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Comparison of blood electrolyte and biochemical parameters between single infections of rotavirus and *Cryptosporidium parvum* in diarrheic Hanwoo calves

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ABSTRACT

Background: Neonatal calf diarrhea is a major problem in the cattle industry worldwide. Rotavirus and *Cryptosporidium parvum* are the primary causative agents, especially during the first three weeks of the calf's life.
Objectives: This study investigated the differences in acid-base, electrolytes, and biochemical parameters of diarrheic calves with infection of either rotavirus or *C. parvum*.
Methods: A total of 61 Korean native calves (≤ 20 days old) were divided into two groups based on rotavirus or *C. parvum* infections: rotavirus infection (n = 44) and *C. parvum* infection (n = 17). The calves with at a specific blood pH range (pH 6.92–7.25) were chosen for comparison. The acid-base, electrolyte, chemistry, and serum proteins were analyzed, Further, fecal examinations were performed.
Results: Compared to *C. parvum*-infected calves, the rotavirus-infected calves showed lower levels of total carbon dioxide, bicarbonate (HCO_3^-), anion gap, total protein, and albumin/globulin ratio, and significantly lower levels of potassium, globulin, and α2-globulin ($p < 0.05$). The *C. parvum*-infected calves ($r = 0.749$) had stronger correlations between pH and HCO_3^- than the rotavirus-infected calves ($r = 0.598$). Compared to rotavirus-infected calves, strong correlations between globulin and α2-globulin, α2-globulin and haptoglobin were identified in *C. parvum*-infected calves.
Conclusions: This study is the first to investigate acid-base, electrolyte, and biochemical parameters in calves in response to infections of rotavirus and *C. parvum*. Although rotavirus and *C. parvum* cause malabsorptive and secretory diarrhea in similar-aged calves, blood parameters were different. This would help establish the diagnostic and treatment strategies.

Keywords: Diarrhea; electrolyte; serum protein; rotavirus; *Cryptosporidium parvum*

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Conflict of Interest

The authors declare no conflicts of interest.

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INTRODUCTION

Owing to its high morbidity and mortality, neonatal calf diarrhea continues to be a major problem for the cattle industry worldwide. Calf diarrhea also causes economic loss through the cost of medical treatment, retarded growth, and human labor. Infectious agents and non-infectious factors influence the pathogenesis of calf diarrhea. Various types of virus, bacteria, and protozoa comprise the enteric pathogens of calf. However, rotavirus, coronavirus, *Escherichia coli*, *Salmonella* spp., and *Cryptosporidium parvum* are primary infectious agents in calf diarrhea [1-3], and the most frequently identified pathogens in fecal samples are rotavirus and *C. parvum* [4].

At 1–3 weeks, both rotavirus and *C. parvum* infections are present in calves [5,6]. Both pathogens induce malabsorptive and secretory diarrhea throughout the small intestine. The mature villous enterocytes are the preferential site for rotavirus and *C. parvum* invasion, which leads to malabsorption by villous atrophy [7]. However, nonstructural glycoprotein 4 in rotavirus infection plays a major role in secretary diarrhea, while prostaglandin-mediated secretion occurs in *C. parvum* infection [7-9]. In addition, the pronounced sites in the intestine are different. The most severe lesions are the duodenum and proximal jejunum in rotavirus infection, but the distal jejunum and ileum in *C. parvum* infection [7,8,10,11].

Diarrhea leads to dehydration, acid-base imbalance, and gas and electrolyte disturbances in calves. Dehydration and low levels of pH, Na^+, total carbon dioxide (tCO_2), bicarbonate (HCO_3^-), and base excess (BE) of the extracellular fluid were observed in Korean native (Hanwoo) diarrheic calves [12].

As for total protein, hepatocytes produce plasma proteins including acute phase proteins. B lymphocytes and plasma cells secret γ-globulin. Intestinal malabsorption and malnutrition are associated with hypoalbuminemia [13]. Haptoglobin is a hemoglobin binding α2-globulin and bacteriostatic by preventing microorganisms from utilizing iron [14]. Low levels of total protein and albumin and high α2-globulin levels were present in diarrheic Korean native calves at the 1 to 20 days of age. In addition, Haptoglobin was synthesized more in neonatal diarrheic calves, and not serum amyloid A [15].

