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Hematological parameters of dogs infected by Ancylostoma spp.

The aim of this study was to measure the hematological parameters in dogs infected by Ancylostoma spp. Fecal and blood samples of 108 naturally infected dogs were used, without age and sex distinction. The animals used in this study were divided in three groups according to the parasite load (eggs per gram of feces): Group A (1 to 500 eggs); Group B (501 to 1000 eggs) and Group C (above 1000 eggs). For the coproparasitological diagnosis, it was carried out the Willis-Mollay technique and to quantify the parasite load of the positives, the Mcmaster technique was used. Through the hemogram was possible to determine the counting of the different blood cells. The main alterations observed were anemia, thrombocytopenia and eosinophilia presented, in 43.5%, 47.2% and 19.4% of the animals, respectively. Based on the results observed, it is concluded that Ancylostoma spp. is related to the development of anemia, eosinophilia and thrombocytopenia in dogs.

Keywords: Diagnosis; Hematophagy; Hemogram; Parasites.

Paramêtros hematológicos de cães parasitados por Ancylostoma spp.

O objetivo deste trabalho foi mensurar os parâmetros hematológicos em cães parasitados por Ancylostoma spp. Foram utilizadas amostras fecais e de sangue de 108 cães naturalmente parasitados, sem distinção de sexo e idade. Os animais utilizados no trabalho foram agrupados em três grupos, de acordo com a carga parasitária (ovos por grama de fezes): Grupo A (1 a 500 ovos); Grupo B (501 a 1000 ovos) e Grupo C (acima de 1000 ovos). Para o diagnóstico coproparasitológico foi realizado a técnica de Willis Mollay, e para quantificar a carga parasitária dos positivos, a de Macmaster. Através do hemograma foi determinada a contagem dos diferentes tipos de células sanguíneas. As principais alterações observadas foram anemia, eosinofilia e trombocitopenia, encontradas, respectivamente, em 43,5%, 47,2% e 19,4% dos animais. A partir destes resultados, conclui-se que Ancylostoma spp. está relacionado com o desenvolvimento de quadros de anemia, eosinofilia e trombocitopenia em cães.

Palavras-chave: Diagnóstico; Hematofagia; Hemograma; Parasitos.

Topic: **Parasitologia**

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INTRODUCTION

The fact that domestic dogs (*canis lupus familiaris*) are important definitive hosts of several parasites with zoonotic potential, has been widely studied and recognized as an important public health problem, as it increases the risk of human exposure to these agents (CAPUANO et al., 2006). Among these parasites, the nematode *Ancylostoma* spp. stands out, responsible for the parasitic zoonosis cutaneous larva migrans, caused by the active penetration of infective larvae (L3) of the parasite in the epidermis, through direct contact (SOUZA et al., 2010). In dogs, the infection can be actively acquired percutaneously or passively orally (ingestion of the infecting larva directly or in paratenic hosts) or by vertical transmission (transmammary and transplacental) (BOWMAN et al., 2010).

The most important hookworms for dogs are *Ancylostoma caninum* and *A. braziliense*, which attach to the small intestine mucosa of the definitive host, where they carry out hematophagy (TRAVERSA, 2012). Clinical signs depend on the pathogenicity of the species involved, the parasite load and age of the infected animals. *A. Caninum* usually generates more severe symptoms than *A. Braziliense* due to its greater hematophagy potential (TRAUB et al., 2004). Regarding the age of the animals, young dogs are more likely to show clinical signs of the disease, such as vomiting, abdominal pain, anemia, bloody mucous diarrhea and growth retardation. In the hematological examination, dogs infected with *Ancylostoma* spp. may present anemia, eosinophilia, hypoproteinemia and thrombocytopenia (SILVA et al., 2010).

