MAE Vet Fak Derg, 8 (2): 55-60, 2023
DOI: 10.24880/maeuvfd.1120634

Research Article

# Etiological examination of neonatal calf diarrhea cases detected in Burdur region

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**Key Words:**
calf
diarrhea
etiology
neonatal
rapid diagnostic test

Received :24.05.2022
Accepted :26.08.2022
Published Online : 31.08.2023
Article Code : 1120634

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## ABSTRACT

This work was conducted to determine the prevalence of *E. coli*, *Cryptosporidium* spp., *Giardia* sp., rotavirus and coronavirus agents involved in the etiology of diarrhea in cases of neonatal calf diarrhea detected in Burdur Mehmet Akif Ersoy University Animal Hospital Clinics and other farms in the region between 2019-2020 years. The material of the study consisted of 50 neonatal calves with diarrhea. Stool samples were taken from calves with diarrhea and examined with immunoch-romatographic rapid test kits. One or more enteropathogenic agents were detected in 70% of the calves. In calves with diarrhea, *Cryptosporidium* spp (42%) and rotavirus (30%) were detected as single and mixed enteropathogen. Mixed enteropathogens (14% *Cryptosporidium* spp. + Rotavi-rus, 2% Rotavirus + coronavirus, 2% *Cryptosporidium* spp. + rotavirus + coronavirus + *E. coli* K99 + *Giardia* spp, 2% *Cryptosporidium* spp. + coronavirus + *E. coli* K99 + *Giardia* spp) were determi-ned in 20% of calves. As a result, it was concluded that in neonatal calf diarrhea, *Cryptosporidium* spp. and rotavirus factors are common and necessary precautions should be taken in enterprises against these factors.

## INTRODUCTION

The neonatal period is the most sensitive period of calf rearing, covering the first 28 days from birth. Diarrhea is one of the most important symptoms threatening the life of calves during this period. Because diarrhea increases the mortality rate in sick calves and causes great economic losses, it is very important to combat diarrhea in newborn calves (Tokgoz et al., 2013).

Diarrhea is a symptom characterized by an increase in the daily amount of stool and the number of stools, and the consistency of the stool is watery. It can be shaped due to infectious and non-infectious causes (Roy, 1980). Diarrhea in studies; Enthropathogenic bacterial species such as *Clostridium perf-ringens* (*Cl. Perfringens*), *Escherichia coli* (*E. coli*), *Salmonella* spp., *Chlamydia* spp., and *Camphylobacter jejuni*, Viruses such as Rotavirus, coronavirus, Coccidia, *Cryptosporidium* spp and *Giardia* spp, environmental factors, care and nutritional disorders, enzyme deficiencies and the immunological status of the calf have been reported to play a role (Kalınbacak, 2003; Citil, 2013).

It has been determined that the risk of diarrhea in neonatal calves varies between 15% and 20% between 0 and 30 days, and mortality rates in calves with diarrhea are between 0.2% and 8% in studies conducted in developed countries (Bendali et al, 1999). It has been reported that 75% to 90% of neonatal calf diarrhea is associated with rotavirus, coronavirus, enterotoxigenic *E. coli* and *Cryptosporidium* spp. (Senturk, 2018).

Rotavirus usually causes diarrhea in 1 to 2 week old calves. The virus has a very short incubation period of 12-24 hours and induces peracute diarrhea in affected calves. The virus replicates in the cytoplasm of small intestinal villus epithelial cells. Viral infection causes villous atrophy and usually affects the caudal part of the small intestine. After being infected, calves shed large amounts of virus in the feces for 5-7 days (Steele et al., 2004).

Coronavirus infection can occur in cattle as three separate clinical pictures. These symptoms are a diarrheal complex consisting of winter dysentery in 1-2 week old calves and hemorrhagic diarrhea in adult animals and a complex of bovine respiratory disease in both young and adult cattle (Liu L, et al., 2006). The pathogenesis of coronavirus and rotavirus is



Bu makaleye atıfta bulunmak için/ To cite this article:
Mamak N, Kıyıcı R, Şahinduran Ş, Şensoy S, Akkan HA, Yıldız R, Musabeşoğlu Y, Gökçe Hİ. Etiological examination of neonatal calf diarrhea cases detected in Burdur region. MAE Vet Fak Derg. 2023; 8 (2) :55-60.

similar. However, atrophy of both the small and large intestines is more common in coronavirus infections (McGavin and Carlton, 1995).

