Evaluation of blood gases in calves with Cryptosporidiosis

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SUMMARY

Neonatal calf diarrhea (NCD) is a widespread disease in calves worldwide. NCD has multifactorial etiology and is mainly caused by aetiological agents by E. coli, *Cryptosporidium* spp., Rotavirus, and Coronavirus. Among these agents, Cryptosporidiosis and rotaviruses are the most common entero-pathogens in our region and all over the world. Cryptosporidiosis is protozoal enteritis caused by *Cryptosporidium* spp., a coccidial parasite belonging to the *Cryptosporidium* genus. Cryptosporidiosis causes malabsorptive and secretory diarrhea in the small intestine. In neonatal calves with diarrhea, fluid losses during diarrhea and electrolyte imbalances may cause metabolic acidosis. Blood gas analysis assesses the degree of severity of metabolic acidosis. The aim of this research, to investigate the effect of cryptosporidiosis on calf metabolic status and compare it with healthy calves. The material of the study consisted of 16 Holstein calves naturally affected by cryptosporidiosis, which were classified as the diseased group. 9 healthy calves with no diarrhea or other problems were classified as the control group. Clinical examination, fecal rapid tests, and fecal microscopic examinations were performed in all calves at the beginning of the study. Calves which were positive only for cryptosporidiosis and had *Cryptosporidium spp.* oocysts at the infection level were selected as the diseased group. Blood gas analyses were performed for both groups. In the diseased group, there was a significant decrease in pH, cHCO3, cBase(B)c, and ctCo2(B)c ($p < 0.001$) compared to the healthy group with a significant increase in cHc. Additionally, a statistically significantly increased Anion Gap (K)c (p 0.001) was detected in the diseased group compared to the healthy group. It was determined that anion gap metabolic acidosis may occur in calves with cryptosporidiosis, although the clinical findings of diarrhea and the degree of dehydration were not very severe. It can be thought that parenteral bicarbonate administration will contribute to the treatment success by compensating for acid-base balance in calves with cryptosporidiosis.

KEY WORDS

Blood gas, calves, cryptosporidiosis, diarrhea.

INTRODUCTION

Owing to its high morbidity and mortality, neonatal calf diarrhea (NCD) continues to be a major problem for the cattle industry worldwide. NCD also causes economic loss through the cost of medical treatment, retarded growth, and human labor. Infectious agents and noninfectious factors influence the pathogenesis of calf diarrhea. Studies emphasize that a single cause does not play a role in the formation of calf diarrhea, and the etiology is often complex (1-3). Various types of viruses, bacteria, and protozoa comprise the enteric pathogens of calves. However, rotavirus, coronavirus, *Escherichia coli, Salmonella spp.*, and *Cryptosporidium parvum (C. parvum)* are primary infectious agents in calf diarrhea (4-6), and the most frequently identified pathogens in fecal samples are rotavirus and *C. parvum* (7).

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Cryptosporidiosis causes malabsorptive and secretory diarrhea in the small intestine, especially in the distal jejunum and ileum. The mature villous enterocytes are the preferential site for cryptosporidiosis invasion, which leads to malabsorption by villous atrophy (8). The incubation period of cryptosporidiosis is 2-5 days. The disease is more common in calves that are 1-2 weeks old. The first findings are varying degrees of depression and anorexia. This is followed by yellow, watery, mucous, jelly-like, and sometimes blood-containing malabsorptive diarrhea. During the diarrhea in calves, varying degrees of dehydration, and imbalances in electrolyte and acid-base status occur. These metabolic imbalances are also the main causes of death in NCD (1,8).

Blood gas analysis is widely regarded as the gold standard test to assess the degree of severity of metabolic acidosis, strong ion difference (SID), and electrolyte derangements in diarrheic calves. In particular, blood gas parameters may help in treatment planning (1, 9, 10). This study aimed to reveal the acid-base and electrolyte imbalance in the blood of calves infected with Cryptosporidium spp without severe diarrhea and to compare it with healthy calves.

