Research Article DOI: 10.17094/vetsci.1799529

Molecular Characterization and Prevalence of Bovine Cryptosporidium Isolates in the Afyonkarahisar Region

Mustafa ESER[1]
Hatice ÇİÇEK[2]
Metin ERDOĞAN[3]
Kürşat KARTAL[4]
Mustafa KÖSE[2]

[1]Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Eskişehir, Türkiye
[2]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Parasitology, Afyonkarahisar, Türkiye
[3]Afyon Kocatepe University, Faculty of Veterinary Medicine, Department of Medical Biology and Genetic, Afyonkarahisar, Türkiye
[4]Gazi Mustafa Kemal Anatolian High School, Biology Teacher, Eskişehir, Türkiye

ABSTRACT

Cryptosporidiosis is a zoonotic protozoan disease that affects humans and animals. This disease, which causes diarrhea, dehydration, weight loss, stunted growth, and death, especially in newborn calves, leads to economic losses. Early diagnosis of disease on cattle farms, and even taking preventive measures against it, is of great importance. This study aimed to identify the cryptosporidiosis species and prevalence in diarrheic cattle in the Afyonkarahisar region, where cattle farming is quite intensive. For this reason, a total of 188 fecal samples were obtained rectally from 152 diarrheic bovine calves (0-6 months) and 36 diarrheic cattle (≥ 6 months) from the farms. The specimens were analyzed for Cryptosporidium oocysts by the modified acid-fast staining (mAF) procedure. Whereas the frequency of bovine cryptosporidiosis was 9.2% (14/152) in diarrheic calves, no Cryptosporidium oocysts were detected in the feces of diarrheic cattle older than six months. In the 14 identified Cryptosporidium infections, 13.6% were in calves aged 0 to 3 months, whereas 3.1% were in calves aged 3 to 6 months. The sequencing from the 14 isolates at the 18S ribosomal RNA (rRNA) loci indicated the detection of two Cryptosporidium species: *C. parvum* (64.2%) and *C. bovis* (35.7%). These findings highlight that C. parvum, one of the most important factors causing zoonoses, is the primary cause of neonatal cryptosporidial diarrhea in calves, and that more comprehensive epidemiological studies are needed to determine the epidemiology and etiology of cryptosporidiosis in this region.

Keywords: 18S rRNA, Afyonkarahisar, Calves, Cattle, *Cryptosporidium spp.,* DNA Sequencing

**This research was presented as an oral presentation at the 20th National Congress on Parasitology (Eskişehir-2017).*

Received 08.10.2025
Accepted 27.03.2026
Publication Date 28.04.2026

Corresponding author: Mustafa Eser
E-mail: meser961@anadolu.edu.tr
Cite this article: Eser M, Çiçek H, Erdoğan M, Kartal K, Köse M. Molecular characterization and prevalence of bovine cryptosporidium isolates in the afyonkarahisar region. *Vet Sci Prac.* 2026;21(1):26-32

INTRODUCTION

Cryptosporidiosis is a zoonotic protozoan disease caused by an apicomplexan parasite that affects both humans and animals.[1,2] To date, 45 *Cryptosporidium* species and over 120 genotypes were reported [3,4] Among these species, cattle have been reported as primary hosts for *C. parvum, C. bovis, C. ryanae*, and *C. andersoni*.[5,6] *Cryptosporidium parvum* and *C. bovis* are responsible for more than 90% of bovine infections.[7] *Cryptosporidium parvum* is the most common species causing diarrhea in newborn calves, dehydration, weight loss, developmental retardation, and death in animals.[8,9] The estimated annual economic losses caused by cryptosporidiosis in calves range from €5.85 to 15.16€ in Belgium, from €5.38 to 10.13€ in France, and from €5.32 to 7.35€ in the Netherlands.[10] Human cryptosporidiosis is mostly triggered by *C. parvum* and *C. hominis*.[11] Infected calves with *C. parvum* serve as a source of the oocyst for human infections and environmental contamination.[2,8,11,12] Microscopy, serological, and molecular diagnostic tests are commonly used in identifying cryptosporidiosis in calves and cattle.[13,14]

In surveys conducted on diarrheic calves in Türkiye, the microscopical prevalence of the bovine cryptosporidiosis was in the ranging from 7.0 to 63.3%[14-21] and the molecular prevalence was in the range of 3.9-68.4%.[13,14,17,21-26] Using the serological methods, diseases prevalence has also been reported at 7.5%[27] and 27.33% with ELISA[28] and at 56.25% with IFA[26], respectively.

MATERIALS AND METHODS

This research received approval by the Afyon Kocatepe University Local Ethics Committee on Animal Experiments (Date: April 18, 2014 Number: 49533702/51).

