

Serum Calprotectin Levels in Dogs with Diarrhea

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ABSTRACT

Background: Diarrhea induced by infectious factors may lead to significant health problems in dogs. Canine parvovirus (CPV), canine coronavirus (CCV), canine distemper virus (CDV), *Giardia* spp., *Escherichia coli* (*E. coli*), and *Salmonella* spp. are the important infectious agents that may induce diarrhea in dogs. The present study aimed to investigate the effect of CPV, CCV, CDV, *Giardia* spp., *E. coli*, and *Salmonella* spp. infections on the change in serum calprotectin (Calp) concentration.

Materials, Methods & Results: A total of 30 dogs were enrolled in the study. The study dogs were divided into 3 groups. Healthy animals as confirmed by clinical examination and animals negative for the specified pathogens were placed in Group 1. Animals infected by one or more agents, including CPV, CCV, CDV, and *Giardia* spp., but negative for *E. coli* or *Salmonella* spp. were placed in Group 2. Finally, animals positive for *E. coli* or *Salmonella* spp. and infected or not infected by one or more agents, including CPV, CCV, CDV, and *Giardia* spp., were placed in Group 3. Stool samples and rectal and conjunctival swab samples were collected to investigate the etiologic agents that induced diarrhea. Blood samples were collected through vena cephalica antibrachii for hematological and biochemical examinations. The samples were obtained via routine clinical examinations at the Prof. Dr. Servet Sekin outpatient clinic at Dicle University Veterinary Faculty. CPV, CCV, CDV, and *Giardia* spp. diagnoses were made based on immunochromatographic test kits. The bacteriological analysis of stool samples was used to diagnose *E. coli* and *Salmonella* spp. infection. Serum Calp concentrations were measured by Enzyme-Linked Immunosorbent Assay (ELISA). The analysis of swab and stool samples by immunochromatographic rapid diagnosis kits and microbiological methods showed that 5 animals were infected with CPV, 10 with CCV, 6 with CDV, 3 with *Giardia* spp., 12 with *E. coli*, and 2 with none of the specified agents. Total leukocyte count (WBC), lymphocyte (Lym - %), and granulocyte (Gra - %) values were higher in the diarrheal dogs when compared with the control group. In the biochemical examination of serum samples, total bilirubin (TBIL) and phosphorus (P) levels were higher and sodium (Na) levels were lower in Group 3 when compared to the control group ($P = 0.025$, 0.024 , and 0.018 , respectively). Total protein (TP) and albumin (Alb) values were lower in Group 2 compared to Groups 1 and 3 [$P = 0.001$ and 0.019 for TP, $P = 0.000$ and 0.01 for Alb, respectively]. There was a statistically significant difference in creatine kinase (CK) levels between Group 1 and Group 2 ($P = 0.013$). Serum Calp level was higher in the *E. coli* infected group (Group 3) compared to the other groups, no significant differences were noted between the groups ($P > 0.05$).

Discussion: In conclusion, to the best of authors knowledge, this study is the first to evaluate serum Calp levels in dogs with diarrhea induced by viral, bacterial, and protozoan infections. The Calp level was higher in the sick dogs that were infected by at least one agent, including CPV, CCV, CDV, and *Giardia* spp., and were at the same time *E. coli* positive when compared with the control group and the group without *E. coli* infections. It was concluded that new studies could be useful to reveal the diagnostic importance of serum Calp concentration in dogs with diarrhea and that these results may contribute to future studies in this area.

Keywords: calprotectin, diarrhea, dog, gastroenteritis, enteropathogens, enterotoxins, intestinal diseases.

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INTRODUCTION

Diarrhea, one of the most prominent clinical findings of intestinal diseases, is a symptom characterized by increased water content and volume of the stool with elevated frequency of defecation [27]. Canine parvovirus (CPV), canine coronavirus (CCV), canine distemper virus (CDV) [9,25,26,28] *Giardia* spp., *Escherichia coli* (*E. coli*), and *Salmonella* spp. [5,9,28] are the important pathogens that may induce diarrhea in dogs.

