The Veterinary Journal 232 (2018) 27–32

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Original Article

Comparison of efficacy of oral and parenteral cobalamin supplementation in normalising low cobalamin concentrations in dogs: A randomised controlled study

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ARTICLE INFO

Article history:
Accepted 11 December 2017

Keywords:
Canine
Chronic enteropathy
Inflammatory bowel disease
Oral supplementation
Vitamin B12

ABSTRACT

The aim of this study was to compare the efficacies of parenteral and oral cobalamin supplementation protocols in dogs with chronic enteropathies and low cobalamin concentrations. It was hypothesised that both treatments would increase serum cobalamin concentrations significantly. Fifty-three dogs with chronic enteropathies and serum cobalamin concentrations < 285 ng/L (reference interval 244–959 ng/L) were enrolled. Dogs were randomised to treatment with either daily oral cobalamin tablets (0.25–1.0 mg cyanocobalamin daily according to body weight) or parenteral cobalamin (0.4–1.2 mg hydroxycobalamin according to body weight). Serum cobalamin concentrations were analysed 28 ± 5 days and 90 ± 15 days after initiation of supplementation. After 28 days, all dogs had serum cobalamin concentrations within the reference interval or above. In the parenteral group ($n = 26$), median (range) cobalamin concentrations were 228 (150–285) ng/L at inclusion, 2107 (725–10,009) ng/L after 28 days and 877 (188–1267) ng/L after 90 days. In the oral group ($n = 27$), median (range) serum cobalamin concentrations were 245 (150–285) ng/L at inclusion, 975 (564–2385) ng/L after 28 days and 1244 (738–4999) ng/L after 90 days. In both groups, there were significant differences in serum cobalamin concentrations between baseline and 28 days, and between 28 days and 90 days ($P < 0.001$). In conclusion, both parenteral and oral cobalamin supplementation effectively increase serum cobalamin concentrations in dogs with chronic enteropathies and low cobalamin concentrations.

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Introduction

The gastrointestinal transport of cobalamin (vitamin B12) is a complex process, involving several carrier proteins, of which the most important is intrinsic factor (IF) (Banerjee, 2006). Final absorption from the gastrointestinal tract occurs in the ileum, where cobalamin bound to IF is absorbed. In human beings, an alternative route of absorption of cobalamin, independent of IF, has been reported (Berlin et al., 1968); using radioactively labelled cobalamin, ~1% free cobalamin was absorbed along the entire intestine by passive diffusion. However, this route of absorption has not been demonstrated formally in dogs.

Diseases thought to cause cobalamin deficiency in dogs are those that affect ileal receptors, those that cause decreased production of IF, and intestinal dysbiosis. The former can occur in chronic enteropathy (CE), intestinal lymphosarcoma (lymphoma), or familial selective cobalamin malabsorption, in which genetically defective cobalamin-IF receptors are expressed (Batt and Morgan, 1982; Fyfe et al., 1989; Cook et al., 2009; Grützner et al., 2010; Lutz et al., 2013). In canine exocrine pancreatic insufficiency (EPI), decreased production of IF causes hypocobalaminaemia (Batt et al., 1989; Batt et al., 1991). Intestinal dysbiosis may cause bacterial competition for nutrients, resulting in decreased amounts of cobalamin available for absorption in the ileum (Ruaux, 2013).

Hypocobalaminaemia results in a range of clinical and metabolic effects (Arvanitakis, 1978; Ruaux, 2013). Clinical signs in dogs with hypocobalaminaemia include anorexia, lethargy,

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https://doi.org/10.1016/j.tvjl.2017.12.010
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weight loss, failure to thrive, and central and/or peripheral neuropathies (Fyfe et al., 1989; Fordyce et al., 2000; Battersby et al., 2005; Lutz et al., 2013). Immunodeficiency and intestinal changes, such as villous atrophy and malabsorption, have been reported. Cobalamin deficiency has been associated with a negative prognosis in dogs with CE or EPI (Allenspach et al., 2007; Batchelor et al., 2007).