Sampling and Identification of Cryptosporidium Oocysts

A total of 188 fecal samples were taken rectally from intensively farmed 152 diarrheic calves (0–6 months) and 36 diarrheic cattle (≥ 6 months) from farms located in Merkez, İhsaniye, Sinanpaşa, İscehisar, and Cay districts of Afyonkarahisar province during the period from May 2014 to April 2015. Farms were visited once during the study period, fecal samples were quickly collected from the rectum of all animals using a latex glove and then transferred into sterile containers. The time, area, location of farm, animal genders, age, and breed were additionally noted. The samples collected were brought to the Department of Parasitology at the Afyon Kocatepe University, Faculty of Veterinary Medicine for rapid analysis. *Cryptosporidium* oocysts in fecal samples were identified with the mAF method as previously described.[29]

DNA Isolation and PCR Amplification

The isolation of the DNA was carried out utilising a modified version of the Boom et al. method [30], which was adapted for use with 500 nm magnetic beads. DNA was obtained from 14 specimens of *Cryptosporidium* spp. oocysts. The GeneJet Genomic DNA extraction kit (Thermo Scientific, K0701) was used for the isolation of DNA. The obtained DNA specimens were stored at -80°C until the PCR stage. The primers were designed by the FastPCR Professional 6.1.2 package program [36] based on the references SSU 18 S RNA gene sequence (AF308600.1, MF074583.1, OP836383.1).

A two-step nested-polymerase chain reaction (nested-PCR) methodology was utilised to amplify the 18S rRNA gene fragments from several *Cryptosporidium* isolates, employing primers that were supplementary to the conserved nucleotide sequences, which were obtained from the GenBank database. A PCR product of approximately 1328 bp was amplified using forward (5′-CGGTTAAACTGCGAATGGCTC-3′) and reverse (5′-CATCTAAGGGCATCACAGACC-3′) primers. The PCR mixture comprised 20 ng of DNA, 5 µM dNTP mix, 1× PCR buffer (including 1.5 mM $MgCl_2$), 2.0 U of Phusion High-Fidelity DNA Polymerases (Thermo Fhisher Scientific, USA, F530S), and 5 µM of each primer in a total volume of 25 µL. The reactions were carried out for 35 cycles, including 30 seconds at 95°C, 30 seconds at 62°C, and 1 minute at 72°C, utilizing the Veriti PCR equipment (Thermo Fisher Scientific, USA), preceded by a first provide heated start at 95°C for 5 minutes and ending with a final extension step at 72°C for 10 minutes. In the following phase of PCR, a fragment of 848 bp was amplified utilizing 2.5 µl of the primary PCR mixture together with a set of nested forward (5′-GGAAGGGTTGTATTTATTAGATAAAG-3′) and reverse (5′-CTCATAAGGTGCTGAAGGAGTA-3′) primers. The parameters for the secondary PCR were identical to those of the first PCR, except for a reduced annealing temperature of 56°C. The PCR product was analyzed by agarose gel electrophoresis and visualized following staining with RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology, 21141).

Sequencing and Phylogenetic Analysis

The secondary-PCR products were purified using ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher Scientific, USA, 78201.1) prior to DNA sequencing and sequenced on an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific, USA) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA, 4337455).

After DNA sequencing, each sample was edited using Sequencher 5.4.6 (Gene Codes Corporation) and subsequently aligned with the BioEdit 7.0.9 Sequence Alignment[32] program.

Phylogenetic analyzes based on aligned sequences were conducted to assess the evolutionary relationships among Cryptosporidium species. A neighbour-joining tree[33] was constructed using the MegaX program[34], with evolutionary distances calculated through Maximum Composite Likelihood analysis. The reliability of the trees was evaluated using the bootstrap method[35] with 1,000 pseudoreplicates. Tajima's Neutrality Test was used to determine whether populations underwent mutation and natural selection.[36,37] The MegaX software package program[34] was used to calculate the nucleotide differences (π), the mutation rate (ϴ), and the Tajima D value.[38,39]

Statistical Analysis

The frequencies of Cryptosporidium infection in the studied bovine populations were compared by age group, animal sex, breed, and farms of origination by the chi-square test. The statistical evaluation of the data used the "SPSS 22.0 (IBM SPSS Corp., Armonk, NY, USA) for Windows" package program. A probability $P < .05$ was accepted as indication of statistical importance.

