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AUTHORS: KA Allenspach; JP Mochel; Y Du; SL Priestnall; F Moore; M Slayter; A Rodrigues; M Ackermann; M Krockenberger; J Mansell; N Luckschander; C Wang; J Suchodolski; N Berghoff; AE Jergens

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

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4 Karin A. Allenspach<sup>1</sup>, Jonathan P. Mochel<sup>1</sup>, Yingzhou Du<sup>1</sup>, Simon L. Priestnall<sup>2</sup>, Frances  
5 Moore<sup>3</sup>, Michael Slayter<sup>4</sup>, Aline Rodrigues<sup>5</sup>, Mark Ackermann<sup>1</sup>, Mark Krockenberger<sup>6</sup>, Joanne  
6 Mansell<sup>5</sup>, WSAVA GI Standardization Working Group\*, Nicole Luckschander<sup>7</sup>, Chong Wang<sup>1</sup>,  
7 Jan Suchodolski<sup>5</sup>, Nora Berghoff<sup>8</sup>, Albert E. Jergens<sup>1</sup>.

8

9 1. College of Veterinary Medicine, Iowa State University, Ames, IA, USA.

10 2. Royal Veterinary College, University of London, London, UK.

11 3. Marshfield Labs, Marshfield, WI, USA.

12 4. Idexx Laboratories, CA, USA.

13 5. College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College  
14 Station, TX, USA.

15 6. University of Sydney, Sydney, AU.

16 7. University of Vienna, Vienna, AT

17 8. College of Veterinary Medicine, Michigan State University, East Lansing, MI, USA.

18 \* WSAVA GI standardization working group members: M. J. Day, T. Bilzer, J. Mansell, B.  
19 Wilcock, E. J. Hall, A. Jergens, T. Minami, M. Willard and R. Washabau

20

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23 Corresponding author: Dr. Albert E. Jergens, Department of Veterinary Clinical Sciences,  
24 College of Veterinary Medicine, Iowa State University, Ames, IA,  
25 50011, USA; [ajergens@iastate.edu](mailto:ajergens@iastate.edu)

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52 **Abbreviations:**

53	IBD	inflammatory bowel disease
54	WSAVA	world small animal veterinary association
55	GI	gastrointestinal
56	cPLI	pancreatic-lipase immunoreactivity
57	cTLI	trypsin-like immunoreactivity
58	H&E	hematoxylin and eosin
59	CIBDAI	canine IBD activity index
60	CCECAI	canine chronic enteropathy activity index
61	LP	lamina propria
62	CI	confidence interval
63	GC	granulomatous colitis

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## 78 ABSTRACT

79 Prior studies have failed to detect a convincing association between histologic lesions of  
80 inflammation and clinical disease activity in dogs with inflammatory bowel disease (IBD). We  
81 hypothesized that use of a simplified histopathologic scoring system would improve the  
82 consistency of interpretation among pathologists when describing histologic lesions of  
83 gastrointestinal inflammation. Our aim was to evaluate the correlation of histopathologic changes  
84 to clinical disease activity in dogs with IBD using this new system. Forty two dogs with IBD and  
85 19 healthy control dogs were enrolled in this retrospective study. Endoscopic biopsies from the  
86 stomach, duodenum, ileum, and colon were independently scored by 8 pathologists. Clinical  
87 disease activity was scored using the canine inflammatory bowel disease activity index (CIBDAI)  
88 or the canine chronic enteropathy clinical activity index (CCECAI), depending on the individual  
89 study center (USA vs. UK, respectively). Summative histopathological scores and clinical disease  
90 activity were calculated for each tissue (stomach, duodenum, ileum, and colon) and each tissue  
91 histologic score (inflammatory/morphologic feature). The correlation between CCECAI/CIBDAI  
92 and summative histopathologic score was significant ( $p < 0.05$ ) for duodenum ( $r = 0.42$ ) and colon  
93 ( $r = 0.33$ ). In evaluating the relationship between histopathologic scores and clinical disease  
94 activity, significant ( $p < 0.05$ ) correlations were observed for crypt dilation ( $r = 0.42$ ), lamina propria  
95 (LP) lymphocytes ( $r = 0.40$ ), LP neutrophils ( $r = 0.45$ ), mucosal fibrosis ( $r = 0.47$ ), lacteal dilation  
96 ( $r = 0.39$ ), and villus stunting ( $r = 0.43$ ). Compared to earlier grading schemes, the simplified  
97 scoring system shows improved utility in correlating histopathologic features (both summative  
98 histology scores and select histologic scores) to IBD clinical activity, at diagnosis, as defined by  
99 CIBDAI/CCECAI.

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

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

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

123

## 124 METHODS

### 125 *Ethical animal use*

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

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

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### 132 *Animals*

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

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

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

155 judged to be healthy on the basis of normal results on physical examination, hematological and  
156 serum biochemical analysis, urinalysis, multiple fecal examinations, and *Dirofilaria* antigen assay.  
157 Mucosal biopsies of the small and large intestines were obtained from each dog as previously  
158 described<sup>2,16,33</sup>.

