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RESEARCH ARTICLE

Detection of Cryptosporidiosis in Diarrheic Calves in The Kars Region of Türkiye Using Microscopy and Coproantigen Tests

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Abstract

Cryptosporidiosis is a parasitic disease that causes diarrhea in vertebrate animals. It is frequently observed in neonatal livestock, particularly in calves. The infection is globally widespread and has been reported in various animal species in Türkiye. This study aimed to assess the prevalence of *Cryptosporidium* spp. in calves suffering from diarrhea using both microscopic staining and coproantigen-based detection techniques. One hundred (100) calves suffering from diarrhea were studied from in Kars region. Oocysts were purified using the flotation technique and subsequently stained with the modified acid-fast method (mAF). All samples were also analyzed using a rapid coproantigen test. Microscopic examination revealed that 8 out of 100 samples (8%) were positive for *Cryptosporidium*, while coproantigen detection identified 20 positive samples (20%).

Key Words: Calves, coproantigen rapid test, cryptosporidiosis, *Cryptosporidium*, Kars, modified acid-fast

Türkiye'nin Kars Bölgesinde İshalli Buzağılarda Mikroskopi ve Koproantijen Testi ile Cryptosporidiosisin Tespiti

Öz

Cryptosporidiosis, omurgalı hayvanlarda ishale neden olan paraziter bir hastalıktır. Yenidoğan hayvanlarda, özellikle buzağılarda sıklıkla görülür. Enfeksiyon dünya çapında yaygındır ve Türkiye'de çeşitli hayvan türlerinde bildirilmiştir. Bu çalışma, mikroskobik boyama ve koproantijen bazlı tespit tekniklerini kullanarak ishal olan buzağılarda *Cryptosporidium* spp. prevalansını değerlendirmek amacıyla yapılmıştır. Kars bölgesinde ishal olan yüz (100) buzağı incelenmiştir. Oositler flotasyon tekniği kullanılarak saflaştırılmış ve ardından modifiye asit dirençli yöntem (mAF) ile boyanmıştır. Tüm örnekler hızlı koproantijen testi ile de analiz edilmiştir. Mikroskobik inceleme, 100 numunenin 8'inde (%8) *Cryptosporidium* pozitifliği saptanırken, koproantijen tespiti ile 20 pozitif numune (%20) saptanmıştır.

Anahtar Kelimeler: Buzağı, cryptosporidiosis, coproantijen test, *Cryptosporidium*, Kars, modifiye asit-fast

INTRODUCTION

Diarrhea is one of the leading causes of mortality in neonatal calves and can be attributed to various bacterial, viral, and parasitic pathogens (1). The highest susceptibility of calves to *Cryptosporidium* spp. infections occurs between the first and third weeks of life (2). Cryptosporidiosis, an important diarrheal disease in calves, is caused by the apicomplexan parasite *Cryptosporidium*, with more than 49 recognized species (3,4). In cattle, the most frequently detected *Cryptosporidium* species include *C. parvum*, *C. bovis*, *C. andersoni*, and *C. ryanae* (5,6). *C. parvum* is primarily associated with clinical cryptosporidiosis in pre-weaned calves, whereas *C. bovis and C. ryanae* are more commonly identified in subclinical infections among weaned calves. (7-9).

Cryptosporidiosis has significant effects on dairy calves, leading to increased mortality, stunted growth, and adverse impacts on female calves by delaying the age at first calving and reducing future milk production. *C. parvum* is considered endemic in cattle worldwide and is among the most common causes of diarrhea in the dairy industry. Calves are at the greatest risk of infection during their first month of life, when they are exposed to high concentrations of pathogens in their environment through the fecal–oral route (10).

Zoonotic *Cryptosporidium* species parasitize young ruminants, especially calves, causing the most significant losses for the livestock industry (1,11). Cryptosporidiosis causes diarrhea and enteritis in humans, while bovine cryptosporidiosis causes bovine enteritis, increased mortality and reduced growth (1). *Cryptosporidium* oocysts are resistant to many disinfectants and treatment options for the disease are limited. An antigen-based vaccine has recently been developed for cattle (Bovilis Cryptium, Merck & Co., Inc., Rahway, NJ, USA), which is administered to pregnant cows to raise anti-*Cryptosporidium* antibody levels in their colostrum and provide passive immunization of calves (12). Although this helps to reduce the clinical signs of cryptosporidiosis in calves, it does not completely prevent infections.

The global prevalence of cryptosporidiosis in dairy calves has been reported to range from 13.2% to 30.7% depending on the region, with an overall worldwide prevalence estimated at 21.9% (13). Studies on the prevalence of cryptosporidiosis in calves in Türkiye have reported rates ranging from 5.1% to 70.3% (14,15).

This study was carried out to determine the presence of *Cryptosporidium* spp. in calves in the Kars Province by microscopic and coproantigen methods.

