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Prognostic Roles of Trace Element and Cobalamin Concentrations in Dogs With Parvoviral Enteritis

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Keywords: cobalamin | dog | hospital | trace elements

ABSTRACT

Background: The trace elements copper (Cu), zinc (Zn), and selenium (Se) have been the focus of research into their potential roles in the prognosis of gastrointestinal disorders in humans.

Objective: Evaluation of the predictive potential serum concentrations of Cu, Zn, Cu/Zn, Se, and cobalamin as possible prognostic indicators in dogs with parvoviral enteritis (CPV).

Animals: Client-owned dogs diagnosed with CPV ($n = 20$) and healthy controls ($n = 10$).

Methods: A case-controlled study. Serum concentrations of Cu and Zn were measured using a spectrophotometric method; serum Se levels were determined by mass spectrophotometry; and serum cobalamin concentrations were assessed using a chemiluminescent immunoassay method. The Mann–Whitney *U* test was employed to compare subgroup medians.

Results: Upon admission, surviving dogs with CPV ($n = 10$) exhibited higher serum Cu concentrations (median = 154.24; range = 60.15–188.46 μg/dL) and Cu/Zn ratios (median = 1.52; range = 0.67–2.45), alongside lower serum Zn concentrations (median = 88.05; range = 51.3–129.2 μg/dL) and cobalamin levels (median = 252.5; range = 111–396 pg/mL), compared to the control group (Cu, median = 72.12; range = 47.04–90.26 μg/dL), Zn (median = 184.2; range = 73.0–262.7 μg/dL), Cu/Zn (median = 0.37; range = 0.26–0.73), cobalamin (median = 638.5; range = 306.0–1016 pg/mL). Additionally, non-surviving dogs ($n = 10$) exhibited markedly higher serum Cu concentrations (median = 193.5; range = 125.0–229.0 μg/dL) and Cu/Zn ratios (median = 5.45; range = 1.95–9.23), and significantly lower serum Zn (median = 37.75; range = 24.8–71.6 μg/dL), Se (median = 52.45; range = 21.27–91 μg/L), and cobalamin levels (median = 52.2; range = 20.0–147.0 pg/mL) compared to both survivors and controls.

Conclusions and Clinical Importance: Statistical variations in serum concentrations of Cu, Zn, and cobalamin, alongside Cu/Zn ratios, were observed among survivors, non-survivors, and controls (control-survivor and survivor-non-survivor: $p < 0.05$ and control-non-survivor: $p < 0.01$), which might suggest their potential prognostic value in CPV.

1 | Introduction

Canine parvovirus (CPV) is a relevant contributor to morbidity and death worldwide, particularly affecting dogs between 6 weeks and 6 months of age [1]. CPV infection is characterized by its ability to damage the gastrointestinal tract, resulting in increased permeability and subsequent malabsorption [2, 3].

Abbreviations: AUC, area under curve; CPV, canine parvovirus; Cu, copper; PPV, positive predictive value; ROC, receiver operating characteristic; Se, selenium; Zn, zinc.

