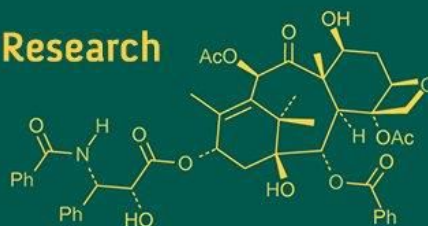
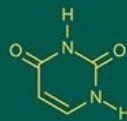
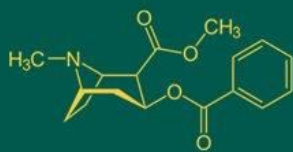


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Clinical, haematological and serum biochemical evaluation in dogs with large intestinal inflammatory bowel disease

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Abstract

Inflammatory Bowel Disease (IBD) encompasses 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. This study aimed to examine the clinical disease activity and haemato-biochemical changes related to large intestinal IBD in dogs. Clinical disease activity in dogs with IBD, was assessed using the Canine IBD Activity Index (CIBDAI) and Canine Chronic Enteropathy Clinical Activity Index (CCECAI) scoring systems. Chronic diarrhoea was the predominant clinical sign exhibited by the dogs with IBD. Haematological findings revealed a statistically significant decrease in Hb and increase in VPRC values and a significant increase in TLC values in dogs with inflammatory bowel disease when compared to the control group. Significant increase in ALP and decrease in TP and albumin values were noted. No statistically significant change was observed in serum folate cyanocobalamin concentrations in dogs with large intestinal IBD.

Keywords: IBD, Dogs, CIBDAI, CCECAI

Introduction

Inflammatory bowel disease (IBD) is a chronic illness that affects their gastrointestinal tract, which includes intestines and stomach. The major factors contributing to this condition are genetics, environment and diet. Dogs with inflammatory bowel disease are diagnosed when they exhibit symptoms of gastrointestinal distress for longer than two or three weeks, show no symptoms of extraintestinal or intestinal diseases, do not respond to dietary or antibiotic treatments, have intestinal biopsy results that confirm chronic inflammation, show histological changes in the lamina propria, and do not respond well to immunosuppressive therapy (Jergens and Simpson, 2012) [20]. The canine IBD Activity Index (CIBDAI) scoring system was used to evaluate the clinical signs in dogs with IBD. It takes into account factors such as general attitude/activity, appetite, frequency of vomiting, consistency and frequency of stools and weight loss (Lee *et al.*, 2021) [25]. The Canine Chronic Enteropathy Clinical Activity Index (CCECAI) is a tool that can be used to rate clinical disease activity in IBD cases. It considers parameters such as attitude/activity, appetite, frequency of vomiting, consistency and frequency of stools, weight loss, albumin levels, ascites/peripheral oedema and pruritus (Bhavani *et al.*, 2023) [4]. This research work thus helps in assessing the clinical signs, haematological and biochemical changes associated with IBD in dogs.

Materials and Methods

A total of 115 dogs with clinical signs such as anorexia, weight loss, vomiting and chronic diarrhoea for more than three weeks were screened for the study. These dogs were subjected to a detailed clinical examination and clinical samples were subjected to laboratory assessments. All the animals in the study group had been subjected to anamnesis and clinical examination with special emphasis on the gastrointestinal system. All the dogs underwent diagnostic investigations, such as examination of faecal samples for ova of parasites. Clinically, these animals were assessed based on CCECAI and CIBDAI. A total of 14 animals with an unknown etiology for chronic large intestinal gastrointestinal disorders and

CCECAI and CIBDAI scores of four and above were selected for the study (Konstantinidis *et al.*, 2020) [24]. Epidemiological data regarding the breed, age, sex, food habits and duration of present illness, were collected from the owners. Whole blood and serum from these dogs were used for evaluating haemato-biochemical changes. Serum cyanocobalamin and folate levels were also estimated. Six apparently healthy dogs formed the control group for recording normal values of vital parameters like respiratory and pulse rate, temperature, hydration status, and other body systems, and to obtain the normal parameters under study. The parameters studied in both the study and control groups included clinical examination findings, haemoglobin, volume of packed red cells, total erythrocyte count, total leucocyte count, platelet count, differential leucocyte count, total protein, albumin, alkaline phosphatase, cyanocobalamin and folate.

