Efficacy and tolerance of oral versus parenteral cyanocobalamin supplement in hypocobalaminaemic dogs with chronic enteropathy: a controlled randomised open-label trial

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Summary

Objectives: Determine comparative tolerance of daily oral and weekly parenteral cobalamin supplementation, in hypocobalaminaemic dogs with chronic enteropathy. Determine whether oral is as effective as parenteral supplementation at achieving eucobalaminaemia, in hypocobalaminaemic dogs with protein-losing enteropathy (PLE), severe hypocobalaminaemia or high Canine Inflammatory Bowel Disease Activity Index (CIBDAI) at inclusion.

Methods: Thirty-seven client-owned dogs with hypocobalaminaemia and clinical signs of chronic enteropathy were prospectively enrolled in three UK referral centres. Dogs were randomly allocated to daily oral for 12 weeks or weekly parenteral cobalamin supplementation for six weeks and one additional dose four weeks later. Serum cobalamin, body condition score, CIBDAI and body weight were assessed at inclusion, week 7 and 13. Serum methylmalonic acid concentration was evaluated at inclusion and at week 13. Owners completed treatment adherence, palatability, tolerance and satisfaction questionnaires at week 13.

Results: Nineteen dogs completed the study. All dogs orally supplemented achieved normal or increased cobalaminaemia at week 7 and 13. There was no statistical difference in cobalamin concentration at week 13 in dogs treated with oral or parenteral supplementation, regardless of presence of PLE, severity of hypocobalaminaemia or CIBDAI at inclusion. Serum methylmalonic acid concentration was not significantly different between oral and parenteral groups, neither were treatment adherence, satisfaction, and tolerance scores at week 13.

Clinical Significance: Oral is as effective and as well-tolerated as parenteral cobalamin supplementation in hypocobalaminaemic dogs with chronic enteropathy and severe clinical or

biochemical phenotypes, and should be considered as a suitable treatment option regardless of disease severity.

Keywords: chronic enteropathy, dog, oral cyanocobalamin, vitamin B12, treatment tolerance

Abbreviations

BCS Body condition score

CI Confidence interval

CIBDAI Canine Inflammatory Bowel Disease Activity Index

EPI Exocrine Pancreatic Insufficiency

PLE Protein-Losing-Enteropathy

QOL Quality-of-life

TLI Trypsin-Like Immunoreactivity

UPCR Urine Protein:Creatinine Ratio

Introduction

Cobalamin, also referred to as vitamin B12, is a water-soluble vitamin derived from animal products, especially red meat, dairy, and eggs (Antony 2003). Cobalamin is ingested bound to animal protein and then released in the stomach under the action of activated pepsin and gastric acid (Qureshi *et al.* 1994). Free gastric cobalamin binds to the R-protein before binding to intrinsic factor. Intrinsic factor is a glycoprotein produced by gastric parietal cells and the canine pancreas. In dogs, only a minor fraction of intrinsic factor is produced in the stomach (Marcoullis & Rothenberg 1981, Batt & Horadagoda 1989). This allows for absorption within the distal ileum (Marcoullis & Rothenberg 1981, Batt & Horadagoda 1989, Steiner 2016). The cobalamin/intrinsic factor complex binds to specific cubam receptors localised within the brush border of the ileal enterocytes. Approximately 1% of dietary cobalamin is absorbed via passive

55 diffusion across the entire length of the intestinal mucosal epithelium, in addition to the
56 receptor-mediated cobalamin uptake by the ileal enterocytes.
57
58 In hypocobalaminaemia, the enzymatic reactions where cobalamin is involved as a cofactor
59 are inhibited (e.g. conversion of L-methylmalonyl-CoA into succinyl-CoA), leading to
60 accumulation of methylmalonic acid which is excreted in the urine. Methylmalonic acid
61 concentrations can be measured either in serum (Vaden *et al.* 1992, Ruaux *et al.* 2009,
62 Berghoff *et al.* 2012) or urine (Fyfe *et al.* 1991, Lutz *et al.* 2012), where in people, its
63 concentration is up to 40-fold higher than in serum (Norman & Cronin 1996). Elevations in
64 methylmalonic acid can hence serve as a marker of cellular cobalamin deficiency (Savage *et*
65 *al.* 1994, Berghoff et al. 2012, Kather *et al.* 2020). Apart from cobalamin deficiency, increased
66 methylmalonic acid concentrations can occur in renal disease, plasma volume contraction and
67 primary abnormalities in hepatic methylmalonyl-CoA mutase activity (Carmel *et al.* 2003,
68 Ruaux *et al*. 2013), but this has never been demonstrated in dogs.
69
70 Hypocobalaminaemia has been reported in dogs with different medical conditions including
71 Imerslund-Gräsbeck syndrome, chronic enteropathies, canine parvovirosis, alimentary or
72 multicentric lymphoma, and exocrine pancreatic insufficiency (EPI) (Kather *et al.* 2020,
73 Engelbrecht *et al*. 2022). In chronic enteropathy, the prevalence of hypocobalaminaemia
74 ranges from 19% to 38% (Heilmann *et al.* 2016a, Heilmann *et al.* 2016b, Volkmann *et al.* 2017,
75 Heilmann *et al.* 2018). Historically, parenteral cobalamin supplementation was recommended
76 over oral supplementation as the first line treatment in dogs with hypocobalaminaemia
77 secondary to chronic enteropathy (Hall & Day 2016), and it has been shown to result in
78 eucobalaminaemia and reduction in methylmalonic acid concentration (Ruaux *et al.* 2005,
79 Toresson *et al.* 2019). However, several studies comparing oral cobalamin supplementation
80 with parenteral cobalamin supplementation in people with hypocobalaminaemia have shown
81 equal efficacy (Kuzminski *et al.,* 1998; Bolaman *et al.,* 2003; Castelli *et al.,* 2011; Kim *et al.,*
82 2011). Moreover, studies have shown that oral cobalamin supplementation was non-inferior to

