

Evaluation of Enteropathogens and Fecal pH Changes in Neonatal Calves with Diarrhea

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ABSTRACT

The aim of this study is to investigate fecal pH changes in diarrheal newborn calves infected with enteropathogens such as *Bovine rotavirus*, *Bovine Coronavirus*, *Cryptosporidium* spp, *Escherichia coli* K99 and *Giardia lamblia*. fecal pH values of calves with identified pathogens were measured using nitrazine paper and digital pH meter, and it was revealed that there was a relationship between the detected factor and the age of the case. The animal material of the study consisted of 96 diarrheal calves aged between 1 and 28 days. Fecal samples were taken from calves with diarrhea. Rapid diagnostic kits were used to diagnose enteropathogens in the stool samples taken. When the pH analysis results of all cases were examined, fecal pH values of both single and co-infected cases were determined to be predominantly between 5-6 and 6.1-7. Fecal pH values of infected calves were determined to be maximum between 6.1-7. When the pH values of all cases were examined, it was determined that cases with 8-15 days of diarrhea were detected more frequently. It was determined that the fecal pH values of these calves with diarrhea varied between 6.1-7. In the analysis of fecal pH values in 12 samples in which only *E. coli* was detected, it was determined that the fecal pH value of 11 of these calves was measured between 5 and 6, and only 1 of them had a pH value above 6. On the other hand, it was determined that the fecal pH value was measured below 6 in 12 of the remaining 84 calves. It was observed that the fecal pH value was between 5-6 in the majority of calves in which *E. coli* was detected. It was determined that the fecal pH value was above 6 in 73 of a total of 96 calves. Additionally, pH was not detected above 7.1 in any of the cases infected with enteropathogens. The pH value of single and co-infected cases was predominantly determined between 6.1 and 7.

As a result, it was determined that the fecal pH values of cases infected with enterogens were not higher than 7, and the fecal pH values of enteropathogens were concentrated in the range of 6.1-7. For faster diagnosis, prevention, control and treatment of enteropathogens that cause diarrhea in newborn calves, attention can be taken in the diagnosis and treatment of the disease when fecal pH values are an easily detected parameter. It is thought that it will shed light on future studies regarding fecal pH.

Keywords: Calf, Diarrhea, Diagnosis, pH

İshalli Yenidoğan Buzağlarda Enteropatojenler ile Dışkı pH Değişimlerinin Değerlendirilmesi

ÖZ

Bu çalışmanın amacı Sığır rotavirüsü, Sığır Coronavirüsü, *Cryptosporidium* spp, *Escherichia coli* K99 ve *Giardia lamblia* gibi enteropatojenlerle enfekte olmuş ishalleri yeni doğan buzağlarda dışkı pH değişikliklerinin araştırılmasıdır. Enteropatojen tespit edilen buzağların dışkı pH değerleri nitrazin kâğıdı ve dijital pH metre kullanılarak ölçüldü ve tespit edilen faktör ile olgunun yaşı arasında bir ilişki olduğu ortaya çıktı. Araştırmanın hayvan materyalini yaşları 1 ile 28 gün arasında değişen 96 ishalleri buzağı oluşturdu. İshalleri buzağlardan dışkı örnekleri alındı. Alınan dışkı örneklerinde enteropatojenlerin tanısının konulması için hızlı tanı kitleri kullanıldı. Tüm vakaların pH analiz sonuçları incelendiğinde hem tek hem de ko-enfekte vakaların dışkı pH değerlerinin ağırlıklı olarak 5-6 ile 6,1-7 arasında olduğu belirlendi. Enfekte buzağların dışkı pH değerlerinin maksimum 6,1-7 arasında olduğu belirlendi. Tüm vakaların pH değerleri incelendiğinde 8-15 gün süren ishal vakalarının daha sık tespit edildiği belirlendi. İshalleri bu buzağların dışkı pH değerlerinin 6,1-7 arasında değiştiği belirlendi. Sadece *E. coli* tespit edilen 12 örnekte dışkı pH değerleri analizinde, bu buzağlardan 11 tanesinin dışkı pH değerinin 5 ile 6 arasında ölçüldüğü, sadece 1 tanesinin pH değerinin 6'nın üzerinde olduğu belirlendi. Öte yandan geri kalan 84 buzağının 12'sinde dışkı pH değerinin 6'nın altında ölçüldüğü belirlendi. *E. coli* tespit edilen buzağların büyük çoğunluğunda dışkı pH değerinin 5-6 arasında olduğu görüldü. Toplam 96 buzağının 73'ünde dışkı pH değerinin 6'nın üzerinde olduğu belirlendi. Ayrıca enteropatojenlerle enfekte olguların hiçbirinde pH 7,1'in üzerinde saptanmadı. Hem tek hem de ko-enfekte vakaların pH değeri ağırlıklı olarak 6,1 ile 7 arasında belirlendi.