To the best of our knowledge, no studies have investigated the differences in acid-base, electrolytes, and biochemical parameters between single natural infections of rotavirus or *C. parvum* in calves. In this study, we hypothesized that calves would respond to rotavirus or *C. parvum* infection in different ways because infection mechanisms and pronounced infection regions were different even though both pathogens induce malabsorptive and secretory diarrhea at a similar age. Thus, the objective of this study was to determine the differences in acid-base, electrolyte, and biochemical parameters in rotavirus- or *C. parvum*-infected diarrheic calves. These findings will help understand the characteristics of diarrhea associated with these pathogens and establish a treatment plan for rotavirus- or *C. parvum*-infected diarrheic calves.

MATERIALS AND METHODS

Ethics approval and consent to participate

All animal procedures were conducted according to ethical guidelines for the use of animal samples, and were approved by the Jeonbuk National University (Institutional Animal Care and Use Committee Decision No. JBNU 2020-052). All cases were naturally occurring infections. Every diarrheic calf was treated after sampling. All procedures and possible consequences were explained to the managers of the surveyed farm, and written consent was obtained.

Sample collection

The current study used a total of 61 diarrheic Hanwoo calves (native Korean cattle breed), which were of ≤ 20 days old and raised in the Republic of Korea from 2019 to 2020. The calves were housed with their dam and fed colostrum and milk by their dam. Blood samples were collected from the jugular vein of the calves in serum-separation tubes (SST) for serum protein analysis. Acid-base, electrolyte, and chemistry analyses were performed immediately after blood sampling. Fecal samples were collected by experienced veterinarians using digital rectal palpation to induce bowel movements. These fecal samples were stored in 50 mL specimen bottles (SPL Life Sciences, Korea) and transported to the laboratory under refrigeration. The sampling procedures were performed 1–2 days after the calves began to show diarrhea (before the treatment).

Fecal examination

Feces were subdivided into solid, semi-solid, loose, and watery stools. Solid and semi-solid stools were classified as normal while loose and watery stools were classified as diarrhea. Fecal samples were initially screened using a rapid diagnosis antigen test kit (BoviD-5 Ag) to identify antigens associated with pathogens, including *C. parvum*, *Giardia duodenalis*, *E. coli*, coronavirus, and rotavirus. These samples were also examined by reverse transcriptase-polymerase chain reaction targeting bovine viral diarrhea virus (BVDV) and *Salmonella* spp., as well as the five pathogens noted above. BVDV, coronavirus, rotavirus, *E. coli*, *Salmonella* spp., *C. parvum*, and *G. duodenalis* were the major pathogens associated with diarrhea in calves [16,17]. To detect *Eimeria* spp., all fecal samples were suspended in a solution of 2.5% potassium dichromate and then transported to the laboratory. In the laboratory, fecal samples were analyzed to detect oocysts using the floatation methods with Sheather's solution (saturated sugar solution; specific gravity 1.28) and examined microscopically (×400 magnification) based on the morphological features of *Eimeria* spp. oocysts.

Experimental group definition

A total of 114 diarrheic calves used in this study had a score over two, based on a previous study [18]. Diarrheic calves (n = 32) that tested positive for at least one of the other pathogens associated with diarrhea, except for rotavirus and *C. parvum* were excluded; none of these pathogens were detected in fecal samples from clinically healthy calves. Calves (n = 10) with concurrent rotavirus and *C. parvum* infections were excluded. To avoid the difference of blood acidity while comparing calves with rotavirus to those with *C. parvum*, we used calves (n = 61) with specific blood pH values (pH 6.92–7.25). The calves were divided into two groups according to the agent: rotavirus infection (n = 44) and *C. parvum* infection (n = 17). The parameters of clinically healthy calves (n = 57) were used as the reference (**Table 1**).

Table 1. Descriptive statistics for Hanwoo calves included in the study

Variable	Rota (n = 44)	Crypt (n = 17)	*p* value	Healthy (n = 57)
Sex			0.580	
Male	22	7		28
Female	22	10		29
Age (day)	10.64 ± 4.41	10.35 ± 3.39	0.813	11.79 ± 4.32

Data are presented as the mean ± SD.
Rota, diarrheic calves infected with rotavirus; Crypt, diarrheic calves infected with *Cryptosporidium parvum*; Healthy, clinically healthy calves without diarrhea.

