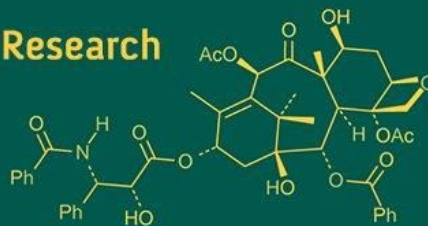
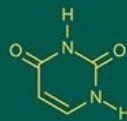
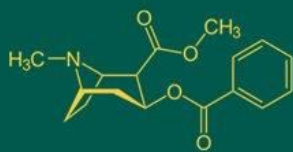


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Pathological evaluation of large intestinal inflammatory bowel disease in dogs

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Abstract

Inflammatory bowel disease (IBD) in dogs is primarily marked by persistent or recurring gastro-intestinal symptoms persisting for over three weeks without a known etiology associated with histologic evidence of inflammatory infiltration of the intestinal mucosa. Endoscopic examination of the intestinal mucosa, along with histologic analysis of biopsy samples, is considered as the gold standard for identifying and measuring intestinal inflammation in IBD. Histologic evaluation helps differentiate between healthy and affected tissue, identify the main cell types involved in the inflammation, assess the severity of the inflammatory response and tissue alterations and offer a precise morphological or etiological diagnosis. This process aids in making an accurate diagnosis and developing an effective treatment strategy and prognosis in canine IBD. Colonoscopic evaluation of the study group showed mild granularity, erythema, and a few erosions associated with the ongoing inflammatory process. The present study revealed lymphocytic-plasmacytic infiltrations in the lamina propria of dogs affected by large intestinal IBD, as observed in histopathological examination. These findings also showed a significant association with the clinical disease activity as determined by the Canine IBD Activity Index (CIBDAI) scoring system.

Keywords: Inflammatory bowel disease, Inflammation, CIBDAI

Introduction

Inflammatory Bowel Disease (IBD) in dogs encompass a cluster of chronic intestinal ailments of uncertain etiology, distinguished by the widespread infiltration of inflammatory cellular elements into the lamina propria of the intestinal mucosa. The Gastrointestinal Standardization group of the World Small Animal Veterinary Association (WSAVA) defines the diagnosis of inflammatory bowel disease as the patient has chronic gastrointestinal symptoms that have persisted or returned for more than three weeks; a histopathological examination confirms the inflammation of the intestinal mucosa; other possible causes of the symptoms are ruled out; the patient does not respond to dietary, antibiotic or antiparasitic interventions and the patient exhibits clinical improvement after receiving anti-inflammatory and immunosuppressive therapy (Washabau *et al.*, 2010) [1]. In dogs, a histopathology investigation yields a conclusive diagnosis of intestinal inflammation linked to IBD. Mucosal inflammation in dogs with IBD was classified according to cellular infiltrations such as lymphocytic and plasmacytic and only occasional infiltration by eosinophils or macrophages. A simplified histopathologic scoring system for canine IBD takes into account factors such as surface epithelial injury, crypt dilatation, fibrosis, goblet cell number and lamina propria infiltrates (Bhavani *et al.*, 2023) [3]. Thus, this study involves the pathological evaluation of inflammatory bowel disease in dogs with reference to histopathological examination of colon mucosa.

Materials and Methods

A total of 14 animals with unknown etiology and Canine IBD Activity Index (CIBDAI) score of four and above were selected for the study after the exclusion of other possible causes of chronic gastro-intestinal disorders. The numerical clinical scoring index, CIBDAI composed of parameters such as attitude or activity, appetite, vomiting, stool consistency, stool frequency and weight loss.

The CIBDAI score system define clinically insignificant disease (score: 0-3) or the presence of mild (score: 4-5), moderate (score: 6-8) or severe (score: ≥ 9) IBD (Jergens and Simpson, 2012) [6]. Dogs in the control group had no gastrointestinal symptoms in the months preceding the diagnostic assessment. From all owners of the 14 dogs in the study group, written informed consent for colonoscopy and biopsy sample collection was obtained. Colonoscopy was performed using a borescope of diameter 8 mm. Histopathological evaluations was carried out in dogs in the study group as follows,

Collection of samples

Biopsy samples from the colon was collected with the guidance of a custom made colonoscope in 10 percent neutral buffered formalin (NBF) for histopathological examination.

Histopathological examination

Tissue samples of colon were fixed in 10 percent, NBF for 48 h and processed by routine paraffin embedding method. Serial sections were cut at five-micron thickness using rotary microtome and sections were stained by routine haematoxylin and eosin procedure. The sections were examined under a light microscope (Zeiss microscope) and microscopical lesions were recorded.

