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Evaluation of serum cobalamin concentrations in dogs of 164 dog breeds (2006–2010)

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Abstract. Altered serum cobalamin concentrations have been observed in dogs with gastrointestinal disorders such as exocrine pancreatic insufficiency (EPI) or gastrointestinal inflammation. The aims of the current study were 1) to identify breeds with a higher proportion of dogs with a decreased serum cobalamin concentration, 2) to determine whether dogs with such decreased concentrations tend to have serum canine trypsin-like immunoreactivity (cTLI) concentrations diagnostic for EPI, and 3) to compare the number of submissions for serum cobalamin analysis by breed to the American Kennel Club (AKC) breed ranking list of 2009. In this retrospective study, results of 28,675 cobalamin tests were reviewed. Akitas, Chinese Shar-Peis, German Shepherd Dogs, Greyhounds, and Labrador Retrievers had increased proportions of serum cobalamin concentrations below the lower limit of the reference interval (<251 ng/l; all $P < 0.0001$). Akitas, Chinese Shar-Peis, German Shepherd Dogs, and Border Collies had increased proportions of serum cobalamin concentrations below the detection limit of the assay (<150 ng/l; all $P < 0.0001$). Akitas, Border Collies, and German Shepherd Dogs with serum cobalamin concentrations <150 ng/l were more likely to have a serum cTLI concentration considered diagnostic for EPI (≤2.5 µg/l; all $P \leq 0.001$). The breed with the highest proportion of samples submitted for serum cobalamin analysis in comparison with the AKC ranking list was the Greyhound (odds ratio: 84.6; $P < 0.0001$). In Akitas and Border Collies, further investigations are warranted to clarify if a potentially breed-specific gastrointestinal disorder is responsible for the increased frequency of decreased serum cobalamin and cTLI concentrations.

Key words: Cobalamin; canine breeds; malabsorption; trypsin-like immunoreactivity.

Introduction

Cobalamin (vitamin B_{12}) is essential for a wide variety of metabolic processes in many tissues and organs. Immunoassays for the measurement of cobalamin concentrations in serum from human beings, cats, and dogs are routinely used to diagnose cobalamin deficiency.

In dogs, cobalamin deficiency can be caused by exocrine pancreatic insufficiency (EPI),[19] severe and longstanding ileal disease, small intestinal dysbiosis, or an inherited condition. Cobalamin deficiency can also be associated with systemic metabolic complications such as central and peripheral neuropathies[6] and immunodeficiencies,[8] and is also associated with intestinal changes, such as villous atrophy[18] or malabsorption of vitamins and other nutrients.

In cases of longstanding ileal disease, low serum cobalamin concentrations have been documented in both human and canine patients with chronic enteropathies such as inflammatory bowel disease.[1,20] Chronic enteropathies have been commonly described in canine patients of different breeds such as the Basenji,[16] Boxer,[12] German Shepherd Dog,[13] Irish Setter,[4,11] and Soft Coated Wheaten Terrier.[15] A comparison with data from the American Kennel Club (AKC), which shows the number of dogs of various breeds that are registered based on popularity, could help identify additional breeds with disproportionately high numbers of serum submissions (e.g., to the Gastrointestinal Laboratory at Texas A&M University [GL-TAMU], College Station, TX) for serum cobalamin analysis.

Low serum cobalamin concentrations have been observed in dogs with EPI, which is recognized as a potential cause of cobalamin deficiency.[19] The measurement of serum canine trypsin-like immunoreactivity (cTLI) is considered the gold standard test for the diagnosis of canine EPI.[3] An investigation of serum cTLI concentrations in dogs with low serum cobalamin concentrations could help to identify breeds where EPI is associated with cobalamin deficiency.

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In the past decade, cases of cobalamin deficiency have been reported in several dog breeds. For instance, a family of Giant Schnauzers,[10] a Beagle,[9] 2 juvenile Border Collies,[6,17] juvenile Australian Shepherds,[17] and Chinese Shar-Peis (Williams DA: 1991, Markedly subnormal serum cobalamin in Shar Pei dogs with signs of gastrointestinal disease. J Vet Intern Med 5:133. Abstract)[7] have been described with selective malabsorption of cobalamin and deficiency of this vitamin. A breed predisposition for hypocobalaminemia has been described for Chinese Shar-Peis in North America.[7] In the United Kingdom, cobalamin deficiency has been described for the Chinese Shar-Pei, Staffordshire Bull Terrier, as well as a group of mixed-breed dogs (Dandrieux JRS, Noble P-JM, Halladay LJ, et al.: 2010, Breed predispositions for severe hypocobalaminemia and relation to folate concentration in dogs with gastrointestinal disease. J Vet Intern Med 24:722. Abstract).

Due to the variety of breeds that were represented at GL-TAMU, serum cobalamin concentrations of 164 breeds (based on the AKC breed ranking list of 2009) were investigated. The first aim of the study was to identify breeds with higher proportions of decreased serum cobalamin concentrations. The second aim was to look for serum cTLI concentrations that were diagnostic for EPI in the dogs with decreased serum cobalamin concentrations to identify breeds in which EPI is associated with cobalamin deficiency. Finally, the study compared the number of serum submissions for cobalamin analysis by breed with the AKC breed ranking list of 2009 to identify breeds with disproportionately high numbers of serum submissions for serum cobalamin analysis. A trend or discovery of a high number of serum submissions for serum cobalamin analysis in a certain dog breed could help to identify a clinical problem in a specific breed perceived by veterinarians and may help to direct future investigations.