The current supplementation protocol for hypocobalaminaemic dogs calls for repeated parenteral injections (Ruaux, 2013). In human beings with cobalamin deficiency, all studies comparing oral and parenteral cobalamin supplementation have shown equal efficacy of oral supplementation compared to parenteral supplementation (Kuzminski et al., 1998; Bolaman et al., 2003; Castelli et al., 2011; Kim et al., 2011). A Cochrane review suggested that '*high oral doses of B12 could be as effective as intramuscular administration*' (Vidal-Alaball et al., 2005).

We have reported a retrospective study demonstrating successful oral cobalamin supplementation in 51 dogs with CE and hypocobalaminaemia (Toresson et al., 2016). However, there are no published studies comparing oral and parenteral cobalamin supplementation in dogs. The aim of the present study was to compare the efficacy of oral and parenteral cobalamin supplementation in dogs with CE and serum cobalamin concentrations at the lower end of the reference interval, or below. The hypothesis was that both protocols would significantly increase serum cobalamin concentrations.

Materials and methods

Animal inclusion

Inclusion criteria for the study were dogs with signs of CE and serum cobalamin concentrations ≤285 ng/L (reference interval 244–959 ng/L), representing the lower end of the reference interval, or below (referred to as 'low cobalamin' in the remaining text). Exclusion criteria were EPI without histologically verified CE, on-going cobalamin supplementation or intestinal neoplasia. Dogs were enrolled from Evidensia Specialist Animal Hospital, Helsingborg, Sweden, and Halsinge Small Animal Clinic, Sweden, from March 2014 to July 2016. The study was approved by the animal ethics committee of Uppsala University, Uppsala, Sweden (approval number C109/13; date of approval 27 September 2013).

Study design, baseline data and laboratory investigation

This was a prospective open, randomised controlled study. Serum cobalamin concentrations were measured at baseline, 28 ± 5 days and 90 ± 15 days after inclusion. The canine inflammatory bowel disease activity index (CIBDAI) was calculated at inclusion and at follow-up visits (Jergens, 2004). The CIBDAIs of the parenteral and oral groups were compared at each time point. Breed, age, medical history, body weight, body condition score (BCS) and findings on physical examination were recorded. Pet food manufacturers of all of the commercial diets that the dogs were fed at inclusion were contacted for information on cobalamin content. However, cobalamin concentrations were not confirmed by chemical analysis. Haematology profiles and selective serum biochemistry profiles were performed, including alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, total protein, albumin, haematocrit, total white blood cell count, folate, trypsin-like immunoreactivity (TLI) and canine pancreatic lipase immunoreactivity (cPL, as measured by Spec-cPL, IDEXX) or SNAP canine pancreatic lipase (SNAP-cPL, IDEXX). Faecal parasitology was performed, comprising three separate samples analysed by the sedimentation-flotation method and the IDEXX SNAP Giardia test or direct immunofluorescence assay. Abdominal ultrasound was performed on 51/53 dogs. Endoscopic biopsies of the stomach, small intestine and large intestine were collected; on average, eight biopsies were collected from each site in each dog. The biopsies were prepared routinely for histopathology at Biovet, Sollentuna, Sweden. Dietary changes and medical treatments during the study were based on clinical judgement (Table 1).

Medication history and diet

Twenty-four dogs were being treated for gastrointestinal disorders at the time of inclusion; corticosteroids ($n = 11$) were the most common medication. At the time of completion of the study, 41/49 dogs were receiving immunomodulatory treatment (Table 1). Three dogs had been supplemented with oral cobalamin previously; supplementation had ended 30–385 days prior to recurrence of low cobalamin levels.

Table 1
Medication and diet in 53 dogs with chronic enteropathy and low cobalamin concentrations; 49 dogs remained in the study after 90 days.