RESULTS

The frequency of bovine cryptosporidiosis in Afyonkarahisar province was found to be 7.4% using microscopy, with *Cryptosporidium* spp. oocysts found in 14/188 of the fecal specimens investigated. Among the 14 observed *Cryptosporidium* infections, 13.6% (12/88) were in calves aged 0 to 3 months, whereas 3.1% (2/64) were in calves aged 3 to 6 months. *Cryptosporidium* oocysts were absent in the fecal samples of diarrheic cattle older than 6 months (Figure 1).

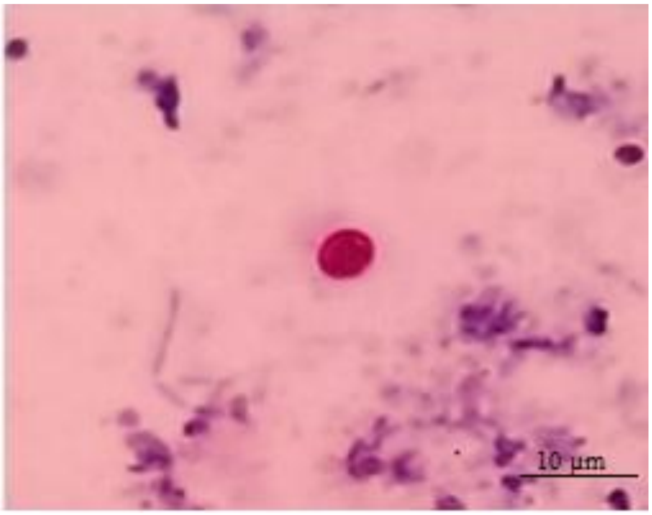

Figure 1. Microscopic inspection of *Cryptosporidium* oocysts in the stools of diarrhoeic calves stained via a modified acid-fast technique. (×40).

The occurrence of *Cryptosporidium* species among age group, gender, and breed in diarrheic calves and cattle is shown in Table 1.

Among the age groups, 0- to 3-month-old calves had the highest (13.6%) infection rate (Table 1). The difference between age groups was found to be statistically significant (*P* < .05). The overall prevalence of bovine cryptosporidiosis in Holstein, Simmental and Brown Swiss was 10.6%, 6.7% and 4.7%, respectively, with a statistically significant difference between the breeds (*P* < .05) (Table 1). The overall prevalence of *Cryptosporidium* in female and male was found to be 8.4% and 6.1%, respectively, with no significant difference between them (*P* > .05).

Table 1. Distribution of *Cryptosporidium* species according to the age, sex, and breed of infected cattle.

Factor	Number of Animals Examined	Infected Animals		*P*
Age Groups (months)		Number	Percentage (%)	
0-3	88	12	13.6^a	< .05
3-6	64	2	3.1^b	
≥ 6	36	0	0^c	
Gender				
Female	107	9	8.4	> .05
Male	81	5	6.1	
Breed				
Holstein	66	7	10.6	> .05
Simmental	59	4	6.7	
Brown Swiss	63	3	4.7	
Total	188	14	7.4	

^{a,b,c}: The difference between groups with different letters in the same column is statistically significant.

The distribution of Cryptosporidiosis according to the research center is given in Table 2.

The highest prevalence was recorded in the Sinanpaşa district (14.6%). No statistically significant distinction was seen among the research centers (*P* > .05; Table 2).

According to the sequence analysis of 14 isolates from the 18S rRNA locus, two *Cryptosporidium* species were identified, including *C. parvum* (64.2%) and *C. bovis* (35.7%). The sequences from the isolates matching to each identified. *Cryptosporidium* species in the study were subsequently submitted in GenBank (Table 3). Among the *Cryptosporidium* isolates, the sequences of three (AF_CR01, AF_CR02, AF_CR04) were not re-uploaded to GenBank due to their 100% similarity with sequences documented in other research. The agarose gel image of the amplicons from some samples using PCR assay targeting 18S rRNA is given in Figure 2. The first-round PCR gel image has been added to illustrate the optimization and validation of the nested PCR protocol.

Table 2. Distribution of cryptosporidiosis according to the research center

Research Center	Number of Animals	Examined Number of Infected Animals (%)
Sinanpaşa	41	6 (14.6)
İscehisar	29	3 (10.3)
Merkez	32	2 (6.2)
Çay	49	2 (4.0)
İhsaniye	37	1 (2.7)
Total	188	14 (7.4)

Table 3. Codes, species and GenBank accession numbers of specimens used for comparative analysis.

