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# Comparison of Histopathologic Findings in Duodenal and Ileal Endoscopic Biopsies in Dogs with Chronic Small Intestinal Enteropathies

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**Background:** The current tendency when investigating dogs with chronic upper gastrointestinal signs is to perform endoscopy and biopsy only the duodenum. This approach could lead to overlooking important ileal lesions and affect the clinical management.

**Objectives:** To compare concurrent duodenal and ileal endoscopic biopsies in dogs with chronic enteropathies and evaluate their correlation with clinicopathologic findings.

**Animals:** Thirty-eight dogs with chronic enteropathies.

**Methods:** Duodenal and ileal biopsies were retrospectively reviewed. Nine histologic variables, 5 structural (villous stunting, epithelial injury, crypt distension, lacteal dilatation, and mucosal fibrosis) and 4 inflammatory (intraepithelial lymphocytes, lamina propria lymphocytes and plasma cells, eosinophils, and neutrophils) were scored. Clinical severity scores and relevant clinicopathologic variables were evaluated.

**Results:** There was only slight agreement between duodenal and ileal histologic scores ($\kappa = 0.003$). There was slight agreement between the presence of any of the morphological and inflammatory variables, with the exception of mucosal fibrosis ($\kappa = 0.44$). Statistically significant correlation was found between clinical severity and duodenal crypt distension ($P = .031$), ileal lacteal dilatation ($P = .038$), and ileal mucosal lymphoplasmacytic inflammation ($P = .035$). A significant correlation was found between hypoalbuminemia and ileal lacteal dilatation ($P = .033$) and number of ileal intraepithelial lymphocytes ($P = .019$). A statistically significant correlation was found between hypocobalaminemia and number of ileal intraepithelial lymphocytes ($P = .012$).

**Conclusions and Clinical Importance:** When investigating dogs with chronic upper gastrointestinal signs, the collection of concurrent duodenal and ileal endoscopic biopsies is recommended.

**Key words:** Duodenum; Enteropathies; Histology; Ileum.

Chronic enteropathies (CE), including food-responsive diarrhea, antibiotic-responsive enteropathy, and inflammatory bowel disease are common causes of persistent or recurrent gastrointestinal signs in dogs.

The diagnosis of canine CE requires an extensive diagnostic evaluation to eliminate other causes of chronic gastrointestinal signs (eg, infectious, metabolic, endocrine, or neoplastic diseases) and it is based on the finding of inflammatory infiltrates within the intestinal lamina propria on histopathologic examination of intestinal biopsies. Since its advent, flexible endoscopy is the preferred method for collecting intestinal biopsies, whereas abdominal surgery and laparoscopy are performed less frequently.[1]

In recent years, the clinical utility of intestinal histopathology in the diagnosis and management of canine CE has come under scrutiny because of evidence that clinical signs, clinicopathologic findings, and response to treatment do not correlate with histopathologic findings.[2–4] Whereas it is possible that histopathology simply does not reflect clinical disease, alternative explanations for this discrepancy include variability in interpretation of the same intestinal biopsy by different pathologists[5] and the impact of poor biopsy quality or inadequate tissue processing on the pathologist's ability to identify specific intestinal lesions.[6–8]

**Abbreviations:**

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| CCECAI | canine chronic enteropathy clinical activity index |
| CE | chronic enteropathies |
| cPLI | canine-specific pancreatic lipase immunoreactivity |
| IEL | intraepithelial lymphocytes |
| TLI | trypsin-like immunoreactivity concentration |
| UPC | urine protein to creatinine |
| WSAVA | World Small Animal Veterinary Association |

Another potential reason for this discrepancy could be failure to biopsy the anatomical region of the intestinal tract that is most affected by the pathologic process during endoscopy or surgery. The lesions of canine CE are multifocal and not necessarily diffuse and potentially affect different regions of the intestinal tract with different degrees of severity in each region.[9,10] The current tendency during the endoscopic investigation of dogs with CE is to biopsy the stomach and duodenum only on the assumption that entering the ileum would be difficult and would prolong anesthetic time without necessarily adding diagnostic or prognostic value. However, this approach could result in overlooking important ileal lesions, which subsequently could impact diagnosis, treatment, and prognosis.

