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Relationship between serum cobalamin concentration and endoscopic ileal appearance and histology in dogs with chronic inflammatory enteropathy

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Abstract

Background: It has not been determined whether ileal appearance differs among dogs with chronic inflammatory enteropathy (CIE) and different serum concentrations of cobalamin.

Objective: To compare endoscopic and histologic ileal findings in dogs with CIE and different serum cobalamin concentrations and then evaluate the correlation of ileal changes to cobalamin serum concentration using updated scoring systems to assess the ileum.

Animals: Sixty-eight dogs with CIE.

Methods: Retrospective study. Frequency of ileal features and ileal histologic and endoscopic scores (IHS and IES) were obtained and compared among CIE dogs with severe hypocobalaminemia (SHC; <200 ng/L), hypocobalaminemia (HC; 200-350 ng/L), or normocobalaminemia (NC; >350 ng/L). The correlation of IHS and IES with cobalamin was evaluated.

Results: Friability, villus atrophy, crypt dilatation, epithelial injury, and intraepithelial lymphocytes were more frequent in SHC than in NC dogs (all $P \leq .01$). Median SHC-IES (2; range, 0-4) was higher than NC-IES (1; range, 0-5; $P = .004$). Median SHC-IHS (6; range, 3-9) was higher than HC-IHS (4; range, 1-7; $P < .001$) and NC-IHS (3; range, 1-8; $P < .001$). Cobalamin concentration correlated negatively with IES ($\rho = -.34$, $P = .005$) and IHS ($\rho = -.58$, $P < .001$).

Conclusions and Clinical Importance: Ileal features and involvement degree markedly differed when cobalamin was <200 or >350 ng/L in CIE dogs. With updated scales to assess the mucosa, greater ileal damage was associated with lower serum cobalamin concentration.

KEYWORDS
canine, endoscopy, histopathology, small bowel disease

Abbreviations: CCECAI, canine chronic enteropathy clinical activity index; CIBDAI, clinical inflammatory bowel disease activity index; CIE, chronic inflammatory enteropathy; HC, hypocobalaminemia; IES, ileal endoscopic score; IEL, intraepithelial lymphocytes; IHS, ileal histologic score; NC, normocobalaminemia; PLE, protein-losing enteropathy; SHC, severe hypocobalaminemia; VTH-UEx, Veterinary Teaching Hospital of the University of Extremadura; WSAVA, World Small Animal Veterinary Association.

1 | INTRODUCTION

Chronic inflammatory enteropathy (CIE) in dogs is a gastrointestinal disorder characterized by histological evidence of inflammation in the small intestine.[1] Endoscopy and biopsy sampling of the digestive tract is a crucial tool in the diagnosis of CIE. During the examination, ileoscopy is recommended due to the extreme disparity between ileal, duodenal, or colonic histopathologic findings.[2,3] Nevertheless, the tendency is to explore and biopsy only the stomach, duodenum, and colon due to the difficulty of ileal intubation.[4,5] In those cases, possible injuries to the ileum might have been overlooked.

It has been postulated that chronic mucosal disease affecting the ileum reduces the epithelial expression or function of the cubam receptor, leading to reduced mucosal uptake of cobalamin.[6] On this basis, serum cobalamin concentration might be expected to be a marker of ileal mucosa damage. However, a single earlier study failed to correlate hypocobalaminemia with increased ileal histologic scores obtained according to the World Small Animal Veterinary Association (WSAVA) criteria.[4] Nevertheless, that system showed a high subjectivity and did not include a specific evaluation of ileal biopsy specimens.[7,8]

A new simplified histological scale was developed that found associations not identified by using the WSAVA scheme.[9] However, the relationship between cobalamin and ileum injury has never been reassessed using this histological scale. An added difficulty is that hypocobalaminemia has been defined by different thresholds.[4,10-14] Similarly, a validated endoscopic activity scale for inflammatory bowel disease in dogs was described a few years ago.[15] Nevertheless, there is no description of the endoscopic appearance of the ileum in hypo- or normocobalaminemic dogs with CIE or of the correlation between ileal endoscopic and histologic findings.

Our hypotheses were that dogs with CIE will have different ileal features according to different serum cobalamin concentrations, and ileum damage assessed using updated scales will correlate with cobalamin concentration. If so, serum cobalamin concentration could provide clinicians with information about the severity of ileal damage in CIE dogs if the ileum is not explored and sampled.

Therefore, there were 3 goals of this study. First, to describe and compare histologic and endoscopic findings in the ileum of dogs with CIE with normal and low concentrations of serum cobalamin assessed using updated and validated scales. Second, to determine whether the association between serum cobalamin concentration and ileal mucosa damage can be detected with those scales different from the traditional WSAVA system. Third, to evaluate the relationship between ileal endoscopic activity, histologic changes, and clinical severity in light of those scoring systems.

2 | MATERIAL AND METHODS

2.1 | Study design and selection criteria

The electronic clinical database of the Veterinary Teaching Hospital of the University of Extremadura (VTH-UEx) was searched from January 2016 to December 2020 to identify retrospective cases of CIE in dogs. Only dogs with CIE (diagnosed based on the histologic finding of inflammatory infiltrates within the lamina propria) and in which full gastrointestinal endoscopy had been performed, ileum endoscopic biopsies had been obtained, and serum cobalamin concentration had been determined, were included in the study.

