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Oral Cobalamin Supplementation in Dogs with Chronic Enteropathies and Hypocobalaminemia

L. Toresson, J.M. Steiner, J.S. Suchodolski, and T. Spillmann

Background: Cobalamin deficiency is commonly associated with chronic enteropathies (CE) in dogs and current treatment protocols recommend parenteral supplementation. In humans, several studies have reported equal efficacy of oral and parenteral cobalamin administration of cobalamin.

Objectives: To retrospectively evaluate whether oral cobalamin supplementation can restore normocobalaminemia in dogs with CE and hypocobalaminemia.

Animals: Fifty-one client-owned dogs with various signs of CE and hypocobalaminemia.

Material and Methods: Retrospective study based on a computerized database search for dogs treated at Evidensia Specialist Animal Hospital, Helsingborg, Sweden during January 2012–March 2014. Inclusion criteria were dogs with signs of CE, an initial serum cobalamin ≤270 ng/L (reference interval: 234–811 ng/L) and oral treatment with cobalamin tablets. Serum cobalamin for follow-up was analyzed 20–202 days after continuous oral cobalamin supplementation started.

Results: All dogs became normocobalaminemic with oral cobalamin supplementation. The mean increase in serum cobalamin concentration after treatment was 794 ± 462 ng/L. Serum cobalamin concentrations were significantly higher after supplementation (mean 1017 ± 460 ng/L; P < .0001) than at baseline (mean 223 ± 33 ng/L).

Conclusion and Clinical Importance: Our results suggest that oral cobalamin supplementation is effective in normalizing serum cobalamin concentrations in dogs with CE. Prospective studies comparing cellular cobalamin status in dogs being treated with parenteral versus oral cobalamin supplementation are warranted before oral supplementation can be recommended for routine supplementation.

Key words: Inflammatory Bowel Disease; Oral supplementation; Vitamin B12.

Common causes for cobalamin deficiency in dogs are chronic enteropathies (CE), exocrine pancreatic insufficiency (EPI) and familial cobalamin deficiency (reported in Chinese Shar Peis, Giant Schnauzers, Border Collies and Beagles). Anecdotal reports of short-bowel syndrome and cobalamin deficiency in dogs also exist, an association that is well documented in humans. Cobalamin deficiency in dogs is commonly associated with canine CE with a reported prevalence of 6–73%. Hypocobalaminemia has also been reported to be a negative prognostic factor in dogs with CE or EPI, associated with an increased risk of euthanasia.

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This study was conducted at Evidensia Specialist Animal Hospital, Helsingborg, Sweden.

Abbreviations:
- BCS: body condition score
- c-PLi: canine pancreatic lipase immunoreactivity
- CE: chronic enteropathy
- CIBDAI: canine inflammatory bowel disease index
- EPI: exocrine pancreatic insufficiency
- IF: intrinsic factor
- TLI: trypsin-like immunoreactivity

Suggested mechanisms of cobalamin deficiency in canine CE are damage to the ileal mucosal receptors for binding of cobalamin–intrinsic factor (IF) complexes or bacterial competition for nutrients in small intestinal dysbiosis resulting in decreased amounts of cobalamin available for absorption. 

Gastrointestinal processing and absorption of cobalamin in mammals are mediated by carrier proteins and IF, which in dogs is mainly produced by the pancreas. The cobalamin-IF complex is absorbed by receptors in the ileum. However, studies in humans using radioactively labeled cobalamin have shown that approximately 1% of free cobalamin was absorbed along the entire intestine by passive diffusion, independently of IF.

