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Correlating Gastrointestinal Histopathologic Changes to Clinical Disease Activity in Dogs with Idiopathic Inflammatory Bowel Disease

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Abbreviations:

IBD	inflammatory bowel disease
WSAVA	world small animal veterinary association
GI	gastrointestinal
cPLI	pancreatic-lipase immunoreactivity
cTLI	trypsin-like immunoreactivity
H&E	hematoxylin and eosin
CIBDAI	canine IBD activity index
CCECAI	canine chronic enteropathy activity index
LP	lamina propria
CI	confidence interval
GC	granulomatous colitis

ABSTRACT

Prior studies have failed to detect a convincing association between histologic lesions of inflammation and clinical disease activity in dogs with inflammatory bowel disease (IBD). We hypothesized that use of a simplified histopathologic scoring system would improve the consistency of interpretation among pathologists when describing histologic lesions of gastrointestinal inflammation. Our aim was to evaluate the correlation of histopathologic changes to clinical disease activity in dogs with IBD using this new system. Forty two dogs with IBD and 19 healthy control dogs were enrolled in this retrospective study. Endoscopic biopsies from the stomach, duodenum, ileum, and colon were independently scored by 8 pathologists. Clinical disease activity was scored using the canine inflammatory bowel disease activity index (CIBDAI) or the canine chronic enteropathy clinical activity index (CCECAI), depending on the individual study center (USA vs. UK, respectively). Summative histopathological scores and clinical disease activity were calculated for each tissue (stomach, duodenum, ileum, and colon) and each tissue histologic score (inflammatory/morphologic feature). The correlation between CCECAI/CIBDAI and summative histopathologic score was significant ($p<0.05$) for duodenum ($r = 0.42$) and colon ($r = 0.33$). In evaluating the relationship between histopathologic scores and clinical disease activity, significant ($p<0.05$) correlations were observed for crypt dilation ($r = 0.42$), lamina propria (LP) lymphocytes ($r = 0.40$), LP neutrophils ($r = 0.45$), mucosal fibrosis ($r = 0.47$), lacteal dilation ($r = 0.39$), and villus stunting ($r = 0.43$). Compared to earlier grading schemes, the simplified scoring system shows improved utility in correlating histopathologic features (both summative histology scores and select histologic scores) to IBD clinical activity, at diagnosis, as defined by CIBDAI/CCECAI.

Diagnosis of canine idiopathic inflammatory bowel disease (IBD) requires histopathologic confirmation of inflammation in intestinal biopsies.[12,16,17,26,33,40] Previous studies have found it difficult to correlate histopathologic findings with clinical disease activity due to a lack of agreement among pathologists when describing histopathologic changes[37] and inconsistent grading schemes reported by different groups[2,3,6,7,9,10,15-18,24,26,29,32,35,40] investigating canine and feline chronic enteropathies. The World Small Animal Veterinary Association (WSAVA)* GI standardization grading scheme[5] was an attempt to rectify some of these problems but even this was associated with poor agreement among pathologists[39] .

Prior studies have failed to detect a convincing association of mucosal histopathology with clinical signs, biomarkers of inflammation, or response to therapy and outcome in dogs with IBD. [2,4,9,13,21,22,27,29,32,41] One explanation for these findings is that histopathology and clinical activity are not associated; whereas, another possibility is that current WSAVA guidelines require refinement to improve diagnostic consistency.[36] Recently, use of a simplified histopathologic scoring system has shown excellent agreement among pathologists in defining duodenal inflammation in dogs with IBD.[14]

We hypothesized that use of the simplified histopathologic scoring system will improve the consistency of interpretation among pathologists when evaluating gastric, duodenal, ileal, and colonic endoscopic biopsies. The aim of the present study was to evaluate the correlation of histopathologic changes to clinical disease activity in dogs with IBD using this new system.

METHODS

Ethical animal use

The collection and analysis of intestinal biopsies obtained endoscopically from healthy dogs and dogs with IBD were previously approved by the University of Giessen and the Iowa State University Institutional Animal Care and Use Committee. Written informed consent was

obtained from all owners of dogs enrolled in separate trials (University of Giessen: V54-19c 20 15 (1) GI 18/17 Nr. 36/2011; ISU IACUC Log numbers: 1-11-7061-K, 12-11-7269-K).