Diarrheal pathogens may stay on the mucosal surface and produce strong enterotoxins that can disrupt fluid flow. Furthermore, the pathogens may penetrate the epithelial cells, leading to inflammatory damage [7].

Calprotectin (Calp), a protein belonging to the S-100 protein family, mainly occurs in neutrophils, monocytes, and macrophages [4,12,20] but is also found in many body tissues and fluids [15]. This protein, which has bacteriostatic and fungistatic properties and protects the intestinal epithelium against infections [11], is actively or passively released into the extracellular fluid following cell lysis [4,8,24].

The serum concentration of Calp can be used as a marker in inflammatory diseases because of increased circulation in certain inflammatory conditions [22,23]. It has been reported that Calp concentration increases in dogs during inflammatory diseases [12,13].

The present study aimed to investigate the serum Calp concentration in diarrheal dogs and to identify the effects of certain enteropathogens (CPV, CCV, CDV, *Giardia* spp., *E. coli*, and *Salmonella* spp.) on the change in serum Calp concentration.

MATERIALS AND METHODS

Animals

The sample of the present study comprised 30 dogs (aged 2-6 months) of different breeds and sexes, including 10 healthy and 20 diarrheal dogs, presented to the Prof. Dr. Servet Sekin outpatient clinic at Dicle University Veterinary Faculty. The study dogs were divided into 3 groups, as follows: Group 1 included healthy animals as confirmed upon clinical examination, which were negative for the abovementioned pathogens (n = 10); Group 2 consisted of animals infected by 1 or more agents, including CPV, CCV, CDV, and *Giardia* spp., which were negative for *E. coli* or *Salmonella* spp. (n = 8); and Group 3 consisted

of animals positive for *E. coli* or *Salmonella* spp. and infected or not infected by 1 or more agents, i.e., CPV, CCV, CDV, and *Giardia* spp. (n = 12).

Sample collection and analysis

Following routine clinical examination of healthy and diarrheal dogs, blood samples were collected through vena cephalica antebrachii for hematological and biochemical examinations in accordance with dedicated techniques. Stool samples and rectal and conjunctival swab samples were collected to investigate the etiologic agents that induced diarrhea.

The samples were obtained via routine clinical examinations at the Prof. Dr. Servet Sekin outpatient clinic at Dicle University Veterinary Faculty.

Using the blood samples, total leukocyte count (WBC), lymphocyte (Lym), monocyte (Mon) and granulocyte (Gra) ratios, and erythrocyte count (RBC), hemoglobin concentration (Hgb), and hematocrit value (Hct) were measured using the hematology device (Mindray BC2800)¹. The alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), blood urea nitrogen (BUN), creatinine (Cre), total protein (TP), albumin (Alb), total bilirubin (TBIL), glucose (GLU), calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na), potassium (K), and chlorine (Cl) concentrations were measured in the serum samples using biochemistry device (Fujifilm NX 500)¹. Serum Calp concentration was measured using the commercial Canine Calprotectin Enzyme Linked Immunosorbent Assay (ELISA) kit² pursuant to the manufacturer's instructions.

CPV, CCV, and *Giardia* spp. and CDV infections were diagnosed in the diarrheal dogs using rectal and conjunctival swab samples with CPV-CCV-*Giardia* Ag Test³ and CDV Ag Test³ rapid diagnosis kits. Salmonellosis and *E. coli* diagnoses were made using bacteriological analysis of the stool samples collected in sterile stool containers from the rectum.

Escherichia coli isolation and identification

Stool samples were inoculated in two 5% sheep blood agar and MacConkey agar, and incubated aerobically and anaerobically (sheep blood agars) at 37°C for 24 h. Lactose-positive and β -hemolytic colonies that were Gram negative were evaluated as suspected *E. coli*. The isolated agents were identified by conventional methods, taking into consideration the

results of biochemical tests, such as triple sugar iron (TSI)/urea, ornithine de-carboxylase (ODC)/tryptone, hydrogen sulfide (H₂S), methyl red, Voges-Proskauer and motility, performed using fully automated identification system (VITEK-2)⁴.