Parameter at inclusion or after 90 days	Variable	Number of dogs (%)
Treatment at inclusion	Corticosteroids[a]	11 (23)
	Cyclosporine[b]	4 (8)
	Antibiotics[c]	6 (11)
	Other[d]	9 (17)
Diet at inclusion	Kibble diet (KD)	41 (77)
	KD: 'Intestinal'	16 (30)
	KD; single protein	11 (21)
	KD; hydrolysed	2 (4)
	Home-cooked	8 (15)
	Raw food (commercial)	4 (8)
Diet change during study		33 (67)
Treatment after 90 days	Corticosteroids[e]	43 (88)
	Cyclosporine	4 (8)
	Chlorambucil[f]	9 (18)
	Antibiotics[g]	2 (49)
	Corticosteroids + miscellaneous[h]	21 (43)
	Miscellaneous[i]	3 (6)
Diet after 90 days	Kibble diet (KD)	45 (85)
	KD 'Intestinal'	14 (26)
	KD single protein	18 (34)
	KD hydrolysed	12 (23)
	Home-cooked	2 (4)
	Raw food (commercial)	2 (4)

[a] Corticosteroids (prednisolone, methylprednisolone or budesonide) ($n = 11$), alone or in combination with olsalazine ($n = 5$).
[b] Three dogs were treated with cyclosporine + corticosteroids.
[c] Metronidazole ($n = 4$), amoxicillin ($n = 1$), unknown antibiotics ($n = 1$).
[d] Omeprazole ($n = 3$), sucralphate ($n = 2$), $n = 1$ each: metoclopramide, chaolin clay, chaolin clay + probiotics, pancreatic enzymes, folate, clomipramine.
[e] Prednisolone/methylprednisolone ($n = 32$), budesonide ($n = 12$).
[f] All dogs were treated with corticosteroids + chlorambucil.
[g] Metronidazole.
[h] Folate ($n = 10$), psyllium ($n = 7$), olsalazine ($n = 7$), pancreatic enzymes ($n = 2$), sucralphate ($n = 2$).
[i] Folate ($n = 1$), psyllium ($n = 1$), metoclopramide ($n = 1$).

At inclusion, 39 dogs received commercial pet food kibble from major pet food companies, two dogs were fed kibble of an unknown brand, four dogs were fed various commercial raw food diets, and eight dogs received primarily a home-cooked meat-based diet (Table 1). Twenty-nine dogs were fed kibble diets labelled 'intestinal', 'single protein', or 'hydrolysed protein'. During the study, 33 dogs were switched to a new diet (Table 1). The cobalamin contents of 38/39 kibble diets were revealed by the manufacturers. On a dry matter (DM) basis, the median cobalamin content was 0.13 mg/kg (0.046–0.35). None of the diets contained less cobalamin than the American Association of Feed Control Officials minimum recommendation of 0.028 mg/kg DM.[1] The raw food manufacturer could not specify the cobalamin content of their diets. Furthermore, the cobalamin content of the home cooked diets was not available.

Cobalamin supplementation

Dogs were supplemented according to a block-randomised schedule designed by an external statistician. Dogs in the 'parenteral group' received one injection per week for 6 weeks, and an additional injection 4 weeks later according to a published protocol (Ruaux, 2013). Depending on body weight, dogs received 0.25–1.2 mg hydroxycobalamin (Behepan, 1 mg/mL, Pfizer) on each occasion. Dogs in the 'oral group' received oral cyanocobalamin (Behepan 1 mg, Pfizer) daily during the study. Dogs with a body weight ≤20 kg received 0.025 mg/kg body weight cyanocobalamin and dogs with a body weight >20 kg received 1 mg cyanocobalamin. Owner instructions included withholding the tablet on the day of the follow-up blood sample and bringing the pill container for tablet counting.

[1] See: http://www.merckvetmanual.com/management-and-nutrition/nutrition-small-animals/nutritional-requirements-and-related-diseases-of-small-animals (accessed 5 December 2017).

Cobalamin analysis

Serum samples were refrigerated within 2 h of collection, frozen at −20 °C for 1–3 days and sent to Evidensia Specialist Animal Hospital, Strömsholm, Sweden, with cold packs using priority delivery. Stable serum cobalamin concentrations have been demonstrated previously under similar storage conditions (Drammeh et al., 2008). The samples were analysed using an automated chemiluminescence immunoassay (Immulite 2000, Siemens Healthcare Diagnostics). Serum cobalamin concentrations were compared within each group. Increases in serum cobalamin concentrations relative to baseline were compared between the groups at 28 and 90 days.

