Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 49, Number 4, p. 471-481, December 2021
DOI: 10.21608/zvjz.2021.107361.1164

RESEARCH ARTICLE

Ultrasound, Hematological and Biochemical Analysis in Canine with Inflammatory Bowel Disease

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Article History: Received: 05/12/2021 Received in revised form: 18/21/2021 Accepted: 31/12/2021

Abstract

Animal feed may contain different phenolic antioxidants, such as butylated hydroxyanisole, Inflammatory bowel disease (IBD) in dogs is a group of disorders characterized by persistent gastrointestinal clinical manifestations. The goal of this study was to investigate the hematobiochemical alterations in association with the clinical outcomes and prognostic markers including cobalamine, folate and vitamin D3 in dogs with IBD. A total of 23 IBD dogs with symptoms of chronic gastrointestinal diseases were chosen for the study, these include vomiting, diarrhea, anorexia, weight loss and abdominal pain. Ultrasonographically, the most common IBD-related abnormalities were dilated intestinal loops with hypermotility, as well as thickening and loss of wall layers in the stomach, duodenum, and jejunum. Hematological findings showed significant increase in the total white blood cell count (14.58 ± 0.49) and neutrophils (69.8 ± 1.6), and significant decrease in hemoglobin (9.83 ± 0.44) and Hematocrit value (30.30 ± 1.24). Biochemical analysis revealed significant increase in serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen and blood creatinine compared to the controls. Whilst significant decrease in serum 25(OH)D, folate and cobalamin levels in IBD dogs was observed which indicate intestinal malabsorption, and these markers are good predictors of IBD.

Keywords: IBD, vitamin D, cobalamin, folate, ultrasonography.

Introduction

Inflammatory bowel disease (IBD) is a long-term condition that affects a dog's gastrointestinal tract, which includes the stomach and intestines. IBD is caused by a complex interaction of several factors, including genetics, environmental, chemical factors, exposure to infected animals, lifestyle, and diet. The imbalance in the intestinal microbial population in IBD dog's intestine is a predisposing factor [1]. The pathogenesis of IBD is thought to include complex interactions of host-related genetic factors, gut microbiota dysbiosis, and damage to immune mediated intestinal microflora [2].

The chronic and regional variation in IBD epidemiology shows that hereditary and environmental variables have a role in the disease's genesis and etiology. The disease usually affects middle-aged dogs and affects both males and females equally; although intermittent symptoms have been noted even in puppies < 6 months of age [3].

A complete clinical examination, laboratory testing for blood, urine, and feces

and histological examination for biopsy are required to rule out possible causes of chronic enteritis and other causes of inflammation. Diet correction is also an important strategy for confirming food-related diarrhea [4].

The mucosa of the gastrointestinal tract become inflamed, and it loses its capacity to digest and absorb nutrients properly. This produces a number of symptoms, including chronic vomiting and/or diarrhea, loss of appetite, weight loss, and poor nutrient absorption, which can impair folate, vitamin B12 and vitamin D absorption. Folate has an effect on how the gut microbiota interacts with systemic immune responses, which may play a role in the etiology of IBD. Vitamin B12 is a coenzyme involved in various metabolic pathways, and vitamin B12 and folate deficiency in IBD dogs has been linked to macrocytic anemia [5, 6]. In addition, serum 25(OH)D in dogs is used as a clinical marker for a variety of illnesses, including irritable bowel syndrome. Dogs with protein-losing enteropathy exhibited considerably lower 25(OH)D concentrations than healthy controls, according to Zafalon *et al.* [7]. According to multiple studies, vitamin D appears to play a role in immune system modulation in dogs. As a result, dysregulation of the immune response to commensal gut bacteria may play a role in IBD pathogenesis [7, 8].

Inflammatory bowel disease cannot be cured, but it can be treated. Because not all dogs respond the same way to the same medication or food, a combination of pharmaceuticals and/or meals will be required. With a confirmed diagnosis of inflammatory bowel disease, the prognosis is generally favorable. Once, the proper medications or diet are determined many dogs will stay on them for the rest of their lives, but the drug dosage may be reduced over time [5, 9-12].