Acid-base, electrolyte, and chemistry analyses

Blood parameters, such as pH, tCO_2, partial pressure of carbon dioxide (pCO_2), HCO_3^-, BE, Na^+, K^+, Cl^-, anion gap (AG), glucose, and blood urea nitrogen (BUN) were measured immediately after obtaining fecal samples using the EC8 + i-STAT cartridge (Abbott, USA). The strong ion difference (SID) was calculated based on the combined electrolyte concentrations as shown below:

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

Serum protein gel electrophoresis and acute phase proteins

Serum samples were separated from blood by centrifugation. Serum was harvested by centrifuging SST tubes at 3,000 rpm (2,600 g) for 10 min. The serum was frozen and stored at −70°C pending analysis. Serum samples (n = 6) with low quality were excluded (rotavirus group, n = 4; *Cryptosporidium* group, n = 2). Subsequently, agarose gel electrophoresis was performed to analyse 5 protein fractions (albumin, α1-globulin, α2-globulin, β-globulin, and γ-globulin) using a semi-automated agarose gel electrophoresis system (HYDRASYS 2; Sebia, UK), following the manufacturer's protocols. Briefly, 30 μL of serum was subjected to the microtechnique assay, electrophoresed for 35 min, stained for 5 min, de-stained for 5 min, and cleared for 30 sec. Excess solution was removed with a glass rod and samples were dried for 10 min and then measured by optical density scanning (HYDRASYS; Sebia). Normal serum was used as a control for measurement accuracy. The results of the serum protein electrophoresis gel were reviewed and interpreted by a laboratory expert.

Analyses for haptoglobin and serum amyloid A were performed using serum samples. Haptoglobin concentrations were assessed using commercial colorimetric kits (Tridelta Development, Ireland) based on haemoglobin binding assay. Serum amyloid A levels were analyzed using sandwich enzyme-linked immunosorbent assay (ELISA) kits (Tridelta Development). The optical densities were read on a microplate reader (BioTek Instruments, USA) at 630 nm for haptoglobin and at 450 nm and 630 nm as a reference for serum amyloid A.

Statistical analyses

Statistical analyses were performed using SPSS software (version 26.0; IBM Corp., USA). The Shapiro-Wilk test and Levene's test were used for normality analysis and equality of variances for independent *t*-test. Data are expressed as the mean ± SD. Statistical significance was set at $p < 0.05$. Pearson's correlation test was used to determine correlations between the parameters. Pearson's correlation coefficient is represented as $r \geq 0.7$, strong correlation; $0.5 \leq r < 0.7$, moderate correlation; $0.3 \leq r < 0.5$, weak correlation; and $r < 0.3$, no correlation.

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RESULTS

Acid-base, electrolyte, and chemistry analysis

Diarrheic calves infected with rotavirus or *C. parvum* showed lower levels of pH, tCO_2, pCO_2, HCO_3^-, BE, Na^+, SID, and glucose, but higher levels of Cl^-, AG, and BUN than clinically healthy calves. The mean levels of tCO_2, pCO_2, HCO_3^-, and BE in blood were different between calves with rotavirus and those with *C. parvum*, despite similar pH. Calves infected with rotavirus showed lower values of tCO_2, pCO_2, HCO_3^-, and BE than those infected with *C. parvum.* The levels of Na^+, Cl^-, and SID were similar between the two diarrheic groups. The difference in AG was over 1.6 mmol/L between calves with rotavirus and *C. parvum*. Calves with *C. parvum* showed lower glucose levels than those with rotavirus, but BUN vice versa, even though the difference was insignificant (**Table 2**).

Stronger correlations of pH were observed with HCO_3^- ($r = 0.749$, $p < 0.001$) and BE ($r = 0.840$, $p < 0.001$) in calves infected with *C. parvum* than in calves infected with rotavirus or clinically healthy calves. Calves infected with *C. parvum* also showed a stronger correlation ($r = 0.788$, $p < 0.05$) between HCO_3^- and SID in both groups. The correlation of Na^+ was positively strong with Cl^-, but negatively moderate with K^+ in both diarrheic calves. Between the two groups, the correlation of Na^+ was stronger with Cl^-, while weaker with K^+ in calves infected with rotavirus (**Table 3**).