Several studies in humans with hypocobalaminemia suggest that the administration of oral cobalamin might be as effective as parenteral administration in restoring serum cobalamin concentrations in patients suffering from pernicious anemia, various gastrointestinal disorders, or for those on a restricted diet (vegetarians and
Oral cobalamin is prescribed to human patients to avoid the discomfort, inconvenience, and cost of monthly injections of vitamin B12. In European countries, oral cobalamin supplementation is widely utilized. Factors influencing the increase in serum cobalamin concentration after oral administration in humans are dose and time. A daily oral intake of 1,000–2,000 µg cyanocobalamin successfully normalized serum cobalamin in all studies available in human patients. Continuous increases of serum cobalamin concentrations during oral cobalamin treatment have been demonstrated in several studies. Current supplementation protocols for cobalamin in dogs call for repeated parenteral injections based on pathophysiologic justification, clinical empiric experience, and specialist opinion. Currently, studies assessing the effect of oral cobalamin supplementation in dogs with CE are lacking. However, due to reports of successful oral cobalamin substitution in humans with various gastrointestinal disorders, one of the authors (L. T.) has started to use oral supplementation. The purpose of this retrospective study was to evaluate whether oral cobalamin supplementation can restore normocobalaminemia in dogs with CE.

Materials and Methods

Study Design

A retrospective study based on the review of medical records of dogs treated with oral cobalamin at Evidensia Specialist Animal Hospital, Helsingborg, Sweden, that commenced between January 2012 and March 2014.

Study Population

Client-owned dogs with signs of CE and hypocobalaminemia treated with oral cobalamin supplementation at standardized doses were identified by searching the electronic patient database of the hospital. The database fields for treatment and prescription were searched for the brand name of cobalamin used for either tablets or injections. All fields were also searched for the brand name as free text. No other brand of cobalamin had been prescribed at the hospital during the last 5 years. All dogs were given oral cobalamin tablets once daily until the day before the collection of the follow-up serum sample for cobalamin determination. If a follow-up sample had been collected on more than one occasion from the same patient, the first sample was chosen. Owners were instructed not to give cobalamin on the day of the blood test and were asked to withhold food from their dogs for at least 8 hours prior to sample collection.

Inclusion Criteria

To be included in the study, a documented physical examination had to be available as well as a dietary and medical history. As far as possible, extra-intestinal diseases were excluded in all dogs by a standardized diagnostic work-up including hematology, serum biochemistry, fecal examination and abdominal ultrasound.

Cobalamin Analysis

Serum samples were refrigerated within 2 hours of collection, sent with refrigerated transport and analyzed within 1–4 days. It has been shown previously that cobalamin is stable during these storage conditions. Serum cobalamin concentrations were determined before and after oral cobalamin treatment. Increases in serum cobalamin concentrations after supplementation were further stratified based on initial serum cobalamin concentrations and CIBDAI at inclusion. Increases in serum cobalamin concentrations were also compared between dogs that had a change in diet or medical treatment during supplementation and dogs that had an unaltered diet and treatment. The cobalamin analyses were performed at IDEXX VetMedLabor, Ludwigsburg, Germany using an automated chemiluminescence immunoassay. Serum samples were sent by refrigerated transport.

Baseline Data

The following data were obtained from case records: breed, sex, age, body weight and body condition score (BCS) at time of diagnosis. Further, medication history, diet, clinical signs, and physical examination at time of diagnosis and at follow-up were noted. CIBDAI was calculated retrospectively for the time when hypocobalaminemia was diagnosed based on information from the medical records. For dogs that were already receiving immunosuppressive therapies at study inclusion, CIBDAI was also retrospectively calculated for the time-point before these dogs had undergone endoscopy and before immunosuppressive treatment being commenced. Other laboratory parameters available for some of the patients included folate, serum tryptophan-like immunoreactivity (TLI), canine pancreatic lipase immunoreactivity (c-PLI, as measured by Spec-c-PLI or SNAP canine pancreatic lipase (SNAP-c-PL), and histopathologic reports from gastrointestinal biopsies. All biopsies were analyzed by board certified pathologists at the veterinary pathology laboratory Biovet in Sollentuna, Sweden.
**Statistical Analyses**

The D’Agostino & Pearson omnibus normality test was used for normality testing. Serum cobalamin concentrations before and after cobalamin supplementation were analyzed with a paired \( t \)-test. CIBDAI in dogs receiving immunosuppressive treatment was compared to that in dogs not receiving immunosuppressive treatment using a Mann–Whitney test. CIBDAI was also compared at the time of initiation of immunosuppressive treatment to that at the time of study inclusion using a Wilcoxon matched-paired signed rank test. Increases in serum cobalamin concentrations stratified after initial serum cobalamin concentrations were analyzed with an unpaired \( t \)-test. Comparison of increases in serum cobalamin concentration based on CIBDAI at inclusion were analyzed with an unpaired \( t \)-test. Comparison of increases in serum cobalamin concentrations in patients with an unaltered treatment plan compared to patients that underwent changes in diet or medical treatment during supplementation. All statistical analyses were performed using a commercially available software package.\(^5\)