Materials and methods

Selection of serum cobalamin data

The current retrospective study covered a period of 4 years (March 1, 2006, through February 28, 2010). Information on canine serum samples in the database of GL-TAMU was reviewed. Serum samples that had been submitted for evaluation of serum cobalamin concentration were selected, but the clinical history and disease status of the dogs were not provided by the referring veterinarian. A total of 28,675 canine submissions (belonging to 164 breeds, representing the AKC ranking list of 2009) for analysis of serum cobalamin concentration were reviewed, and gender and age were identified where reported on the submission form. Resubmissions and duplicates were excluded. The concentrations of serum cobalamin had been measured using an automated chemiluminescence assay.[a] The reference interval for canine serum cobalamin concentration had previously been established as 251–908 ng/l.[b] The frequency of decreased (<251 ng/l) and undetectable (<150 ng/l) serum cobalamin concentrations recorded in the GL-TAMU database were compared between breeds by calculating the odds ratio (OR) and the 95% confidence interval (CI) for 164 breeds that were listed in the AKC breed ranking list of 2009.[c] Serum cobalamin concentrations between 251 ng/l and 150 ng/l were excluded for the proportion analyses of dogs with serum cobalamin concentration <150 ng/l. Only breeds with at least 30 submitted samples were included in the calculation. However, the 5 breeds, Giant Schnauzer, Beagle, Border Collie, Australian Shepherd, and Chinese Shar-Pei, that had been mentioned in case reports of cobalamin deficiency over the past 2 decades (1990–2010) were reported regardless of the OR.

Breeds with a significantly higher odds of having samples with serum cobalamin concentrations <150 ng/l were subsequently investigated for proportions of dogs with a serum cTLI concentration that is considered diagnostic for EPI (≤2.5 µg/l as measured at roughly the same time as serum cobalamin concentration; i.e., serum cTLI may have been measured up to 48 hr before or after serum cobalamin concentration due to logistical reasons, but would have been measured on the same serum sample). The concentration of serum cTLI was measured using a commercially available radioimmunoassay,[d] and the reference interval has previously been established as 5.7–45.2 µg/l.[e] Breeds that were identified as having a significant OR (95% CI) for a serum cTLI concentration ≤2.5 µg/l were considered to have an association of undetectable serum cobalamin concentration and EPI. Only breeds that showed a significant OR were reported for all analyses. However, data for the 5 breeds that had been mentioned in case reports of cobalamin deficiency over the past two decades were reported regardless of the OR.

Comparison of submissions for serum cobalamin measurement with the 2009 AKC breed ranking list

Due to possible annual variation of submissions, the average number of serum samples submitted for cobalamin analysis to GL-TAMU over a 4-year period was calculated for each breed. Thus, a total of 7,203 canine submissions, the calculated average number of serum samples submitted for cobalamin analysis to GL-TAMU for 1 year, were compared by calculating the OR and the 95% CI to the AKC breed ranking list of 2009 to identify breeds with higher proportions of submissions for serum cobalamin analysis. Again, only breeds with at least 30 sample submissions were included in the calculation. The AKC ranking list of 2009 contained a total of 649,677 registered dogs. Subsequently, for all breeds with a higher proportion of serum sample submissions for serum cobalamin analysis, the serum cobalamin concentrations and age were compared among the breeds (averaged across the 4 years). Also, serum cTLI concentrations were subsequently

Table 1. Over- and underrepresented dog breeds with regard to serum cobalamin concentration below the lower limit of the reference interval (<251 ng/l) using data from the Gastrointestinal Laboratory (Texas A&M University, College Station, Texas) database.

Breed*	2009 American Kennel Club ranking position	Age†	Cobalamin‡		Odds ratio§
			<251 ng/l	251–908 ng/l	
A. Odds ratio > 1					
Chinese Shar-Pei	47	6.0 (8.5)	82/5,646	71/21,428	4.4 (3.2–6.0)¦
Akita	50	7.0 (3.3)	43/5,685	59/21,440	2.8 (1.9–4.1)¦
Greyhound	140	9.0 (5.1)	174/5,554	262/21,237	2.5 (2.1–3.1)¦
German Shepherd Dog	2	5.0 (8.8)	1,095/4,633	2,767/18,732	1.6 (1.5–1.7)¦
Labrador Retriever	1	7.0 (6.9)	738/4,990	2,098/19,400	1.4 (1.3–1.5)¦
B. Odds ratio < 1					
Golden Retriever	4	8.0 (8.5)	224/5,504	1,152/20,347	0.7 (0.6–0.8)¦
Boxer	6	6.0 (5.6)	126/5,602	930/20,569	0.5 (0.4–0.6)¦
Great Dane	21	5.0 (10.0)	40/5,688	310/21,189	0.5 (0.4–0.7)¦
Standard Poodle	9	7.0 (1.8)	56/5,672	469/21,030	0.4 (0.3–0.6)¦
Miniature Schnauzer	11	8.0 (11.3)	62/5,666	597/20,902	0.4 (0.3–0.5)¦
Belgian Malinois	81	9.0 (0.0)	3/5,725	73/21,426	0.2 (0.1–0.5)¦
C. Case reports					
Australian Shepherd	28	8.0 (6.3)	64/5,664	187/21,312	1.3 (1.0–1.7)¶
Beagle	5	8.0 (9.0)	89/5,639	357/21,142	0.9 (0.7–1.2)¶
Giant Schnauzer	89	8.0 (0.0)	7/5,721	9/21,490	2.9 (1.1–7.9)¶
Border Collie	52	5.0 (4.8)	104/5,624	295/21,204	1.3 (1.1–1.7)#
Chinese Shar-Pei	47	See above			