Animals

Two groups of dogs were studied. Group 1 comprised a cohort of 42 dogs diagnosed with idiopathic IBD according to previously published criteria.[2,12,16,33] Dogs with IBD were enrolled from two study centers: the Royal Veterinary College (Allenspach) and Iowa State University (Jergens) from January 2010 to May 2012. Over this 2.5 year period, approximately 150 dogs at ISU and 300 dogs at the RVC were referred for diagnostic evaluation of chronic gastrointestinal signs. In many instances, individual dogs underwent upper, lower, or both upper and lower GI endoscopy with collection of mucosal biopsies as part of the diagnostic investigation for their chronic GI signs. The inclusion criteria included persistent (> 3 weeks duration) GI signs, failure to respond to appropriate dietary trials (elimination diet fed exclusively for at least 3 weeks) and antimicrobial therapy (metronidazole or tylosin administered exclusively for 14 days or more), failure to document other causes for gastroenteritis by thorough diagnostic testing, and histopathologic evidence of mucosal inflammation in biopsy specimens.

The diagnostic evaluation in all dogs with IBD dogs consisted of extensive medical histories taken over one or more clinical examinations, hematological and serum biochemistry analyses, urinalysis, fecal examinations for parasites, diagnostic imaging (abdominal radiographs in all and abdominal sonography performed in 31/42 dogs), and histopathologic examination of GI mucosal biopsy specimens. In some dogs, samples were additionally collected for a measurement of serum pancreatic-lipase immunoreactivity (cPLI), trypsin-like immunoreactivity (cTLI), cobalamin, and/or folate concentrations.

Group 2 dogs served as controls and were comprised of 19 young (<2 years old) healthy laboratory-reared beagles (n=12) and beagle cross mongrels (n=7). Each of these dogs was free of GI signs over several months preceding diagnostic evaluation. Moreover, control dogs were

judged to be healthy on the basis of normal results on physical examination, hematological and serum biochemical analysis, urinalysis, multiple fecal examinations, and Dirofilaria antigen assay. Mucosal biopsies of the small and large intestines were obtained from each dog as previously described[2,16,33].

Simplified histopathologic scoring system for gastrointestinal inflammation[14]

The simplified histopathologic scoring system for GI inflammation in dogs was based on the morphologic and inflammatory features contained in the original WSAVA GI standardization data set graded by the 4 individual pathologists who participated in that study. In brief, Chi-square analysis of these data was performed and compared the extent of agreement among study pathologists for each inflammatory and morphologic feature. The resultant *P*-values were then used to determine the inter-observer agreement among study pathologists for each single inflammatory or morphologic feature. The 3-5 histopathologic features that showed the least inter-observer variability (based on Chi-square analyses) for GI inflammation involving the stomach, small intestine, and colon comprised the simplified scoring system.

The current study independently validates use of the simplified scoring system first tested on the original WSAVA study population. We now report use of this same system in a separate study population with tissues graded by a different group of pathologists. This allowed for quantification of parameters resulting in the derivation of summative histopathologic scores and individual histopathologic scores which could then be correlated to clinical disease activity in dogs with IBD.

Histopathologic assessment

Hematoxylin and eosin (HE) stained tissue sections of formalin-fixed, paraffin-embedded endoscopic biopsies from the stomach, duodenum, ileum, and/or colon of each dog were evaluated for histologic lesions using a simplified scoring system for defining GI histopathological

changes. Only tissue sections of adequate diagnostic quality were used in the study.[38] Poor quality tissues (those graded as marginal or inadequate) were excluded. Individual glass slides from the different regions of the GI tract of healthy and diseased dogs were scanned on-site using an Aperio digital pathology whole slide scanning system housed at Texas A&M University. Digital tissue images were then sent to each pathologist separately for review. Pathologists were blinded to the dogs' status as a control dog or IBD patient. Up to five morphologic/inflammatory features in randomly sorted, HE-stained digital slides from each area of the GI tract were independently scored by the 8 study pathologists (Table 1). Individual pathologists' lesion scores for each parameter were graded using a 4-point scale (i.e., 0 = normal, 1 = mild, 2 = moderate, 3 = marked histopathologic change). This resulted in the generation of both region-specific scores and summative histopathologic scores used for statistical analysis. The same histopathologic grading criteria used for duodenal biopsy specimens were used for diagnostic interpretation of ileal tissue specimens.

