Short communication

Effects of oral cobalamin supplementation on serum cobalamin concentrations in dogs with exocrine pancreatic insufficiency – a pilot study

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Abstract

The objective of this retrospective study was to evaluate serum cobalamin concentrations before and after oral cobalamin supplementation in dogs with low serum cobalamin concentrations and exocrine pancreatic insufficiency (EPI). Eighteen dogs with serum trypsin-like immunoreactivities between <1.0-2.7 µg/L (reference interval: 5.2-35 µg/L) and serum cobalamin concentrations ≤ 350 ng/L (reference interval: 244–959 ng/L) were enrolled. All dogs were treated with oral cyanocobalamin according to a previously described protocol (0.25-1.0 mg daily, depending on body weight). Median (range) serum cobalamin concentrations at inclusion was 188 ng/L (<111-350 ng/L) which increased significantly to 1000 ng/L (794-2385 ng/L; $P < 0.001$) after cobalamin supplementation for 19-199 days (median: 41 days). Oral cobalamin supplementation is a potential alternative to parenteral supplementation in dogs with EPI.

Key words: Cobalamin deficiency, Dog, Exocrine pancreatic insufficiency, Vitamin B12

Cobalamin deficiency is a common sequel to exocrine pancreatic insufficiency (EPI) in dogs, with a reported prevalence of 74-84 % (Hall et al., 1991; Batchelor et al., 2007). Cobalamin deficiency has been reported to be a negative prognostic factor in dogs with EPI, associated with a shorter survival (Batchelor et al., 2007; Soetart et al., 2019). Several mechanisms behind cobalamin deficiency in dogs with EPI have been proposed, of which decreased synthesis and secretion of intrinsic factor (IF) from the exocrine pancreas is likely the most important one (Batt et al., 1989; Simpson et al 1989).

Since EPI is irreversible in almost all cases, maintenance cobalamin supplementation is often required after initial cobalamin deficiencies have been corrected. The traditional protocol for cobalamin supplementation calls for multiple parenteral injections (Toresson et al., 2018). However, recent reports in dogs with chronic enteropathies and low serum cobalamin concentrations have shown that oral cobalamin supplementation is an effective alternative to parenteral supplementation (Toresson et al., 2016; Toresson et al., 2018). The aim of this retrospective study was to evaluate the effects of oral cobalamin supplementation on serum cobalamin concentrations in dogs with EPI and low serum cobalamin concentrations.

A computerized database search for dogs treated at Evidensia Specialist Animal Hospital (ESAH), Helsingborg, Sweden during 2010-2019 was performed. Due to the retrospective nature of the study, no ethical permit was available. Inclusion criteria were dogs diagnosed with EPI based on a serum trypsin-like immunoreactivity ≤ 2.7 µg/L (TLI; reference range: 5.2-35 µg/L), an initial serum cobalamin concentration ≤ 350 ng/L (reference interval: 244-959 ng/L), a follow-up blood sample for serum cobalamin concentration within 200 days, and daily treatment with cyanocobalamin tablets (Behepan, Pfizer) according to a previously described protocol (1 mg/tablet; ¼ tablet for dogs with a body weight (BW) of 1-10 kg, ½ tablet for dogs with a BW of >10-20 kg, and dogs with a BW >20 kg received 1

tablet daily) (Toresson et al., 2016; Toresson et al., 2018). Exclusion criteria were failure to comply with the treatment protocol or parenteral cobalamin supplementation in parallel with oral supplementation. Dog owners were asked to withhold cobalamin on the day of the follow-up blood sample. Serum cobalamin concentrations were measured using an automated chemiluminescence immunoassay (Immulite 2000). Serum cobalamin and TLI concentrations were measured at the Laboratory Department at ESAH, Strömsholm, Sweden. Due to the retrospective nature of the study, leftover serum samples for analysis of methylmalonic acid (MMA) were only available from four dogs. Serum MMA concentrations were analysed at the Gastrointestinal Laboratory at Texas A&M University, College Station, Texas, using gas chromatography-mass spectrometry as previously described (Ruaux et al., 2001).