The diagnosis can be done by the coproparasitological exam, by the identification parasitological in different stages, such as eggs and larvae (LOPES et al., 2021). In addition, the use of hematological exams is important in the screening, diagnosis and follow-up of patients, being widely used in the clinic for companion animals (SILVA et al., 2010). Thus, the coproparasitological exams of feces (EPF), together with the evaluation of the blood count, make it possible to diagnose a large number of parasitic diseases.

Therefore, the aim of this study was to measure the hematological parameters in dogs infected by *Ancylostoma* spp.

MATERIALS AND METHODS

Fecal and blood samples of 108 naturally infected dogs by *Ancylostoma* spp., without sex, race and age distinction were used. Animals that presented association with others genders of parasites were not included in this study.

The animals were divided in three groups according to the parasite load, being attributed a scale to express the egg's number found: Group A (+) (1 to 500 eggs), Group B (++) (501 to 1000 eggs) and Group C (+++) (above 1000 eggs).

For the coproparasitological diagnosis, the Willis Mollay technique (1921) was performed, which consists of the spontaneous flotation of light helminth eggs and protozoan oocysts in a hypersaturated solution. To carry out the technique, 5g of feces from each sample were used, which were homogenized with 20 ml of hypersaturated glucose solution (d=1.230). The material resulting from this mixture was filtered

through a sieve and transferred to a 10 ml tube, which was filled until a meniscus formed, where a coverslip was placed. After 15 minutes, all samples were analyzed under an optical microscope at 100x magnification to identify the eggs of *Ancystoloma* spp.

The technique used to measure the parasite load of positive animals was Macmaster's (GORDON et al., 1939), a flotation technique to determine the number of eggs per gram of feces (epg) and oocysts per gram of feces (opg). To carry out the technique, 4 grams of feces from each animal were used, diluted in 56ml of hypersaturated glucose solution (d=1.230). The content was poured into a sieve to remove solids and the filtrate used to fill the chamber. The samples were analyzed after 5 minutes in an optical microscope, at 100x magnification. The samples were made in duplicate, and the epg value was given from the average between them. The EPG calculus was performed through the following formula:

$$EPG = \frac{Number of Eggs Counted on the Chamber of Macmaster}{2} \times 100$$

Blood samples from the studied dogs were collected by venipuncture of the jugular or cephalic vein and placed in a tube with EDTA anticoagulant. The hemogram was performed with the aid of the automatic Sysmex pocH-100 iv DiFF® equipment and the leukocyte differential was obtained by morphological evaluation and cell count, performed manually by means of a blood smear stained with rapid panoptic, evaluation in optical microscopy (magnification of 1000x).

The blood parameters values were analyzed by GraphPad prism 7.0 program. The data were evaluated by variance analysis (Anova), followed by the Tukey test to determinate the difference between the averages, considering the level of significance α equal to 5%. First, to verify the homoscedasticity of the data, Bartlett's test was performed. This study was approved by the Ethics in Animal Research Comitte of UFPel, under the number 23110.002060/2017-71, according to the ethical principles of animal experimentation.

RESULTS AND DISCUSSION

Of the 108 animals evaluated, 43.5% presented anemia (47/108). Analyzing between the groups by parasite load, 33.3% (group A), 38.8% (group B) and 58.3% (group C) presented anemia. The mean and minimum and maximum values of the hematological parameters of the animals in each group are shown in Table 1 and 2.

Table 1: Mean of hematological parameters of the three groups of naturally infected dogs by *Ancylostoma* spp.

	GROUPS				
	A	**B**	**C**		
Hemogram	**+ (1-500)**	**++ (501-1000)**	**+++ (>1000)**	**P**	**Reference Value**
Red Blood Cells	6.6[a]	5.8[ab]	5.4[b]	0.0333[*]	5.5-8.5 milhões/ul
Hemoglobin	13.4	12.7	11.2	0.0805	12.0-18.0 g/dL
Hematocrit	40.2	38.7	35.2	0.1781	37-55 %
MVC	61.7	66.9	65,0	0.1810	60-77 fL
MCHC	32.8	32.4	32.0	0.2725	32-36 %
Platelets	350.157	332.545	411.208	0.3916	200-500 (mil/uL)
Leukocytes	11.682	12.567	13.198	0.9551	6.000-17.000/uL