The first 4 days after birth is the period when newborn calves are most susceptible to *Escherichia coli* infection (Foster and Smith, 2009). *E. coli* septicemia is common in neonatal cases. The most common cause of diarrhea in calves is entero-toxigenic *Escherichia coli* (ETEC) (Nataro and Kaper, 1998).

Infected calves with *Cryptosporidium* spp may be asymptomatic or develop severe diarrhea leading to dehydration (Fayer R., 2010). The disease causes severe villous atrophy and causes long-term malnutrition and growth retardation in infected animals (Nydam and Mohammed, 2005). Although calves can be infected with giardiasis from the age of 4 days, the 4-12 week period is the period in which stool cyst distribution is most common (O'Handley et al., 2003).

Neonatal calf diarrhea is a complex condition due to its multifactorial nature. Therefore, considering the risk factors of each pathogen that detects pathogens with laboratory tests and ensuring correct farm management may be beneficial in controlling calf diarrhea. With the rapid test kits developed in recent years, the diagnosis of some of the factors that cause diarrhea in calves can be determined quite easily under field conditions, without the need for a laboratory environment and experienced personnel.

This study was carried out to determine the prevalence of E coli, cryptosporidium spp, giardia, rotavirus and coronavirus agents in newborn calves brought to Burdur Mehmet Akif Ersoy University Animal Hospital Clinics and diagnosed with diarrhea in farms in the center and districts of Burdur between 2019-2020.

## MATERIALS AND METHOD

The animal material of the study was collected from 20 Simmental, 24 Holstein, and 6 other breeds, at the age of 1-30 days, with neonatal calf diarrhea detected in Burdur Mehmet Akif Ersoy University Animal Hospital Clinics and other farmers in the region between 2019-2020. (Table 1).

**Table 1.** Gender distribution of calves with neonatal diarrhea.

| Gender | Number of Calves (n) | Ratio to Total Number of Calves (%) |
|---|---|---|
| Female | 21 | %42 |
| Male | 29 | %58 |
| Total | 50 | |

General clinical examinations of calves with diarrhea were performed. In stool samples, Commercial in vitro Rapid Diagnostic Test (BoviD-5 Ag Test kit, Bionote, Inc. Korea) was used for the detection of rotavirus, coronavirus, cryptosporidium, *E. coli* (K99) and *Giardia* spp. The tests were performed according to the manufacturer's test procedure.

## RESULTS

In this study, while no agent could be detected in 30% (15/50) of calves with diarrhea, some enteropathogenic agents were detected in 70% (35/50) of them. Of these enteropathogenic agents, 24% (12/50) of the calves were *Cryptosporidium* spp., 12% (6/50) rotavirus, 6% (3/50) *E. coli* K99, 6% (3/50) of the calves *Giardia* spp, 2% (1/50) coronavirus, 14% (7/50) *Cryptosporidium* spp. + Rotavirus, 2% (1/50) Rotavirus + coronavirus, 2% (1/50) *Cryptosporidium* spp. + rotavirus + coronavirus + *E. coli* K99 + *Giardia* spp, *Cryptosporidium* spp. + coronavirus + *E. coli* K99 + *Giardia* spp were detected (Table 2). Mixed enteropathogen was detected in 20% (10/50) of the calves. In calves with diarrhea, *Cryptosporidium* spp. at the rate of 42% (21/50) and rotavirus at the rate of 30% (15/50) were detected as single and mixed enteropathogens.

**Table 2.** Distribution rates of enteropathogen agents in calves with neonatal diarrhea

| Agent | Number of Animals (n=50) | Ratio (%) |
|---|---|---|
| **Positive** | **35** | **70** |
| *Cryptosporidium* spp | 12 | 24 |
| Rotavirus | 6 | 12 |
| *E.Coli* K99 | 3 | 6 |
| *Giardia* spp | 3 | 6 |
| Coronavirus | 1 | 2 |
| *Cryptosporidium* spp + Rotavirus | 7 | 14 |
| Rotavirus+ Coronavirus | 1 | 2 |
| *Cryptosporidium* spp + Rotavirus+ Coronavirus+*E.Coli* K99+Giardia spp | 1 | 2 |
| *Cryptosporidium* spp + Coronavirus+*E.Coli* K99+Giardia **Negative** | 1 | 2 |
| | **15** | **30** |

The incidence of *E. coli* as a single and mixed enteropathogen was 10%, the rate of *Giardia lamblia* as a single and mixed enteropathogen was 10%, and the rate of coronavirus as a single and mixed enteropathogen was determined as 8%. Treatment for the agent detected and symptomatic treatment protocols were applied to the calves with diarrhea. The treatment results of the calves were followed up by calling the owners, 26 calves recovered and 11 died. 13 patient owners could not be contacted. The data of the calves used in the study are given in Table 3.