Salmonella spp. isolation and identification

Although the stool samples were directly inoculated onto xylose-lysine-tergitol-4 (XLT4)⁵ agar, 1 g of stool sample was also inoculated into 10 mL of selective pre-enrichment medium, Selenite F broth. The cultured media were incubated at 37°C for 24 h under aerobic conditions. Subsequently, black colored colonies were suspected to be *Salmonella* and were subjected to TSI, urea, and motility tests and lysine de-carboxylase (LDC)/tryptone and *Salmonella* “O” agglutination tests for the purpose of identification. If growth on XLT4 agar was negative for *Salmonella*, a loopful of selective pre-enrichment was collected and incubated on XLT4 agar and evaluated using the abovementioned procedure.

Statistical analysis

Data analysis was performed using the SPSS (Statistical Packages for the Social Sciences)⁶ version 22.0. Analysis of variance was used for group comparisons and the Tukey test was used to investigate the differences between the groups. A *P*-value of < 0.05 was considered statistically significant.

RESULTS

The study dogs were not vaccinated and had not received any prior treatment against the specified infections and presented to the clinic with complaints of vomiting and diarrhea. During the clinical examination, the dogs were found to have a lack of appetite, decreased interest in their surroundings, and a high body temperature (> 39°C). The analysis of swab and stool samples by immunochromatographic rapid diagnosis kits and microbiological methods showed that 5 animals were infected with CPV, 10 with CCV, 6 with CDV, 3 with *Giardia* spp., 12 with *E. coli*, and 2 with none of the specified agents; furthermore, all the dogs were negative for *Salmonella* spp. (Figure 1).

The percentages of viral alone and bacterial alone infections in diarrheal dogs were 15% and 10%, respectively, whereas no animal was infected by protozoans. The percentage of dogs infected with more than 1 virus but not simultaneously with bacteria and

protozoa was 5%. The percentages of viral + bacterial infection, viral + protozoan infection, and viral + bacterial + protozoan infections were 35%, 10%, and 15%, respectively (Figure 2).

In the hematological examination, WBC, Lym (%), and Gra (%) values were higher in the diarrheal dogs when compared with the control group (Table 1).

In the biochemical examination of serum samples, TBIL and P levels were higher and Na levels were lower in Group 3 when compared to the control group (*P* = 0.025, 0.024, and 0.018, respectively). TP and Alb values were lower in Group 2 compared to Groups 1 and 3 (*P* = 0.001 and 0.019 for TP, *P* = 0.000 and 0.001 for Alb, respectively). There was a statistically significant difference in CK levels between Group 1 and Group 2 (*P* = 0.013). Although the serum Calp level was higher in the *E. coli* infected group (Group 3) compared to the other groups, no significant differences were noted between the groups (*P* > 0.05) [Table 2].

DISCUSSION

Puppies aged <1 year are highly susceptible to gastrointestinal infections [5]; hence, viral, bacterial, and parasitic agents can induce disease alone or concomitantly. In cases of infection with more than one agent, pathogens involved in coinfection may enhance the virulence of each other, leading to more severe diarrhea compared to infections caused by a single pathogen [7]. In the present study, coinfection was detected in 13 (65%) dogs, whereas infections caused by CPV alone was detected in 1 (5%) dog, CCV alone in 2 (10%) dogs, and *E. coli* alone in 2 (10%) dogs. Dogs with coinfection had more severe diarrhea than the ones with a single infection.