A recent study investigated agreement between the histopathologic diagnosis from concurrent duodenal and ileal biopsies (both endoscopically and surgically

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obtained) in dogs with mixed intestinal diarrhea of different causes and found a high percentage of cases in which duodenal and ileal histopathologic lesions differed substantially.[11] In addition, the same study determined that histopathologic abnormalities often were more readily detected in the ileum than in the duodenum. In view of the diversity of histologic diagnoses found in the study and the absence of any clinicopathologic information, the practical relevance of these conclusions and their application in the diagnosis and management of canine CE remain unclear. Nevertheless, the findings of the study raised doubts regarding the reliability of duodenal histopathology alone in dogs with CE. At present, pictorial or descriptive templates of abnormal ileal mucosa are not included in the Consensus Statement of the World Small Animal Veterinary Association (WSAVA) Gastrointestinal Standardization Group[12,13] and often endoscopic ileal biopsies are only collected in dogs suffering from both small and large intestinal diarrhea.

The aim of this study, therefore, was to compare the histopathologic findings of concurrent endoscopically obtained duodenal and ileal biopsies from dogs with CE. In addition, we aimed to evaluate the correlation between ileal and duodenal histopathologic findings with clinical signs, abdominal ultrasonographic findings, and clinicopathologic findings of known prognostic value in canine CE, including serum albumin concentration, serum cobalamin concentration, and serum canine-specific pancreatic lipase concentration (cPLI).[3,14–16]

## Material and Methods

The electronic clinical database of the Queen Mother Hospital for Animals (QMHA) of the Royal Veterinary College was searched to identify cases of canine CE between January 2007 and April 2011. Only dogs with clinical signs consistent with small intestinal disease (eg, anorexia, weight loss, melena, increased fecal volume, normal frequency) and for which both duodenal and ileal endoscopic biopsies had been obtained were included in the study. The presence of mild concurrent large intestinal signs did not warrant exclusion from the study. For each case, any other known causes of chronic small intestinal diarrhea and vomiting had been ruled out by routine hematology and serum biochemistry findings, fecal parasitology, fecal bacterial culture (for detection of *Campylobacter* spp., *Salmonella* spp., *Yersinia* spp., and enteropathogenic *Escherichia coli)*, abdominal ultrasonography, serum ACTH stimulation test, and measurement of serum canine trypsin-like immunoreactivity concentration (TLI). In each case, the diagnosis of CE was based on the histologic finding of lymphoplasmacytic infiltrates, eosinophilic infiltrates, or both within the lamina propria of endoscopically collected duodenal or ileal biopsies. All cases included in the study were retrospectively reviewed and the following data were recorded: signalment, body weight, body condition score (BCS, from 1 to 9 of 9) at presentation, serum albumin concentration (g/L), serum cobalamin concentration (ng/L), and serum cPLI concentration (μg/L). The clinical severity of disease for each dog was determined as being absent, mild, moderate, severe, or very severe according to a previously published scoring system (canine chronic enteropathy clinical activity index, CCECAI).[3] For the majority of cases (32/38), the CCECAI score was available at the time of presentation based on a questionnaire completed by the owner. When not available, the score was retrospectively calculated from information in the clinical history. The abdominal ultrasonography report of each case was reviewed and the presence or absence of the following findings was recorded: mesenteric lymphadenomegaly, mucosal hyperechogenicity, small intestinal wall thickening, and ascites. For each case, the presence or absence of hypocobalaminemia (serum cobalamin concentration <200 ng/L), clinically relevant hypoalbuminemia (serum albumin concentration <20 g/L), and abnormal cPLI concentration (serum cPLI concentration >200 μg/mL) were recorded.