Specimen Codes	Species	Accession Numbers
AF_CR01	*Cryptosporidium bovis*	Not submitted
AF_CR06		MT416391
AF_CR07		MT416392
AF_CR08		MT416393
AF_CR09		MT416394
AF_CR02	*Cryptosporidium parvum*	Not submitted
AF_CR03		MT416389
AF_CR10		MT416395
AF_CR11		MT416396
AF_CR04		Not submitted
AF_CR05		MT416390
AF_CR12		MT416397
AF_CR13		MT416398
AF_CR14		MT416399

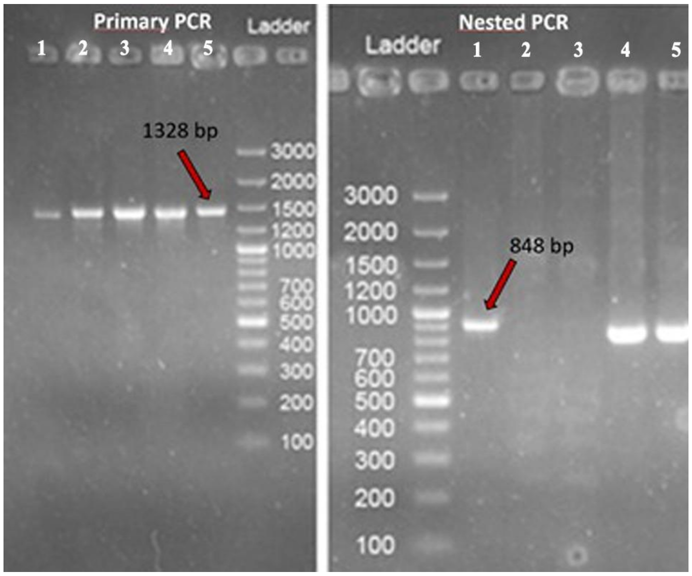

Figure 2. Figure 2. Agarose gel electrophoresis of PCR products targeting the SSU rRNA gene of Cryptosporidium spp. (Left) Primary PCR amplification showing products of approximately 1300–1500 bp. (Right) Nested PCR amplification yielding products ranging from approximately 800 to 1000 bp, depending on Cryptosporidium species. Variations in amplicon size reflect species-specific sequence length polymorphisms within the SSU rRNA gene.

Prevalence of infection in 0–3-month-old calves (13.6%) was higher than the 3–6-month-old calves (3.1%). The prevalence of *C. parvum* (66.6 %) was higher than *C. bovis* (33.3%) in 0–3-month-old calves (Table 4).

Tajima's Neutrality test results in positive fecal samples are given in Table 5.

Table 5 shows that the 14 positive samples had 27 distinctive polymorphic areas. The nucleotide variance (π) of the 18S rRNA region analyzed among the strains was estimated to be 1.7%. According to Tajima's test statistic, new mutations (D = 2.442337) were not observed in the examined populations.

Table 4. Distribution of *Cryptosporidium* species according to age groups in diarrheic calves and cattle

Age groups (months)	Number of examined animals	Number of infected animals (%)	*Cryptosporidium* species	
			C. parvum	*C. bovis*
			Infected animals (%)	
0-3	88	12 (13.6)	8 (66.6)	4 (33.3)
3-6	64	2 (3.1)	1 (50.0)	1 (50.0)
≥ 6	36	0	0	0

Table 5. Tajima's Neutrality test results in positive fecal samples

M	S	p_s	Θ	π	D
14	27	0.03567	0.011216	0.017638	2.4423378

m: Number of positive samples, S: Number of different nucleotides, ps: S/M, Θ: Mutation rate, π: Nucleotide differences, D: Tajima value

Phylogenetic analysis results of *Cryptosporidium* isolates are given in Figure 3.

The phylogenetic tree shows that the source of infection has different genetic structures in different animals (Figure 3).