Statistical analysis

Data were analysed using a commercial statistical software package (GraphPad Prism 6.0). All data sets were tested for normal distribution using the D'Agostino and Pearson omnibus normality test. None of the data sets (weight, BCS, CIBDAI, serum cobalamin concentrations) were normally distributed. The Mann–Whitney *U* test was used for CIBDAI comparisons and for comparisons of improvement of serum cobalamin concentrations between groups. The Wilcoxon matched-pairs signed rank test was used for comparisons of serum cobalamin concentrations by body weight and BCS. Statistical significance for all tests was set at $P < 0.05$.

Results

Baseline data and clinical signs

Initially, 53 dogs aged 1.5–13.1 (median 6.2) years were enrolled in the study (Table 2); 49 dogs remained at the second follow-up visit; reasons for drop-out were non-compliance with the treatment protocol ($n = 3$) or euthanasia ($n = 1$). Twenty-eight different breeds were included, of which the most common were Labrador retrievers ($n = 8$) and mixed breed dogs ($n = 7$) (Table 2). At the time of inclusion, the median (range) weight at inclusion was 14.1 (3.1–49.0) kg and the median (range) BCS was 4/9 (2/9–7/9). At the last follow-up, the median (range) body weight and BCS had increased significantly to 15.2 kg (4.2–50.7; $P < 0.003$) and 5/9 (1/9–7/9; $P < 0.001$; Table 2), respectively. The dog with a BCS of 1 at follow-up had a BCS of 2 at inclusion. The most common clinical signs were diarrhoea ($n = 30$), anorexia ($n = 24$) and vomiting ($n = 21$). Most dogs (34/53, 64%) had shown clinical signs of gastrointestinal disease for more than 1 year.

Table 2
Baseline data at inclusion for 53 dogs with chronic enteropathy and low cobalamin concentrations.

Parameter at inclusion or after 90 days	Variable	Range (median) or number of dogs (%)
Age at inclusion (years)	–	1.5–13.1 (6.2)
Body weight at inclusion (kg)	–	3.1–49.0 (14.1)
Body condition score at inclusion	–	2/9–7/9 (4/9)
Body weight at 90 days (kg)	–	4.2–50.7 (15.2)
Body condition score after 90 days	–	1/9–7/9 (5/9)
Breed	Labrador retriever	8 (15)
	Mixed breed	7 (13)
	German shepherd	6 (11)
	Bernese mountain dog	3 (6)
	Miscellaneous[a]	29 (55)
Major clinical signs	Diarrhoea	31 (58)
	Anorexia	24 (45)
	Vomiting	23 (43)
	Lethargy	18 (34)
	Weight loss	12 (23)
	Pica	5 (9)
Duration of clinical signs	Up to 1 month	1 (2)
	1 month to 1 year	14 (26)
	>1 year	38 (72)

[a] Twenty-four additional breeds, each represented by one or two individuals.

Haematology, serum biochemistry and ancillary laboratory data

Selected haematology and serum biochemistry data are detailed in Table 3. The most common changes were leucocytosis and decreased total protein and albumin concentrations. Two dogs had subnormal serum TLI concentrations of 1.0 and 2.7 μg/L (reference interval, 5.5–35 μg/L); both of these dogs had histologically verified chronic enteritis. Ten dogs had Spec cPL concentrations above the reference interval (0–200 μg/L), ranging from 213 to 795 μg/L. Results of parasitology, ultrasonography and histopathology of gastrointestinal biopsies are available as supplementary material (see Appendix).