All duodenal and ileal histologic specimens were retrieved and independently reviewed by a single board-certified pathologist who was blinded to the clinical information of each case. All slides were examined and scored according to the histopathologic scoring system developed by the WSAVA Gastrointestinal Standardization Group.[12] First, the quality of the slide was assessed and determined to be inadequate, marginal, or adequate.[7] Biopsies were inadequate if only villi or subvillous lamina propria, but not both, were present. Marginal samples had at least 1 or 2 sections with subvillous lamina propria, but did not clearly have the full thickness of the subvillous lamina propria extending to the muscularis mucosa. Biopsies were adequate if at least 3 sections contained both villous and subvillous lamina propria that extended to the border of the mucosa and muscularis mucosa.[7] Only cases with concurrent marginal or adequate duodenal and ileal biopsies were included in the statistical analysis. Secondly, 9 histologic parameters, 5 representing morphological changes (villous stunting, epithelial injury, crypt distension, lacteal dilatation, and mucosal fibrosis) and 4 representing inflammatory changes (intraepithelial lymphocytes, lamina propria lymphocytes and plasma cells, lamina propria eosinophils, and lamina propria neutrophils) were scored as absent = 0, mild = 1, moderate = 2, or severe = 3 according to pictorial and descriptive templates included in the guidelines of the WSAVA Gastrointestinal Standardization Group.[12,13] In addition, the presence or absence (regardless of severity) of each histologic lesion was recorded. Finally, the independent duodenal and ileal total histologic severity scores (consisting of the sum of the 9 histologic parameter scores) were recorded and determined to be normal (WSAVA score 0), mild (score 1–6, equivalent to $\leq$25% of the maximal WSAVA score of 27), moderate (score 7–13, between 25 and 50% of the maximal WSAVA score), severe (score 14–20, between 50 and 75% of the maximal WSAVA score), and very severe (score >20, equivalent to >75% of the maximal WSAVA score). Because specific templates for the interpretation of ileal biopsies are not provided by the guidelines of the WSAVA Gastrointestinal Standardization Group, the ileal biopsies were scored using the guidelines provided for the interpretation of duodenal biopsies.[12,13]

### *Statistical Analysis*

Normally distributed variables are expressed as mean (±SD). Variables that were not normally distributed are indicated as median and interquartile range (25–75%). Correlations between variables were evaluated with the chi-square and with the Kendall's tau-b tests for ordinal variables. Comparison between the frequency/ratio of duodenal and ileal biopsies of diagnostic quality was performed by the chi-square test. Agreement between the histologic findings of concurrent duodenal and ileal biopsies was made by κ analysis. Agreement was evaluated first on the total duodenal and ileal histologic severity score and secondly on the presence or absence of each histologic lesion (morphological and inflammatory) regardless of severity. The interpretation of κ values was extrapolated from a previous study[11] as follows: κ values of <0.2 indicated slight agreement, values between 0.2 and 0.4

indicated fair agreement, values between 0.4 and 0.6 indicated moderate agreement, values between 0.6 and 0.8 indicated substantial agreement, and values between 0.8 and 1.0 indicated almost perfect agreement. Statistical significance was set at a $P$ value of <.05.

## Results

### *Descriptive Analysis*

Thirty-eight cases were included in this study. Thirty-three of 38 dogs suffered exclusively from upper gastrointestinal signs. The remaining 5 dogs suffered from concurrent but mild large intestinal signs. All 38 dogs had received antihelmintic treatment, a combination of exclusion diets and antibiotic treatments (as prescribed by the referring veterinarians) with no clinical response before undergoing endoscopy. None of the 38 dogs had received corticosteroid treatment in the 7 days before endoscopy.

