JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Prevalence of *Rotavirus*, *Coronavirus*, *Cryptosporidium* spp., *Escherichia coli* K99 and *Giardia lamblia* in neonatal calves with diarrhea in Burdur and its districts

Veysel Çelik[1], Süleyman Kozat[1]

1. Department of Internal Medicine, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Zeve Campus, Tuşba/Van-Turkey

Çelik, V. ORCID ID: 0009-0003-6406-2905; Kozat, S. ORCID ID: 0000-0001-5089-2623

Research Article

Volume: 8, Issue: 1
April 2024
Pages: 82-91

ABSTRACT

The aim of this study is to determine the prevalence of pathogens such as *Rotavirus*, *Coronavirus*, *Cryptosporidium* spp, *Escherichia coli* K99, and *Giardia lamblia* in neonatal calves with diarrhea in Burdur and its districts. The study material consisted of 96 diarrheic calves aged between 1 and 28 days from different cattle farms in the Burdur region. Fecal samples were collected, and the causative agents were identified using rapid diagnostic kits (BoviD-5 Ag Test Kit- BIONOTE). In the study, among the 96 diarrheic calves, a single enteropathogen was detected in 61 (63.54%), and 25 (26.04%) of these calves were positive for *Cryptosporidium*, 12 (12.5%) for *E. coli* K99, 11 (11.45%) for *Rotavirus*, 7 (7.29%) for *Coronavirus*, and 6 (6.25%) for *Giardia lamblia*, respectively. Multiple enteropathogens were responsible for diarrhea in 18 calves, and 2 (2.08%) of them were *Cryptosporidium* spp.+*Giardia lamblia*, 8 (8.33%) *Cryptosporidium* spp.+*Rotavirus*, 3 (3.12%) *Coronavirus*+*Rotavirus*, with 1 (1.04%) *Coronavirus*+*Cryptosporidium* spp., 2 (2.08%) *Rotavirus*+*E. coli*, and 1 (1.04%) of them was *E. coli*+*Cryptosporidi*um spp.+*Rotavirus*. Among the 96 diarrheic calves with mixed infections or a single enteropathogen, 38 (39.58%) had *Cryptosporidium*, 15 (15.62%) had *E. coli* K99, 26 (27.08%) had *Rotavirus*, 12 (12.50%) had *Coronavirus*, and 8 (8.33%) had *Giardia lamblia*. However, in 17 of the 96 diarrheic calves, neither mono- nor multiple enteropathogens were detected, suggesting that other factors might have caused diarrhea. Finally, this research provides valuable information for faster diagnosis, prevention, control, and treatment of enteropathogens causing diarrhea in neonatal calves in the Burdur region, contributing to reducing calf losses. It is believed that the research findings will shed light on future studies.

Article History

Received: 21.02.2024
Accepted: 28.04.2024
Available online: 30.04.2024

Keywords: calf, diagnosis, diarrhea, enteropathogen

DOI: https://doi.org/10.30704/http-www-jivs-net.1440871

To cite this article: Çelik, V., & Kozat, S. (2024). Prevalence of *Rotavirus*, *Coronavirus*, *Cryptosporidium* spp., *Escherichia coli* K99 and *Giardia lamblia* in neonatal calves with diarrhea in Burdur and its districts. ***Journal of Istanbul Veterinary Sciences, 8*(1), 82-91. Abbreviated Title*: J. İstanbul vet. sci.**

Introduction

Diarrhea is a symptom that occurs due to infectious and non-infectious causes and is characterized by the volume of the stool, the amount of liquid it contains, and the increase in the frequency of excretion (Kozat, 2000; Geletu et al., 2021; Özbek et al., 2024). Neonatal calf diarrhea (NCD) poses a significant problem in cattle breeding, especially in newborn calves. Diarrhea causes loss of productivity and deaths in newborn calves and negatively affects the livestock sector economically (Khan and Khan, 1991; Hall et al., 1992; Radostits et al., 1994; Lorenz et al., 2011a). During pregnancy, ruminants do not transfer immunoglobulin from the mother to the fetus. For this reason, calves are usually born hypogammaglobulinemic or

*Corresponding Author: Süleyman Kozat
E-mail: skozat@yyu.edu.tr

agammaglobulinemic and receive the necessary immunoglobulins through colostrum and milk after birth (Arda, 1994; Yılmaz and Akgül, 2014; Kozat, 2019).

Neonatal calves begin to produce their immunoglobulins on the 10th day after birth and reach normal plasma immunoglobulin levels by 60 days of age. This may cause infectious diarrhea to be common in newborn calves (Çakıroğlu et al., 2010; Yılmaz and Akgül, 2014; Kozat, 2019). During the first 28 days of newborn calves' lives, enteropathogens such as *Rotavirus*, *Coronavirus*, *Escherichia coli* (*E. coli*) and *Cryptosporidium*, alone or as mixed infections, are common factors that cause diarrhea in neonatal calves (Kozat, 2000). Among these enteropathogens, it has been reported that *Bovine Rotavirus* is the most common viral agent in neonatal calf diarrhea, along with *Bovine Coronavirus* (Woode et al., 1982; Snodgrass et al., 1986; Clark, 1993). Among bacterial factors, *E. coli* is an important enteropathogen in neonatal calves. Furthermore, *Cryptosporidium* spp. leads to a protozoal infection. The prevalence and zoonotic importance of parasitic agents such as *Giardia* spp. are increasing day by day. In addition to viral, bacterial and parasitic factors, non-infectious factors such as unfavorable shelter conditions, animal breeders with low education levels, insufficient colostrum intake and neglect of umbilical cord care are also effective in neonatal calf diarrhea (Kozat, 2000). In Turkey, many important studies are carried out to obtain healthy calves and protect them from diseases. In this context, it has been observed that research on the causes and factors that cause diarrhea, especially in calves, has increased rapidly in recent years (Kozat, 2000; Özkan and Akgül, 2004; İçen et al., 2013).

Rotaviruses destroy enterocytes during their journey from the oral tract to the small intestine, causing shedding in the intestinal cavity (Geletu et al., 2021). As a result of the replication of these viruses, disruptions occur in the digestion and absorption mechanisms (Snodgrass et al., 1980; Saklı, 2017).

Coronaviruses cause diarrhea due to decreased absorption and digestion, resulting in water and electrolyte loss (Clark, 1993). Diarrhea caused by Coronaviruses has a more severe course compared to Rotaviruses.