The definite diagnosis is based on disease symptoms, and the most commonly used diagnostic tests include ultrasonography, gastrointestinal endoscopy with intestinal biopsy, and IBD-related biomarkers. Ultrasonography is a noninvasive, low-cost method of evaluating the gastrointestinal system in small animals with gastrointestinal problems. According to Bhavani *et al.* [6], ultrasonography is often used as the first diagnostic method to distinguish inflammatory from neoplastic infiltration, which is critical for determining successful treatment approaches. In humans, gastrointestinal ultrasonography provides for a more objective assessment of inflammation, particularly in Crohn's disease patients, where the most apparent and significant indicator of inflammatory activity is increased intestinal wall thickness [13]. The current study was designed and carried out to investigate the clinical, ultrasonographic, and hematobiochemical changes as prognostic indicators in dogs with IBD.

Materials and Methods

The present study was carried out on 33 dogs with different ages and breeds (including German shepherd, Golden retriever, Griffon). All dogs included in the current study were collected from a private Pets Clinic and Animal Rescue Shelter at Ash Sharkia province. Ten dogs were clinically healthy, and their ages ranged from 5 to 15 years, dogs were selected to be free of gastrointestinal diseases based on the normal physical examination findings, complete blood count (CBC), serum biochemistry and fecal examination.

The remaining 23 dogs had gastrointestinal problems (mostly inflammatory bowel disease IBD), including chronic diarrhea and failure to react to dietary and symptomatic treatments. Other GIT illnesses were excluded from this investigation following a negative fecal examination, as fecal swabs were collected from diseased dogs and analyzed macro- and microscopically [14]. This study not includes experimental work, so the Institutional Animal Care and Use Committee, Zagazig University (ZU -IACUC) was not required. Owners of the dogs have approved to include their dogs in the current study.

Physical examination was performed for all dogs including rectal temperature, heart and respiration rates. Clinical disease activity was assessed according to the alterations from normal dogs and these include: attitude/activity, appetite, vomiting, stool consistency, stool frequency and weight loss.

Ultrasonographic examination was performed for all dogs in the present study, especially the small intestine "duodenum and jejunum". Ultrasound scanner *(WED, WELLD®,Shenzhen WellD Medical Electronics Co., Ltd., China)* equipped with trans-abdominal scanning head (7·5 MHz mechanical transducer) was used. The duodenum was identified either from a substernal or a right intercostal approach. The intestinal loops located in the mid-abdomen were identified from a ventral approach. The wall thickness, incorporating all layers, was measured using the method previously reported [15].

To carry out hematological and biochemical tests before beginning any therapy, 7 ml of blood was collected from the cephalic vein, 1-2 ml of blood was placed in vacuum Ethylene diaminetetraacetic acid (EDTA) coated tubes for hematological analysis. The remaining blood samples were divided into 3-4 ml sterile heparinized tubes and 2 ml plain tubes for plasma and serum sample collection. Plasma and serum were separated immediately after collection by centrifuging for 10 minutes at 3000 rpm. Hemoglobin (Hb), Hematocrit (Ht), total erythrocytic count (TEC) and total and differential leucocytic counts were determined using Sysmex XN1000 analyzer (*Sysmex, USA*) using standard methods [16]. Serum samples were harvested for biochemical analysis of serum cobalamin, vitamin D3, blood folate and other blood parameters including total protein, albumin, AST, ALT, urea and creatinine. All measurements were applied using Beckman AU5800 analyzer (Beckman Coulter, California, USA). Serum cobalamin and vitamin D3 concentration were measured using Siemens ADVIA Centaur XP analyzer (Siemens Medical Solutions, Malvern, USA). In addition, blood folate was measured by Beckman DXI-800 analyzer (Beckman Coulter, California, USA). Blood was also collected from ten healthy dogs for establishing control values to compare the data.

Statistical Analysis

The data were statistically analyzed using SPSS program, version 17.0 (Version 17.0 released 2008, SPSS Inc., Chicago). The data were tested for normal distribution using Shapiro Wilks W Test and were found normally distributed. Descriptive data of clinical observations (rectal temperature, heart rate and respiration rate) were presented as mean ± S.E and the clinical signs were presented as absolute number and its percentage (%). Hematological and biochemical data were compared using one way ANOVA test where age, sex and breed are considered covariance. All data are listed as mean ± SE. Differences between parameters were tested for significance at probability levels of $P<0.05$ and $P<0.01$.