Sonuç olarak enteropatojenlerle enfekte olguların dışkı pH değerlerinin 7'den yüksek olmadığı, enteropatojenlerin dışkı pH değerlerinin ise 6,1-7 aralığında yoğunlaştığı belirlendi. Yeni doğan buzağlarda ishale neden olan enteropatojenlerin daha hızlı teşhisi, önlenmesi, kontrolü ve tedavisi için dışkı pH değerlerinin kolay tespit edilen bir parametre olduğu için hastalığın teşhis ve tedavisinde dikkat edilebilir. Dışkı pH'ı ile ilgili ileride yapılacak çalışmalara ışık tutacağı düşünülmektedir.

Anahtar Kelimeler: Buzağı, İshal, pH, Tanı

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INTRODUCTION

Diarrhea is one of the most common causes of morbidity and mortality in young calves, especially in both dairy and beef calves younger than one month (Torche et al. 2020; Chen et al. 2022). In ruminants, they do not transfer immunoglobulin from the mother to the fetus during pregnancy. For this reason, calves are usually born hypogammaglobulinemic or agammaglobulinemic and receive the necessary immunoglobulins through colostrum and milk after birth (Yılmaz and Akgül 2014; Kozat, 2019). Calves start producing their immunoglobulin on the 10th day after birth and reach normal plasma immunoglobulin levels when they are 60 days old. This may cause infectious diarrhea to be common in neonatal calves (Yılmaz and Akgül 2014; Çakıroğlu et al. 2010). Neonatal calf diarrhea (NCD) is one of the most common multifactorial diseases (Probo and Veronesi, 2022). Virus, bacteria, and protozoa infection, as well as immunological status and management factors (housing, feeding, and hygienic conditions) play an important role as determinants and predisposing factors, respectively. During the first 28 days of their lives, enteropathogens such as *Bovine rotavirus*, *Bovine coronavirus*, *Escherichia coli* (*E. coli*) and *Cryptosporidium* are common factors that cause diarrhea in neonatal calves, either alone or as mixed infections (Kozat and Voyvoda, 2006). Among these enteropathogens, it has been reported that *Bovine rotavirus* is the most common viral agent in neonatal calf diarrhea, along with *Bovine coronavirus* (Snodgrass et al., 1986; Ramig, 2004). Among bacterial factors, *E. coli* is an important enteropathogen in neonatal calves. Additionally, *Cryptosporidium* spp. The prevalence and zoonotic importance of parasitic agents such as *Giardia* 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, 2019). In Turkey, 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 (Al and Balıkçı, 2012; Kozat and Voyvoda, 2006)

Calves with diarrhea may show signs like loss of appetite, weight loss, and poop that is loose, bloody, and sometimes contains mucus. In severe cases, research shows that the poop can be very watery, bloody, and even have bits of lining from the intestines. Animals in this condition may also become thin, dehydrated, weak, and uninterested in their surroundings (Kozat and Voyvoda, 2006; Lorenz et al. 2011; Yang et al. 2017; Ganapathy et al. 2023). In calves with diarrhea, changes occur in biochemical and hematological parameters as well as clinical findings. Studies on newborn calves with diarrhea indicate that

