samples, 17.4% and 43.5% of the 69 lambs, 25.5% and 21.8% of the 55 calves in the study were found to be shedding *Cryptosporidium spp*. and *G. duodenalis* oocysts/cysts, respectively. Molecular analysis identified *Cryptosporidium* species as *C. parvum, C. xiaoi*, and *C. ubiquitum* in lambs and *C. parvum, C. bovis,* and *C. ryanae* in calves. Assemblages A and E from genotype of *G. duodenalis* were detected in both lambs and calves. The detection of species and genotypes with zoonotic characteristics in asymptomatic lambs and kids concluded that the *Cryptosporidium spp*. and *G. duodenalis* oocysts/cysts shed by lams and calves are important for animal and human health.

Keywords: *Calves, Cryptosporidium, Giardia, Lamb, SSUrRNA*

Introduction

Cryptosporidium and *Giardia duodenalis* are intestinal protozoan parasites found various hosts, including humans, domestic animals and wild animals. Both of these parasites are important from a medical and veterinary viewpoint. In ruminants, infections caused by these parasites are typically associated with diarrhea outbreaks, primarily occurring in young animals [1, 2].

Cryptosporidiosis is a widespread disease that affects cattle and sheep globally. It is also a significant cause of gastroenteritis in newborn calves and lambs [3]. The prevalence of *Cryptosporidium* has been reported at different rates, 8.3-35.7% in calves and 11.7-32.4% in lambs in the worldwide [4, 5]. The four main species of *Cryptosporidium* that can infect cattle are *Cryptosporidium parvum*, *C. bovis, C. ryanae*, and *C. andersoni* [2, 6]. The species *C. parvum* is typically found in pre-weaned calves, while *C. bovis* and *C. ryanae* are commonly found in post-weaned calves and young cattle [7]; while, *C. andersoni* is the most frequently occurring Cryptosporidium species occurs in adult cattle. The most prevalent *Cryptosporidium* species in lambs are *C. parvum*, *C. ubiquitum*, and *C. xiaoi*. Research shows that *C. parvum* is frequently found in clinically ill lambs, while *C. ubiquitum* and *C. xiaoi* are commonly observed in healthy lambs in Europe [8-13].

The prevalence of *G. duodenalis* in calves varied between 1.1% and 74.2% in recent publications in

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the word [14]. The prevalence of *G. duodenalis* infection in lambs varied from 1.5 to 42% among studies conducted in different countries [1, 13, 15-17]. The most common genotype of *G. duodenalis* in calves is assemblage E, while assemblages A and B are only found sporadically. Other assemblages are rare, with only a few cases of assemblage D in China and assemblage F in Spain being reported [18, 19]. Assemblage E is the most dominant genotype of *G. duodenalis* in lambs, followed by assemblage A. Assemblages B and D have rarely been detected in recent years while assemblages A and B are zoonotic and can be found in humans and many animal species [20, 21].

This study aimed to assess the prevalence of *Cryptosporidium* spp. and *G. duodenalis* in lambs and calves up to three month ages, determine their genotypes, and estimate their public health significance in Southeastern Anatolia, Türkiye.

Material and Methods

Sample Collection

A total of 124 fecal samples were collected from asymptomatic l69 lambs and 55 calves under three month ages in Diyarbakır city, southeastern Anatolia, Türkiye. Fecal samples were collected from the lamb and calf using sterile gloves and stored at 4°C. Microscopic analyses were carried out within 24 hours.

Microscopic Fecal Analysis

To detect the presence of *Cryptosporidium* oocysts and *Giardia* cysts, the Crypto/Giardia-Cel FITC Staining Kit (Cellabs Inc., Brookvale, Australia) was used. One g of faecal material was processed according to the manufacturer's instructions. A fluorescence microscope examined each sample under 200×, 400×, and 1000× magnification.

DNA Extraction

Total DNA was extracted from fresh fecal samples using the QIAamp DNA Stool Mini Kit (QIAGEN Inc. Valencia, CA) and DNA extracts were stored at -20°C.

Molecular detection of Cryptosporidium spp. and Giardia duodenalis

To identify the species of *Cryptosporidium,* a nested PCR protocol was used to amplify a fragment of the *SSU rRNA* gene was done using published primers (Table 1) as per the method Xiao *et al.* [22]. A nested PCR was utilised to amplify a 130 bp region of the *SSU rRNA* gene of *Giardia* using four primers (Table 1) as previously described by Hopkins *et al.,* and Read *et al.,* [23, 24].

TABLE 1. Oligonucleotide primers used in this study

	Primer Sequence (5'-3')	Amplicon size (bp)	Reference
Cryptosporidium spp.	TTCTAGAGCTAATACATGCG CCCTAATCCTTCGAAACAGGA	1325	[22]
	GGAAGGGTTGTATTTATTAGATAAAG AAGGAGTAAGGAACAACCTCCA	825	[22]
Giardia duodenalis	CAT CCGGTCGATCCTGCC AGTCGAACCCTGATTCTCCGCCCAGG	292	[23]
	GACGCTCTCCCCAAGGAC CTGCGTCACGCTGCTCG	130	[24]

The secondary PCR products of all positive samples were sequenced in one direction on an automated sequencer. Nucleotide sequence analysis was performed by BLAST alignment using the National Center for Biotechnology Information database.