**Results**

**Baseline Characteristics**

Fifty-one dogs met the inclusion criteria, of which 34 were males (67%); 26 intact and 8 neutered males) and 17 were females (33%); 15 intact and 2 spayed females). Thirty different breeds were represented, including mixed breed dogs (7/51; 14%), followed by German Shepherd (3), Labrador Retriever (3), Soft Coated Wheaten Terrier (3), Cavalier King Charles Spaniel (3), and Miniature poodle (3). Of the remaining 29 dogs, there were 2 Giant Schnauzers present, a breed for which familial cobalamin deficiency has been reported.\(^2\) No other breeds for which familial cobalamin deficiency has been reported were noted.

All dogs were older than 1 year of age, ranging from 1.3 to 12.8 years (median 4.9). Body weights ranged from 2.6 to 52.0 kg (median 14.0) at the time of diagnosis and BCSs ranged from 2–7/9 (median 5/9).

**Medication History and Diet**

Twenty-two of the 51 dogs were receiving immunosuppressive treatment at the time of study inclusion when hypocobalaminemia was documented (Table 1). Median duration of immunosuppressive treatment was 566 days (range 9–2,811 days) before study inclusion. Miscellaneous treatments in this group consisted of olsalazin (6), metoclopramide (3), folate (2), or metronidazole (1).

The remaining 29 dogs were not treated with immunosuppressive drugs when hypocobalaminemia was diagnosed. However, 4 dogs had received miscellaneous treatments for gastrointestinal disease, such as folate (2), sucralfate (1), and pancreatic enzymes (1). Of those 29 dogs, 8 were started on immunosuppressive drugs during oral cobalamin supplementation. Six of those 8 dogs had endoscopically collected biopsies confirming chronic gastrointestinal inflammation. CE was also suspected in the remaining 2 dogs that were concurrently diagnosed with chronic pancreatitis, but the owners declined further work-up. Immunosuppressive treatment was hence started without endoscopy in those dogs and led to clinical improvement.

Previous parenteral cobalamin supplementation had been administered to 12/51 dogs (24%, Table 1). The supplementation ended 37–1,788 days before study inclusion. Eight dogs had received parenteral supplementation alone (1 injection/week during 4–6 weeks), while 4 dogs had received both parenteral and enteral supplementation concurrently (1–4 injections parallel with oral treatment during 100–200 days). In all dogs, recurrence/persistence of hypocobalaminemia following withdrawal of cobalamin supplementation had been documented before study inclusion.

Forty of 51 dogs were fed commercial pet food kibbles from a major pet food company, 5 dogs were fed various commercial raw food diets, 4 dogs received kibbles mixed with a home-cooked diet, and 2 dogs were exclusively fed a home-cooked diet. When the second serum cobalamin sample was collected, 19/51 dogs were fed a new diet (Table 1). In total, 26/51 dogs had no change in medical treatment or diet compared to baseline at follow-up aside from the addition of oral cyanocobalamin (20/26) or cyanocobalamin and folate (6/26).

**Table 1.** Selected data and treatment of 51 dogs with hypocobalaminemia.

<table>
<thead>
<tr>
<th>Variable at Inclusion(a) or During Cobalamin Supplementation(b)</th>
<th>Range (Median) or Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)(a)</td>
<td>1.3–12.8 (4.9)</td>
</tr>
<tr>
<td>CIBDAI(a)</td>
<td>1–13 (5)</td>
</tr>
<tr>
<td>IS tx(a)</td>
<td>22/51</td>
</tr>
<tr>
<td>IS tx started before inclusion (days)</td>
<td>9–2,811 (566)</td>
</tr>
<tr>
<td>Previous cbl tx(a)</td>
<td>1/2</td>
</tr>
<tr>
<td>Previous cbl tx ended</td>
<td>37–1,788 (146)</td>
</tr>
<tr>
<td>Concurrent GI diseases(a)</td>
<td>Pancreatitis 4(^a)/51, EPI 1/51</td>
</tr>
<tr>
<td>IS tx started/changed(a)</td>
<td>14/51</td>
</tr>
<tr>
<td>Diet change(b)</td>
<td>19/51</td>
</tr>
<tr>
<td>Unaltered diet/tx(b)</td>
<td>26/51</td>
</tr>
</tbody>
</table>