Histopathological parameters evaluated in dogs with inflammatory bowel disease were surface epithelial injury, crypt hyperplasia, crypt dilation or distortion, mucosal fibrosis or atrophy, lymphocytic-plasmacytic infiltration in lamina propria, neutrophilic infiltration in lamina propria, eosinophilic infiltration in lamina propria and macrophage infiltration in lamina propria. Each parameter was scored as 0 (normal), 1 (mild), 2 (moderate) and 3 (marked) according to Day *et al.* (2008) [4]. Based on the total score obtained from the histopathological examination, IBD was graded as mild (total score = 1-6), moderate (total score = 7-12) and severe (total score >13) (Lee *et al.*, 2021) [8].

Results

A total of 14 dogs clinically found to have large intestinal associated chronic gastro-intestinal disorders of unknown etiology and CIBDAI score of four and above were selected as the study group and underwent detailed diagnostic investigations. Colonoscopic parameters assessed in the study group were friability, granularity and erosions. No abnormalities were observed in the dogs in the control group. Friability was absent in all dogs in the study group with a score of zero and mild granularity was observed in 28.6 percent (four out of 14) of the dogs in the study group, with a score of one indicating increased mucosal texture of the colon. Mucosal erythema was observed in 85.7 percent of dogs (12 out of 14) with IBD. Few erosions were observed in 35.7 percent (five out of 14) of the dogs in the study group. Microscopic examination of the surface epithelium of the colon revealed moderate epithelial injury characterized by multifocal areas of attenuation and loss of epithelial cells (Plate 1) to severe surface epithelial injury with diffuse epithelial necrosis (Plate 2 and Plate 3). The severity of crypt hyperplasia varied from mild to moderate degree with more basophilic and thickened crypt lining. Some crypts showed multiple layers of epithelial cells. (Plate 4). Mild crypt dilation and distortion with perpendicular arrangement of crypts and increased diameter

of the crypt lumen (Plate 5) and moderate degree of crypt dilation and distortion with many irregularly oriented crypts and crypts with markedly increased diameter of the lumen were recorded (Plate 6). Mucosal fibrosis varied from no fibrosis to mild to moderate degree in severity. Moderate mucosal fibrosis was characterised by loss of crypts and presence of prominent collagenous matrix widely separating the remaining apparently normal and/or atrophied crypts (Plate 7). Mild degree of inflammatory cell infiltration was observed in the lamina propria of colon in all animals which consisted predominantly of lymphocytes and plasma cells (Plate 8). Apart from these findings, mucosal and submucosal congestion and haemorrhage, dilated lymphatics and focal herniation of the mucosa into the submucosa were also observed (Plate 9). Based on the total score obtained from the histopathological examination, IBD in dogs in the study group was graded as mild in 35.7 percent (five out of 14) and moderate in 64.3 percent (nine out of 14). Histopathological and CIBDAI evaluations in dogs in the study group were statistically analysed using the Chi-square test. The statistical analysis revealed a significant association ($p < 0.05$) between the histopathological and CIBDAI scoring evaluations (Table 1).

Discussion

In the present study, microscopic examination of the surface epithelium of the colon of dogs in the study group revealed moderate epithelial injury characterized by multifocal areas of attenuation and loss of epithelial cells to severe surface epithelial injury with diffuse epithelial necrosis. The severity of crypt hyperplasia varied from mild to moderate degree with more basophilic and thickened crypt lining. Some crypts showed multiple layers of epithelial cells. Mild crypt dilation and distortion with perpendicular arrangement of crypts and increased diameter of the crypt lumen and moderate degree of crypt dilation and distortion with many irregularly oriented crypts and crypts with markedly increased diameter of the lumen were recorded. Mucosal fibrosis varied from no fibrosis to mild to moderate degree in severity. Moderate mucosal fibrosis was characterised by loss of crypts and presence of prominent collagenous matrix widely separating the remaining apparently normal and/or atrophied crypts. Day *et al.* (2008) [4] reported that the gastro-intestinal inflammation in dogs with IBD can be histologically graded as normal, mild, moderate or marked based on changes in parameters like surface epithelial injury, crypt hyperplasia, crypt dilation or distortion and mucosal fibrosis or atrophy. Priya *et al.* (2023) [9] also explained that dogs with IBD may show mild degeneration of mucosal epithelium, crypts epithelial cell hyperplasia with and very few mononuclear cell infiltrations in the lamina propria of colon on histopathologic examinations. In the present study, inflammatory cell infiltration observed in the lamina propria of colon of dogs with IBD as lymphocytes and plasma cells with mild degree of infiltration. This finding was in agreement with the findings of Kleinschmidt *et al.* (2006) [7], who conducted a study on 64 dogs with IBD. Their study revealed histopathological findings of villous blunting and shortening with infiltrations of lymphocytes and plasma cells in the lamina propria of five dogs, resembling lymphocytic-plasmacytic colitis, a variant of canine IBD. In their research, eosinophilic inflammatory alterations were also observed in 16 percent of dogs on histologic examination. Day *et al.* (2008) [4] also

