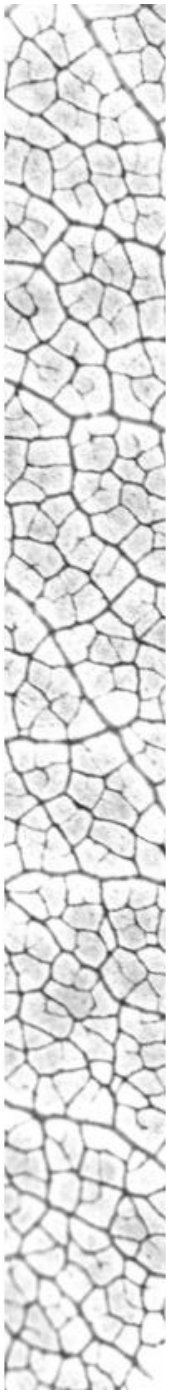

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Pathological influence of concomitant infestation of *Cystoisospora* SP. and *Giardia* SP. in dogs

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Abstract

Research on the clinical analysis of protein and cobalamin malabsorption in dogs with protozoal pathology in 83 animals of various breeds and ages has been carried out. There were 44 dogs under the age of 3 years with established intestinal protozoa. 19 of them were found to be parasitized by *Giardia* sp., 18 by *Cystoisospora* sp., and 7 dogs were found to be parasitized by *Giardia* sp. and *Cystoisospora* sp., as well as 39 dogs from 8 to 12 years old, with confirmed intestinal protozoosis: in 22 Giardia sp., in 11 - *Cystoisospora* sp. Combined invasion of *Giardia* sp. and *Cystoisospora* sp. observed in 6 dogs.

Changes in blood biochemical parameters, blood folate levels were evaluated, and structural changes in the mucous membrane of the small intestine in dogs with combined invasion of *Cystoisospora* sp. and *Giardia* sp.

Young dogs and animals 8-12 years old, invaded by intestinal protozoa, had a decrease in the level of urea, total protein, albumin and globulin, and cholesterol. The bile acid level remained within the normal range.

Among dogs with confirmed giardiosis, a decrease in vitamin B12 levels was noted in 73.7%, with isosporosis - in 88.8%, and with combined invasion in 100% of animals.

Biopsy specimens of 6 young dogs parasitized by *Giardia* sp. showed predominantly duodenitis with moderate changes in accordance with the recommendations of the WSAVA Gastrointestinal Standardization Group (2008). Two dogs showed marked duodenitis in the samples. In older animals, a pronounced process became predominant. In dogs with *Cystoisospora* sp. was established duodenitis with moderate changes.

Keywords: malabsorption, folate, mucosal pathology, *Cystoisospora sp.*, *Giardia* sp., Dogs

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INTRODUCTION

Diseases of the gastrointestinal tract are the most common pathologies observed in dogs (Sparks et al., 2014). Among the diseases of the digestive system, gastroenteritis is predominant. Protozoa are the leading causes of acute gastroenteritis in many species worldwide.

Giardia sp.'s involvement in the development of chronic gastrointestinal upset has been previously reported. This can lead to malabsorption and developmental delays in young animals, which could decrease their resistance and make them susceptible to other infections (Troeger et al., 2007; Cotton et al., 2011).

Malabsorption syndrome is a clinical symptom complex caused by impaired intake of nutrients into the body due to disorders of absorption processes in the small intestine. Intestinal absorption is a complex process that depends on many variables, including the digestion of nutrients in the intestinal lumen, the absorbent surface of the small intestine, membrane transport systems, and epithelial absorbing enzymes. Acquired causes of malabsorption are classified by focusing on three phases of digestion and absorption: 1) the luminal / digestive phase, 2) the mucous / absorbing

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phase, and 3) the transport phase (van der Heide, F. 2016).

Acquired causes of malabsorption are classified by focusing on the three phases of digestion and absorption, and these include gastroenteritis, motility disorders and deficiencies in digestive enzymes or bile acids (van der Heide, F. 2016). In some cases, malabsorption can be implicit, manifested by the pathology of other organs of the systems: digestion (liver, pancreas), respiration (lungs) and hematopoietic (anemia). Also, systemic problems - growth retardation. As a result of a violation of the intake of macro- and micronutrients into the body, “deficiency” states develop, the clinical manifestations of which create difficulties for the timely assessment of the secondary pathological process, worsen the prognosis and contribute to a long recovery period (Sparks, E., Jean-Philippe, K. 2014).

