Serum cobalamin concentrations in dogs infected with canine parvoviral enteritis

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OBJECTIVE
To compare the serum cobalamin concentrations in canine parvovirus (CPV)–infected dogs with those of healthy control dogs.

ANIMALS
45 dogs with CPV enteritis and 17 healthy age-matched control dogs.

PROCEDURES
Infection was confirmed by visualization of CPV-2 through fecal electron microscopy. All dogs received supportive care. Serum samples taken at admission were used to determine cobalamin, C-reactive protein, and albumin concentrations.

RESULTS
Serum cobalamin concentrations were significantly lower in the CPV-infected group (median [interquartile range], 173 pmol/L [< 111 to 722 pmol/L]) than in healthy control dogs (379 pmol/L [193 to > 738 pmol/L]). There was no association between cobalamin concentration and C-reactive protein or albumin concentration.

CLINICAL RELEVANCE
While hypocobalaminemia was common in CPV-infected dogs, the clinical relevance of this finding remains to be determined. Studies assessing markers of cellular cobalamin deficiency in dogs with CPV infection appear warranted.

In mammalian species, cobalamin, a water-soluble vitamin (B12), is an essential cofactor for 2 enzyme systems, and adequate quantities are necessary for nucleic acid synthesis, hematopoiesis, and other vital functions. Omnivorous or carnivorous animals, including dogs and cats, are unable to synthesize cobalamin, and it is mainly ingested with foods of animal origin. Cobalamin has a complex mechanism of absorption, involving both the pancreas and small intestine. Following release from dietary animal-derived protein in the stomach, cobalamin is bound to gastric and salivary-produced R-proteins as it is carried into the duodenum. At the level of the duodenum, the R-protein–cobalamin complex is broken down by pancreatic proteases, and cobalamin is then complexed with intrinsic factor, produced by the pancreas. Once in the ileum, absorption from the gastrointestinal lumen involves a cubam receptor–mediated mechanism, which occurs exclusively in this gut segment in dogs and cats. Disorders of cobalamin metabolism are increasingly recognized in small animal medicine. Hypocobalaminemia in dogs and cats has been associated with a range of clinical and metabolic effects.

Clinical signs of hypocobalaminemia are nonspecific and include anorexia, lethargy, weight loss, failure to thrive, and central or peripheral neuropathies. Supplementation of oral or parenteral cobalamin in cases with hypocobalaminemia is recommended. Due to the restricted occurrence of the cobalamin receptor on ileal enterocytes and the abundance of cobalamin in companion animal diets, the usefulness of cobalamin as both a clinical marker for disease and a possible therapeutic option appears to be most relevant to conditions of the gastrointestinal tract.

Canine parvovirus (CPV) is an important worldwide pathogen and a significant cause of viral gastroenteritis in dogs. Transmission is via the fecal-oral route, followed by viral replication within lymphoid tissue and subsequent spread to rapidly dividing cells. Puppies from 6 weeks to 6 months of age are most commonly affected. The initial clinical signs of CPV infection appear warranted.
tion and may improve patient outcomes. Although there is an increasing awareness of the prevalence and prognostic implications of cobalamin deficiency in small animal medicine, to the authors’ knowledge there are no studies investigating normal cobalamin levels in puppies or changes in animals with CPV. Multiple studies in people have shown that infants have lower serum cobalamin concentrations than adults and it is therefore likely that a cobalamin deficiency is common in otherwise healthy infants. This, in addition to a lack of reference intervals for cobalamin in this age group, makes interpretation of cobalamin status in infants impossible.22 Cobalamin concentrations in puppies have not been investigated, and it is not known whether these limitations also exist in dogs.