Clinical disease activity

Severity of clinical disease (activity) at diagnosis was scored using either the canine chronic enteropathy clinical activity index (CCECAI)[2] or the canine IBD activity index (CIBDAI)[18] at the different clinical trial centers.

Statistical methods

Correlations between histopathologic variable(s) and clinical disease activity were calculated for both cohorts. For each dog, the average summative histopathologic score in each area of the GI tract (i.e., averaged over the 3-5 histopathologic parameters) and individual histopathologic scores at each tissue location (stomach, duodenum, ileum and colon) were calculated for correlation to clinical activity indices. Pearson's correlation coefficients between histopathological scores and clinical disease activity were calculated for each tissue location, and combination of

tissue and histologic score. Significance of the correlation was determined by Fisher Z-transformation tests using PROC CORR in SAS version 9.4. Prior to this, differences among pathologists on those correlations were evaluated using F-tests, after verifying the model assumptions of independence, homoscedasticity, and normality. For all tests, p-values < 0.05 were considered statistically significant.

Inter-observer agreement among pathologists by histopathologic variable

When no significant differences in the (histopathological/clinical) correlations were found among pathologists, Cohen's κ statistics were used to assess the extent of agreement among individual raters for each histopathologic variable.[31] This was performed using a binary or 2-point scale (i.e., normal/mild and moderate/severe) as previously described.[39] The resulting κ statistic was interpreted as <0 less than chance agreement; 0.01-0.20 slight agreement; 0.21-0.40 fair agreement; 0.41-0.60 moderate agreement; 0.61-0.80 substantial agreement; 0.81-0.99 almost perfect agreement. This output generated both kappa statistics and p-values to assess the significance of the kappa scores.

RESULTS

Of the 42 dogs with IBD assessed for clinical disease severity, 25 were enrolled at the RVC and scored using CCECAI while 17 were enrolled at ISU and scored using CIBDAI. For the RVC cohort, 11 dogs had mild clinical activity, 8 dogs had moderate clinical activity, and 6 dogs had severe clinical disease. For the ISU cohort, 2 dogs had mild clinical activity, 7 dogs had moderate clinical activity, and 8 dogs had severe clinical disease. Overall, 14 of 42 (33%) dogs with IBD had severe clinical disease (i.e., CIBDAI/CCECAI score >9) with 7/42 (17%) dogs having hypoalbuminemia and/or ascites at IBD diagnosis. Thus, overall, 35/42

(83%) of study dogs had identical CIBDAI and CCECAI scores for correlation to histopathologic indices.

The areas of the GI tract available for diagnostic evaluation in the two dog groups are presented in Table 2. The collection of endoscopic biopsies from dogs with IBD was guided by localization of the predominant GI signs to the small intestine (enteritis, n = 8 dogs), large intestine (colitis, n = 4 dogs), or both small and large intestines (enterocolitis, n = 30 dogs). Digital images contained 6-15 individual endoscopic tissue specimens per glass slide depending on the area sampled. For some dogs, tissue samples from all four areas of the GI tract were not obtained due to anesthetic complications and time constraints, difficulty with endoscopic examination (retrograde ileoscopy), and/or clinical disease localization to a single area of the GI tract (e.g., signs of colitis alone).

Results of the F-test comparing the histopathological/clinical correlations among the 8 pathologists did not show any significant differences when averaging summative histopathologic scores for the stomach, duodenum, ileum, or colon. Moreover, there was no significant difference among pathologists in the correlation of individual histopathological scores at each GI location and the clinical activity indices. In this instance, the histopathologic scores from all 8 pathologists were pooled together to calculate Pearson's correlation coefficients and increase the power of the study for detection of statistically significant differences between histopathologic changes assigned by the 8 pathologists.

