The Veterinary Journal 191 (2012) 41–45

Contents lists available at ScienceDirect

The Veterinary Journal

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

# Partial characterization of cobalamin deficiency in Chinese Shar Peis

Micah A. Bishop *, Panagiotis G. Xenoulis, Nora Berghoff, Niels Grützner, Jan S. Suchodolski, Jörg M. Steiner

*Gastrointestinal Laboratory, Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843-4474, USA*

ARTICLE INFO

*Article history:*
Accepted 7 May 2011

*Keywords:*
Cobalamin
Shar Pei
Methylmalonic acid
Vitamin B12
Dog

ABSTRACT

A total of 22,462 serum sample results from dogs being evaluated for gastrointestinal disease at the Gastrointestinal Laboratory, College of Veterinary Medicine, Texas A&M University were evaluated retrospectively. The proportion of dogs with serum cobalamin concentrations below the reference interval and median serum concentrations were compared between Shar Peis and other dog breeds. Serum samples were also obtained prospectively from 22 healthy and 32 Shar Peis with chronic gastrointestinal disease and 59 healthy dogs of other breeds, and serum concentrations of cobalamin, folate, and methylmalonic acid were determined and compared.

Overall, 64.0% (89/139) of serum samples from Shar Peis showed serum cobalamin concentrations below the limit of the reference interval and 38.1% (53/139) of these were below the detectable limit for the assay. The median serum cobalamin concentration in Shar Peis was significantly lower than in other breeds. Shar Peis with gastrointestinal disease had significantly lower serum cobalamin and higher serum methylmalonic acid concentrations compared to healthy Shar Peis. Healthy Shar Peis had significantly increased serum methylmalonic acid concentrations compared to healthy dogs of other breeds. There were no meaningful differences in folate concentrations between groups. In conclusion, Shar Peis have a high prevalence of cobalamin deficiency compared to other breeds and healthy Shar Peis may have subclinical cobalamin deficiency.



## Introduction

Cobalamin (vitamin B12) is a water-soluble vitamin, which is produced exclusively by microorganisms and is incorporated into dietary protein of animal origin. Cobalamin is initially released from dietary protein by pepsin and gastric acid in the stomach (Fowler, 1998). The free cobalamin molecules are then bound by haptocorrin (R-protein), a family of glycoproteins with a high affinity for cobalamin (Qureshi et al., 1994; Fowler, 1998; Fairbanks and Klee, 1999). Binding of cobalamin to haptocorrin is thought to prevent utilization of the vitamin by bacteria in the proximal gastrointestinal tract (Qureshi et al., 1994). Once the vitamin has entered the proximal duodenum, pancreatic proteases cleave haptocorrin from cobalamin (Qureshi et al., 1994).

Cobalamin is immediately bound to intrinsic factor (IF), which in the dog, is mainly secreted by the exocrine pancreas (Batt et al., 1989; Simpson et al., 1993). A specific receptor found in the brush border of the ileum, known as cubam, recognizes the IF-cobalamin complex and facilitates receptor-mediated endocytosis of the complex (Fyfe et al., 2004). Cobalamin is then released into the bloodstream where the biologically available cobalamin is transported to the cells by transcobalamin II (TCII) (Fowler, 1998; Simpson et al., 2001). Once it has reached the cell, cobalamin acts as a cofactor for the synthesis of methionine and the conversion of propionyl-CoA to succinyl-CoA (Qureshi et al., 1994; Fairbanks and Klee, 1999). These reactions are important for DNA synthesis and the intermediary metabolism of fatty acids and sulfur-containing amino acids (Solomon, 2007).

Our clinical experience indicates that Shar Peis with or without clinical signs of gastrointestinal disease often have decreased serum cobalamin concentrations, and this is supported by anecdotal observations by others (Williams, 1991). Therefore, the hypothesis of this study was that Shar Peis have a high prevalence of cobalamin deficiency compared to dogs of other breeds.

### Methods and materials

*Retrospective study*

The database of the Gastrointestinal Laboratory at Texas A&M University was searched for results of serum cobalamin and folate concentrations assayed in dogs over a 4-year period (October 2002–October 2006). The clinical history and disease status of the dogs from which the serum samples had been collected were unknown. Samples for which the breed type was not specified were excluded from analysis. Follow-up or duplicate samples from Shar Peis were excluded from analysis. Due to the large number of samples from other breeds, duplicate accessions could not be identified and excluded for other breeds.

