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# RELATIONSHIP BETWEEN THE PARASITE LOADS WITH EOSINOPHILIA OCCURRENCE IN DOGS

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## ABSTRACT

The aim of this study was to relate the parasite load with eosinophilia in dogs. It was used fecal and blood samples of 86 naturally infected dogs, without sex and age distinction. Animals that presented history of stimulating eosinophilia disease were not included on the study. The animals used in this study were separated into three groups according to the parasite load: Group A (1 to 500 eggs); Group B (501 to 1000 eggs) and Group C (above 1000 eggs). For the coproparasitological diagnosis, it was performed the Willis Mollay technique and the Mcmaster technique to qualification the parasite load of the positive animals, a flotation technique used to determinate de amount of eggs per gram of feces (EPG) and oocysts per gram of feces (OPG). The blood samples were obtained from jugular venipuncture or cephalic veins, the differential leukocyte count was obtained through cell counting, manually performed by blood smears stained by fast panoptic. Of the 86 naturally infected dogs, 40.7% presented eosinophilia, being this blood parameter, the only one to present a statistically significant difference between the groups (p=0.0498), indicating that the increase of the parasite load is related to the eosinophilia cases. It was concluded from the obtained results that the eosinophils counting may be increased on the infected dogs and the parasite load is straightly related with the eosinophilia rate.

**Keywords:** eosinophils. endoparasites. diagnosis.

# RELAÇÃO DO GRAU DA CARGA PARASITÁRIA COM EOSINOFILIA EM CÃES

## RESUMO

O objetivo deste trabalho foi relacionar o grau da carga parasitária com eosinofilia em cães. Foram utilizadas amostras fecais e de sangue de 86 cães naturalmente parasitados, sem distinção de sexo e idade. Animais que apresentavam histórico de doenças estimuladoras de eosinofilia não foram incluídos no estudo. Os animais utilizados no trabalho foram agrupados em três grupos, de acordo com a carga parasitária: 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, uma técnica de flutuação utilizada para determinar a quantidade de ovos por grama de fezes (opg) e oocistos por grama de fezes (Oopg). As amostras de sangue foram colhidas por venopunção da veia jugular ou cefálica, e o diferencial leucocitário foi obtido pela contagem das células, realizado manualmente por meio de esfregaço sanguíneo corado por panótico rápido. Dos 86 animais naturalmente parasitados, 40,7% apresentaram eosinofilia, sendo este parâmetro sanguíneo, o único que apresentou diferença estatística significativa entre os grupos (p=0,0498), indicando que o aumento da carga parasitária está relacionada com quadros de eosinofilia. Conclui-se que a contagem de eosinófilos pode estar aumentada nos cães parasitados, e a carga parasitária está diretamente relacionada com grau de eosinofilia.

**Palavras-Chave:** eosinófilos. endoparasitos. diagnóstico.

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## INTRODUCTION

The coexistence between human beings and animals exists for a long time, this can be explained due to the benefits that the animals can provide to people, as the increase on physical and emotional health (ALVES, 2005; YOUSSEF, 2020). This bond grows more every time and currently Brazil has 55.1 million dogs and 24.7 million cats population (ABINPET, 2019). Based on this, stands out the significance of guiding the population about basic health care and hygiene, which animals need. The lack of health care can predispose the emergence of diseases with zoonotic potential, among them the parasitic diseases (VASCONCELLOS, 2006).

The prevalence of parasitic diseases is related to some factors, such as geographic location, climate, seasons of the year, health care, animal management and socio-environmental conditions as water supply, dejects and garbage destination (BUSNELLO, 2009; FERREIRA et al., 2020). The most common parasitic diseases reported in dogs and cats according to the literature, are caused by Ancylostoma spp., Toxocara spp., Trichuris spp. and Giardia spp. infections. (SILVA, 2010; FERREIRA et al., 2020; YOUSSEF, 2020). The infected animals may be asymptomatic or present clinical signs as weight loss, dehydration, enteritis, vomiting, among others. Furthermore, may cause hematological alterations as, for example, eosinophilia (SILVA, 2010).