As *Cystoisospora* sp. grow naturally in the physiological system of dogs, they penetrate directly into the epithelial cells of mucous membrane. In this case, extensive lesions develop that result in impaired absorption function and inflammation. *Cystoisospora canis*, *C. ohioensis*, and *C. burrowsi* cause diarrhea in puppies (6 months or younger) and in dogs with weakened immunity (Buehl et al., 2006). In adult animals, diarrhea may be absent as a leading symptom. Although *C. canis* is the predominant species that causes cystoisosporosis in dogs, *C. ohioensis* is also often identified in dogs by polymerase chain reaction (He et al., 2012).

Based on clinical symptoms, it is difficult for veterinarians to include giardiosis and cystozosporiosis in the list of differential diagnoses and continue with the process further in dogs. Diarrhea may not be constant or pronounced, and sometimes, it can even be absent (Ballweber et al., 2010; He et al., 2012; Matsubayashi et al., 2011). There are many problems when it comes to diagnosing these protozoans (Kurnosova et al., 2017). In this regard, it seems relevant to study the pathological effects of protozoa on the mucous membrane of dogs’ small intestines, and to track the changes in blood serum indices.

This study aimed to evaluate changes in blood biochemical parameters and blood folate levels and assess structural changes in the small intestine mucosa of dogs with combined parasitization of *Cystoisospora* sp. and *Giardia* sp.

Studies have shown that the clinical manifestations of the invasion of *Giardia* spp. vary greatly, from an acute to a chronic course, but invasion in dogs can also be asymptomatic (Ballweber et al., 2010). Clinical signs may cause nausea, weight loss, abdominal pain, and diarrhea. At the same time, giardia was recognized as one of the most important protozoal pathogens causing diarrhea (Cotton et al., 2011). However, in a study conducted by Kotloff (2013), researchers found no relationship between the parasitism of *Giardia* sp. and diarrhea (Kotloff et al., 2013), and in some cases, constipation was recorded (Morken et al., 2008).

MATERIALS AND METHODS

A clinical analysis for malabsorption of proteins and cobalamin in dogs with protozoal pathology was conducted in 83 dogs of various breeds, and their owners were allowed to visit the veterinary clinic. Of the dogs tested, 44 dogs were under the age of 3 years, and had intestinal protozoa. In 19 of these, parasitization of *Giardia* sp. was found; in 18 of these, *Cystoisospora* sp. was seen; in 7 of these, the combined invasion of *Giardia* sp. and *Cystoisospora* sp., as well as in 39 dogs from 8 to 12 years old, were confirmed to have intestinal protozoans. Of those with protozoans, *Giardia* sp. was found in 22 of them, and 11 were confirmed to have *Cystoisospora* sp. Concomitant invasion of *Giardia* sp. and *Cystoisospora* sp. was observed in 6 dogs.

Giardiasis was diagnosed by combining feces with a formalin-ether deposition, and with the use of immunochromatographic analysis using VetExpert Giardia Ag rapid tests (BioNote, Inc., Republic of Korea). Isosporosis was confirmed by flotation.

Blood samples were taken from animals before feeding after hours of fasting, in the morning, blood samples were taken from the vena saphena of the animals before feeding them, and the samples were collected into two plastic tubes with a volume of 2 mL. These samples were used for biochemical studies and to measure the levels of cobalamin and folate in the dogs. Blood serum was obtained after assaying the blood samples and retracting a blood clot, followed by centrifugation at 2000 rpm for 10-15 min. Serum was examined for 4 hours. Hemolyzed and chylous samples were not used. The biochemical composition of the blood serum was studied using a HumaLyzer Junior automatic biochemical analyzer (HUMAN, Germany).

To exclude differential diagnoses, urine protein measurements, urine protein/creatinine ratios, and serum bile acid measurements were performed.

A total of 22 dogs were selected for conducting a histological examination of the mucous membrane of the small intestine. *Cystoisospor*a sp. was found in 7 animals, while the remaining 15 were diagnosed with *Giardia* sp. The animals were kept in a shelter in the Moscow region.