in cases of diarrhea, there is a decrease in sodium and chloride concentrations in the blood plasma and an increase in the potassium concentration in the blood plasma (Dincer et al. 2021; Ganapathy et al. 2023, Guzelbektes et al. 2007). Regardless of the cause, diarrhea can cause dehydration, electrolyte imbalance, metabolic acidosis and hypovolemia (Dincer et al. 2021; Ganapathy et al. 2023; Torche et al. 2020). Hypovolemia results in kidney failure and heart block due to hyperkalemia and septicemia due to secondary bacterial overgrowth in the small intestine. The kidney reduces urine production to compensate for the increased fluid losses caused by diarrhea (Shehta et al. 2022). Several studies (Kozat and Voyvoda, 2006; Saleh et al. 2022) found differences in these parameters compared to healthy calves. These differences include lower levels of total protein, albumin, and the albumin-globulin ratio, along with unchanged levels of beta globulin and potentially higher levels of alpha, gamma globulins, and total globulins. Other research (Subhash Malik et al. 2012) suggests that dehydrated calves with diarrhea also have higher red blood cell counts and white blood cell counts, indicating dehydration. Additionally, the severity of these changes depends on the type, duration, and intensity of the diarrhea (Nayak et al. 2019). While there are many studies on changing hematological and biochemical parameters in cases of diarrhea, there are few studies on changes in fecal pH value (Turgut and Ok 1998). In cases of diarrhea, enteropathogens disrupt the intestinal mucosal barrier system and cause damage to the intestinal epithelium (Kozat, 2023). It is thought that the pH of the stool will change as a result of the deterioration of the intestinal mucosal barrier system. Although the severity of clinical findings varies, changes in stool pH value are observed depending on enteropathogens. *Enterobacteriaceae* causes organic acid production, and the organic acids formed cause to decrease pH value of fecal. However, the same study stated that as a result of the damage to the gastrointestinal system during diarrhea, the normal flora changes and the beneficial bacteria decrease, the fecal pH value may increase, and the resulting alkaline environment may lead to the proliferation of harmful bacteria in human medicine (Osuka et al. 2012).

In the literature review, no research was found in the field of veterinary medicine regarding enteropathogens and changes in fecal pH in cases of diarrhea. This research will be to reveal the relationship between fecal pH and enteropathogens in cases of diarrhea. In this study, the objective of the present study was to evaluate fluctuations in fecal pH levels in diarrheic and normal fecal samples from neonatal calves, while also revealing whether there is a relationship between the factors that play a role in diarrhea and the fecal pH value.

Animals

Within the scope of this research, 96 newborn calves with diarrhea, 47 male and 49 female, were used as study material. During the clinical examination of the sick calves included in the research; the consistency, odor, content and color of the stool and the frequency of defecation were noted in detail.

Examination protocol of calves

Detailed systemic clinical examinations were executed 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.

Taking stool samples and determining the causative agents

Stool samples were collected in stool containers or by swab or by swab following the technique. After the stool was collected, the BoviD-5 Ag rapid test kit (RC1302DD) originating from the Republic of Korea was used for agent analysis. Before the test, the box of the kit 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 in the test device.

pH determination from stool samples

Stool pH values were determined by dipping nitrazine paper into the stool samples taken into a sterile container and using a digital pH meter (AD11 Waterproof pH-TEMP Pocket Tester with replaceable electrode).

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.

Clinical findings

In the study, when we looked at the color, content and consistency of diarrhea, it was determined that they were yellow watery, yellow gruel-like, yellow-green watery, green watery, green gruel-like, grey-white, brown and bloody and mucous.