Vomiting and diarrhea in animals with gastroenteritis may lead to changes in the hematological parameters [21]. In the present study, the mean WBC values and lymphocyte and granulocyte ratios in Groups 2 and 3 were higher than in Group 1 [Group 2: *P* = 0.000, 0.000, and 0.034; Group 3: *P* = 0.000, 0.001, and 0.013, respectively]. The higher mean WBC values and lymphocyte and granulocyte ratios in the sick dogs compared to the healthy dogs suggested that the elevated levels were associated with inflammation in the gastrointestinal tract, as reported in other studies [3,17].

Although the serum CK level is higher in young dogs compared to the adults [32], it has been reported that there might be an increase in serum CK

Table 1. Hematological parameters of the 3 groups of dogs.

Parameter	Group 1 (n=10)	Group 2 (n=8)	Group 3 (n=12)
WBC (x10 ³ /μL)	12.55 ± 0.84 ^a	26.56 ± 0.72 ^b	24.63 ± 1.89 ^b
Lym (%)	1.81 ± 0.17 ^a	8.53 ± 0.10 ^b	6.23 ± 0.95 ^b
Mon (%)	0.42 ± 0.06	0.49 ± 0.06	0.48 ± 0.04
Gra (%)	10.32 ± 0.84 ^a	17.55 ± 1.37 ^b	17.91 ± 2.3 ^b
RBC (x10 ⁶ /μL)	5.80 ± 0.40	5.89 ± 0.39	5.26 ± 0.28
HGB (g/dL)	11.44 ± 0.57	13.24 ± 0.95	11.55 ± 0.65
HCT (%)	37.13 ± 1.67	39.94 ± 2.79	35.08 ± 1.99

n: Number; WBC: White Blood Cells; RBC: Red Blood Cells; Hgb: Haemoglobin; Htc: Haematocrit. ^{a,b}Means with different superscripts in the same line differ significantly. In the WBC: lymphocyte (Lym), monocyte (Mon) and granulocyte (Gra) % values were higher in the diarrheal dogs when compared with the control group (1).

Table 2. Serum biochemical parameters of the 3 groups of dogs.

Parameter	Group 1 (n=10)	Group 2 (n=8)	Group 3 (n=12)
ALP (U/L)	111.50 ± 17.51	228.37 ± 55.98	199.92 ± 42.26
AST (U/L)	36.40 ± 2.48	45.25 ± 9.96	39.83 ± 4.03
ALT (U/L)	41.90 ± 5.05	25.62 ± 1.27	36.42 ± 5.29
LDH (U/L)	500.00 ± 48.67	291.12 ± 62.98	420.75 ± 84.00
CK (U/L)	242.90 ± 31.09 ^a	454.87 ± 66.14 ^b	302.25 ± 42.67 ^{ab}
BUN(mg/dL)	14.88 ± 0.91	14.52 ± 2.78	10.69 ± 1.70
Cre (mg/dL)	0.70 ± 0.06	0.45 ± 0.11	0.51 ± 0.12
TP (g/dL)	6.22 ± 0.26 ^a	4.62 ± 0.22 ^b	5.65 ± 0.23 ^a
Alb (g/dL)	2.88 ± 0.14 ^a	1.26 ± 0.36 ^b	2.73 ± 0.22 ^a
TBIL (mg/dL)	0.26 ± 0.04 ^a	0.28 ± 0.05 ^{ab}	0.54 ± 0.09 ^b
Glu (mg/dL)	85.60 ± 3.55	88.50 ± 9.64	79.08 ± 5.94
Ca (mg/dL)	10.39 ± 0.25	9.56 ± 0.31	10.21 ± 0.31
Mg (mg/dL)	1.72 ± 0.09	1.71 ± 0.72	1.77 ± 0.07
P (mg/dL)	5.30 ± 0.52 ^a	6.75 ± 0.58 ^{ab}	7.20 ± 0.43 ^b
Na (mEq/L)	144.10 ± 1.05 ^a	138.62 ± 2.30 ^{ab}	137.92 ± 1.40 ^b
K (mEq/L)	4.63 ± 0.13	4.91 ± 0.27	4.64 ± 0.15
Cl (mEq/L)	110.10 ± 1.64	106.50 ± 2.26	105.17 ± 1.78
Calp (ng/mL)*	284.74 ± 36.39	283.46 ± 28.73	352.30 ± 42.84