reported that the type of IBD in dogs can be categorised based on the cellular infiltrations in the lamina propria like lymphocytic-plasmacytic infiltration, neutrophilic infiltration, eosinophilic infiltration and macrophage infiltration. According to Allenspach *et al.* (2019) [1], the most common type of IBD in dogs was reported to be due to the cellular infiltrations such as lymphocytic and plasmacytic. Also in this study, simplified histopathologic scoring system for canine IBD was utilised considering the parameters like crypt dilation, fibrosis, goblet cell number, surface epithelial injury and lamina propria infiltrates. Suchodolski *et al.* (2012) [10] and Bhavani *et al.* (2023) [3] also reported that in dogs with inflammatory bowel disease, lymphocytes and plasma cells were the predominant cellular infiltrates observed within the mucosa of the colon. Other research observations were also noted on histopathological examinations in canine IBD condition like Washabau *et al.* (2010) [11] stated that histopathologically, the principal characteristic of canine inflammatory bowel disease is found to be mucosal inflammation, which may be accompanied by epithelial injury, elevated intraepithelial lymphocytes, stunting of villi, dilation of lacteals and diverse extents of disruption to mucosal architecture. Faray *et al.* (2020) [5] reported that for histologic evaluation for IBD condition in dogs, from small bowel samples, the primary morphological characteristics assessed included villous atrophy, epithelial damage, crypt dilatation, lacteal dilation, metaplastic squamous changes and mucosal fibrosis. In large bowel samples, the features evaluated were epithelial damage, crypt dilatation, lacteal dilation, mucosal fibrosis and metaplastic squamous changes. In the study of Faray *et al.* (2020) [5], the cellular composition in mucosal samples from suspected IBD cases primarily consisted of lymphocytes and plasma cells, occasionally with small numbers of eosinophils, neutrophils and macrophages. In the study of Lee *et al.* (2021) [8], the morphological changes associated with IBD in dogs were reported to be epithelial damage, crypt expansion, dilated lacteals and mucosal scarring, along with histological changes such as the presence of plasma cells, lamina propria lymphocytes, eosinophils and neutrophils. Also, these parameters were scored as normal (0), mild (1), moderate (2) and severe (3). Based on these scores, inflammatory bowel disease was classified histologically as mild (total score of one to six), moderate (total score of seven to twelve) or severe (total score > 13) according to Lee *et al.* (2021) [8]. In the present study, based on the total score obtained from the histopathological examination, IBD was graded as mild in 35.7 percent and moderate in 64.3 percent. According to Bhavani *et al.* (2023) [3], biopsy samples from dogs affected by IBD should be examined for inflammatory alterations such as intraepithelial lymphocytes, lamina propria lymphocytes/plasma cells, lamina propria eosinophils, lamina propria neutrophils, gastric lympho-follicular hyperplasia and lamina propria macrophages. Morphological changes assessed in their study included surface epithelial damage, gastric pit epithelial injury, fibrosis/glandular changes, nesting/mucosal atrophy, villous stunting, epithelial injury, crypt distension, lacteal dilation, mucosal fibrosis, surface epithelial injury, crypt hyperplasia and crypt dilation and distortion. Also, they assigned severity grading for the IBD cases as 0 for normal, 1 for mild, 2 for moderate and 3 for marked changes. The statistical analysis of histopathological and CIBDAI

evaluations in dogs in the present study group revealed a significant association between the histopathological and CIBDAI scoring evaluations. This observation is in agreement with the findings of Bhavani *et al.* (2023) [3]. Their study concluded that CIBDAI scoring system will serve as a dependable gauge for assessing clinical indicators of inflammation in IBD, while histopathology provides a definitive diagnosis of intestinal inflammation associated with IBD in dogs. So, before opting an invasive diagnostic method like biopsy sample collection for the IBD diagnosis, assessment of clinical disease activity among dogs with chronic gastro-intestinal disorders persisting for more than three weeks with an unknown etiology can be done using CIBDAI score system as it is having some significant association with the histopathological observations.

