

***Leishmania chagasi* in dogs from the city of Jaciara, Mato Grosso, Brazil**

***Leishmania chagasi* em cães no município de Jaciara, Mato Grosso, Brasil**

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ABSTRACT

*The aim of this study was to identify the **Leishmania** species in Jaciara dogs; visceral leishmaniasis (VL) is endemic in the county of Jaciara. A comparison among the PCR results for the spleen, bone marrow, skin and blood of 101 dogs with VL-reactive serum were evaluated. Spleen tissue showed the highest detection percentage, followed by bone marrow. In 97 (96,04%) dogs, the **Leishmania** DNA detected was confirmed as **L. chagasi**, emphasizing the importance of aetiological identifications in sympatric areas with other trypanosomatids.*

Key words: dog, visceral leishmaniasis, PCR, different tissues.

RESUMO

*Com o objetivo de identificar a espécie de **Leishmania** circulante em cães de Jaciara, município endêmico para leishmaniose visceral (LV), e comparar pela PCR a detecção em baço, medula óssea, pele e sangue, foram avaliados 101 cães sororreagentes para LV. O baço foi o tecido que apresentou melhor percentual de detecção, seguido da medula óssea. Em 97 (96,04%) cães, foi detectado DNA de **Leishmania**, confirmada como **L. chagasi**, ressaltando a relevância da identificação etiológica em área de simpatria com outros tripanosomatídeos.*

Palavras-chave: cão, leishmaniose visceral, PCR, distintos tecidos.

Visceral leishmaniasis (VL) is a zoonotic disease that is transmitted by sand flies and caused by **L. chagasi** (syn. **L. infantum**) in the Americas. The dog is considered the primary reservoir of the aetiological agent of VL, and dogs have an important role in maintaining the disease transmission cycle in urban areas because

of the parasitism of their skin, regardless of the dogs clinical presentation and proximity to man (BRASIL, 2006; BRITO et al., 2014a, b).

Various tissues, such as blood, skin, spleen, bone marrow, liver, and lymph nodes, have been identified as good sites for the parasitological confirmation of canine visceral leishmaniasis (CVL) (LACHAUD et al., 2002; ALMEIDA et al., 2013; RAMOS et al., 2013). The ease of obtaining the sample, the clinical status of the animal and the laboratory technique should be considered for the diagnosis (MARTÍNEZ et al., 2011). In accordance to this list of considerations, the Polymerase Chain Reaction has emerged as an important alternative diagnostic technique (LACHAUD et al., 2002; GOMES et al., 2007), which has adequate sensitivity to detect the DNA of the agent in host tissue prior to the occurrence of seroconversion, providing an early diagnosis of the infection (REIS et al., 2013).

In areas of sympatry with other trypanosomatid agents, the identification of circulating **Leishmania** species in dogs is an important component in the epidemiological study of leishmaniasis, and diagnosis by PCR has been used for this purpose (ALMEIDA et al., 2013). The municipality of Jaciara has presented VL cases since 2003 with a prevalence of 54.7% CVL (BRITO et al., 2014a; 2014b; LOPES et al., 2014) without the confirmation of **Leishmania**

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species in dogs. In this context, the aim of this study was to identify the *Leishmania* species in domestic dogs in the urban Jaciara area by PCR and to evaluate the use of this diagnostic technique in different tissues.

This study was conducted in the municipality of Jaciara (15 57' 22" S and 54 57' 48" W), which is located in the Cerrado of Mato Grosso (IBGE, 2014). The dogs (which were of different breeds, ages and sexes and lived in the urban area) that were identified as seropositive by Enzyme-Linked Immunosorbent Assay (ELISA) and Indirect Immunofluorescence Reaction (IFA) as part of a routine public health screening were forwarded to the Municipal Health Department for euthanasia.

The evaluated dogs were clinically classified as symptomatic, asymptomatic or oligosymptomatic (ALMEIDA et al., 2013). Blood samples were collected by venipuncture (cephalic or jugular). After the euthanasia (CFMV, 2012), bone marrow (the region of the iliac crest), skin fragments (scapular region) and spleen fragments were collected.

The sample DNA was extracted according to GOMES et al. (2007) by phenol/chloroform method. The PCR primers 150 and 152 were used for *Leishmania* sp. (DEGRAVE et al., 1994) and RV1 and RV2 (LACHAUD et al., 2002) were used for *L. chagasi*. A DNA reference strain of *L. infantum* (MHOM / BR / 1974 / PP75) was used as positive control, and a DNA-free reaction was employed as negative control. Amplified products were fractionated by electrophoresis on a 2% agarose gel, stained with red gel and visualized by UV transillumination.