159

#### 160 *Simplified histopathologic scoring system for gastrointestinal inflammation*<sup>14</sup>

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

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

176

#### 177 *Histopathologic assessment*

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



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

194

#### 195 *Clinical disease activity*

196 Severity of clinical disease (activity) at diagnosis was scored using either the canine  
197 chronic enteropathy clinical activity index (CCECAI)<sup>2</sup> or the canine IBD activity index (CIBDAI)<sup>18</sup>  
198 at the different clinical trial centers.

199

#### 200 *Statistical methods*

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

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

212

### 213 *Inter-observer agreement among pathologists by histopathologic variable*

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

222

## 223 RESULTS

224 Of the 42 dogs with IBD assessed for clinical disease severity, 25 were enrolled at  
225 the RVC and scored using CCECAI while 17 were enrolled at ISU and scored using CIBDAI.  
226 For the RVC cohort, 11 dogs had mild clinical activity, 8 dogs had moderate clinical activity,  
227 and 6 dogs had severe clinical disease. For the ISU cohort, 2 dogs had mild clinical activity,  
228 7 dogs had moderate clinical activity, and 8 dogs had severe clinical disease. Overall, 14 of  
229 42 (33%) dogs with IBD had severe clinical disease (i.e., CIBDAI/CCECAI score >9) with 7/42  
230 (17%) dogs having hypoalbuminemia and/or ascites at IBD diagnosis. Thus, overall, 35/42

231 (83%) of study dogs had identical CIBDAI and CCECAI scores for correlation to  
232 histopathologic indices.

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

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

250 The correlation between CCECAI/CIBDAI and the summative histology score was  
251 significant ( $p < 0.05$ ) for duodenum ( $r = 0.42$ , 95% CI = [0.08-0.65]) and colon ( $r = 0.33$ , 95% CI =  
252 [0.04-0.57]). The correlation was nearly significant for ileum ( $p = 0.06$ ,  $r = 0.29$ , 95% CI = [-0.02-  
253 0.55]) but non-significant for stomach ( $p = 0.7$ ,  $r = 0.05$ , 95% CI = [-0.24-0.34]). Evaluation of  
254 individual histopathologic scores showed numerous significant correlations between histology  
255 and clinical disease activity for all areas of the GI tract, and in particular, the duodenum, ileum

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

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

266

## 267 DISCUSSION

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

279         Unfortunately, significant inter-observer variability in the diagnostic interpretation of  
280 endoscopic mucosal specimens exists even with the use of the original WSAVA standardization  
281 criteria.<sup>36,39</sup> Moreover, the original WSAVA criteria do not consider some parameters, such as

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

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

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

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

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

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

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

357 As noted by Roth et al, however, changes in goblet cell numbers are not always accompanied by  
358 clinical signs since 28/48 samples from dogs with a clinical diagnosis of chronic diarrhea and/or  
359 colitis had histopathologic normal tissues (e.g., colitis grade of  $\leq 2.0$ ).<sup>26</sup>

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

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

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382



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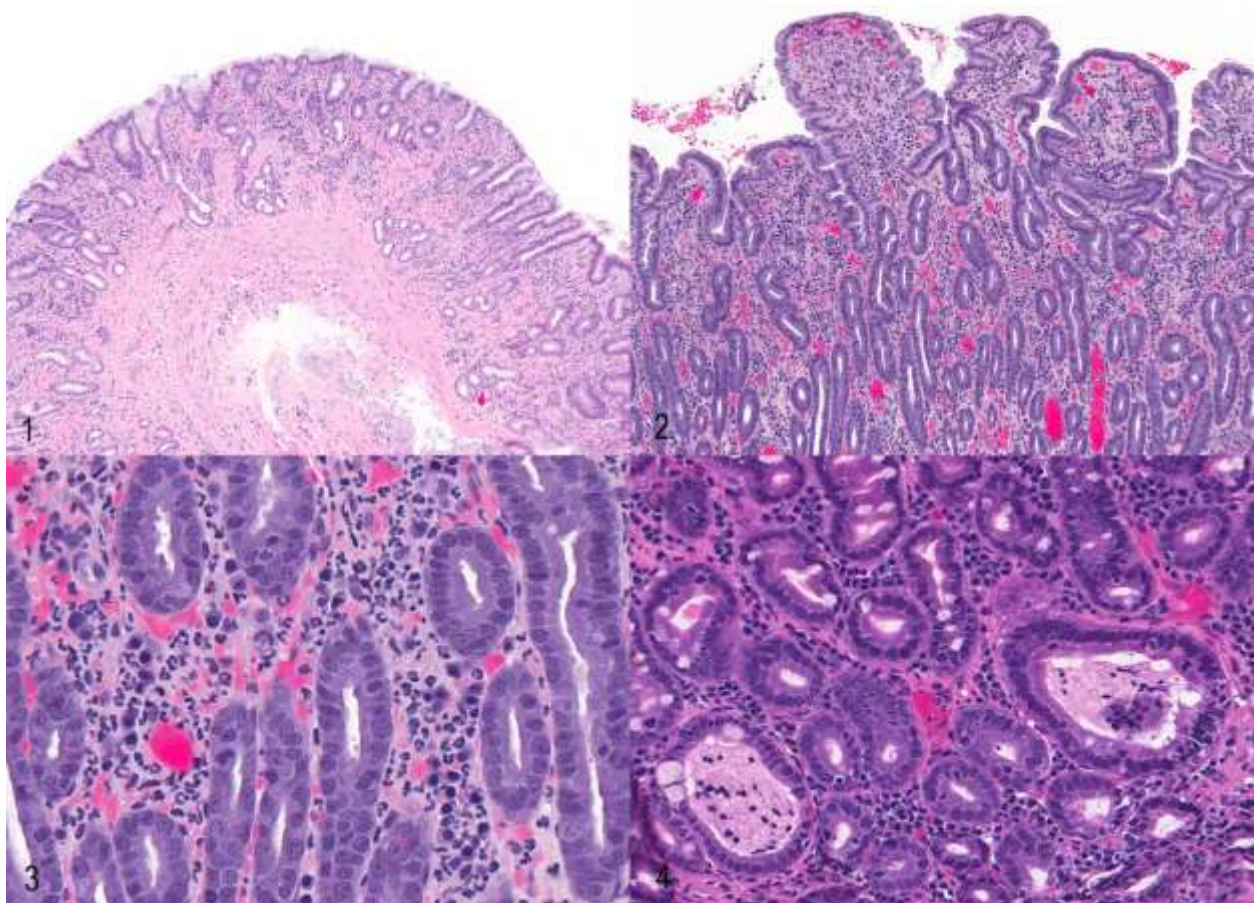
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## 554 FIGURE LEGEND