For each case, other causes of persistent signs of gastrointestinal disease (>3 weeks) were ruled out by routine hematologic and serum biochemical findings, fecal parasitology, abdominal ultrasonography, measurement of serum canine trypsin-like immunoreactivity concentration, and histopathological review of endoscopically obtained mucosal biopsy specimens. Proper food (hydrolyzed or selected protein diets not previously prescribed as Hill's z/d, and Royal Canin Hypoallergenic or Royal Canin GI Low Fat and Hill's i/d Low Fat in cases of protein-losing enteropathy [PLE] for a minimum of 4 weeks) and antibiotic trials (metronidazole 10 mg/kg, PO, q 12 hours for a minimum of 3 weeks) were completed before recommending endoscopic examination. Dogs with clinically relevant concurrent extragastrointestinal disease (pancreatitis, hepatic dysfunction, kidney disease, leishmaniasis, and ehrlichiosis), with causes of gastrointestinal disease other than inflammatory enteritis or for which a complete medical record could not be obtained, were excluded. Dogs were excluded if they had received corticosteroids or other anti-inflammatory or immunosuppressant medication within 2 weeks before undergoing endoscopy and sampling.

Recorded data included age, breed, sex, and the clinical severity of disease for each dog according to 2 previously published scoring systems: the Clinical Inflammatory Bowel Disease Activity Index (CIBDAI)[16] and the Canine Chronic Enteropathy Clinical Activity Index (CCECAI).[17] For each case, the presence of hypoalbuminemia (serum albumin concentration <3 g/dL) was also recorded.

Cobalamin values were analyzed by an external laboratory (Laboklin, Madrid, Spain). Dogs were placed in either the CIE with normal serum cobalamin concentration (>350 ng/L) group (NC), the CIE with hypocobalaminemia (cobalamin concentration 200-350 ng/L) group (HC), or the CIE with severe hypocobalaminemia (cobalamin concentration <200 ng/L) group (SHC). The lower cutoff of 200 ng/L was chosen because it has been proven to be significantly associated with disease outcome[17] and was used in a referential study[4] whose results will be compared to ours. The upper cutoff of 350 ng/L was chosen based on the increased likelihood of clinically important cobalamin deficiency below this concentration; in veterinary practice, cobalamin supplementation is commonly initiated whenever serum cobalamin concentration is <350 ng/L.[10,12,13]

2.2 | Ileum assessment

The ileal endoscopic examination was carried out using a Fuji 2200 or a Storz PV-SG28-140 flexible video endoscope. The endoscopy video recordings of each case were reviewed, assessed, and scored independently by 2 experienced endoscopists using a validated quantitative endoscopic activity score for inflammatory bowel disease in dogs.[15]

This scale grades ileum granularity, friability, erosions, and lymphatic dilatation from 0 to 2 (0, absent; 1, moderate; 2, severe) with a maximum lesion score of 8. Each dog was assigned an ileal endoscopic score (IES; the mean of the scores of the 2 endoscopists).

Flexible endoscopic biopsy forceps with 2.5-mm smooth-edged oval cups were used to collect 8 to 10 mucous membrane specimens from the ileum for histopathological evaluation. All ileal histologic preparations were re-examined and reviewed by a single board-certified pathologist blinded to the endoscopy exam and clinical information for each case. To obtain the ileal histologic score (IHS) for each dog, different morphologic/inflammatory features (villus stunting, crypt dilatation, lacteal dilatation, surface epithelial injury, and lamina propria infiltrates: lymphocytes, eosinophils, and neutrophils, or neutrophils) were scored 0 (absent), 1 (mild), 2 (moderate), or 3 (severe), according to a simplified histopathologic scoring system.[9] The maximum possible score was 21. The presence of intraepithelial lymphocytes (IEL) was independently scored in the same way.

2.3 | Statistical analysis

Data were collected and imported into SPSS Statistics for Windows, version 26 (IBM Corp, Armonk, New York). Categorical data were described using frequencies and proportions. Normality was assessed using a Shapiro-Wilk test. Most data were not normally distributed, so their descriptive statistics were reported as median and range (min-max).

Kruskal-Wallis and Mann-Whitney post hoc tests were used for between-group comparisons of quantitative variables (albumin and cobalamin concentrations, IHS, IES, CIBDAI, and CCECAI). Categorical variables (histologic and endoscopic features) were compared by the chi-squared or Fisher's exact test. For such analyses, ordinal categorical variables were converted into binary variables where its absence was scored as 0 points and its presence as 1 point. The correlations between variables were evaluated by Spearman's correlation analysis. Statistical significance was set at $P < .05$.

3 | RESULTS

3.1 | Study sample

Sixty-eight dogs were included in this study. There were 20 mixed-breed dogs, 4 German Shepherd dogs, 3 each of Cocker Spaniel, Labrador Retriever, and West Highland White Terrier, 2 each of American Staffordshire Terrier, English Beagle, English Setter, Boxer, Shar Pei, Siberian Husky, Medium Poodle, Spanish Hound, French Bulldog, Epagneul Breton, Pyrenean Mastiff, and Weimaraner and 1 each of Argentine Dogo, Bernese Mountain Dog, Dalmatian, Border Collie, Greyhound, Belgian Shepherd Malinois, Giant Schnauzer, Chow Chow, Portuguese Water Dog, Staffordshire Bull Terrier, and Standard Schnauzer. Forty-one dogs were male (4 castrated), and 27 were female (10 spayed). The study sample median age was 5 years (range, 1-14 years), and there was no significant difference in age between groups ($P = .53$).