The Canine Inflammatory Bowel Disease Activity Index and Canine Chronic Enteropathy Clinical Activity Index was assigned to all dogs to evaluate disease activity. This numerical clinical scoring indices composed of parameters such as attitude or activity, appetite, vomiting, stool consistency, stool frequency, weight loss, albumin levels, ascites and peripheral oedema and pruritus. Both CIBDAI and CCECAI scores defines 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) [20]. Blood was collected from the peripheral (cephalic/saphenous) veins of the animals under study in vacutainers containing Ethylene Diamine Tetra Acetic Acid (EDTA) as an anticoagulant for haematological investigation and in clot activator vials for serum biochemical analysis. Parameters including Haemoglobin (Hb), Volume of Packed Red Cells (VPRC), Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Differential Leucocyte Count (DLC) and Platelet count (PLT) were estimated. A three-part fully automated haematological analyser (Mindray BC-2800Vet) was used for analysis. Serum was separated by centrifugation. Biochemical parameters like alkaline phosphatase, total protein and albumin were estimated using semi-automatic biochemical analyzer (MISPAVIVA 2578-10/17). Commercially available standard diagnostic kits from Agappe diagnostics Pvt. Ltd. Kochi were used to estimate the above biochemical parameters.

Serum cyanocobalamin (CNCbl) and folate (FA) were estimated in dogs with chronic gastro-intestinal disorders of unknown etiology using Enzyme-Linked Immunosorbent Assay (ELISA) (Canine specific ELISA kit supplied by bioelsa, New Delhi), as they are markers for determining the region of the gastro-intestinal tract involved in the inflammatory process (Lyon *et al.*, 2020) [26]. Frozen serum was thawed and then subjected to ELISA test in 96 well plates using an ELISA plate reader (Bio-rad iMark™ Microplate reader). An inverse correlation between CNCbl concentration and the assay signal activity was determined by regression analysis of standard curve obtained. Brought all kit components and samples to room temperature (18-25 °C) before use. Diluted the 25x wash buffer into 1x working concentration with double steaming water. For standard working solution, reconstituted the Standard with 1.0 mL of Standard Diluent, kept it for 10 minutes at room temperature, shook gently (not to foam). Prepared seven tubes containing 0.5 mL Standard Diluent and used the

diluted standard to produce a double dilution series. Mixed each tube thoroughly before the next transfer. In order to guarantee the experimental results validity, please used the new standard solution for each experiment. Briefly spun the stock Biotinylated Antibody and Streptavidin-HRP before use. Diluted them to the working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively. Aspirated the needed dosage of the TMB substrate solution with sterilized tips. All the reagents and samples were brought to room temperature before use. All samples and standards were assayed in duplicate. A plate layout was provided to record standards and samples assayed. Determined wells for diluted standard, blank and sample. Prepared seven wells for standard and one well for blank. Added 100 µL each of standard working solution or 100 µL of samples into the appropriate wells. Covered with the Plate sealer. Incubated for 80 min at 37 °C. Removed the liquid from each well. Aspirated the solution and washed with 200 µL of 1x Wash Solution in each well and let it sit for 1-2 min. Removed the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Washed a total of three times. After the last wash, removed any remaining Wash Buffer by aspirating. Inverted the plate and blotted it against absorbent paper. Added 100 µL of Biotinylated Antibody working solution to each well, covered the wells with the plate sealer and incubated for 50 min at 37 °C. Repeated the aspiration and wash process for total three times as conducted in step 2. Added 100 µL of Streptavidin-HRP working solution to each well, covered the wells with the plate sealer and incubated for 50 min at 37 °C. Repeated the aspiration and wash process for total 5 times as conducted in step 2. Added 90 µL of TMB Substrate Solution to each well. Covered with a new Plate sealer. Incubated for 20 min at 37 °C (Don't exceed 30 min). Protected from light. The liquid was turned blue by the addition of TMB Substrate Solution. Added 50 µL of Stop reagent to each well. The liquid turned yellow by the addition of Stop reagent. Mixed the liquid by tapping the side of the plate. The insertion order of the Stop reagent was the same as that of the TMB Substrate Solution. Removed any drop of water and fingerprint on the bottom of the plate and confirmed there were no bubbles on the surface of the liquid. Then, ran the microplate reader and conducted measurement at 450 nm immediately.