The primary aim of this study was to compare the serum cobalamin concentrations of CPV-infected dogs with those of healthy control dogs. Other serum biochemistry abnormalities are nonspecific but often include a hypoalbuminemia and an increased serum C-reactive protein (CRP) secondary to intestinal inflammation.14-16 CRP is classified as a major positive acute-phase protein (APP) in dogs due to the magnitude of its response.23,24 The circulating concentration of APPs is related to the severity of the underlying condition and is elevated in dogs with CPV enteritis.25 A secondary aim was therefore to determine whether there was a correlation between serum cobalamin concentration and age, serum cobalamin and CRP values, and serum cobalamin and albumin concentrations. We hypothesized that the serum cobalamin concentrations of the CPV-infected dogs would be significantly lower than those of healthy control dogs and that these would correlate with age, CRP concentration, and albumin concentration.

Materials and Methods

This project was part of a prospective study that used samples from 3 other studies,26-28 using clinical cases of dogs naturally infected with CPV presented to the Onderstepoort Veterinary Academic Hospital, over a period of 11 months between November 2018 and September 2019. University of Pretoria Faculty of Veterinary Science Research Committee and Animal Ethics Committee approval was granted for the study (certificate Nos. V073-18 and V090-18).

Forty-five client-owned dogs that presented to the Onderstepoort Veterinary Academic Hospital that were diagnosed with CPV enteritis were included in the study. A control group of 17clinically healthy dogs that presented for routine vaccination or elective surgical procedures, such as orchidectomy or ovariohysterectomy, was also used. A consent form was signed by each owner prior to inclusion in the study.

Inclusion criteria for the CPV-infected group included dogs of any breed or gender, between the ages of 8 weeks and 12 months, weighing > 3 kg, and demonstrating clinical signs associated with CPV infection (eg, depression, vomiting, hemorrhagic diarrhea, anorexia, and dehydration). Initial diagnosis of CPV enteritis was made based on a positive result from a validated, rapid patient-side immunoassay (Idexx Laboratories). Infection was confirmed by visualization of CPV-2 through fecal negative staining transmission electron microscopy (CM 10 transmission electron microscope; Philips Electron Optical Division). Dogs were not included in the study if they had received prior treatment for parvoviral enteritis. All dogs in this group received standardized treatment for CPV infection as set out by the isolation unit, Onderstepoort Veterinary Academic Hospital.

The healthy control dogs were only included once the dogs with CPV enteritis were chosen to age-match the control group with the affected group. The control dogs were regarded as healthy based on the absence of any abnormalities in their history, a physical examination, gravitational fecal flotation, peripheral blood smear evaluation, CBC, and negative results of CPV-2 fecal transmission electron microscopy testing.

Detailed history taking, physical examination, peripheral blood smear, and gravitational fecal flotation were performed for all the CPV-infected and control dogs at presentation. A fecal sample was collected via syringe at admission. The fecal sample was refrigerated once collected and submitted for fecal transmission electron microscopy within 12 hours of collection to be examined for the presence of CPV and screened for other viral pathogens. Blood was collected at presentation, prior to any treatment, by careful venipuncture from the jugular vein with 21-gauge needles directly into EDTA and serum blood collection tubes. The anticoagulated EDTA sample was used to perform a CBC on a hematology analyzer (Siemens Healthcare Diagnostics) within 30 minutes of collection. The anticoagulated EDTA sample was used to perform a blood smear on all dogs. Serum samples were left to clot at room temperature and then centrifuged at 1,520 X g for 8 minutes. The sample was used to immediately determine the serum CRP and albumin concentration, and the remaining sample was stored at -80 °C for serum cobalamin measurement.

Serum CRP concentration was measured using a canine-specific immunoturbidimetric CRP method (Gentian AS), and serum albumin concentration was measured using the colorimetric bromocresol green assay on an automated wet chemistry analyzer (Cobas Integra 400 Plus; Roche Products Ltd). The working range of the CRP assay was reportedly 10 to 300 mg/L, and the laboratory reference interval was < 15 mg/L. Any samples with an initial CRP result > 300 mg/L were diluted 1:2 with saline (0.9% NaCl) solution and rerun.