Twenty-nine dogs (29/68, 43%) were normocobalaminemic (NC group). Thirty-nine (39/68, 57%) showed low concentrations of cobalamin, among which 19/68 (28%) were assigned to the HC group and the remaining 20/68 (29%) to the SHC group.

3.2 | Clinical indices and laboratory findings

Significant differences were found in CIBDAI among the 3 groups, and CCECAI was significantly different between the SHC and the other 2 groups. However, CCECAI scores were similar between the HC and NC groups (Table 1). As anticipated by group definitions, differences in cobalamin concentration among the 3 groups were statistically significant (Table 1). In the NC group, 7/29 (24%) dogs showed hypoalbuminemia (median albumin concentration, 3.2 g/dL; range, 1.70-4.20 g/dL). In groups HC (median albumin concentration, 3.2 g/dL; range, 1.40-3.80 g/dL) and SHC (median albumin concentration, 2.79 g/dL; range, 1.7-3.7 g/dL), 5/19 (26%) and 13/20 (65%) dogs, respectively, showed low concentrations of cobalamin and albumin simultaneously. Albumin concentration did not differ significantly among the 3 groups ($P = .09$).

TABLE 1 Data of the indices analyzed in the three groups of dogs with CIE according to the cobalamin serum concentration

	SHC	HC	NC	P
Cobalamin concentration (ng/L)	145 (95-198)[a]	288 (200-343)[b]	415 (354-804.40)[c]	<.0001
CIBDAI	10 (6-14)[a]	7 (1-8)[b]	5 (2-11)[c]	<.0001
CCECAI	8 (8-15)[a]	7 (2-10)[b]	5 (2-14)[b]	<.0001
IHS	6 (3-9)[a]	4 (1-7)[b]	3 (1-8)[b]	<.0001
IES	2 (0-4)[a]	1 (0-4)[ab]	1 (0-5)[b]	.009

Note: Data are represented by median (min-max range). Reported *P* values are for the Kruskal-Wallis test. Within a row, data without a common superscript differ ($P < .05$).

Abbreviations: CCECAI, canine chronic enteropathy clinical activity index[17]; CIBDAI, clinical inflammatory bowel disease activity index[16]; CIE, chronic inflammatory enteropathy; HC, hypocobalaminemia; IES, ileal endoscopic score[15]; IHS, ileal histologic score[9]; NC, normocobalaminemia; SHC, severe hypocobalaminemia.

TABLE 2 Comparative of the endoscopic findings in the ileum of dogs with CIE between the different cobalamin groups

	SHC	HC	NC	*P*
Friability	8/20 (40%)[a]	3/19 (15.8%)[ab]	3/29 (10.3%)[b]	.03
Granularity	13/20 (65%)[ab]	14/19 (73.7%)[a]	12/29 (41.4%)[b]	.03
Erosions	3/20 (15%)[a]	0/19 (0%)[a]	3/29 (10.3%)[a]	.23
Lymphangiectasia	8/20 (40%)[a]	6/19 (31.6%)[a]	5/29 (17.2%)[a]	.42

Note: *P* values correspond to chi-squared test. Within a row, data without a common superscript differ ($P < .05$).
Abbreviations: CIE, chronic inflammatory enteropathy; HC, hypocobalaminemia; NC, normocobalaminemia; SHC, severe hypocobalaminemia.

TABLE 3 Comparative of the histopathologic findings in the ileum of dogs with CIE between the different cobalamin groups

	SHC	HC	NC	*P*
Villus atrophy	18/20 (90%)[a]	12/19 (63.2%)[ab]	11/29 (37.9%)[b]	<.0001
Epithelial injury	8/20 (40%)[a]	6/19 (31.6%)[ab]	3/29 (10.3%)[b]	.04
Crypt dilatation	16/20 (80%)[a]	13/19 (68.4%)[a]	8/29 (27.6%)[b]	.002
Lacteal dilatation	11/20 (55%)[a]	6/19 (31.6%)[a]	8/29 (27.6%)[a]	.11
LP lymphocites	20/20 (100%)[a]	19/19 (100%)[a]	29/29 (100%)[a]	1
LP eosinophils	4/20 (20%)[a]	3/19 (15.8%)[a]	4/29 (13.8%)[a]	.84
LP neutrophils	8/20 (40%)[a]	3/19 (15.8%)[a]	7/29 (24.1%)[a]	.39
IEL	14/20 (70%)[a]	3/19 (15.7%)[b]	3/29 (10.3%)[b]	<.0001

Note: *P* values correspond to chi-squared test. Within a row, data without a common superscript differ ($P < .05$).
Abbreviations: CIE, chronic inflammatory enteropathy; HC, hypocobalaminemia; IEL, intraepithelial lymphocites; LP, lamina propria; NC, normocobalaminemia; SHC, severe hypocobalaminemia.

3.3 | Endoscopic findings

Endoscopic intubation and exploration of the ileum were achieved in all dogs. The most common endoscopic abnormality recorded, present in 39/68 (57%) cases, was the increase in mucosal texture (granularity), mostly to a mild degree, followed by lymphangiectasia in 19/68 (28%) cases. Friability was noted in 14/68 (20%) dogs, and 6/68 (9%) dogs exhibited mucosal erosions.

The frequency of each endoscopic lesion according to severity degree and group is reported in Table S1. Friability, erosions, and lymphangiectasia were most frequent in the SHC group, and granularity was most common in the HC group. However, the only significant differences were in the proportion of friability between SHC and NC dogs and the proportion of granularity between HC and NC dogs (Table 2). Furthermore, the SHC group showed the highest frequency for every severity category of each endoscopic lesion, except for the mild increase in granularity, which was found most often in the HC group.