Endoscopy was performed using a Leyte Medical Equipment Co., ETV150 apparatus. The biopsy material for testing was collected from eight sites of the small intestine mucosa of each animal. Biopsies were fixed in a 10% aqueous solution of neutral formalin, and then those were poured into paraffin. Histological sections were stained with Ehrlich hematoxylin and eosin (Goralsky et al., 2008). The resulting preparations were studied using a UNICO G304 binocular microscope with magnifications from ×100 to ×1000. The degree of

Table 1. Changes in the biochemical parameters of blood in dogs aged 1 year to 3 years, invaded by protozoa

Indicators	Units measurements	**Infestation Giardia sp**	**Infestation Cystoisospora sp.**	**Concomitant Infestation Giardia sp. и Cystoisospora sp.**	**Control group**	Type average
Bilirubin total (Tbil)	μmol / l	2,4±2,6	4,6±2,34	2,2±2,58	7,71±2,51	<13.5
Direct bilirubin (Dbil)	μmol / l	2,1±0,03	1,8±0,11	0,6±0,00	0,00±0,00	<5.5
AST (GOT)	U / L	37±13,8	43±12,9	29±14,6	30,08±29,65	over 6 months: 8-42 (up to 6 months: <70)
ALT (GPT)	U / L	22±15,3	31±16,7	44±12,6	46,24±14,203	10 - 58
Ritis coefficient	estimated indicator	1,1±0,26	1,2±0,26	1,2±0,26	1,21±0,26	1.1 - 1.3
Urea (Urea)	mmol / l	3,9±1,90	2,8±17,29	3,2±1,34	6,14±1,54	3.5 - 9.2
Creatinine (Creat)	μmol / l	134±14,4	121±15,2	136±16,7	93,17±17,29	54-138 (44-90 dogs up to 10 kg)
Total protein (Prot, total)	g / l	56±5,1	43±3,41	49±4,1	66,24±5,1	over 6 months: 55-73 (up to 6 months: 44-56)
Albumin (Alb)	g / l	25±3,45	23±4,65	20±4,21	28,2±4,35	25 - 39
Alkaline phosphatase (ALP, IFCC)	U / L	51±23,35	42±22,41	38±22,64	48,87±23,64	over 8 months: 10-70 (up to 8 months: 80-230)
Alpha Amylase, total (ά-Amylase, total)	U / L	831±144,58	1102±204,65	945±231,18	1474,58±256,5	300-1500 (over 4 months)
Glucose (Glu)	mmol / l	5,8±0,94	5,5±0,92	5,3±0,93	5,65±0,92	3.3 - 6.3
Cholesterol	mmol / l	2,6±1,77	2,3±1,87	2,3±1,67	7,33±1,83	2.5-6.0
Triglycerides	mmol / l	0,44±0,13	0,41±0,14	0,42±0,13	0,77±0,16	0.15-0.84
LDH (LDH, IFCC)	U / L	182±42,31	164±51,31	196±41,68	247,38±62,0	23 - 220
Globulin (Glob)	g / l	24±4,3	28±4,1	26±4,8	37±4,8	26 - 44
Albumin / Globulin (Alb / Glob)	estimated, g / l	1,04±0,18	0,82±0,22	0,76±0,12	38±4,8	0.65 - 1.49
Bile acids:	μmol / l	2,1±0,11	2,4±0,13	2,3±0,12	0,8±0,14	0-5
on an empty stomach	μmol / l	2,9±0,13	2,6±0,14	1,8±0,12	1,1±0,16	0-5

Note. P <0.05

Table 2. Changes in blood biochemical parameters in elderly dogs invaded by protozoa