The breed distribution of the study population consisted of 5 Labrador Retrievers, 3 German Shepherd Dogs, 3 Golden Retrievers, 2 Beagles, 2 Border Terriers, 2 Boxers, 2 Rottweilers, 2 Rough Collies, and 1 each of the following breeds: American Bull Dog, Border Collie, Cavalier King Charles Spaniel, Cockapoo, English Bull Terrier, Greyhound, Griffon, Labradoodle, Lurcher, Miniature Schnauzer, Miniature Poodle, Portuguese Water Dog, Scottish Terrier, Staffordshire Bull Terrier, Tibetan Terrier, Weimaraner, and cross breed. There were 20 male dogs and 18 female dogs. Median age of the study population was 64.0 months (26.7–99.0 months). Median body weight was 23.2 kg (13.5–30.3 kg). The median BCS was 4.3/9 (3–5/9; ideal 4–5/9), with 13/38 (34%) dogs in less than ideal body condition (BCS <4/9).

Median CCECAI score was 8 (6–11), equivalent to clinically moderate disease. There were 8/38 (21%) dogs with mild clinical disease (CCECAI score 4–5), 16/38 (42%) dogs with moderate clinical disease (CCECAI score 6–8), 6/38 (16%) dogs with severe clinical disease (CCECAI score 9–11), and 8/38 (21%) dogs with very severe clinical disease (CCEAI score $\geq 12$).

The median serum albumin concentration was 32.5 g/L (23.2–36.6 g/L; reference interval, 26–38 g/L). There were 7/38 (18.4%) dogs with clinically relevant hypoalbuminemia (serum albumin concentration <20 g/L). In all dogs with hypoalbuminemia, the urine protein-to-creatinine (UPC) ratio was available and was not consistent with proteinuria (UPC ratio <0.5), suggesting that the protein loss occurred through the bowel. In addition, the combination of normal pre- and postprandial bile acid concentrations (available for 3/7 hypoalbuminemic dogs) and the absence of concurrent hypoglycemia, decreased urea concentration, and hypocholesterolemia in the remaining dogs excluded the presence of severe hepatic dysfunction as cause of the hypoalbuminemia. Median serum cobalamin concentration was 393.9 ng/L (161.8–579.3 ng/L; reference interval, >200 ng/L). There were 11/38 (28.9%) dogs with hypocobalaminemia (serum cobalamin concentration <200 ng/L) at the time of presentation of which 7/11 (64%) had undetectable (<150 ng/L) serum cobalamin concentration. Serum cPLI concentration was available in 32/38 dogs. Median serum cPLI concentration was 52.5 μg/L (<30–228 μg/L; reference interval, <30–200 μg/L). Serum cPLI concentration was abnormal (>200 μg/L) in 9/32 (28%) dogs, of which 6/9 had an equivocal serum cPLI concentration (>200 but <400 μg/L) and 3/9 had a serum cPLI concentration suggestive of pancreatitis (>400 μg/L). None of the dogs with abnormal serum cPLI concentration had ultrasonographic evidence of pancreatitis.

All dogs in this study had undergone abdominal ultrasonography performed by a residency trained radiologist, board-certified radiologist, or both. Mesenteric lymphadenomegaly was observed in 9/38 (24%) dogs. Small intestinal thickening was recorded in 10/38 (26%) dogs. Small intestinal mucosal hyperechogenicity was present in only 3/38 (8%) of dogs. Finally, ascites was recorded in 6/38 (16%) dogs. None of the dogs with ascites had peripheral edema noted on physical examination.