* Table shows the dog breeds with a higher (A) or lower (B) proportion of decreased serum cobalamin concentrations (<251 ng/l). Also shown are data for 5 breeds that had previously been reported in case reports describing cobalamin deficiency in a group of dogs of a single breed (C).
† Median age (in years) for all dogs of each breed. Dogs where age was not reported is shown in parentheses (value in percentages).
‡ Number of dogs of a particular breed/number of dogs of the remaining dog breeds in which decreased serum cobalamin concentrations (<251 ng/l) and normal serum cobalamin concentrations (251–908 ng/l) were identified.
§ Calculated odds ratio, 95% confidence interval (in parentheses) for each breed, and the corresponding *P* values (¦ = <0.0003, # = <0.05, ¶ = >0.05).

investigated in breeds with significantly higher proportions of serum samples submitted for cobalamin analysis.

Statistical analyses

A commercially available software[f] was used to perform statistical analyses. All variables, the breed proportion of dogs with a serum cobalamin concentration of <251 ng/l, those with a serum cobalamin concentration <150 ng/l, and those with a cobalamin concentration within the reference interval, were compared by using a 2-sided Fisher exact test. Breeds for which the 95% CI of breed distribution of submissions to the GL-TAMU database population and those in the AKC ranking list of 2009 differed were considered potentially overrepresented or underrepresented in the population of dogs for the respective group. Because of multiple comparisons between 164 dog breeds of the AKC ranking, statistical significance level for a difference was adjusted from $P < 0.05$ to $P < 0.0003$ using a Bonferroni correction for multiple statistical comparisons.[g] Breeds with significantly higher proportions of samples with undetectable serum cobalamin concentrations and those with higher proportions of serum sample submissions for cobalamin analysis were subsequently investigated for proportions of dogs with a serum cTLI diagnostic for EPI using a Fisher exact test; statistical significance was set at $P < 0.05$. A Kruskal–Wallis test with a Dunn post test was used to compare serum cobalamin concentrations and age in breeds with a higher proportion of submissions for serum cobalamin analysis over the 4-year period ($P < 0.05$).

Results

Data from the GL-TAMU database showed that the Akita, Chinese Shar-Pei, German Shepherd Dog, Greyhound, and Labrador Retriever had significantly higher proportions of dogs with serum cobalamin concentrations <251 ng/l (OR > 1; all $P < 0.0001$; Table 1). In contrast, the Belgian Malinois, Boxer, Golden Retriever, Great Dane, Miniature Schnauzer, and Standard Poodle had significantly lower proportions of dogs with serum cobalamin concentrations <251 ng/l (OR < 1; all $P < 0.0001$; Table 1).

Furthermore, the Akita, Border Collie, Chinese Shar-Pei, and German Shepherd Dog had significantly higher proportions of dogs with serum cobalamin concentrations <150 ng/l (OR > 1; all $P < 0.0001$; Table 2). In contrast, the Boxer, Golden Retriever, Miniature Schnauzer, and Standard Poodle had significantly lower proportions of dogs with serum

Table 2. Over- and underrepresented dog breeds with regard to undetectable serum cobalamin concentrations (<150 ng/l) using data from the Gastrointestinal Laboratory (Texas A&M University, College Station, Texas) database.*

Breed*	2009 American Kennel Club ranking position	Age†	Cobalamin‡		Odds ratio§
			<150 ng/l	251–908 ng/l	
A. Odds ratio > 1					
Chinese Shar-Pei	47	5.0 (1.6)	63/1,670	71/21,428	11.4 (8.1–16.0)¦
Akita	50	6.8 (22.2)	18/1,715	59/21,440	3.8 (2.2–6.5)¦
Border Collie	52	4.5 (4.0)	50/1,683	295/21,204	2.1 (1.6–2.9)¦
German Shepherd Dog	2	5.0 (7.3)	354/1,379	2,767/18,732	1.7 (1.5–2.0)¦
B. Odds ratio < 1					
Golden Retriever	4	8.0 (6.8)	44/1,689	1,152/20,347	0.5 (0.3–0.6)¦
Boxer	6	7.0 (12.1)	33/1,700	930/20,569	0.4 (0.3–0.6)¦
Miniature Schnauzer	11	8.0 (20.0)	20/1,713	597/20,902	0.4 (0.3–0.6)¦
Standard Poodle	9	7.0 (0.0)	10/1,723	469/21,030	0.3 (0.1–0.5)¦
C. Case reports					
Australian Shepherd	28	7.0 (0.0)	19/1,714	187/21,312	1.3 (0.8–2.0)¶
Beagle	5	8.5 (7.7)	26/1,707	357/21,142	0.9 (0.6–1.3)¶
Giant Schnauzer	89	6.5 (0.0)	1/1,732	9/21,490	1.4 (0.2–11.0)¶
Border Collie	52	See above			
Chinese Shar-Pei	47	See above			