Indicators	Units measurements	**Infestation Giardia sp**	**Infestation Cystoisospora sp.**	**Concomitant Infestation Giardia sp. и Cystoisospora sp.**	**Control group**	Type average
Bilirubin total (Tbil)	μmol / l	2,4±2,6	4,6±2,34	1,9±2,51	7,71±2,51	<13.5
Direct bilirubin (Dbil)	μmol / l	2,1±0,03	1,8±0,11	0,3±0,08	0,00±0,00	<5.5
AST (GOT)	U / L	37±13,8	43±12,9	41±13,4	30,08±29,65	over 6 months: 8-42 (up to 6 months: <70)
ALT (GPT)	U / L	22±15,3	31±16,7	40±13,7	46,24±14,2	10 - 58
Ritis coefficient	estimated indicator	1,1±0,26	1,2±0,26	1,0±0,23	1,21±0,26	1.1 - 1.3
Urea (Urea)	mmol / l	3,2±1,63	2,9±1,39	3,9±1,4	6,14±1,54	3.5 - 9.2
Creatinine (Creat)	μmol / l	134±14,4	121±15,2	136±16,21	93,17±17,29	54-138 (44-90 dogs up to 10 kg)
Total protein (Prot, total)	g / l	53±4,81	40±4,11	48±4,73	66,24±5,1	over 6 months: 55-73 (up to 6 months: 44-56)
Albumin (Alb)	g / l	23±2,54	22±3,65	21±3,45	28,2±4,35	25 - 39
Alkaline phosphatase (ALP, IFCC)	U / L	51±23,35	42±22,41	33±21,41	48,87±23,64	over 8 months: 10-70 (up to 8 months: 80-230)
Alpha Amylase, total (ά-Amylase, total)	U / L	831±144,58	1102±204,65	842±218,34	1474,58±256,5	300-1500 (over 4 months)
Glucose (Glu)	mmol / l	5,8±0,94	5,5±0,92	4,8	5,65±0,92	3.3 - 6.3
Cholesterol	mmol / l	2,4±2,67	2,2±2,71	2,2±2,6	7,33±1,83	2.5-6.0
Triglycerides	mmol / l	0,44±0,13	0,41±0,14	0,30	0,77±0,16	0.15-0.84
LDH (LDH, IFCC)	U / L	182±42,31	164±51,31	212	247,38±62,0	23 - 220
Globulin (Glob)	g / l	23±3,8	26±3,4	33±3,6	37±4,8	26 - 44
Albumin / Globulin (Alb / Glob)	estimated, g / l	1,0±0,17	0,84±0,22	0,63±0,12	38±4,8	0.65 - 1.49
Bile acids:	μmol / l	2,1±0,11	2,4±0,13	2,3±0,12	0,8±0,14	0-5
on an empty stomach	μmol / l	2,9±0,13	2,6±0,14	2,9±0,17	1,1±0,16	0-5

Note. P <0.05

pathological changes was assessed in accordance with the recommendations of the WSAVA Gastrointestinal Standardization Group (2008).

The results were subjected to statistical analysis, using the Student's confidence criterion, and $p \leq 0.05$ was considered statistically significant.

RESULTS

The studies have shown that the clinical manifestations of the invasion of *Giardia* sp. vary greatly - from acute to chronic course, but also the invasion in dogs can be asymptomatic. If clinical signs are present, nausea, weight loss, abdominal pain and diarrhea may occur.

It should be noted that young dogs invaded by intestinal protozoa had lower levels of urea, total protein, albumin, and globulin, as well as cholesterol. The level of bile acids remained within the normal range. The results of the changes in biochemical blood parameters of young and elderly dogs are shown in **Tables 1** and **2**, respectively.

The biochemical study of the blood of elderly dogs invaded by protozoa revealed that the animals had lower levels of urea, total protein, albumin, globulin, and

Table 3. Changes in the level of vitamins B12 and B9 in the blood of dogs invaded by Giardia sp. and Cystoisospora sp.

Indicator	Units measuring	Infestation Giardia sp	Infestation Cystoisospora sp.	Concomitant Infestation Giardia sp. и Cystoisospora sp.	Norm [11]
B12 Cobalamin	ng/L	186.2	147.3	132.5	251-908
B9 Folate	µg/L	29	22.7	28.1	7.7-24.4

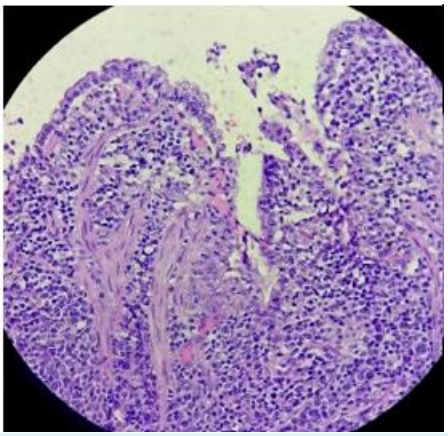

Fig. 1. Inflammatory reaction in a *Giardia* sp.-infected dog duodenum. Infiltration by small lymphocytes, plasmocytes, and neutrophils is visible. Ehrlich hematoxylin and eosin staining SW ×100