E. coli is another agent and is usually transmitted to calves orally during the neonatal period, but rarely it can also be transmitted through the umbilical cord. One of the most common enteropathogens that cause diarrhea is Enterotoxigenic *E. coli* (ETEC) (Şen et al., 2013). *Cryptosporidium* is transmitted to animals via the fecal-oral route (Olson et al., 1997). Infected calves expel oocysts along with their feces. Oocysts cause villous atrophy, degeneration and subsequent inflammatory changes, causing diarrhea (Sanford and Josephson, 1982). Giardia infection usually occurs at the end of the neonatal period and usually causes acute diarrhea (O'Handley and Olson, 2006).

The aim of this research was to identify specific enteropathogens and their prevalence in diarrhea occurring in neonatal calves in Burdur districts, and to provide a guide to veterinarians on prevention and control strategies. Besides evaluating the possible relationship between factors and fecal pH value, as well as investigating the existence of a potential correlation between fecal pH and calf ages were also aimed. The results of the study were intended to contribute to an observable reduction in deaths from neonatal calf diarrhea.

Material and Method

Animal material

This research was conducted on calves with neonatal diarrhea, which were reported to veterinary clinics operating in Burdur province and its districts (Ağlasun, Bucak, Çavdır, Gölhisar, Karamanlı, Kemer, Merkez, Tefenni, Yeşilova) between December 2022 and July 2023, and were determined by visiting various enterprises in the region. A total of 96 calves with neonatal diarrhea were evaluated, 47 of which were male and 49 were female. The calves included in the study consist of Holstein, Montofon, Simmental, Jersey and crossbreeds. When calves were included in the study, their previous illnesses and medication treatments were taken into consideration. During the clinical examination of the sick calves, the consistency, odor, content and color of the feces and the frequency of defecation were noted in detail. In addition, important factors such as the calves' age, breed, whether they received colostrum, body temperature, heart rate, dehydration degree, and diarrhea duration were evaluated.

Animals and ethical approval

Approval for this study was obtained from the Animal Experiments Local Ethics Committee of the Van Yüzüncü Yıl University (Date: 01.12.2022, Number: 2022/12-05).

Detection of enteropatogens in stool samples

Detailed systemic clinical examinations were performed on the diarrheal calves used in the study. During the examination, the consistency and content of the stool, the condition of the mucosa and the color of the conjunctiva were evaluated. In addition, clinical characteristics such as body temperature, skin elasticity, position of the eyeball in the orbit and

sucking reflex of the calves were examined. Rectal faecal samples were taken with a new plastic glove from each individual calf in accordance with the technique. Rapid test kits (Rapid BoviD-5 Ag Test kit; Cat. No: RC1302DD-Republic of Korea) were used to detect enteropathogens in stool samples. Before the test, kit box was carefully opened and materials such as test devices, assay diluent, and dropper were removed and checked for completeness. Then, a sample was taken from the diarrheal calf using a sterile fecal container and wearing gloves. The feces taken with the sampling apparatus were mixed with the assay diluent liquid and a homogeneous mixture was obtained by ensuring that the sample was completely dissolved. The sample taken from the resulting mixture with a dropper was carefully added to the sample section of the testing device and the process was completed. The test result was obtained by waiting 5-10 minutes for the sample to react with the test device. The test result was seen as agent negative or positive (Figure 1).

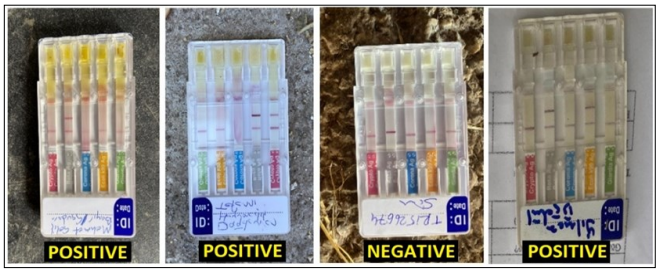

Figure 1. Positive and negative test results

Statistical Analysis

The obtained data were used to determine the rate and frequency of occurrence of the factors for descriptive statistics. SPSS (version-21) statistical package program was used in the calculations.

Results

Clinical findings

In the study, yellow watery, yellow gruel-like, yellow-green watery, green watery, green gruel-like, grey-white, brown, bloody, and mucous appearances were detected when the color, content and consistency of diarrhea were examined. The relationship between the appearance of feces and the factors is shown in Table 1.

Biochemical findings

In 17 of the diarrheal calves, none of the enteropathogens that could be determined by the test kit used were detected and this group was defined as other factors. While a single enteropathogen was detected in 61 of the remaining 79 calves, more than one enteropathogen was found in 18. Among the 61 calves in which a single enteropathogen was detected, the most common ones were *Cryptosporidium* spp. in 25, *E. coli* K99 in 12, *Rotavirus* in 11, *Coronavirus* in 7, and *Giardia lamblia* in 6 calves with diarrhea. According to the test results, mixed enteropathogens were detected in this study: *Cryptosporidium* spp.+ *Giardia lamblia* in 2 calves, *Cryptosporidium* spp. +

Table 1. The relationship between the color appearance of feces and the etiological agent

Agent	Yellow juicy	Yellow gruel-like consistency with blood	Yellow green liquid	Yellow green gruel-like consistency	Grayish-yellow liquid	grayish-dun liquid	other color	Total
Bovine Bovine rotavirus	5	3	1				2	11
Bovine Bovine coronavirus	2	1			2		2	7
E. coli	10	2						12
Giardia lamblia	2		1				3	6
Cryptosporidium spp	3	4	7	8	1	2		25
Bovine coronavirus + Bovine rotavirus	2				1			3
Bovine rotavirus + Cryptosporidium spp			2	1	4	1		8
Cryptosporidium spp + *Giardia lamblia*		1	1					2
Cryptosporidium spp+*Bovine coronavirus*	1							1
Bovine rotavirus +E. coli	1				1			2
Bovine rotavirus+Bovine coronavirus + Cryptosporidium spp.	1							1
Bovine rotavirus + Cryptosporidium +E. coli		1						1
Other factors (Unknown)	2	5	1	1	2	1	5	17

Rotavirus in 8 calves, *Coronavirus* + *Rotavirus* in 3 calves, *Coronavirus* + *Cryptosporidium* spp. in 1 calf, *Rotavirus* + *E. coli* in 2 calf, *E. Coli* + *Cryptosporidium* spp. + *Rotavirus* in 1 calf and *Coronavirus* + *Rotavirus* + *Cryptosporidium* spp in 1 calf. In addition, *Rotavirus* was detected in 27.08%, *Coronavirus* in 12.5%, *E. coli* in 15.62%, *Cryptosporidium* spp. in 39.58% and *Giardia lamblia* alone or simultaneously with other enteropathogens in 8.33% of the 96 examined calves (Table 2). The relationship between age and the prevalence of enteropathogens detected in calves with neonatal diarrhea in the Burdur region. The distribution of infectious agents detected in the study according to the ages of newborn calves with diarrhea is shown in Table 3 and Table 4. The age ranges where enteropathogens are most commonly seen are given in Table 5 and Figure 2. The relationship between age and the prevalence of enteropathogens detected in calves with neonatal diarrhea in the Burdur region.