After study subject recruitment was complete, stored serum samples for cobalamin measurement were thawed as a batch and remixed, and serum cobalamin was measured using a solid-phase competitive chemiluminescent immunoassay (Vitamin B12, Immulite 2000; Siemens Healthcare Diagnostics). This assay had a working range of 111 to 738 pmol/L, and the laboratory’s reference interval for dogs was 200 to 646 pmol/L.

For all clinical pathology assays, internal quality control was performed daily, and results were within the laboratory’s performance goals. Serum CRP, albumin, and cobalamin concentrations were measured.
once, on the admission serum sample, and records of hospitalization were not included in the study.

Statistical analysis
Statistical analysis was performed using a commercial software package (MedCalc Software Ltd). Cobalamin results below and above the working range of the assay were set at 111 and 738 pmol/L respectively, for the purposes of the statistical analyses. Results for CRP < 10 mg/L were set at 10 mg/L. The Shapiro-Wilk test was used to assess data distribution. Regardless of data distribution and due to the small sample size of the control group, the Mann-Whitney U test for nonparametric data was used to compare serum cobalamin, albumin, and CRP concentrations between CPV-infected dogs and control dogs. The correlations between cobalamin concentration and age, cobalamin and CRP concentration, cobalamin and albumin concentration, and cobalamin and duration of illness at presentation were determined by calculation of the Spearman rank correlation coefficient (ρ). The level of significance was set at \( P < 0.05 \).

Results
There was no significant difference in age between the CPV-infected group (n = 45) and healthy control group (n = 17; median [range] age, 4.0 years [1.0 to 12.0 years] and 4.0 years [2.0 to 12.0 years], respectively). The CPV group included 32 males and 13 females, and the control group included 11 males and 6 females. Various breeds were represented. Clinical findings and laboratory results for all animals enrolled in the study were also summarized (Supplementary Table S1). The median duration of illness for the CPV-infected dogs was 1 day (range, 1 to 5 days).

Serum cobalamin, albumin, and CRP measurements were compared between the CPV-infected and control groups (Supplementary Table S2; Figure 1). The median (interquartile range) duration of serum storage for cobalamin measurements in the CPV-infected group was 300 days (67 to 348 days) and in the control group was 189 days (130 to 228 days). Serum cobalamin and albumin concentrations were significantly lower in the CPV-infected group than in healthy control dogs. Serum CRP concentration was significantly higher in CPV-infected dogs than in healthy control dogs.

Overall, 26 of the 45 (58%) CPV-infected dogs had low serum cobalamin values: 19 had serum cobalamin values that were detectable but below the lower reference limit (hypocobalaminemia), and a further 7 had serum cobalamin results below the detection limit of the assay (cobalamin deficiency). The range of cobalamin results for the control dogs was generally similar to the laboratory’s reference interval for adult dogs, although 1 dog in the control group had a mild hypocobalaminemia and 4 dogs had results above the upper limit of the working range.

No significant correlations were found between serum cobalamin and CRP concentrations (\( \rho = -0.24; P = 0.13 \)), cobalamin and albumin concentrations (\( \rho = 0.22; P = 0.16 \)), and cobalamin concentration and dog age (\( \rho = -0.03; P = 0.83 \)) or duration of illness (\( \rho = 0.15; P = 0.33 \)).
Discussion

This study demonstrated that CPV-infected dogs had significantly lower serum cobalamin concentrations at presentation than do healthy dogs and that a significant proportion of dogs with this disease have hypocobalaminemia or cobalamin deficiency. Hypocobalaminemia and cobalamin deficiency are caused by a variety of conditions in dogs, including exocrine pancreatic insufficiency, chronic severe ileal disease, and small intestinal dysbiosis. Furthermore, primary cobalamin disorders have also been described in various dog breeds but are considered to be rare. In people, strict vegetarians and those who consume a lacto-ovo vegetarian diet are at high risk of nutritional vitamin B12 deficiency because vitamin B12 is found exclusively in animal products, including meat, eggs, fish, and milk. Contrarily, the authors are unaware of any reports of dietary origin deficiencies of cobalamin in cats and dogs in peer-reviewed literature.