Clinical diagnosis

Thirty-six of 49 (73.5%) dogs had CE, without protein-losing enteropathy (PLE) or EPI, responsive to immunomodulatory treatment. Ten of 49 (20.4%) dogs were classified as having PLE based on a serum albumin concentration < 20 g/L, of which two were also diagnosed with EPI. Six of 49 (12.2%) dogs had food-responsive diarrhoea and one dog (2.0%) had severe *Toxocara* sp. infestation and food-responsive diarrhoea.

Canine inflammatory bowel disease activity index

The median CIBDAI was 8 (range 2–14) in the parenteral group and 6.5 (range 2–13) in the oral group at the time of inclusion,

Table 3
Selected haematology and serum biochemistry data at inclusion in 53 dogs with chronic enteropathy and low cobalamin concentrations.

Parameter	Reference interval	Range (median) and number of dogs (%)
Increased total leukocyte count[a]	$6.2–11.4 \times 10^9$/L	$11.5–31.9 \times 10^9$/L (15.0) 23 (44)
Increased total neutrophil count[a]	$3.0–11.0 \times 10^9$/L	$12.1–22.0 \times 10^9$/L (13.6) 13 (30)
Increased total lymphocyte count[a]	$1.0–4.8 \times 10^9$/L	$5.0–5.3 \times 10^9$/L (5.2) 2 (5)
Increased total eosinophil count[a]	$0–1.25 \times 10^9$/L	$1.44–6.48 \times 10^9$/L (3.96) 2 (5)
Increased total monocyte count[a]	$0–1.35 \times 10^9$/L	$1.36–3.43 \times 10^9$/L (1.61) 10 (23)
Decreased haematocrit[a]	43–52%	25–42% (39) 15 (29)
Increased haematocrit[a]	43–52%	53–60% (53.5) 4 (8)
Decreased total protein[a]	65–75 g/L	26–64 g/L (57.5) 42 (81)
Decreased albumin[a]	29–39 g/L	14–28 g/L (23) 22 (42)
Increased spec cPL[b,c]	0–200 μg/L	213–795 μg/L (582) 10 (19)
Decreased TLI[d]	5.5–35 μg/L	1–2.7 μg/L (1.9) 2 (4)
Decreased folate	7–20 ng/mL	2–6 ng/mL (5) 15 (28)
Increased folate	7–20 ng/mL	21–>24 ng/mL (22) 6 (11)
Increased alanine aminotransferase	0–80 U/L	108–301 U/L (176) 5 (9)
Increased creatinine[e]	0.7–1.2 mg/dL	1.3–1.5 mg/dL (1.4) 2 (4)

[a] $n = 52$ (for all other data, $n = 53$ if not specified).
[b] Canine pancreatic lipase.
[c] Snap cPL was measured in 19/53 dogs and was normal in all dogs tested. Spec cPL was measured in 34/53 dogs; six dogs had Spec cPL concentrations > 400 μg/L (the cut-off limit for a suspected diagnosis of pancreatitis) and four dogs had Spec cPL concentrations of 200–400 μg/L.
[d] Trypsin-like immunoreactivity.
[e] Both dogs had normal creatinine and blood urea nitrogen concentrations, and normal urine specific gravity at next follow-up.

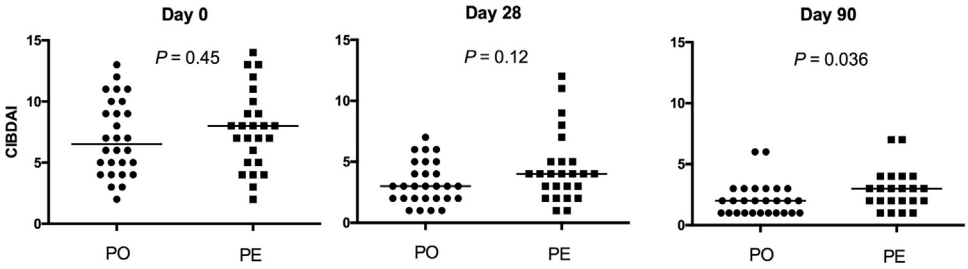

Fig. 1. Canine inflammatory bowel disease activity index (CIBDAI) on days 0, 28 and 90. Long horizontal lines represent medians. Data for the oral (peroral, PO) group are shown on the left in each panel ($n = 27$ at each time point) and data for the parenteral (PE) group are shown to the right ($n = 26$ on days 0 and 28; $n = 22$ on day 90). According to the CIBDAI interpretation, the PO group had moderate disease activity at inclusion, which decreased to clinically insignificant at days 28 and 90. The PE group had moderate disease activity at inclusion, mild disease at 28 days and clinically insignificant findings after 90 days.