Finally, concerning IES, the maximum score obtained was 5 (out of a possible 8), and 59/68 (87%) dogs obtained scores between 0 and 3. The IES was significantly higher in the SHC group than in the NC group. There were no other differences among the groups (Table 1).

3.4 | Histopathologic findings

Ileal biopsy samples were obtained by endoscopy in all cases. All dogs had primary lymphoplasmacytic inflammation and showed some alteration in the studied ileal histologic features, to varying extents. An increased number of lamina propria lymphocytes was observed in all dogs, to a moderate degree in 37/68 (54%) cases. The second and third most frequent features present in the ileal biopsies were villous atrophy, found in 41/68 (60%) dogs, and crypt dilatation, found in 37/68 (54%) dogs, followed by lacteal dilatation in 25/68 (37%) dogs and an increase in IEL in 20/68 (29%) dogs. Infiltration of neutrophils in the lamina propria (18/68, 26%), epithelial injury (17/68, 25%), and infiltration of eosinophils in the lamina propria (11/68, 16%) were the less common abnormalities.

The SHC group had a significantly greater proportion of dogs with villus atrophy and epithelial injury than the NC group and a greater proportion of cases with increased IEL than both the NC and HC groups. The occurrence of crypt dilatation was significantly higher in the SHC and HC groups than in the NC group. Epithelial injury, lacteal dilatation, and lamina propria infiltration were more frequently observed in the SHC group, but the differences among the groups for all 3 features were not significant (Table 3).

The frequency of each histopathologic feature according to severity degree and group is presented in Table S2. Moderate and severe degrees of histologic features were observed most frequently in the SHC group. The highest IHS obtained was 9 (out of a maximum possible of 21), and 48/68 (65%) dogs had scores ≤5. Among the 3 groups, IHS was significantly higher in the SHC group. No differences in IHS were found between the HC and NC groups (Table 1).

TABLE 4 Spearman correlation coefficients between the different variables analyzed in CIE dogs

		IES	IHS	IEL	CIBDAI	CCECAI	ALB
Cobalamin		$\rho = -.34$ $P = .005$	$\rho = -.58$ $P < .001$	$\rho = -.46$ $P < .001$	$\rho = -.58$ $P < .001$	$\rho = -.51$ $P < .001$	$\rho = .21$ $P = .08$
All population	IES		$\rho = .49$ $P < .001$	$\rho = .11$ $P = .33$	$\rho = .25$ $P = .03$	$\rho = .31$ $P = .009$	
	IHS			$\rho = .46$ $P < .001$	$\rho = .67$ $P < .001$	$\rho = .70$ $P < .001$	
NC	IES		$\rho = .50$ $P = .006$		$\rho = .24$ $P = .90$	$\rho = .03$ $P = .85$	
	IHS				$\rho = .25$ $P = .18$	$\rho = .27$ $P = .15$	
HC	IES		$\rho = .18$ $P = .44$		$\rho = .43$ $P = .06$	$\rho = .41$ $P = .77$	
	IHS				$\rho = .52$ $P = .01$	$\rho = .67$ $P = .002$	
SHC	IES		$\rho = .08$ $P = .73$		$\rho = .24$ $P = .30$	$\rho = .09$ $P = .68$	
	IHS				$\rho = .56$ $P = .009$	$\rho = .55$ $P = .01$	

Abbreviations: ALB, albumin; CCECAI, canine chronic enteropathy clinical activity index[17]; CIBDAI, clinical inflammatory bowel disease activity index[16]; CIE, chronic inflammatory enteropathy; HC, hypocobalaminemia; IEL, intraepithelial lymphocites; IES, ileal endoscopic score[15]; IHS, ileal histologic score[9]; NC, normocobalaminemia; SHC, severe hypocobalaminemia.

3.5 | Correlation study

According to Spearman's test, cobalamin concentration showed a weak negative association with IES and a moderate negative correlation with IHS. An increased presence of IEL was also moderately correlated with lower serum cobalamin. Similarly, a moderate negative correlation of cobalamin with CCECAI and CIBDAI was observed. No correlation was found between cobalamin and albumin concentrations. Endoscopic and histologic scores were somewhat correlated. IEL showed a moderate positive correlation with the histologic score but not with the endoscopic score. CCECAI and CIBDAI were directly and moderately correlated with IHS but poorly with IES (Table 4).

The within-group correlations showed no correlation between the clinical indices and histological or endoscopic indices in the NC group. However, IHS and IES were significantly positively correlated. On the contrary, a significant positive correlation was detected between the clinical indices and IHS in the HC and SHC groups, but not with IES nor between histologic and endoscopic indices (Table 4).

4 | DISCUSSION

This study compares the endoscopic and histologic abnormalities in the ileum of CIE dogs with different serum cobalamin concentrations. The only 3 studies that previously addressed ileal findings in dogs with chronic enteropathy focused on potential associations with the duodenal mucosa histology[2-4] or possible correlations between histological changes and clinical activity.[9] However, none provided any description of ileal features and their frequency in dogs with enteropathy, and only 1 secondarily addressed the cobalamin impact.[4]

The gross endoscopic appearance of the ileum has been traditionally assessed using the endoscopic guidelines developed by the WSAVA Gastrointestinal Standardization Group.[8] During endoscopy, the report forms provided are extremely useful in helping to ensure that examinations are complete. However, the endoscopic activity score used in this study is the only validated scale in veterinary medicine to assess endoscopy activity in dogs with inflammatory bowel disease.[15] It was designed to exclude the more infrequent or useless parameters and select those features with the best interobserver agreement and reproducibility among experienced endoscopists. However, neither the WSAVA guidelines nor the scale used in this study provided any representative image of the peculiarities of the ileal mucosa in dogs with CIE.