It is likely that the causes of lower concentrations of cobalamin found in the CPV-infected dogs were multifactorial and involved changes in intestinal mucosal integrity, the gut microbiome, pancreatic function, and increased tissue utilization. Serum cobalamin concentration is used as a marker for small intestinal dysfunction in dogs due to the strict localization of the cubam cobalamin receptor to the ileum and the abundance of cobalamin in companion animal diets. Although no studies have investigated the expression levels of the cubam ileal receptors in dogs with enteropathies, it has been hypothesized that mucosal disease affecting the ileum diminishes epithelial expression or function of the cubam receptors, leading to decreased mucosal uptake of cobalamin and a reduced serum cobalamin concentration. With time, body stores of cobalamin become depleted, and serum cobalamin deficiency follows. Characteristic histological findings in CPV include necrosis of the intestinal crypt epithelium, shortening or obliteration of villi, and dilation of intestinal crypts with necrotic cellular debris. This severe, diffuse mucosal disease would result in a decreased number of functional cubam receptors and could be 1 reason for reduced serum cobalamin in CPV-infected dogs.

A healthy microbiome is of vital importance for host health. Intestinal dysbiosis is defined as the alteration in composition or richness of the intestinal microbiota. Major functions of the intestinal microbiota that contribute to the preservation of gastrointestinal health include important trophic effects on immune structure and function, protection against enteropathogens, and metabolic activities that cultivate energy and nutrients and provide nutritional benefits. The presence of bacteria is also important for the development of proper gut structure as demonstrated by altered epithelial architecture in germ-free mice. Sudden variations in diet and changes in the architecture of the intestine with consequent changes in intestinal motility may lead to variations in the microbial populations. With changes in the microbial composition, microbiota may compete with the host for nutrients and produce deleterious metabolites causing further inflammation. Cobalamin coupled to intrinsic factor can be absorbed by anaerobic intestinal bacteria. Therefore, when the numbers of these bacteria are increased, they may compete for cobalamin, which may lead to decreased serum cobalamin concentrations.

It is vital to understand virus-bacterial interactions, as microbiota can influence viral infection and vice versa. Despite CPV being a major cause of viral enteritis, there is a lack of information regarding parvovirus-bacterial interactions. A recent study aimed to evaluate the gut microbiota in dogs with CPV before and after infection compared with that in healthy dogs. Despite no significant differences in overall species richness and diversity between healthy and CPV-infected dogs, there were significant differences in microbial community structure and membership, indicating a difference between healthy and CPV-infected dogs.

Another indication of microbiome disruption in CPV is that fecal microbial transplantation, whereby fecal suspensions from a healthy donor are administered to an individual with disease, have been shown to be associated with more rapid clinical recovery and decreased time of hospitalization in survivor dogs with acute hemorrhagic diarrhea caused by CPV. Despite indications that the intestinal microbiota may play a part in the pathogenesis of CPV, the actual mechanisms of host-microbiome interactions remain elusive. It is therefore possible that an upset in the balance of the microbial ecosystem that occurs in CPV-infected dogs may result in a competition for nutrients, including cobalamin.

In dogs and cats, the exocrine pancreas is a major site of intrinsic factor synthesis, and therefore hypocobalaminemia is common in association with exocrine insufficiency in both these species. Failure to absorb cobalamin in dogs with pancreatic disease may be caused by the following proposed mechanisms: reduced or absent pancreatic secretion of intrinsic factor, an absence of digestive enzymes causing an impaired release of cobalamin from R-factor and thus no binding of cobalamin to intrinsic factor, secondary small intestinal dysbiosis compromising the endogenous production of cobalamin, and potential intestinal mucosal compromise due to the presence of toxic metabolites as a result of small intestinal dysbiosis. A study investigating the prevalence of increased canine pancreas-specific lipase concentrations in dogs with CPV demonstrated that increased serum canine pancreas-specific lipase is relatively common in dogs with CPV enteritis. CPV can result in severe dehydration and subsequent tissue hypoperfusion, resulting in pancreatic ischemia. This may in turn affect pancreatic exocrine function, but has not specifically been investigated in dogs with CPV enteritis.