The data were analysed with Epi Info software 3.3.2 (CDC, Atlanta, USA) by chi-square or Fisher's exact tests to assess the association between the presence of *Leishmania* sp. DNA in different sampled tissues and the clinical status of the dogs, with a significance level of 5%.

Of the 101 dogs evaluated by PCR for *Leishmania* sp., parasite DNA was detected in the samples from 97 dogs (96.03%) and identified as

L. chagasi. In analysing the collected tissues, DNA amplification occurred in 92 (91.08%) spleen samples, 78 (78%) bone marrow samples, 69 (68.31%) skin samples and 45 (44.55%) blood samples (Table 1). In comparison with positive results between tissues, the spleen was statistically superior to *Leishmania* DNA detection in bone marrow ($P=0.003$, $OR=9.38$ [1.80 to 54.04]). The marrow was statistically superior to the skin for detection ($P=0.004$; $OR=4.49$ [1.50 to 13.70]). There was no significant difference among the other tissues ($p>0.05$).

In considering the clinical condition of the dogs, 1 (0.99%) was rated asymptomatic, 14 (13.86%) were oligosymptomatic and 86 (85.14%) were symptomatic. Of these dogs, 01 (100%), 12 (85.71%) and 84 (97.67%) were positive according to the PCR.

Several regions in Brazil are endemic for CVL, with Jaciara being one of the cities with the highest serological prevalence in Mato Grosso (BRITO et al., 2014a; LOPES et al., 2014). The observation that 96.04% of PCR positives were associated with seropositive dogs confirms the occurrence of the infection, despite of the possibility of cross-reactivity in serological tests as recommended by the Ministry of Health (BRAZIL, 2006; MADEIRA et al., 2009). This finding also enabled the identification of the *L. chagasi* species in domestic reservoirs in Jaciara, which were previously found only in the vector (BRITO et al., 2014b).

Although there is variability in the PCR results because of the type of primers used and the target of the amplified DNA (ALMEIDA et al., 2013; REIS et al., 2013), in the present study it was used the kinetoplast DNA sequences to increase the test sensitivity by presenting a large number of parasite copies (REIS et al., 2013), which combined the use of different samples to allow a higher positivity rate.

Previous studies described an increased percentage of *L. chagasi* DNA detection in symptomatic dogs (ALMEIDA et al., 2013), a finding that was not observed in this study. However, this discrepancy should be analysed with caution because the symptomatic dogs were numerically superior to and oligosymptomatic and

Table 1 - *Leishmania chagasi* detection by PCR in different tissues according to clinical status of dogs.

Clinical Status	-----Sampled Tissues-----				+ / n (%)
	Spleen (n=101)	Bone Marrow (n=100)	Skin (n=101)	Blood (n=101)	
Asymptomatic (n=01)	01	01	01	00	01/01 (100)
Oligosymptomatic (n=14)	12	11	08	04	12/14 (85,7)
Symptomatic (n=86)	79	66	60	41	84/86 (97,7)
TOTAL (%)	92 (91,1)	78 (78)	69 (68,3)	45 (44,5)	97/101 (96)

+ - total number of positive dogs, n - sampling.

asymptomatic, preventing a reliable statistical analysis. In this study, skin, bone marrow, blood and spleen in agreement with previous studies (MADEIRA et al., 2009; ALMEIDA et al., 2013), and the spleen showed a higher percentage of *L. chagasi* DNA detection than the bone marrow. According to REIS et al. (2013), the spleen is considered one of the major organs affected by *Leishmania* sp.; however, its invasive collection hampers its applicability at a large scale. The superiority of detecting the infection in bone marrow compared with skin differs from that described by ALMEIDA et al. (2013). However, the positive difference between the sampled tissues may be related to *Leishmania*'s tropism for lymphoid organs (RAMOS et al., 2013).

In summary, the spleen presented the strongest detection of *Leishmania* confirmed as *Leishmania chagasi*, followed by bone marrow tissue, emphasizing the importance of aetiological identification in areas with sympatric species.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The consent of the dog owners was obtained and indicated by signatures on the science terms/euthanasia agreement, and the procedures were approved by the Ethics Committee on Animal Use at the Universidade Federal de Mato Grosso (CEUA/UFMT) under protocol number 23108.018081/12-0.

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