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Research Note

Seroprevalence of anti-*Leishmania* spp. antibodies in rural dogs from the city of Monte Negro, State of Rondônia, Brazil

Soroprevalência de anticorpos anti-*Leishmania* spp. em cães rurais do município de Monte Negro, Estado de Rondônia, Brasil

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Abstract

The present study assessed the prevalence of anti-*Leishmania* spp. antibodies in dogs from the city of Monte Negro, State of Rondônia, Brazil. ELISA (NE ≥ 3) and IFAT (≥1:40) were used to evaluate 161 serum samples collected from rural dogs from Monte Negro. Forty-five (27.9%) dogs were positive by ELISA tests and five (3.1%) were positive by IFAT. The present study showed for the first time the frequency of exposure to *Leishmania* spp. in dogs in the State of Rondônia, Amazon Region.

Keywords: *Leishmania* spp., prevalence, dogs, ELISA, IFAT.

Resumo

O presente estudo determinou a prevalência de anticorpos anti-*Leishmania* spp. em cães do município de Monte Negro, Estado de Rondônia, Brasil. Foram utilizados os testes de ELISA (NE ≥ 3) e RIFI (≥1:40) para avaliar 161 amostras de soro de cães da zona rural do município. Quarenta e cinco cães (27,9%) reagiram no teste de ELISA e cinco (3,1%) na RIFI. O presente estudo demonstra pela primeira vez a freqüência de exposição por *Leishmania* spp. em cães de Rondônia, Região Amazônica.

Palavras-chave: *Leishmania* spp., prevalencia, cães, ELISA, RIFI.

Leishmaniasis is a worldwide infection disease of humans and animals, caused by intra-mononuclear protozoans of the genus *Leishmania*, which are transmitted to susceptible hosts by phlebotomines of the genus *Lutzomyia* in Brazil (DANTAS-TORRES, 2008). Visceral leishmaniasis is one of the most important zoonotic diseases in South America, mainly in Brazil, where it is caused by *Leishmania* (*Leishmania*) *chagasi* (ARIAS, 1996). The presence of *Leishmania* spp. or antibodies against *Leishmania* spp. has been reported nationwide (DANTAS-TORRES, 2008), but some regions such as rural area of the city of Monte Negro, State of Rondônia, Northern Brazil, has no information about it.

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In an effort to investigate the frequency of antibodies against *Leishmania* spp., serum samples from dogs collected in Rondônia were tested by indirect immunofluorescence antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA).

Dogs serum samples were obtained in the rural area of the city of Monte Negro, State of Rondônia, in the western Amazonian Region of Brazil (10° 18' S and 63° 14' W). These serum samples were collected during Aguiar et al. study (2006) who evaluated the prevalence of antibodies against *Neospora caninum* in cattle and dogs. The rural area of Monte Negro comprised 722 cattle farms and most of them had at least one dog. The number of sampled farms was determined with an estimated prevalence of 50%, absolute desired precision of 10 and 95% confidence interval. Thus, 86 farms were randomly selected and all dogs found in these farms were sampled, totaling 161 dogs (range of one to six dogs per farm) from 70 farms.

All serum samples (n = 161) were tested by IFAT and ELISA using *L. chagasi* antigen preparations and methods described by

Oliveira et al. (2008). To calculate a measure of agreement between ELISA and IFAT, the results were assessed using the Cohn's Kappa coefficient with 95% confidence interval.

Of 161 rural dog samples tested, 45 (27.9%) were positive against *L. chagasi* antigens by ELISA and only 5 (3.1%) were positive by IFAT (titers between 40 and 1280). Among the 45 ELISA positive sera, only three (6.6%) were positive by IFAT. The Kappa test showed a lower agreement of 0.248 (95% CI: 0.171–0.325).

The positive sera (27.9%) found in the ELISA compared to IFAT (3.1%) are probably due to differences in the antigens used. ELISA methods using crude antigens of *L. chagasi* are considered less specific than IFAT, and can result in false-positive and negative reactions (SALOTRA et al., 2002). ELISA and IFAT methods using crude antigens are recommended by Brazilian Ministry of Health, but they show highly variable effectiveness to detect canine *L. chagasi* infection, mainly due to cross-reactivity of samples from dogs infected with other trypanosomatids such as *L. braziliensis* and *Trypanosoma cruzi* and low ability to detect seropositivity in asymptomatic dogs (PALATNIK-DE-SOUSA et al. 2001). The study region is also characterized by the endemic occurrence of other *Leishmania* species (SHAW et al., 2007) and Trypanosomatidae (*Trypanosoma* spp.) (UMEZAWA et al., 2009), which may have contributed to the difference observed between the two tests. According to the Brazilian Ministry of Health, in 2004, 2,131 cases of cutaneous leishmaniasis were reported in humans in Rondônia, confirming the importance of this infection in the western Amazonian region of Brazil (BRASIL, 2006). However, recent observations made by Gil et al. (2003) suggest there is a risk that visceral leishmaniasis could become an endemic disease in Rondônia because the presence of *Lutzomyia longipalpis* was identified in this region.

Many authors have reported differences between results obtained by ELISA and IFAT (BRAGA et al. 1998; METTLER et al., 2005). These differences are expected, and the use of both methods is interesting. ELISA allows simultaneous analysis of a large number of samples in a short time and IFAT can be used to confirm the specificity of positive samples, which is essential to an efficient diagnosis of canine visceral leishmaniasis. In conclusion, the results found in the present study are the first confirmation of the presence of antibodies against *Leishmania* spp. in dogs from Monte Negro, State of Rondônia.

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