Results

Chronic gastro-intestinal disorders in dogs during 2022 and 2024 were found to be 5.8 percent (115 out of 1989 dogs). Out of 115 dogs, 58 (50.4 percent) were found to be infected with intestinal parasites. Among the 58 dogs, 44.8 percent (26 out of 58) with ova of *Ancylostoma* spp., 20.7 percent (12 out of 58) with ova of *Dipylidium* spp., 18.9 percent (11 out of 58) with *Toxocara* spp., 10.3 percent (six out of 58) with mixed infection of ova of both *Ancylostoma* spp. and *Toxocara* spp. and 5.3 percent (three out of 58) with *Diphylobothrium* spp. Clinical disease activity in 115 dogs with anorexia, weight loss, vomiting and chronic diarrhoea was assessed using the Canine IBD Activity Index (CIBDAI) and Canine Chronic Enteropathy Clinical Activity Index (CCECAI) scoring systems. Among the 115 dogs, 43 (37.4 percent) dogs were found to have clinically insignificant disease with both the CIBDAI and CCECAI scores ranging between zero and three and 72 (62.6 percent) dogs had CIBDAI and CCECAI scores above four. Among

the 72 dogs, mild clinical disease severity was shown by 21 (29.2 percent) dogs on the CIBDAI score system and nine (12.5 percent) dogs on the CCECAI scoring system. Dogs exhibiting moderate disease severity were 63.9 percent (46 out of 72) and 75 percent (54 out of 72) on the CIBDAI and CCECAI scoring systems, respectively. Severe clinical disease activity was shown by 6.9 percent (five out of 72) of dogs on the CIBDAI and 12.5 percent (nine out of 72) of dogs on the CCECAI score system.

Out of 72 dogs, parasitic infections were seen in 39 (54.2 percent), food allergies were found in seven (9.7 percent), foreign body obstruction was seen in three (4.2 percent), parvo viral enteritis in three (4.2 percent), paralytic ileus was observed in one (1.4 percent) and 19 (26.3 percent) had chronic gastro-intestinal signs with no apparent etiology. The 72 dogs were also subjected to gelatin digestion test for faecal proteolytic activity and the digestion of the gelatin coating on X-ray film strips incubated in faecal homogenate ruled out exocrine pancreatic insufficiency. The 19 dogs with unknown etiology exhibited a lack of response following a two week period of antimicrobial therapy, followed by a two week dietary trial using hypoallergenic diets. Clinical signs exhibited by the 19 dogs with unknown chronic gastro-intestinal disorders included chronic diarrhoea, mucoid faeces, haematochezia, melena, anorexia, vomiting, flatulence, tenesmus and weight loss. The predominant clinical sign observed in these dogs was chronic diarrhoea. In cases of diarrhoea, characteristics such as increased frequency, tenesmus and a small volume of faeces containing mucus and blood were indicative of large intestinal disease and were observed in 14 out of 19 dogs. A small intestinal disease associated with a diarrhoea of large volume, melena and weight loss was found in five out of 19 dogs.

The measurement of serum cyanocobalamin and folic acid levels was done in 19 dogs with chronic gastro-intestinal disorders of unknown etiology. The concentrations of CNCbl and FA were estimated using canine-specific ELISA kits. A standard curve was plotted using various concentrations of the standard at 450 nm for CNCbl and FA (Figures 1 and 2). The comparison of serum cyanocobalamin and folic acid concentrations between the control group and dogs in the study group was done by using an independent t-test. The mean CNCbl concentration in 14 dogs and the control group was 0.44 ± 0.009 ng/mL and 0.523 ± 0.026 ng/mL, respectively and the mean folate concentration in the control group and study group was 8.05 ± 0.37 ng/mL and 7.62 ± 0.36 ng/mL, respectively. There was no statistically significant change in the concentrations of CNCbl and FA in study group dogs. The mean FA and CNCbl concentrations in five dogs with small intestinal chronic gastro-intestinal disorder of unknown etiology were 15.11 ± 2.62 ng/mL and 0.392 ± 0.014 ng/mL, respectively. A statistically significant increase in FA concentrations ($p < 0.05$) and a significant decrease in CNCbl concentrations ($p < 0.01$) were obtained in dogs with small intestinal chronic gastro-intestinal disorders of unknown etiology.