Copper (Cu), zinc (Zn), and selenium (Se) are primarily absorbed in dogs through the small intestine, a process extensively explored in recent research [4–6]. Within this intricate system, intestinal enterocytes emerge as key players, particularly in Cu absorption. These cells not only detect circulating Cu but also aid its uptake by converting it into a more absorbable form through brush-border reductase enzymes. Once transformed, Cu is efficiently transported across the enterocyte's apical membrane by specialized Cu transporter proteins [7]. Similarly, Zn absorption predominantly occurs in the small intestine of dogs, with the duodenum taking the lead, followed by the distal ileum and proximal jejunum [8]. This absorption process relies heavily on apical transport mechanisms, facilitated by Zn transporters positioned on the membrane [9]. In addition to absorption processes, recent studies have shown that Cu and Zn are also closely associated with the immune system [10, 11], and levels might vary depending on the immune response [12, 13]. In contrast, our understanding of Se metabolism in dogs remains limited. However, absorption pathways in the small intestine exhibit variability depending on the Se form. Organic Se utilizes an active transport mechanism, whereas the inorganic form is thought to be absorbed by simple diffusion, even if at a slower rate [14]. However, these absorption mechanisms could potentially be compromised in the presence of intestinal damage. Furthermore, while human research has extensively characterized trace element abnormalities caused by enteritis [3, 15, 16], trace elements play a prognostic role in inflammation in dogs [17]. Moreover, a previous study emphasized the role of Cu and Zn concentrations as relevant inflammatory markers in dogs with CPV [18]. However, the prognostic value of these markers in predicting the outcomes of CPV cases remains unclear. Additionally, the review of the literature revealed a lack of studies examining Se concentrations in dogs with CPV, pointing to an important gap in the current research.

Cobalamin serves as a critical cofactor for numerous enzyme systems in mammals and is essential for nucleic acid synthesis [19]. In dogs, dietary cobalamin binds to intrinsic factor before absorption, forming a complex that is subsequently recognized and absorbed by specific receptors in the ileum [20]. However, similar to trace elements, impaired intestinal absorption also affects cobalamin concentrations, and diseases related to cobalamin metabolism are increasingly recognized in small animal medicine, as highlighted by recent studies [21, 22]. In this context, cobalamin has become an important marker for dogs with CPV [22, 23], and its prognostic role has recently been highlighted, particularly in the clinical setting [24].

In this study, we hypothesized that the levels of Zn, Cu, Se, cobalamin, and the Cu/Zn ratio at hospital admission are important prognostic indicators of disease outcomes in dogs with CPV. Furthermore, this study aimed to determine the prognostic significance of these biomarkers at the time of hospital admission.

2 | Materials and Methods

2.1 | Animals

A case-controlled, prospective, observational study was conducted at the Animal Hospital of the Faculty of Veterinary Medicine between February and May 2024, with a particular focus on diarrhea cases in dogs. The study was approved by the University Local Animal Experimentation Ethics Committee (Decision Number: 2024/129). The dogs were included in the study in two stages, in accordance with the established inclusion criteria. The initial stage entailed the identification of unvaccinated dogs that were positive for CPV but negative for other potential diseases, including canine coronavirus, canine distemper virus, and parasitic infections. The study excluded cases in which CPV was unconfirmed, cases with CPV co-infection, or both. The second stage of the process involved the confirmation of CPV antigen positivity through molecular detection. Dogs with negative PCR results were excluded from the study. Furthermore, the dogs included in the study were required to demonstrate positive results at both stages.

In this context, the first stage included dogs of any breed or sex presenting with hemorrhagic diarrhea, lethargy, vomiting, and anorexia, as well as other clinical signs indicative of CPV infection, and who had not received previous CPV vaccinations or undergone medical intervention. The first stage involved a comprehensive evaluation, including a thorough review of the dog's medical records (vaccination status, any diseases diagnosed in the past month, and any treatments received for other conditions), a physical examination, and fecal sample analysis that was carried out using passive flotation methods without centrifugation using a saturated sodium chloride solution. Dogs suspected of CPV infection were screened using a reliable and rapid CPV antigen test (iVET, Dubai). Following the initial CPV screening, further examinations were conducted to detect other potential infections, including canine coronavirus and canine distemper virus. The presence of canine coronavirus and canine distemper virus was detected through a rapid diagnostic ELISA kit (iVET, Dubai, LiliF, South Korea). Dogs diagnosed with any additional diseases alongside CPV were excluded from the study.