Biochemical Findings

According to the stool analysis results, enteropathogens were detected in 79 of 96 diarrheal cases, while no enteropathogens were detected in 17 cases. Of the 79 cases in which enteropathogens were detected, single enteropathogens were detected in sixty-one, double enteropathogens were detected in sixteen, and triple enteropathogens were detected in two (Table 1). Among the 61 calves were *Cryptosporidium* spp. in 25, *E. coli* K99 in 12, *Bovine rotavirus* in 11, *Bovine coronavirus* in 7 and *Giardia lamblia* in 6. Additionally, 2 calves had *Cryptosporidium* spp.+*Giardia lamblia*, 8 cases *Cryptosporidium* spp. + *Bovine rotavirus*, 3 cases of *Bovine coronavirus*+*Bovine rotavirus*, 1 case of *Bovine coronavirus* + *Cryptosporidium* spp., 2 cases of *Bovine rotavirus*+*E. coli*, 1 case of *E. coli*+*Cryptosporidium* spp.+*Bovine rotavirus*, 1 in *Bovine coronavirus*+*Bovine rotavirus* + *Cryptosporidium* spp. the factors were detected as co-infection. Of the 96 calves within the scope of the research, 27.08% had *Bovine rotavirus*, 12.5% had *Bovine coronavirus*, 15.62% had *E. coli*, 39.58% had *Cryptosporidium* spp. and in 8.33%, *Giardia lamblia* was detected alone or simultaneously with other enteropathogens.

Fecal pH results

Within the scope of the research, fecal pH values of 96 calves were measured. When the pH analysis results in all cases were examined, the pH values of both single and co-infected cases were determined in two groups as 5-6 and 6.1-7. The pH values of infected calves were detected in the density group between 6.1-7. When the pH values of all cases were examined, cases with 8-15 days of diarrhea were detected more frequently and the pH value of the calves was determined to be 6.1-7. In the analysis of fecal pH values in 12 samples in which only *E. coli* was detected, it was determined that the fecal pH value of 11 of these calves was measured between 5 and 6, and only 1 of them had a pH value above 6. On the other hand, it was determined that the fecal pH value was measured below 6 in 12 of the remaining 84 calves. It was determined that the fecal pH value was above 6 in 73 of a total of 96 calves (Table 1, Table 2). Additionally, pH of none of the cases infected with enteropathogens was detected above 7.1. The pH value of both single and co-infected cases was predominantly determined between 6.1 and 7. In this study, single enteropathogens were detected in 61 cases among 96 diarrheal cases. Fecal pH values of cases with single enteropathogens (n=61) were detected between 5-6 in 17 cases and 6.1-7 in 44 cases.

Table 1. Distribution of calf fecal pH value according to infectious factors

	Agent (s)	pH 5- to- 6	pH 6.1-to-7	pH 7.1 and above	Total
Single (n=61)	<i>Bovine rotavirus</i>	2	9	0	11
	<i>Bovine coronavirus</i>	0	7	0	7
	<i>E. coli</i>	11	1	0	12
	<i>Giardia lamblia</i>	2	4	0	6
	<i>Cryptosporidium spp.</i>	2	23	0	25
	<i>Bovine coronavirus</i> +	0	3	0	3
	<i>Bovine rotavirus</i>				
Double (n=16)	<i>Bovine rotavirus</i> + <i>Cryptosporidium</i> spp.	1	7	0	8
	<i>Cryptosporidium</i> spp.+ <i>Giardia</i> <i>lamblia</i>	1	1	0	2
	<i>Cryptosporidium</i> spp.+ <i>Bovine</i> <i>coronavirus</i>	0	1	0	1
	<i>Bovine rotavirus</i> + <i>E.coli</i>	2	0	0	2
	<i>Bovine rotavirus</i> +	0	1	0	1
Triple (n=2)	<i>Cryptosporidium</i> spp. + <i>E.coli</i>				
	<i>Bovine rotavirus</i> + <i>Bovine</i> <i>coronavirus</i> + <i>Cryptosporidium</i> spp..	0	1	0	1
Unknown (n=17)		2	14	1	17

Table 2. Distribution of pH value determined in stool samples according to age

pH	pH between 5-6 (n=22)	pH between 6.1-7 (n=73)	pH 7.1 and above (n=1)	Total
Age (s)				
1-7 days	17	18	0	35
8-15 days	4	41	1	46
16-28 days	1	14	0	15

DISCUSSION

It is a fact that calf diarrhea still causes economic losses in cattle farms, despite modern veterinary practices. In addition to diarrhea treatments, determining etiological factors and taking preventive measures is becoming increasingly important. As stated by Walker (1998), diarrhea in newborn calves with high morbidity and mortality constitutes a serious problem in the cattle industry. Worldwide, one of the main causes of calf deaths and financial losses to the cattle industry is losses due to diarrhea (Kozat, 2019, Von Buenau et al. 2005; Wudu et al. 2008). Precautions taken 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 et al., 2013; Kozat, 2019). 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 and Voyvoda, 2006).