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*	

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566 \*Quantity of lymphocytes/plasma cells, eosinophils, neutrophils, and/or macrophages as  
567 individual cellular infiltrates

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570 Table 2. Number of Tissues Sampled From Healthy Dogs and Dogs With Inflammatory Bowel  
571 Disease (IBD).  
572

GI Organ	Stomach	Duodenu m	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 ( $\kappa$  Statistics) for Histopathologic Scoring of Lesions  
 581 in Dogs With Inflammatory Bowel Disease.<sup>a</sup>  
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Subscore Parameter	Stomach	Duodenum	Ileum	Colon
Surface epithelium	-	0.14	0.03	<i>0.25</i>
Villus stunting	-	<i>0.33</i>	0.09	-
Crypt dilatation	-	<i>0.30</i>	0	<i>0.44</i>
Lacteal dilatation	-	<i>0.36</i>	0.10	-
Colonic goblet cells	-	-	-	<i>0.40</i>
Mucosal fibrosis/atrophy	<i>0.23</i>	-	-	0.16
Intraepithelial lymphocytes	0.16	0.01	0	-
LP lymphocytes and plasma cells	<i>0.43</i>	<i>0.25</i>	0.07	<i>0.34</i>
LP eosinophils	<i>0.42</i>	<i>0.46</i>	0.09	<i>0.54</i>
LP neutrophils	-	-	0	0.16
LP macrophages	0	0.17	0	<i>0.50</i>

583 Abbreviations: LP, lamina propria; -, not performed/not applicable

584 <sup>a</sup>Italicized values denote a  $\kappa$  statistic of slight to fair agreement.

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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



		<b>Surface epithelial injury</b> (% 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
	<b>Inflammatory parameters</b>	<b>Lamina propria lymphocytes and plasma cells</b> (% area of one x40 villous field <u>or</u> cells between crypts)	≤25, ≤2	26-50, 3-5	51-75, 6-10	≥76, ≥11
		<b>Lamina propria eosinophils</b> (cells per x40 field)	≤3	4-10	11-20	≥21
		<b>Lamina propria neutrophils</b> (cells per x40 field)	0	≤10	11-30	≥31
<b>Colon</b>	<b>Morphologic parameters</b>	<b>Crypt dilation and distension</b> (% of crypts per section)	0	≤25	26-50	≥51
		<b>Fibrosis</b> (fibrocytes separating crypts)	≤2	3-5	6-10	≥11
		<b>Goblet cell numbers</b> (% reduction from normal)	0	≤25	26-50	≥51
		<b>Surface epithelial injury</b> (% of villi per section)	As duodenum and ileum			
	<b>Inflammatory parameters</b>	<b>Lamina propria lymphocytes and plasma cells</b> (cells between crypts)	≤5	6-10	11-20	≥21
		<b>Lamina propria eosinophils</b> (cells per x40 field)	≤2	3-10	11-20	≥21
		<b>Lamina propria neutrophils</b> (cells per x40 field)	As duodenum and ileum			
		<b>Lamina propria macrophages</b> (cells per x40 field)	≤2	3-20	21-50	≥51

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