* Table shows the dog breeds with a higher (A) or lower (B) proportion of undetectable serum cobalamin concentrations (<150 ng/l). Also shown are data for 5 breeds that had previously been reported in case reports describing cobalamin deficiency in a group of dogs of a single breed (C).
† Median age (in years) for all dogs of each breed. Dogs where age was not reported is shown in parentheses (value in percentages).
‡ Number of dogs of a particular breed/number of dogs of the remaining dog breeds with undetectable serum cobalamin concentrations (<150 ng/l) and normal serum cobalamin concentrations (251–908 ng/l) were identified.
§ Calculated odds ratio, 95% confidence interval (in parentheses) for each breed, and the corresponding *P* values (¦ = <0.0003, # = <0.05, ¶ = >0.05).

cobalamin concentrations <150 ng/l (OR < 1; all $P < 0.0001$; Table 2). Also, for the Akita, Border Collie, and German Shepherd Dog, but not for the Chinese Shar-Pei, submissions with undetectable serum cobalamin concentrations were more likely associated with a serum cTLI concentration considered diagnostic for EPI than those submissions with a normal serum cobalamin concentration (all $P \leq 0.001$; Table 5).

A total of 19 breeds were found to have disproportionately higher proportions of serum samples submitted for serum cobalamin analysis (all $P < 0.0001$, Table 3) relative to the AKC breed ranking list of 2009. The breed with the highest proportion of serum samples submitted for serum cobalamin analysis was the Greyhound (Table 3). In contrast, 7 breeds were found to have disproportionately lower proportions of serum samples submitted for serum cobalamin analysis (all $P < 0.0001$, Table 3). For the Cairn Terrier, Cardigan Welsh Corgi, Cocker Spaniel, Dalmatian, Wire Fox Terrier, West Highland White Terrier, and Australian Shepherd (1 of the 5 breeds previously reported in a case series with cobalamin deficiency), submissions with undetectable serum cobalamin concentrations were more likely to be associated with serum cTLI concentrations considered diagnostic for EPI than those with normal cobalamin concentrations (all $P < 0.05$; Table 5).

Among the 19 breeds with higher proportion of serum sample submissions, serum cobalamin concentrations as well as ages were significantly different (both: $P < 0.0001$; Figs. 1 and 2, respectively; Table 4). Dunn post test showed that serum cobalamin concentrations in the Greyhound were significantly lower than those in the other 18 breeds (all $P \leq 0.01$; Fig. 1). Also, the ages differed significantly among the 19 breeds ($P < 0.0001$; Fig. 2). Post test revealed that the ages in the German Shepherd Dog differed significantly from those in 17 other breeds, but not the Irish Setter (all $P \leq 0.05$; Fig. 2). Furthermore, Dunn post test showed that the age in the Irish Setters differed significantly from those in 16 other breeds, but not the German Shepherd Dog or Soft Coated Wheaten Terrier (all $P \leq 0.05$; Fig. 2). The American Eskimo Dog, Dalmatian, and Keeshond had a median age of 10 years, which differed significantly from the Border Collie, Cairn Terrier, Cardigan Welch Corgi, German Shepherd Dog, Irish Setter, and Soft Coated Wheaten Terrier (all $P \leq 0.05$; Fig. 2).

Discussion

In the present retrospective study, 5 breeds (the Akita, Chinese Shar-Pei, German Shepherd Dog, Greyhound, and Labrador Retriever) were observed to be overrepresented, and 6 breeds (Belgian Malinois, Boxer, Golden Retriever, Great Dane, Miniature Schnauzer, and Standard Poodle) were underrepresented with regard to a serum cobalamin concentration below the lower limit of the reference interval. Furthermore, 4 breeds (Akita, Border Collie, Chinese

Table 3. Breeds with proportions of serum samples submitted to the Gastrointestinal Laboratory (GL-TAMU; Texas A&M University, College Station, Texas) for serum cobalamin analysis when compared with the American Kennel Club (AKC) ranking list of 2009.

Breed*	AKC ranking position	GL-TAMU† (*n*)	AKC† (*n*)	Odds ratio‡
A. Odds ratio > 1				
Greyhound	140	110/7,093	119/649,358	84.6 (65.2–110.0)
Parson Russell Terrier	87	121/7,082	691/648,786	16.0 (13.2–19.5)
Standard Schnauzer	99	58/7,145	559/648,918	9.4 (7.2–12.4)
American Eskimo Dog	118	23/7,180	318/649,159	6.5 (4.3–10.0)
Cardigan Welsh Corgi	83	43/7,160	818/648,659	4.8 (3.5–6.5)
Border Collie	52	102/7,101	2,009/647,468	4.6 (3.8–5.7)
Wire Fox Terrier	94	28/7,175	622/648,855	4.1 (2.8–6.0)
Soft Coated Wheaten Terrier	62	58/7,145	1,367/648,110	3.8 (3.0–5.0)
Keeshond	102	20/7,182	542/648,935	3.3 (2.1–5.2)
Irish Setter	73	39/7,164	1,044/648,433	3.4 (2.5–4.7)
English Setter	95	22/7,181	622/648,855	3.2 (2.1–4.9)
Dalmatian	75	35/7,168	1,001/648,476	3.2 (2.3–4.4)
Cairn Terrier	56	54/7,149	1,791/647,686	2.7 (2.1–3.6)
Bichon Frise	35	113/7,090	4,161/645,316	2.5 (2.1–3.0)
Australian Shepherd	67	34/7,169	1,271/648,206	2.4 (1.7–3.4)
German Shepherd Dog	2	976/6,227	40,938/608,539	2.3 (2.2–2.5)
Lhasa Apso	54	49/7,154	1,932/647,545	2.3 (1.7–3.1)
West Highland White Terrier	36	98/7,105	4,096/645,381	2.2 (1.8–2.7)
Cocker Spaniel	23	171/7,032	8,282/641,195	1.9 (1.6–2.2)
B. Odds ratio < 1				
Labrador Retriever	1	722/6,481	89,599/559,878	0.7 (0.6–0.8)
Standard Poodle	9	138/7,065	18,601/630,876	0.7 (0.6–0.8)
Pomeranian	14	76/7,127	11,415/638,062	0.6 (0.5–0.8)
French Bulldog	24	36/7,167	7,381/642,096	0.4 (0.3–0.6)
Beagle	5	117/7,086	30,672/618,805	0.3 (0.3–0.4)
English Springer Spaniel	29	21/7,182	5,896/643,581	0.3 (0.2–0.5)
Bulldog	7	54/7,149	23,248/626,229	0.2 (0.2–0.3)