CIBDAI, canine inflammatory bowel disease index. IS, immunosuppressive; cbl, cobalamin; tx, treatment.

\(a\)At inclusion.

\(b\)During cobalamin supplementation.

\(^1\)1 dog had borderline PLI.

**Historical Findings and CIBDAI**

Presenting complaints at time of diagnosis included anorexia (21), diarrhea (17), lethargy (16), weight loss (14), vomiting (13), increased frequency of defecation (9), signs of abdominal pain (6), pica (3), borborygmus (3), excessive licking of the mouth, paws, or the floor (3), halitosis (2), retching (2), melena (2), bad hair coat
(1), syncope (1), seizures (1), and polyuria and polydipsia (1).

The median CIBDAI for all dogs at study inclusion was 5 (range 1–13; Table 1). The CIBDAI scores at inclusion were significantly lower (P = .0005) for dogs receiving immunosuppressive therapies (n = 22; CIBDAI = 3, range 1–13) compared to the remaining dogs (n = 29; CIBDAI = 6, range 2–14).

For the 22 dogs already receiving immunosuppressive treatment at study inclusion, the CIBDAI had progressively improved following initiation of immunosuppressive treatments, and was significantly lower (P = .0059) by the time of study inclusion (CIBDAI = 3, range 1–13) compared with that calculated retrospectively and before medical intervention (CIBDAI = 6, range 2–10).

Cobalamin

Mean serum cobalamin concentrations at inclusion was 223 ± 33 ng/L (reference interval: 234–811). At follow-up 20–202 days (median 72) after initiation of continuous oral cobalamin supplementation, the mean serum cobalamin concentration was 1,017 ± 460 (Fig 1). The difference between serum cobalamin concentrations before and after treatment was statistically significant (P < .0001). The mean increase in serum cobalamin concentration was 794 ± 462 ng/L. The smallest increase was 73 ng/L. The dog with the smallest response, a German Shepherd dog sampled 54 days after start of supplementation, had previously been treated with parenteral cobalamin supplementation achieving an equally poor response. This dog was unfortunately lost to further follow-up. The dogs with the second and third smallest response had follow-up blood samples collected at 64 and 76 days, respectively. Both dogs were continued on oral cobalamin supplementation. At the next follow-up, serum cobalamin concentrations had continued to increase by 122 and 128% as compared to serum cobalamin concentration during the first follow-up, respectively. Increases in serum cobalamin concentrations were stratified based on serum cobalamin concentrations at time of inclusion (Fig 2) or CIBDAI at time of inclusion (Fig 3). There were no statistically significant differences between the groups (P = .98 and .83, respectively). Furthermore, there was no statistically significant difference between the increase in serum cobalamin concentrations between the dogs that had an unaltered diet or medical treatment during supplementation compared with the dogs that had a change in diet or medical treatment (P = .27; Fig 4).

Folate and Pancreatic Function Tests

Serum folate concentrations at time of inclusion were available for 49 of 51 dogs. Two of these 49 dogs were treated with oral folate because of previously diagnosed folate deficiency. Of the remaining 47 dogs, subnormal serum folate concentrations were noted in 20 of 47 dogs (43%), while 7 dogs (15%) had
increased levels of serum folate concentrations (reference interval 9.3–24 ng/mL).

Serum TLI concentrations were available for 31/51 dogs. One dog, a mixed-breed, had a TLI of 1.6 µg/L consistent with EPI (reference interval 8.5–35 µg/L, IDEXX VetMed Labor). Of the remaining dogs, 28/30 had serum TLI concentrations within the reference interval while 2/30 had a serum TLI above 50 µg/L. The 2 dogs with increased serum TLI concentrations were further tested for pancreatitis by measurement of serum Spec-cPL concentration or by running a SNAP-cPL test, which were normal or negative, respectively.