For the second stage, sterile swabs were used to collect fecal samples, which were then immediately stored in phosphate-buffered saline with a pH of 7.3 ± 0.1. PCR was used to confirm the presence of CPV infection, specifically targeting the VP2 gene of CPV. Viral DNA isolation was achieved by centrifuging fecal samples at 1500 g for 5 min at 4°C, followed by the extraction of 200 μL of the supernatant using the High Pure Viral Nucleic Acid Kit (Roche, Germany) as per the manufacturer's protocol. CPV infection diagnosis was confirmed through direct detection of the viral genome in fecal samples using PCR primers (Parvovirus H-forward [5′-CAGGTGATGAATTTGCTACA-3′] and H-reverse [5′-CATTTGGATAAACTGGTGGT-3′]), targeting the VP2 gene fragment, consistent with established procedures [25]. Following PCR amplification, amplicons measuring 629 bp were deemed positive. Dogs were excluded from the study if they were positive for CPV antigen but negative using PCR.

After inclusion of the CPV-infected group, control dogs were included to verify that there was an equal age distribution between the control and CPV-infected groups. The control dogs received a thorough assessment, which included a review of their medical history, a physical examination, an analysis of fecal samples, a complete blood count (CBC), and confirmation of their health condition by negative findings on a CPV test. Moreover, no abnormalities were allowed in the medical history, physical examination, or CBC and stool samples were then analyzed using the flotation

technique. To be included in the study, all CBC values had to fall within the reference range, and all other tests had to be negative.

2.2 | Laboratory Analysis

Before treatment initiation, blood samples were obtained via jugular venipuncture with 21-gauge needles. The obtained blood was partitioned into anticoagulant (EDTA) and serum tubes. Whole blood counts were evaluated with an automated hematology analyzer (Abacus Junior Vet5, Hungary). After collecting the blood, the serum samples were left to coagulate at room temperature and then spun in a centrifuge at a force of 1008 g for 10 min. Subsequently, the serum samples were stored at a temperature of −80°C until further analysis.

The serum concentrations of Cu and Zn were determined with a spectrophotometric technique (ADVIA 1800). The detectable range for Cu concentration was 4 to 600 μg/dL, whereas for Zn concentration, it was 3 to 750 μg/dL. The ratio of Cu to Zn was determined by dividing the concentration of Cu in the serum by the concentration of Zn in the serum. Serum cobalamin levels were determined via chemiluminescent immunoassay (ADVIA Centaur CP), with a measurable range of 45–2000 pg/mL. Additionally, Se levels were assessed using a mass spectrophotometric method (iCAP RQ), with a measurable range of 5–270 μg/L.

2.3 | Statistical Analysis

The G*Power program, version 3.1.9.4, developed by Franz Faul at the University of Kiel, Germany, was used to determine the minimum sample size for each group based on Zn concentration data. The calculation was performed using information from a previous study [26] conducted on dogs with CPV, with the following parameters: effect size = 1.8454540, significance level = 0.05, and power = 0.95. The analysis determined that at least eight dogs should be included in each group. Data from the study were analyzed with the Kruskal–Wallis test, a non-parametric method suitable for use with small sample sizes [27]. Additionally, the Mann–Whitney *U* test was employed to compare subgroup medians when the Kruskal–Wallis test indicated significant differences among the primary groups (control, CPV survivors, and CPV non-survivors). To determine a prognostic cut-off for distinguishing CPV survivors from non-survivors, a receiver operating characteristic (ROC) curve analysis was conducted. This analysis included calculating the specificity, sensitivity, and area under the curve (AUC) for each variable. The AUC values were classified according to their predictive ability, which was subsequently categorized as fail, poor, fair, good, or excellent. In particular, the AUC values were classified into the following categories: fail (0.50–0.60), poor (0.60–0.70), fair (0.70–0.80), good (0.80–0.90), and excellent (0.90–1.00) [28]. Statistical analyses were performed using SPSS 27.0 software, with significance set at $p < 0.05$ to detect meaningful differences between groups.