* Shown are 19 dog breeds with a higher proportion of samples submitted for serum cobalamin analyses that were considered overrepresented (A), and 7 breeds with a lower proportion of samples submitted for serum cobalamin analysis that were considered underrepresented (B).
† Number of dogs of a particular breed/number of dogs of the remaining dog breeds that had been identified by the GL-TAMU database and in the AKC ranking list of 2009.
‡ Calculated odds ratio and 95% confidence interval (in parentheses) for each breed (*P* values for all <0.0003).

Shar-Pei, and German Shepherd Dog) were overrepresented with regard to undetectable serum cobalamin concentrations, and 4 breeds (Boxer, Golden Retriever, Miniature Schnauzer, and Standard Poodle) were underrepresented in this regard.

The GL-TAMU database also revealed that the Akita, Border Collie, and German Shepherd Dog, but not the Chinese Shar-Pei, with undetectable serum cobalamin concentrations, were more likely to also have a serum cTLI concentration considered diagnostic for EPI than dogs with a normal cobalamin concentration. Of the 5 breeds that were mentioned in previous reports regarding cobalamin deficiency during the past two decades, the Border Collie and the Chinese Shar-Pei were the only breeds in the current study that showed an association with undetectable serum cobalamin concentrations. Of these 5 breeds, the Border Collie and Australian Shepherd, with undetectable serum cobalamin concentrations, revealed an association with serum cTLI concentrations considered diagnostic for EPI. In contrast, the Beagle showed no such association in the present study, which could indicate that the case report of cobalamin deficiency was an isolated case and not a reflection of a breed predilection. Because there were less than 30 serum submissions for the Giant Schnauzer (1 dog with undetectable serum cobalamin concentration), that breed was excluded from the analysis.

The findings of the current study suggest that the Akita, Australian Shepherd, Border Collie, and German Shepherd Dog, but not the Chinese Shar-Pei, may have a higher prevalence of cobalamin deficiency due to exocrine pancreatic insufficiency. Pancreatic secretions play an important role in the intestinal absorption of cobalamin in the dog.[19] Intrinsic factor, which is essential for cobalamin absorption, is secreted mainly from pancreatic acinar cells in dogs.[5] Therefore, in the Akita, Australian Shepherd, Border Collie, and German Shepherd Dog with decreased serum cobalamin concentration and serum cTLI concentration considered