Diarrhea and changes in gut bacteria create a two-way street of health problems in dehydrated calves. This highlights the interconnectedness of the issues and emphasizes the importance of considering both the gut bacteria and blood tests for a complete understanding of the calf's health (Li et al. 2020). According to recent research, cases of diarrhea in calves in the first four weeks after birth have been linked to various pathogens. Enteropathogens such as *Bovine rotavirus*, *Coronavirus*, *E. coli*, *Cryptosporidium* and *Giardia* are stated as the most important causes of calf diarrhea (De la Fuente et al. 1998; Langoni et al. 2004; Yang et al. 2017). 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). Studies have shown that calves with diarrhea have a decrease in intestinal microbiota diversity, significant changes in the fecal microbial composition, and dysbiosis (Gomez et al., 2017). They reported that they detected a significant decrease in the alpha diversity of bacterial and fungal communities, especially in the intestine of calves (Jang et al. 2019; Liu et al. 2022). At the same time, diarrhea is characterized by a decrease in beneficial bacteria that produce short-chain fatty acids (SCFAs), which play a role in reducing the risk of diarrhea (Li et al. 2023). In this study, the fecal pH value of *bovine rotavirus*-infected calves with diarrhea was between 6.1 and 7 in 9 calves and between 5 and 6 in 2 calves. The maximum change in fecal pH values is between 6.1-7, which can be interpreted as being caused by disorders caused by the agent in the intestines.

When a calf gets infected with bovine coronavirus (BCoV), it first attacks the beginning of the small intestine and often spreads throughout the entire small and large intestines. BCoV mainly targets mature cells on the surface of tiny finger-like structures called villi, but it can also damage cells deeper in the gut lining (crypt cells). This damage takes longer to heal, making the illness last longer. Infected villi in the small intestine and cells in the large intestine get replaced by immature cells, and the ridges within the large intestine shrink (Cho and Yoon, 2014). In this research, the fecal pH value of 7 cases with *Bovine coronavirus* infection values was detected between 6.1-7.

Cryptosporidium species are zoonotic protozoans that cause gastrointestinal infections in various species. In particular, *Cryptosporidium parvum* is considered one of the main causes of calf diarrhea and is important as a potential zoonotic factor (Trotz-Williams et al. 2008; Chalmers et al. 2011). In studies conducted in other countries around the world, *Cryptosporidium* spp. different rates have been obtained regarding its prevalence. 47.9% in newborns in Spain (Castro-Hermida et al., 2002), 17.9% in France, 33.5% in Vietnam (Nguyen et al., 2007), 13% in Canada, 27.9% in England (Brook et al. 2008), 35% in America (Santín et al., 2004) and 11% in Sweden (Lefay et al. 2000; Björkman et al. 2003; McAllister et al. 2005). In this study, only *Cryptosporidium* infection was detected in 25 of 96 calves with neonatal diarrhea aged 1-28 days, while 38 (39.58%) were found to have other enteropathogens along with cryptosporidium infection. This shows that *Cryptosporidium* can be effective alone or in combination with other factors to cause diarrhea. *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 (Kozat and Voyvoda, 2006). *Enterotoxigenic E. coli* (ETEC) strains from calves are mediated by adhesin antigens, mainly F5 (K99) and F41 fimbriae. The distal part of the small intestine is most suitable for ETEC colonization due to low pH (less than 6.5). It binds to the intestinal epithelium and proliferates in the enterocytes of the intestinal villi (Foster and Smith, 2009). *E. coli* K99 rates vary around the world, for example 0.3% in Switzerland (Torsein et al. 2011), 2.6% in Germany (Bartels et al., 2010). In a study conducted in Spain, the mixed ratio of *E. coli* K99 and *C. parvum* was found to be 27.8% (De la Fuente et al. 1998). In this study, it was determined that the fecal pH value in patients with *E. coli* infection was 6 and lower than 6 in 11 out of 12 calves.