Table 2. Ratio of etiological factors

Agent	Presence rate (%)
Cryptosporidium spp	25/96 = %26.04
E. coli	12/96 =%12.5
Rotavirus	11/96 = %11.45
Coronavirus	7/96 = %7.29
Giardia lamblia	6/96 = %6.25
Cryptosporidium+Rotavirus	8/96 = %8.33
Cryptosporidium+Giardia lamblia	2/96 = %2.08
Rotavirus+Coronavirus	3/96 = %3.12
Rotavirus + Coronavirus + Cryptosporidium spp	1/96 = %1.04
Rotavirus+Cryptosporidium+E.coli	1/96 = %1.04
Cryptosporidium spp+Coronavirus	1/96 = %1.04
Rotavirus+E. coli	2/96 = %2.08
Other factors	17/96 = %17.70

Table 3. Occurrence rate of infectious factors alone

Agent	Count	Percentage distribution
Rotavirus	26	%27.08
Coronavirus	12	%12.5
E. coli	15	%15.62
Cryptosporidium spp.	38	%39.58
Giardia lamblia	8	%8.33

Table 4. Age distribution according to factors in calves with diarrhea

Agent \ Age distribution	1-7 days	7-15 days	15-28 days	Total
Rotavirus	9	2	0	11
Coronavirus	6	1	0	7
Giardia lamblia	0	2	4	6
Cryptosporidium spp.	7	17	1	25
E. coli	11	1	0	12
Rotavirus + Cryptosporidium spp.	1	5	2	8
Cryptosporidium spp. + *Giardia lamblia*	0	1	1	2
Cryptosporidium spp. + *Coronavirus*	0	0	1	1
Coronavirus + Rotavirus	2	1	0	3
Rotavirus + E. coli	2	0	0	2
Rotavirus + Cryptosporidium spp. + E. coli	1	0	0	1
Rotavirus + Coronavirus + Cryptosporidium spp.	0	1	0	1
Other factors (unknown)	4	10	3	17
Total	42	41	13	96

Table 5. Minimum and maximum age range at which infectious agents are seen

Agent	Youngest age (d) (1-7)	Oldest age (d) (15-28)
Rotavirus	2	10
Coronavirus	2	10
E. coli	2	8
Giardia lamblia	12	26
Cryptosporidium spp.	6	16
Coronavirus + Rotavirus	2	8
Rotavirus + Cryptosporidium spp.	8	20
Cryptosporidium spp.+Giardia lamblia	9	16
Cryptosporidium spp+Coronavirus	17	17
Rotavirus + E. coli	3	4
Rotavirus + Coronavirus + Cryptosporidium spp.	14	14
Rotavirus + Cryptosporidium spp.+ E. coli	3	3
Other factors	2	25

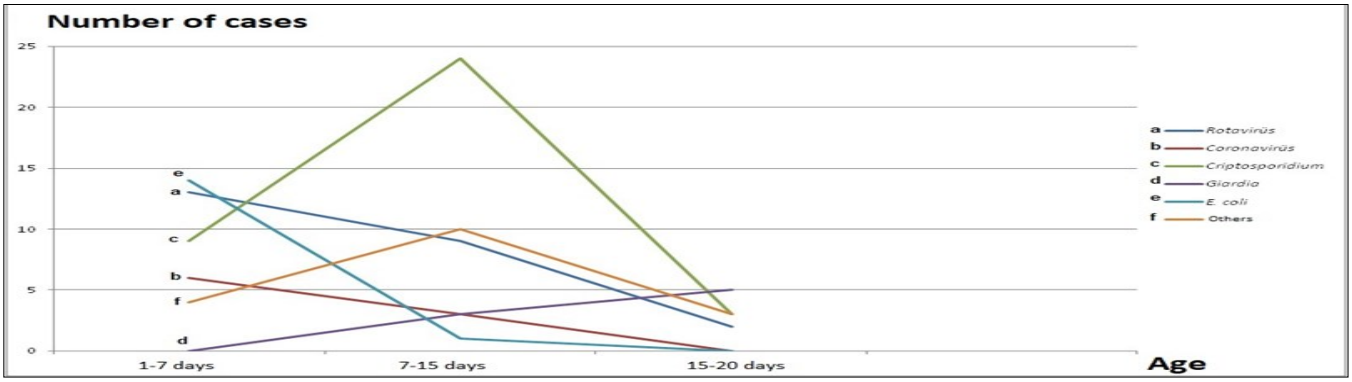

Figure 2. Relationship between age and prevalence of enteropathogens

The distribution of infectious agents detected in the study according to the ages of newborn calves with diarrhea is shown in Table 3 and Table 4. The age ranges where enteropathogens are most commonly seen are given in Table 5 and Figure 2.

Discussion

Despite modern veterinary practices, it is a fact that calf diarrhea still causes economic losses in cattle farms. In addition to diarrhea treatments, determining etiological factors and taking preventive measures is becoming increasingly important.

This study aims to provide a perspective on treatment strategies by determining the etiological factors affecting neonatal calf diarrhea in Burdur province and its districts. Diarrhea in newborn calves is a serious problem in the cattle industry, with high morbidity and mortality (Walker, 1998). Worldwide, one of the main causes of calf deaths and financial losses to the cattle industry is losses due to diarrhea (Wudu et al., 2008; Kozat, 2019). It is stated in many studies that various factors play a role in the emergence of diarrhea and that environmental factors, the effects of infectious agents, nutrition and many factors affecting the immune system interact in a complex manner (Waltner-Toews et al., 1986).