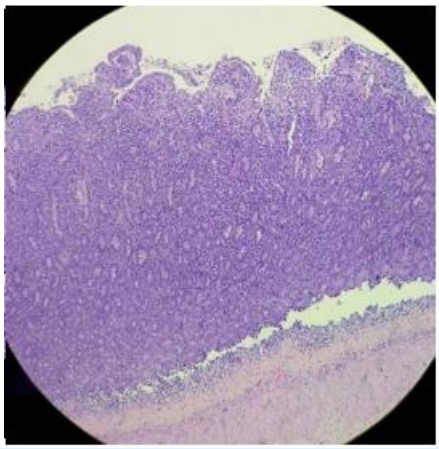

Fig. 2. Inflammatory reaction in a *Giardia* sp.-infected dog duodenum. Ehrlich hematoxylin and eosin staining, ×100

cholesterol. The level of bile acids also remained within the normal range (**Table 2**).

In all dogs included in the experiment, the urine protein/creatinine ratio remained within the normal range.

We assumed that along with the protein, there was also a violation of the absorption of cobalamin in the small intestine. Therefore, we conducted a blood test to determine the relative levels of cobalamin and folate in the dogs. As a result, in 84% (37 animals) of the studied dogs with confirmed protozoans, we noted a decrease in the level of vitamin B12, with a minimum value of 102 ng/mL. Among dogs with confirmed giardiosis, a decrease was noted in 73.7% (14 animals); in those with isosporosis, there was a decrease in 88.8% (16 animals), and in those with combined invasion, 100% of the animals showed a decrease in vitamin B12 (**Table 3**).

Clinical signs of changes in stool consistency were noted in 72.7% of all infested young dogs, and in 27.3% of dogs, such signs were absent. Among older dogs, 66.6% had diarrhea or other changes in stool (increased frequency, change in consistency towards softening, the appearance of a fetid odor). In 33.4% of animals, no changes in stool were observed.

In histological slides of the small intestinal mucosa in dogs with *Giardia* sp. fragments and muscle membranes of the intestinal tube were performed. The histoarchitectonics of the organs were partially disturbed; the layers were delimited quite well, but the villi were shorter and wider. Some crypts were stretched. The mammary lymphatic vessels in most cases were not visible. The epithelial lining was represented by a single layer of core-containing cells with low cylindrical and On average, there were 3-5 goblet cells per hundred enterocytes. The nuclei of the round-shaped epithelial cells were rather small, sometimes of medium size; in most cases, nucleoli were not clearly visible. Mitotic figures were rare. The owner plate was edematous with marked infiltration by inflammatory cells, mainly small lymphocytes; plasmocytes were sometimes visible, neutrophils were relatively rare, and the eosinophils were single per field of view. The infiltration practically did not spread beyond the muscle plate. The fibrotic changes were moderate. Some vessels were dilated and blood-filled, and there were no major hemorrhages. There was no evidence of neoplasia within the slide.

When studying the biopsy specimens of dogs with *Cystoisospora* sp., surface fragments of the intestinal mucosa were present in the preparations (integral longitudinally oriented villi are few in numbers). The histoarchitectonics of the organ was partially disturbed, and some villi were shortened. Crypts were few in number, and were sometimes slightly elongated (**Figs. 3** and **4**).

Some mammary lymphatic vessels were mildly dilated. The epithelial lining was represented by a single layer of nucleated cells with cylindrical shape and had mild signs of degenerative changes. On average, there were 1-3 goblet cells per hundred enterocytes. Intraepithelial lymphocytes were rare. The nuclei of round/ovoid epithelial cells were rather small, sometimes of medium size, and anisokaryosis was mild/moderate. In most cases, the nucleoli are not clearly visible. Mitotic figures were rare.The lamina propria was swollen, with moderate infiltration by inflammatory cells, mainly small lymphocytes. Plasmocytes were sometimes visible, neutrophils were