Giardia lamblia is becoming increasingly important as it causes growth retardation in farm animals, reducing feed utilization and causing economic losses by causing diarrhea (O'Handley et al., 2003). In this study, *Giardia lamblia* infection was detected in 6 cases. The fecal pH of two of the 6 cases was found to be between 5-6, and the fecal pH of four calves was found to be between 6.1-7.

The pH value of a stool sample is usually measured using nitrazine paper. Normally, fecal pH varies between 7.0-7.5; However, lower than 5.5 indicates an acidic stool (Eherer and Fordtran, 1992). Higher or lower than normal fecal pH value is related to the severity of infection and can be used as an evaluation tool for the clinical course of diseases (Osuka et al. 2012). In the research conducted on fecal pH changes in diarrheal and healthy calves, the average pH after birth increased from the 1st week (pH 5.39), decreased in the 2nd week (6.02) and 3-4 weeks (6.30). In the same study, in the diarrhea group, week 1 showed a higher pH (6.18) and week 3-4. They reported that they detected lower pH (5.77) in diarrheal stools in weeks (Walker et al. 1998). In this study, the pH value of the feces was measured and it was determined that the pH value of 23 of 96 neonatal calves with diarrhea was between 5-6 and 72 of them was between 6.1-7. The highest fecal pH value was found to be 6.1-7 in all age groups. *E. coli* infection was detected in 11 of the calves whose fecal pH value was between 5-6. In addition, it was determined that the fecal pH value of 17 (48.57%) of 35 calves with diarrhea aged 1-7 days was between 5-6, and 18 (51.43%) was between 6.1-7; It was determined that the fecal pH value of 4 (8.69%) of 46 calves with diarrhea, aged 8-15 days, was between 5-6, and 41 (91.31%) were between 6.1-7.

These findings suggest that stool pH may be related to both *E. coli* infections and age. It has been stated that fecal pH value is related to the severity of infection and measuring fecal pH value can be used as an evaluation tool for the course of diseases (Osuka et al. 2012). In another study, in the comparison of fecal pH values in diarrheal and normal calves, it was reported that there were wide differences especially between Lactate and Succinate concentrations of both groups, but the average fecal pH value in healthy first week old calves was 5.39, 6.02 in 1-2 week old calves and 6.30 in 3-4 week old calves (Sato and Koiwa, 2008).

A fecal pH value of 5-6 suggests the presence of an acidic environment, potentially increasing the chances of pathogenic bacteria such as *E. coli* to proliferate. This situation was reported by Osuka et al (2012) that organic acids produced by Enterobacteriaceae may cause the fecal pH value to decrease. The findings on this subject suggest that fecal pH value can be used as an important parameter in evaluating gastrointestinal health status and diagnosing the disease agent. However, more studies are needed on the relationship between infectious factors and fecal pH values.

CONCLUSION

In summary, in this study, the pathogens *Bovine rotavirus*, *Bovine coronavirus*, *Cryptosporidium* spp., *E. coli* K99 and *Giardia lamblia* were rapidly detected in calves with neonatal diarrhea. A difference was detected between the rates of occurrence of these factors alone and in mixed forms. Additionally, the fecal pH values of calves identified as the causative agent were

measured. Considering that there was a relationship between the measured fecal pH values and the factors and that the factors were single and mixed in all calves, single and mixed factors were detected to be concentrated between fecal pH values of 6.1-7. It was concluded that there was a connection between fecal pH value changes and the factors. It is thought that this study will shed light on future research on fecal pH values.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: VÇ and SK contributed to the project idea, design and execution of the study. VÇ contributed to the acquisition of data. VÇ wrote the manuscript. SK reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

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). "This study is not subject to the permission of Van YUHADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees". The data, information and documents presented in this article were obtained within the framework of academic and ethical rules."

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Explanation: We have not presented as a oral, poster, and abstract vs.

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