Eosinophils are leukocytes, with usually a bilobed nucleus and characterized by the presence of intracytoplasmic granules with high affinity for eosin. They are cells which primary function is to protect the host against relatively large organisms, as the helminths (BEHM and OVINGTON, 2000). The eosinophilia occurs in result of the evolution cycle of the parasite, which means that as more complex the cycle is, the greater will be the number of circulating eosinophils. However, the increase on the number of eosinophils during the larval stages migration may variate according to each individual, being more common after 20 days of infection (SKUBITZ, 2004; SILVA, 2010). The eosinophilia in dogs is closely related to the presence of ecto and endoparasites, mainly Dirofilaria, Ancylostoma, Ascaris, Spirocerca, Strongyloiedes, Trichuris and Paragonimus (JERICÓ et al., 2015).

Therefore, main goal of this study was to relate the relationship between the parasite loads with eosinophilia occurrence in dogs.

## MATERIAL AND METHODS

For this study, it was used fecal and blood samples of 86 naturally infected dogs, without age and sex distinction. Animals that presented history of eosinophilia stimulating diseases, as allergic and immune mediated diseases were not included on the study.

The animals were divided in three groups according to the parasite load, it was attributed a scale to express the number of eggs that were found: 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 (1921), which consists on the spontaneous flotation of light eggs of helminths and protozoan oocysts on supersaturated solution. For the technique, it was used 5g of feces of each sample, which were homogenized with 20 ml of glucose supersaturated solution (d=1.230), and the resulting material of this mix was transferred to a 10 ml tube with the help of a filtration sample sieve, until the liquid created a meniscus on the edge of the tube, for the placement of a coverslip. The reading was carried out in optical microscopy after 15 minutes.

The technique used to measure the parasite load of the positive animals was the Macmaster (GORDON and WHITLOCK, 1939), a flotation technique that determinates the amount of eggs per gram of feces (epg) and oocysts per gram of feces (opg). For this technique, it was used 4 grams of feces of each animal, diluted in 56 ml of glucose

supersaturated solution (d=1.230). The content was poured into a sieve for the removal of solids, and the filtrated was used to fill the chamber. The reading was carried out after 5 minutes on optical microscopy, at 100x magnification. The samples were made in duplicate, and the epg value came from the average between them.

The EPG calculus was performed through the following formula:

$$EPG = \frac{\text{Number of eggs counted on the chamber of Macmaster x100}}{2}$$

The blood samples of the dogs were collected by venipuncture of the cephalic or jugular vein and conditioned on tubes with the EDTA anticoagulant. The haemogram was performed with the help of the Sysmex pocH-100 iv DiFF® automatic equipment and the leukocyte differential was obtained through the morphological evaluation and counting of cells, manually performed by blood smear colored by rapid panoptic, with optical microscopy evaluation (magnification at 1000x). The reference value used for the eosinophils counting in dogs was 150-1250 /uL. (Schalm's, 2010).

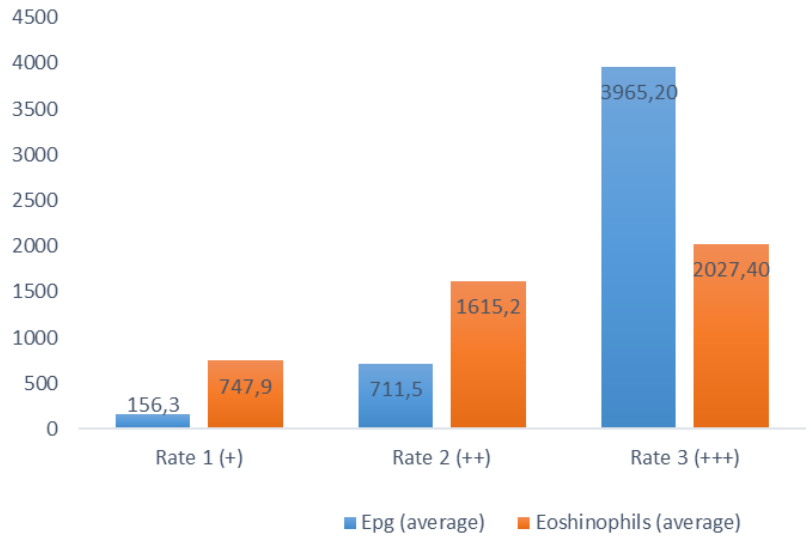
The blood parameters values were analyzed by GraphPad prism 7.0 program. The data were evaluated by variance analysis, followed by the Tukey test to determinate the difference between the averages, considering the level of significance $\alpha$ equal to 5%.