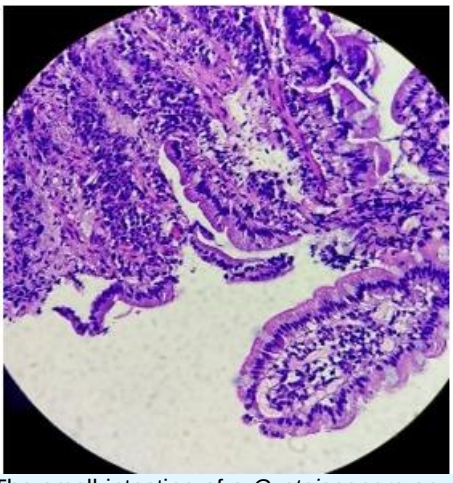

Fig. 3. The small intestine of a *Cystoisospora* sp. infected dog. In small, partially fragmented sections of the surface fragments of the mucous membrane, some villi are shorter, with moderate infiltration by inflammatory cells. Ehrlich hematoxylin and eosin staining, ×100

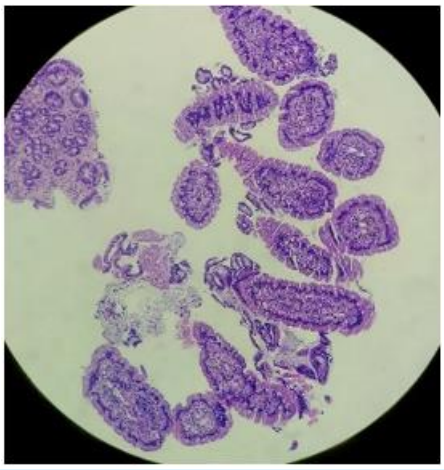

Fig. 4. The small intestine of a *Cystoisospora* sp. infected dog. Moderate infiltration by inflammatory cells: lymphocytes, plasmocytes, neutrophils, and eosinophils. Some vessels are dilated and blood-filled. Ehrlich hematoxylin and eosin staining, ×100

relatively rare, and eosinophils are single per field of view. Fibrotic changes were mild/moderate. Some vessels were dilated, blood-filled, and there were no major hemorrhages. There was no evidence of neoplasia within the slide.

DISCUSSION

Malabsorption syndrome is a complex clinical symptom, caused by a disruption of the intake of nutrients in the body and disorders of the absorption processes in the small intestine. Intestinal absorption is a complex process that depends on many variables, such as the digestion of nutrients in the intestinal lumen, the absorbent surface of the small intestine, membrane transport systems, and epithelial absorbing enzymes. Acquired causes of malabsorption are classified by focusing on three phases of digestion and absorption: 1) the luminal/digestive phase, 2) the mucous absorbent phase, and 3) the transport phase (van der Heide, 2016).

The causes of malabsorption are classified by focusing on the three phases of digestion and absorption, including gastroenteritis, impaired motility, and deficiencies in digestive enzymes or bile acids (van der Heide, 2016). In some cases, malabsorption can be implicit, manifested by the pathology of other organs of the respective systems, digestive (liver, pancreas), respiratory (lungs), and hematopoietic (anemia). Along with systemic problems, stunted growth can also occur. Due to the violation of the intake of macro- and micronutrients, deficient conditions develop, the clinical manifestations of which create difficulties for the timely assessment of the secondary pathological process, worsening the prognosis, and contributing to a long recovery period (Sparks et al., 2014).

The proximal part of the small intestine absorbs nutrients extremely efficiently. After perfusion of nutrients into the duodenum, physiological processes absorb up to 80% of triglycerides, 60% of carbohydrates, and 50% of proteins (Henderson, 1997). Intestinal absorption is a complex process that depends on many variables, such as insufficient intake of vitamin A from food, impaired digestion of nutrients in the lumen of the intestine, and the gastrointestinal tract (GIT). It also depends on the pathologies of the small intestine's absorbent surface, membrane transport systems, and epithelial absorbent enzymes. Diseases of the small intestine can lead to impaired absorption of proteins, destruction of cobalamin receptors, and impaired absorption of cobalamin (Shen, 2009). Moreover, with a cobalamin deficiency, gastrointestinal dysfunction, including atrophy of intestinal villi and infiltration of the intestinal mucosa by cells with inflammatory processes, develops and further decreases the absorption of cobalamin and other nutrients (Hall et al., 2016).