In our study, the most frequent findings were increased mucosal texture (granularity) and multifocal white granular foci present in the ileal mucosa. Granularity has never been reported as a feature of the ileum affected by CIE, but ileal lymphangiectasia has been described previously in 3 dogs from a small sample of 10 with gastrointestinal disorders.[18]

We showed that endoscopic lesions, such as erosions, lymphangiectasia, and particularly mucosal friability, were more likely, and gross ileal injury was significantly greater in dogs with CIE when the cobalamin concentration was <200 ng/L than when it was >350 ng/L. In addition, intermediate concentrations provided little useful information, and the endoscopic ileum involvement could resemble that of 1 or other extreme. However, because cobalamin and endoscopic activity demonstrated a weak inverse correlation in

our study, it might be expected that as cobalamin approaches the upper margin, the characteristics of the ileum resemble more closely those of normocobalaminemic dogs and vice versa.

According to a recent study, ileoscopy can be challenging and involve certain risks. Iatrogenic ileocecocolic perforation is a rare but serious complication that might occur, mainly when lower endoscopy is performed by a novice endoscopist.[19] This might be a deterrent to performing routine ileoscopy and could be a possible reason for the lack of knowledge on ileum appearance.

It has been stated that low serum cobalamin concentration suggests a focal or diffuse mucosal disorder affecting absorption in the distal (ileum) small intestine and is an indication for endoscopy.[5] The WSAVA International Gastrointestinal Standardization Group recommended obtaining ileal biopsies in animals whenever gastroduodenoscopy or colonoscopy seems indicated because ileal biopsy is recognized as providing valuable information not always found in duodenal or colonic biopsies.[8] However, the decision to perform ileoscopy is not usually determined by the cobalamin concentration. In fact, cobalamin concentration is often unknown at the time of the endoscopy, as the results of the gastrointestinal panel might be pending when the procedure is performed based on the client appointment and the hospital schedule. Then, the final decision is dependent mainly on the endoscopist's experience, clinical signs, or even on anesthetic considerations because of the increased exploration time.[4,5] Our study demonstrated that cobalamin might be a suitable indicator of ileal involvement in CIE dogs without ileoscopy. Nevertheless, the decision to perform ileoscopy should be based on the diagnostic value of the ileum exploration and sampling. An early study in dogs found that the concordance of histologic diagnosis between duodenal and ileal sites was high for lymphoplasmacytic enteritis (73%) but low for eosinophilic enteritis (17%).[3] The same study also confirmed the discordance between upper and lower gastrointestinal biopsies for small-cell lymphoma in dogs.[3] In our opinion, these are compelling reasons to perform ileoscopy. Nevertheless, in those cases in which CIE was the diagnosis and endoscopy did not include ileoscopy, this study and cobalamin concentration might provide the clinician with insight into the ileum's condition.

The simplified histopathologic model[9] used in this study was designed and later refined as an extension of the original WSAVA criteria[7,8] to reduce interobserver variability and improve consistency in the diagnostic interpretation of gastrointestinal inflammation between pathologists. Furthermore, the WSAVA system does not include ileal templates for histopathologic evaluation of ileal biopsies, although there is a general agreement on the need to explore and sample the ileum.[7-9] Consequently, the ileum has usually been assessed and scored as the duodenum. Our study now offers a description of the specific characteristics of the ileal mucosa of dogs with CIE.

All dogs in this study showed some histopathological abnormality. In a previous study, microscopic lesions were found in 80% of ileal samples of dogs with enteropathy.[2] Accordingly to 1 study, the likelihood of obtaining adequate mucosal samples and identifying pathology is better in the ileum than in the duodenum due to its relatively thinner mucosa.[5]

As noted macroscopically, histopathological changes in the ileal mucosa are more frequent and severe when the cobalamin serum concentration is <200 ng/L, and significantly more scarce and milder abnormal features can be observed when concentrations are >350 ng/L. However, our study detected an increased presence of crypt dilatation in the intermediate range when the cobalamin fell <350 ng/L.

The presence of IEL in the small intestine was not included as a parameter in the simplified histopathologic scoring system.[9] However, because it has been correlated with hypocobalaminemia,[4] we decided to analyze it separately. This was valuable as it confirmed the relationship between lower cobalamin concentration and the rise of IEL. Moreover, the proportion of increased IEL proved to be the only parameter that differed between the 2° of hypocobalaminemia in our study. Furthermore, dogs with SHC had a greater proportion of villous stunting, epithelial injury, crypt dilatation, and IEL and to a more severe degree than NC dogs.

In our study, morphologic features, except for lacteal dilatation, vary significantly between cobalamin concentrations, whereas inflammatory infiltrates, except for IEL, were not different between groups. This phenomenon was also observed in the small intestine when histopathologic features were compared between hypo- vs normoalbuminemic dogs diagnosed with CIE.[20] Another study argued that morphological features appeared to be more important than the intensity of duodenal inflammation in the assessment of lymphoplasmacytic enteropathy in dogs.[21]

Our data shows that ileal involvement can be found more frequently when cobalamin is <200 ng/L, with features distinct from those observed when the concentration is >350 ng/L. Furthermore, the severity of the histologic injury is significantly greater when the cobalamin concentration is <200 ng/L than >200 ng/L. These findings support previous studies in which dogs with chronic enteropathy and initial serum cobalamin concentrations below the cutoff value of 200 ng/L had a markedly higher chance of a negative outcome.[17] Thus, 200 ng/L is the value below which hypocobalaminemia should be considered in studies on CIE in dogs.