MATERIAL AND METHODS

Ethics Committee Approval

This study was approved by the Kafkas University Animal Experiments Local Ethics Committee (Approval no: KAU-HADYEK/2025-117).

Sample Collection

The study material consisted of fecal samples from 100 diarrheic calves aged 1 to 25 days, brought to the Veterinary Faculty Hospital of Kafkas University during the winter-spring season of 2025. Of these samples, 47 were from female calves and 53 from male calves. Based on age, the fecal samples were divided into three groups: 1–3 days old (18 samples), 4–10 days old (53 samples), and 11–25 days old (28 samples).

Microscopic Examination

For microscopic diagnosis, direct sedimentation was performed on diarrheic samples, and smears were prepared from the obtained sediment. After air-drying, the smears were stained using the modified Acid-Fast (mAF) method and examined under a light microscope at 100× objective magnification based on morphological characteristics. At least 20 fields were scanned for each slide.

Coproantigen Screening

For antigen detection in feces, all samples were subjected to a commercially available rapid test kit (Vetdiagnostix, China). All procedures were performed according to the manufacturer's instructions.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 25.0; IBM Corp., Armonk, NY, USA). Differences in positivity rates between diagnostic methods were evaluated using the Chi-square test with Yates' correction and Fisher's Exact test. Statistical significance was considered at the $P<0.05$ level.

RESULTS

In the examinations performed using the modified acid-fast (mAF) staining method, *Cryptosporidium* spp. oocysts were detected in 8% of the samples, whereas the coproantigen rapid test yielded 20% positive results. Statistical analysis confirmed that the difference between the two diagnostic methods was significant (Chi-square test, $\chi^2 = 5.02$, P=0.025; Fisher's Exact test, P=0.024), demonstrating the higher sensitivity of the coproantigen test. Based on age distribution, mAF staining identified 2 positive samples in calves aged 1–3 days (11.1%), 6 in the 4–10-day group (11.1%), and 2 in the 11–25-day group (7.1%), while the coproantigen rapid test detected 3 positives in the 1–3-day group (16.6%), 13 in the 4–10-day group (24.1%), and 9 in the 11–25-day group (32.1%). However, statistical analysis revealed no significant differences in positivity among the age groups for either method (mAF: $\chi^2 = 0.353$, P=0.838; coproantigen test: $\chi^2 = 1.453$, P=0.484). According to sex, mAF staining detected *Cryptosporidium* spp. in 3 of 47 female calves (6.4%) and 5 of 53 male calves (9.4%), with no significant association between sex and positivity (Chi-square test, $\chi^2 = 0.037$, P=0.848; Fisher's Exact test, P=0.719). In contrast, the coproantigen rapid test identified 14 positives among females (29.8%) and 6 among males (11.3%), and this difference was statistically significant (Chi-square test, $\chi^2 = 4.218$, P=0.040; Fisher's Exact test, P=0.026), indicating a higher prevalence of infection in female calves (Table 1).

Based on age distribution, mAF staining identified 2 positive samples in calves aged 1–3 days (11.1%), 6 in the 4–10-day group (11.1%), and 2 in the 11–25-day group (7.1%). The coproantigen rapid test detected 3 positives in the 1–3-day group (16.6%), 13 in the 4–10-day group (24.1%), and 9

in the 11–25-day group (32.1%). According to sex, mAF staining detected *Cryptosporidium* spp. in 3 of 47 female calves (6.4%) and 5 of 53 male calves (9.4%), while the coproantigen rapid test identified 14 positives among females (29.8%) and 6 among males (11.3%).

Table 1. Detection of *Cryptosporidium* spp. in calves by diagnostic method, age group

Diagnostic Method	Group	Positive (n)	Negative (n)	Total	Positivity (%)
mAF staining	1–3 days	2	16	18	11.1
	4–10 days	6	48	54	11.1
	11–25 days	2	26	28	7.1
	Female	3	44	47	6.4
	Male	5	48	53	9.4
	Total	8	92	100	8.0
Coproantigen rapid test	1–3 days	3	15	18	16.6
	4–10 days	13	41	54	24.1
	11–25 days	9	19	28	32.1
	Female	14	33	47	29.8
	Male	6	47	53	11.3
	Total	20	80	100	20.0

DISCUSSION AND CONCLUSION

Escherichia coli, *Cryptosporidium* spp., coronavirus, and rotavirus are among the most common pathogens responsible for neonatal diarrhea in calves. Diarrhea in newborn calves represents a major health problem and leads to substantial economic losses worldwide. Numerous studies have demonstrated that the incidence of diarrhea in calves is strongly associated with the prevalence of *Cryptosporidium* spp. (16). Cryptosporidiosis in neonatal calves can be transmitted from multiple sources, including dams, other animals on the farm, caretakers, and the environment. The parasite's life cycle allows rapid multiplication within the host, facilitating swift spread of the disease within susceptible animal groups (17). Millions of infectious oocysts excreted into the environment by infected animals are resistant to many farm disinfectants and chlorination, making control and eradication of the disease particularly challenging. Currently, no vaccine is available, and treatment options remain limited. Considering these constraints, *C. parvum* may lead to severe outbreaks in susceptible calves and, in more serious cases, result in substantial economic losses to farm productivity (17,18).

