

EQUINE INFECTION WITH LEISHMANIA SP: HEMATOLOGICAL STATUS

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Resumo:

In this study, we detected *Leishmania infantum* infection in equines and evaluated hematological status. A total of 48 equines were selected between November and December 2016, from Uruguaiana, Rio Grande Do Sul, Brazil. All animals underwent general and dermatologic examinations and followed by blood samples collected to molecular diagnosis and hematological tests. *Leishmania infantum* was detected in two animals; the hematological analyses did not show statistical differences between the infected and non- infected groups. Although the infected horses showed slight alterations in the leukogram. The results may indicate that the equines were asymptomatic due to the absence of apparent clinical signs and the impossibility of relating hematological alterations between infected and non-infected groups.

Palavras-chave: DIAGNOSIS; CLINICAL EVALUATION; HEMATOLOGY

Modalidade de Participação: Iniciação Científica

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1. INTRODUCTION

Leishmaniasis is an zoonotic disease distributed worldwide that has the potential to cause significant public health impacts. It has high lethality if untreated, as well as impossibility of vector eradication and, consequently, a proven tendency to expand (CEVS, 2011). In addition the disease affecting humans and dogs, wild and domestic mammals have already been identified with the infection in other regions, including the equines (ROQUE & JANSEN, 2014; MÜLLER et al., 2015).

Infection in horses, as well as in other species, may cause a variety of clinical manifestations (nodules, skin lesions) that may be spontaneously cured or show an immune response without apparent clinical signs, keeping the host asymptomatic making initial clinical evaluation and suspicion of infection difficult (KOEHLER et al., 2002; MÜLLER et al., 2009 FEITOSA et al., 2012; SOARES et al., 2013).

It is already clear that the infection can alter hematological and biochemical markers in dogs, however, the changes that can occur in horses are not elucidated. Therefore the objective of this study was to analyze the leukocyte pattern in horses infected with *Leishmania infantum*.

2. METHODS

The study was carried out in strict accordance with the recommendations in the Ethical Principles of the Brazilian College of Animal Experimentation. The protocol was approved by the Federal University of Pampa Ethics Committee on Animal Use (CEUA - UNIPAMPA) permit number: CEUA- 016/2016.

Forty eight mixed-breed equines from an endemic region for visceral leishmaniasis was evaluated to determine hematological alterations in equine leishmaniasis.

Blood samples from jugular venipuncture were used for molecular (conventional PCR) and hematological analyzes. DNA from blood samples was extracted using the technique by Sambroock et al., (1989). The DNA purified was submitted to PCR using primers for *Leishmania* that had been designed to amplify a 145bp fragment of the conserved region of kDNA minicircles (ALMEIDA et al., 2013).

The parameters evaluated in the Leukogram: white blood cell count (WBC), granulocytes (Neutrophils, Eosinophils, Basophils), lymphocytes and monocytes were performed by an automatic cell counter (Sysmex KX-21N) followed by differential count blade using the Giemsa method with the Panótico Rápido kit.

The animals were separated into two groups (*Leishmania* infected Group and non-infected Group) according to the results obtained in the PCR for diagnosis of *Leishmania* sp.

The values are expressed as mean $\pm$ SE for the *Leishmania* infected Group and the non-infected Group separately. The quantitative variables were submitted to the normality test and T Student Test was applied to normal distribution variables. Wilcoxon test was applied for comparison of two means when the variables remained non-parametric after transformation by Log (x + 1). Data were analyzed using the

SPSS® for Windows computing program (Version 11.0), significance was set at p-value <0.05.

3. RESULTS AND DISCUSSION

In this study the equine population was compound by 48 animals, 33 males and 15 females, with the average age 7.5 and 6.7 years old respectively. Detection of *Leishmania* infection by the molecular method of cPCR in peripheral blood samples resulted in 2/48 (4.16%) equine positive, through the amplification of kinetoplast DNA regions of the parasite.

Statistical analysis performed on the leukogram data showed that the means of WBC values was increased in non-infected group in comparison with positive group. The granulocyte counts (neutrophil, eosinophil and basophil) and monocytes values were acceptable both groups, although the neutrophil rod showed an increase values in both groups. Lymphocytes were an increased value in *Leishmania infantum* positive equines in comparison with the non-infected ones. No significant difference occurred in leukogram analysis (Table 1).

Table 01 – Hematological profile in non-infected and infected equines with *Leishmania infantum* on PCR diagnostic technique.

Parameters	Samples		p- value
	Infected Group (X±SE)	Non-infected Group (X±SE)	
WBC (10 ³ /μL)	12.5 ±3.11	12.8 ± 2.59	0.87
Neutrophils Rod (μL)	125.00± 22.00	198.91± 20.47	0.43
Neutrophils (μL)	5.441± 291.50	6.828± 287.45	0.32
Eosinophils (μL)	669± 360	551.50± 44.03	0.74
Basophilss (μL)	147± 147	84.98± 19.81	0.53
Lymphocytes (μL)	5.566± 1.343	4.557± 242.94	0.33
Monocytes (μL)	551.50±36.50	619.63±51.61	0.43

Leucogram values expressed as mean ± standard error. WBC: 5.500-12.500; Neutrophil Rods: 0-100; Neutrophils: 2.700-6.700; Eosinophil: 0-925; Basophil: 0-170; Lymphocytes: 1.500-5.500; Monocytes: 0-800;

Several cases of equine cutaneous leishmaniosis caused by *L. infantum* had been described. There have been reports that horses had temporary clinical signs, which included nodules of variable size in sometimes covered by eroded or ulcerated epidermis(KOEHLER et al., 2002; ROLÃO et al., 2005; SOARES et al., 2013).

In Brazil, there are few reports describing the hematological status of *Leishmania infantum* naturally infected horses. Interestingly, the cases described here were parasitized but the evaluation of hematological parameters did not present significant variations between the groups.

Apparent lymphocytosis may be due to infection by *Leishmania infatum* or also by some type of stress caused to the animal. Several studies point to the importance of the role of lymphocytes cells in the immune response against leishmaniasis, CD8+ T cells are important for controlling infections caused by protozoa, due to their ability to lyse infected cells and stimulate cytokine production (PINELLI et al., 1995; MILLER et al., 2006; MARTIN et al., 2010). Assymtomatic *Leishmania* infected dogs showed an increase in lymphocytes CD8+ T cells with low *Leishmania* spp. burden, suggesting that high levels of CD8+ T cells may be the key to an effective immune

response against the parasite, since the destruction of parasites is associated with a high number of lymphocytes (REIS et al., 2006).

4. FINAL CONSIDERATIONS

Although the infected horses showed slight alterations in the leukogram. The results may indicate that the equines were asymptomatic due to the absence of apparent clinical signs and the impossibility of relating hematological alterations between infected and non-infected groups. It's suggested that the infected animals have a greater clinical follow-up during the course of the infection added to the quantitative of parasite load techniques to assess whether hematological changes remain during the infection and identify which lymphocytes are acting on the immune response of the infection.

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