Table 2. Acid-base, electrolyte, and biochemical analyses of the blood from rotavirus or *Cryptosporidium parvum* infected calves

Parameter	Rota (n = 44)	Crypt (n = 17)	*p* value	Healthy (n = 57)
pH	7.09 ± 0.08	7.09 ± 0.12	0.956	7.39 ± 0.04
tCO_2 (mmol/L)	11.07 ± 3.94	13.12 ± 5.85	0.119	31.98 ± 3.29
pCO_2 (mmHg)	32.67 ± 10.36	36.35 ± 11.58	0.233	50.24 ± 3.92
HCO_3^- (mmol/L)	10.16 ± 3.73	11.81 ± 5.50	0.181	30.50 ± 3.21
BE (mmol/L)	−19.70 ± 4.75	−17.82 ± 6.94	0.230	4.79 ± 3.47
Na^+ (mmol/L)	134.98 ± 8.19	133.00 ± 7.37	0.389	140.65 ± 3.77
K^+ (mmol/L)	4.80 ± 1.42[a]	5.71 ± 1.85[a]	0.044	5.45 ± 0.72
Cl^- (mmol/L)	106.95 ± 8.35	105.88 ± 7.88	0.650	98.86 ± 2.31
AG (mmol/L)	22.67 ± 3.71	21.02 ± 3.39	0.116	16.74 ± 0.56
SID (mmol/L)	32.83 ± 4.64	32.83 ± 4.37	0.997	47.24 ± 3.61
Glucose (mg/dL)	96.89 ± 52.61	89.47 ± 26.29	0.601	108.95 ± 16.18
BUN (mg/dL)	47.02 ± 36.18	58.47 ± 34.78	0.267	8.28 ± 2.95

Data are presented as the mean ± SD.
Rota, diarrheic calves infected with rotavirus; Crypt, diarrheic calves infected with *Cryptosporidium parvum*; Healthy, clinically healthy calves without diarrhea; tCO_2, total carbon dioxide; pCO_2, partial pressure of carbon dioxide; HCO_3^-, bicarbonate; BE, base excess; AG, anion gap; SID, strong ion difference; BUN, blood urea nitrogen.
[a]$p < 0.05$.

Table 3. Pearson's correlation analysis of the acidity, and electrolyte in the blood samples from rotavirus or *Cryptosporidium parvum* infected calves

Parameter	Rota (n = 44)	Crypt (n = 17)	Healthy (n = 57)
pH – HCO_3^-	0.598[c]	0.749[c]	0.720[c]
pH – BE^-	0.759[c]	0.840[c]	0.814[c]
HCO_3^- – SID	0.626[c]	0.788[a]	0.245 ($p = 0.066$)
Na^+ – K^+	−0.501[c]	−0.551[a]	0.365[b]
Na^+ – Cl^-	0.847[c]	0.783[c]	0.487[c]

Rota, diarrheic calves infected with rotavirus; Crypt, diarrheic calves infected with *Cryptosporidium parvum*; Healthy, clinically healthy calves without diarrhea; HCO_3^-, bicarbonate; BE, base excess; SID, strong ion difference.
[a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$.

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Table 4. Serum protein parameters of the rotavirus- or *Cryptosporidium parvum*- infected calves

Parameter	Rota (n = 40)	Crypt (n = 15)	*p* value	Healthy (n = 57)
Total protein (g/dL)	6.02 ± 1.03	6.50 ± 0.94	0.119	6.24 ± 0.85
Albumin (g/dL)	2.43 ± 0.55	2.23 ± 0.39	0.207	3.19 ± 0.47
Globulin (g/dL)	3.60 ± 0.88[a]	4.27 ± 1.05[a]	0.018	3.06 ± 0.92
Albumin/Globulin ratio	0.71 ± 0.23	0.57 ± 0.27	0.061	1.15 ± 0.53
α1-globulin (g/dL)	0.93 ± 0.38	0.94 ± 0.39	0.923	0.80 ± 0.04
α2-globulin (g/dL)	0.95 ± 0.57[a]	1.51 ± 0.86[a]	0.030	0.37 ± 0.02
β-globulin (g/dL)	0.99 ± 0.34	0.96 ± 0.28	0.717	0.93 ± 0.03
γ-globulin (g/dL)	0.73 ± 0.58	0.86 ± 0.42	0.403	0.96 ± 0.09
Haptoglobin (mg/dL)	104.2 ± 71.0	130.0 ± 80.2	0.238	11.95 ± 0.59
Serum amyloid A (mg/L)	227.4 ± 89.7	246.5 ± 68.9	0.447	152.6 ± 95.4

Data are presented as the mean ± SD.
Rota, diarrheic calves infected with rotavirus; Crypt, diarrheic calves infected with *Cryptosporidium parvum*; Healthy, clinically healthy calves without diarrhea.
[a]$p < 0.05$.