parenteral cobalamin supplementation in normalising serum cobalamin concentration in dogs with chronic enteropathy (Toresson *et al.* 2018, Toresson *et al.* 2019, Chang *et al.* 2022) and dogs with EPI (Toresson *et al.* 2021, Chang *et al.* 2022). More recently, the efficiency of oral cobalamin supplementation at treating hypocobalaminaemic dogs with hereditary intestinal cobalamin malabsorption was also demonstrated in a study published by Kook & Hersberger (2019). Despite promising results, it remains unclear whether treatment efficiency and tolerance is comparable between oral cobalamin supplementation and parenteral cobalamin supplementation in dogs suffering with severe chronic enteropathy (severe clinical presentation, severe hypocobalaminaemia or protein-losing enteropathy; PLE).

The primary aim of this study was to prospectively determine whether oral cobalamin supplementation is as effective as parenteral cobalamin supplementation at restoring eucobalaminaemia in dogs with PLE, severe chronic enteropathy (as defined by severity of clinical signs) or severe hypocobalaminaemia secondary to chronic enteropathy. We hypothesised that oral cobalamin supplementation is non-inferior to parenteral cobalamin supplementation in each setting. The secondary aim of this study was to evaluate ease of administration and tolerance for both types of administration by assessing pet owners' opinions on the protocols used. We hypothesised that oral cobalamin supplementation protocols are perceived as less stressful and better tolerated compared to parenteral cobalamin supplementation protocols.

Materials & Methods

Study design, cobalamin supplementation and inclusion/exclusion criteria: This controlled, randomised, multicentric, non-inferiority study was conducted in dogs with hypocobalaminaemia secondary to chronic enteropathy. Three UK-based small animal referral centres participated in the study.

Dogs with clinical signs of chronic enteropathy and hypocobalaminaemia (serum cobalamin concentration <250 ng/L (reference interval: 240-590 ng/L) were recruited in a prospective manner. Cases were enrolled from August 2018 to April 2020. Dogs with chronic enteropathy were characterised by chronic persistent or recurrent clinical signs of gastrointestinal disease (such as vomiting, diarrhoea, weight loss, or a combination of those) for at least three weeks. Written informed consent was obtained from owners or authorised agents for each dog to participate in the study. Owners received an information form to optimise their understanding of the study protocols.

The study was approved by an ethics committee in March 2018 (reference number SN1702). Local ethical approval was obtained by participating centres in accordance with their local regulations.

At the time of presentation, each dog underwent a mandatory workup which included a serum biochemistry, complete blood cell count, serum cobalamin concentration, trypsin-like immunoreactivity (cTLI), and serum methylmalonic acid concentrations, all assessed by the same commercial laboratory. The canine inflammatory bowel disease activity index (CIBDAI) was also completed for each dog (Jergens *et al.* 2003). Additional investigations, clinical management and treatment were determined at the discretion of the clinician in charge of each respective case. Diet was not standardised prior to the study or during the study. Dogs were fasted for at least 12 hours prior to blood sampling.

Using block randomisation (Excel®, Microsoft Office 2016), dogs were randomly assigned to oral cobalamin supplementation (Cobalapex®, Protexin, UK) or parenteral cobalamin supplementation (Vitbee 250®, Dechra, UK).

Dogs enrolled in the oral cobalamin supplementation group received cyanocobalamin based on their weight orally once daily for 12 weeks as recommended by the manufacturer, which is equivalent to a minimum dose of 25ug/kg (Table 1). Dogs enrolled in the parenteral cobalamin supplementation group received a weekly subcutaneous cyanocobalamin injection for 6 weeks and a seventh dose 4 weeks later, at a minimum dose of 25ug/kg of cyanocobalamin per injection (Table 1).

In all dogs, physical examination, body weight, serum folate concentration, serum cobalamin concentration and CIBDAI score were repeated at week 7. Physical examination, body weight, body condition score, serum biochemistry, complete blood count, serum folate concentration, serum cobalamin concentration, CIBDAI score, and serum methylmalonic acid concentration were repeated at week 13. Treatment failure was defined by recurrence of hypocobalaminaemia at week 13.

At the end of data collection, dogs were additionally grouped by several clinical characteristics to allow statistical analysis: presumptive PLE chronic enteropathy (defined as dogs with gastrointestinal signs and serum albumin below the reference interval, absence of azotaemia, absence of significant proteinuria, and absence of hyperbilirubinaemia) compared to non-PLE chronic enteropathy, moderate to severe hypocobalaminaemia (defined as a serum cobalamin concentration of <200ng/L) compared to mild hypocobalaminaemia (defined as a serum cobalamin between 200 and 250 ng/L), severe clinical disease based on CIBDAI score categories (CIBDAI >9 compared to CIDAI score ≤9).

Dogs were excluded from the study if they had received cobalamin administration in the 12 weeks preceding the study, or if there was a known hypersensitivity to active ingredients and/or excipients of the oral or injectable cobalamin products. Enrolment to the study was terminated if recruited dogs developed concomitant disease, if oral or injectable treatment was interrupted, if cobalamin supplement dosing errors occurred, or upon owner’s withdrawal of consent.