ALP: Alkaline Phosphatase; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; LDH: Lactate Dehydrogenase; CK: Creatine Kinase; BUN: Blood Urea Nitrogen; Cre: Creatinine; TP: Total Protein; Alb: Albumin; Glu: Glucose; Ca: Calcium; P: Phosphorus; Na: Sodium; K: Potassium; Cl: Chlorine; Calp: Calprotectin. ^{a,b}Means with different superscripts in the same line differ significantly. *Serum Calp level was higher in the *E. coli* infected group (Group 3).

levels due to muscular damage, infection, and anorexia [1,32]. In the present study, the mean serum CK level was significantly higher ($P = 0.013$) in Group 2 compared to the control group, which may be due to anorexia, as reported by researchers [1,32].

It has been suggested that hypoproteinemia might develop in dogs with enteritis owing to the leakage of serum protein from the capillaries of damaged

intestinal villi and decreased absorption from damaged villi [18,30]. It has been reported that CPV [19] and *Giardia* [29] infections cause intestinal protein loss and that CCV and *Giardia* spp. increase the severity of the disease in dogs infected by CPV [19]. In the present our study, mean serum TP and Alb levels were significantly lower in Group 2 than in Groups 1 and 3 [TP, $P = 0.001$ and 0.019; Alb, $P = 0.000$ and

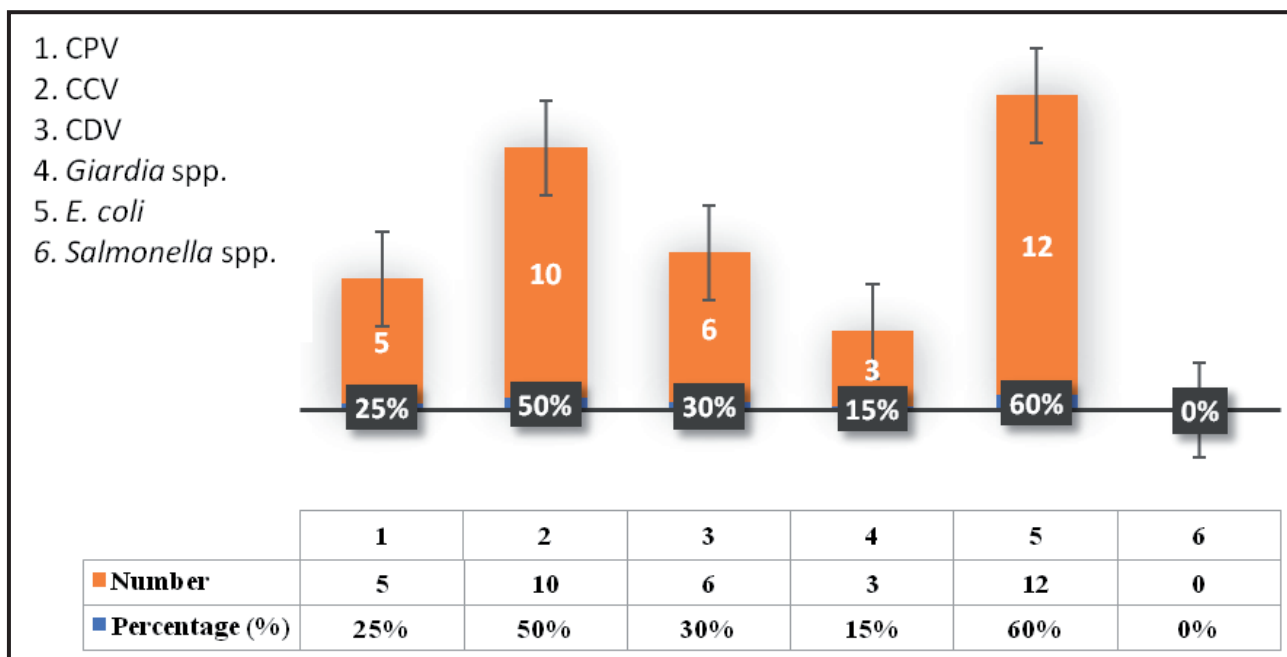


Figure 1. The number of dogs infected with CPV, CCV, CDV, *Giardia* spp., *Escherichia coli* and *Salmonella* spp.