Serum protein profile analysis

Calves infected with *C. parvum* had higher levels of total protein than those infected with rotavirus due to globulin. Albumin levels were similar, but Globulin levels were significantly different. Both diarrheic groups had low albumin level (< 2.5 g/dL), and high levels of globulins (α1-, α2-, and β-globulin). However, the levels of globulin were 1.18 times and α2-globulin were 1.62 times higher in calves infected with *C. parvum* than those infected with rotavirus ($p < 0.05$). The albumin/globulin ratio was lower in calves infected with *C. parvum* than in those infected with rotavirus. The values of haptoglobin and serum amyloid A in both diarrheic groups were higher, but were not significantly different between the two groups (**Table 4**).

Total protein was strongly correlated with globulin in calves ($r > 0.7$, $p < 0.001$). However, a strong correlation between globulin and α2-globulin was identified only in calves infected with *C. parvum*, correlation between α2-globulin and haptoglobin was strong in calves infected with *C. parvum* ($r = 0.805$, $p < 0.001$), but was moderate in calves infected with rotavirus ($r = 0.542$, $p < 0.001$). Meanwhile, the correlation between globulin and γ-globulin in calves infected with *C. parvum* ($r = 0.656$, $p < 0.01$) was moderate and lower than that in calves infected with rotavirus ($r = 0.760$, $p < 0.001$; **Fig. 1**).

DISCUSSION

This study aimed to identify the differences of acid-base, electrolyte, and biochemistry of the blood and serum proteins in diarrheic Hanwoo calves that were severely infected with rotavirus or *C. parvum*. Despite the similar pH, the levels and correlations of acid-base, electrolyte, and serum proteins were different. Calves infected with *C. parvum* showed high levels of tCO_2, pCO_2, HCO_3^-, BE, and K^+, compared to those infected with rotavirus. Calves infected with *C. parvum* had stronger correlations between pH and HCO_3^-, and pH and BE, and Na^+ and K^+, but a weaker correlation between Na^+ and Cl^- than those infected with rotavirus. In terms of serum proteins, the level of total protein was markedly high in calves infected with *C. parvum*, which was associated with significantly elevated globulin and α2-globulin.

Rotavirus and *C. parvum* evoke diarrhea through malabsorption and secretory components [7,9,19]. Both pathogens cause intestinal villus atrophy, and stunted intestinal villi lead to malabsorption [7,19,20]. As rotavirus or *C. parvum* changes the villus epithelium functions, the crypt cell undergoes hyperplasia [8,9]. The mechanism by which rotavirus causes