Our study confirms the correlation between hypocobalaminemia and the clinical severity of disease (CCECAI) reported previously.[4,22] Contrarily, however, while the presence of hypocobalaminemia could not be related to the ileal histologic WSAVA scores,[4] we found that cobalamin concentration was inversely associated not only with the ileal histopathologic damage but also with endoscopy activity in our study. The assessment scales used in our study have likely contributed to achieving these results.

Previously, it was claimed that of the 3 mechanisms that reduce cobalamin availability from the small intestine (congenital disorders of receptor function, excess competition from the intestinal microbiome or decreased mucosal absorptive capacity) the latter was likely to be the most important in clinical veterinary medicine.[23] Cobalamin is essential for many cell functions and mucosal regeneration, and a deficiency can contribute to mucosal inflammatory infiltration and villous atrophy.[24,25] On this basis, and the correlation between hypocobalaminemia and the increased number of IEL and lacteal

dilatation in the ileal mucosa, an association between ileal mucosal inflammatory changes and hypocobalaminemia in dogs with chronic enteropathy has been expected.[6,21] Our results confirmed the relationship and showed that cobalamin might be considered an indicator of ileal mucosa involvement in dogs with CIE. This exploratory research should be complemented by an evaluation of the expression of cobalamin-receptors in dogs with CIE.

Our third aim was to review the relationships of clinical severity with endoscopic and histologic ileal activities using updated scales rather than the WSAVA's, which could not establish any association between clinical activity and ileal histology.[4] More recently, a nearly significant positive correlation between the CCECAI and the IHS was found using the simplified scoring system.[9] The present study goes a step further, definitively establishing a moderate positive correlation between the IHS and clinical activity. The reasons for this improved correlation might lie in the larger sample of this study and the study sample cobalamin serum concentrations. We noted that clinical and histologic ileal indices directly correlated in the dogs with cobalamin concentrations <350 ng/L but not in the NC group. Thus, the correlation between ileal histology and clinical activity improves at lower cobalamin concentrations. Conversely, the study referred to earlier[9] did not provide data concerning cobalamin concentration.

A few studies have attempted to associated endoscopic lesions with clinical severity and histopathologic features.[16,26-28] Each employed a different endoscopic scale, but all agreed on the lack of a relationship among them. The absence of a validated scale and the differences in operator experience have been suggested to explain these discordant results. With the use of a validated endoscopic scale and a simplified histologic scale, a slight positive correlation between endoscopic and histopathologic involvement of the ileum was found in our study. Nevertheless, we did not observe a relationship between clinical and endoscopy activity.

The within-group correlations showed how histology and endoscopy are only associated in dogs with normal cobalamin concentrations, probably due to the scarcity of lesions in the ileum at both the microscopic level and the macroscopic level. On the contrary, as cobalamin decreases, the microscopic changes occur with greater intensity than the macroscopic ones, thus resulting in a lack of correlation between the endoscopic and histological indices.

In this study, the more severe ileal damage correlated not only with lower cobalamin concentration but also with higher clinical indices. Low cobalamin concentration and high clinical activity indices have been previously associated with poor prognosis.[17] Thus, it could be inferred that high endoscopic and histologic ileal scores together with high clinical indices and low cobalamin concentration could worsen the prognosis of dogs with CIE. Future research that includes the outcome of dogs with CIE is required to determine the effect of the endoscopic and histologic ileal damage on the prognosis of the disease.

In this study, hypoalbuminemia and hypocobalaminemia coexisted more frequently in animals with higher clinical activity and more severe and extensive intestinal involvement, as noted previously.[17,21,29] Although some studies associated hypocobalaminemia with hypoalbuminemia in dogs with chronic enteropathy,[17,22,30] no correlation between the trend of cobalamin and albumin concentrations was found in the present study. Despite this, it is indisputable that the proportion of hypoalbuminemic individuals was highest in the group of dogs with SHC. The prevalence of hypocobalaminemia ranges from 19% to 54% in dogs with chronic enteropathies[14] but reportedly increases to 43%-75% in dogs with nonneoplastic, noninfectious causes of PLE.[6] However, a review showed that low serum cobalamin was described in only 8 of 23 studies on PLE dogs.[31] Despite this, a clear limitation of our study is the lack of a group containing dogs with PLE. Consequently, future research to investigate and compare the ileal changes in hypo- and normocobalaminemic PLE and non-PLE dogs is recommended.