The correlation between CCECAI/CIBDAI and the summative histology score was significant ($p<0.05$) for duodenum ($r = 0.42$, 95% CI = [0.08-0.65]) and colon ($r = 0.33$, 95% CI = [0.04-0.57]). The correlation was nearly significant for ileum ($p=0.06$, $r = 0.29$, 95% CI = [-0.02-0.55]) but non-significant for stomach ($p=0.7$, $r = 0.05$, 95% CI = [-0.24-0.34]). Evaluation of individual histopathologic scores showed numerous significant correlations between histology and clinical disease activity for all areas of the GI tract, and in particular, the duodenum, ileum

and colon (Table 3). These lesions encompassed a variety of morphologic and inflammatory changes including mucosal fibrosis, villus stunting, and crypt dilation, as well as changes in the character of the mucosal cellular infiltrate in dogs with IBD (Figure 1).

The kappa agreement among pathologists for histopathologic scores varied across regions of the GI tract (Table 4). The best agreement for lesions was seen for colonic scores and lamina propria (LP) eosinophils across 3 of 4 areas of the GI tract. Those histopathologic lesions having at least fair-to-moderate agreement (>0.21-0.40) based on κ statistics included: stomach (fibrosis, LP lymphocytes/plasma cells, LP eosinophils); duodenum (villus stunting, crypt dilation, lacteal dilation, LP lymphocytes/plasma cells, LP eosinophils); and colon (crypt dilation, LP eosinophils and macrophages).

DISCUSSION

Characterizing the extent and severity of GI inflammation in endoscopic biopsy specimens from dogs and cats is difficult. Several studies from different institutions have shown that a variety of factors influence histopathologic interpretation of intestinal biopsies including (1) the correct area of the GI tract to be sampled[36], (2) the number and quality of tissue samples submitted to the laboratory[38], (3) the quality of sample processing by the laboratory[39], and (4) the lack of consistency in interpretation of histopathologic changes among pathologists[5,37]. Complicating these potential limitations are various grading schemes for interpretation of intestinal histopathology which have failed to gain uniform acceptance in different laboratories. In response to these concerns, the WSAVA GI Standardization Group designed a histopathologic template for characterizing the nature and severity of mucosal inflammatory and morphologic changes. It was hoped that this might reduce variation among different pathologists and different institutions.[5]

Unfortunately, significant inter-observer variability in the diagnostic interpretation of endoscopic mucosal specimens exists even with the use of the original WSAVA standardization criteria.[36,39] Moreover, the original WSAVA criteria do not consider some parameters, such as

changes in the number of colonic goblet cells and their content of mucus, although this parameter may be decreased in granulomatous colitis (GC) and other forms of colitis[28,30], and may be significantly increased post-treatment[20]. A recent study evaluated a simplified histopathologic scoring system using select WSAVA template criteria (i.e., those indices having the greatest agreement among WSAVA pathologists) to reduce variability in the diagnostic interpretation of GI inflammation among pathologists.[14] However, this investigation only graded inflammation in canine duodenal endoscopic biopsies and, like the original WSAVA study, did not include evaluation of ileal biopsy specimens.

The present study aimed to build on earlier observations using the simplified histopathologic scoring system, now extending its use to other areas of the GI tract (stomach, ileum, and colon) and correlating quantifiable histopathologic changes to clinical disease activity in dogs with IBD. Numerous studies from different groups have failed to show a consistent association between mucosal histopathologic changes and clinical disease severity; either at diagnosis or in response to different treatments. Jergens et al[18] showed that clinical scores (CIBDAI) correlated best to a combination of histopathologic inflammation and serum C-reactive protein at IBD diagnosis; post-treatment histopathologic assessment was not performed in these dogs. In another study, clinical signs (CIBDAI scores) improved with medical treatment in dogs with IBD, but this did not result in significant changes in the severity of gastric or duodenal histologic inflammatory lesions.[9] In separate studies, Allenspach *et al* showed that total lymphocyte numbers in the duodenal mucosa of dogs with IBD did not change in response to successful cyclosporine treatment[1], and that histopathologic scores were not correlated with CCECAI scores, endoscopy scoring, or long-term outcome in dogs with IBD over 3 years[2]. In this latter paper (Allenspach), the presence of severe mucosal lesions in the duodenum (observed during duodenoscopy), hypoalbuminemia, and low serum cobalamin were significantly associated with negative outcome in dogs with chronic enteropathy. Munster et al[22] failed to demonstrate a strong correlation between treatment response (CIBDAI) and severity of