Results

From a total of 33 dogs, 23 dogs with varying ages (8 to 17 years) and breeds (4 German shepherd, 11 Golden retriever, 8 Griffon) had inflammatory bowel illness, while the remaining 10 were healthy dogs aged from 5 to 15 years and of various breeds (3 German shepherd, 4 Golden retriever, 3 Griffon). IBD was more usually detected in middle-aged (8 - 17 years) and male dogs (65%), with female dogs (35%). IBD dogs had a highly significant increase in the mean body temperature of 40.2 ± 0.12°C ($p<0.05$), a mean heart rate of 126.9 ± 2.3 per minute ($p<0.05$), and a significant increased mean respiratory rate of 54.8 ± 2 breaths per minute ($p<0.01$). The most common clinical indicators in dogs with inflammatory bowel disease, based on clinical examination, were gastrointestinal problems. They include vomiting (35%), diarrhea (hematochezia (48%), or watery (52%), anorexia (70%), weight loss, and abdominal pain. The remaining 10 dogs (controls) showed no signs of GIT disorders, and the physical examination was normal, the mean body temperature was 38.4 ± 0.08 °C, a mean heart

rate was 77.1 ± 1.1 per minute, and a mean respiratory rate of 26 ± 1.4 breaths per minute (Tables 1 and 2).

Table 1: Clinical observations in IBD and control dogs, the data are represented as Mean ± S.E

Parameters	Control group (n = 10)	IDB group (n = 23)	Reference values [#]
Age (year)	9.5 ± 0.92 (5 - 15)	9.8±0.54 (8 - 17)	-
Weight (Kg)	25.4 ± 1.2	21.6 ± 1.4	-
Rectal temperature	38.4 ± 0.08 [a]	40.2 ± 0.12 [b]	38.2 - 39.2
Heart rate (per min.)	77.1 ± 1.1 [a]	126.9 ± 2.3 [b]	70 - 90
Respiration rate (per min.)	26 ± 1.4 [a]	54.8 ± 2 [b]	15 - 35

NB.: Means carrying different superscripts (a and b) in the same row are significantly different at P<0.05, [#] [16].

Table 2: Frequencies of the dog characteristics and clinical signs in IBD and healthy dogs, the data are represented as n (%).

Clinical signs	Control group (n = 10)	IDB group (n = 23)
Gender:		
Male	6 (60%)	15 (65%)
Female	4 (40.00)	8 (35%)
Breed:		
German shepherd	3 (30%)	4 (17%)
Golden retriever	4 (40%)	11 (48%)
Griffon	3 (30%)	8 (35%)
Anorexia	None	16 (70%)
Vomiting	None	8 (35%)
Diarrhea:		
Non hemorrhagic	None	12 (52%)
Hemorrhagic		11 (48%)
Dehydration:		
Nil		5 (22%)
Mild	None	11 (48%)
Moderate		7 (30%)

Hematological and biochemical findings

The mean values of hematological and biochemical indices in dogs with IBD are summarized in Table (3). Complete blood count showed highly significant increase in the total white blood cell count (14.58 ± 0.49), neutrophils (69.8 ± 1.6) and nonsignificant decrease in red blood cell count (4.43 ± 0.12), significant decrease in hemoglobin (9.83 ± 0.44) and hematocrit (Ht) (30.30 ± 1.24) compared to the control group. The serum activities of AST and ALT (178.7 ± 23.68 and 76.5 ± 5.9, respectively) showed significant increase compared to the control dogs. As well as there was a highly significant increase in blood urea nitrogen (51.6 ± 2.8) and blood creatinine (3.03 ± 0.2). While, the mean values of serum total proteins, albumin, globulin were not significantly changed in IBD dogs compared to the controls. Serum 25(OH)D, folate and cobalamin levels in IBD dogs were significantly lower than in controls (83.1 ± 4.1, 4.61 ± 0.14, 300.4 ± 11.1, respectively).

Table 3: Mean values of hematological and serum parameters in dogs with IBD in comparison to control group, data are presented as Mean ± SE.