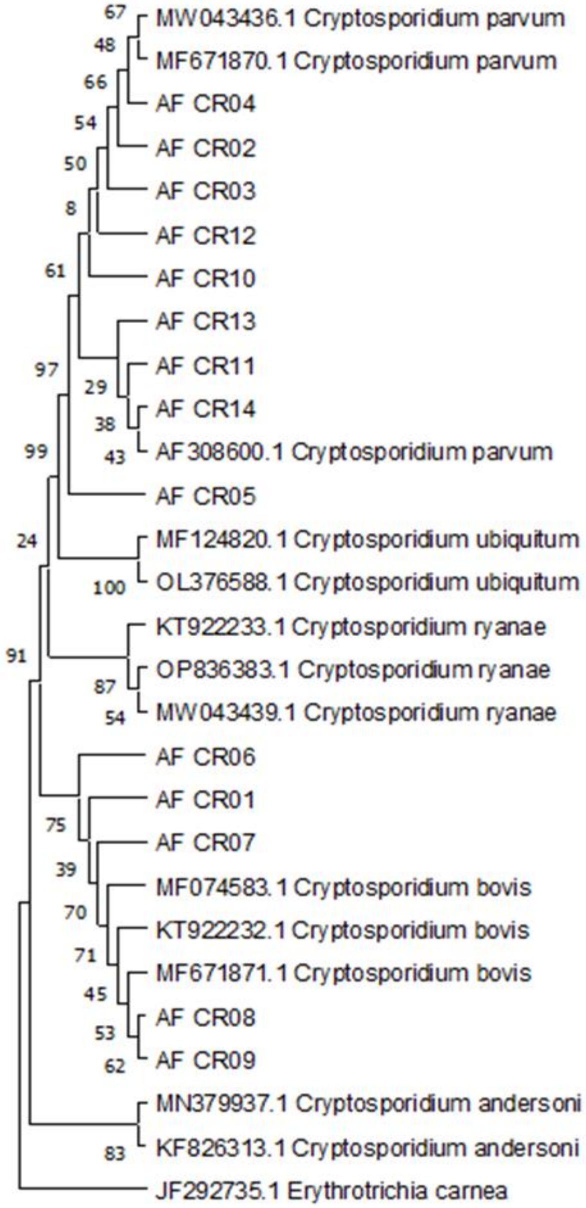

Figure 3. Phylogenetic classification of Cryptosporidium parasites revealed from neighbor-joining evaluation of 18S rRNA gene nucleotide sequences

DISCUSSION

Cryptosporidium is a major protozoan pathogen responsible for acute diarrhea in ruminant livestock, leading to severe illnesses and fatalities in neonatal animals. The global rates of *Cryptosporidium* infection in calves and pre-weaned cattle range between 27.0% and 37.5%.[1]

The most frequently occurring species in cattle include: *C. parvum*, *C. andersoni, C. bovis,* and *C. ryanae. C. parvum* is extremely harmful in neonatal calves, although *C. bovis* and *C. ryanae* may also cause serious diarrhea. *Cryptosporidium parvum* and *C. hominis* are responsible for human cryptosporidiosis. Consequently, infected cattle are regarded as significant reservoirs of *Cryptosporidium* for human diseases.[7,11] It is emphasized that *C. parvum*, identified as the most common species globally, particularly in cattle experiencing diarrhea, can be a significant disease agent for humans.[1]

Ouakli et al.[40] found *C. parvum* at 72.4%, *C. bovis* at 13.8%, *C. andersoni* at 3.4%, and *C. ryanae* at 3.4% of neonatal calves. Lichtmannsperger et al.[41] reported *C. parvum* in 77.4%, *C. ryanae* in 11.8%, and *C. bovis* in 7.5% of calves with diarrhea under 6 months of age. In studies conducted on cattle and calves in different regions within Türkiye, *C. parvum* was 3.9%-91.7%, *C. bovis* was 0.3-43.2%, and *C. ryanae* was 5.3%-57.1%.[21,23-26] In the research carried out in Afyonkarahisar province, *C. parvum* was detected in 66.6% of diarrheic calves aged 0-3 months and *C. bovis* in 33.3%. *Cryptosporidium parvum* was the predominant species in neonatal calves, which is consistent with previous studies.[21,23-26,40,41] The diagnosis of cryptosporidiosis in farm animals was made using microscopic, immunological, and molecular techniques. In recent years, the use of molecular techniques has become widespread due to their greater sensitivity and specificity.[13,42,43]

Prevalence of *Cryptosporidium* in molecular studies conducted in diarrheic calves in different age groups in various countries have been reported to be 88.6% in France[44], 38% in Iraq[45], 32.3% in India[46], 18.24% in China[47], 3.9%-68.4% in Türkiye.[13,14,17,21-26] The variations in total *Cryptosporidium* prevalence among studies may be ascribed to changes in ecology, research design, seasonal factors, management systems, age, herd size, and laboratory methodologies employed.[48] Alba et al.[49] reported that prevalence of dairy calf cryptosporidiosis in the age group of ≤20 days (25.2-42.5%) was higher than prevalence of 16.3–25.5% in the age group of under 90 days. Fayer et al.[5] indicated that prevalence of cryptosporidiosis was 41% in un-weaned calves, 26% in weaned calves, 12% in heifers, and 5.7% in cows, respectively. The prevalence of *Cryptosporidium* spp. infection was reported to be 58.2% and 57.5% in un-weaned calves, 45.5% in weaned calves, and 25.3% in cows and 18.5% in heifers, respectively[21] In the present study, cryptosporidiosis infection was detected to be 13.6% in 0-3 month-old calves and 3.1% in 3-6 month-old calves, while no oocysts were identified in diarrheic cattle above 6 months old. This supports previous studies indicating that neonatal calves are particularly susceptible due to immature immune systems.[5,21,46,49]

Certad et al.[50] determined the rate of cryptosporidiosis infection to be 30% in their research on postpartum cows and heifers. It has been reported that *C. andersoni* causes infection in cows, while *C. ryanae*, *C. bovis*, and *C. andersoni* cause infection in heifers. In this study, no infection was found in cattle older than 6 months. Additionally, only the species *C. bovis* and *C. parvum* were identified. These differences in research results suggest that they are related to changes in age, season, and geographical region.