histopathologic lesions of IBD. Finally, Rossi et al[25] showed reduced histopathologic lesion scores, accompanied by a positive clinical response (CIBDAI), following an 8-week course of combination probiotic therapy, while White et al[34] did not despite using the same probiotic protocol. Difficulties in showing associations in these earlier studies may relate to the use of non-standardized histopathologic scoring systems and/or differences in study design.

Use of the simplified scoring system was repeatable among the different pathologists in the current study. The results comparing histopathological/clinical correlations among pathologists did not show significant differences when averaging summative histopathologic scores for the stomach, duodenum, ileum or colon. Moreover, there was no significant difference among pathologists in the correlation of individual histopathological scores within separate areas of the GI tract and clinical disease activity. These findings suggest that histologic scores of mucosal inflammation and morphologic change can be consistently applied to the scoring system.

We also observed significant correlations between some histologic scores and clinical indices. Clinical activity in dogs with IBD was positively correlated to the summative histopathologic scores for the duodenum and colon. While the correlation between clinical disease and the summative histopathologic score approached significance ($p = 0.06$) for ileal biopsies, it was clearly not significantly correlated for the stomach. The association between histologic scores and clinical severity scored by internists was also significantly positive for several mucosal inflammatory/morphologic changes, including increased numbers of LP lymphocytes and neutrophils (duodenum), mucosal fibrosis (stomach and colon), crypt dilation (ileum and colon), and villus stunting (duodenum and ileum).

The histologic score of gastric fibrosis was correlated with clinical disease activity in our dogs with IBD. This was an interesting observation considering that the total gastric histopathology score was not correlated to disease activity in affected dogs. In the original WSAVA study, gastric fibrosis was combined with other morphologic changes indicative of mucosal injury (including glandular nesting and mucosal atrophy) to define the presence and extent of gastric inflammation.[5] The system used to grade gastritis in the present study (the simplified scoring system) was adapted from that proposed by the WSAVA standardization group and also included gastric fibrosis/mucosal atrophy as one parameter of morphologic injury. One standardized photographic grading scheme for evaluating gastric atrophy, fibrosis, and cellular infiltrates has also been proposed for characterizing gastritis in dogs.[11] In this previous study, expression of IL-1β and the presence of mast cells and atrophy were related to gastric fibrosis. While the association of cytokines and cellular infiltrates with architectural changes was not investigated in the present study, it is possible that IL-1β and other inflammatory mediators in the lesions of IBD were related to gastric fibrosis.[8,19,23]

There was no correlation between the numbers of colonic goblet cells, intra-epithelial lymphocytes, LP eosinophils, and macrophages with IBD clinical disease activity. Changes in the numbers of goblet cells in dogs with colitis have been previously described in separate clinical studies. Roth et al[26], using a colitis score comprised of LP cellular infiltrates, epithelial architecture, intra-epithelial lymphocytes, and number of goblet cells, showed that dogs with moderate-to-severe IBD colitis were more likely to have reduced/absent numbers of goblet cells versus heathy dogs. Mansfield et al[20] showed that decreased numbers of goblet cells were present in a cohort of Boxers with GC and observed an increase in goblet cells and improved histopathological abnormalities following eradication of invasive intra-mucosal *Escherichia coli* with enrofloxacin administration. Colonic goblet cell numbers in dogs with IBD of the present study were not reduced compared to the numbers of goblet cells in colonic biopsies obtained from healthy dogs.