A total of 14 dogs clinically found to have large intestinal associated chronic gastro-intestinal disorders of unknown etiology were selected as the study group and underwent detailed diagnostic investigations. Five dogs with clinical signs specific to the small intestine were excluded from this study due to limitations in performing upper gastro-intestinal endoscopy and subsequent biopsy sample

collection for histopathologic examination. Dogs in the control group had no gastrointestinal symptoms in the months preceding the diagnostic assessment. Additionally, these dogs were healthy based on normal findings from physical examination, haematological and serum biochemical analyses, as well as examinations of faecal samples.

The prevalence of large intestinal inflammatory bowel disease among dogs with chronic gastro-intestinal disorders accounts for 12.2 percent (14 out of 115 dogs). Among 14 dogs, severe clinical disease activity was shown by 35.7 percent (five out of 14) of dogs on the CIBDAI score system and 64.3 percent (nine out of 14) of dogs on the CCECAI scoring system. Moderate clinical disease severity was shown by 42.8 percent (six out of 14) of dogs on the CIBDAI score system and 35.7 percent (five out of 14) of dogs on the CCECAI scoring system. Mild clinical disease severity was shown by 21.4 percent (three out of 14) of dogs on the CIBDAI score system. At the time of presentation, the median CIBDAI score was 8 (range: 5-10) and the median CCECAI score was 10.5 (range: 6-13). In the present study, large intestinal inflammatory bowel disease was highly prevalent with 64.3 percent in the age group of three to five years followed by 28.6 percent in the age group of one to three years and 7.1 percent in the age group of five to seven years. The prevalence of IBD was 57.1 percent (eight out of 14 dogs) in females and 42.9 percent (six out of 14 dogs) in males. The prevalence of IBD was highest in Rottweiler with 43 percent (six out of 14 dogs), both in German Shepherd and Non-descript with 14.3 percent (two out of 14 dogs) and Boxer, Labrador Retriever, Golden Retriever and Siberian Husky with 7.1 percent each (one out of 14 dogs), respectively. In the present study, vital parameters like respiratory rate, pulse rate and temperature of dogs affected with inflammatory bowel disease were recorded. The mean respiratory rate (27.13 ± 1.02 /min), pulse rate (88 ± 0.51 /min) and temperature (101.27 ± 0.14 °F) were recorded and found to be in the normal range. Chronic diarrhoea with mucus and blood was the chief complaint reported in 100 percent of dogs with inflammatory bowel disease. Other clinical signs reported were anorexia in 71.4 percent (10 out of 14), flatulence in 64.3 percent (nine out of 14), tenesmus in 57.1 percent (eight out of 14), vomiting in 21.4 percent (three out of 14) and weight loss in 14.3 percent (two out of 14) of dogs with IBD.

Haematological parameters of control group animals in this study showed a mean $\pm$ S.E values of Haemoglobin of 16.03 ± 0.70 g /dL, VPRC of 48.18 ± 1.89 percent, TEC count of $6.87 \pm 0.26 \times 10^6/\mu\text{L}$, TLC count of $11.85 \pm 0.66 \times 10^3/\mu\text{L}$ and Platelet count of $274.67 \pm 25.28 \times 10^3/\mu\text{L}$. The mean $\pm$ S.E values of neutrophil, lymphocyte, monocyte and eosinophil counts were 76.75 ± 1.66 percent, 16.97 ± 1.79 percent, 4.55 ± 0.44 percent and 1.73 ± 0.38 percent respectively. In the study group, the mean $\pm$ S.E values of haemogram include Hb of 13.34 ± 0.70 g /dL, VPRC of 57.97 ± 2.52 percent, TEC count of $5.92 \pm 0.30 \times 10^6/\mu\text{L}$, TLC count of $21.59 \pm 1.96 \times 10^3/\mu\text{L}$, Platelet count of $314.07 \pm 30.63 \times 10^3/\mu\text{L}$, neutrophil count of 80.83 ± 1.93 percent, lymphocyte count of 13.76 ± 1.82 percent, monocyte count of 3.38 ± 0.28 percent and eosinophil count of 2.04 ± 0.21 percent (Table 1). Comparison between study and control animals was done by using independent t-test. There was no statistically significant finding in TEC, platelet count, neutrophil, lymphocyte, monocyte and eosinophil