Eighteen dogs aged 1.0-9.2 years (median: 3.3 years) were included in the study (table 1). The most common breed was German shepherd (n = 5), followed by mixed breed dog (n = 4). Remaining breeds, represented by 1 individual each, were ♭Basenji, Cairn Terrier, Cavalier King Charles Spaniel, Eurasian, German Spaniel, Japanese Spitz, Malinois, Miniature Dachshound and Welsh Corgi Cardigan. Median serum TLI concentration was 1.0 µg/L (range: <1.0–2.7 µg/L). Five dogs were already being treated with pancreatic enzymes and had previously been supplemented with parenteral cobalamin, but were enrolled when a recurrence of low serum cobalamin concentration was detected. The last cobalamin injections were given 28-60 days prior to the blood tests demonstrating recurrence of a low serum cobalamin concentration. The remaining dogs started supplementation with pancreatic enzymes during the study period.

Serum cobalamin concentrations at inclusion were <150-350 ng/L (median 188 ng/L; reference interval: 244–959 ng/L). The follow-up blood sample was collected 19-199 (median 41) days after initiation of cobalamin supplementation. By that time, serum cobalamin concentrations had increased to 794-2385 ng/L (Fig. 1, median: 1000 ng/L; P <

0.001, Wilcoxon matched-pairs signed rank test). Two of 18 dogs had serum cobalamin concentrations within the upper quartile of the reference interval at follow-up. The remaining dogs all had supranormal serum concentrations. Samples were diluted to exact numbers in all but 9 dogs, for which the laboratory reported a serum cobalamin concentration > 1000 ng/L. For statistical analysis values > 1000 ng/L were truncated to 1000 ng/L. Thus, the exact magnitude of the increase could not be accurately determined for all dogs. Body condition score increased significantly from 1-5/9 (mean: 3.3/9) to 2–6/9 (mean: 3.9/9, $P = 0.02$, Student's *t* test), comparing baseline and follow-up. Body weight also increased significantly, from 3.1–35.0 kg (mean: 17.7 kg) to 4.6–41.5 kg (mean: 19.2 kg, $P = 0.02$, Student's *t* test), comparing baseline and follow-up.

Baseline serum MMA concentrations ($n = 4$) were 1165–2317 nmol/L (median: 1771 nmol/L; reference interval: 415-1193 nmol/L) at inclusion, which decreased to 962–1650 nmol/L (median: 1022 nmol/L) after supplementation (Fig. 2). Statistical comparisons (i.e., before and after supplementation) for serum MMA concentrations were not possible due to the small sample size.

This is the first study reporting a significant increase in serum cobalamin concentrations after oral cobalamin supplementation in dogs with EPI and low serum cobalamin concentrations. A direct uptake of cobalamin, independent of IF, has been proven in humans by using radioactively labeled cobalamin (Berlin et al., 1968). Approximately 1% of the oral dose administered was absorbed via this direct route. The exact mechanism behind the uptake is not known, but a passive diffusion process was suggested. The response to oral cobalamin in dogs with EPI is especially interesting as it can be assumed that the majority of these dogs are lacking IF. The gastric output of IF is very minute compared to the output of IF from the pancreas (Batt et al., 1989).

The pancreatic enzyme supplements available in Sweden should not contain any IF, besides possible negligible traces due to contamination (personal communication, Malin Lindgren, Mylan). However, even if porcine IF were present, it is not known whether dogs can utilize it. It has been shown that dogs cannot use IF of bovine origin (Simpson et al., 1989). Our data support the theory that dogs also have an alternative absorptive pathway for cobalamin, independent of IF. Additionally, a recent report on successful oral cobalamin supplementation in dogs with Imerslund-Gräsbeck syndrome further suggests the presence of an alternative absorptive pathway beyond ileal receptor recognition (Kook and Hersberger, 2019). Further studies regarding this alternative absorptive pathway are warranted.