As noted by Roth et al, however, changes in goblet cell numbers are not always accompanied by clinical signs since 28/48 samples from dogs with a clinical diagnosis of chronic diarrhea and/or colitis had histopathologic normal tissues (e.g., colitis grade of ≤2.0).[26]

There are some potential limitations to this study. We utilized a retrospective study design of archived gut specimens for diagnostic investigation from only 2 study centers. The healthy dog cohort was difficult to find at any one institution, hence this cohort was realized by pooling samples from laboratory reared animals from different (ISU and RVC) institutions. These dogs were younger than the majority of dogs with IBD but the availability of age and breed-matched control tissues for comparison to dogs with IBD was difficult due to the retrospective study design with limited availability of tissue blocks. It would have been ideal to study post-treatment biopsy samples from these same dogs with IBD to evaluate changes in disease activity and histopathology versus baseline values, but this was not possible in all cases. Lastly, endoscopic biopsy specimens from the stomach, duodenum, ileum, and colon were not available from all dogs for histopathologic interpretation and may have reduced the overall power of our study.

In conclusion, the simplified histopathologic scoring system provides objective and descriptive information on the extent of mucosal inflammation in the GI tract of dogs. This scoring system incorporates key WSAVA morphologic and inflammatory features which can now be applied to diagnostic interpretation of endoscopic specimens obtained from the stomach, duodenum, ileum, and colon. Compared to earlier grading schemes, the simplified scoring system shows improved utility in correlating histopathologic features (both summative histology scores and select histologic scores) to IBD clinical activity, at diagnosis, as defined by CIBDAI/CCECAI. We now provide a quantitative simplified scoring system for use by pathologists and clinicians alike in future studies (Table 5). Finally, gastric biopsies would appear to be less clinically useful versus duodenal and colonic biopsies for defining intestinal inflammation in dogs with IBD.

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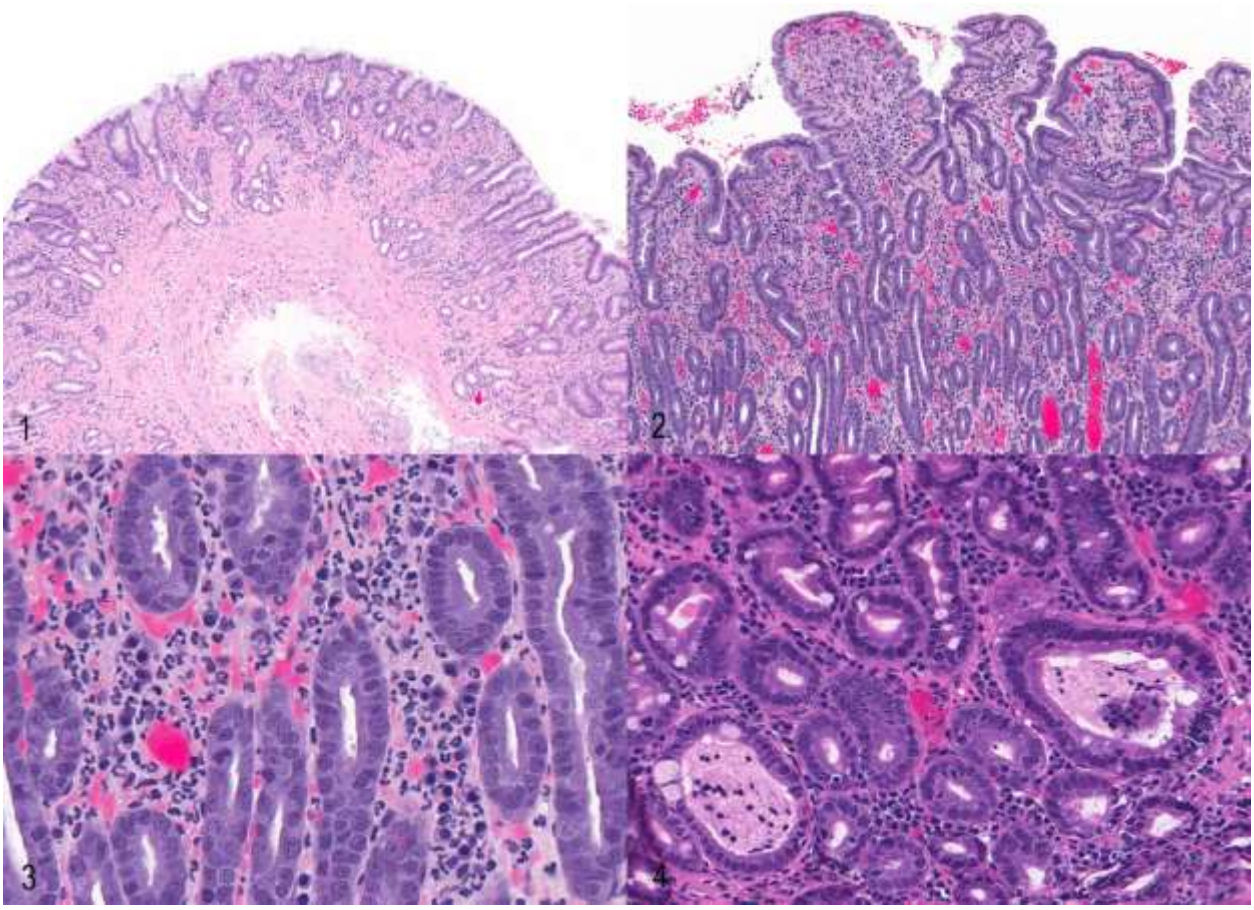
554 FIGURE LEGEND