Results and Discussion

Microscopic analysis of 69 lamb fecal samples revealed the presence of *Cryptosporidium* oocysts and *Giardia* cysts in 12 samples (17.39%) and 30 samples (43,47%), respectively. *Cryptosporidium* and *G. duodenalis* oocysts/cysts were determined in 25.45% and 21.81% of the 55 calves, respectively (Table 2).

TABLE 2. Occurrence of *Cryptosporidium* spp. and *Giardia duodenalis* in lambs and calves

	No. of samples examined	No. of positives	
		Cryptosporidium spp.	***Giardia duodenalis***
Lambs	69	12(17.39%)	30(43.47%)
Calves	55	14(25.45%)	12 (21,81%)

Cryptosporidium spp. and *G. duodenalis* are two common enteric pathogens that can infect animals and humans worldwide. These parasites threaten animal health, leading to economic losses due to infection. However, their impact is not limited to animal health alone. There is also a public health concern as humans can be exposed to environmental contamination of *Cryptosporidium* spp. oocysts and *G. duodenalis* cysts that originate from animals [1, 2]. Results of the study have demonstrated the occurrence of *Cryptosporidium spp*. and *G. duodenalis* in asymptomatic lambs and calves in southeastern Türkiye. The infection rate of *Cryptosporidium* in lambs in the present study was 17,39%, which is lower than the rates reported in Spain (44.8%) [25], in Serbia (42.1%) [26], in Spain (74.4%) [8], in French (45.6%) [27], in Türkiye (38.8%) [28] in Australia (24.5%) [29]. Similar results have been reported in Poland (19.2%) [9], in Türkiye (19.4%) [30]. However, the infection rate assigned in this study is higher than investigations from other countries such as Greece (5.1%) [13], Belgium (13.1%) [15], Türkiye (13.63%) [32], and Brazil (3.7%) [31]. Several epidemiological studies have been conducted to identify the prevalence of cryptosporidiosis in calves across the globe. In this study, it was discovered that 25.5% of the examined calves were infected with *Cryptosporidium*. This finding is consistent with previous studies which reported prevalence rates of 22.5% in Poland [33] and 27.4% in Türkiye [30] for *Cryptosporidium* infection in calves. However, the infection rate assigned in this study is higher than investigations reported prevalence rates of 18.7% in Jordan [34], 14.7% in Iran [35], 9.7% in Egypt [36], but lower than the reported prevalence rate of 47.9% in Iraq [37], 58.3% in Sudan [38], 84% in Algeria [39], 36.7% in Sweden [40], 33.1% in Kuwait [41], 38.8% in Italy [42], and 53.6% in Türkiye [43]. These variations in the *Cryptosporidium* infection rates in lambs and calves could be due to geographical differences, farm management, hygiene, agroecological differences, sample size, health situation and diagnostic methods.

A fragment of the *SSU rRNA* gene was amplified from the 26 samples in which *Cryptosporidium* oocysts were microscopically identified. Sequence analysis revealed the presence of *C. xiaoi, C. ubiquitum, C. parvum* in lambs and *C. bovis, C. parvum, C. ryanae* in calves (Tables 3, 4).

TABLE 3. *Cryptosporidium* and *Giardia* species/genotypes identified in lambs and calves

	***Cryptosporidium* spp.**	***Giardia duodenalis***
Lambs	*C. xiaoi(41.6%)* *C. ubiquitum(33.3%)* *C. parvum(25%)*	*AssemblageE(92.03%)* *Assemblage A(7.69%)*
Calves	*C. bovis (57.4%)* *C. parvum (21.42%)* *C. ryanae (21.42%)*	*AssemblageE(90.09%)* *Assemblage A(9.09%)*

TABLE 4. Comparison of results of the study samples generated using the NCBI Basic Local Alignment Search Tool

Species/Genotypes	**Access codes of the most similar sample**	
	Lambs	**Calves**
C. xiaoi	JX258864, KT235701	
C. ubiquitum	KM199752, EU827382	
C. parvum	KJ917579, AH006572, DQ656355	KU198182, KJ917579, KU987671
C. bovis		KC618620, KT922232
C. ryanae		KJ020910
Assmblages E	MF069070, MF069058	MF163433, MF069070
Assmblages A	KP716565	KP716565

In our study, the most common species in lambs was *C. xioai* (41,66%), followed by *C. ubiquitum* (33.33%) and *C. parvum* (25%). Studies suggest that *C. parvum* is more commonly determined in clinically affected lambs, while *C. ubiquitum* and *C. xiaoi* are frequently observed in healthy lambs in Europe [10, 11, 44-46]. *C. xiaoi,* was also the most common species in this study, while *C. ubiquitum and C. parvum* were the other two species. *C. xiaoi* was reported as dominant species in sheep in Australia [47, 48]. The fact that *C. xiaoi* was the most common species in asymptomatic lambs in this

study supports previous studies. In previous studies conducted in Türkiye, *C. parvum, C. ryanae* and *C. andersoni* species were reported in lambs and sheep [30, 49]. In our study, *C. xiaoi* and *C. ubiquitum* species were first reported in lambs in Türkiye.