### *Comparison of Diagnostic Quality of Biopsies from Duodenum and Ileum*

Of the duodenal biopsies, 32/38 (84%) were of diagnostic quality (marginal or adequate quality), of which 23/32 (72%) were adequate and 9/32 (28%) were marginal. There were 6/38 (16%) inadequate duodenal biopsies. Of the ileal biopsies, 29/38 (76%) were of diagnostic quality, of which 19/29 (65%) were adequate and 10/29 (35%) were marginal. There were 9/38 (24%) inadequate ileal biopsies. There was no statistically significant difference in the number of duodenal biopsies of diagnostic quality as compared with ileal biopsies of diagnostic quality ($P = .194$). The number of cases with concurrent duodenal and ileal biopsies of diagnostic quality that were eligible for statistical analysis of agreement was 24/38 (63%). From the 14 cases with inadequate biopsies from at least 1 anatomic region, in 8/14 (57%) cases, a duodenal biopsy of diagnostic quality corresponded to an inadequate ileal biopsy, and in 5/14 (38%) cases, an ileal biopsy of diagnostic quality corresponded to an inadequate duodenal biopsy. Only in 1/38 (3%) case were both the duodenal and ileal biopsies of inadequate diagnostic quality.

Median duodenal WSAVA score was 4 (3–8), equivalent to histologically mild disease. There were 4/32 (13%) cases with histologically normal duodenum (score WSAVA score 0). There were 19/32 (60%) cases with histologically mild duodenal disease (WSAVA score 1–6). There were 8/32 (25%) cases with histologically moderate duodenal disease (WSAVA score 7–12). There was only 1/32 case (3%) with histologically severe duodenal disease (WSAVA score 13–20), and no cases had histologically very severe (WSAVA score >20) duodenal disease.

Median ileal WSAVA score was 4 (1.5–6), equivalent to histologically mild disease. There were 2/29 (7%) cases with histologically normal ileum. There were 21/29 (72%) cases with histologically mild ileal disease. There were 5/29 (17%) cases with histologically moderate ileal disease and 1/29 (3%) case with histologically severe ileal disease. There were no cases with histologically very severe ileal disease.

### *Agreement between Duodenal and Ileal Histologic Biopsy Scores*

There was only slight agreement between the histologic severity of duodenal and ileal disease based on the total WSAVA scores ($\kappa = 0.003$). In 4/24 (17%) cases, histologically normal duodenum corresponded to histologically mild ileal disease, whereas in 1/24 (4%) cases, histologically normal ileum corresponded to histologically mild duodenal disease. In 3/24 cases (12%), histologically mild duodenal disease corresponded to moderate ileal disease, whereas in 4/24 cases (16%), histologically mild ileal disease corresponded to histologically moderate duodenal disease. Reciprocal concurrent duodenal and ileal histologic severity was detected in only 12/24 (50%) of cases. In particular, in 10/24 (42%), there were concurrent histologically mild duodenal and ileal disease, whereas in 2/24 cases (8%), there were concurrent histologically moderate duodenal and ileal disease.

When considering the presence of villus blunting regardless of its severity, there was only slight agreement between duodenal and ileal biopsies ($\kappa = 0.05$). In 3/24 cases (12%), villous blunting was present in the ileal, but not in the duodenal biopsies. In 9/24 (38%), villous blunting was present in the duodenal, but not in the ileal biopsies. Agreement was present in the remaining 12/24 (50%) cases, in 5 cases, villous blunting was absent in both duodenal and ileal biopsies and in 7 cases, villous blunting was present in both duodenal and ileal biopsies.

There was only slight agreement on the presence of villous epithelial injury ($\kappa = 0.068$). In 5/24 (21%) cases, this lesion was absent in both duodenal and ileal biopsies. In 6/24 (25%) cases, this lesion was present in both duodenal and ileal biopsies. In 5/24 cases (21%), epithelial injury was present in the ileum, but not in the duodenum, whereas in 8/24 (33%), villous epithelial injury was present in the duodenal, but not in the ileal biopsies.

There was only slight agreement on the presence of crypt distension ($\kappa = 0.008$). In 12/24 (50%) cases, crypt distension was present in the ileum, but not in the duodenum, whereas in 5/24 cases (21%), cryptal disease was present in the duodenum, but not in the ileum. Agreement on the concurrent absence or presence of cryptal disease between duodenum and ileum was found in only 7/24 (29%) cases (absent in 5/24 and present in 2/24 cases).