3 | Results

The number of dogs subjected to screening for potential inclusion in the study, along with the rationale for their exclusion, is illustrated in Figure 1. The dogs included in the study received complete veterinary care in accordance with the hospital's approved treatment procedures. Medical care included intravenous administration of fluids, immunostimulants, antibiotics, and antiemetics. Ten of the treated dogs responded positively and comprised the CPV survivor group. Conversely, 10 dogs failed to react to therapy and were categorized as CPV non-survivors. Unfortunately, all of the 10 non-survivors died due to disease progression, characterized by worsening clinical signs including severe dehydration, persistent vomiting, and lethargy, as well as the development of secondary complications

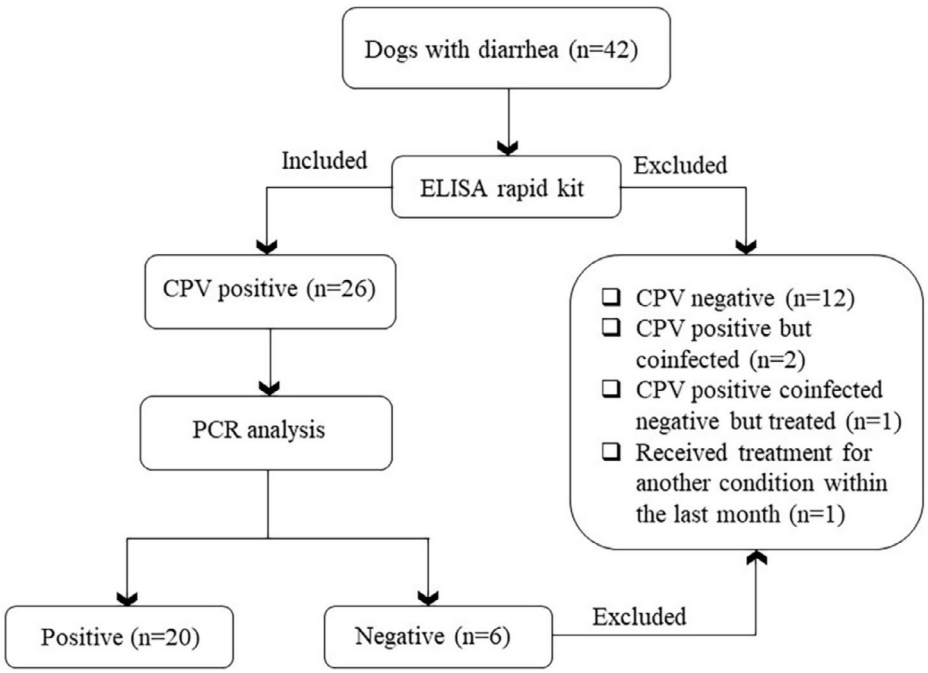

FIGURE 1 | Number of dogs subjected to screening and reasons for inclusion and exclusion. CPV: canine parvovirus.

like systemic inflammatory response syndrome. None of the non-survivors were euthanized due to clinical deterioration or financial constraints.

The results of the study indicated that there was no significant difference in age between the CPV-infected group ($n = 20$) and the control group ($n = 10$). The median age of the CPV-infected group was 4 months (range: 2.0–6.0 months), while that of the control group was 4.5 months (range: 2.0–6.0 months). The CPV group comprised 10 males and 10 females, while the control group included 5 males and 5 females.

Figure 2 outlines the concentrations of Cu, Zn, cobalamin, Se, and the Cu/Zn ratio in the control group, CPV survivors, and CPV non-survivors. Notably, Cu concentrations were significantly increased in both CPV survivors (median, 154.24 μg/