Four main *Cryptosporidium* species cause cattle infections: *C. parvum, C. andersoni*, *C. bovis* and *C. ryanae* [50-53]. Our data showed that calves mainly were infected with *C. bovis* (57.14%) followed by *C. ryanae* (21.42%), and *C. parvum* (21.42%). Similarly, several recent studies in China, India, Malaysia, Australia, Sweden, and Canada, however, have demonstrated the common occurrence of *C. bovis* and *C. ryanae* in calves in the absence or low occurrence of *C. parvum* [54-58]. In contrast, previous studies reported the predominance of *C. parvum* in calves globally [41, 43, 59]. This difference in the distribution of *Cryptosporidium* species in lambs and calves might have resulted from various environmental, host, and management factors.

In the present study, the infection rate of *G. duodenalis* in lambs was 43.37% (30/69) (Table 2). Similar results to our results have been reported in lambs in China (59.4%), Algeria (32.1-69%), Brazil (34%), Greece (37.3%), Spain (42%) [4, 13, 21, 60-62] while a lower prevalence was observed in the USA (4%) [44], Ethiopia (2.6%) [17], Algeria (6.9%) [61], Belgium (25.5%) [15], Türkiye (8.3% and 10.2%) [49, 63].

Among the 55 calves fecal samples, 21,81% were positive for *G. duodenalis*. Earlier studies reported infections rates of 11.2 - 64.7% for *G. duodenalis* in calves in Türkiye [49, 64-66]. Our finding is lower than the rate reported from Türkiye (64,7%, 30.2 %) [64, 66] Germany (72.4%) [67], in Norway (48%) [69], in Nepal (44.79%) [70], in Canada (42%) [71], higher than in Türkiye (16.67%) [65], in Egypt (13.3%) [36], in Vietnam (13.8%) [68], in Korea (10%) [72]. The different rates reported in lambs and calves are thought to be due to geographical conditions, management systems, population density, and hygiene of water sources.

Based on PCR analysis of the *SSU rRNA* gene, 42 out of 124 samples produced amplicons of the expected size for *G. duodenalis*. Sequence analysis of the *SSU rRNA* locus was successful for 37/42 (Table 3,4). The most prevalent genotype of *G. duodenalis* in both sheep and cattle is typically assemblage E, with assemblages A and B being found sporadically. In the current study, we obtained partial *SSU rRNA* gene sequences, most identified as assemblage E (92.03%), while a minority belonged to assemblage A (7.69%) in lambs. Similarly, studies conducted on sheep from different countries worldwide have consistently shown the predominance of assemblage E, aligning with our findings [13, 15, 17, 29, 62, 73]. Assemblage A, which holds zoonotic significance, was identified as our study's second most common genotype, mirroring findings from many studies worldwide [15, 16, 74].

In this study, the livestock specific assemblage E (90.09%) was found to be the predominant *G. duodenalis* genotype in calves, while another genotype, assemblage A (9,09%), was identified at a lower rate. Assemblages E, A, and B have been previously reported in cattle in Türkiye [64-66, 75]. Consistent with our findings, assemblage E is also commonly observed in calves in various regions, including China, Türkiye, and Ethiopia [16, 17, 66, 76, 77]. Assemblage A is the primary group of *G. duodenalis* that encompasses genotypes capable of causing human infections with zoonotic characteristics [78]. It is also the second most common assemblage A found in calves [66, 79]. Similarly, in this study, assemblage A was identified as the second most prevalent genotype. The presence of the identical zoonotic genotypes in both lambs and calves in the region serves as evidence that farm animals pose a zoonotic risk.

Conclusions

At the end of our study, new information has been revealed regarding the prevalence and genetic characterization of *Cryptosporidium* spp and *G. duodenalis* in calves and lambs in the southeastern region of Türkiye. This study determined which zoonotic importance in both calves and lambs. We found *G. duodenalis* genotypes (assemblages A and E) that zoonotic characteristics possess. As a result, we have concluded that the cysts and oocysts released by calves and lambs are significant for animal and human health. To assess the zoonotic potentials and species/genotype distributions of *Cryptosporidium* spp and *Giardia duodenalis* in cattle and sheep, epidemiological studies are needed in different regions of Türkiye.

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Conflict of Interest

No conflict of interest was declared by the authors.

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