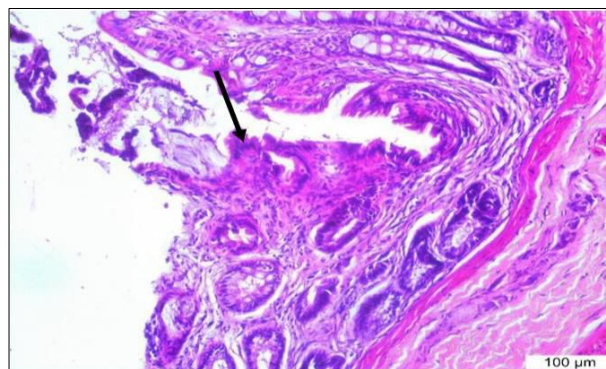


Plate 1: Moderate epithelial injury - Necrosis and loss of columnar epithelial cells (arrow) (H&E, X200)

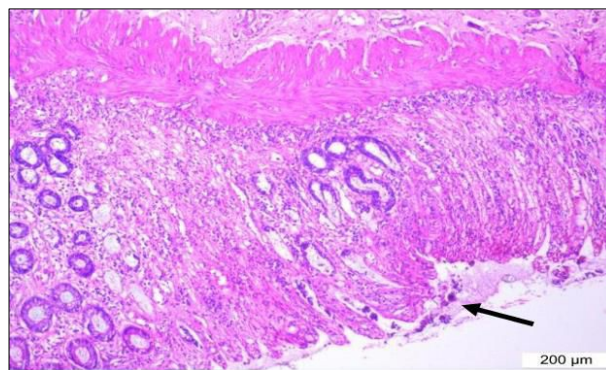


Plate 2: Severe epithelial injury - Diffuse necrosis and loss of columnar epithelial cells (arrow) (H&E, X200)

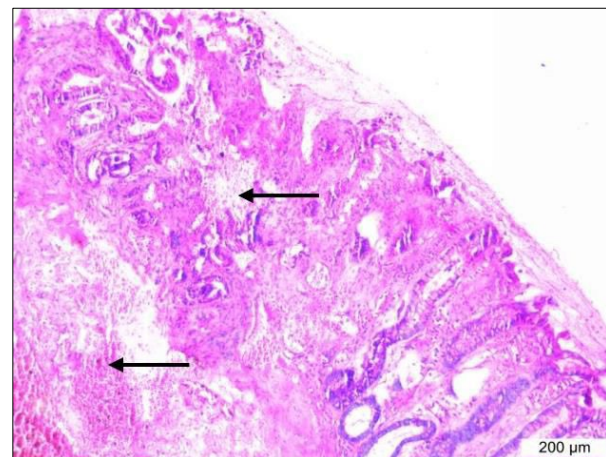


Plate 3: Severe epithelial injury - Diffuse epithelial necrosis, loss of crypts, mucosal and submucosal haemorrhage (arrows) (H&E, X200)

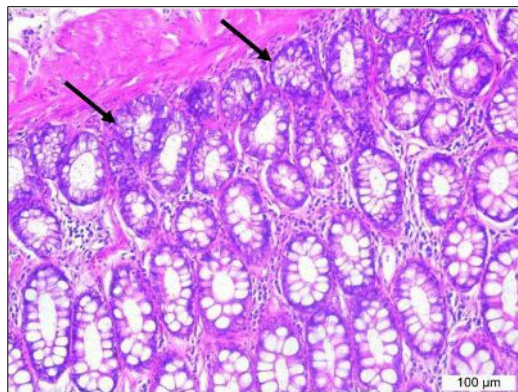


Plate 4: Moderate crypt hyperplasia - Increased basophilia and thickening of crypt lining. Note multiple epithelial layers in crypts (arrows) (H&E, X200)

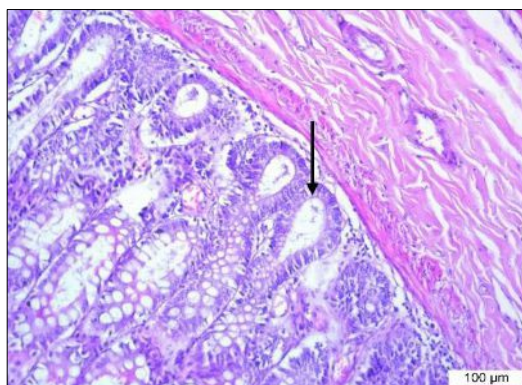


Plate 5: Mild crypt dilation - Mild increase in diameter of crypt lumen (arrow), (H&E, X200)