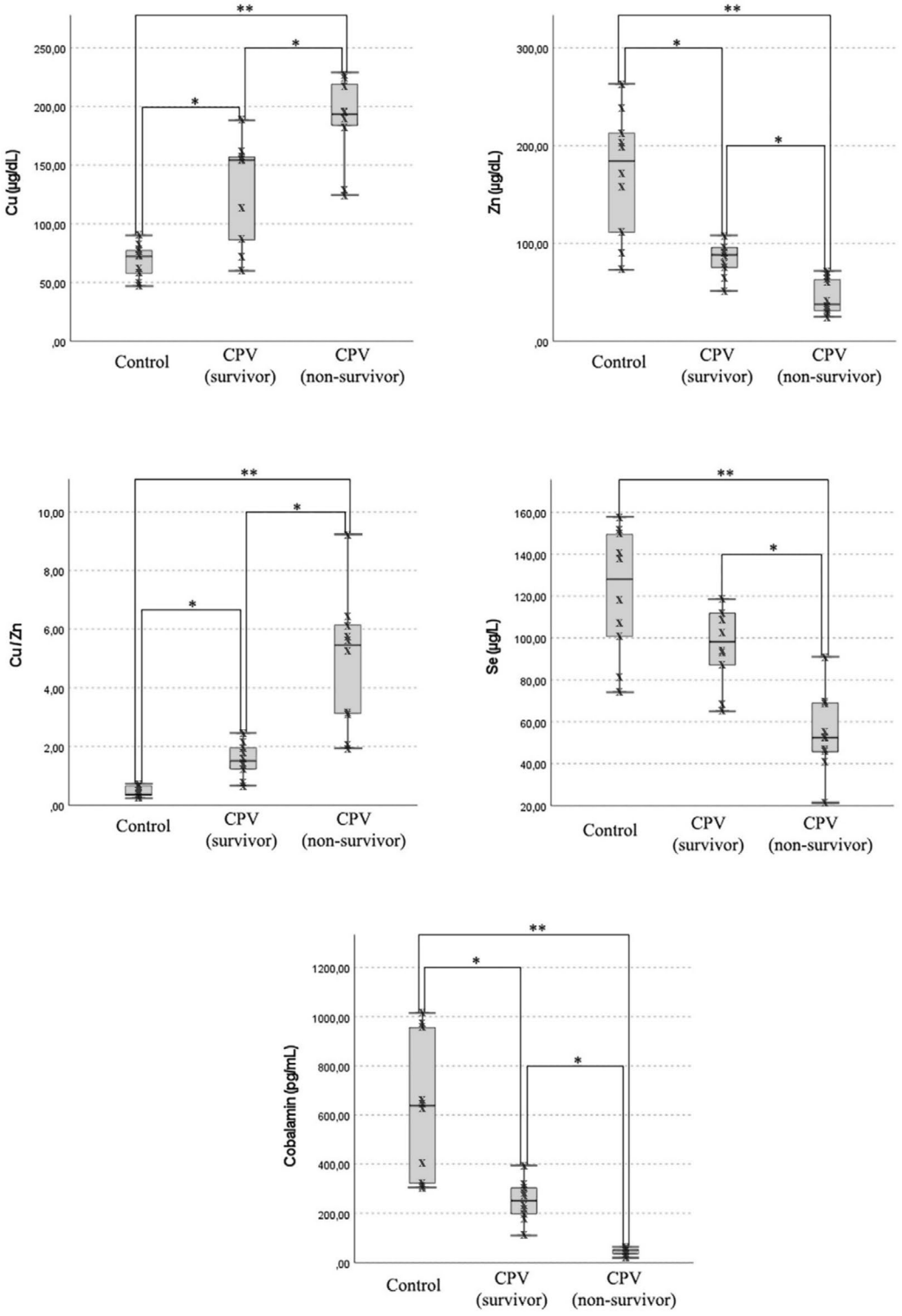

FIGURE 2 | Scattered box plots of copper, zinc, copper/zinc ratio, selenium, and cobalamin data of dogs in control ($n = 10$), canine parvovirus-infected survivor ($n = 10$) and non-survivor ($n = 10$) groups that are being statistically compared (*$p < 0.05$, **$p < 0.01$).