MATERIALS AND METHODS

This study was carried out with the approval of the Bursa Uludag University Animal Experiments Local Ethics Committee (2022-15/01). In the study period (between June and August), 60 calves were evaluated daily for the presence of diarrhea, and diarrhea was diagnosed in 35 calves. All the calves in the study were born from healthy mothers without dystocia and were fed colostrum at the rate of approximately 5% of their body weight in the first 2 hours after birth. The calves were 10-15 days old and kept in individual boxes according to farm procedures. The study consisted in16 Holstein calves (9 male and 7 female) that were naturally affected by only cryptosporidiosis and kept in Bursa Uludag University Veterinary Faculty Research and Application Center. Also, 9 healthy Holstein calves (5 male and 4 female) in the same farm were selected for the control group.

During the routine daily farm visitings, diarrhoeic calves were determined and stool samples were taken from them by rectal touch or during spontaneous defecation. The physical appearance of fecal samples was noted. The fecal scores of diarrhoeic calves were evaluated in a five-scoring system (1:normal fecal consistency and 5:watery diarrhea) (11, 12). Fecal smears were prepared with carbol fuchsin and then were examined for the presence of *Cryptosporidium spp.* oocysts in light microscopy (11). In the calves' stools in which *Cryptosporidium* oocysts were positive, fecal rapid tests (Anigen Bovid-5 Calf Diarrhea Ag Test Kit, Bionote, South Korea) were performed. Also, body posture and sucking reflexes of calves were evaluated regarding symptoms of depression.

Eight-milliliter blood samples of the diseased group were taken only once after the just diagnosis, into tubes (with heparin) from the vena jugularis of all calves. Similarly, the same blood samples were taken from healthy calves on any day. Blood gas analyses were performed with an automatic blood gas analyzer (Radiometer ABL9, Hasvet, Turkey) in the blood samples with heparin. In addition to blood gas profiles in the calves, the strong ion difference (SID) was calculated based on the combined electrolyte concentrations as shown below:

$$SID = (Na^+ + K^+) - Cl^-.$$

Information about calves obtained from the medical records included sex, rectal body temperature, and sucking reflexes.

Statistical analyzes were performed with Sigma statistical software program. The normality of all parameters were tested with the Shapiro-Wilk test. The 't-test' was used to compare the results between healthy and diseased groups. In all evaluations of statistical results, $p<0.05$ indicated statistical significance.

RESULTS

The physical appearance of feces of 16 diarrheal calves; (1) 4 of them were yellow, watery, and mucous, (2) 4 of them were yellow and watery, (3) 3 of them were yellow, mucous and slightly bloody, (4) 5 of them were brown and mucous. The fecal scores of all calves infected only with *Cryptosporidium spp.* were determined as 3 and 4. Also, none of these calves were lying down and their sucking reflexes were normal. Fecal analysis revealed that 45.71% (n:16), 28.57% (n:10), 14.28% (n:5), 5.71% (n:2), and 5.71% (n:2) calves were infected with only *Cryptosporidium*, *Cryptosporidium*, and Rotaviruses, only Rotaviruses, only Coronavirus, and no infective agents, respectively. There was no death in calves in the study period.

Blood gasses and electrolyte panels of calves in the healthy group and diseased group (calves with Cryptosporidiosis) were compared in this study. Changes in blood gasses parameters in each group are summarized in Table 1. Among them, some parameters including, Ph, pH(T)c, cHc, cHCO3(P)c, cBase(B)c, ctCO2(B)c, ctCO2(P)c and Anion Gap(K)c were statistically significantly (p 0.001) different from each other. But some parameters such as pCo2, HCT, K, Na, Ca, Cl, Lac, pCO2(T)c, and pO2(T)e did not differ in

Table 1 - Blood gas and some laboratory parameters in control and diseased groups.