When the distribution of neonatal diarrheal calves with enteropathogenic agents according to age groups is examined, 13 (37.14%) of diarrhea cases are at the age of 1-7 days, 11 (31.43%) at the age of 8-14 days, 7 (20%) at the age of 15-21 days, and at the age of 15-21 days. It was determined that 4 of them (11.43%) were 22-30 days old (Table 3). Of the causative neonatal calves with diarrhea, 17 (48.57%) were female and 18 (51.43%) were male. When the recovery rates of the calves with neonatal diarrhea were examined, the information that 7 of the calves died and 19 of them recovered was obtained from the owners of the patients, and the owners of 9 calves could not be reached. 18 of these calves were from Holstein, 14 of them were from Simmental and 3 of them were from other breeds.

## DISCUSSION

Detection of enteropathogens in stool with rapid diagnostic kits has advantages compared to other diagnostic methods, such as rapid results in field conditions, inexpensive, simple and easy application, analysis of more than one factor at the same time, and determination of treatment strategies in a short time, without the need for a laboratory environment (Kaya and Coşkun, 2018).

Many researchers in our country, in various provinces, conducted studies to reveal the etiological factors in diarrhea cases of calves in the neonatal period with different methods such as agent isolation, search for parasites in the stool, serological

**Table 3.** Data of calves with detected enteropathogen

| | Current status of the patient | Patient's race | Age (days) | Gender | Agent | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1. | Died | Holstein | 20 | Male | *Cryptosporidium* spp | | | | |
| 2. | Healed | Holstein | 11 | Female | *Cryptosporidium* spp | Rota virus | | | |
| 3. | Healed | Norvegian red | 6 | Female | | Rota virus | Coronavirus | | |
| 4. | Healed | Simmental | 8 | Female | *Cryptosporidium* spp | Rota virus | | | |
| 5. | Not reached | Holstein | 22 | Female | | | | | *Giardia* spp |
| 6. | Healed | Simmental | 2 | Male | | | | *E.coli* | |
| 7. | Not reached | Simmental | 10 | Male | *Cryptosporidium* spp | Rota virus | | | |
| 8. | Healed | Hybrid | 10 | Male | | | Coronavirus | | |
| 9. | Healed | Simmental | 10 | Female | *Cryptosporidium* spp | | | | |
| 10. | Healed | Simmental | 7 | Male | *Cryptosporidium* spp | Rota virus | | | |
| 11. | Not reached | Simmental | 24 | Male | | Rota virus | | | |
| 12. | Died | Holstein | 10 | Female | | | | | *Giardia* spp |
| 13. | Died | Belgian blue | 10 | Female | *Cryptosporidium* spp | | Coronavirus | E.*coli* | *Giardia* spp |
| 14. | Not reached | Simmental | 11 | Male | *Cryptosporidium* spp | | | | |
| 15. | Died | Holstein | 15 | Female | *Cryptosporidium* spp | Rota virus | Coronavirus | | *Giardia* spp |
| 16. | Healed | Holstein | 3 | Female | | Rota virus | | | |
| 17. | Not reached | Holstein | 11 | Female | | Rota virus | | | |