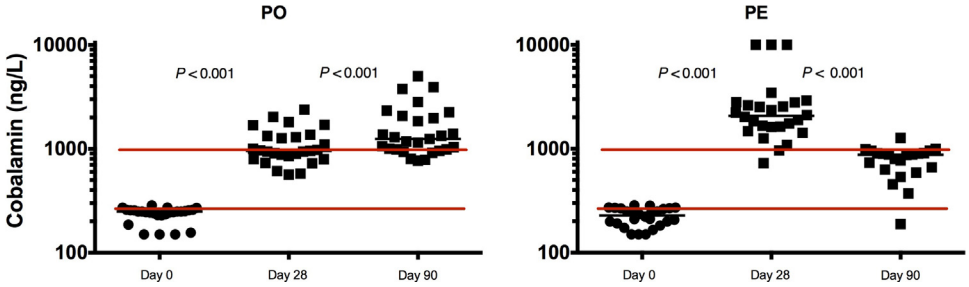

Fig. 2. Serum cobalamin concentrations on days 0, 28 and 90. PO, peroral (oral) group ($n = 27$). PE (parenteral) group ($n = 26$ on days 0 and 28; $n = 22$ on day 90). Long horizontal lines represent reference interval; shorter horizontal lines median, Log_{10} scale.

representing moderate clinical disease activity in both groups. In both groups, CIBDAI decreased during the study (Fig. 1). The median CIBDAI was 4 (range 1–12) in the parenteral group and 3 (range 1–7) in the oral group after 4 weeks, which decreased to 3 (range 1–7) and 2 (range 1–6), respectively, after 3 months. There were no statistically significant differences between the parenteral and oral groups at baseline or after 4 weeks ($P = 0.45$ and $P = 0.12$, respectively), whereas, after 3 months of treatment, the oral group had a significantly lower CIBDAI than the parenteral group ($P = 0.036$).

Serum cobalamin concentrations

Compliance with oral cobalamin supplementation, based on tablet count, was satisfactory in 22/27 patients after 28 days and 19/27 after 90 days (see Appendix: Supplementary material). Six dogs had undetectable serum cobalamin at time of enrolment (<150 ng/L). In the parenteral group, the median serum cobalamin concentration was 228 ng/L (range 150–285 ng/L) at the time of inclusion, 2065 ng/L (range 725–10,009 ng/L; $P < 0.001$) after 28 days and 874 ng/L (range 188–1267 ng/L; $P < 0.001$) after 90 days (Fig. 2). In the oral group, the median serum cobalamin concentration was 249 ng/L (range 150–285 ng/L) at the time of inclusion, 955 ng/L (range 564–2385 ng/L; $P < 0.001$) after 28 days, and 1244 ng/L (range 768–4999 ng/L; $P < 0.001$; Fig. 2) after 90 days. The increase in serum cobalamin concentration (Δ) was significantly higher in the parenteral group (median 1799 ng/L, range 575–9827 ng/L) than the oral group (696 ng/L, range 316–2235 ng/L; $P < 0.001$) after 4 weeks, while Δ was significantly lower in the parenteral group (600 ng/L, range 38–997 ng/L) than the oral group (1045 ng/L, range 516–4849 ng/L; $P < 0.001$) after 90 days of treatment (Fig. 3).