Parameters		Control group (n = 10)	IBD group (n = 23)	Reference values [#]
Hemoglobin (g/dl)		14.53 ± 0.28 [b]	9.83 ± 0.44 [a]	12 - 18
Hematocrit (%)		39 ± 0.91 [b]	30.30 ± 1.24 [a]	37 - 55
Total erythrocyte ($\times 10^6$/l)		5.67 ± 0.12	4.43 ± 0.12	5.5 - 8.5
Total leukocyte ($\times 10^3$/l)		9.47 ± 0.29 [a]	14.58 ± 0.49 [b]	6 - 16
DLC (%)	**Neutrophils**	65.1 ± 1.17 [a]	69.8 ± 1.6 [b]	60 - 70
	Lymphocytes	21.1 ± 1.4	20.8 ± 1.3	15 - 30
	Monocytes	4.7 ± 0.57	4.6 ± 0.36	3 - 8
	Eosinophils	2.9 ± 0.31	3.4 ± 0.29	2 - 10
Totla protein (g/dl)		6.3 ± 0.26	6.7 ± 0.16	5 - 7
Albumin (g/dl)		3.4 ± 0.21	3.1 ± 0.15	3.1 - 4.5
Globulin (g/dl)		3.1 ± 0.18	4.6 ± 0.17	2.8 - 4.5
BUN (mg/dl)		14.7 ± 1.6 [a]	51.6 ± 2.8 [b]	7 - 25
Creatinine (mg/dl)		1 ± 0.08 [a]	3.03 ± 0.2 [b]	0.4 - 1.8
AST (IU/l)		39.2 ± 2.9 [a]	57.8 ± 5.3 [b]	5 - 55
ALT (IU/l)		38.6 ± 2.7	76.5 ± 5.9	5 - 60
Vit. D3 (ng/ml)		114.1 ± 1.9 [b]	83.1 ± 4.1 [a]	100 - 120
Cobalamin (ng/l)		525 ± 35.3 [b]	300.4 ± 11.1 [a]	251 - 908
Folate (µg/l)		7.78 ± 0.12 [b]	4.61 ± 0.14 [a]	7.7 - 24.4

NB.: Means carrying different superscripts (a and b) in the same row are significantly different at $P<0.05$, [#] [16].

Ultrasonographic findings

In this investigation, the most common IBD-related alterations in the ultrasonographic image were distended intestinal loops with hypermotility in 7 cases (Figure 1), the intestinal loops appeared hyperechogenic with increased its thickness, the intestinal contents were fluid in nature with presence of swirling movement suggest intestinal hypermotility. There was mild thickening with loss of wall layers in the descending duodenum in 2 cases, as well as lumen dilation; luminal contents (Figure 2) revealed the presence of moderate inflammation, which is the most common cause of persistent diarrhea in dogs and is described as inflammatory cell infiltration into the intestinal walls. The jejunum in 2 cases showed increased wall thickness with ill-defined layering (Figure 3). The stomach in 3 cases appeared as thick-walled viscus that appeared hyperechogenic with increased lumen contents, the hyperechoic wall appeared corrugated (Figure 4).

Figure 1. Ultrasonography of cross-sectional view of the **intestine**, the intestinal loops appeared dilated with loss of layering and increased wall thickness (line). The intestinal contents are anechoic with presence of excessive swirling movement of the intestinal contents due to hypermotility (Arrow). Ventral is at the top of the image, with the right side of the body at the left side of the image.

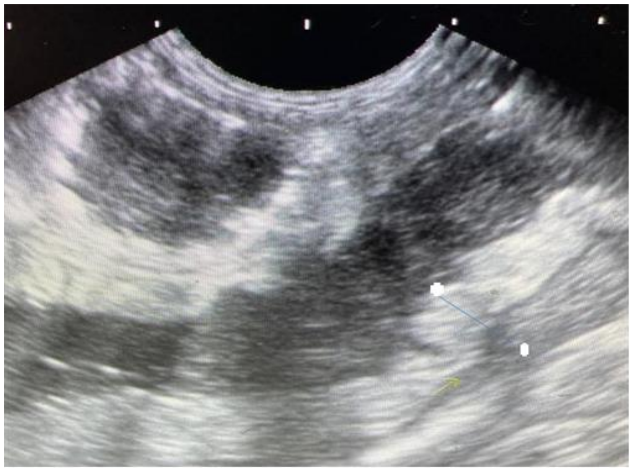

Figure 2. Ultrasonography of the **descending duodenum** (long axis view) in a dog with IBD, showed increased wall thickness (line). The lumen is distended with hypoechoic content and increased swirling movement related to hypermotility. Ventral is at the top of the image, with the right side of the body at the left side of the image.