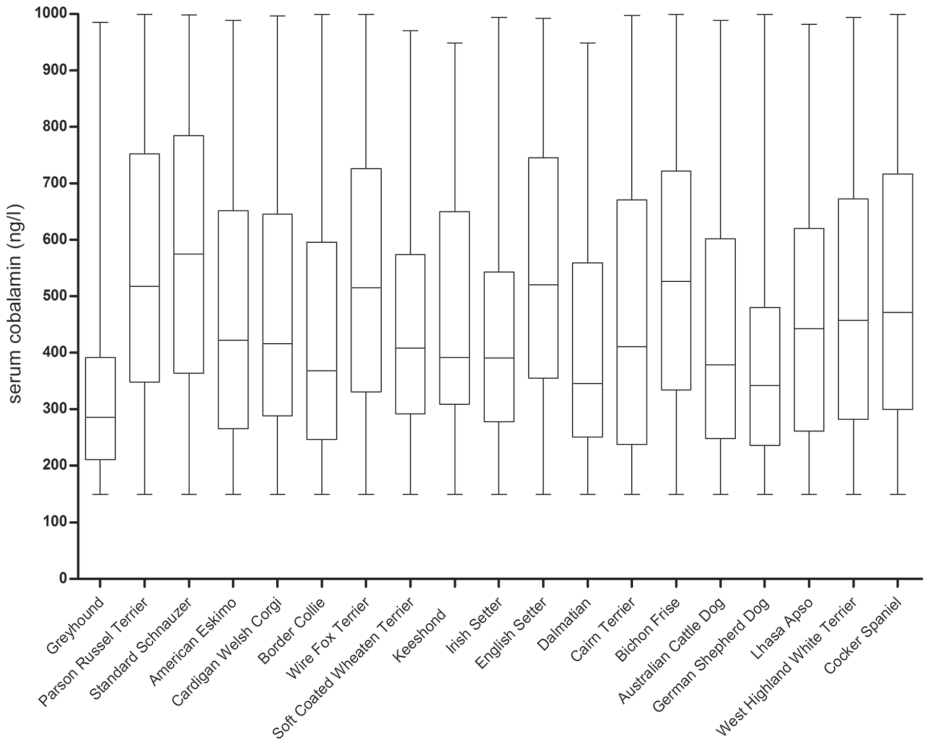

Figure 1. Serum cobalamin concentrations in 19 dog breeds. Serum cobalamin concentrations differed significantly among these 19 breeds ($P < 0.0001$). Furthermore, serum cobalamin concentrations in Greyhounds differed significantly from those in the other 18 dog breeds ($P \leq 0.01$). Order of listed breeds in the figure is the same as in Tables 3 and 4.

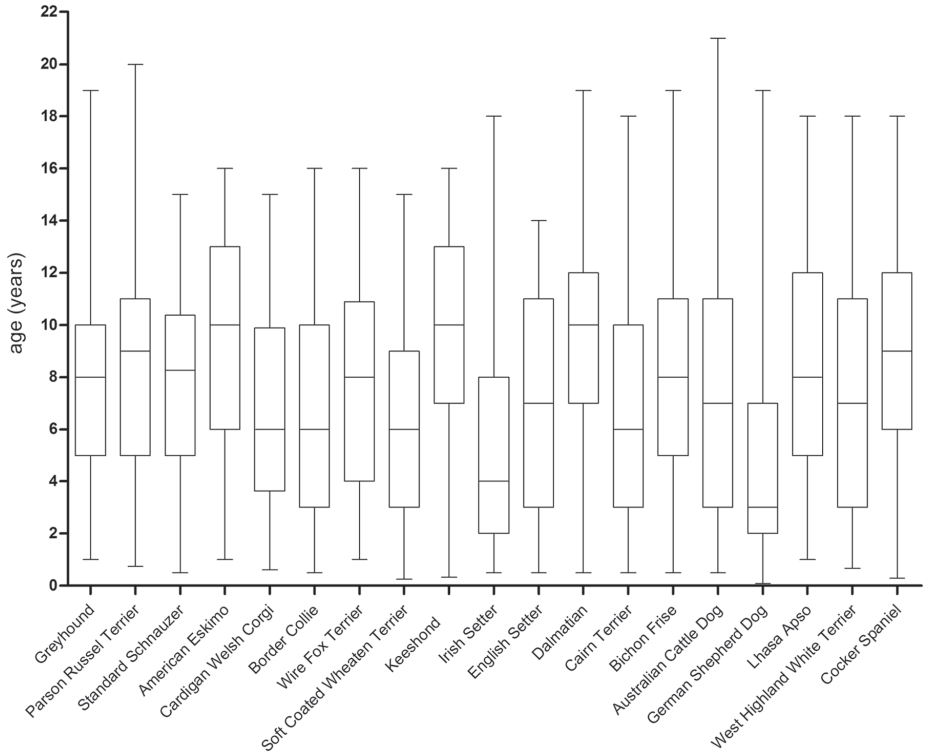

Figure 2. Ages of dogs of 19 dog breeds. The ages among the 19 breeds differed significantly ($P < 0.0001$). Also, the ages of German Shepherd Dogs differed significantly from those of the 17 dog breeds other than the Irish Setter ($P \leq 0.05$). The American Eskimo Dog, Keeshond, and Dalmatian (median age: 10 years) were significantly older than the Cardigan Welsh Corgi, Border Collie, Soft Coated Wheaten Terrier, Cairn Terrier, Irish Setter, and German Shepherd Dog ($P \leq 0.05$). Order of the listed breeds in the figure is the same as in Figure 1 and Tables 3 and 4.

Table 4. Gastrointestinal Laboratory (GL-TAMU; Texas A&M University, College Station, Texas) data set for a period of 4 years (2006–2010) for 19 breeds with their corresponding gender, median age, and median serum cobalamin concentration.*

Breed*	2009 American Kennel Club ranking position	GL-TAMU† (*n*)	Age‡	Cobalamin§
A. Odds ratio > 1				
Greyhound	140	441	8.0 (4.5)	286
Parson Russell Terrier	87	484	8.8 (7.8)	518
Standard Schnauzer	99	231	8.0 (6.0)	575
American Eskimo	118	91	10.0 (11.0)	422
Cardigan Welsh Corgi	83	171	6.0 (4.1)	416
Border Collie	52	409	6.0 (8.3)	368
Wire Fox Terrier	94	111	8.0 (9.9)	515
Soft Coated Wheaten Terrier	62	230	5.6 (9.6)	409
Keeshond	102	80	10.0 (7.5)	392
Irish Setter	73	154	4.0 (6.5)	391
English Setter	95	86	7.0 (3.5)	520
Dalmatian	75	141	10.0 (3.5)	346
Cairn Terrier	56	214	6.0 (5.1)	411
Bichon Frise	35	452	8.0 (7.3)	526
Australian Cattle Dog	67	136	7.0 (7.4)	379
German Shepherd Dog	2	3,905	3.0 (8.9)	342
Lhasa Apso	54	195	8.0 (9.2)	443
West Highland White Terrier	36	393	7.0 (5.3)	458
Cocker Spaniel	23	684	9.0 (7.9)	472

* Table shows the dog breeds with a higher (A) proportion of serum samples submitted to GL-TAMU.
† Number of dogs per breed in which higher proportion of serum samples were submitted to GL-TAMU.
‡ Median age (in years) for all dogs of each breed. Dogs where age was not reported is shown in parentheses (value in percentages).
§ Median serum cobalamin concentrations (in ng/l) for all breeds.

diagnostic for EPI, further investigations of the findings are warranted.