Şimşek et al.[23] revealed that the overall prevalence of *Cryptosporidium* was 32.6%, 25% and 11.1% in Holstein, Simmental and Brown Swiss breeds, respectively, with a statistically significant difference ($P < .05$) between the Brown Swiss and Holstein breeds. In the present study, the overall prevalence of *Cryptosporidium* was 10.60%, 6.78%, and 4.77% in the Holstein, Simmental, and Brown Swiss breeds, respectively, with no significant difference between them ($P > .05$).

Oğuz et al.[14] found the molecular prevalence of Cryptosporidiosis in cattle to be 9.5% using PCR technique. They reported three different *Cryptosporidium* species (*C. bovis*, *C. ryanae* and *C. parvum*), and *C. parvum* was detected at the highest rate in calves aged 0-6 months (17.31%). The infection rate determined in their study was similar to our results, but *C. ryanae* was not found in this study. This indicates that *C. ryanae* can be observed in specific geographical areas, age groups, and seasons, and can be locally dominant in those regions.

In conclusion, this study determined the prevalence and genetic diversity of *Cryptosporidium spp*. found in diarrheic calves in the city center of Afyonkarahisar province and 4 different districts, which is of great importance for cattle breeding. Our findings indicate that neonatal cryptosporidial diarrhea in calves is primarily associated with *C. parvum*. However, broader biological and molecular epidemiological research is needed to determine the epidemiology and transmission dynamics of cryptosporidiosis in this region. The absence of positive and negative controls in PCR tests is a limitation that could affect the reliability of amplification results. Therefore, this situation should not be overlooked in the comprehensive studies to be planned.

Ethics Committee Approval: This research received approval by the Afyon Kocatepe University Local Ethics Committee on Animal Experiments (Date: April 18, 2014 Number: 49533702/51).

Author Contributions: Concept-M.E., K.K.; Design- M.E, H.Ç., M.E., M.K., K.K.; Supervision- M.E., K.K.; Resources- M.E., K.K.; Data Collection and/or Processing- M.E., H.Ç., M.E., K.K.; Analysis and/or Interpretation- M.E., H.Ç., M.E., M.K., K.K.; Literature Search- M.E., H.Ç., K.K; Writing Manuscript- M.E., H.Ç., K.K.; Critical Review- M.E., H.Ç., M.E., M.K., K.K.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors declare no conflict of interest.

Financial Disclosure: Afyon Kocatepe University Scientific Projects Fund (Project No. 14.VF.01) has financially supported this study.

Use of Artificial Intelligence: No artificial intelligence program was used in this article.