dL; range, 60.15–188.46 μg/dL) and non-survivors (median, 193.5 μg/dL; range, 125.0–229 μg/dL) in comparison to the control cohort (median, 72.12 μg/dL; range, 47.04–90.26 μg/dL). Additionally, non-surviving CPV-infected dogs exhibited significantly higher Cu levels than surviving dogs. A cut-off threshold of > 154.6 μg/dL at the time of enrollment yielded an AUC of 0.93 (95% confidence intervals [CI], 0.84–1), a specificity of 80% (95% CI, 22.3%–98.2%), and a sensitivity of 80% (95% CI, 45.5%–95%) for survival in cases of CPV (Table 1).

Conversely, Zn concentrations were significantly lower in non-surviving CPV-infected dogs (median, 37.75 μg/dL; range, 24.8–71.6 μg/dL) than in both the control group (median, 184.2 μg/dL; range, 73–262.7 μg/dL) and the surviving group (median, 88.05 μg/dL; range, 51.3–129.2 μg/dL). The Zn levels were also significantly lower in survivors compared to the control group. At admission, a Zn cut-off value of < 69.05 μg/dL provided an AUC of 0.97 (95% CI, 0.92–1), specificity of 90% (95% CI, 27.9%–99.5%), and sensitivity of 90% (95% CI, 55.6%–98.5%) for survival in CPV cases.

The Cu/Zn ratio was significantly elevated in both the CPV-survivor group (median, 1.52; range, 0.67–2.45; 95% CI, 1.14–1.95) and the CPV-non-survivor group (median, 5.45; range, 1.95–9.23; 95% CI, 3.24–6.51) compared to the control group (median, 0.37; range, 0.26–0.73; 95% CI, 0.32–0.56). Furthermore, dogs that did not survive CPV infection had significantly elevated Cu/Zn ratios compared to survivors. Moreover, a Cu/Zn ratio exceeding 1.87 at enrollment demonstrated an AUC of 0.98 (95% CI, 0.94–1), sensitivity of 100% (95% CI, 70.1%–100%), and specificity of 95% (95% CI, 18.7%–99.9%).

The cobalamin concentrations in both CPV survivors (median, 252.5 pg/mL; range, 111–396.0 pg/mL) and non-survivors (median, 52.2 pg/mL; range, 20–147 pg/mL) were significantly lower than in the control group (median, 638.5 pg/mL; range, 306–1016 pg/mL). In addition, concentrations of cobalamin were markedly decreased in non-surviving dogs compared to surviving dogs. A cobalamin threshold of < 129 pg/mL at presentation was found to predict CPV survival with a sensitivity of 90% (95% CI, 57.7%–98.3%), a specificity of 95% (95% CI, 18.3%–99.9%), and an AUC of 0.99 (95% CI, 0.98–1).

The Se concentrations were significantly decreased in non-surviving CPV-infected dogs (median, 52.45 μg/L; range, 21.27–91 μg/L) compared to both the control group (median, 128.1 μg/L; range, 74.25–157.6 μg/L) and the survivor group (median, 98.19 μg/L; range, 65.24–118.7 μg/L). The Se cut-off value of < 71.84 μg/L at admission resulted in an AUC of 0.95 (95% CI, 0.89–1) for survival, with a sensitivity of 90% (95% CI, 55.6%–98.5%) and specificity of 90% (95% CI, 27.9%–99.5%).

4 | Discussion

In our investigation, we examined the complex dynamics between Cu, Zn, Cu/Zn, cobalamin, and Se in dogs affected by CPV, with an emphasis on unraveling the pathogenesis of the disease and determining the optimal cut-off values tailored exclusively for CPV survivors.