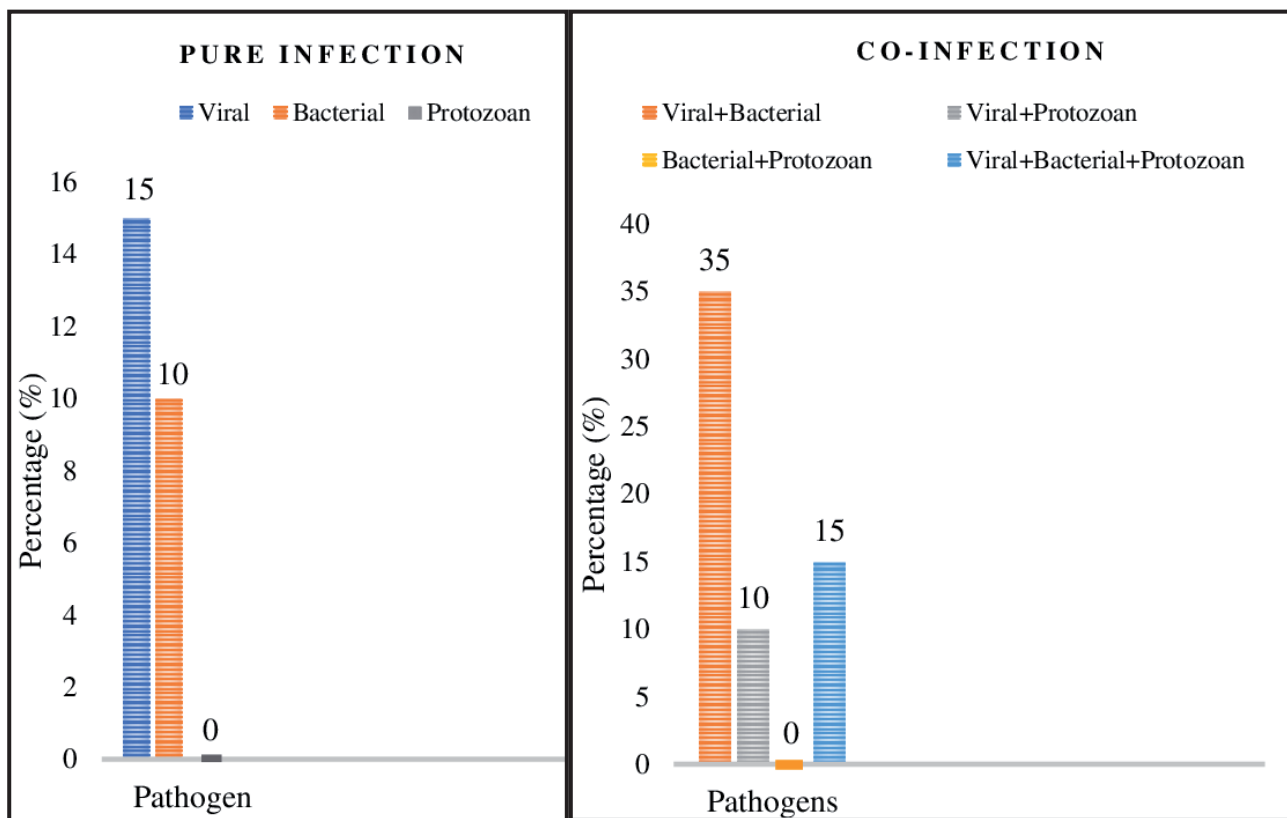


Figure 2. Virus, bacteria and protozoan association in dogs with diarrhea.