Protective measures starting from the pregnancy period are of great importance to reduce the frequency of calf diarrhea (Kozat, 2019). It has been emphasized in many studies that taking the right precautions in cases of diarrhea, especially giving colostrum on time and sufficiently, plays a critical role in strengthening the immune system of calves (Göncü, 2013; Şen et al., 2013). In addition to colostrum, improving the housing conditions of enterprises and ensuring hygiene can make significant contributions to the growth of calves in a healthier and more disease-resistant manner (Kozat, 2000). Within the scope of this study, we evaluated the reasons why some enterprises encounter calf diarrhea less frequently during our field visits to enterprises. They stated that the vaccination practices they carried out especially when the mother was in the dry period were effective in this case. It has been observed that in farms where these vaccination applications are carried out successfully, the immune systems of calves are strengthened and health problems such as diarrhea are prevented. It has been determined that adequate colostrum intake, as well as appropriate shelter conditions and balanced nutrition, are of great importance in raising healthy and disease-resistant calves during the neonatal period.

Breeders should be informed about the amount and timing of colostrum to be given to calves with neonatal diarrhea. However, despite these positive practices, it was determined that the housing conditions of the calves were not sufficient in most of the farms where the disease was detected during the field study.

Living conditions cause calves to decrease their resistance to diseases and cause health problems such as diarrhea, as they are not sheltered in a hygienic environment and proper care. These research findings are in line with the statements of previous researchers (Waltner-Toews et al., 1986; Göncü, 2013; Şen et al., 2013) about the importance of colostrum, as well as the findings of researchers about care and hygiene conditions (Kozat, 2000; Kozat and Tuncay, 2018).

Findings of this study support the data and emphasize that raising a healthy calf requires a multi-factor approach.

According to statistical data on diarrhea cases, the rate of diarrhea cases in newborn calves is over 50%, and the calf mortality rate varies between 1.5 and 8% depending on these cases (Frank and Kaneene, 1993). Similarly, in Turkey, calf diarrhea is accompanied by high levels of morbidity and mortality. This situation is still an important problem today due to reasons such as high treatment costs, poor performance and death which cause economic losses (Uzlu et al., 2010; Taş and Kozat, 2023).

According to recent research, cases of diarrhea in calves in the first four weeks after birth have been associated with various pathogens (Table 1). Microorganisms such as *Rotavirus*, *Coronavirus*, *E. coli*, *Cryptosporidium* and *Giardia* are stated as the most important causes of calf diarrhea (Khan and Khan, 1991; de La Fuente et al., 1998; Langoni et al., 2004; Lorenz et al., 2011b). It is emphasized that there is no single reason for the occurrence of neonatal calf diarrhea, instead, more than one factor plays a role, and regular and accurate fluid-electrolyte treatment is as important as an effective chemotherapy treatment to reduce high mortality rates due to diarrhea (Cho and Yoon, 2014).

According to the analysis results of this study, 61 of 96 calves had a single enteropathogen. Among these calves, *Cryptosporidium* spp. was detected in 25 (26.04%) of them, *E. Coli* K.99 in 12 (12.5%), *Bovine rotavirus* in 11 (11.45%), *Bovine coronavirus* in 7 (7.29%), and *Giardia lamblia* was detected in 6 (6.25%) of these calves.

Additionally, it was revealed that mixed infections played a role in 18 (18.75%) of 96 calves with diarrhea (Table 3). In this study, it was revealed that the enteropathogens detected in neonatal calves with diarrhea were similar to the infectious agents identified by previous researchers (Khan and Khan, 1991; de La Fuente et al., 1998; Langoni et al., 2004;

Lorenz et al., 2011b; Cho and Yoon, 2014).

It has been determined that providing appropriate hygiene conditions, as well as considering multiple etiological factors, plays a critical role in controlling and preventing the disease. These findings emphasize the need for a holistic approach to effectively manage diarrheal cases. Diarrhea occurring in neonatal calves significantly affects animal productivity losses and calf mortality rates. Therefore, it is of great importance to quickly identify the factors that cause diarrhea and apply effective treatment methods in order to reduce diarrhea-related losses and alleviate its effects (Kalınbacak, 2003; Murat and Balıkçı, 2012; Kozat and Tuncay, 2018). For this purpose, rapid diagnostic test kits are widely used (Al and Balıkçı, 2012; Kozat and Tuncay, 2018; Bal, 2019).

Immunochromatographic ready-made diagnostic kits help quickly and effectively detect the causes of diarrhea in newborn calves. These kits have an important role in obtaining rapid results under field conditions, being low-cost, easy to apply, and in detecting more than one enteropathogenic agent (Kozat ve Tuncay, 2018; Taş ve Kozat, 2023).

In this study, a commercial immunochromatographic test called Rapid Diagnostic Test (BoviD-5 Ag Test Kit - BIONOTE) was used to quickly determine the factors that cause diarrhea in newborn calves in the Burdur region. This test delivered the opportunity to detect *Rotavirus*, *Coronavirus, Cryptosporidium*, *E. coli* K99 and *Giardia lamblia* from fresh stool samples in a short time of 5-10 minutes.

According to the results of the study, in cases where a single enteropathogen was detected in 61 calves with diarrhea, the most common ones were *Cryptosporidium* in 25 (26.04%), *E. coli* K99 in 12 (12.5%), *Rotavirus* in 11 (11.45%), and *Rotavirus* in 7 (11.45%). In 6 cases (7.29%), *Coronavirus* and 6 cases (6.25%) were caused by *Giardia lamblia* (Table 3). In addition, among the 96 calves with diarrhea, 38 (39.58%) had *Cryptosporidium*, 15 (15.62%) had *E. coli* K99, 26 (27.08%) had *Rotavirus*, 12 (12.50%) had Coronavirus (12.50%) had *Cryptosporidium* (8.33%) and *Giardia lamblia* agents were detected (Table 4).

Many studies have been conducted on *Rotavirus,* which causes diarrhea in newborn calves in Turkey. Burgu et al. (1995) reported in a study that the rotavirus rate was determined to be 33.6%. In the research of Al and Balıkçı (2012), this rate was found to be 30%. In a different study, Alkan (1998) detected 53% *Rotavirus* in Ankara. In another study conducted in Ankara, Alkan et al. (1992) detected *Rotavirus* at a rate of 26.8%. In the study conducted by Erdoğan (2003) in Kars, this rate was found to be 26.9%. In a study conducted in Manisa, the rate determined by Bal (2019) was reported as 14%. In the study conducted in Van, Çabalar et al. (2007) detected *Rotavirus* at a rate of 17.97%. In this study, the *Bovine rotavirus* incidence rate was found to be similar compared to other researchers, but it was determined to be higher than the results obtained from some researchers (Çabalar et al., 2000; Bal, 2019). It is thought that these differences may result from regional climate and environmental conditions. Additionally, it is taken into consideration that differences in the age range of calves may also affect these results. *Rotavirus* infections can often occur as mixed infections (Garcia et al., 2000). In this study, mixed *Rotavirus* infection was detected in 26 of 96 calves (27.08%) in total. The single infection rate was found to be 11.45%.