Cyanocobalamin (Behepan) was used in this study, as in our previous studies on oral cobalamin supplementation in dogs (Toresson et al., 2016; Toresson et al., 2018). This product was also used in the groundbreaking research by Berlin and co-workers, in which an alternative absorptive pathway of cobalamin in humans was shown in 1968 ((Berlin et al., 1968).

In previous studies, 3-83% of dogs with EPI and hypocobalaminemia were supplemented with cobalamin (Batchelor et al., 2007; Soetart et al., 2019). Oral cobalamin supplementation may be a more convenient and cost-effective treatment option for dog owners in several countries (Kook and Hersberger, 2019), and could potentially increase the number of dogs receiving supplementation. Supranormal serum cobalamin concentrations was seen in 16/18 dogs, indicating that lower doses of cobalamin could be used. Since cobalamin has a very high safety profile, oversupplementation is not considered a problem.

One limitation is the time point for the follow-up blood sample, which varied substantially in this study. Six dogs had already had a follow-up blood sample collected between 19 and 29 days after supplementation was started. However, serum cobalamin concentration was supranormal in 5/6 of those dogs even after this short treatment period.

Although the number of dogs is low, it suggests a quick response to oral cobalamin. Other limitations are lack of control group and that metabolic markers of cobalamin, such as MMA, could only be analysed in four dogs. Despite these limitations, our results suggest that oral cobalamin supplementation appears effective in treating dogs with EPI and low serum cobalamin concentrations.

Conflict of interest statement

Joerg Steiner, Jan Suchodolski, and Jonathan Lidbury work at the Gastrointestinal Laboratory at Texas A&M University that performs measurement of cobalamin, TLI, and MMA on a fee for service basis. Dr. Steiner also serves as a paid consultant for Nutramax Laboratories Veterinary Sciences INC, the manufacturer of an oral cobalamin supplement.

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Table 1. Selected baseline data and serum biochemistry at inclusion and follow-up after oral cobalamin supplementation in 18 dogs with exocrine pancreatic insufficiency

Parameter	Reference interval	Range (median)
Age (years)	-	1.0-9.2 (3.4)
Body weight (kg)	-	3.1-35.0 (15.7)
Body condition score	-	1/9-5/9 (4/9)
Time to follow-up (days)	-	19-199 (41)
Body weight at follow-up (kg)	-	4.6-41.5 (16.3)
Body condition score at follow-up	-	2/9-6/9 (4/9)
Serum TLI[a] (µg/L)	5.5-35	<1.0-2.7 (1.2)
Serum cobalamin concentration (ng/L)	234-811	<111-350 (188)
Serum cobalamin concentration (ng/L) at follow-up	234-811	794-2385 (1000)
Serum MMA[b] concentration (nmol/L)[c]	415-1193	1165–2317 (1771)
Serum MMA[b] concentration (nmol/L) at follow-up[c]	415-1193	962–1650 (1022)

[a] Trypsin-like immunoreactivity. [b] Methylmalonic acid. [c] $n = 4$ (for all other data, $n = 18$)

Figure legend

Fig. 1. Serum cobalamin concentrations at baseline (T0) and follow-up (T1) after oral cobalamin supplementation in 18 dogs with exocrine pancreatic insufficiency and low serum cobalamin concentrations. Dotted lines represent the limits of the reference interval and medium horizontal lines the median.

Fig. 2. Serum methylmalonic acid (MMA) concentrations at baseline (T0) and follow-up (T1) after oral cobalamin supplementation in 4 dogs with exocrine pancreatic insufficiency and low serum cobalamin concentrations. Dotted lines represent the limits of the reference interval and medium horizontal lines the median.