Figure 3. Ultrasonography of the **jejunum** (longitudinal axis view) in a dog with IBD, it showed increased wall thickness with ill-defined layering. Note the severe jejunal wall thickening (calipers), measuring 0.5 cm. Ventral is at the top of the image, with the right side of the body at the left side of the image.

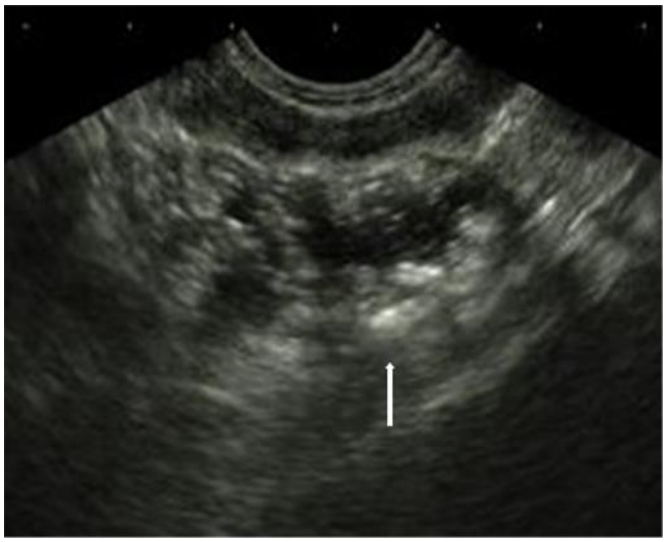

Figure 4. Ultrasonography of the **stomach** in a dog with IBD, it showed increased wall thickness that appeared hyperechoic with presence of corrugated wall. Ventral is at the top of the image, with the right side of the body at the left side of the image.

Discussion

IBD is most commonly seen in middle aged and male dogs [17]. Similarly, in our study 8-17 years aged male dogs (15/23) were affected. However, the reason for highest prevalence in male dogs might be due to over representation of population and owners' preference for male dogs. In the current study, there is a significant increase in temperature, heart rate, respiration, in addition to anorexia and weight loss and this agreed with previous study by Bhat *et al.* [18]. These signs resulted from the release of interleukins and catecholamine which are important mediators of inflammation [19].

In this study, the most common presenting symptoms were hemorrhagic diarrhea (48%), watery diarrhea (52%), vomiting (35%), anorexia (70%), weight loss and abdominal pain indicating the severity of the condition, and this was in agreement with Bhavani *et al.* [6]. IBD is characterized by the infiltration of

inflammatory cells into the intestinal walls, which is the prevalent cause of chronic diarrhea in dogs.

Hematological analysis showed highly significant increase in the total white blood cell count, neutrophils while there was significant decrease in hemoglobin and hematocrit compared to the control group. Similarly, Ristic and Stidworthy [20] noticed the same alterations and hypothesized that prolonged gastrointestinal blood loss could culminate in non-regenerative iron deficiency anemia and that neutrophilic leukocytosis was linked to stress and chronic inflammation.

The biochemical changes such as blood urea nitrogen and creatinine levels were considerably higher in the IBD group, confirming the findings of Bhavani *et al.* [6] in diarrheic dogs. Renal involvement has been linked to a variety of pathological processes. Kidney damage can be caused by the disease itself, as well as consequent extra intestinal problems (malnutrition) and therapeutic side effects [21]. Increased blood urea nitrogen indicates pre-renal uremia, which is caused by a reduced glomerular filtration rate and tissue catabolism as a result of fever [22]. When compared to the control group, the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were considerably high. This rise could be attributed to reactive hepatopathy [23]. While, the mean values of serum total proteins, albumin, globulin were not significantly changed in IBD dogs indicating that the IBD dogs in this study have not progressed to Protein losing enteropathy [24].

Cobalamin (vitamin B12) is a water-soluble vitamin that has diagnostic and therapeutic use. It is used as a diagnostic marker for GIT disease in companion animal medicine. Allenspach and Gaschen [25] discovered 13 hypocobalaminemic dogs in a study of 70 dogs with various enetropathies, including IBD. Folate is another B vitamin that is water soluble (vitamin B9). The decline in both vitamins is caused by either a decrease in folate absorption or a change in the gut microbiota. In the present study, reduced serum cobalamin and folate levels in IBD dogs were comparable to previous studies by Heilmann and Steiner [26]. Reduced serum cobalamin and folate levels together can indicate malabsorption and, as a result, be a sign for inflammation and approximately the inflammation involve the small intestinal wall [23].