The results of our study indicated a notable elevation in Cu concentration, which might be indicative of a poor prognosis in dogs infected with CPV. This finding is in line with the results of a few studies investigating Cu levels in dogs with CPV and cats with feline panleukopenia virus infection [18, 19, 29]. Nevertheless, it is important to note that contradictory findings have also been reported [26]. Elevated Cu levels observed in dogs with CPV might be attributed to the heightened inflammatory response associated with the increased acute-phase protein levels [18]. This is a highly plausible rationale. However, as acute-phase proteins were not evaluated in this article, it is not possible to definitively attribute this to an inflammatory response. In contrast, the decrease in Cu levels in dogs with CPV could be attributed to the oxidative process [26]. However, there are studies reporting elevated and reduced Cu levels in dogs with CPV [18, 26]. In light of the disease's pathogenesis, the intestinal tract is the primary site of infection with CPV, where necrosis of the intestinal crypt epithelium results in the shortening or disappearance of the villi and the enlargement of the intestinal crypts with necrotic cellular debris [30]. These pathological changes result in increased intestinal permeability [3, 31]. Research indicates that a compromised intestinal barrier might facilitate the leakage of Cu into the bloodstream [32]. In light of these findings, it is plausible that CPV-induced impairment of intestinal barrier function in this study might have facilitated the translocation of Cu into the bloodstream, potentially resulting in elevated systemic Cu concentrations.

The results of the study demonstrated markedly lower serum Zn concentrations in dogs with CPV, with the lowest concentrations observed in non-surviving dogs. The lower Zn concentrations in dogs with CPV are consistent with previous reports [18, 26, 33]. The reduction in Zn concentration observed in

TABLE 1 | Prognostic potential of trace element and cobalamin concentrations in the CPV survivors ($n = 10$), CPV non-survivors ($n = 10$), and control group ($n = 10$) at enrollment.

Variables	AUC	SE	Sensitivity (%)	Specificity (%)	Cut-off value
Cu (μg/dL)	0.93 (95% CI, 0.84–1)	0.046	80 (95% CI, 45.5–95)	80 (95% CI, 22.3–98.2)	154.6
Zn (μg/dL)	0.97 (95% CI, 0.92–1)	0.027	90 (95% CI, 55.6–98.5)	90 (95% CI, 27.9–99.5)	69.05
Cu/Zn	0.98 (95% CI, 0.94–1)	0.020	100 (95% CI, 70.1–100)	95 (95% CI, 18.7–99.9)	1.87
Cobalamin (pg/mL)	0.99 (95% CI, 0.98–1)	0.008	90 (95% CI, 57.7–98.3)	95 (95% CI, 18.3–99.9)	129
Se (mg/L)	0.95 (95% CI, 0.89–1)	0.034	90 (95% CI, 55.6–98.5)	90 (95% CI, 27.9–99.5)	71.84

Abbreviations: AUC, area under curve; CI; confidence intervals; Cu, copper; Se, selenium; SE, standard error; Zn, zinc.

dogs with CPV might be attributed to the utilization of Zn in the antioxidant synthesis stages of the oxidative process [20]. Oxidative damage can occur in dogs with CPV [34, 35]. In light of the aforementioned evidence, it is reasonable to suggest that there might be a reduction in the oxidative damage-induced immune response process in dogs with CPV as a result of Zn consumption. Furthermore, reduced Zn levels observed in dogs with CPV could result from malabsorption due to disrupted intestinal surface integrity [33]. It could also be appropriate to consider malabsorption as a factor in this study. Nevertheless, it is essential to consider the potential effects of elevated Cu concentrations on the outcomes of this study. There is competition between the absorption of trace elements, in particular between the absorption of Cu and Zn [36, 37]. Therefore, an increase in Cu absorption can result in a reduction in Zn concentration. The competition between these two trace elements, as indicated by the Cu/Zn ratio that was identified, might be the reason for its prognostic relevance in CPV dogs that survive. This result is similar to the findings of a few human research studies, which have reported an elevated Cu/Zn ratio in cases of intestinal damage [3, 38].