In addition, it has been determined that the stool in diarrhea due to *Rotavirus* infection is generally yellow and watery. These findings show that *Rotavirus* infection may begin at an early age in neonatal calves and that the risk of disease continues at young ages. The findings of this study support the data of previous researchers (Rodger et al., 1982; Walker et al., 1998).

In many studies conducted on *Bovine Coronavirus* in Turkey, different rates were obtained in different regions. While the *Coronavirus* rate was determined as 13% in Al and Balıkçı's (2012) study in Elazığ. In other studies, *Coronavirus* rates are as follows: 7% in the study conducted in the Siirt region (Kozat and Tuncay, 2018), 9.35% in the Tokat region (Kaya and Coşkun, 2018), 9% in the Manisa region (Bal, 2019), 9% in the Sivas region (Kuliğ and Coşkun, 2019), 25% in the Muş region (Taş and Kozat, 2023). These results show that geographical differences and climatic conditions in Turkey may affect the spread of *Bovine Coronavirus*.
In this study, the diarrheal conditions of neonatal calves aged 1-28 days were examined in detail, and in total, *Coronavirus* was detected alone in 5 of 96 neonatal calves with diarrhea, and the number of infected calves reached 12 with mixed cases. In this context, while the rate of *Coronavirus* being found alone was 5.20%, the rate of mixed infection increased to 12.5%. These results reveal that *Coronavirus* rates may vary in studies conducted in different regions. In this research, it was observed that the smallest Coronaviruses were seen in calves that were 2 days old and the largest were 17 days old. This highlights that *Coronavirus* is a significant health threat to calves and the importance of preventive and therapeutic interventions initiated early in the disease. Additionally, diarrhea in *Coronavirus* infected animals has been observed to be yellow-green in color. In this study, it was determined that 7 of the stool colors of

12 sick animals diagnosed with *Coronavirus* were yellow, 3 were green and the other 2 were different colors. This information represents an important observation in terms of evaluating the clinical symptoms.

Cryptosporidium species are zoonotic protozoans that cause gastrointestinal infections in various species (Guerrant, 1997; Nguyen et al., 2007). Many studies have been conducted in different regions regarding the prevalence of *Cryptosporidium* spp in Turkey. 26.7% in Karacabey-Bursa (Burgu, 1984), 7% in Sivas (Kuliğ and Coşkun, 2019), 5.12% in Siirt (Kozat and Tuncay, 2018), 10.7% in Aydın (Özlem et al., 1997), 7.2% in Elazığ (Özer et al., 1990), 25.7% in Kars (Arslan et al., 2003), 27.33% in Konya (Sevinç et al., 2003), 35.8% in Ankara. (Sahal et al., 2005), 22.8% in Erzurum (Sarı et al., 2008), 20.7% in Nevşehir (Şimşek et al., 2012), 11.21% in Tokat (Kaya and Coşkun, 2018), 5.12% in Muş (Taş and Kozat, 2023).

In this study, while only *Cryptosporidium* infection was detected in 13 (13.54%) of 96 calves with neonatal diarrhea aged 1-28 days, it was determined that 38 (39.58%) had other factors along with *Cryptosporidium* infection. This shows that *Cryptosporidium* can be effective alone or in combination with other factors to cause diarrhea. It is stated that there are many risk factors affecting the prevalence of *Cryptosporidium* spp. Factors such as the number of animals in the enterprise, the age of the animals, whether they have diarrhea or are healthy, shelter type, suckling status, litter type and water source. It may have an impact on the prevalence of *Cryptosporidium* infections (Brook et al., 2008; Trotz-Williams et al., 2008). When we look at *Cryptosporidium* infections in Turkey and around the world, we see that the prevalence rates are different. It is thought that the reasons for these differences may be related to the geographical and climatic characteristics of the region where the studies were conducted, animal breeding practices, isolation of sick animals and the effect of environmental factors.

In addition, it was stated in the study that *Cryptosporidium* infection had been seen in certain enterprises before, but the disease was prevented by using a drug called halofuginone. Although *Cryptosporidium* infection has been seen in calves between the ages of 3 and 20 days, it has generally been observed that the calves are between the ages of 7-15 days. These findings show that *Cryptosporidium* infection can be seen in neonatal calves in the first week and is more common in calves in the first week or two. *Escherichia coli* infections are considered one of the leading causes of calf diarrhea, which usually occurs within 2-10 days after birth, and can rarely be seen within the first 24 hours after birth (Bilal, 2007). *Escherichia coli*, which is frequently encountered in the neonatal period, especially in 1-7 day(s) old calves, stands out as one of the main causes of diarrhea worldwide (Rodostitis et al., 2007). It has also been stated that enteropathogenic *E. coli* can be seen in neonatal calves within the first 30 days (Rodostitis et al., 2007).

E. coli infection rates also vary in studies conducted in Turkey. For example, in a study conducted in Siirt and its surroundings, the incidence of *E. coli* alone was reported as 6%, while the incidence rate in mixed was reported as 18% (Kozat and Tuncay, 2018). In a study conducted in the Tokat region, the rate of *E. coli* in calves with diarrhea was found to be 7.48% (Kaya and Coşkun, 2018). In Elazığ, the prevalence of *E. coli* in calves with diarrhea was determined as 16.66% (Al and Balıkçı, 2012). In this study, only *E. coli* infection was detected in 12 of 96 calves with neonatal diarrhea aged 1-28 days, and other factors were observed in 3 of them along with *E. coli* infection. While the rate of *E. coli* being found alone was 12%, the rate of being found together with other enteropathogens increased to 15.62% (Table 3, Table 4). It was determined that the majority of the feces were yellow and watery (Table 1) and the age range of 14 out of 15 *E. coli* infected calves was 1-7 days old (Table 5). This study confirms that the prevalence of *E. coli* infection varies between countries and regions and is frequently seen in the neonatal period, especially in calves 1-7 days old. Additionally, it was determined that *E. coli* infection was seen in calves that were at the youngest age of 2 days old and in calves that were at the oldest age of 8 days old (Table 5). These findings show that *E. coli* is a common infectious agent in calves during the neonatal period and that the risk of infection is higher in the first week.