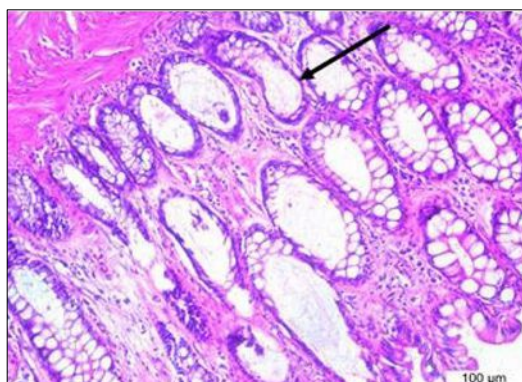


Plate 6: Moderate crypt dilation - Marked increase in diameter of the crypt lumen. Note the irregularly oriented crypt (arrow) (H&E, X200)

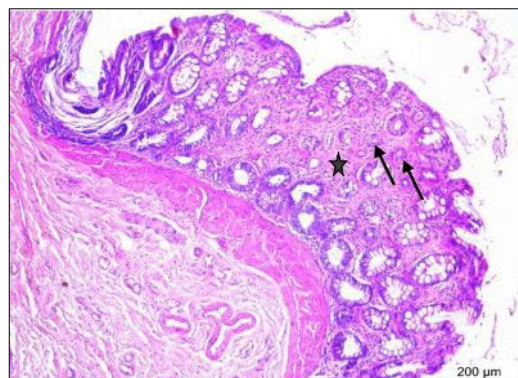


Plate 7: Moderate mucosal fibrosis- Prominent collagen deposition (star) between atrophied (arrows) and apparently normal crypts (H&E, X100)

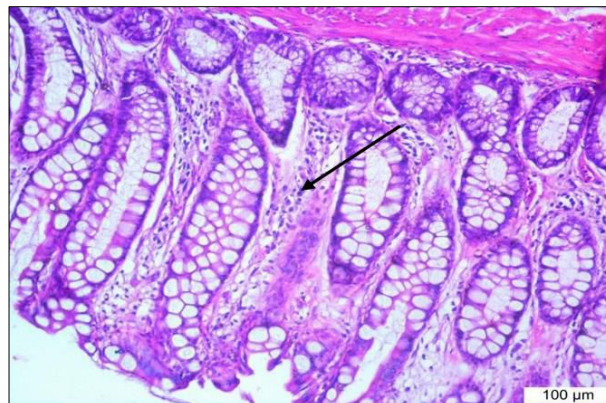


Plate 8: Mild cellular infiltration- Lymphoplasmacytic infiltration in lamina propria (arrow) (H&E, X200)

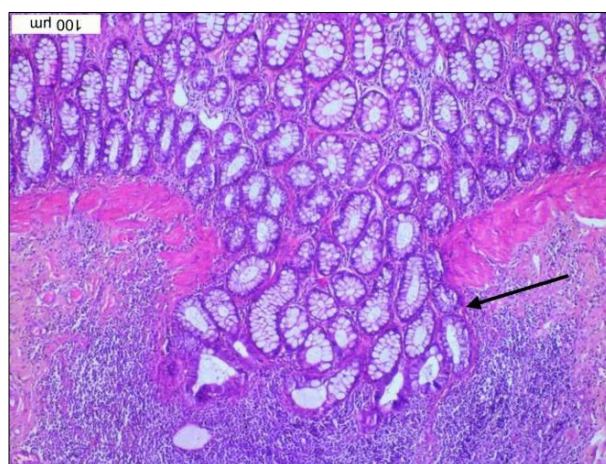


Plate 9: Focal crypt herniation- Note the presence of crypts in the submucosa (arrow) (H&E, X200)

Table 1: Comparison of histopathological and CIBDAI scoring evaluations in dogs in the study group

Histopathology	CIBDAI			Total	p-value (Chi-square value)
	Mild	Moderate	Severe		
Mild	3	2	0	5	0.017* (8.193)
Moderate	0	4	5	9	
Total	3	6	5	14	

* Significant at 0.05 level

Conclusion

In conclusion, colonoscopic examination of the large intestinal mucosa, along with histologic analysis of biopsy samples, was instrumental in definitively identifying and measuring intestinal inflammation associated with IBD. The clinical disease activity in dogs affected by IBD in the present study was effectively assessed using the CIBDAI scoring system and a significant association was observed between the histopathological findings and the CIBDAI scores. This research highlights the importance of the CIBDAI scoring system in evaluating clinical disease activity and additionally, it underscores the utility of colonoscopy and histopathological examination for the definitive diagnosis of IBD in dogs with chronic gastrointestinal disorders of unknown etiology.

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