Results for canine pancreatic lipase testing were available for 22 dogs and a canine SNAP-cPL was available for 13 dogs. Eighteen of 22 dogs had a cPLI concentration within the reference interval of 0–200 µg/L, one mixed-breed dog had a cPLI of 251 µg/L, and 3 dogs (a Miniature Poodle and 2 Cavalier King Charles Spaniels) had cPLI concentrations above 400 µg/L.

**Histopathology**

Histopathology reports from gastrointestinal biopsies were available for 33/51 dogs (65%). Biopsies were collected endoscopically in 32 dogs and surgically via laparotomy in 1 dog. Endoscopic biopsies were retrieved from the gastric mucosa, duodenum, and colon in 28/32 dogs and only from the gastric mucosa and duodenum in 4/32 dogs. Ileal biopsies were available only from the dog undergoing laparotomy. Lymphocytic-plasmacytic inflammation was seen in 28 dogs while 5 dogs had a predominantly eosinophilic inflammation of the gastrointestinal tract. The inflammation of the small intestine and colon was characterized as mild to moderate in all dogs. Evidence for gastrointestinal neoplasia was not found in any of the biopsies.

**Discussion**

In this study, serum cobalamin concentrations increased significantly and normocobalaminemia was restored in all dogs with CE and hypocobalaminemia after daily oral cobalamin supplementation for 20–202 days. These results suggest that oral cobalamin supplementation might be an alternative to parenteral administration in dogs with CE and cobalamin deficiency. Daily oral cobalamin supplementation is usually a cheaper, simpler, and pain-free alternative to weekly cobalamin injections. Parenteral cobalamin supplementation has been the only recommended route of cobalamin administration in dogs with cobalamin deficiency. Studies validating parenteral supplementation protocols in dogs are however lacking. Similarly, to our knowledge, no studies evaluating the efficacy of oral cobalamin supplementation in dogs with CE and hypocobalaminemia have been published. This is in contrast to human medicine where oral supplementation has already been widely studied in various conditions associated with cobalamin deficiency. In humans, parenteral cobalamin is administered intramuscularly or subcutaneously, which has been associated with adverse effects such as pain and, rarely, adverse reactions including localized scleroderma or tissue necrosis. Parenteral cobalamin administration might have other disadvantages compared to oral supplementation. In some countries, such as Sweden or Finland, pet owners are not legally allowed to give injections to their pets aside from insulin and hypoglycemic treatment. Parenteral cobalamin injections thus have to be given by a veterinary health care provider, increasing inconvenience, time, and costs. Even in countries where pet owners are allowed to give injections, not all owners are comfortable performing this task. Dogs might show pain and some people are very reluctant to handle hypodermic needles and syringes. In such a setting, the injectable form of cobalamin was more than double the price of the oral supplement for a 3-month treatment period, without the costs for veterinary consultation and injections.

One possible explanation why oral cobalamin might be effective in restoring normocobalaminemia in dogs with CE is the possibility of an alternative absorptive pathway of cobalamin beyond receptor-mediated transport in the ileum, which has been reported in humans. This might explain why oral cobalamin supplementation has been effective in normalizing serum cobalamin concentrations as well as serum MMA and homocysteine concentrations in humans lacking IF or an intact IF pathway. Factors known to influence serum cobalamin concentrations in humans treated with oral cobalamin supplementation are dose and time. When oral cobalamin was given to human patients at 500 µg/day, it produced satisfactory responses in the majority of patients but caused bordering cobalamin concentrations in a few patients. A daily oral starting dose of 1,000–2,000 µg/day is currently recommended for humans. Using this dose, there has been no statistical difference between oral and parenteral supplementation in serum cobalamin concentrations during and post treatment.
ies. However, it is possible that some dogs require higher doses, or that not all dogs in the study received their daily dose as prescribed. Regarding increase in serum cobalamin concentration over time, 5 separate studies demonstrate that serum cobalamin concentrations increase with continuous oral cobalamin supplementation in humans. Serum cobalamin concentrations were only measured at one time-point post treatment during our study. Thus, we cannot determine if a long-term oral cobalamin supplementation in dogs would be associated with a continuous increase in serum cobalamin concentration. Further studies are needed to answer this question.