counts in study group. Significant decrease in Hb ($p < 0.05$) and a significant increase in TLC ($p < 0.01$) and VPRC ($p < 0.05$) values were obtained in study group dogs when compared to the control group. The mean ALP in control and study group animals was 77.16 ± 7.19 IU/L and 406.58 ± 88.71 IU/L, respectively. Significant increase ($p < 0.05$) in ALP was observed in the study group. The mean TP values in control and study group animals were 6.11 ± 0.12 g/dL and 3.44 ± 0.24 g/dL, respectively. The mean albumin

values in the control and study groups were 2.88 ± 0.08 g/dL and 1.53 ± 0.06 g/dL, respectively. There was a highly significant decrease in TP and albumin ($p < 0.01$) values in the study group when compared to the control group. Comparison between the study and control group animals was done using an independent t-test. The mean serum biochemistry values of the study and control groups are given in Table 2.

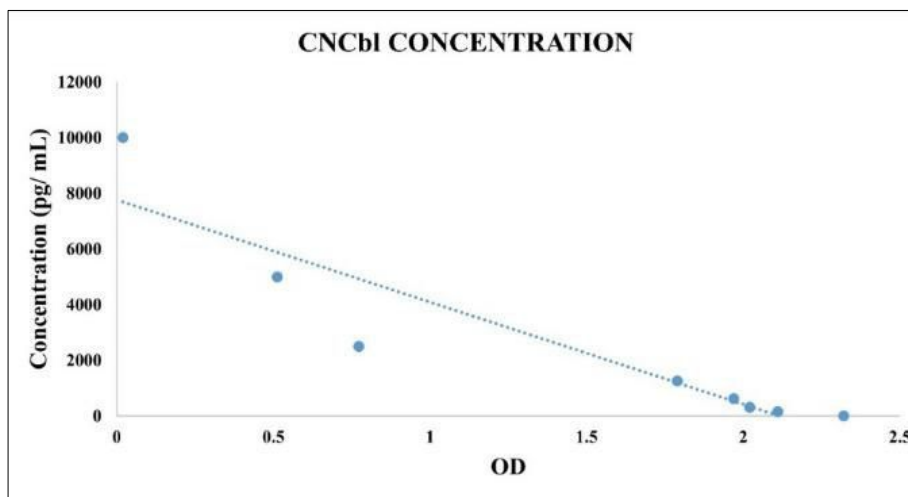


Fig 1: Standard curve obtained for various concentrations of Canine CNCbl

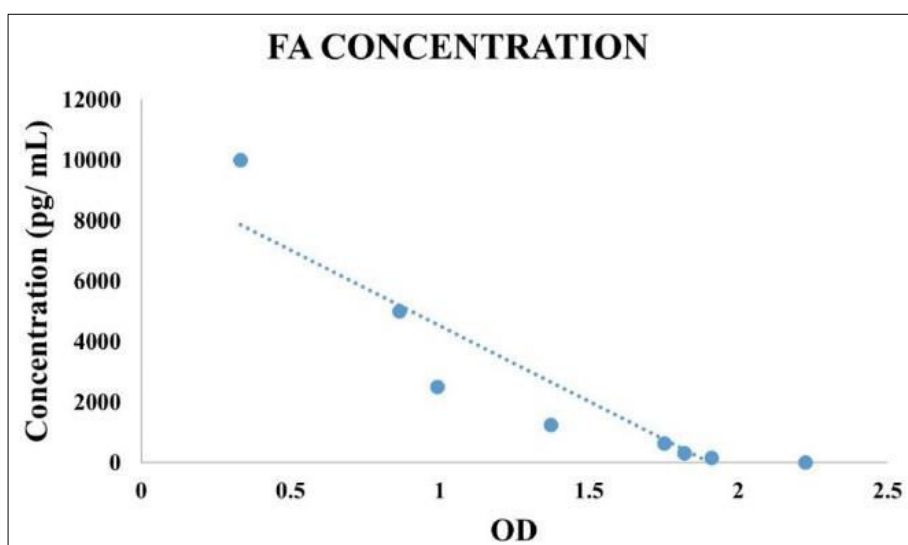


Fig 2: Standard curve obtained for various concentrations of Canine FA

Table 1: Comparison of haematology of dogs with inflammatory bowel disease and control animals