Giardia lamblia is becoming increasingly important as it causes growth retardation in farm animals, reduces feed utilization and causes economic losses by causing diarrhea (O'Handley et al., 2003). *Giardia lamblia*, which causes significant economic losses in calf diarrhea in Turkey, is among the etiological factors that are generally ignored in some studies (Ayan et al., 2016). In another study, the presence and prevalence of *Giardia* in the feces of 10,672 cattle between the ages of 1-730 days was evaluated and *Giardia* was detected in 1,236 feces. It was determined that *Giardia* agents continued to spread as cysts in 1,184 of the animals in which *Giardia* was detected (Mark-Carew et al., 2010). In studies conducted in Turkey, Göz et al. (2006) detected *Giardia* at a rate of 14.7% in 231 newborn and young calves aged between 1 day

231 newborn and young calves aged between 1 day and 8 months in Van, and Kaya and Coşkun (2018) detected *Giardia* at a rate of 16.82% in 107 neonatal calves with diarrhea in Tokat. In this study, *Giardia* infection was detected in 6 of 96 diarrheal neonatal calves aged 1-28 days, and other factors were observed in 2 of them along with *Giardia* infection. While the rate of *Giardia* being found alone was 6.25%, the rate of being found together with the mixture increased to 8.33% (Table 3, Table 4). In this study, it was observed that the *Giardia* agent was seen in neonatal calves between 9 and 26 days old, but was mostly seen in calves older than 15 days (Table 4, Table 5). These results suggest that *Giardia* infection may be a cause of diarrhea in neonatal calves in Burdur and its districts and that the risk of infection may increase, especially after 15 days of age.

This research examined the cases of diarrhea in newborn calves in Burdur and its surroundings and identified the important enteropathogens that cause diarrhea. It was concluded that diarrhea in newborn calves poses a significant problem for the cattle industry, causing high mortality and yield losses. Rapid Diagnostic Test (BoviD-5 Ag Test Kit - BIONOTE), an immunochromatographic test, was used to detect the factors that cause diarrhea. Thanks to this test, the pathogens *Rotavirus, Coronavirus, Cryptosporidium, E. coli* K99 and *Giardia lamblia* were quickly detected. According to the research results, the infectious agents were determined to be *Cryptosporidium, Rotavirus, E. coli* K99, *Coronavirus* and *Giardia lamblia*, respectively. *Cryptosporidium, Rotavirus, E. c*oli K99, *Coronavirus* and *Giardia lamblia* infections were determined to be the most common factors in neonatal calf diarrhea. A difference was observed between the rates of occurrence of these factors alone and in mixed forms. *Cryptosporidium* and *Rotavirus* infections were determined to be the most common factors in neonatal calf diarrhea. Considering that these factors can be seen in the first two weeks of the neonatal period, the importance of preventive measures in the emergence of diarrhea in neonatal calves was emphasized. The study pointed out that the shelter conditions and hygiene of businesses are critical in the emergence of diarrhea.

As a result, in order to control the cases of diarrhea in newborn calves in Burdur and its districts, and to reduce economic losses, the factors must be identified quickly and accurately. The application of effective treatment methods, producer training for raising healthy and productive animals in the cattle industry, animal care and nutrition, and standardization of shelter conditions should be taken. It was emphasized that it is important to make this possible and take protective measures. It is thought that this study will shed light on future research.

Conflict of interest

In this study, I declare that there are no conflicts of interest among the authors.

References

Al, M., & Balıkçı E. (2012). Neonatal ishalli buzağılarda rotavirus, coronavirus, E. coli K99 ve Cryptosporidium parvum'un hızlı test kitleri ile teşhisi ve enteropatojen ile maternal immünite ilişkisi. *Fırat Üniversitesi Sağlık Bilimleri Dergisi. 26* (2), 73-78.

Alkan F. (1998). Buzağı ishallerinde rotavirus ve coronavirusların rolü. *Ankara Üniversitesi Veteriner Fakültesi Dergisi. 45*, 29-37.

Alkan, F., Pulat, H., Yazıcı, Z., & Burgu İ.(1992). Ters (reverse) pasif hemaglutinasyon testi ile ishalli buzağıların gaitalarında rotavirusların tespiti. *Ankara Ankara Üniversitesi Veteriner Fakültesi Dergisi. 39*, 238-246.

Arslan, M.Ö., Erdoğan, H.M., & Tanrıverdi S. (2003). Neonatal buzağılarda Cryptosporidiosis'in epidemiyolojisi. *13. Ulusal Parazitoloji Kongresi, Program ve Özet Kitabı; 08-12 Eylül,* Konya; SB6-01, s.186.

Ayan, A., Ural, K., Aysul, N., Gültekin, M., Erdoğan, H., Balıkçı, C., & Toros, G. (2016). Giardia duodenalis ile infekte buzağılarda doğal kist saçılımı. *Journal of Advance VetBio Science and Techniques, 1*(1), 14-19.

Bal, D. (2019). Manisa yöresinde neonatal buzağı ishalleri üzerine etiyolojik araştırmalar, *Yüksek lisans tezi*. Afyon, Kocatepe Üniversitesi.

Bilal, T. (2007) *Yenidoğanların iç hastalıkları.* İstanbul Ünv. Basım ve Yayın Evi Müdürlüğü, İstanbul; s. 158-69.

Brook, E., Hart, C. A., French, N., & Christley, R. (2008). Prevalence and risk factors for *Cryptosporidium* spp. infection in young calves. *Veterinary Parasitology, 152*(1-2), 46-52.

Burgu, A. (1984). Türkiye'de buzağılarda *Cryptosporidium*'ların bulunuğu ile ilgili ilk çalışmalar. *Ankara Üniversitesi Veteriner Fakültesi Dergisi, 31*(03), 573-585.

Burgu, İ., Akça, Y., Alkan, F., Özkul, A., & Karaoğlu, T. (1995). Yenidoğan ishalli buzağılarda rotavirusların electron microskopi (EM), enzyme linked immunosorbent assay (ELISA) ve polyacrylamide gel electrophorosis (PAGE) teknikleri ile çabuk teşhisi ve antijenik karakterizasyonu. *Ankara Üniversitesi Veteriner Fakültesi Dergisi,* 42, 491-498.