**Table 3 Continue.** Data of calves with detected enteropathogen

| | Current status of the patient | Patient's race | Age (days) | Gender | Agent | | | |
|---|---|---|---|---|---|---|---|---|
| 18. | Not reached | Holstein | 15 | Male | *Cryptosporidium* spp | | | |
| 19. | Healed | Simmental | 3 | Male | | Rota virus | | |
| 20. | Healed | Holstein | 10 | Male | *Cryptosporidium* spp | Rota virus | | |
| 21. | Died | Holstein | 4 | Male | *Cryptosporidium* spp | | | |
| 22. | Healed | Simmental | 8 | Male | *Cryptosporidium* spp | | | |
| 23. | Died | Holstein | 15 | Female | *Cryptosporidium* spp | | | |
| 24. | Not reached | Simmental | 7 | Male | *Cryptosporidium* spp | | | |
| 25. | Not reached | Simmental | 5 | Female | *Cryptosporidium* spp | | | |
| 26. | Healed | Holstein | 15 | Female | *Cryptosporidium* spp | Rota virus | | |
| 27. | Healed | Simmental | 4 | Male | *Cryptosporidium* spp | Rota virus | | |
| 28. | Healed | Holstein | 15 | Male | *Cryptosporidium* spp | | | |
| 29. | Healed | Simmental | 2 | Female | | | E.*coli* | |
| 30. | Healed | Holstein | 15 | Male | *Cryptosporidium* spp | | | |
| 31. | Died | Simmental | 28 | Female | | | | *Giardia* spp |
| 32. | Not reached | Holstein | 2 | Female | | | *E.coli* | |
| 33. | Healed | Holstein | 7 | Male | *Cryptosporidium* spp | | | |
| 34. | Healed | Holstein | 7 | Male | | Rota virus | | |
| 35. | Healed | Holstein | 11 | Female | | Rota virus | | |

examinations or the use of rapid test kits (Kaya and Coşkun, 2018).

In this study, calf stool samples were taken and immunochromatographic test kits were used, which can diagnose different enteropathogens within 10-15 minutes and give rapid results (Klein et al., 2009). Mixed enteropathogens, in which at least two of these disease factors were found together, were diagnosed in 10 of 35 patients evaluated in the study. 5 enteropathogens were diagnosed together in 1 of these 10 patients, 4 enteropathogens were diagnosed together in 1, and 2 enteropathogens were diagnosed together in 8 of them. Diarrhea caused by a single enteropathogen was detected in 25 patients. Of these 25 patients, 12 of them were cryptosporidium spp., 6 of them rotavirus, 3 of them *Giardia lamblia*, 3 of them *E. coli* K99 and 1 of them coronavirus, alone caused diarrhea (Table 2). Diarrhea in mixed infections can develop as osmotic and secretory. In such cases, rotavirus, coronavirus, *Cl. Perfringens*, *Cryptosporidium* and *E. coli* two or more factors may coexist, leading to the development of osmotic and secretory diarrhea. It has been reported that mixed infections increase the severity of the disease (Baljer and Wieler, 1989).

Among the parasitic agents of neonatal diarrhea, *Cryptosporidium* spp constitutes a zoonotic disease with diarrhea, with a high probability of death in young and immune-compromised patients (Birdane, 2017). In studies conducted in our country, ((Kulig and Coskun, 2019) determined *Cryptosporidium* spp at a rate of 7% by rapid ELISA method (Citil, 2003; Al and Balikci, 2012), but they could not detect *Cryptosporidium* spp in calves with diarrhea with the same method. Emre and Fidancı (Emre and Fidanci, 1998) detected *Cryptosporidium* spp infection in feces with a rate of 63.3% using the safranin methylene blue technique. Çitil et al. (2003) found 32.9% *Cryptosporidium* spp. in stool samples in a study they conducted in Kars region. Kaya and Coşkun (2018) found

the rate of *Cryptosporidium* spp to be 11.21% in the province of Tokat. Külig and Coşkun (21) detected 7% of cryptosporidi-um spp in calves with diarrhea in Sivas. In the calves used in this study, *Cryptosporidium* spp the incidence as a single and mixed enteropathogen was found to be 42%. This rate differs from the values reported by other researchers. This difference may be due to the different methods used.

Rotavirus infections are one of the important causes of diarrhea in the neonatal period. This virus usually causes infection in calves aged 4-21 days, with 100% morbidity and 2% mortality (Sahal, et al., 2018). In studies conducted in our country in calves with neonatal diarrhea (Duman R ve Aycan AE., 2010), rotavirus infections were found between 8.5-53%. Kaya and Coşkun (Kaya and Coskun, 2018) found rotavirus infection in Tokat province to be 44.86%. Kulig and Coskun (Kulig and Coskun, 2019) found rotavirus infection at a rate of 22% in calves with diarrhea in Sivas. In studies related to the subject abroad, it has been reported that the incidence of this factor varies between 7-80% (Azkur and Aksoy, 2018). The incidence of rotavirus as a single and mixed enteropathogen in the calves used in this study was found to be 30%. This rate is between the percentiles reported by other researchers. In studies conducted in Turkey, the prevalence of rotavirus infections in calves with diarrhea at the age of 1-28 days was found to be between 0-53% (Alkan, 1998). The fact that a total of 35 calves with diarrhea included in this study were in this age range was found to be compatible with the average age range determined in previous studies.