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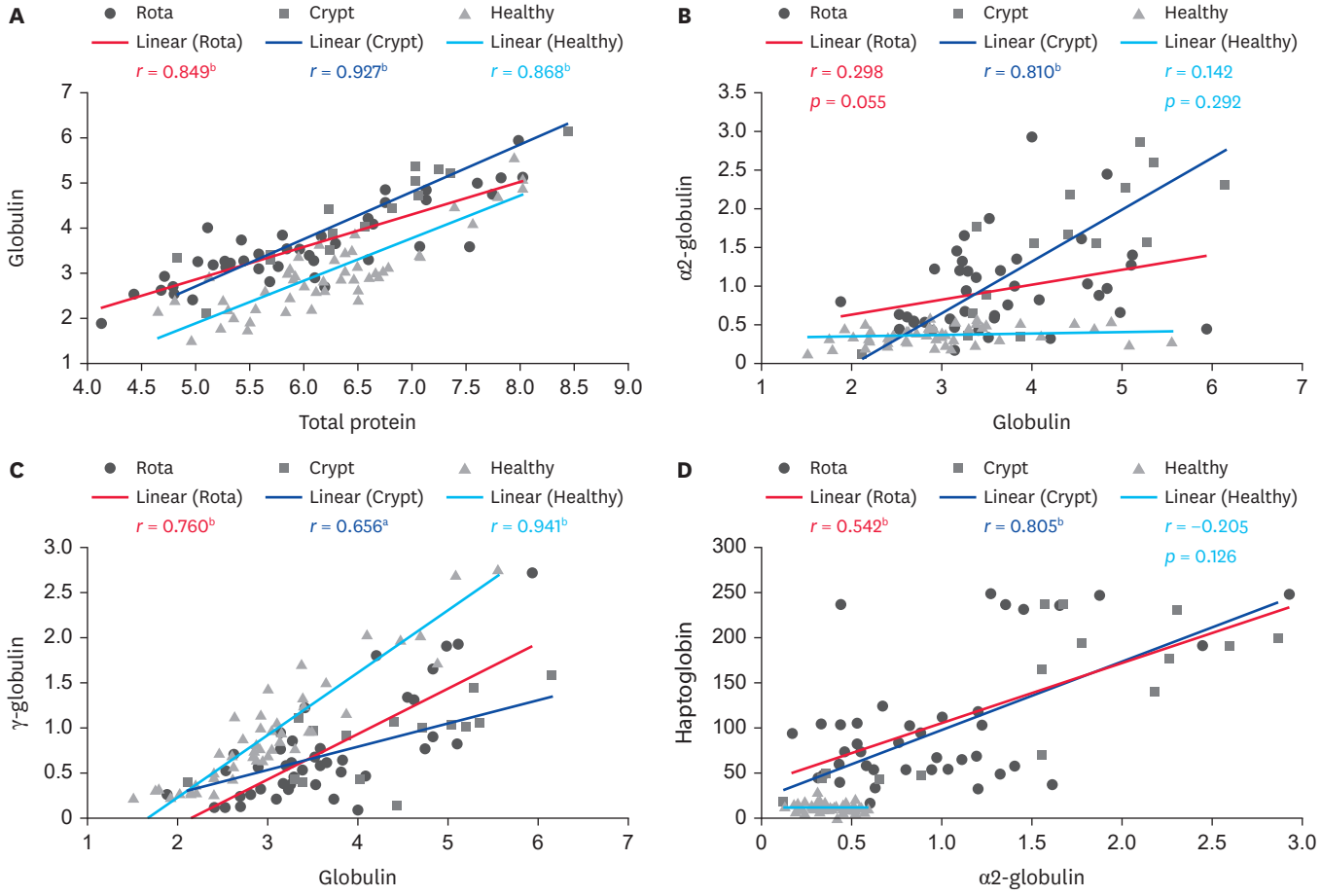

Fig. 1. Correlations among the various types of serum proteins (Rota: 40, Crypt: 15, Healthy: 57). (A) Correlation between total protein and globulin. (B) Correlation between globulin and α2-globulin. (C) Correlation between globulin and γ-globulin. (D) Correlation between α2-globulin and haptoglobin. Rota, diarrheic calves infected with rotavirus; Crypt, diarrheic calves infected with *Cryptosporidium parvum*; Healthy, clinically normal calves without diarrhea. [a]$p < 0.01$; [b]$p < 0.001$.

secretory diarrhea is unclear, but the enteric nervous system and viral enterotoxin play central roles [7,9]. The absorption of Na^+ and glucose decreases and the secretion of Cl^- increases due to the rotaviral secretory mechanism, which exacerbates the dehydration of the host [7,21]. Elevated prostaglandin production, acting on the enteric nervous system and enterocytes, mediates secretory diarrhea by *C. parvum*, in which the secretion of Cl^- or HCO_3^- increases and the absorption of NaCl decreases [7,8,19,22]. In the current study, calves infected with rotavirus showed low levels of tCO_2, pCO_2, HCO_3^-, BE, and correlation coefficients of pH – HCO_3^- and pH – BE, compared to those infected with *C. parvum*, despite increased HCO_3^- secretion. The result is even more interesting, considering that much of the HCO_3^-, remaining in the intestine lumen after the neutralization of stomach HCl, is reabsorbed in the ileum and colon [23]. The reason for this is unclear, but two mechanisms are thought to lead to lower bicarbonate levels in rotavirus infection than in *C. parvum* infection. One might be the difference in their preferential sites. Rotavirus infection is concentrated in the proximal small intestine, whereas *C. parvum* infection is most pronounced in the distal small intestine [8,21,24]. Glucose, amino acids, and lipids are mainly absorbed in the duodenum and jejunum [23,25-27]. Rotavirus infection reduces the activity of digestive enzymes [28-30]. Therefore, rotavirus may cause severe malnutrition in calves compared to *C. parvum*. Malnutrition increases the reaction of non-volatile acids with bicarbonate [23]. Non-