Sl. No.	Parameters	Mean $\pm$ SE		t-value (P-value)
		Study group (n = 14)	Control group (n = 6)	
1.	Hb (g/dL)	13.34 ± 0.70	16.03 ± 0.70	2.309* (0.033)
2.	VPRC (%)	57.97 ± 2.52	48.18 ± 1.89	2.502* (0.022)
3.	TEC ($\times 10^6 / \mu\text{L}$)	5.92 ± 0.30	6.87 ± 0.26	1.917 ^{ns} (0.071)
4.	TLC ($\times 10^3 / \mu\text{L}$)	21.59 ± 1.96	11.85 ± 0.66	3.179** (0.005)
5.	PLT ($\times 10^3 / \mu\text{L}$)	314.07 ± 30.63	274.67 ± 25.28	0.789 ^{ns} (0.442)
6.	N (%)	80.83 ± 1.93	76.75 ± 1.66	1.284 ^{ns} (0.215)
7.	L (%)	13.76 ± 1.82	16.97 ± 1.79	1.054 ^{ns} (0.306)
8.	M (%)	3.38 ± 0.28	4.55 ± 0.44	2.243 ^{ns} (0.05)
9.	E (%)	2.04 ± 0.21	1.73 ± 0.38	0.704 ^{ns} (0.501)

** Significant at 0.01 level ($p < 0.01$); * Significant at 0.05 level ($p < 0.05$);

ns Non-significant ($p > 0.05$)

Table 2: Comparison of serum biochemical values of dogs with inflammatory bowel disease and control animals

Sl. No.	Parameters	Mean $\pm$ SE		t-value (P-value)
		Study group (n = 14)	Control group (n = 6)	
1	TP (g/dL)	3.44 $\pm$ 0.24	6.11 $\pm$ 0.12	7.016** (<0.001)
2	Alb (g/dL)	1.53 $\pm$ 0.06	2.88 $\pm$ 0.08	13.256** (<0.001)
3	ALP (IU/L)	406.58 $\pm$ 88.71	77.16 $\pm$ 7.19	2.392* (0.028)

** Significant at 0.01 level ($p < 0.01$); * Significant at 0.05 level ($p < 0.05$);

ns Non-significant ($p > 0.05$)

Discussion

In the present study, the overall prevalence of chronic gastro-intestinal disorders in dogs was 5.8 percent. The prevalence of large intestinal inflammatory bowel disease among dogs with chronic gastro-intestinal disorders accounts for 12.2 percent. According to Allenspach *et al.* (2016) [1], 15-20 percent of dogs with chronic gastro-intestinal disorders were diagnosed with IBD. This might be due to the excessive immune response triggered by certain factors in dogs with genetic predispositions and due to environmental influences. In inflammatory bowel disease (IBD), a breakdown in tolerance to the endogenous microflora, food antigens or endogenous antigens can result in persistent inflammation of the gastrointestinal tract. Additionally, disruptions in the permeability of the gastrointestinal mucosa and abnormalities in the immune system can lead to inappropriate immune responses, as explained by Malewska *et al.* (2011). Inflammatory bowel disease was highly prevalent, with 64.3 percent in the age group of three to five years, followed by 28.6 percent in the age group of one to three years and 7.1 percent in the age group of five to seven years. These observations were consistent with the earlier reports by Kathrani *et al.* (2011) [23], Yogeshpriya *et al.* (2017) [32] and Kalenyak *et al.* (2018) [21], all of whom reported a higher incidence of IBD in middle-aged dogs and they also explained that this higher incidence could stem from a combination of genetic predisposition and environmental factors. In the present study, the prevalence of inflammatory bowel disease was 57.1 percent in females and 42.9 percent in males, of which two and three were neutered, respectively. In contrast, Marchesi *et al.* (2017) [27] and Hemida *et al.* (2021) [17] reported a higher incidence of IBD in male dogs than female dogs. Yogeshpriya *et al.* (2017) [32] found no gender predisposition for the development of IBD in dogs. According to Calalang *et al.* (2021) [6], in spayed or neutered dogs affected by inflammatory bowel disease, gender did not yield a notable variance in the composition of the gut microbiota contributing to the disease's pathogenesis. The findings observed in the present study were not comparable with above-mentioned studies. The prevalence of inflammatory bowel disease was highest in Rottweiler with 43 percent, both German Shepherd and Non-descript with 14.3 percent and Boxer, Labrador Retriever, Golden Retriever and Siberian Husky with 7.1 percent each. These findings were in agreement with the observations made by Simpson and Jergens (2011) [30], Peiravan *et al.* (2018) [28] and Felix *et al.* (2022) [12], all of them reported a higher incidence of IBD in breeds like Rottweilers followed by German Shepherds and Boxers. The findings in the present study might be due to the genetic susceptibility of the above-mentioned dog breeds to IBD through polymorphisms in TLR4 and TLR5 in German Shepherd breeds or through polymorphisms in the NCF2 gene in Boxer dogs. These explanations were in accordance with