This study has other limitations. The first is the low quality of biopsied samples obtained endoscopically because of the potential to miss lesions deep within the submucosal and muscularis layers.[32] As mentioned above, biopsy sample quality is influenced by operator experience, the endoscopic technique of sample collection, the number of specimens collected, and the biopsy specimen handling. Superior endoscopic specimen quality is obtained by using an endoscope that allows the largest forceps possible (2.8 better than 2.2). Fenestrated forceps cause fewer crush artifacts and yield larger biopsy specimens than nonfenestrated models.[5] Six adequate or 10 to 15 marginal samples should be collected from the canine duodenum, depending on the lesion being sought. The exact number of recommended endoscopic specimens from the ileum is unknown, although 3 to 5 adequate biopsies seem to be enough.[33] To be considered adequate, a biopsy sample should contain the full thickness of the mucosa and be wide enough to have at least 3 to 4 intact and preferably contiguous villi. Fewer biopsy samples are needed to establish a diagnosis as the quality of the tissue increases. However, it is typically easy to obtain high-quality biopsies from the ileum because ileal mucosa is relatively thin, allowing for full-thickness specimens with minimal effort.[5] In general, skilled endoscopists must take fewer samples than less-skilled endoscopists to achieve the same number of adequate samples.[8] Tissue samples should be carefully removed from the biopsy forceps and placed directly into 10% formalin or collected in a foam-lined tissue cassette before fixation.[5,8] In an effort to maximize the quality of the histopathologic analysis and to fulfill the recommendations mentioned, 2.5-mm smooth-edged fenestrated oval-cupped biopsy forceps were used, and a minimum of 8 to 10 good-quality biopsies were collected by experienced endoscopists in this study. Samples were submitted in 2 forms: free-floating in formalin; in commercial cassettes with sponges.

Another limitation of this study is that mainly medium- and large-sized dogs, which allowed ileal intubation with an endoscope of 8 to 9 mm in diameter and a 2.8-mm channel, were included. Thus, breeds highly predisposed to PLE, like the Yorkshire Terrier, are underrepresented in this study. No purebred Yorkshire Terrier, Bichon Frise, or Maltese was included but only crosses of those and similar breeds in which ileoscopy could be performed with the endoscope described.

Finally, a breed predisposition for hypocobalaminemia has been identified.[11] Predisposed dogs, like German Shepherd dogs (n = 4),

Labrador Retrievers (n = 3), Shar Peis (n = 2), Greyhounds (n = 1), and Border Collies (n = 1), represented 16% (11/68) of the study sample in our study. Although we cannot rule out potential selection bias, other studies on CIE described cohorts with a similar proportion of those breeds.[3,4,10,17,21,22,25,29,34-37] In those in which cobalamin concentration was also detailed, the percentages of hypocobalaminemic dogs[4,10,21,22] were similar to that of our study. Only 2 studies reported percentages of dogs with cobalamin <200 ng/L higher (42%)[3] or lower (19%)[17] than that we have observed (30%).

Therefore, this study sample can be considered representative of what could be found in routine clinical practice.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES

1. Simpsons KW, Jergens AE. Pitfalls and progress in the diagnosis and management of canine inflammatory bowel disease. *Vet Clin Small Anim*. 2011;41:381-398.
2. Casamian-Sorrosal D, Willard MD, Murray JK, Hall EJ, Taylor SS, Day MJ. Comparison of histopathologic findings in biopsies from the duodenum and ileum of dogs with enteropathy. *J Vet Intern Med*. 2010;24:80-83.
3. Caulfield S, Priestnall S, Kathrani A. Concordance of the histopathologic diagnosis of concurrent duodenal and ileal biopsy specimens in dogs. *Animals*. 2021;11:2938.
4. Procoli F, Mõtsküla SV, Keyte S, et al. Comparison of histopathologic findings in duodenal and ileal endoscopic biopsies in dogs with chronic small intestinal enteropathies. *J Vet Intern Med*. 2013;27:268-274.
5. Jergens AE, Willar MD, Allenspach K. Maximizing the diagnostic utility of endoscopic biopsy in dogs and cats with gastrointestinal disease. *Vet J*. 2016;214:50-60.
6. Kather S, Grützner N, Kook PH, Dengler F, Heilmann RM. Review of cobalamin status and disorders of cobalamin metabolism in dogs. *J Vet Intern Med*. 2020;34:13-28.
7. Day MJ, Bilzer T, Mansell J, et al. Histopathological standards for the diagnosis of gastrointestinal inflammation in endoscopic biopsy samples from the dog and cat: a report from the World Small Animal Veterinary Association Gastrointestinal Standardization Group. *J Comp Pathol*. 2008;138(Suppl 1):S1-S43.
8. Washabau RJ, Day MJ, Willard MD, et al. Endoscopic, biopsy, and histopathologic guidelines for the evaluation of gastrointestinal inflammation in companion animals. *J Vet Intern Med*. 2010;24:10-26.
9. Allenspach KA, Mochel JP, Du Y, et al. Correlating gastrointestinal histopathologic changes to clinical disease activity in dogs with idiopathic inflammatory bowel disease. *Vet Pathol*. 2019;56:435-443.
10. Berghoff N, Suchodolski JS, Steiner JM. Association between serum cobalamin and methylmalonic acid concentrations in dogs. *Vet J*. 2012;191:306-311.
11. Grützner N, Cranford SM, Norby B, Suchodolski JS, Steiner JM. Evaluation of serum cobalamin concentrations in dogs of 164 dog breeds (2006-2010). *J Vet Diagn Invest*. 2012;24:1105-1114.
12. Toresson L, Steiner JM, Suchodolski JS, Spillmann T. Oral cobalamin supplementation in dogs with chronic enteropathies and hypocobalaminemia. *J Vet Intern Med*. 2016;30:101-107.
13. Stanley E, Appleman E, Schlag A, Siegel A. Relationship between cobalamin and folate deficiencies and anemia in dogs. *J Vet Intern Med*. 2019;33:106-113.
14. Heilmann RM, Steiner JM. Clinical utility of currently available biomarkers in inflammatory enteropathies of dogs. *J Vet Intern Med*. 2018;32:1495-1508.
15. Slovak JE, Wang C, Sun Y, et al. Development and validation of an endoscopic activity score for canine inflammatory bowel disease. *Vet J*. 2015;203:290-295.
16. Jergens AE, Schreiner CA, Frank DE, et al. A scoring index for disease activity in canine inflammatory bowel disease. *J Vet Intern Med*. 2003;17:291-297.
17. Allenspach K, Wieland B, Gröne A, Gaschen F. Chronic enteropathies in dogs: evaluation of risk factors for negative outcome. *J Vet Intern Med*. 2007;21:700-708.
18. Malancus RN, Tofan CM. Assessment of ultrasonographic and endoscopic changes in dogs with gastrointestinale disorders. *Arq Bras Med Vet Zootec*. 2017;69:1451-1455.
19. Woolhead VL, Whittemore JC, Stewart SA. Multicenter retrospective evaluation of ileocecocolic perforations associated with diagnostic lower gastrointestinal endoscopy in dogs and cats. *J Vet Intern Med*. 2020;34:684-690.
20. Wennogle SA, Priestnall SL, Webb CB. Histopathologic characteristics of intestinal biopsy samples from dogs with chronic inflammatory enteropathy with and without hypoalbuminemia. *J Vet Intern Med*. 2017;31:371-376.
21. Moser K, Mitze S, Teske E, Von Bomhard W, Stockhaus C. Correlation of clinical, diagnostic and histopathological parameters in dogs with chronic lymphocytic-plasmacytic enteropathy. *Tierarztl Prax Ausg K Kleintiere Heimtiere*. 2018;46:15-20.
22. Volkmann M, Steiner JM, Fosgate GT, Zentek J, Hartmann S, Kohn B. Chronic diarrhea in dogs—retrospective study in 136 cases. *J Vet Intern Med*. 2017;31:1043-1055.
23. Ruaux CG. Cobalamin in companion animals: diagnostic marker, deficiency states and therapeutic implications. *Vet J*. 2013;196:145-152.
24. Weiss DJ. Congenital dyserythropiesis. In: Weiss JD, Wardrop KJ, eds. *Schalm's Veterinary Hematology*. Vol 6. Ames, IA: Wiley; 2010:196-197.
25. Berghoff N, Steiner JM. Laboratory tests for the diagnosis and management of chronic canine and feline enteropathies. *Vet Clin North Am Small Anim Pract*. 2011;41:311-328.