Vitamin D levels were also significantly lower in IBD dogs as compared to controls, which were consistent with prior research by Zafalon *et al.* [7]. Because fat-soluble vitamins require optimal absorption of dietary fat, malabsorptive intestinal diseases can obstruct vitamin D absorption, resulting in hypovitaminosis D. Hypovitaminosis D, on the other hand, may change the immune response via dysregulated colonic antimicrobial activity and enteric bacteria balance, resulting in intestinal protein loss [25].

The most common IBD-related alterations in this study were distended intestinal loops and intestinal loop hypermotility, according to ultrasonographic evaluation of IBD dogs (7 cases). There was modest thickening with loss of wall layers in the stomach (3 cases), duodenum (2 cases), and jejunum (2 cases). In addition to lumen dilatation, the presence of mild inflammation was identified in the luminal contents. These findings corroborated those of Bhavani et al. [6], who found that ultrasonography is frequently employed as the first diagnostic tool to distinguish inflammatory from neoplastic infiltration, which is critical for selecting effective treatment methods. In humans, gastrointestinal ultrasound allows for an objective assessment of inflammation, particularly in people suffering from Crohn's disease. Increased intestinal wall thickness is the most visible and crucial indicator of inflammatory activity in human IBD [13].

Conclusions

In conclusion, the hematological indices including total leukocyte count and neutrophils showed significant increase, while hemoglobin and hematocrit showed significant decrease. Reduced serum cobalamin and folate levels in IBD dogs suggested intestinal malabsorption, which was thought to be an indication of small intestine inflammation and they act as good predictors in IBD. Furthermore, IBD dogs had lower serum vitamin D concentrations than healthy controls, which could be a risk factor for IBD in dogs. **We advise** routine screening of dogs with IBD for vitamin D, vitamin B12, and folate insufficiency to assess whether supplements should be offered to assure IBD patients' nutritional status and enhance their health.

Conflict of interest

None of the authors have any conflict of interest to declare

Acknowledgements

We would like to thank Dr. Tarek Allam, Professor at Animal Health Research Institute, Zagazig, Egypt for his support and help in collecting and analysis of blood samples.

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الملخص العربي

الفحص بالموجات فوق الصوتية و تحليل الدم والكيمياء الحيوية في الكلاب المصابة بمرض إلتهاب الأمعاء

هبه الزهار، زينب عبدالرحمن ، عباس النجار

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مرض التهاب الأمعاء (IBD) في الكلاب هو مجموعة من الاضطرابات التي تتميز بالاعراض السريرية المعوية المزمنة. الهدف من هذه الدراسة هو إلقاء نظرة على التغيرات الكيميائية الحيوية في الدم بالمقارنة بالنتائج السريرية والعلامات التنبؤية في الكلاب المصابة بمرض التهاب الأمعاء. تم تحديد 23 كلبًا مصابا بالتهاب الامعاء مع وجود أعراض أمراض الجهاز الهضمي المزمنة. وتشمل القيء والإسهال وفقدان الشهية وفقدان الوزن وآلام البطن. بالموجات فوق الصوتية ، كانت النتائج الأكثر شيوعًا المرتبطة بمرض التهاب الأمعاء هي اتساع الحلقات المعوية وزيادة الحركة، بالإضافة إلى سماكة وفقدان طبقات الجدار في المعدة والاثني عشر والصائم. أظهرت النتائج الكاملة لتعداد الدم زياده معنويه في إجمالي عدد خلايا الدم البيضاء ، العدلات ، مع نقص في تركيزات الهيموجلوبين والهيماتوكريت. كشف الفحص البيوكيميائي عن زيادة معنوية في نشاط AST، ALT، نيتروجين اليوريا في الدم وكرياتينين الدم مقارنة بالكلاب السليمة. بينما لوحظ انخفاض كبير في مستويات فيتامين (د) وحمض الفوليك والكوبالامين في الكلاب المصابة بالتهاب الأمعاء نتيجه سوء امتصاص الأمعاء. وهذه العلامات هي مؤشرات جيدة للتنبؤ بمرض التهاب الأمعاء.