555 Figures 1-4. Inflammatory bowel disease, dog. Hematoxylin and eosin. Figure 1. Stomach,
556 antrum. Within the lamina propria there is multifocal glandular atrophy and
557 replacement with mature fibrosis. Figure 2. Duodenum. There is diffuse
558 moderate blunting and shortening (atrophy) of villus profiles. Figure 3.
559 Duodenum. Within the lamina propria there are markedly increased numbers of
560 neutrophils. Figure 4. Ileum. There is multifocal dilation of crypts.



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563 Table 1. Parameters included in the simplified histopathologic scoring system for canine
564 inflammatory bowel disease.

Location	Histopathologic Parameter	Grade
Stomach (fundus or antrum)	Fibrosis Intraepithelial lymphocytes Lamina propria infiltrates*	Grade (0; normal, 1; mild, 2; moderate and 3; marked) was assessed for each parameter according to the descriptors given in Table 5.
Duodenum	Villus stunting Crypt dilation Lacteal dilation Surface epithelial injury Lamina propria infiltrates*	
Ileum	As duodenum	
Colon	Crypt dilation Fibrosis Goblet cell number Surface epithelial injury Lamina propria infiltrates*	

565
566 *Quantity of lymphocytes/plasma cells, eosinophils, neutrophils, and/or macrophages as
567 individual cellular infiltrates
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569
570 Table 2. Number of Tissues Sampled From Healthy Dogs and Dogs With Inflammatory Bowel
571 Disease (IBD).
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GI Organ	Stomach	Duodenum	Ileum	Colon
Healthy dogs (n=19)	7	5	9	10
IBD dogs (n=42)	38	29	31	35

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576 Table 3. Correlations Between Organ-Specific Histopathologic Scores and Clinical Disease
577 Activity in Dogs With Inflammatory Bowel Disease.

Histopathologic Variable	Correlation, (confidence interval [CI])	p-value
Stomach - fibrosis	r = 0.36, 95% CI = (0.07-0.59)	0.014
Duodenum - LP lymphocytes	r = 0.40, 95% CI = (0.07-0.02)	0.017
Duodenum - LP neutrophils	r = 0.45, 95% CI = (0.13-0.01)	0.007
Duodenum - lacteal dilation	r = 0.39, 95% CI = (0.06-0.65)	0.019
Duodenum - villus stunting	r = 0.40, 95% CI = (0.05-0.02)	0.024
Ileum - crypt dilation	r = 0.32, 95% CI = (0.01-0.57)	0.041
Ileum - villus stunting	r = 0.33, 95% CI = (0.02-0.58)	0.034
Colon - fibrosis	r = 0.47, 95% CI = (0.21-0.67)	0.001
Colon - crypt dilation	r = 0.42, 95% CI = (0.14-0.64)	0.003