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volatile acids are not measured, while they lower bicarbonate level in the blood [27]. This might cause lower bicarbonate levels in calves infected with rotavirus than in those infected with *C. parvum*. The other mechanism might be the elevated secretion of secretin. Secretin in the duodenum and upper jejunum, stimulates bicarbonate secretion [23]. A delayed gastric emptying time, which is observed in rotavirus infection, might be induced by elevated gastrointestinal hormone secretions, including secretin [31]. Increased secretion of secretin might lead to increased bicarbonate loss.

All diarrheic calves lose potassium in the feces, which results in potassium deficiency throughout the body [32]. In general, hyperkalemia is most often observed in neonatal calves with diarrhea due to metabolic acidosis and dehydration, where increased hydrogen ion exchange with potassium ions and reduced renal elimination of potassium occurs [27,33]. However, excessive loss of potassium due to diarrhea can lead to hypokalemia and metabolic acidosis [1]. In this study, compared to clinically healthy calves, calves with rotavirus showed lower potassium levels despite metabolic acidosis, whereas those with *C. parvum* showed higher potassium levels. The difference might be due to the degree of potassium loss in the feces. Secretion of K^+ at high rates was observed in piglet jejunum infected with rotavirus [34]. In human children, rotavirus-positive diarrhea had higher fecal K^+ losses than non-rotavirus diarrhea [35]. The imbalance between the secretion and absorption of K^+ in the crypt-type epithelium is speculated to lead to high K^+ loss in the feces [34]. Calves might have similar mechanisms to those of children and piglets in terms of fecal K^+ secretion, which contributes to hypokalemia in rotavirus infection.

Total protein levels should be interpreted with the albumin/globulin ratio. Dehydration results in hyperproteinemia with the reference interval of the albumin/globulin ratio [13,36]. In this study, diarrheic calves showed a decreased albumin/globulin ratio (low levels of albumin and high levels of globulin), compared to clinically healthy calves. Hypoalbuminemia is associated with intestinal malabsorption, malnutrition, and inflammation, and hyperglobulinemia with increased alpha globulin is associated with infection and inflammation. Albumin is a negative acute phase protein while alpha globulin is a positive acute phase protein in inflammation [13,37]. Our finding in this study corresponds to previous studies. Diarrheic calves infected with either rotavirus or *C. parvum* led to hypoalbuminemia and hyperglobulinemia with elevated α-globulin levels. In addition, acute-phase proteins such as haptoglobin and serum amyloid A were synthesized following infection with either of the pathogens. Interestingly, calves infected by *C. parvum* had higher α2-globulin levels, including haptoglobin than those infected with rotavirus. The magnitude and type of immune responses depend on the pathogen, and the type of globulins produced also differs according to the type of pathogen [38]. Acute phase responses are more pronounced in bacterial infections than in viral infections [39]. More production of α2-globulin including haptoglobin may be an immunological characteristic of *C. parvum* infection.

The current study has a few limitations. Mild cases of rotavirus and *C. parvum* infection were not included. More cases should have been investigated to compare single infection with concurrent infections of rotavirus and *C. parvum*. In addition, diarrheic calves by the degree of clinical symptoms (dehydration or duration of manifestation) were not subdivided. Further studies are required to elucidate the impact of these two pathogens in details. However, to the best of our knowledge, the current study is the first field study that compares acid-base, electrolyte, and biochemical parameters in calves with severe diarrhea caused by rotavirus or

C. parvum infections. Even though rotavirus and *C. parvum* cause malabsorptive and secretory diarrhea in similar-aged calves, blood parameters were different.

In conclusion, the rotavirus-infected calves significantly low levels of potassium, globulin, and α2-globulin and weak correlations between pH and HCO_3^-, pH and base excess, HCO_3^- and SID, globulin and α2-globulin, α2-globulin and haptoglobin, compared to *C. parvum*-infected calves. Our findings would contribute to understandings of the host responses to rotavirus and *C. parvum* infections in the field, differential diagnosis, and the establishment of well-informed treatment strategies for both pathogens in diarrheic neonatal calves.

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