Owner questionnaire design:

Owners were asked to complete a Treatment Adherence & Satisfaction Questionnaire (Table S1) and a Treatment Tolerance Questionnaire (Table S2 & S3) at week 13. The Treatment Adherence & Satisfaction Questionnaire was identical for both groups, the Treatment Tolerance Questionnaire was different as questions specific to the type, protocol and duration of cobalamin were required (Table S2 & S3). An additional Treatment Palatability Questionnaire was also completed by owners of dogs in the oral cobalamin supplementation group (Table S4). In the absence of pre-existing validated scores, the questionnaires and the scoring system used were designed for the purpose of this study. For each questionnaire designed, a high score signified an excellent satisfaction/ treatment tolerance. Inversely, a low score implied a poor satisfaction/ treatment tolerance.

The Treatment Adherence & Satisfaction Questionnaire included 8 questions divided into 3 categories aiming at assessing the ease of treatment administration (administration, treatment planning, observance), any perceived stress caused by treatment administration (to the owner and to the dog), and the owner's overall satisfaction. A score from 0 to 4 was allocated to each answer, which provided a Treatment Adherence & Satisfaction Score rated out of 32 (Table S1). A score of 32 was consistent with perceived perfect ease of administration. Additionally, owners were asked whether they would choose the same treatment should their dog require cobalamin supplementation in the future. By extracting replies to a subgroup of questions within the Treatment Adherence & Satisfaction Questionnaire, an Owner Satisfaction Score out of 8 was also calculated (Table S2).

The Treatment Tolerance Questionnaire and the Treatment Palatability Questionnaire for dogs receiving oral cobalamin supplementation (Table S3 & S4) included 22 questions scored via a 5-point Likert scale, divided into 4 groups aimed at assessing tolerance of taking capsules or tablets before the study (5 questions), assessing tolerance of taking cobalamin capsules during

the study (5 questions), assessing behavioural signs of stress while taking the cobalamin capsules during the study (11 questions), and finally, 1 question regarding the technique owners used to administer the cobalamin capsules. Following completion, a score ranging from 0 to 4 or from 0 to 8 was allocated to each answer. The Oral Cobalamin Supplementation Tolerance Score was a mean of subscores from questions related to dog's response to taking cobalamin capsules and behavioural changes when being given the cobalamin supplement, providing a final score rating out of 72, the highest score indicating perfect tolerance to treatment. To determine whether the cobalamin capsules were well tolerated compared to other types of capsules/tablets administered prior to this trial, we compared the Oral Capsule Tolerance Score Before Trial designed from the 5 questions related to tolerance taking capsules or tablets before the study to the Oral Capsule Tolerance Score During Trial designed from the 5 questions related to tolerance of taking Cobalaplex® capsules during the study. Both scores were calculated to obtain a final score rated out of 10, a score of 10 indicating complete tolerance to treatment. The nature of the tablets or capsules administered prior to this trial was not documented.

The Treatment Tolerance Questionnaire for dogs receiving parenteral cobalamin supplementation (Table S5) included 23 questions divided into three categories as follows: dog's response and tolerance to visiting the veterinarian before this trial (11 questions), dog's response to visiting the veterinarian for the cobalamin injections (12 questions), including 11 questions about the dog's behaviour at the veterinarian before the injection and 1 question about the dog' response to the cobalamin injections. A score ranging from 0 to 4 was allocated to each answer. The Parenteral Cobalamin Supplementation Tolerance Score was a mean of subscores from questions related to dog's response to visiting the veterinarian for the cobalamin injections, which provided a total score rated out of 48, the highest score indicating perfect tolerance to treatment. To determine tolerance of the visits at the veterinary clinic to administer cobalamin injections, the Veterinarian Visit Tolerance Score Before Trial designed from 11 questions, were compared to the Veterinarian Visit Tolerance Score During Trial

designed from 12 questions. Both scores were calculated to obtain a number out of 10 (Table S5), a score of 10 indicating complete absence of anxiety during veterinary visits. The purpose of previous visits at a veterinary clinic and/or the nature of injections undertaken during consultations prior to this trial were not documented.

Blood sample processing: Routine bloods and serum collected for methylmalonic acid assessment were sent to the same commercial laboratory by each participating centre within 48h of collection using priority delivery (Veterinary Pathology Group, Exeter, United-Kingdom). Serum collected for methylmalonic acid assessment was refrigerated within 2 hours of collection, frozen at -20°C on the same day upon arrival at this laboratory, and later sent as a batch on dry ice, to a different branch for analysis (Synlab laboratory, Augsburg, Germany).

Assays: Serum cobalamin concentration was assessed using a chemiluminescent assay (Immulite 2000 Vitamin B12, Siemens Healthcare Diagnostics) which has been validated in dogs (Grutzner *et al.* 2016) and has proved good analytical performance (McLeish *et al.* 2019). Methylmalonic acid was analysed using a liquid chromatography–mass spectrometry method. The reference interval used for canine serum methylmalonic acid was 415 to 1193 nmol/L, as previously determined (Berghoff *et al.* 2012).

Data analysis:

A commercially available statistical software (R 4.1.3, R Core Team, 2022) was used for all data analyses. Continuous data were assessed for normality using the Shapiro Wilk test and presented as mean ± sd if normally distributed or median +/- range if not. Mann–Whitney U test was used for non-normally distributed variables and t-test used for normally distributed variables comparisons. The Wilcoxon rank sum test was used for age and weight comparison and the Fisher's exact test was used for duration of symptoms comparison. Statistical significance for all tests was set at $P<0.05$. A Chi-squared test was used to assess gender distribution.