Parameter	Healthy Control Group			Diseased Group			Significance (p)
	Mean±Std. Error	Min.	Max.	Mean ± Std. Error	Min.	Max.	
ph	7,384 ± 0,009	7,34	7,43	7,221 ± 0,018	7,12	7,34	0.001
pCo2	42,089 ± 2,961	25,1	53,7	36,369 ± 1,883	26,6	53,3	0.101
K	4,581 ± 0,184	3,9	5,74	4,364 ± 0,204	3,12	6,23	0.485
Na	132,111 ± 2,756	111	138	130,25 ± 2,281	119	151	0.618
Lac	1,033 ± 0,167	0,7	1,2	1,279 ± 0,293	0,4	4,6	0.899
cHc	41,289 ± 0,881	36,8	45,3	60,8 ± 2,468	45,8	75,8	0.001
cHCO3(P)c	25,433 ± 2,195	14,2	36	15,456 ± 1,339	9	28,3	0.001
cBase(B)c	0,378 ± 2,126	-10,2	10,7	-11,225 ± 1,465	-18,3	2,1	0.001
ctCO2(B)c	24,489 ± 2,106	13,4	33,9	15,244 ± 1,276	9,2	27,1	0.001
Anion Gap(K)c	13,678 ± 1,613	7,1	20,8	21,356 ± 1,288	13,7	31,3	0.001
SID	39,139 ± 0,997	33,340	42,90	36,682 ±0,863	31,390	42,610	0.087
HCT	27,111 ± 1,982	17	36	29 ± 1,573	20	40	0.470
Ca	1,29 ± 0,028	1,12	1,39	1,253 ± 0,026	1,1	1,44	0.378
Cl	97,556 ± 3,046	76	109	97,938 ± 2,676	86	124	0.929

the groups ($p>0.05$). When comparing the SID values in groups, it was revealed that SID values were lower in the diseased group than in the control group (36,68 ± 0,86 & 39,13 ± 0,99) but not statistically significant ($p=0.087$).