Discussion

This study compared oral and parenteral cobalamin supplementation in dogs with low-normal or decreased serum cobalamin concentrations and signs of chronic enteropathy; both treatment protocols resulted in significantly increased serum cobalamin concentrations. All dogs had serum cobalamin concentrations that were within or above the reference interval after 4 weeks of treatment. The increase in serum cobalamin concentration was significantly greater in the parenteral group than in the oral group after 4 weeks of treatment, but significantly greater in the oral group than in the parenteral group after 90 days of treatment. At this time point, the dogs in the parenteral group had the last injection 3–4 weeks earlier, which is the recommended time point for re-evaluating the efficacy of parenteral supplementation (Ruaux, 2013). One dog in the parenteral group was hypocobalaminaemic at this time. Although the response in the parenteral group was significantly better after 4 weeks, all except one dog in the oral group were in the upper half of the reference interval, or above, signalling a satisfactory response to oral supplementation in most dogs.

Parenteral administration bypasses the diseased intestine and would be predicted to be more effective than oral supplementation. However, the oral supplementation protocol in this study used high daily oral doses to override the malfunctioning intestine. A similar design has been used in several comparative cobalamin studies in human beings (Kuzminski et al., 1998; Castelli et al., 2011; Kim et al., 2011). Increased serum methylmalonic acid concentrations, signalling intracellular deficiency, have been shown in 19% of dogs with serum cobalamin concentrations in the low end of the reference interval (Berghoff et al., 2012). Furthermore, cobalamin deficiency can be associated with a negative prognosis and supplementation is safe; therefore, some authors (Dossin, 2011) and centres[2] recommend early intervention. The decision to include dogs with signs of CE and serum cobalamin concentrations in the low end of the reference interval was based on the same reasoning.

[2] See: http://vetmed.tamu.edu/gilab/research/cobalamin-information#dosing (accessed 5 December 2017).

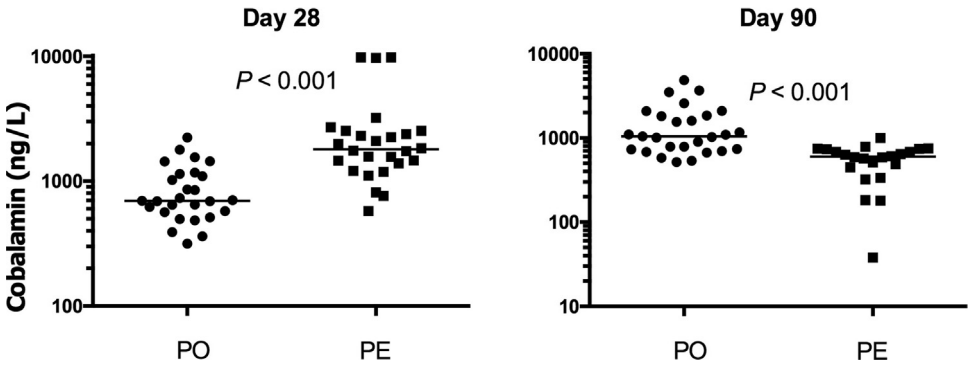

Fig. 3. Difference (Δ) in serum cobalamin concentrations compared to baseline over time after oral (peroral, PO; $n = 27$) and parenteral (PE; $n = 26$ after 28 days, $n = 22$ after 90 days) cobalamin supplementation. Log_{10} scale, long horizontal lines represent median.

The cobalamin tablets used in this study in Sweden at the time of writing are less than a third of the price than the injectable form over a 3 month period of administration, without adding the cost for syringes and hypodermic needles. Similarly, our observation is that the most cost-effective option in the USA, using the same time frame, is to let the dog owner give cobalamin injections at home.

In human beings, the alternative absorptive route of cobalamin independent of IF accounts for about 1% of the total absorption. Although not formally demonstrated, this alternative uptake may also exist in dogs, which could explain why oral cobalamin is effective in dogs with CE and low cobalamin concentrations. The dog in the oral group with the highest serum cobalamin concentration after 90 days had CE, EPI and PLE. Furthermore, in our recent retrospective cobalamin study, another dog with EPI and CE also showed an excellent response to oral cobalamin (Toresson et al., 2016). Only negligible amounts of IF may be present in the pancreatic enzymes available in Sweden (M. Lindgren, personal communication); thus, the good response to oral cobalamin in those dogs may support the theory of an alternative uptake independent of IF. Further studies, for example using radioactively labelled cobalamin, are needed to investigate whether this route of absorption exists in dogs.