Multivariable mixed-effects linear models were run in the oral cobalamin supplementation and parenteral cobalamin supplementation dogs included in the study, and also in each dog category, for each of the seven following outcome variables: cobalamin, body weight, body condition score (BCS), CIBDAI score, serum folate concentration, serum methylmalonic acid concentration, and every scores from the different questionnaires (Oral Cobalamin Supplementation Tolerance Score, Oral Capsule Tolerance Score Before Trial, Oral Capsule Tolerance Score During Trial, Parenteral Cobalamin Supplementation Tolerance Score, Treatment Adherence & Satisfaction Questionnaire, Treatment Adherence & Satisfaction Score, Veterinarian Visit Tolerance Score Before Trial, Veterinarian Visit Tolerance Score During Trial). In addition, changes were assessed as absolute values for each outcome from baseline to week 7 (where available), and baseline to week 13.

Results

Case recruitment

Thirty-seven dogs were enrolled in the study and randomly assigned treatment as follows: n=18 in the oral cobalamin supplementation group, and n=19 in the parenteral cobalamin supplementation group. Nineteen dogs completed the study: n=11 in the oral cobalamin supplementation group, and n=8 in the parenteral cobalamin supplementation group. From the oral cobalamin supplementation group, seven dogs were excluded from analysis, due to lack of follow-up (n=4), or euthanasia due to clinical deterioration (n=3). From the parenteral cobalamin supplementation group, 11 dogs were excluded from analysis, due to lack of follow-up (n=8), euthanasia due to clinical deterioration (n=2), or euthanasia due to a comorbidity (n=1).

Signalment, clinical signs, body weight, body condition score, CIBDAI score

The 19 dogs completing the study represented 13 different breeds. Labrador retrievers (n=5), Cairn Terriers (n=2), and mixed breed dogs (n=2) were the most commonly represented breeds (Table 2). There were 9 female neutered dogs (47.4%), 2 entire male dogs (10.5%) and 8 neutered male dogs (42.1%) (Table 2). There was no difference in gender distribution amongst treatment groups (P=0.156).

The mean (+/-sd) age was 6.62 years (+/-3.55 years). Dogs in the oral cobalamin supplementation group received a median daily cobalamin dose of 33.2 mg/kg (range: 28.1-42.2 mg/kg), and dogs in the parenteral cobalamin supplementation group received a median weekly cobalamin dose of 30.5 mg/kg (range: 22.7-43.9 mg/kg). There was no significant difference of cobalamin dose per kg body weight per administration between the two groups (P=0.3856).

The most common clinical signs were diarrhoea (n=18), vomiting (n=15), inappetence/anorexia (n=13), weight loss (n=11) and lethargy (n=11) (Table 2). Other clinical signs included

abdominal distension (n=8), borborygmi (n=3), prayer stance (n=2), constipation (n=2) and pica (n=1). Most dogs (11/19, 57.9%) had shown clinical signs of gastrointestinal disease for one month to one year. However, a large proportion of dogs also presented signs for more than three weeks but less than one month (7/19, 36.8%) (Table 2).

At inclusion, the median (range) body weight was 16.9 kg (6.1-44 kg), and the median (range) body condition score was 4/9 (2/9-7/9). Body weight and body condition score were not significantly different between dogs assigned to receive oral cobalamin supplementation and dogs assigned to receive parenteral cobalamin supplementation (P=0.103 and P=0.933, respectively).

At week 13, body condition score was not significantly different in the oral cobalamin supplementation group (median 5 +/- 1.27) and in the parenteral cobalamin supplementation group (median 5 +/- 1.11) (P=0.377). The change in body weight from baseline to week 13 was +2 kg in the oral cobalamin supplementation group (95% CI [0.62, 3.38]) and +0.51 kg in the parenteral cobalamin supplementation group (95% CI [-1.28, 2.31]), and was not significantly different (+1.49 kg, 95% CI [-0.63, 3.6], P=0.21). The change in body condition score from baseline to week 13 was also not significantly different in the oral cobalamin supplementation group (P=0.07), and in the parenteral cobalamin supplementation group (P=0.511).

Both treatment groups included, dogs with a CIBDAI score >9 at inclusion gained significantly more weight than dogs with a CIBDAI score ≤9 (+2.81 kg, 95% CI [1.35, 4.27], P=0.005), with no significant difference between dogs with a CIBDAI score >9 in the oral cobalamin supplementation and in the parenteral cobalamin supplementation groups (P=0.076). There was no significant change in body condition score between baseline and week 13 in dogs with a CIBDAI score >9 (P=0.139), and also in dogs with a CIBDAI score ≤9 (P=0.135).

At inclusion, the median CIBDAI score was 8 (range: 3–17) in the oral cobalamin supplementation group and 10 (range: 5–17) in the parenteral cobalamin supplementation

group, and was not statistically different between the oral cobalamin supplementation and the parenteral cobalamin supplementation groups (P=0.77). The change in CIBDAI score between week 0 and week 7 was -7.27 (95% CI [-8.49, -6.06]) in the oral cobalamin supplementation group and -7.38 (95% CI [-8.7, -6.01]) in the parenteral cobalamin supplementation group. The change in CIBDAI score between week 0 and week 13 was -8.27 (95% CI [-9.49, -7.06]) in the oral cobalamin supplementation group and -8.38 (95% CI [-9.74, -7.01]) in the parenteral cobalamin supplementation group. There was no significant difference in reduction of CIBDAI between the two treatment groups at week 7 (P=0.932) or week 13 (P=0.49). Eight dogs had severe CIBDAI scores at the start of the study (CIBDAI>9), including 4 dogs in the oral cobalamin supplementation group and 4 dogs in the parenteral cobalamin supplementation group.