Cho, Y. I., & Yoon, K. J. (2014). An overview of calf diarrhea-infectious etiology, diagnosis, and intervention. *Journal of Veterinary Science, 15*(1), 1-17.

Clark, M.A. (1993). Bovine coronavirus. *British Veterinary Journal, 149*(1), 51-70.

Çabalar, M., Kaya, A., & Arslan, S. (2007). Yeni doğan buzağıların ishal olgularında rotavirus ve coronavirus araştırılması. *Veteriner Bilimleri Dergisi, 23*(3-4), 103-106.

Çabalar, M., Voyvoda, H., & Sekin, S. (2000). Virologic and serologic examinations for rotaviruses in diarrhoeic calves. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi,* 11(1), 18-21.

Çakıroğlu, D., Meral, Y., Pekmezci, D., Onuk, E.E., & Gökalp, G. (2010). Yenidoğan buzağılarda çeşitli hematolojik ve biyokimyasal parametreler ile kolostral immun globulinler arasındaki ilişkinin belirlenmesi. *Fırat Üniversitesi Sağlık Bilimleri Dergisi, 24*(1), 43-46.

De la Fuente, R., Garcıa, A., Ruiz-Santa-Quiteria, J. A., Luzon, M., Cid, D., García, S., & Gómez-Bautista, M. (1998). Proportional morbidity rates of enteropathogens among diarrheic dairy calves in central Spain. *Preventive Veterinary Medicine, 36*(2), 145-152.

Erdoğan, H.M., Ünver, A., Güneş, V., & Çitil, M. (2003). Frequency of rotavirus and coronavirus in neonatal calves in Kars district. *Kafkas Universitesi Veteriner Fakultesi Dergisi. 9*(1), 65-68.

Frank, N. A., & Kaneene, J. B. (1993). Management risk factors associated with calf diarrhea in Michigan dairy herds. *Journal of Dairy Science, 76*(5), 1313-1323.

Garcia, A., Ruiz-Santa-Quiteria, J. A., Orden, J. A., Cid, D., Sanz, R., Gómez-Bautista, M., & De La Fuente, R. (2000). Rotavirus and concurrent infections with other enteropathogens in neonatal diarrheic dairy calves in Spain. Comparative Immunology, Microbiology and Infectious *Diseases, 23*(3), 175-183.

Geletu, U. S., Usmael, M. A., & Bari, F. D. (2021). Rotavirus in calves and its zoonotic importance. *Veterinary Medicine International*, 1-18.

Göncü, S., Mevliyaoğulları, E., & Koluman, N. (2013). Siyah alaca inek ve düvelerde kolostrum kalitesi ve buzağıların bağışıklık düzeyleri. *Çukurova Üniversitesi Ziraat Fakültesi Dergisi, 28*(1), 31-38.

Göz, Y., Altug, N., Yuksek, N., & Ozkan, C. (2006). Parasites detected in neonatal and young calves with diarrhoea. *Bulletin-Veterinary Institute in Pulawy, 50* (3), 345.

Guerrant, R. L. (1997). Cryptosporidiosis: an emerging, highly infectious threat. *Emerging Infectious Diseases, 3*(1), 51-57.

Hall GA, Jones PW, Morgan JH. Calf diarhoea. Andrews AH, Blowey RW, Boyd H, Eddy RG, (1992). *Bovine Medicine Diseases and Husbandry of Cattle*, 1st ed. Oxford, Blackwell Science Ltd.

İçen, H., Arserim, N. B., Işık, N., Özkan, C., & Kaya, A. (2013). Prevalence of four enteropathogens with immunochromatographic rapid test in the feces of diarrheic calves in east and southeast of Turkey. *Pakistan Veterinary Journal, 33*(4), 496-99.

Kaya, U., & Coşkun, A. (2018). Tokat Bölgesindeki neonatal buzağı ishallerinin etiyolojisinin belirlenmesi. *Manas Journal of Agriculture, Veterinary and Life Science, 8*(1), 75-80.

Khan, A., & Khan, M.Z. (1991). Aetiopathology of neonatal calf mortality. *Journal of Islamic Academy of Sciences, 4*(2), 159-165.

Kozat, S. (2000). İshalli buzağılarda kristalloid (laktatli ringer) ve kolloid + kristalloid (% 6 dekstran-70 + laktatli ringer) infüzyon solüsyonlarinin rehidratasyon etkinliği. *Doktora tezi*. Yüzüncü Yil Üniversitesi. Van, Türkiye.

Kozat, S. (2019). Yenidoğan buzağılarda kolostrum yönetiminin önemi. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 14*(3), 343-353.

Kozat, S., & Tuncay, İ. (2018). Siirt yöresindeki yenidoğan ishalli buzağılarda Rotavirus, Coronavirus, Cryptosporidium spp, *Escherichia coli* K 99 ve Giardia lamblia etkenlerinin prevalansı. *Van Veterinary Journal, 29*(1), 17-22.

Kuliğ, C.C., & Coşkun, A. (2019). Sivas ve ilçelerindeki neonatal ishalli buzağılarda E. coli, Cryptosporidium, Clostridium perfringens, Rotavirus ve Coronavirus Prevalansı. *Turkish Veterinary Journal, 1*(2), 69-73.

Langoni, H., Linhares, A. C., Avila, F. A. D., Da Silva, A. V., & Elias, A. O. (2004). Contribution to the study of diarrhea etiology in neonate dairy calves in São Paulo state, Brazil. *Brazilian Journal of Veterinary Research and Animal Science, 41*, 313-319.

Lorenz, I., Fagan, J., & More, S. J. (2011a). Calf health from birth to weaning. II. Management of diarrhoea in pre-weaned calves. *Irish Veterinary Journal, 64*, 1-6.

Lorenz, I., Mee, J. F., Earley, B., & More, S. J. (2011b). Calf health from birth to weaning. I. General aspects of disease prevention. *Irish Veterinary Journal, 64*(1), 1-8.

Mark-Carew, M. P., Khan, Y., Wade, S. E., Schaaf, S., & Mohammed, H. O. (2010). Incidence of and risks associated with Giardia infections in herds on dairy farms in the New York City Watershed. *Acta Veterinaria Scandinavica, 52*(1), 1-8.

Murat, A., & Balıkçı, E. (2012). Neonatal ishalli buzağılarda rotavirus, coronavirus, E. coli K99 ve Cryptosporidium parvum'un hızlı test kitleri ile teşhisi ve enteropatojen ile maternal immünite ilişkisi. *Fırat Üniversitesi Sağlık Bilimleri Dergisi, 24* 26(2), 73-78.