This study was approved by the Ethics in Animal Research Comitte of Federal University of Pelotas (CEEA-UFPEL), under the number 23110.002060/2017-71, according to the ethical principles of animal experimentation.

## RESULTS AND DISCUSSION

Of the 86 evaluated animals, 40.7% (35/86) presented eosinophilia. The average of the parasite load and eosinophils of the animals in each group are shown in Figure 1.

**Figure 1. Average of the parasite load and eosinophils percentual on the three groups of naturally infected dogs.**




Some authors consider that the production of eosinophils is influenced by the genus and number of parasites, location on the host and the infection period (MAWHORTER, 1994). Therefore, the helminth diseases that lead to eosinophilia with greater

occurrence are those that cause tissue invasion, being eosinophilia more pronounced during larval development and migration. When the parasites limit themselves to the digestive tube, eosinophilia is milder. (SKUBITZ, 2004).

Of the evaluated animals, 64 were infected by only one genus of parasite (74.4%) and 22, an association between different parasite genus (25.6%). Eggs of Ancylostoma spp. were the most prevalent on the analyzed samples, being observed in 71.6% of the samples (61/85), of these, 41 as only agents and 20, associated to other genus of parasites. (Table 1).

Table 1. Prevalence of the parasitic genus found in the coproparasitological examination and the frequency of cases of eosinophilia.

| Parasite | Positive Samples | Eosinophilia n(%) |
|---|---|---|
| *Ancylostoma* spp. | 41 | 17(41.5) |
| *Toxocara* sp. | 09 | 1(11.1) |
| *Trichuris vulpis* | 08 | 3(37.5) |
| *Cystoisospora* spp. | 05 | 2(40.0) |
| *Capillaria* spp. | 01 | - |
| *Ancylostoma* spp + *Trichuris vulpis* | 15 | 7(46.7) |
| *Ancylostoma* spp. + *Toxocara* sp. | 02 | 1(50.0) |
| *Toxocara* sp. + *Trichuris vulpis* | 02 | 2(100) |
| *Ancylostoma* spp. + *Toxocara* sp. + *Trichuris vulpis* | 02 | 1(50.0) |
| *Ancylostoma* spp. + *Cystoisospora* spp. | 01 | 1(100) |
| **Total** | 86 | 35(40.7) |

Of the blood parameters analyzed, only the eosinophils counts presented statistical difference between the groups (p=0.0498) (Table 2), indicating that the increase on the parasite load is related to eosinophilia cases. Between the groups, there was a significant results on the average comparison of eosinophils between group A (+) and group C (+++) (p=0.0422). The comparison between group A (+) and group B (++) (p=0.04429) and group C (+++) (p=0.8191), did not present a significant statistical difference (P>0,05) (Table 3).

Table 2. Mean of hematological parameters of the three groups of naturally infected dogs.

| | GROUPS | | | | |
|---|---|---|---|---|---|
| | A | B | C | | |
| Leukogram | + | ++ | +++ | P | Reference |
| | (1–500) | (501-1000) | (>1000) | | Value |
| Leukocytes | 12.455 | 13.432 | 13.892 | 0.6073 | 6.000-17000/uL |
| Neutrophils | 8.716 | 8.621 | 8.986 | 0.9079 | 3.000-11.500/uL |
| Lymphocytes | 2.573 | 2.727 | 2.381 | 0.8544 | 1.000-4.800/uL |
| Eosinophils | 747,9 | 1.615 | 2.027 | 0.0498* | 150-1.250/uL |
| Monocytes | 365,3 | 431,8 | 439,5 | 0.7271 | 150-1.350/uL |