Staining methods, immunological methods, antigen detection methods, and molecular diagnostic methods are used in the diagnosis of cryptosporidiosis. Of these, routine staining methods, direct fluorescent antibody (DFA), enzyme immunoassay (EIA, ELISA), and rapid diagnostic tests are used in studies to determine the prevalence of the parasite. Immunological methods have been reported to be more sensitive than staining methods (15,18,19). In recent years, commercial immunochromatographic tests have been used for the rapid detection of *C. parvum* or *C. parvum* and other important intestinal pathogens in calf feces. These tests enable the detection of *C. parvum* antigens in direct feces within a few minutes (17,20-22).

Many enteropathogens in neonatal calves exhibit similar clinical manifestations, which often leads to misdiagnosis. Therefore, before associating a specific agent with clinical findings, diagnostic tests should be performed to exclude other enteric pathogens that may cause similar symptoms. Although specialized tests that detect oocysts, antigens, or DNA of *C. parvum* in feces are practical, their major limitations are the lengthy stool examination procedures and high costs (17). In contrast, the coproantigen test used in our study offers clear advantages, including ease of use, rapid results, and relatively lower cost.

This study determined a prevalence of 8% and 20% using modified acid-fast and coproantigen test, respectively. Globally, the prevalence of bovine cryptosporidiosis has been reported to range from a high of 74.8% (Portugal) to a low of 4.4% (Korea) (23,24).

In recent studies conducted in Türkiye, the prevalence of cryptosporidiosis in calves was 34% in Kayseri (25), 37.2% in Burdur (25), 25.5% in Diyarbakır (9), 8% in Siirt (26), and 3.9–22.8% in Erzurum (11,27). In the Kars region, the prevalence of cryptosporidiosis in calves has been reported to range from 4 to 32.9%. (15,28-31). In our study, the prevalence of cryptosporidiosis in calves with diarrhea was found to be 20%. Although this rate is relatively lower than some previously reported prevalence values for cryptosporidiosis in Türkiye, it is comparable to those reported in the Kars region.

In our study, the infection rate was higher in females (29.8%) than in males (11.3%) based on the coproantigen rapid test, and this difference was statistically significant. Previous studies (26,27) have also reported a higher prevalence in females, which is consistent with our findings.

In the present study, the prevalence of *Cryptosporidium* spp. was higher according to the coproantigen test (20%) compared to the mAF method (8%), and this difference was found to be statistically significant (χ^2 = 5.02, P=0.025; Fisher's Exact test, P=0.024). Although staining methods are often considered the gold standard, the reliability of fecal antigen detection tests is also high (17). The detection of antigens in animals that are infected but have not yet started oocyst shedding is important for the diagnosis of the disease.

Rapid diagnosis of cryptosporidiosis is essential for preventing the spread of infection to other animals and for reducing the risk of zoonotic transmission through the isolation of affected animals and disinfection of contaminated farms. In our study, the coproantigen rapid test demonstrated a higher detection rate (20%) compared to the mAF staining method (8%), highlighting the diagnostic value of antigen-based assays. In addition to their higher sensitivity, rapid tests offer practical advantages, including the ability to process a large number of samples efficiently with minimal effort, the clarity of the results, and the limited need for specialized laboratory equipment. These features make commercially available rapid tests a convenient and reliable tool for field use in determining the infection status of calves with diarrhea caused by *C. parvum* (17).

In this study, the prevalence of *Cryptosporidium spp.* in diarrheic calves in the Kars region was determined to be 8%

by the mAF staining method and 20% by the coproantigen rapid test. These findings demonstrate that cryptosporidiosis is an important causative agent of diarrhea in calves. If left untreated, the infection can lead to high mortality, and due to its zoonotic nature, it also poses a serious threat to public health. Overall, the results emphasize the significance of the disease in both veterinary and human medicine and highlight the necessity of employing rapid and reliable diagnostic methods for effective control and prevention.

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There was no funding from any organization to conduct this research.

CONFLICT OF INTEREST

There is no conflict of interest to be declared by the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, N.Ö.; methodology, N.Ö. and E.A.; sample collection, E.A. and M.S.; writing - original draft preparation, N.Ö. N.A. and G.T.T.; writing - review and editing, N.Ö. and B.S. The writing and final checks of the study were carried out with the contributions of all authors.

ETHICAL STATEMENT

The ethical committee approval required for this study was obtained from the Kafkas University Local Animal Experiments Ethics Committee with decision number 2025-117.

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