There was only slight agreement on the presence of lacteal dilatation ($\kappa = 0.07$). In 6/24 (25%) cases, lacteal dilatation was observed in the ileum, but not in the duodenum, whereas in 4/24 (17%), lacteal dilatation was present in the duodenum, but not in the ileum. Agreement on the absence or presence of lacteal dilatation between duodenal and ileal biopsies was found in only 14/24 (58%) cases (absent in 11/24 and present in 3/24 cases).

There was moderate agreement on the presence of mucosal fibrosis ($\kappa = 0.44$). Agreement on the concurrent absence or presence of mucosal fibrosis in the duodenum and ileum was observed in 18/24 (75%) cases (absent in 13 cases and present in 5 cases). In 4/24 (17%) cases, mucosal fibrosis was observed in the ileum, but not in the duodenum, whereas in 2/24 (8%) cases, mucosal fibrosis was present only in the duodenum.

There was only slight agreement on the presence of increased number of intraepithelial lymphocytes, IEL ($\kappa = 0.17$). In 1/24 (4%) cases, an increased number of IEL was found in the ileum, but not in the duodenum, whereas in 9/24 (37%) cases, increased IEL were observed in the duodenum, but not in the ileum. Agreement on the absence or presence of an increased number of IEL was found only in 14/24 (59%) cases (absent in 11/24 and present in 3/24 cases).

There was only slight agreement on the presence of lymphoplasmacytic infiltrates of the mucosal lamina propria ($\kappa = 0.03$). In 3/24 (12%) cases, lymphoplasmacytic inflammation was observed in the ileum, but not in the duodenum, whereas in 9/24 (37%) cases, lymphoplasmacytic inflammation was observed in the duodenum only. Agreement on the concurrent absence or presence of lymphoplasmacytic inflammation in duodenal and ileal biopsies was observed in only 12/24 (50%) cases (absent in 8/24 and present in 4/24 cases).

There was only slight agreement on the presence of mucosal eosinophilic inflammation ($\kappa = 0.14$). In 6/24 (25%) cases, mucosal eosinophilic inflammation was present in the ileum, but not in the duodenum, whereas in 2/24 (8%) cases, eosinophilic inflammation was observed only in the duodenal mucosa. Agreement on the concurrent absence or presence of mucosal eosinophilic inflammation was found in 16/24 (67%) cases (absent in 12/24 and present in 2/24 cases).

Finally, there was only slight agreement on the presence of mucosal neutrophilic inflammation ($\kappa = 0.11$). However, in the majority of cases (19/24, 79%), this lesion was not detected in either the duodenal or the ileal biopsies. The presence of concurrent duodenal and ileal mucosal neutrophilic inflammation was not found in any case. In 3/24 (12%) cases, neutrophilic inflammation was observed in the ileal, but not in the duodenal biopsies, whereas in 2/24 (8%) cases, neutrophilic inflammation was only found in the duodenal biopsies.

One of the original aims of the study was to additionally assess agreement on the severity of each histologic lesion when present concurrently in duodenal and ileal biopsies (considering a different severity as a different disease) by kappa analysis. Unfortunately, because some severity scores were not represented for each histologic lesion, the kappa analysis (based on a cross-tabulation) could not be performed.

However, the Kendall's tau-b test for correlation between ordinal variables was performed instead and did not identify a correlation between the severity of reciprocal duodenal and ileal histologic lesions (results not shown).

### *Correlation between Histologic Scores and Clinical Severity, Serum Albumin, Cobalamin, and cPLI Concentrations*

There was no statistically significant correlation between clinical (CCECAI score) and histologic duodenal and ileal disease (WSAVA scores; $P = .42$ and $P = .66$, respectively). There was no correlation between duodenal and ileal histologic WSAVA scores and the presence of hypoalbuminemia ($P = .60$ duodenum and $P = .38$ ileum), hypocobalaminemia ($P = .65$ duodenum and $P = .66$ ileum), or increased serum cPLI concentration ($P = .87$ duodenum and $P = .22$ ileum). There was no correlation between the duodenal and ileal total histologic WSAVA scores and any of the abdominal ultrasonographic findings.