Nguyen, S. T., Nguyen, D. T., Le, D. Q., Le Hua, L. N., Van Nguyen, T., Honma, H., & Nakai, Y. (2007). Prevalence and first genetic identification of Cryptosporidium spp. in cattle in central Viet Nam. *Veterinary Parasitology, 150*(4), 357-361.

O'Handley, R. M., & Olson, M. E. (2006). Giardiasis and cryptosporidiosis in ruminants. *Veterinary Clinics: Food Animal Practice, 22*(3), 623-643.

Olson, M. E., Guselle, N. J., O'Handley, R. M., Swift, M. L., McAllister, T. A., Jelinski, M. D., & Morck, D. W. (1997). Giardia and Cryptosporidium in dairy calves in British Columbia. *Canadian Veterinary Journal, 38* (11), 703.

Özbek, M., Özkan, C., & Altan, Y. (2024). Effect of fluid therapy and oxygen application on venous blood parameters of diarrheic calves with hyperkalemia. *Journal of the Hellenic Veterinary Medical Society, 75* (1), 6757-6764.

Özer, E., Erdoğmuş, S. Z., & Köroğlu, E. (1990). Elazığ yöresinde buzağı ve kuzularda bulunan Cryptosporidium'un yayılışı üzerinde araştırmalar. *Turkish Journal of Veterinary and Animal Sciences, 14*, 439-445.

Özkan, C., & Akgül, Y. (2004). Neonatal ishalli buzağılarda hematolojik, biyokimyasal ve elektrokardiyografik bulgular. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi,* 15(1), 123-129.

Özlem, M. B., Eren, H., & Kaya, O. (1997). Aydın yöresi buzağılarında *Cryptosporidium*'ların varlığının araştırılması. *Bornova Veteriner Kontrol ve Araştırma Enstitüsü Dergis, 22*(36), 15-22.

Radostits OM, Blood DC, Gay CC. (1994). *Veterinary medicine. A textbook of the diseases of cattle, sheep, pigs, goats and horses. 8th ed*. Bailliere Tindall Ltd.

Rodger, S. M., Bishop, R. F., & Holmes, I. H. (1982). Detection of a rotavirus-like agent associated with diarrhea in an infant. *Journal of Clinical Microbiology, 16*(4), 724-726.

Sahal, M., Karaer, Z., Cizmeci, S., & Tanyel, B. (2005). Cryptosporidiosis in newborn calves in Ankara region: clinical, haematological findings and treatment with Lasalocid-NA. DTW. *Deutsche tierarztliche Wochenschrift, 112*(6), 203-208.

Saklı, G. U. (2017). İshalli buzağı dışkılarında sığır coronavirusu ve sığır rotavirusunun hızlı tanı kiti ve RT-PCR ile araştırılması. *Doktora Tezi*. Selçuk University. Konya. Türkiye.

Sanford, S. E., & Josephson, G. K. A. (1982). Bovine cryptosporidiosis: clinical and pathological findings in forty-two scouring neonatal calves. *Canadian Veterinary Journal, 23*(12), 343.

Sarı, B., Aktaş, M. S., & Arslan, M. Ö. (2008). Erzurum yöresinde buzağılarda Cryptosporidium türlerinin prevalansı. *Türkiye Parazitoloji Dergisi, 32*(2), 116-119.

Snodgrass, D. R., Fahey, K. J., Wells, P. W., Campbell, I., & Whitelaw, A. (1980). Passive immunity in calf rotavirus infections: maternal vaccination increases and prolongs immunoglobulin G1 antibody secretion in milk. *Infection and Immunity, 28*(2), 344-349.

Snodgrass, D. R., Terzolo, H. R., Sherwood, D., Campell, I., Menzies, J. D. (1986). Aetiology of diarrhoea in young calves. *Veterinary Record, 119*(2), 31-34.

Şen, İ., Güzelbekteş, H., &Yıldız, R. (2013). Neonatal buzağı ishalleri, Patofizyoloji, epidemiyoloji, klinik, tedavi ve koruma. *Turkiye Klinikleri Journal of Veterinary Science, 4*(1), 71-78.

Şimşek, A. T., İnci, A., Yıldırım, A., Çiloğlu, A., Bişkin, Z., & Düzlü, Ö. (2012). Nevşehir yöresindeki yeni doğan ishalli buzağılarda cryptosporidiosis' in real time PCR ve Nested PCR yöntemleri ile saptanması. *Erciyes Üniversitesi Veteriner Fakültesi Dergisi, 9*(2), 79-87.

Taş, Ş., & Kozat S. (2023). Muş ve ilçelerinde yenidoğan ishalli buzağılarda rotavirus, coronavirus, Cryptosporidium spp., *Escherichia coli* K99 ve Clostridium perfringens etkenlerinin prevalansı. *Van Veterinary Journal, 34*(2), 86-92.

Uzlu, E., Karapehlivan, M., Çitil, M., Gökçe, E., & Erdoğan, H. M. (2010). İshal semptomu belirlenen buzağılarda serum sialik asit ile bazı biyokimyasal parametrelerin araştırılması. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi,* 21(2), 83-86.

Walker, P. G., Constable, P. D., Morin, D. E., Drackley, J. K., Foreman, J. H., & Thurmon, J. C. (1998). A reliable, practical, and economical protocol for inducing diarrhea and severe dehydration in the neonatal calf. *Canadian Journal of Veterinary Research, 62*(3), 205.

Waltner-Toews, D., Martin, S. W., & Meek, A. H. (1986). The effect of early calfhood health status on survivorship and age at first calving. *Canadian Journal of Veterinary Research,* 50(3), 314.

Woode, G. N., Reed, D. E., Runnels, P. L., Herrig, M. A., & Hill, H. T. (1982). Studies with an unclassified virus isolated from diarrheic calves. *Veterinary Microbiology, 7*(3), 221-240.

Wudu, T., Kelay, B., Mekonnen, H.M., &Tesfu, K. (2008). Calf morbidity and mortality in smallholder dairy farms in Ada'a Liben district of Oromia, Ethiopia. *Tropical Animal Health and Production,* 40(5), 369-76.

Yılmaz, N., & Akgül, Y. (2014). İmmünglobulinler ve septisemi. *Uludağ Üniversitesi Veteriner Fakültesi Dergisi*, 33(1-2), 33-42.