* Corresponding author. Tel.: +1 979 458 3303.
*E-mail address:* mbishop@cvm.tamu.edu (M.A. Bishop).

1090-0233/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tvjl.2011.05.008

The numbers of dogs with serum cobalamin or folate concentrations below the lower limit of the respective reference intervals (cobalamin <249 ng/L; folate <6.5 μg/L) were determined for Shar Peis and also for dogs of all other breeds combined. In addition, in order to determine whether the decreased serum concentrations of cobalamin were unique to Shar Peis, serum cobalamin concentrations of Shar Peis were compared to 11 randomly selected dog breeds with an equal to or greater number of sample submissions (>139 dogs) as well as a mixed breed dog group.

*Prospective study*

Serum samples from clinically healthy Shar Peis and Shar Peis with signs of chronic gastrointestinal (GI) disease were obtained. Dogs classified as clinically healthy had to be free of clinical illness and have no history of chronic disease with the exception of minor dermatological issues at the time of blood collection. Serum samples from healthy Shar Peis were requested from breeders and owners through the Shar Pei Breed Association.

Owners who decided to participate in this study were sent a package containing materials necessary for blood collection and were asked to schedule an appointment with their veterinarian to obtain the serum samples. The owners were instructed not to feed their dogs for at least 12 h prior to the scheduled blood collection. In addition, they were asked to complete a questionnaire for each dog. The protocol of this part of the study was reviewed and approved by the Clinical Research Review Committee at the College of Veterinary Medicine at Texas A&M University and owners were asked to sign an informed consent form.

Shar Peis with clinical signs of chronic GI disease were located by searching the database of the Gastrointestinal Laboratory at Texas A&M University for recent serum samples submitted from Shar Peis. This research was carried out from January 2005 until January 2008. The veterinarians submitting these samples were contacted and the presence of clinical signs of chronic GI disease was confirmed for all dogs enrolled in this group. Veterinarians were also asked to submit a second sample to replace the original sample if the dog had not received any cobalamin treatment and/or not responded to therapy.

For the purpose of this study, clinical signs of chronic GI disease were defined as the presence of small bowel diarrhea for a minimum of 2-week duration prior to sample collection, the lack of response to common anthelmintic agents, and a normal serum canine serum trypsin-like immunoreactivity (cTLI) concentration. The enrolled dogs came from a variety of private and referral practices and were not based on specific diagnoses. Therefore, dogs were grouped based only on the presence of clinical signs of chronic GI disease and on specific diagnoses. Dogs diagnosed with exocrine pancreatic insufficiency (EPI) based on a serum cTLI concentration <5 μg/L were excluded from analysis as it has been shown that dogs with EPI commonly have low serum cobalamin concentrations as an indirect consequence of inadequate function of the exocrine pancreas (Simpson et al., 1989; German et al., 2003).

Serum samples were also collected from healthy dogs of other breeds using the same criteria as above for healthy Shar Peis. These samples were collected from students and staff at the College of Veterinary Medicine and Biomedical Sciences at Texas A&M University.

Serum cobalamin and folate concentrations were measured on all samples using commercially available chemiluminescent assays validated for use in dogs (Immulite 2000 Vitamin B12, Siemens; Immulite 2000 Folic Acid, Siemens). Serum cTLI concentrations were measured in all Shar Peis with clinical signs of chronic GI disease using a commercially available radioimmunoassay (Canine TLI, Siemens).

*Serum methylmalonic acid (MMA) assay*

Serum samples from the group of healthy dogs of other breeds as well as all the recently saved and prospectively obtained Shar Pei serum samples were assayed for serum MMA concentrations using a stable isotope dilution gas chromatography–mass spectrometry (GC/MS) assay (Ruaux et al., 2001). Briefly, 0.25 nmol of trideuterated MMA (methyl-$d_3$-malonic acid, CDN Isotopes) were added as internal standard to a 500 μL serum sample aliquot in order to give a final isotope concentration of 500 nmol/L. The sample was then acidified with 500 μL 3 M HCl, vortexed, and centrifuged for 5 min. The supernatant from each sample was applied to a liquid–liquid extraction column (Chem Elut 1 mL, Varian) eluted with ethyl acetate, and subsequently dried under a stream of nitrogen.