Nineteen breeds had disproportionately high numbers of serum samples submitted for cobalamin analysis relative to the AKC breed ranking list of 2009. For some breeds, such as the Cairn Terrier, Cardigan Welsh Corgi, Cocker Spaniel, Dalmatian, West Highland White Terrier, and Wire Fox Terrier, submissions with undetectable serum cobalamin concentrations were associated with a serum cTLI concentration considered diagnostic for EPI, suggesting that in these breeds cobalamin deficiency is due to EPI. In contrast, 10 breeds with undetectable serum cobalamin concentrations showed no association with a serum cTLI concentration considered diagnostic for EPI. Consequently, this suggests that hypocobalaminemia in these breeds was most likely independent of EPI.

The ages of dogs for which serum was submitted for cobalamin analysis differed significantly among the 19 breeds for which disproportionate numbers of samples were submitted. Veterinarians requested serum cobalamin analysis more frequently in younger German Shepherd Dogs and Irish Setters, which suggests that, early in life, both breeds are susceptible to gastrointestinal disease. It has been shown in North America and in Europe that EPI in German Shepherd Dogs[2] is a disease that occurs early in life and is suspected to be hereditary. The same applies for the sensitive enteropathy in Irish Setters, but this condition has been reported only in the United Kingdom.[4,11] In contrast, American Eskimo Dogs, Dalmatians, and Keeshonds were significantly older than Border Collies, Cairn Terriers, Cardigan Welsh Corgis, German Shepherd Dogs, Irish Setters, and Soft Coated Wheaten Terriers, which could suggest that the former breeds have a predilection to late-onset gastrointestinal disease that is associated with cobalamin malabsorption.

The Greyhound, which was 1 of 19 breeds with a higher proportion of serum sample submissions for cobalamin measurement, had by far the highest proportion of serum samples submitted for serum cobalamin analysis and the lowest serum cobalamin concentration, suggesting that cobalamin deficiency is frequently suspected in this breed. It also suggests that cobalamin deficiency is common in this breed or that serum cobalamin concentrations in Greyhounds are lower than those in other breeds and that a breed-specific reference interval should be investigated.

It should be noted that there were several limitations of the current study. For instance, mixed-breed dogs might have been included if a dog owner reported the dog to be pure bred. Also, it is possible that animal hospitals did not correctly report the dog breeds. In addition, veterinarians might have submitted samples from certain breeds more frequently because a disease is associated with a particular breed. Also, it might be possible that dog-breeding clubs are aware of certain gastrointestinal diseases in their breed of interest and due to breed-club initiatives submissions rates by breeds can be influenced.

Table 5. Comparison of dog breeds with a higher proportion of dogs with decreased serum canine trypsin-like immunoreactivity (cTLI; ≤2.5 µg/l) concentrations and undetectable serum cobalamin concentrations (<150 ng/l) and those with a higher proportion of decreased serum cTLI (≤2.5 µg/l) concentrations but a normal serum cobalamin concentration (251–908 ng/l).

Breed*	2009 American Kennel Club ranking position	cTLI ≤2.5 µg/l and cobalamin <150 ng/l†	cTLI ≤2.5 µg/l and cobalamin 251–908 ng/l†	Odds ratio‡	*P* value‡
A. Cobalamin < 150 ng/l					
Chinese Shar-Pei	47	0/53	3/49	NA	1.0
Akita	50	10/16	7/50	8.6 (2.3–31.3)	0.001
Border Collie	52	9/37	8/198	7.6 (2.7–21.4)	0.0002
German Shepherd Dog	2	131/316	335/2,024	3.6 (2.8–4.6)	<0.0001
B. Cobalamin submissions					
Greyhound	140	0/1	29/209	NA	1.0
Parson Russell Terrier	87	2/14	17/214	1.9 (0.4–9.3)	>0.05
Standard Schnauzer	99	1/7	1/78	12.8 (0.7–231.8)	>0.05
American Eskimo Dog	118	1/4	1/31	10.0 (0.5–204.1)	>0.05
Cardigan Welsh Corgi	83	4/7	9/76	9.9 (1.9–51.7)	<0.05
Border Collie	52	See above	See above		
Wire Fox Terrier	94	2/4	2/55	26.5 (2.4–296.7)	<0.05
Soft Coated Wheaten Terrier	62	0/5	0/138	NA	1.0
Keeshond	102	0/1	0/42	NA	1.0
Irish Setter	73	0/6	1/91	NA	1.0
English Setter	95	0/3	0/55	NA	1.0
Dalmatian	75	2/7	1/77	30.4 (2.3–395.4)	<0.05
Cairn Terrier	56	12/20	24/91	4.2 (1.5–11.5)	<0.01
Bichon Frise	35	0/16	0/180	NA	1.0
Australian Cattle Dog	67	3/8	7/57	4.2 (0.8–22.0)	>0.05
German Shepherd Dog	2	See above	See above		
Lhasa Apso	54	1/10	6/88	1.5 (0.2–14.1)	>0.05
West Highland White Terrier	36	8/22	30/200	3.2 (1.3–8.4)	<0.05
Cocker Spaniel	23	4/34	8/331	5.4 (1.5–18.9)	<0.05
C. Case reports					
Australian Shepherd	28	4/18	4/109	7.5 (1.7–33.4)	<0.05
Beagle	5	0/22	3/235	NA	1.0
Giant Schnauzer	89	NA	NA	NA	NA
Border Collie	52	See above	See above		
Chinese Shar-Pei	47	See above	See above		