It should be noted that the etiological causes of acquired malabsorption are radiation, certain medications (cytostatic), infectious, parasitic, and other factors (van der Heide, 2016).

Giardia sp. and *Cystoisospora* sp. are often the causes of disorders in gastrointestinal tract, particularly those relating to the absorption processes in the small intestine. An important clinical presentation is that *Isospora* sp., in its development cycle, penetrate directly into the epithelial cells of dogs' mucous membrane in the small intestine. In this case, extensive lesions develop with impaired function and inflammation. Clinically pronounced courses often develop in young puppies, which are kept in conditions of crowding, unsanitary conditions, and high parasitic infection (Sparks, 2014).

Giardia, during invasion, does not penetrate into the cells of the small intestine, but parasitizes the surface of the mucosa; however, it causes dysfunction. Attachment of giardia causes microtraumas of enterocytes,

disturbance of parietal digestion, intensification of fermentation processes, and acceleration of the evacuation of food substrates (Buret, 2007). Because of these disruptions, inflammation can develop. In giardiosis, malabsorption syndrome plays a leading role; however, the issues of correcting impaired intestinal absorption are not adequately addressed in scientific literature.

To date, the pathophysiological stages of giardiosis include, increased enterocyte apoptosis, intestinal barrier dysfunction, lymphocyte activation, reduction of microvilli with or without atrophy, disaccharidase deficiency, and malabsorption in the small intestine (Cotton et al., 2011). Although most invasive animals have no clinical signs of intestinal inflammation, some may develop "a microscopic inflammation of the duodenum" during the chronic course (Morken et al., 2008; Morken et al., 2009), which subsequently develops into a secondary inflammatory process with a clinical manifestation. In addition to the above symptoms, there is now evidence that *G. duodenalis*-infected animals can develop post-infectious disorders that require additional diagnosis (Morken et al., 2008; Morken et al., 2009; Cotton et al., 2011).

A study of dog giardia in the world showed a prevalence of 2.61% (the authors compiled a review of 150 studies, based on 4,309,451 samples of feces from dogs, of which 112,513 were positive) (Bouzid et al., 2015). In studies performed in Russia, it was revealed that, on average, *Giardia* sp. accounts for 29.6% of infections in all dogs studied. In different cities, this value varies. In the central zone of Russia, *Giardia* sp. parasitism accounts for 78.5% of infections in puppies and 4.6-7.05% in adult dogs (Konyaev et al., 2015; Kurnosova et al., 2019).

Studies have shown a high prevalence of canine cystoisosporosis in Austria (8.7%), Italy (5.7%), and the UK (5.1%) (Buehl et al., 2006). *C. canis* is a common species in the UK, while *C. ohioensis* is prevalent in Australia, Japan, and China (Buehl et al., 2006; Matsubayashi et al., 2011; He et al., 2012).

The urine protein/creatinine ratio remained within the normal range for all dogs, included in our study. According to the results obtained, it is possible to compare the mucous membrane's lowering efficiency in dogs with the history of protozoa parasitization in the intestine. As a result of invasion by protozoa, the process of protein assimilation by the proximal part of the small intestine was disrupted. Secondary enteropathy with loss of proteins also developed due to the parasitization, because all the other possible causes for the protein loss were exluded, such as skin damage due to renal failure and liver disease.

According to these results, it is possible to judge a decrease in the functioning of the mucous membrane of the proximal small intestine in dogs against the background of parasitization of protozoa in the intestine. As a result of the invasion was that the process of vitamin B12 assimilation was disrupted and cobalamin malabsorption had developed. These data are also confirmed by an increase in folate levels, while in the background a decrease in cobalamin level was observed.

Measuring the concentration of folates and cobalt-containing, biologically active substances (vitamin B12) has long been used to evaluate intestinal absorption in dogs. Such tests are most informative for confirming malabsorption due to intestinal or pancreatic disease (Fyfe, 2004).

Vitamin B12 (cobalamin, covers all forms of cobalamins, not just cyanocobalamin, which is vitamin B12), is a water-soluble molecule that functions as an important coenzyme for two enzymes (Nielsen et al., 2012). B12 deficiency, which most often occurs as a result of vitamin malabsorption, is a serious illness, characterized by megaloblastic anemia and/or neurological disorders in a chronic course. If left untreated, these disorders can cause irreversible damage, even death of an animal (Reynolds, 2006). In dogs, cobalamin malabsorption can be life threatening, as it may result in vitamin deficiency and catastrophic metabolic disturbances. Therefore, it is important to schedule parenteral cobalamin administration over time (Battersby et al., 2005; He et al., 2005; Lutz et al., 2013).