Haematology, serum biochemistry, and other investigative procedures

The most common haematological changes were neutrophilia (n=7) and leucocytosis (n=4). Anaemia (n=2), eosinopenia (n=2), monocytosis (n=2), eosinophilia (n=1), thrombocytopenia (n=1) and thrombocytosis (n=1) were also identified (Table S4). The most common biochemical changes were panhypoproteinaemia (n=7/19, mean total protein concentration in these 7 hypoproteinaemic dogs = 37.9 +/- 9.15 g/L, reference interval: [54-77 g/L]), hypoalbuminaemia (n=7/19, mean albumin concentration in these 7 hypoalbuminaemic dogs = 13.8 +/- 3.8 g/L, reference interval: [25-40 g/L]), hypocholesterolaemia (n=6, reference interval: [3.8-7 mmol/L]), and increased ALT (n=6, reference interval: [5-66 U/L]). Among hypoalbuminaemic dogs, serum albumin concentration was below 20g/L in all dogs (range: 9-19 g/L). Increased ALP (n=3, reference interval: [0.1-150 U/L]) and azotaemia (n=2, creatinine reference interval: [40-150 umol/L], urea reference interval: [3-9 mmol/L]) were also documented (Table S6).

Two dogs had subnormal serum TLI concentrations of 3.2 and 4 ng/mL (reference interval: 5-40 ng/mL). TLI concentration in these dogs was not reassessed during the study. Hypofolataemia, suggestive of proximal small intestinal malabsorption, was more frequent

(n=7, mean 4.4 +/- 1.7 ug/L, reference interval: [7.2-23.8 ug/L]) than hyperfolataemia (n=1). Basal cortisol and ACTH stimulation testing were conducted in 5 dogs and 1 dog respectively. Results of faecal parasitology testing (n=2), ultrasonography (n=11), abdominal Computed Tomography scan (n=1) and histopathology of endoscopy-guided intestinal biopsies (n =9) were available in 13 dogs. Full urinalysis including urine protein:creatinine ratio (UPCR) was available in 3 dogs, UPCR alone was available in 4 additional dogs, and a bile acid stimulation test was performed in 1 dog. Based on these results, 7 dogs were diagnosed with presumptive PLE chronic enteropathy, including 3 dogs in the oral cobalamin supplementation group and 4 dogs in the parenteral cobalamin supplementation group. Among the remaining non-PLE chronic enteropathy dogs, 8 dogs received oral cobalamin supplementation and 4 dogs received parenteral cobalamin supplementation.

Definitive diagnosis and concurrent diseases

A definitive diagnosis of inflammatory chronic enteropathy was achieved with endoscopy-guided intestinal biopsies in 9 dogs. Concurrent diseases included chronic kidney disease (CKD) (n=3), pulmonary carcinoma (n=1), primary hyperadrenocorticism (n=1), immune-mediated haemolytic anaemia (n=1), stump pyometra (n=1), and idiopathic hyperlipidaemia (n=1).

Concurrent treatments

Details of concurrent treatments are available for 17/19 dogs, and information on dietary recommendations is available for 15/19 dogs (Table S7).

Serum cobalamin concentration

At inclusion, mean serum cobalamin concentration was 188 ng/L (sd +/- 33) in the oral cobalamin supplementation group and 204 ng/L (sd +/- 30) in the parenteral cobalamin supplementation group, and there was no significant difference between the two treatment groups (CI 95% [-3.78, 4.22], P=0.919). Twelve dogs had severe hypocobalaminaemia at

inclusion (cobalamin ⩽200 ng/mL), including 7 dogs in the oral cobalamin supplementation group and 5 dogs in the parenteral cobalamin supplementation group.

At week 7, serum cobalamin concentrations were significantly higher in the oral cobalamin supplementation group (mean 1931 ng/L; sd +/- 167) compared to the parenteral cobalamin supplementation group (mean 914 ng/L; sd +/- 427) (P<0.001).

At week 13, serum cobalamin concentrations were also significantly higher in the oral cobalamin supplementation group (mean 1750 ng/L; sd +/- 517) compared to the parenteral cobalamin supplementation group (mean 515 ng/L; sd +/- 227) (P<0.001) (Figure 1).

In the parenteral cobalamin supplementation group, the mean increase in serum cobalamin concentration was 644 ng/L (95% CI [410.81, 878.14]) between week 0 and week 7, and 372 ng/L (95% CI [138.23, 605.56]) between week 0 and week 13. In the oral cobalamin supplementation group, the mean increase in serum cobalamin concentration was 1791 ng/L (95% CI [1583.52, 1998.15]) between week 0 and week 7, and 1518 ng/L (95% CI [1310.94, 1725.57]) between week 0 and week 13. When comparing both groups, the mean increase in cobalamin between week 0 and week 7 was significantly higher in the oral cobalamin supplementation group compared to the parenteral cobalamin supplementation groups (P<0.001) (Figure 1). The mean increase in cobalamin between week 0 and week 13 was significantly higher in the oral cobalamin supplementation group compared to the parenteral cobalamin supplementation group (P<0.001) (Figure 1).

Treatment failure was identified in one dog in the parenteral cobalamin supplementation group, despite reaching eucobalaminaemia at week 7.

Methylmalonic acid concentrations

On baseline, 10/11 samples were available for serum methylmalonic acid assessment in the oral cobalamin supplementation group and 6/8 in the parenteral cobalamin supplementation group. At week 13, only 9/11 samples were available in the oral cobalamin supplementation group and 5/8 in the parenteral cobalamin supplementation group.