Kathrani *et al.* (2010) [22] and Craven (2011) [7]. Chronic diarrhoea with mucus and blood was the chief complaint reported in 100 percent of dogs with inflammatory bowel disease. Other clinical signs reported were anorexia in 71.4 percent, flatulence in 64.3 percent, tenesmus in 57.1 percent, vomiting in 21.4 percent and weight loss in 14.3 percent of dogs with IBD. These observations were consistent with the earlier reports by Garraway *et al.* (2018) [13], who reported the main clinical manifestations in dogs with IBD such as diarrhoea, melena, haematochezia, weight loss, vomiting and anorexia. Dandrieux (2016) [9] also stated that dogs with IBD would develop chronic enteropathy characterized by gastrointestinal clinical signs such as vomiting, diarrhoea, abdominal pain and weight loss during at least 3 weeks. The clinical presentation of IBD in dogs mainly correlates with the inflamed section of the gastrointestinal tract. Gastric symptoms stem from inflammation in the stomach and upper intestines, while diarrhoea results from inflammation affecting the large intestine or chronic small intestinal issues. The presence of blood in vomit or stool signifies a more advanced condition, often seen in eosinophilic inflammations. Severe inflammation of the GI tract is often associated with anorexia. These explanations were in accordance with the observations made by Wdowiak *et al.* (2014) [31]. According to Bhavani *et al.* (2021), the canine IBD activity index (CIBDAI) and canine chronic enteropathy clinical activity index (CCECAI) scoring systems were useful in preliminary classification of the severity of inflammatory changes in dogs with IBD. In this study, among 14 dogs, severe clinical disease activity was shown by 35.7 percent of dogs on the CIBDAI score system and 64.3 percent of dogs on the CCECAI scoring system. Moderate clinical disease severity was shown by 42.8 percent of dogs on the CIBDAI score system and 35.7 percent of dogs on the CCECAI scoring system. Mild clinical disease severity was shown by 21.4 percent of dogs on the CIBDAI score system. At the time of presentation, the median CIBDAI score was 8 (range: 5-10) and the median CCECAI score was 10.5 (range: 6-13). In the present study, a highly significant increase in TLC ($P < 0.01$) was obtained in dogs with inflammatory bowel disease when compared to the control group. This observation was consistent with the findings made by Hotel and Tams (2016) [19]. They explained that leukocytosis in dogs with IBD ranging from 20,000 to 50,000 cells/ μ L, without a left shift, indicates an ongoing chronic inflammatory process. A statistically significant decrease in Hb and increase in VPRC ($P < 0.05$) values was observed in dogs with IBD when compared to the control group. This was in concordance with Bhargavi *et al.* (2017) [3], whose work explained that increased VPRC levels in IBD dogs might be due to severe dehydration and fluid losses through vomiting and diarrhoea. El-Zahar *et al.* (2021) [10] also reported a notable decrease in haemoglobin (9.83 $\pm$ 0.44) levels coupled with an increase in total white blood cell