However, when the single histopathologic lesions (morphological and inflammatory) of both duodenum and ileum were considered, a statistically significant correlation was found between clinical severity (CCECAI score) and the presence of cryptal distension in the duodenum ($P = .031$), ileal lacteal dilatation ($P = .038$), and ileal mucosal lymphoplasmacytic inflammation ($P = .035$). In addition, a statistically significant correlation was found between the presence of hypoalbuminemia and ileal lacteal dilatation ($P = .033$) and increased number of ileal intraepithelial lymphocytes ($P = .019$). Finally, a statistically significant correlation was found between the presence of hypocobalaminemia and an increased number of ileal intraepithelial lymphocytes ($P = .012$).

A statistically significant correlation was found between clinical severity of disease (CCECAI score) and the presence of hypoalbuminemia ($P = .019$), hypocobalaminemia ($P = .033$), and ascites ($P = .005$). No correlation was found between CCECAI score and increased serum cPLI concentration ($P = .783$).

## Discussion

To our knowledge, this study is the first to compare the histologic findings of endoscopically obtained duodenal and ileal biopsies in dogs with CE and to evaluate their correlation with known indicators of clinical disease and predictors of prognosis.

Our study indicated poor agreement between the total WSAVA histologic score of concurrent duodenal and ileal biopsies, with only 50% of cases showing reciprocal severity of histologic disease ($\kappa = 0.003$). Interestingly, in 5/24 (21%) cases, histologic disease was found in only 1 of the 2 small intestinal anatomical sites. Furthermore, histologic disease (defined as a total WSAVA score >0) was more readily observed in the ileal than in the duodenal biopsies in as much as 4 of the 5 cases had histologically normal duodenal mucosa, but abnormal ileal mucosa. This finding could have had an impact on the correct diagnosis in these 5 cases.

When investigating the presence of the 9 histologic lesions included in the Consensus Statement of the WSAVA Gastrointestinal Standardization Group[12,13] for the diagnosis of inflammatory gastrointestinal diseases, poor agreement was found for all lesions with the exception of mucosal fibrosis for which moderate agreement ($\kappa = 0.43$) was found between ileal and duodenal biopsies.

With regard to the remaining morphological lesions, cryptal dilatation and lacteal dilatation were more readily detected in the ileal biopsies. On the contrary, villous blunting and epithelial injury were more readily detected in the duodenal biopsies.

With regard to inflammatory changes (for all of which, no significant agreement could be detected between duodenal and ileal biopsies), increased numbers of IEL and mucosal lymphoplasmacytic infiltrates were more readily detected in the duodenum, whereas eosinophilic infiltrates were more readily detected in the ileal biopsies. No difference was found for the detection of neutrophilic infiltrates. However, this histologic lesion was not a common feature in the study populations.

Overall, the finding of poor agreement between histologic findings in duodenal and ileal biopsies was consistent with findings of recent studies of both canine and feline CE[11,17] and further supports the current opinion of most veterinary gastroenterologists that ileal endoscopic biopsies also should be collected whenever possible in patients suffering only from or predominantly from upper gastrointestinal signs.[8,11] The collection of concurrent duodenal and ileal endoscopic biopsies in dogs with upper gastrointestinal signs recently has become a procedural standard of care at the QMHA. Our data show that, in many cases, taking ileal biopsies is of clinical importance and therefore pictorial and descriptive templates of ileal mucosa should be included in the WSAVA Standardization Group Consensus Statement for the diagnosis of gastrointestinal inflammatory diseases in dogs.

The second aim of this study was to evaluate the correlation of ileal and duodenal histologic findings (both total WSAVA score and single histologic lesions) with the CCECAI score, an accepted indicator of clinical disease in canine CE.