26. Roth L, Leib MS, Davenport DJ, Monroe WE. Comparisons between endoscopic and histologic evaluation of the gastrointestinal tract in dogs and cats: 75 cases (1984-1987). *J Am Vet Med Assoc*. 1990;196:635-638.
27. Garcia-Sancho M, Rodriguez-Franco F, Sainz A, et al. Evaluation of clinical, macroscopic, and histopathologic response to treatment in nonhypoproteinemic dogs with lymphocytic-plasmacytic enteritis. *J Vet Intern Med*. 2007;21:11-17.
28. Schreiner NMS, Gaschen F, Grone A, et al. Clinical signs, histology, and CD3-positive cells before and after treatment of dogs with chronic enteropathies. *J Vet Intern Med*. 2008;22:1079-1083.
29. Craven M, Simpson JW, Ridyard AE, Chandler ML. Canine inflammatory bowel disease: retrospective analysis of diagnosis and outcome in 80 cases (1995-2002). *J Small Anim Pract*. 2004;45:336-342.
30. Heilmann RM, Volkmann M, Otoni CC, et al. Fecal S100A12 concentration predicts a lack of response to treatment in dogs affected with chronic enteropathy. *Vet J*. 2016;215:96-100.
31. Craven M, Washbau R. Comaparative pathophysiology and management of protein-losing enteropathy. *J Vet Intern Med*. 2019;33:383-402.
32. Larson RN, Ginn JA, Bell CM, Davis MJ, Foy DS. Duodenal endoscopic findings and histopathologic confirmation of intestinal lymphangiectasia in dogs. *J Vet Intern Med*. 2012;26:1087-1092.
33. Willard J, Mansell GT, Fosgate M, et al. Effect of sample quality on the sensitivity of endoscopic biopsy for detecting gastric and duodenal lesions in dogs and cats. *J Vet Intern Med*. 2008;22:1084-1089.
34. Wennogle S, Priestnall SL, Suárez-Bonnet A, et al. Lymphatic endothelial cell immunohistochemical markers for evaluation of the intestinal lymphatic vasculature in dogs with chronic inflammatory enteropathy. *J Vet Intern Med*. 2019;33:1669-1676.
35. Wennogle S, Priestnall SL, Suárez-Bonnet A, et al. Comparison of clinical, clinicopathologic, and histologic variables in dogs with chronic inflammatory enteropathy and low or normal serum 25-hydroxicholecalciferol concentrations. *J Vet Intern Med*. 2019;33:1995-2004.
36. Karlovits S, Manz A, Allenspach K, et al. Ki-67/CD3 ratio in the diagnosis of chronic inflammatory enteropathy in dogs. *J Vet Intern Med*. 2019;34:92-97.
37. Manz A, Allenspach K, Kummer S, et al. Upregulation of signal transducer and activator of transcription 3 in dogs with chronic inflammatory enteropathies. *J Vet Intern Med*. 2021;35:1288-1296.

SUPPORTING INFORMATION

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