On admission, methylmalonic acid concentration was increased in 3/6 dogs in the parenteral cobalamin supplementation group and 4/10 in the oral cobalamin supplementation group. At week 13, methylmalonic acid concentration was persistently increased in 1/5 dogs in the parenteral cobalamin supplementation group and 1/9 in the oral cobalamin supplementation group (Figure 2).

From baseline to week 13, dogs receiving oral cobalamin supplementation experienced a decrease in methylmalonic acid concentration of 801.9 nmol/L (95% CI [-1065.7, -539.3]), and dogs receiving parenteral cobalamin supplementation a decrease of 632.8 nmol/L (95% CI [-1032.7, -232.7]). This was not significantly different between treatment groups (-169.9 nmol/L, 95% CI [-655.5, 309.1], P=0.454).

Questionnaires

Treatment Adherence & Satisfaction Questionnaires, Treatment Tolerance Questionnaires and Treatment Palatability Questionnaire were completed by owners in 16/19 dogs (oral cobalamin supplementation group n = 9, parenteral cobalamin supplementation group n = 7) at week 13.

Treatment Adherence & Satisfaction Score and Owner Satisfaction Score: At week 13, dogs in the oral cobalamin supplementation group had a median Treatment Adherence & Satisfaction Score of 32/32 (range: 27-32) compared to a median of 31/32 (range: 25-32) for dogs in the parenteral cobalamin supplementation group, which was not significantly different (2.22, 95% CI [-0.42, 4.86], P=0.093). At week 13, the Treatment Adherence & Satisfaction Score from dogs with severe hypocobalaminaemia at inclusion (<200 ng/L) (median 32/32, range 25-32) was compared to the Treatment Adherence & Satisfaction Score from dogs with mild to moderate hypocobalaminaemia (median 27/32, range 25-31), regardless of treatment group. The former rated 2.37/32 points higher which was statistically significant (95% CI [0.22-4.52], P=0.033). The Treatment Adherence & Satisfaction Score from dogs with severe hypocobalaminaemia at inclusion (<200 ng/L) in the oral cobalamin supplementation group

was significantly higher than in the parenteral cobalamin supplementation group (+3.11/32 points, 95% CI [0.81-5.41, P=0.012]). There was no statistical difference of the Owner Satisfaction Score between supplementation groups (P=0.55). Only one owner notified that they would have preferred the “other treatment modality”, should another cobalamin supplementation protocol be necessary. This dog belonged to the parenteral cobalamin supplementation group.

Oral Cobalamin Supplementation Tolerance Score, Oral Capsule Tolerance Score Before Trial, Oral Capsule Tolerance Score During Trial: Dogs treated with oral cobalamin had a median Oral Cobalamin Supplementation Tolerance Score of 8.6/10 (range: 4.4-10) compared to a median Parenteral Cobalamin Supplementation Tolerance Score of 7.7/10 (range: 5-9.2) in dogs treated with parenteral cobalamin, which was not significantly different (P=0.22). Dogs in the oral cobalamin supplementation group treated with oral tablets and/or capsules before this trial, had a median Oral Capsule Tolerance Score Before Trial of 6.4/10 (range: 0-10). The same dogs given oral cobalamin capsules at home had a median Oral Capsule Tolerance Score During Trial of 8.6/10 (range: 0-10). There was no significant difference between the Oral Capsule Tolerance Score Before Trial and the Oral Capsule Tolerance Score During Trial (P=0.44). Owners reported the technique they used to administer the “Cobalaplex®” capsules in 9 dogs. One capsule administration technique was used in 6 dogs, and 2 different techniques were used in 3 dogs, as follows: capsule unopened (entire) hidden in the dog’s regular food (n=5), the entire unopened capsule given alone (n=3), wrapped in a treat (n=3), capsule opened and sprinkled on food (n=1). Although reduced appetite was documented in most dogs at inclusion (13/19), major difficulties at administering the cobalamin capsules were reported in only 1 dog (1/11) in the oral cobalamin supplementation group. In this dog, the Oral Capsule Tolerance Score Before Trial (4/28) was however similar to the Oral Cobalamin Tolerance Score During Trial (4/28), its serum cobalamin concentration normalised at week 13, and its owner satisfaction was excellent (32/32).

Parenteral Cobalamin Supplementation Tolerance Score, Veterinarian Visit Tolerance Score Before Trial, and Veterinarian Visit Tolerance Score During Trial: Dogs in the parenteral cobalamin supplementation group who had already attended visits and injections at a veterinary clinic before the trial, had a median Veterinarian Visit Tolerance Score Before Trial of 6.4 (range: 5.5-8.6). The same dogs given VitBee 250 injections at the veterinary practice had a median Veterinarian Visit Tolerance Score During Trial of 7.7 (range: 5-9.2). There was no significant difference between the Veterinarian Visit Tolerance Score Before Trial and the Veterinarian Visit Tolerance Score During Trial (P=0.60).

Comparison of clinical phenotypes by severity

Presumptive PLE chronic enteropathy versus non-PLE chronic enteropathy dogs at inclusion: At baseline, in dogs with PLE, serum cobalamin concentrations were not statistically different between dogs assigned to the oral cobalamin supplementation group (median 170 ng/L (range 150-242)) compared to dogs assigned to the parenteral cobalamin supplementation group (median 199 ng/L (range 197-252)) (P=0.372). At week 13, all dogs with PLE achieved eucobalaminaemia, regardless of treatment group. In dogs with PLE at week 13, the median cobalamin concentration was significantly higher in the oral cobalamin supplementation group (median 2000 ng/L (range: 2000-2000)), compared to the parenteral cobalamin supplementation group (median 614 ng/L (range: 505-786)) (P=0.043).