Another factor potentially influencing response to cobalamin supplementation in dogs is breed. Two Giant Schnauzers were included in our study, a breed in which familial cobalamin deficiency has been reported. These dogs were 6 and 8.7 years old, respectively, at the time of inclusion. Both were diagnosed with chronic eosinophilic enteritis. Histopathology reports and age of onset in the other breeds with familial cobalamin deficiency are less likely than cobalamin deficiency as a result of CE. No other breeds known for familial cobalamin deficiency were represented in this study.

As the main source of IF in dogs is the exocrine pancreas, exocrine pancreatic function is also likely to influence the effect of cobalamin absorption. However, exocrine function was not assessed in all dogs. Only 1 dog in our study had confirmed EPI and CE. This dog responded well to oral cobalamin substitution. Whether all dogs with EPI and cobalamin deficiency would respond to oral cobalamin supplementation remains to be determined.

Most dogs responded very well to oral cobalamin supplementation, but some responded less well than others. When specifically looking for similarities in the 3 dogs with the smallest response to oral cobalamin substitution regarding history, dose in mg/kg, diet and type and degree of intestinal inflammation, none could be identified. Why some dogs do not respond as satisfactorily to oral treatment is unknown and requires further investigation, as does the optimal dosing regimen (higher or lower) to restore normocobalaminemia.

The inclusion criterion for this study was a serum cobalamin concentration of ≥270 ng/L (reference interval: 234–811 ng/L). The majority of the patients had a serum cobalamin concentration below the reference interval, but it might be argued that dogs with a serum cobalamin concentration between 234 and 270 ng/L were not truly deficient. However, hypocobalaminemia can result in severe metabolic consequences and is associated with a negative outcome. Therefore, some centers recommend starting cobalamin supplementation when serum cobalamin concentration is in the low reference range (see www.vetmed.tamu.edu/gilab). A recent study assessing MMA in dogs with different levels of serum cobalamin concentrations demonstrated that 19% of all dogs with serum cobalamin concentrations in the lower reference range had an increased MMA. This suggests that some of these dogs were cobalamin deficient on a cellular level, and supports the recommendation to start treatment in the low reference range. It also parallels recommendations in human medicine, where supplementation often is started at the lowest end of normal reference interval, if serum homocysteine and/or MMA concentrations are concurrently increased.

This study has several limitations. The time-point for the follow-up blood sample varied substantially from 20 to 202 days, which might influence the results as serum cobalamin levels have been shown to increase over time with continuous treatment in humans. Serum TLI concentrations were not measured in this study, which is a prerequisite in many countries to diagnose and treat cobalamin deficiency in humans. Whether such an approach would also be favorable for canine patients still needs to be studied. Despite several study limitations, our results suggest that oral cobalamin appears effective in restoring normocobalaminemia in dogs with CE, although 3 dogs had only a modest increase in their serum cobalamin concentration in response to oral cobalamin treatment. To further evaluate the efficacy of oral cobalamin supplementation, a randomized prospective study comparing serum cobalamin and cellular cobalamin status in dogs treated with oral or parenteral cobalamin supplementation would be warranted.

**Off-Label Antimicrobial Declaration:** Authors declare no off-label use of antimicrobials.

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**Footnotes**

a Behepan (1 mg), Pfizer, Sollentuna, Sweden  
b Behepan (1 mg/mL), Pfizer  
c ADVIA Centaur, Siemens Healthcare Diagnostics, Erlangen, Germany  
d GraphPad Prism 6.0, GraphPad Software, Inc., La Jolla, CA

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**Conflict of Interest Declaration:** At time of submission, February 7, 2015, Dr Toresson had stock or stock options, travel and accommodations covered or reimbursed from the Evidensia Specialist Animal Hospital, Helsingborg, Sweden or from the Swedish Veterinary Care Foundation.

**Off-Label Antimicrobial Declaration:** Authors declare no off-label use of antimicrobials.
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