Neutrophils	7.754	8.254	8.683	0.8123	3.000-1.500/uL
Lymphocytes	2.130	2.493	2.601	0.8362	1.000-4.800/uL
Monocytes	325.4	384.7	405.4	0.6785	150-1.350/uL
Eosinophils	1.131	1.387	1.443	0.6913	150-1.250/uL

* Significant statistics value with a 95% of confidence intermission (p<0,05)
Means with different letters indicate a statistically significant difference (Tukey, p<0.05)

Table 2: Minimum and maximum values of hematological parameters in the three groups of dogs naturally infected by *Ancylostoma* spp.

	GROUPS						
Hemogram	**A**		**B**		**C**		**Reference Value**
	Min.	**Max.**	**Min.**	**Max.**	**Min.**	**Max.**	
Red Blood Cells	3.4	9.9	4.0	7.1	2.4	8.2	5.5-8.5 milhões/ul
Hemoglobin	6.0	18.7	8.4	15.9	2.4	16.8	12.0-18.0 g/dL
Hematocrit	21.0	55.1	26.1	48.7	14.7	49.2	37-55 %
MVC	40.6	74.1	55.3	73.1	44.6	75.5	60-77 fL
MCHC	28.2	35.2	29.4	33.4	27.9	34.5	32-36 %
Platelets	65000	646000	50000	550000	96000	907000	200- 500 (mil/uL)
Total Leukocytes	8300	16300	7700	19400	5792	22900	6000-7000/uL
Neutrophils	5792	12798	4081	11830	4081	15114	3000-11500/uL
Lymphocytes	1342	4401	1604	3999	624	3900	1000-4800/uL
Monocytes	0	1.063	0	507	0	977	150-1350/uL
Eosinophils	0	3.893	0	4.077	0	5.208	150-1250/uL

A percentage similar to that found in the present study was observed by Campos et al. (2017), who correlating hematological changes with parasitism by *Ancylostoma* spp. in dogs, they observed anemia in 42% of the animals. Anemia occurs due to the hematophagy habit of this parasite, and adults can ingest up to 0.5 mL of blood per day (EPE, 2009). At the beginning of the infection, the anemia is usually normocytic and normochromic, with chronicity iron deficiency occurs and the anemia evolves to microcytic and hypochromic (CURY et al., 2002). In our study, 30.4% of cases of anemia were normocytic/normochromic and 26.1% were microcytic/hypochromic.

Eosinophilia was observed in 47.2% of the animals (51/108), 30.6% (11/36) in group A, 52.8% (19/36) in group B and 58.3% (21/36) in group C. Eosinophilia happens due to the evolutionary cycle of the parasite, that is, the more complex the cycle, the greater the number of circulating eosinophils, being more significant in helminths that perform larval migration (PEZZI et al., 2008). A similar percentage was found by Silva et al. (2010) (48%) and higher by Campos et al. (2017) (66%).

In addition, thrombocytopenia was also detected in 19.4% of the dogs (21/108), 22.2% in group A, 19.4% in group B and 16.6% in group C. During hematophagy, *Ancylostoma* spp. secretes peptides with antithrombotic activity such as AcAP5 (*A. caninum* anticoagulant peptide 5) and AcAPc2, which are potent inhibitors of coagulation factors and prothrombin activity (MIESZCZANEK et al., 2004), and may thus occur a decrease in total platelets. In the work carried out by Silva et al. (2010), it was observed that 45% of dogs infected by *Ancylostoma* spp., had thrombocytopenia, indicating that parasitism by this type of parasite can be included in the differential diagnosis of thrombocytopenia.

CONCLUSIONS

It was concluded that *Ancylostoma* spp. is related to the development of anemia, eosinophilia and

thrombocytopenia in dogs and that the parasite load is related to the severity of the condition.

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