REFERENCES

1. Buchanan R, Wieckowski P, Matechou E, Katzer F, Tsaousis AD, Farré M. Global prevalence of Cryptosporidium infections in cattle: A meta-analysis. *Curr Res Parasitol Vector Borne Dis*. 2025;100264.
2. Weldemariam DT, Awoke MG, Aklilu MA. Review on bovine cryptosporidiosis, its associated risk factors and diagnostics methods. *Sm Trop Med J*. 2024;6:1-8.
3. Huang J, Chen M, He Y, Chen H, Huang M, Li N, Guo Y. Cryptosporidium equi n. sp.(Apicomplexa: Cryptosporidiidae): biological and genetic characterisations. *Int J Parasitol*. 2023;53(10):545-554.
4. Ryan UM, Feng Y, Fayer R, et al. Taxonomy and molecular epidemiology of Cryptosporidium and Giardia–a 50 year perspective (1971–2021). *Int J Parasitol*. 2021;51(13-14):1099-1119.
5. Fayer R, Santin M, Trout JM. Prevalence of Cryptosporidium species and genotypes in mature dairy cattle on farms in eastern United States compared with younger cattle from the same locations. *Vet Parasitol*. 2007;145:260-266.
6. Fayer R, Santín M,Trout, JM. Cryptosporidium ryanae n. sp.(Apicomplexa: Cryptosporidiidae) in cattle (Bos taurus). *Vet Parasitol*. 2008;156(3-4):191-198.
7. Widmer G, Carmena D, Kváč M, et al. Update on Cryptosporidium spp.: Highlights from the seventh international Giardia and Cryptosporidium conference. Parasite. 2020;27:14.
8. Santín M, Trout JM, Fayer R. A Longitudinal study of cryptosporidiosis in dairy cattle from birth to 2 years of age. *Vet Parasitol*. 2008;155(1-2):15-23.
9. Imre K, Lobo LM, Matos O, Popescu C, Genchi C, Dărăbuş G. Molecular characterisation of Cryptosporidium isolates from pre-weaned calves in Romania: Is there an actual risk of zoonotic infections? *Vet Parasitol*. 2011;181(2-4):321-324.
10. Roblin M, Canniere E, Barbier A, et al. Study of the economic impact of cryptosporidiosis in calves after implementing good practices to manage the disease on dairy farms in Belgium, France, and the Netherlands. *CRPVBD*. 2023;4:100149.
11. Razakandrainibe R, Diawara EHI, Costa D, et al. Common occurrence of Cryptosporidium hominis in asymptomatic and symptomatic calves in France. *PLoS Negl Trop Dis*. 2018;12:e0006355.
12. Reif JS, Wimmer L, Smith JA, Dargatz DA, Cheney JM. Human cryptosporidiosis associated with an epizootic in calves. *Am J Public Health*. 1989;79(11):1528-1530.
13. Karakavuk M, Can H, Can Ş, Karakavuk T, Döşkaya M, Döşkaya AD. Development of a "Rapid-Crypto Colorimetric LAMP Test" to detect cryptosporidiosis in feces of newborns calves. *Acta Parasitol*. 2024;69:691-699.
14. Oğuz EF, Kilinç OÖ, Ayan A, et al. Cryptosporidiosis in cattle and buffaloes in Türkiye- molecular analysis and public health significance. *Acta Sci Vet*. 2025;53:1995
15. Arslan MÖ, Gıcık Y, Erdoğan HM, Sarı, B. Prevalence of Cryptosporidium spp. oocysts in diarrhoeic calves in Kars province, Türkiye. *Turk J Vet Anim Sci*. 2001;25(2):161-164.
16. Çitil M, Arslan MÖ, Güneş V, Erdoğan HM. Neonatal buzağı ishallerinde Cryptosporidium ve Eimeria enfeksiyonlarının rolü. *Kafkas Üniv Vet Fak Derg*. 2004;10(1):59-64.
17. Sungur T, Kar S, Güven E, Aktaş M, Karaer Z, Vatansever Z. Cryptosporidium spp'nin dışkıdan Nested PCR ve carbol fuchsin boyama yöntemi ile teşhis edilmesi. *Türkiye Parazitol Derg*. 2008;32(4):305-308.
18. Sarı B. Aktaş MS, Arslan MÖ. Erzurum yöresinde buzağılarda Cryptosporidium türlerinin prevalansı. *Türkiye Parazitol Derg*. 2008;32(2):116-119.
19. Ekinci Ö, Sevinç F, Coşkun A, Işık N, Sevinç N. İshalli buzağılarda cryptosporidiosisin yaygınlığı. *Eurasian J Vet Sci*. 2011;27(2):123-126.
20. Çelik ÖY, Şahin T, Çelik BA. Prevalence and molecular characterization of Cryptosporidium spp. in calves in the Siirt Province, Türkiye. *Acta Veterinaria Brno*. 2023;92(3):243-250.
21. Yıldırım A, Adanır R, Inci A, et al. Prevalence and genotyping of bovine Cryptosporidium species in the Mediterranean and Central Anatolia Region of Türkiye. *Comp Immunol Microbiol Infect Dis*. 2020;69:101425.
22. Sayın İpek DN, Sarı B. Occurrence and molecular characterization of Cryptosporidium spp. and Giardia duodenalis in lambs and calves in Southeastern Anatolia, Türkiye. *Egyptian J Vet Sci*. 2024;55(2):599-606.
23. Şimşek A, İnci A, Yıldırım A, Çiloğlu A, Bişkin Z, Düzlü Ö. Nevşehir yöresindeki yeni doğan ishalli buzağılarda Cryptosporidiosis'in Real Time PCR ve Nested PCR yöntemleri ile saptanması. *Erciyes Üniv Vet Fak Derg*. 2012;9(2):79-87.
24. Güven E, Avcioglu H, Balkaya I, Hayırlı A, Kar S, Karaer Z. Prevalence of cryptosporidiosis and molecular characterization of Cryptosporidium spp. in calves in Erzurum. *Kafkas Univ Vet Fak Derg*. 2013;19(6):969-974.
25. Kabir MHB, Ceylan O, Ceylan C, et al. Molecular detection of genotypes and subtypes of Cryptosporidium infection in diarrheic calves, lambs, and goat kids from Türkiye. *Parasitol Int*. 2020;79:102163.
26. İpek DNS. Diyarbakır yöresinde ishalli buzağılarda Cryptosporidium spp. yaygınlığı ve moleküler karakterizasyonu. *Dicle Üniv Vet Fak Derg.* 2022;15(2):9-13.
27. Özçelik S, Poyraz Ö, Kalkan K, Malatyalı E, Değerli S. Hayvancılıkla uğraşanlarda ve sığırlarda Cryptosporidium spp. yaygınlığının ELISA ile araştırılması. *Kafkas Univ Vet Fak Derg*. 2012;18(Suppl-A):A61-A62
28. Gündüz N, Arslan, MÖ. Kars yöresinde buzağılarda cryptosporidium enfeksiyonları prevalansının asit fast boyama (mAF) ve ELISA yöntemleriyle belirlenmesi. *Türkiye Parazitol*