In our study, we excluded all other differential diagnoses that could cause the observed symptoms, including impaired functioning of the kidneys, liver, and lesions of the skin. In these pathologies, protein loss is also noted. As a result of invasion by the protozoa, the process of protein assimilation by the proximal part of the small intestine was disrupted, and secondary enteropathy with protein loss in the intestine developed.

When analyzing the relationship between the type of diarrhea and the etiological factors, we noted that not all dogs, diagnosed with protozoa, had signs of stool disturbance. In animals, invaded by *Giardia* sp., we did not observe a direct relationship between the severity of the small bowel lesions during protozoa parasitization and the clinical manifestations, especially in reference to the severity and frequency of diarrhea, which is consistent with the findings of the current study (van der Heide, 2016). This is important for understanding the pathological process and for practitioners to keep in mind. Protozoal invasion can occur without visible clinical manifestations, but at the same time, it may cause inflammatory changes in the mucous membrane of the duodenum. The absence of diarrhea can be explained by normal absorption processes in the large intestine, and these processes are not affected by the inflammatory changes.

In young dogs with *Giardia* sp. parasitization, duodenitis with moderate changes was determined mainly using biopsy samples, performed in accordance with the recommendations of the WSAVA

Gastrointestinal Standardization Group (2008) (6 animals). Two dogs showed pronounced duodenitis in the samples. In dogs infected with *Cystoisospora* sp., moderate duodenitis was determined in accordance with the recommendations of the WSAVA Gastrointestinal Standardization Group (2008).

Studies of secondary disorders allow for the correction of therapies to improve prognoses and shorten recovery periods, which are very important. Diagnosis of disorders of the gastrointestinal tract should include detection of an etiological factor, which may include protozoa, even in the absence of an obvious clinical sign (change in stool consistency). The simplest diagnosis of elderly animals indicates changes that can be associated with age-related issues, such as lowering natural physiological resistance, and cause a pathology with secondary disorders.

Young dogs, invaded by intestinal protozoa, had lower levels of urea, total protein, albumin, and globulin, as well as cholesterol. A biochemical study of the blood of elderly dogs, affected by protozoa, revealed low urea, total protein, albumin, globulin, and cholesterol. The level of bile acids remained within the normal range. In all dogs, included in the experiment, the urine protein/creatinine ratio remained within the normal range. In 37 (84%) dogs with confirmed protozoans, decrease in vitamin B12, with a minimum value of 102 ng/L, was observed. Among all dogs with confirmed giardiosis, in 14 cases (73.7%) vitamin B12 fall was noted, and of all dogs with isosporosis, 16 (88.8%) had this problem. The issue of B12 fall was noted in all canines with combined invasion. These decreases indicate the need for a directed correction algorithm for the body of animals, affected by protozoa.

A clinical evaluation of the protozoal pathology showed that 72.7% of young dogs showed pronounced signs of changes in stool consistency, while 27.3% of dogs had no signs. Among older dogs, 66.6% had diarrhea or other changes in stool. In 33.4% of the animals, no changes in stool was noted.

CONCLUSIONS

Parasitization of *Giardia* sp. causes the development of disorders of the gastrointestinal tract, including signs of a moderate and severe inflammatory process. The histoarchitectonics of the mucosa and muscle membranes of the intestinal tube was partially disturbed, the epithelial lining was represented by one layer with significant signs of degenerative changes, and the owner plate was edematous, with severe infiltration by inflammatory cells (lymphocytes, plasmocytes, neutrophils, and eosinophils). The infiltration practically did not spread beyond the muscle plate. The fibrotic changes were moderate.

Parasitization of *Cystoisospora* sp. results in duodenitis with moderate changes such as, the villi of the duodenal mucosa are shortened or slightly enlarged; the epithelial lining is represented by one layer of cells with signs of degenerative changes; the lamina propria is swollen, with moderate infiltration by inflammatory cells; and single lymphocytes, single plasmocytes, and neutrophils are apparent. The fibrotic changes were mild.

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