* Significant statistics value with a 95% of confidence intermission (p<0.05)

Table 3. Comparision of the eosinophils average between the groups of naturally infected dogs.

| Groups | P | |
|---|---|---|
| A vs B | 0.4429 | Non significant |
| A vs C | 0.0422 | * p<0.05 |
| B vs C | 0.8191 | Non significant |

* Significant statistics value with a 95% of confidence intermission (p<0.05)

There are only few studies about the relation of eosinophilia and the parasite load in dogs. Silva et al. (2010), evaluating the hematological alterations on the parasite infections in dogs, observed eosinophilia (average of 1.302/uL) in 22;49 naturally infected animals by helminths, and Lunardon et al. (2016) evaluating the presence of eosinophilia in domiciled dogs in the city of Curitiba, observed that 30,7% of the analyzed dogs presented an increase on the eosinophils counting. On the present study, 40.7% (35/86) dogs presented eosinophilia.

Campos et al. (2017) correlating hematological alterations with coproparasitological tests on infected dogs with Ancylostoma spp., observed eosinophilia in 66% (33/50), indicating a correlation between eosinophilia and epg (p=0.0017). In our study, 41.5% (17/41) of the infected dogs only by Ancylostoma spp. presented eosinophilia, a percentage near of the 48% observed by Silva et al. (2010).

In humans, Robertson and Thompson (2002), described in Australia, cases of eosinophilic enteritis due to A. caninum infection characterized by abdominal symptomatology and accentuated eosinophilia. The presence of the adult form of A. caninum on the human intestinal lumen would be a consequence of the ingestion of infective larvae of these parasites (Landmann and Prociv, 2003).

Of the nine cases of infection only by Toxocara spp. , found on the present study, it was observed eosinophilia in 11.1 % (1/9), meanwhile in the two animals that presented Toxocara spp., associated with Ancylostoma spp. the percentage was 50% (1/2). On the developed study by Silva et al. (2010), none of the three infected dogs only by Toxocara spp. presented eosinophilia, however, in the two cases of association between the genus Toxocara and Ancylostoma, it was observed an increase on the eosinophils counting on both. A study performed with 208 children from 1 to 14 years old in the peripheries of São Paulo, Figueiredo et al. (2005), observed that 67.6% of the positive serological tests for Toxocara canis, presented eosinophilia.

Trichuris vulpis, a parasite that affects the large intestine of dogs, was observed in eight animals, of these, three presented eosinophilia (37.5%). Meireles (1989), on a study with ambulatory patients carrying intestinal helminths, diagnosed that 37.5% of the infected by Trchuris trichiuria, presented eosinophilia, the same percentage found in our study.

Oliveira et al (2013), analyzing the hematological and biochemical profile of 53 dogs infected by the Dirofilaria immitis nematode, observed a 2.047 uL eosinophils count on this group, characterizing eosinophilia case on these animals.

The fact that eosinophilia is more significant on larval migration, is more common on the helminth infections, not occurring regularly on protozoan infections (PEZZI and TAVARES, 2008). However, some studies in humans, describe a relation between protozoans and eosinophilia (MELO-REIS et al., 2007; NUTMAN, 2007). On our study, of the five positive animals only infected by the Cystoisosopora spp. protozoan, 40% (2/5) presented eosinophilia.

It was observed eosinophilia in 35.9% of the animals with mono-parasitism (23/64) and in 54.5% (12/22) of those who presented association of parasites. This evidences that the association of two or more parasite genus can generate a more accentuated eosinophilia when compared to mono-parasitic infections.

The absence of eosinophilia in the blood counts can be explained by the low or nonexistent migration of the parasites on the tissues on the moment of the blood collection or the establishment of the parasites on the small intestine (SILVA et al., 2010). Besides that, there are other causes that can lead to an increase on the eosinophils counting.

## CONCLUSIONS

It was concluded that the eosinophils counting might be increased in infected dogs, and the parasite load is directly related with the eosinophilia degree. However, besides the eosinophils mensuration being an indicative of parasite infection, the definitive diagnosis and the identification of the responsible genus of the infection must be performed through coproparasitological tests.

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