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580 Table 4. Agreement Among 8 Pathologists (κ Statistics) for Histopathologic Scoring of Lesions
581 in Dogs With Inflammatory Bowel Disease.[a]
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Subscore Paramater	Stomach	Duodenum	Ileum	Colon
Surface epithelium	-	0.14	0.03	*0.25*
Villus stunting	-	*0.33*	0.09	-
Crypt dilatation	-	*0.30*	0	*0.44*
Lacteal dilation	-	*0.36*	0.10	-
Colonic goblet cells	-	-	-	*0.40*
Mucosal fibrosis/atrophy	*0.23*	-	-	0.16
Intraepithelial lymphocytes	0.16	0.01	0	-
LP lymphocytes and plasma cells	*0.43*	*0.25*	0.07	*0.34*
LP eosinophils	*0.42*	*0.46*	0.09	*0.54*
LP neutrophils	-	-	0	0.16
LP macrophages	0	0.17	0	*0.50*

583 Abbreviations: LP, lamina propria; -, not performed/not applicable
584 [a]Italicized values denote a κ statistic of slight to fair agreement.
585

Table 5. Correlations Between Organ-Specific Histopathologic Scores and Clinical Disease Activity in Dogs With Inflammatory Bowel Disease.

Location		Histopathologic parameter	Grade			
			0 (Normal)	1 (Mild)	2 (Moderate)	3 (Marked)
Stomach (fundus)	**Morphologic parameter**	**Fibrosis** (fibrocytes separating glands)	≤2	3-5	6-10	≥11
	Inflammatory parameters	**Intraepithelial lymphocytes** (lymphocytes per stretch 50 epithelial cells)	≤2	3-10	11-20	≥21
		Lamina propria lymphocytes and plasma cells (cells per x40 field)	≤20	21-50	51-100	≥101
		Lamina propria eosinophils (cells per x40 field)	≤2	3-20	21-50	≥51
		Lamina propria neutrophils (cells per x40 field)	0	≤20	21-50	≥51
Stomach (antrum)	**Morphologic parameter**	**Fibrosis** (fibrocytes separating gastric pits or mucous glands)	≤10	11-15	16-20	≥21
	Inflammatory parameters	**Intraepithelial lymphocytes** (lymphocytes per stretch 50 epithelial cells)	≤2	3-5	4-10	≥11
		Lamina propria lymphocytes and plasma cells (cells per x40 field)	As fundus			
		Lamina propria eosinophils (cells per x40 field)	≤2	3-10	11-50	≥51
		Lamina propria neutrophils (cells per x40 field)	As fundus			
Duodenum and ileum	**Morphologic parameters**	**Villous stunting** (as % of normal length)*	100	75	50	<25
		Crypt dilation (% of crypts in a section dilated, distorted or contain eosinophilic material/degenerate neutrophils ('crypt abscess'))	≤2	3-10	11-25	≥26
		Lacteal dilation (as % of villous width)	≤25	26-50	51-75	≥76

		Surface epithelial injury (% of villi per section)	No erosion or ulceration	≤10 erosion, no ulceration	11-25 erosion and/or ≤10 ulceration	≥26 erosion and/or ≥11 ulceration
	Inflammatory parameters	**Lamina propria lymphocytes and plasma cells** (% area of one x40 villous field **or** cells between crypts)	≤25, ≤2	26-50, 3-5	51-75, 6-10	≥76, ≥11
		Lamina propria eosinophils (cells per x40 field)	≤3	4-10	11-20	≥21
		Lamina propria neutrophils (cells per x40 field)	0	≤10	11-30	≥31
Colon	**Morphologic parameters**	**Crypt dilation and distension** (% of crypts per section)	0	≤25	26-50	≥51
		Fibrosis (fibrocytes separating crypts)	≤2	3-5	6-10	≥11
		Goblet cell numbers (% reduction from normal)	0	≤25	26-50	≥51
		Surface epithelial injury (% of villi per section)	As duodenum and ileum			
	Inflammatory parameters	**Lamina propria lymphocytes and plasma cells** (cells between crypts)	≤5	6-10	11-20	≥21
		Lamina propria eosinophils (cells per x40 field)	≤2	3-10	11-20	≥21
		Lamina propria neutrophils (cells per x40 field)	As duodenum and ileum			
		Lamina propria macrophages (cells per x40 field)	≤2	3-20	21-50	≥51

- N.B. accurate assessment only possible with well-oriented endoscopic samples