Diffuse small intestinal inflammation, the possible presence of pancreatic disease, and an altered gut microbiome with subsequent competition for nutrients and microbial production of deleterious metabolites may all contribute to a decreased ab-
sorption of cobalamin in CPV. Decreased absorption alone does not however explain the findings in this study, as the half-life of cobalamin, in healthy dogs at least, is approximately 6 to 16 weeks. The dogs in our study had acute disease, and it is unlikely that disruption of intestinal cobalamin absorption was present for much longer than 3 days before blood collection, at the most. It is possible that increased utilization of cobalamin in CPV infection may also contribute to decreased serum levels. Cobalamin has been shown to have potential antioxidant properties, with a subclinical cobalamin deficiency being implicated in many age-related diseases, such as Alzheimer’s and Parkinson’s disease, which share a commonality in that oxidative stress is thought to be a crucial factor in their pathophysiology. Oxidative stress occurs when pro-oxidant compounds such as reactive oxygen species exceed any available antioxidant buffering capacity. Both endogenous and exogenous antioxidants, such as selenium, vitamin C and E, flavonoids, coumarins, and possibly cobalamin, can aid in reducing overall oxidative stress. Oxidative damage has been shown to occur in CPV and the increase in reactive oxygen species in CPV-infected dogs may necessitate a greater utilization of cobalamin, further contributing to decreased serum levels.

Other proposed mechanisms for a decrease in serum cobalamin include an alteration in gastrointestinal transit time as well as gastrointestinal and renal losses. Pediatric humans with human immunodeficiency virus have similar histopathological changes to that of CPV patients and have been shown to have hypocobalaminemia associated with alterations in gastrointestinal transit time. Another explanation for hypocobalaminemia is the direct loss of transcobalamin II–cobalamin complex and mediates tubular reabsorption to minimize urinary loss. Following renal glomerular filtration of the transcobalamin II–cobalamin complex, tubular reabsorption occurs to minimize urinary losses of cobalamin. An endocytic receptor in the proximal renal tubules, megalin, has a high affinity for the transcobalamin II–cobalamin complex and mediates renal reabsorption and subsequent retention of cobalamin. Dogs with CPV have to been shown to be at risk of developing acute kidney injury at both the tubular and glomerular level. Acute kidney injury has been proposed to be a result of several factors, including severe dehydration, hypotension, systemic inflammatory response syndrome, and sepsis.

A recent study found that dogs with leishmaniosis had lower serum cobalamin than healthy dogs. The authors hypothesized that this finding may be multifactorial and due to compromised gastrointestinal absorption, increased renal loss, increased systemic requirements and/or increased utilization by the parasite, similar to the proposed mechanisms for a decrease in serum cobalamin in the present study. Like CPV, canine leishmaniosis is typically a multisystemic disease; however, in contrast to CPV, it is usually a chronic condition.