0.001; respectively]. The number of animals infected by *Giardia* spp., CPV, and CCV were 2/8, 3/8 and 5/8 in Group 2 and 1/12, 2/12, and 5/12 in Group 3,

respectively. The low mean serum TP and Alb levels in Group 2 may be associated with the higher rate of *Giardia*, CPV, and CCV infections in this group.

The mean serum TBIL level was significantly higher ($P = 0.025$) in Group 3 compared to the control group. It has previously been reported that the increase in TBIL level might be associated with cellular damage in the spleen and liver [18].

Although the mean serum P level was significantly higher ($P = 0.024$) in Group 3 compared to the control group, the mean serum ALP level and P level were also higher in the sick dogs (Groups 2 and 3) compared to the control group. Therefore, this result is considered normal for young dogs, as reported by the researcher [32].

The mean serum Na level in Group 3 was significantly lower ($P = 0.018$) when compared to Group 1. Relevant studies have documented that vomiting and diarrhea may cause hyponatremia in dogs with gastroenteritis [10,16,18].

It has been stated that Calp could be used as a potential biomarker of infectious diseases due to its extracellular secretion and role in innate immune response [2]. In addition, previous studies have shown that serum Calp concentration in dogs is a significant biomarker to determine inflammation and increase in idiopathic inflammatory bowel disease (IBD), systemic inflammatory response syndrome (SIRS), sepsis, and pancreatitis [12,13]. Equilino *et al.* [6] recorded increased serum Calp concentrations in dogs with protein-losing enteropathy and in dogs with food responsive diarrhea. Bartakova *et al.* [2] reported that the serum Calp levels were higher in patients with bacterial sepsis compared to those with viral infections and that serum Calp level was a reliable biomarker of bacterial sepsis.

In the present study, the mean serum Calp levels of Groups 1 and 2 were highly similar [284.74 ± 36.39 ng/mL; 283.46 ± 28.73 ng/mL, respectively], whereas the mean serum Calp levels in Group 3 were higher when compared to the other groups [352.30 ± 42.84 ng/mL].

Thames *et al.* [31] showed that serum Calp level was higher in dogs with sepsis when compared to those with SIRS, but no significant differences were noted between the groups. Heilmann *et al.* [12] suggested that the mean serum Calp concentration was higher in dogs with idiopathic IBD at baseline (431.1 µg/L) as well as 3 weeks after treatment (676.9 µg/L) when compared to the dogs in the control group (219.4

µg/L). Upon literature review, there was no study that had investigated different etiological agents and serum Calp levels in diarrheal dogs.

Although there was no statistically significant difference in the mean serum Calp levels among Groups 1, 2, and 3 in the present study, the Calp level was higher in the *E. coli* infected group compared to the other groups. In addition, given that the Gra ratio was also higher in this group compared to the other groups, it could be suggested that the high Calp level might be induced by granulocyte and that bacterial lipopolysaccharides might lead to the release of Calp from neutrophils, consistent with other studies [14].

CONCLUSION

In conclusion, to the best of our knowledge, this study is the first to evaluate serum Calp levels in dogs with diarrhea induced by viral, bacterial, and protozoan infections. The Calp level was higher in the sick dogs that were infected by at least 1 agent, including CPV, CCV, CDV, and *Giardia* spp., and were at the same time *E. coli* positive when compared with the control group and the group without *E. coli* infections. It was concluded that new studies could be useful to reveal the diagnostic importance of serum Calp concentration in dogs with diarrhea and that these results may contribute to future studies in this area.

MANUFACTURERS

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Ethical approval. The samples were obtained via routine clinical examinations at the Prof. Dr. Servet Sekin outpatient clinic at Dicle University Veterinary Faculty. Ethical approval from a committee was not necessarily required.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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