Dose and duration affect the response of oral cobalamin supplementation in human beings (Berlin et al., 1968; Kuzminski et al., 1998; Bolaman et al., 2003; Eussen et al., 2005; Castelli et al., 2011; Kim et al., 2011). Similar to findings in human beings, serum cobalamin concentrations in the oral group were significantly higher after 90 days than after 28 days (Kuzminski et al., 1998; Kim et al., 2011). The range of oral doses in the current study was 0.025–0.08 mg/kg. A dose-response curve was calculated, but the correlation was weak ($R^2 = 0.23$; data not shown). However, serum cobalamin concentrations after supplementation varied substantially between individuals in both groups. This emphasises the need for checking serum cobalamin concentrations after supplementation, since some dogs may need long-term supplementation (Ruaux, 2013).

One 10 year old Border Collie was included in the study, but no other breeds known for familial cobalamin deficiency were included. However, the age of this dog and short history of clinical signs (6 weeks) make familial cobalamin deficiency less likely than CE.

Six dogs in the present study had evidence of intestinal parasitism, but only the dog with a heavy burden of *Toxocara canis* was believed to have clinical signs related to parasite infestation. This dog responded to treatment with fenbendazole, but continued to relapse with diarrhoea until the diet was changed. Three dogs with evidence of intestinal parasitism did not have diarrhoea when the results from parasitology were available, one dog did not improve after deworming and one dog had negative parasite samples at inclusion but became positive for parasites during the study.

In this study, 6/49 (12.2%) of the dogs had food-responsive diarrhoea, which is lower than in several other studies of canine CE (Luckschander et al., 2006; Allenspach et al., 2007; Burgener et al., 2008; Heilmann et al., 2016). However, 39/53 (73.6%) dogs were being treated with an 'intestinal', elimination or hydrolysed protein diet at the time of inclusion in the study. Furthermore, only dogs with low-normal or low serum cobalamin concentrations were included. Thus, they are likely represent a cohort affected with more severe intestinal disease than in the other studies.

One limitation of the study is that not all dogs had a full diagnostic work-up, mainly for financial reasons; in addition, some owners were reluctant to proceed with endoscopy. Another limitation of the study is that the intestinal biopsies were not graded according to the World Small Animal Veterinary Association gastrointestinal standardisation template (Washabau et al., 2010). No Swedish laboratory at the time of the study used these guidelines, and the project budget was too limited for re-evaluation of the biopsies. Lastly, intracellular markers of cobalamin deficiency need to be studied and compared between both groups, since serum cobalamin concentrations may not reflect intracellular cobalamin concentrations (Berghoff et al., 2012).

Conclusions

Parenteral and the oral supplementation protocols effectively increased serum cobalamin concentrations in dogs with CE and low cobalamin levels. After 4 weeks of treatment, the increase in serum cobalamin concentrations was significantly higher in the parenteral group compared to the oral group. In contrast, after 90 days of treatment, serum cobalamin concentrations were significantly higher in the group receiving cyanocobalamin orally. Oral cobalamin supplementation is a simple and, in some countries, cost-effective alternative to injections in dogs with low cobalamin concentrations.

Conflict of interest statement

None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

We would like to thank clinicians and laboratory staff at Evidensia Specialist Animal Hospital, Helsingborg, Sweden, as well

as Annelie Rundquist and Louise Baurén, in the Laboratory Department of Evidensia Specialist Animal Hospital, Strömsholm, Sweden. This work was supported by the Swedish Veterinary Care Foundation, Agria and the Swedish Kennel Club Research Foundation and the Ulla Yard Foundation. Preliminary results were presented as an Abstract at the 2016 American Congress of Veterinary International Medicine Forum, Denver, Colorado, USA, 8–11 June 2016.

Appendix: Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tvjl.2017.12.010.

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