The study demonstrated considerably lower serum cobalamin concentrations in dogs infected with CPV, with the lowest levels observed in those that did not survive. This finding is consistent with reported cases of hypocobalaminemia in dogs with intestinal damage [22, 23]. In studies investigating cobalamin levels in dogs with CPV, malabsorption, increased intestinal permeability, and dysbiosis are the causes of decreased cobalamin concentrations [22]. This reduction could be multifactorial, involving factors such as intestinal mucosal integrity, the intestinal microbiome, pancreatic function, and increased tissue utilization [23]. Lower cobalamin levels in dogs with CPV do not reflect cellular cobalamin deficiency, as evidenced by the lack of correlation with methylmalonic acid concentrations [22]. However, non-surviving dogs with CPV have significantly higher methylmalonic acid levels compared to survivors, indicating pronounced cellular cobalamin deficiency [24]. This finding aligns with the results of our study, where the lowest cobalamin levels were observed in the non-surviving CPV group. Oxidative stress occurs in CPV [26, 39], and it is reasonable to propose that the hypocobalaminemia observed in CPV-infected dogs is due to greater cobalamin utilization during the inflammatory response.

The Se is a microelement in the dog's body that is essential for the normal functioning of the metabolism, with an antioxidant function and a role in thyroid metabolism [6]. Although studies in veterinary medicine have focused more on the immunostimulatory effects of Se supplementation in dogs [40–42], and no studies investigating Se levels in dogs with CPV were found in the literature. Research in human medicine has demonstrated that this Se might act as an important inflammatory marker in patients with gastrointestinal damage [43–45]. In our study, we observed a decrease in serum Se concentrations in non-surviving CPV-infected dogs. Our results were consistent with studies reporting reduced Se levels in humans and animal models with gastrointestinal damage [46, 47] and with the relatively small number of studies reporting reduced Se levels in dogs with gastrointestinal damage [48]. One possible mechanism for reduced Se levels might be that the daily requirement of Se is not met due to reduced food intake. Moreover, the American Association of Feed Control Officials recommends a minimum of 0.35 mg of Se for each kilogram of body weight for adult dogs. In this respect, the decrease in daily Se intake due to reduced appetite caused by the nature of parvovirus infection in dogs might be the cause of the decrease in serum Se concentration. Another plausible mechanism could be the transcapillary escape of Se due to increased vascular permeability in dogs with CPV. This escape could lead to a decrease in serum Se concentration by facilitating the transfer of selenium from the blood to the interstitial compartment [49, 50].

This study had several limitations. The first is the relatively small sample size. However, approximately 15% of the dogs that were admitted to the hospital with diarrhea met all criteria and were included in the study. Another limitation is the variation in drugs used during treatment between surviving and non-surviving dogs. In both groups, treatment primarily involved intravenous fluid administration, immunostimulants, antibiotics, and antiemetics. However, in the non-surviving group, additional emergency drugs such as adrenaline and atropine, requiring urgent intervention, were also administered. An additional limitation is that we could not evaluate the oxidative status due to economic constraints.

5 | Conclusions

CPV non-survivors had considerably elevated concentrations of Cu and Cu/Zn ratios and markedly lower concentrations of Zn, cobalamin, and Se than survivors. Furthermore, all of these variables might have prognostic potential, as reflected by the specificity, sensitivity, and AUC values obtained from ROC curve analyses. However, the Cu/Zn ratio showed the highest efficacy with 100% sensitivity, 95% specificity, and an AUC of 0.98, as determined by ROC analysis. The findings of this study suggest that the levels of Zn, Cu, Se, cobalamin, and the Cu/Zn ratio at the time of hospital admission hold prognostic potential in dogs with CPV.

Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

Approved by the Atatürk University Local Animal Experimentation Ethics Committee (Decision Number: 2024/129). Authors declare human ethics approval was not needed.

Conflicts of Interest

The authors declare no conflicts of interest.

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