*Escherichia coli* is one of the leading factors among the bacteria that cause neonatal diseases in calves. It is a factor that causes fatal diarrhea in the first week of neonatal life from one day old. It mostly causes diarrhea in calves younger than 4 days old (Sahal, et al., 2018). In studies conducted in our country, E. coli infections were found between 11.88-69.3% (Sen, et al., 2013). Kaya and Coskun (Kaya and Coskun, 2018) detected 7.48% of E. coli in Tokat province. Küliğ and Coşkun (Kulig and Coskun, 2019) determined E. coli at a rate of 26% in mixed form in calves with diarrhea in Sivas. The incidence of E. coli as a single and mixed enteropathogen in the calves used in this study was determined as 10%. This rate is lower than what many researchers have reported. This difference may be due to the different methods used.

*Giardia lamblia* is another parasitic agent of parasitic diarrhea in newborns. It is a protozoan that can be found in the small intestines during the 4-day to 12-week period of the neonatal period (Kaya and Coskun, 2018). In studies conducted in our country, *Giardia lamblia* infections were detected between 1.98% and 16.82% (Sen et al., 2013). Kaya and Coskun (Kaya and Coskun, 2018) found giardia to be 16.82% in Tokat pro-vince. In studies related to the subject abroad, it is stated that the incidence of this factor varies between 17-73% (Sen et al., 2013). The incidence of *Giardia lamblia* as a single and mixed enteropathogen in the calves used in this study was determi-ned as 10%. This rate is among the percentiles reported by researchers in our country.

Coronavirus is another important viral diarrhea agent in the neonatal period. It mostly causes approximately 100% morbidity and 50% mortality in 4-21-day-old calves (Şahal M, et al., 2018), (Gül Y., 2016). In studies conducted in our country, coronavirus infections were detected between 1.04% and 37.1% (Uyunmaz et al., 2019). Kaya and Coskun (Kaya and Coskun, 2018) found the rate of coronavirus in Tokat to be 9.35%. Küliğ and Coşkun (Kulig and Coskun, 2019) detected 9% mixed coronavirus infection in calves with diarrhea in Sivas. In studies related to the subject abroad, it is stated that the incidence of this factor is between 3 and 80.8% (Citil et al., 2003). The incidence of coronavirus as a single and mixed enteropathogen in the calves used in this study was determined as 8%. This rate is in line with the studies conducted.

Diarrhea factors detected in this study are found at different rates, both in our country and abroad, it can be affected by many factors such as the number of materials used, the variety of methods, the diversity of the causative population in barns or businesses, the awareness and sensitivity of animal owners to neonatal calf diarrhea, the season in which the study was conducted, and the geographical region. The results obtained in this study, when compared with other studies, Cryptosporidium spp. and E. coli rates are similar. (Baljer and Wieler 1989; Kulig and Coskun, 2019)

As a result, in neonatal calf diarrhea, *Cryptosporidium* spp and rotavirus agents are common and necessary precautions should be taken in enterprises against these agents, attention should be paid to the care and nutrition of calves against these enteropathogens as well as other enteropathogens, colostrum management for calves to receive adequate colostrum and vaccination programs for pregnant cows and calves concluded that it should be implemented.

## DECLARATIONS

### Ethics Approval

Burdur Mehmet Akif Ersoy University Experimental Animals Local Ethics Committee Presidency, E-93773921-770-160273, Etiological examination of neonatal calf diarrhea cases detected in Burdur region, due to the use of fecal material, Animal Experiments Ethics Committees dated 15 February 2014 and numbered 28914. According to article 8 of the Regulation on Working Procedures and Principles, Ethics Committee Approval is not required.

### Conflict of Interest

There is no conflict of interest.

### Consent for Publication

N/A

### Author contribution

Idea, concept and design: HAA, MK

Data collection and analysis: RK, SS, YM

Drafting of the manuscript: RY, HIG, NM, SSD

Critical review: HAA, MK

### Data Availability

The data is available from the corresponding author on reasonable request.

**Acknowledgements**

N/A

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