* Shown are dog breeds (A) from Table 2: with a higher proportion of undetectable serum cobalamin concentrations (<150 ng/l), (B) from Table 4: with a higher proportion of samples submitted for serum cobalamin analysis, and (C) dog breeds reported in case reports of cobalamin deficiency and their calculated proportion of low serum cTLI concentrations (≤2.5 µg/l) diagnostic for exocrine pancreatic insufficiency when compared to normal cobalamin concentrations (251–908 ng/l).

† Number of dogs that had a serum cTLI concentration ≤2.5 µg/l and serum cobalamin concentrations <150 ng/l/total number of dogs or a serum cTLI concentration ≤2.5 µg/l and serum cobalamin concentrations 251–908 ng/l/total number of dogs. NA = not applicable.

‡ Calculated odds ratio, 95% confidence interval (in parentheses) for each breed, and the corresponding *P* values. NA = not applicable.

Serum cobalamin concentrations below the lower limit of the reference interval have previously been described in the German Shepherd Dog[18] and Chinese Shar-Pei[7] but not in the Akita, Greyhound, or Labrador Retriever. Undetectable serum cobalamin concentrations have been reported in the Border Collie,[17] Chinese Shar-Pei,[7] and German Shepherd Dog,[3] but not in the Akita. An association of undetectable serum cobalamin concentration and a serum cTLI concentration considered diagnostic for EPI has been previously identified in the German Shepherd Dog,[3] but not in the Akita or Border Collie. In contrast, the Chinese Shar-Pei did not show an association with a serum cTLI concentration considered diagnostic for EPI. Therefore, it appears that only some breeds with undetectable serum cobalamin concentration have an association with EPI. In the Chinese Shar-Pei, for which a high prevalence of cobalamin deficiency has previously been described in North America[7] and the United Kingdom (Dandrieux JRS, et al.: 2010, Breed predispositions for severe hypocobalaminemia), it appears that the cobalamin deficiency is not associated with EPI but rather with a defect in cobalamin metabolism[7] (Grützner N, Stupka KC,

Suchodolski JS, et al.: 2011, Evaluation of serum methylmalonic acid concentrations in Chinese Shar Peis and dogs of six other breeds with cobalamin deficiency. J Vet Intern Med 25:692. Abstract).

Nineteen breeds had higher proportions of samples submitted for serum cobalamin analysis. Breeds such as the American Eskimo, Keeshond, and Standard Schnauzer with undetectable serum cobalamin concentrations did not show an association with EPI. Those breeds might have been overrepresented in the present study because they have been identified in another study as having abnormal findings on Spec cPL® testing (Bishop MA, Xenoulis PG, Suchodolski JS, et al.: 2010, Association between breeds and increased serum Spec cPL. J Vet Intern Med 24:752–753. Abstract). Spec cPL is a test used to diagnose pancreatitis in dogs,[14] and therefore veterinarians might have submitted serum samples from those breeds more frequently to GL-TAMU for concurrent serum cobalamin analysis.

The Chinese Shar-Pei, Staffordshire Bull Terrier, and a mixed-breed dog have been described in the United Kingdom as having a higher risk of low serum cobalamin concentration, while the Boxer, Bullmastiff, English Setter, Flat-Coated Retriever, Golden Retriever, Old English Sheepdog, and Weimaraner have a low risk for low serum cobalamin concentration (Dandrieux JRS, et al.: 2010, Breed predispositions for severe hypocobalaminemia). In the current retrospective study, the Staffordshire Bull Terrier did not show a higher risk of low serum cobalamin concentration. On the other hand, breeds such as the Boxer and Golden Retriever were underrepresented in both North America and the United Kingdom with regard to decreased cobalamin concentration, which suggests that neither breed is predisposed to cobalamin deficiency.

In conclusion, results of the present retrospective study indicate that the Akita, Chinese Shar-Pei, German Shepherd Dog, Greyhound, and Labrador Retriever had an increased proportion with regard to a serum cobalamin concentration below the lower limit of the reference interval. Akitas, Chinese Shar-Peis, German Shepherd Dogs, and Border Collies had an increased proportion of serum cobalamin concentrations below the detection limit of the assay. Furthermore, undetectable serum cobalamin concentrations were associated with a serum cTLI concentration considered diagnostic for EPI in the Akita, Australian Shepherd, Border Collie, German Shepherd Dog, Cairn Terrier, Cardigan Welsh Corgi, Cocker Spaniel, Dalmatian, West Highland White Terrier, and Wire Fox Terrier. However, in the Chinese Shar-Pei, undetectable serum cobalamin concentrations were not associated with serum cTLI concentrations suggestive of EPI. Greyhounds had the highest proportion of serum samples submitted for serum cobalamin analysis. Further investigations are warranted in the breeds identified in this study to clarify if any breed-specific gastrointestinal disorders may exist.

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