Regardless of treatment modality, the decrease in methylmalonic acid from baseline to week 13 was significantly lower in non-PLE dogs compared to PLE dogs (-70.56 mg/L, 95% CI [13.89, 127.24], P =0.020). The number of available results was insufficient to compare serum methylmalonic acid concentration in PLE dogs between oral cobalamin supplementation and parenteral cobalamin supplementation groups at baseline and week 13.

Severe versus moderate hypocobalaminaemia at inclusion: At week 13, all dogs with severe hypocobalaminaemia at inclusion achieved eucobalaminaemia, regardless of treatment group. At that time point, serum cobalamin concentration was significantly higher in dogs with severe

hypocobalaminaemia at inclusion in the oral cobalamin supplementation group (median 2000 ng/L (range: 412-2000)) compared to the parenteral cobalamin supplementation group (median 402 ng/L (range: 320-786)) (P=0.009). At week 13, there was no significant difference in serum methylmalonic acid concentration between oral cobalamin supplementation (median: 88.2 mg/L (range: 58.4-216)) and parenteral cobalamin supplementation (median: 79.1 mg/L (range: 58.4-82.6)) groups in dogs with severe hypocobalaminaemia at inclusion (P=0.45).

CIBDAI score ≤9 *versus >9 at inclusion:* In dogs with CIBDAI score >9, there was no difference in serum cobalamin concentration at baseline (P=0.885). The oral cobalamin supplementation group had a median of 201.5 ng/L (range: 159-242), and the parenteral cobalamin supplementation group a median of 212 ng/L (range: 150-252). At week 13, dogs with a CIBDAI score >9 within the oral cobalamin supplementation group (median 2000 ng/L (range: 2000-2000)) had a significantly higher serum cobalamin concentration than dogs in the parenteral cobalamin supplementation group (median 562.5 ng/L (range: 348-805)) (P=0.021). At week 13, there was no significant difference in serum methylmalonic acid concentration between oral cobalamin supplementation (median: 110.1 mg/L (range: 86.8-216)) and parenteral cobalamin supplementation (median: 112.7 mg/L (range: 58.4-167)) groups in dogs with CIBDAI >9 at inclusion (P=0.8).

Discussion

This study established non-inferiority of oral cobalamin supplementation compared to parenteral cobalamin supplementation, at restoring eucobalaminaemia and for treatment tolerance, when administered in hypocobalaminaemic dogs with chronic enteropathy. In particular, this included the subpopulation of dogs with severe clinical and/or biochemical presentation (“severe biochemical presentation” meaning “severe hypocobalaminaemia”).

However, we failed to demonstrate superiority at normalising serum cobalamin in the oral cobalamin supplementation group in this study. Although comparable performance of oral cobalamin supplementation and parenteral cobalamin supplementation at normalising serum cobalamin concentration had already been reported in previous studies (Toresson *et al.* 2018, Toresson *et al.* 2019), treatment tolerance and efficacy had never been specifically examined in dogs with high CIDBAI scores (>9), severe hypocobalaminaemia (<200 ng/L) or PLE. As reported in *Toresson et al.* publication (2019), our study also demonstrated efficacy and non-inferiority of oral cobalamin supplementation compared to parenteral cobalamin supplementation at improving cobalamin deficiency at a cellular level. We showed a significant decrease in serum methylmalonic acid concentration from baseline to week 13 in oral cobalamin supplementation and parenteral cobalamin supplementation groups, with no significant difference between treatment groups overall, including dogs with severe hypocobalaminaemia or high CIBDAI scores.

Dogs' and owners' QOL and owners' satisfaction during treatment were not significantly different between treatment groups, indicating that both are equally acceptable from a welfare perspective. Only one owner in the parenteral cobalamin supplementation group notified that they would have preferred the "other treatment modality", should another cobalamin supplementation protocol be necessary.

In both treatment groups, dogs with severe hypocobalaminaemia (<200 ng/L) had a significantly higher Treatment Adherence & Satisfaction Score compared to dogs with moderate hypocobalaminaemia. However, cobalamin supplementation was not the only treatment change undertaken during the study period, as diet and/or medications were also adjusted alongside based on clinicians' judgment. Therefore, it remains unclear whether cobalamin supplementation alone was the reason for higher dogs' and owners' QOL in dogs with severe hypocobalaminaemia. Nevertheless, the randomised design of the study would mitigate for this confounding factor.

This study also demonstrates that dogs' tolerance to oral cobalamin capsules was similar to their tolerance to other oral medication. Although poor oral treatment compliance was initially feared, as reduced appetite was documented in most dogs at inclusion, only 1 dog was described to show resistance to administering cobalamin capsules. Regardless of this, serum cobalamin concentration normalised in this dog at week 13 and owner satisfaction was excellent.

Although the mean increase in cobalamin between week 0 and week 13 was significantly higher in the oral cobalamin supplementation group compared to the parenteral cobalamin supplementation group, the decrease in serum methylmalonic acid was not significantly different between treatment groups. The decrease in serum methylmalonic acid was lower in the oral cobalamin supplementation group compared to the parenteral cobalamin supplementation group, however, this later difference did not reach statistical significance. We hypothesised that the low number of cases might have led to this result. Moreover, oral cobalamin supplementation delivered a large surplus of cobalamin. This could explain the significantly greater increase in cobalamin in the oral cobalamin supplementation group at week 7 and at week 13 compared to the parenteral cobalamin supplementation group. Interestingly, in a similar study conducted by Toresson et *al.* (2018), the increase in serum cobalamin concentration was significantly higher in the parenteral group than the oral group after 4 weeks, while the increase in cobalaminaemia was significantly lower in the parenteral cobalamin supplementation group than the oral cobalamin supplementation group at week 12. The reason for this different outcome halfway through the study remains unclear. As the first serum cobalamin reassessment was performed at week 4 in this study, compared to week 7 in our study, we hypothesize that serum cobalamin levels may be slower to increase with oral cobalamin compared to parenteral supplementation.