As cobalamin-dependent metabolic reactions are localized to the intracellular compartment, serum cobalamin concentrations are not a good measure of whole-body cobalamin status. Other markers, such as homocysteine and methylmalonic acid (MMA), more closely reflect the intracellular availability of cobalamin. Despite serum cobalamin concentrations being a reflection of tissue stores, it is not well defined at what serum concentration overt whole-body, tissue, or cellular cobalamin deficiency develops, although it has been suggested that dogs with undetectable levels of serum cobalamin have whole-body cobalamin deficiency. It is likely that the same holds true for tissue stores; it is unknown at what level of tissue depletion serum cobalamin concentrations begin to decrease. It is unknown whether the dogs with decreased serum cobalamin in this study had cobalamin deficiency at a cellular level. This has been shown not to be the case in hyperthyroid cats, for example. Hypocobalaminemia was identified in a significant proportion of hyperthyroid cats in 1 study but was later shown in a second study to have no clinical significance, as no hyperthyroid cats with a low serum cobalamin concentration had increased MMA and cobalamin concentrations normalized without supplementation as cats become euthyroid. Given the acute nature of CPV enteritis, it does seem unlikely that these puppies have a cobalamin deficiency at the cellular level. Nevertheless, this does warrant confirmation through measurement of MMA, as dogs recovering from CPV are likely have an increased demand for cobalamin. Massive cell division takes place in the recovery phase of CPV, particularly in intestinal and myelopoietic tissue. Cobalamin is a vital enzyme for DNA synthesis via its role in the methylation of homocysteine to methionine, and inadequate cellular stores may slow recovery. Unfortunately, measurements of both homocysteine and MMA are currently not widely available and therefore are not routinely performed in companion animals.

The range of serum cobalamin concentrations in the control group of the present study was similar to the reference interval for adult dogs in our laboratory, and there was no correlation between serum cobalamin concentration and age, suggesting that puppies do not have lower concentrations of cobala-
min than adult dogs, which is different from what is described for people.18–21

Hypoalbuminemia has been previously reported to be associated with poor outcome in dogs with chronic enteropathies.31,77 In a study32 where risk factors for negative outcome in dogs with chronic enteropathies were evaluated, the authors found that cobalamin and albumin were strongly correlated and suggested that both analytes should be assessed together. This is in disagreement with the finding of the present study, in which there was no correlation between serum cobalamin and albumin concentrations. Causes of hypoalbuminemia in CPV infection include reduced intake, malabsorption due to a decrease in intestinal surface area, loss due to hemorrhage or exudation of protein into the gastrointestinal tract, increased intestinal permeability, and inflammation (albumin is a negative APP).77 Importantly, serum albumin concentration is also closely related to the hydration status of the patient.31 Dogs with CPV often present with severe dehydration, which may cause hemoconcentration and a relative increase in albumin. This effect may have confounded the results of the correlation analysis, although theoretically dehydration would have a similar masking effect on cobalamin and CRP too.

CRP, the major APP in dogs, has been described as a marker of disease severity in dogs with chronic enteropathies18 as well as in those suffering from CPV.25 In the present study, there was no correlation between serum cobalamin and CRP concentrations. This implies that serum cobalamin levels do not reflect disease severity or the degree of inflammation present, but this needs to be investigated further, in larger groups of animals.

One control dog in the present study had a CRP value of 50 mg/L, which was unexpectedly high for a control value. Dogs were deemed clinically healthy based on the absence of any abnormalities in their history, a physical examination, gravitational fecal flotation, peripheral blood smear evaluation, CBC, and negative results for CPV-2 fecal transmission electron microscopy testing. It is possible that this dog had an underlying condition not picked up by the routine testing performed.

There were several limitations to the study reported here. First, the study sample size was small. Second, the lack of measurement of serum MMA and homocysteine concentrations may have provided additional evidence of cobalamin deficiency at a cellular level. Third, blood collection and analysis were only performed once at admission, and investigators had no control over when in the course of the disease process the dogs were presented and diagnosed with CPV. Lastly, neither serum trypsin-like immunoreactivity nor lipase activity was measured to assess the presence of pancreatic disease and its contribution to low serum cobalamin.

In conclusion, this study showed that the serum cobalamin concentration in CPV-infected dogs was significantly lower than that of healthy control dogs. While hypocobalaminemia is common in CPV-infected dogs, the clinical relevance of this finding remains to be determined. Studies assessing markers of cellular cobalamin deficiency, such as MMA, in dogs with CPV infection appear warranted.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org