Each sample was then derivatized using 50 μL of acetonitrile (Acetonitrile, Allied Signal) and 50 μL of N-methyl-N-(*tert*-butyl-dimethylsilyl)-trifluoroacetamide (MTBSTFA, Pierce) followed by incubation at 64 °C for 15 min. Finally, an aliquot of the derivatized sample was injected onto the GC/MS (Agilent 6890 GC and 5975 Series GC/MS, Agilent Technologies) in splitless mode for analysis on a 100% dimethylpolysiloxane column (DB-1 ms column, Agilent Technologies). An initial column equilibration temperature of 75 °C was held for 2 min following injection. The oven was heated to 215 °C at a rate of 12 °C/min. Ultra-pure helium was used as the carrier gas at a constant flow rate of 2 mL/min. The mass spectrometer was used in a selected ion monitoring mode, scanning for ions with mass to charge ratios 289 and 292 for endogenous MMA and trideuterated MMA, respectively. Quantification of MMA was achieved by calculation of the ratio of the peak area (area under the curve) of the endogenous to the isotopic MMA.

*Statistical analysis*

Data were tested for normal distribution by the Kolmogorov–Smirnov test and the Shapiro–Wilk normality test. For the retrospective part of the study, proportions of Shar Peis and dogs of other breeds that had serum cobalamin and folate concentrations below the lower limit of the reference interval or below the detection limit of the assay (cobalamin only) were compared using the Fisher's exact test, and odds ratios (OR) with their 95% confidence interval (CI) were calculated. The Mann–Whitney *U* test was used to compare two non-parametric data sets. Data sets from the results of serum cobalamin, folate, and MMA measurement in the three groups of dogs as well as the difference in median serum cobalamin concentrations among individual breeds were compared using a Kruskal–Wallis test followed by Dunn's Multiple Comparison post-test. Significance was set at $P < 0.05$ for all analyses. All statistical analyses were performed using a commercially available statistical software package (PRISM 5th Version, GraphPad).

## Results

*Retrospective study*

A total of 22,462 canine serum samples had been submitted to the Gastrointestinal Laboratory for measurement of cobalamin concentrations over the 4-year period. Of these samples, 139 belonged to Shar Peis and were not duplicates. Upon evaluation of the results for these 139 serum samples, 89 (64.0%) samples had serum cobalamin concentrations below the lower limit of the reference interval (249 ng/L), while 53/139 (38.1%) had serum cobalamin concentrations below the detection limit for the assay (<100 ng/L). Shar Peis had significantly higher odds of having a serum cobalamin concentration below the reference interval (OR, 7.6; $P < 0.0001$; 95% CI, 5.4–10.8) and a serum cobalamin concentration below the detection limit of the assay (OR, 55.6; $P < 0.0001$; 95% CI, 37.3–83.1) than dogs of all other breeds combined.

The difference in median serum cobalamin concentrations between Shar Peis (149 ng/L) and the combined median cobalamin concentration of all other breeds (415 ng/L) was statistically significant ($P < 0.0001$; Fig. 1). Serum cobalamin concentrations of Shar Peis were compared to 11 randomly selected dog breeds as well as a mixed breed dog group (Fig. 2). The Kruskal–Wallis test re-

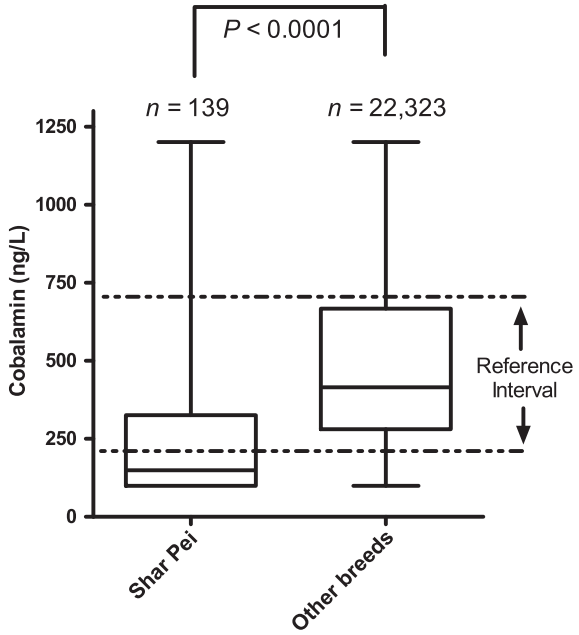


**Fig. 1.** Boxplot of serum cobalamin concentrations (ng/L) for Shar Peis and other dog breeds from the Gastrointestinal Laboratory database. The line in the rectangle corresponds to the median, the upper and lower limits of the rectangle correspond to the 25th and 75th percentile, respectively, and the whiskers correspond to the lowest and highest values.