Derg. 2017:41;5-8.

29. Turgay N. Özel Boyama Yöntemleri. Korkmaz M, Ok ÜZ. (Eds) Parazitolojide laboratuvar. Türkiye Parazitoloji Derneği. Yayın No: 2011;23:37-38.
30. Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, Noordaa JVJ. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol*. 1990;28(3):495-503.
31. Kalendar R, Lee D, Schulman AH. FastPCR software for PCR primer and probe design and repeat search. *Genes Genomes Genomics*. 2009;3:1-14.
32. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: Nucleic acids symposium series. 1999;95-98.
33. Saitou N, M Nei. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*. 1987;4(4):406-25.
34. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol*. 2018;35(6):1547-1549.
35. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985;39(4):783-791.
36. Innan H, Stephan W. The coalescent in an exponentially growing metapopulation and its application to Arabidopsis thaliana. *Genetics*. 2000;155:2015-2019.
37. McVean GA. A genealogical interpretation of linkage disequilibrium. *Genetics*. 2002;162(2):987-991.
38. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*. 1989;123(3):585-595.
39. Nei M, Kumar S. Molecular evolution and phylogenetics. Oxford university press. 2000.
40. Ouakli N, Belkhiri A, Lucio A, et al. Cryptosporidium-associated diarrhoea in neonatal calves in Algeria. *Vet Parasitol*. 2018;12:78-84.
41. Lichtmannsperger K, Harl J, Freudenthaler K. Cryptosporidium parvum, Cryptosporidium ryanae, and Cryptosporidium bovis in samples from calves in Austria. *Parasitol Res*. 2020;119:4291-4295.
42. Aboelsoued D, Megeed KNA. Diagnosis and control of cryptosporidiosis in farm animals. *J Parasit Dis*. 2022;46(4):1133-1146.
43. Guy RA, Yanta CA, Bauman CA. Molecular identification of Cryptosporidium species in Canadian post-weaned calves and adult dairy cattle. *Vet Parasitol*. 2022;34:100777.
44. Mammeri M, Chevillot A, Chenafi I, et al. Molecular characterization of Cryptosporidium Isolates from diarrheal dairy calves in France. *Vet Parasitol: Reg Stud Rep*. 2019;18:100323.
45. Alali F, Abbas I, Jawad M, Hijjawi N. Cryptosporidium infection in humans and animals from Iraq: A review. *Acta Trop*. 2021;220:105946.
46. Paul S, Chandra D, Tewari AK, et al. Comparative evaluation and economic assessment of coprological diagnostic methods and PCR for detection of Cryptosporidium spp. in bovines. *Vet Parasitol.* 2009;164(2-4):291-295.
47. Zhao L, Wang M, Wang L, et al. Prevalence and molecular characterization of Cryptosporidium spp. in dairy and beef cattle in Shanxi, China. *Parasitol Res*. 2024;123:8.
48. Bamaiyi PH, Redhuan NEM. Prevalence and risk factors for cryptosporidiosis: A global, emerging, neglected zoonosis. *Asian Biomed*. 2016;10:309-325.
49. Alba P, Garro C, Florin-Christensen M. Prevalence, risk factors and molecular epidemiology of neonatal cryptosporidiosis in calves: The Argentine perspective. *Curr Res Parasitol Vector Borne Dis*. 2023;4:100147.
50. Certad G, Gantois N, Merlin S, et al. Frequency and molecular identification of Cryptosporidium in adult Prim'Holstein dairy cattle farms in the North of France. *Microorganisms*. 2024;12(2):335.