count (14.58 ± 0.49) in canine IBD. El-Zahar *et al.* (2021) [10] and Gori *et al.* (2021) explained that inflammatory bowel disease in dogs involves long-term inflammation of the GI tract, which can cause ulcers and erosion of the intestinal lining, leading to occasional blood loss. Continuous blood loss over time can gradually lower haemoglobin levels. Also, chronic inflammation in IBD hinders the absorption of nutrients, such as iron, from the gut. This deficiency can result in anaemia because iron is crucial for haemoglobin production. In the present study, a significant increase ($p < 0.05$) in ALP was observed in the study group when compared to the control group. This finding was similar to the observations of Feldman *et al.* (2000) [11], Craven *et al.* (2004) [7] and Lee *et al.* (2021) [25], all of them elucidated that dogs suffering from IBD may experience an increased presence of bacteria in the intestine. This bacterial overgrowth can result in the production of toxins that impact the liver and cause elevated ALP levels. Also, they suggested that persistent inflammation and poor nutrient absorption in IBD can cause deficiencies in essential nutrients like vitamin D. This deficiency can impact liver function and contribute to increased ALP levels. A highly significant decrease of TP and Albumin ($p < 0.01$) values were noted in the study group when compared to the control group. Cabral *et al.* (2001) [5] explained the pathophysiology of hypoproteinaemia in canine IBD, which might be contributed by reduced appetite, malabsorption caused by a reduction in intestinal surface area, haemorrhage or exudation of protein into the digestive tract and increased intestinal permeability. Craven *et al.* (2004) [7], Pugliese *et al.* (2012) [29] and Holmberg *et al.* (2022), in their study, observed similar findings in serum-biochemistry of dogs with IBD, like hypoproteinaemia, hypoalbuminaemia and hypoglobulinaemia.

The present study found no statistically significant change ($p > 0.05$) in the concentrations of CNCbl and FA in dogs affected by large intestinal IBD. A statistically significant increase in FA concentrations ($p < 0.05$) and a significant decrease in CNCbl concentrations ($p < 0.01$) were obtained in dogs with small intestinal chronic gastro-intestinal disorders of unknown etiology. These observations were consistent with the findings of Heilmann *et al.* (2018) [16], Lyon *et al.* (2020) [26] and Gueant *et al.* (2022) [15]. Heilmann *et al.* (2018) [16] reported that hypocobalaminaemia is a common occurrence in dogs afflicted with inflammatory bowel disease, with an incidence ranging from 19 percent to 54 percent. This phenomenon is thought to stem from secondary dysbiosis in the small intestine, characterized by heightened utilization of cobalamin by the intestinal microbiota. Lyon *et al.* (2020) [26] described that low levels of vitamin B12 and folic acid indicate poor absorption and point to localized disease in the distal (ileum) or proximal (duodenum and proximal jejunum) parts of the gastrointestinal tract, where these nutrients are absorbed, respectively. Hence, serum CNCbl and FA concentrations can be evaluated as markers to localize the intestinal segment involved in canine IBD. Furthermore, low vitamin B12 and high folic acid levels suggest dysbiosis in the small intestine due to bacterial consumption of vitamin B12 and excessive production of folic acid. Gueant *et al.* (2022) [15] also explained that low serum cobalamin levels can be linked to conditions affecting the small intestine, exocrine pancreatic insufficiency and imbalanced gut flora.

Conversely, dogs with dysbiosis may exhibit elevated serum folate levels.

Conclusion

In conclusion, the haematological indices, including haemoglobin and haematocrit, demonstrated a significant decrease and haematocrit revealed a significant increase, while the total leukocyte count exhibited a significant increase in dogs with large intestinal IBD. In dogs with large intestinal IBD, no significant change was observed in CNCbl and FA concentrations. The reduced serum cyanocobalamin levels in dogs with chronic small intestinal gastro-intestinal disorders of unknown etiology in the present study indicated small intestinal malabsorption, whereas the elevated folate levels suggested dysbiosis in the small intestine. Consequently, CNCbl and FA serve as markers for identifying the inflamed segment of the intestine in dogs with chronic gastro-intestinal disorder. This research highlights the significant prevalence and clinical impact of IBD in dogs, with variations in disease severity and associated symptoms. Further investigation into the genetic and environmental factors contributing to IBD and its management strategies is warranted.

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