We did not find any correlation between clinical severity (CCECAI) and histologic (WSAVA) severity scores of both the duodenum and ileum, consistent with several earlier studies.[2–4,14,15] However, a correlation was found between CCECAI score and the presence of duodenal cryptal distension, and the presence of ileal lacteal dilatation and lymphoplasmacytic infiltration of the ileal mucosal lamina propria. Although the importance of these findings remains unclear, this represents the first evidence of an association between the severity of clinical disease and ileal histologic findings in dogs with small intestinal CE.

In addition, we aimed to assess the correlation between histologic findings and serum albumin, serum

cobalamin, and serum cPLI concentrations (known biochemical markers of prognosis in canine CE) as a way of indirectly assessing their prognostic potential. Interestingly, a statistically significant correlation was found between hypoalbuminemia and duodenal cryptal distension and ileal lacteal dilatation. A correlation also was found between hypocobalaminemia and increased number of IEL in ileal biopsies. No correlation was found between increased serum cPLI concentration and any of the histologic lesions.

Crypt injuries and lacteal dilatation have long been considered to be the histopathologic lesions resulting in intestinal protein loss in patients with CE[18–20] and yet, with the exception of 1 study,[21] such an association has never been demonstrated. In addition, their association with the severity of clinical disease has never been identified. With regard to the correlation found between hypocobalaminemia and increased number of ileal IEL, this finding represents the first evidence of an association between ileal inflammatory mucosal changes and hypocobalaminemia in dogs with CE. Its clinical relevance remains unclear given that no association was found between structural mucosal changes and hypocobalaminemia, a finding that was anticipated given that the ileum is the small intestinal site known to be associated with the absorption of cobalamin.

Another point of discussion is assessment of the diagnostic quality of endoscopically collected intestinal biopsies in this study. Although the number of biopsies collected in each dog from each anatomical site was not recorded, at the QMHA, it is routine to collect no fewer than 15 duodenal and 10 ileal biopsies and to request that all biopsies be processed. In this study, however, the quality of the biopsies for each anatomical site was assessed collectively. Despite the fact that no statistically significant differences were found between the number of duodenal and ileal biopsies of diagnostic quality, the absolute number was higher for duodenal than ileal biopsies. This finding was not expected because of the expectation that the ileum (because of its thinner mucosa) is easier to biopsy than the duodenum. One likely explanation for our finding is that direct endoscopic visualization of the ileum was not possible in all dogs and blind biopsies were collected in some instances, which could have affected the quality of the samples. Nevertheless, despite the difference in diagnostic quality, several histologic lesions (especially lesions with higher clinical relevance such as crypt distension and lacteal dilatation) were more readily detectable in ileal biopsies.

The main limitations of the study included its retrospective nature and the small number of cases with concurrent duodenal and ileal biopsies of diagnostic quality available, which could have led to overlooking additional possible significant correlations.

In view of the retrospective nature of the study and the absence of consistent information regarding the overall outcome of each case, the prognostic value of the histologic findings could not be directly assessed. Finally, information regarding the response to treatment of the dogs in this study was not available, and the effect of treatment on the histologic findings could not be assessed.

In summary, our results confirm substantial variability between the histologic findings of endoscopically obtained duodenal and ileal biopsies in dogs with upper gastrointestinal signs caused by CE. In addition, some morphological lesions of known clinical relevance were more readily detected in ileal than in duodenal biopsies. A significant correlation was found between ileal lacteal dilatation and ileal lymphoplasmacytic mucosal infiltration with the severity of clinical disease (CCECAI score) and with the presence of hypoalbuminemia. Finally, a significant correlation was found between increased number of ileal IEL and the presence of hypocobalaminemia. In light of the findings of this study, it is recommended that unless contraindicated by the potential risks of prolonged procedural and anesthetic times, ileal biopsies should be obtained in addition to duodenal and gastric biopsies in all dogs suffering from upper gastrointestinal signs undergoing endoscopy.

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*Conflict of Interest Declaration*: Authors disclose no conflict of interest.

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