DISCUSSION

NCD is a widespread disease in calves all over the world. E. coli, *Cryptosporidium* spp., Rotavirus, and Coronavirus are the four main isolated agents in neonatal calf diarrhea (2, 3). It was stated in many reports that *Cryptosporidium* spp. and rotavirus are the most common entero-pathogens among them (13-15). In the presented study, *Cryptosporidium spp., Cryptosporidium spp.* and rotavirus infections in calves with diarrhea were found to be 45.71%, 28.57% and 14.28%, respectively. These results, as in other studies, showed that *Cryptosporidium spp.*, alone or together with rotavirus, is one of the most common etiological agents causing calf diarrhea (2,3,13-15).
When comparing the groups, there was a significant decrease ($p < 0.001$) in pH, cHCO3, cBase(B)c, and ctCo2(B)c, but a significant increase ($p < 0.001$) in cHc in the diseased group than in the healthy group. Additionally, although not at a statistical level, the decreasing concentration of PCO2 in the diseased group confirms the metabolic acidosis in diarrheal calves with cryptosporidiosis. The Anion Gap can be an extremely helpful tool for categorizing causal factors in acid-base imbalances and may prove a useful prognostic guide in animals with severe calf diarrhea (5, 15). In the evaluation of Anion Gap (K)c, it was determined that there was a significant anion gap ($p<0.001$) in cryptosporidiosis-infected diarrheal calves compared to healthy calves. This shows that metabolic acidosis with an anion gap occurs in diarrheal calves infected with cryptosporidiosis. The anion gap may be related to the fact that milk reaches the large intestine without being absorbed due to malabsorption and that it undergoes bacterial fermentation here, and Lactic acid, especially D-Lactate increases (16, 17). It was presented also in the previous studies that calves with cryptosporidiosis have lower pH values than healthy ones (18, 19).
It is well known that in calves with cryptosporidiosis, destruction of intestinal epithelial cells, villous atrophy, shortening and degeneration of microvilli, and hyperplasia of crypts occur. This causes the loss of ciliary enzymes in the intestinal mucosa and a decrease in the surface area of the villi (20). It has also been reported that in relation with an increase in the secretion of endogenous prostaglandins such as PGE2 and PGI2, an increase in the secretion of chloride and bicarbonate from the crypts, and a decrease in the absorption of sodium chloride from the villi (20, 21). Thus, diarrhea associated with maldigestion and malabsorption occurs in affected calves, followed by fluid electrolyte and acid-base imbalances (15, 17, 20). Both the loss of bicarbonate with diarrhea and the increase in D lactic acidemia by exposure of nutrients to bacterial fermentation in the colon as a result of malabsorptive diarrhea may cause metabolic acidosis to be higher than expected in diarrheal calves infected with cryptosporidiosis, even if their general condition is good (17). In this study, although the sucking reflexes and general conditions of the calves infected with cryptosporidiosis were good, the reflection of metabolic acidosis at varying degrees was determined in all of them. Therefore, regardless of the severity of the disease in calves infected with cryptosporidiosis, it is important to examine the blood gas parameters in the determination of fluid therapy, treatment plan, and prognosis in calves with cryptosporidiosis.
It is known that electrolyte loss and acid-base imbalances occur in calves with diarrhea due to malabsorption and hypersecretion (10, 15, 22). For this reason, it is seen that ions such as Na, K, and Cl are evaluated in many studies on calves with diarrhea (10, 15, 17). When comparing with the control group, it was observed that Na and Cl values decreased ($p>0.05$) but K levels increased ($p>0.05$). The results of this study suggested that hyponatremia and hypochloremia occurring in cryptosporidiosis are associated with intestinal loss of Na^+ and Cl^- ions. At the same time, there may be also enteric losses of K in diarrheal cases. Although expected K losses in diarrhea, hyperkalemia may occur by shifting K from intracellular to extracellular space. Also related to decreasing glomerular filtration rate in dehydration can contribute to K retention in calf diarrhea. In this study, when calves with cryptosporidiosis and healthy calves were compared, no statistically significant difference in Na, K, and Cl values was associated with the fact that the calves' fecal scores were not too bad (scores 3 and 4) and the calves were able to stand.
It was detected that although nonsignificant pCo2 levels were lower in the diseased group than in the control group. Generally, lower pCo2 levels than in references are indicative of metabolic acidosis. In this research expected pCo2 levels were calculated in each group by Winter's formulation (Expected PCo2 = (1.5 x HCO3) + 8 ±2) (23). So expected pCo2 level in the diseased group was calculated that it should have been 29.1- 33.1 but it was detected higher than this. So with these findings, it was suggested that there was an uncompensated metabolic acidosis in diseased calves.
Lactic acid levels were measured as higher in the diseased group than in the control group ($p>0.05$). It is known that villous atrophy occurring in calves with cryptosporidiosis causes malabsorption (15). So with our knowledge, it was suggested that elevated lactic acid levels were related to d-lactatemia, which is a result of the passage of milk or food into the large intestine without being digested and the bacterial fermentation occurring there. Also, a significant anion gap was detected in diseased calves (p 0.001) and it was thought that is associated with increased organic acids and lactic acids in the diseased group.

CONCLUSIONS

In conclusion, in the presented study it was determined that anion gap metabolic acidosis may occur in calves infected with cryptosporidiosis, although the clinical findings of diarrhea and the degree of dehydration were not very severe. In this respect, it can be said that parenteral bicarbonate administration to the initial fluid treatments will provide significant support to the treatment to compensate for the acid-base balance in diarrheal calves with cryptosporidiosis.

Author's contribution

SŞ, SK, and YK conceptualization, investigation, data curation, writing-original draft preparation, formal analysis. All authors read and approved the final version of the manuscript.

Ethics Statement

This study was carried out with the approval of the Bursa Uludag University Animal Experiments Local Ethics Committee (2022-15/01).

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Declaration of Competing Interest

The authors declare no conflict of interest.

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