Although all dogs were hypocobalaminaemic at inclusion, only around half had evidence for cobalamin deficiency on a cellular level. The presence of dogs with normal serum

584 methylmalonic acid concentration at inclusion could be explained by a lack of genuine cellular
585 cobalamin deficiency (either because of mild or short duration cobalamin deficiency), a true
586 cellular cobalamin deficiency with an inadequate reference range of serum methylmalonic acid
587 concentration at determining an abnormal result, or because cobalamin metabolism in dogs
588 differs from that of humans, such that elevation in methylmalonic acid is not indicative of a
589 cellular cobalamin deficiency.
590
591 Serum cobalamin concentration was above the normal range in 8/11 dogs in the oral cobalamin
592 supplementation group at week 7 and 13, while they were above the reference in 0/8 dogs in
593 the parenteral cobalamin supplementation group at weeks 7 and 13. These results suggest
594 that a lower oral cobalamin dosage might be effective as demonstrated in people with Crohn's
595 disease (Gomollon *et al.* 2017). As the optimal dose of oral cyanocobalamin in
596 hypocobalaminaemic dogs with chronic enteropathy has not yet been fully determined, future
597 studies of possible dosing ranges are warranted.
598
599 Limitations of this study included small sample size, presumptive rather than definitive
600 diagnosis of CE, and non-controlled diets or adjunctive treatments.
601 Gastrointestinal biopsies and histopathology were lacking in 10 dogs and faecal parasitology
602 was lacking in 17 dogs. Similarly, a definitive diagnosis of PLE was hampered by incomplete
603 availability of diagnostic tests to rule out hepatopathies and protein-losing nephropathy in most
604 dogs. The multicentric nature of the study, and the permission for adjunctive treatments to be
605 non-standardised resulted in variations in case management. Despite the prospective nature
606 of the study, numerous data points were missing which reduced the number of dogs included
607 in the final analysis.
608
609 Although cobalamin supplementation, either oral or parenteral, is suspected to remain the main
610 parameter contributing to changes in serum cobalamin concentration during the study period,

other factors such as dietary trials or concurrent medication may also have affected cobalaminaemia.

Mean serum cobalamin concentration was 163 pg/mL higher in dogs fed a standard dry commercial diet than dogs fed a standard raw diet in one prospective study. This highlights the possibility that diet at inclusion could have affected cobalaminameia and also that a dietary change undertaken during the study period might have contributed to the changes in serum cobalamin concentration (Anturaniemi et *al.* 2020). As the diet provided before the start of the trial was only documented in a few dogs, dietary cobalamin deficiency could not be fully excluded as a cause of hypocobalaminaemia. However, most commercial foods and non-vegetarian/non-vegan home-made foods are not restricted in cobalamin which makes dietary cobalamin deficiency unlikely.

As adjunctive treatments were not controlled at inclusion and during the study period, they could have had a potential effect on cobalaminaemia. Some of them, such as proton pump inhibitors or probiotics could have contributed to a decrease in serum cobalamin concentration, as shown in people (Lam *et al.* 2013) and dogs (Lucena *et al.* 2018) respectively. Antibiotics could also have interfered with serum cobalamin concentration by altering intestinal microbiota resulting in intestinal dysbiosis (Suchodolski 2016). We also hypothesise that steroids could affect cobalamin intestinal absorption by reducing ileal inflammation.

As well as containing cobalamin, Cobalaplex® capsules contain folate and a prebiotic (fructo-oligosaccharide) which could have provided an additional clinical benefit to the oral cobalamin supplementation group.

In the absence of pre-existing validated scores to assess owners' satisfaction and treatment palatability, the questionnaires and the scoring system used were designed for the purpose of this study, and these scores have not been validated in clinical studies. Reliability on subjective owners' assessment is also a significant limitation of these questionnaires, partly as owners were not blinded to treatment group.

Methylmalonic acid concentration was assessed by Synlab laboratory (Augsburg, Germany) which did not establish its own reference interval. Instead, we used the reference interval established from 43 healthy dogs ([414.7–1192.5nmol/L]), published by *Berghoff et al.* (2012), referenced in *Toresson et al.* (2019) publication. Both laboratories use the same assay (chromatography - mass spectrometry method). The only other methylmalonic acid reference interval published was established from 48 healthy dogs ([393-1,476nmol/L]), referenced in *Kook et al.* publication (2019). A mild difference in methylmalonic acid reference interval exists between laboratories. ~~At the present time, the Gastrointestinal laboratory provides results from the largest healthy canine population (43 healthy dogs).~~

Lastly, the lack of long-term follow-up did not allow assessment of the sustainability of treatment efficacy.

Conclusion

This study has demonstrated that oral cobalamin supplementation was well tolerated and non-inferior to parenteral cobalamin supplementation at normalising serum cobalamin concentration and decreasing serum methylmalonic acid concentration in dogs with hypocobalaminaemia due to chronic enteropathy, including subgroups with severe clinical or biochemical abnormalities. Oral cobalamin supplementation and parenteral cobalamin supplementation yielded similar tolerance and owners' satisfaction scores, even in severely affected dogs. This emphasises that the severity of chronic enteropathy should not preclude the use of oral cobalamin supplementation in these dogs.

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