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Short Communication

Characterization of a municipality as free of canine visceral leishmaniasis in the context of One Health

Caracterização de um município como livre de leishmaniose visceral canina em um contexto de Saúde Única

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Abstract

Dogs are the main urban reservoir of *Leishmania infantum*, the causative agent of visceral leishmaniasis (VL), which is transmitted by sand flies. In the state of Paraná, the first detection of a positive dog for VL was in 2014, this year Paraná lost free status for this disease (VL). The objectives of this study were to determine the prevalence of canine visceral leishmaniasis in Palotina, the occurrence of vectors that may transmit *Leishmania infantum*, and the number of notifications of human visceral leishmaniasis cases from period 2010 to 2020. To determine the occurrence of canine visceral leishmaniasis, blood samples from 204 dogs were analyzed using the rapid test DPP® to detect anti-*L. infantum* antibodies. To investigate the occurrence of potential vectors, monthly collections were made at 18 points within the urban area of the municipality. The number of human visceral leishmaniasis cases was investigated from Epidemiological Surveillance records. None of the serologically tested dogs showed positive titration. Only two specimens of *Lutzomyia neivai*, one of *Lutzomyia* sp. and four of *Brumptomyia brumpti* specimens were collected. No human visceral leishmaniasis cases were reported. These results suggest that there is no evidence of circulation of *L. infantum* in Palotina.

Keywords: *Leishmania infantum*, sand fly, serology, vectors.

Resumo

Os cães são os principais reservatórios urbanos da *Leishmania infantum*, agente causador da leishmaniose visceral (VL), transmitida por vetores conhecidos como flebotomíneos. No Paraná, a primeira detecção de casos positivos caninos ocorreu em 2014, ano em que o Paraná perdeu o status de estado indene. O objetivo deste estudo foi determinar a prevalência da leishmaniose visceral canina no município de Palotina, a ocorrência de vetores que possam transmitir *Leishmania infantum* e o número de notificação de casos de leishmaniose visceral humana, no período de 2010 a 2020. Para determinar a ocorrência da leishmaniose visceral canina, amostras de sangue de 204 cães foram analisadas, utilizando-se o teste rápido (DPP®) para detectar anticorpos anti-*L. infantum*. Com o objetivo de investigar a ocorrência de potenciais vetores, coletas foram realizadas mensalmente em 18 pontos na área urbana do município. O número de casos de leishmaniose visceral humana foi investigado a partir de registros da Vigilância Epidemiológica. Nenhum cão testado foi positivo no teste sorológico. Apenas dois espécimes de *Lutzomyia neivai*, uma de *Lutzomyia* sp. e quatro de *Brumptomyia brumpti* foram coletados. Nenhum caso de leishmaniose visceral humana foi notificado. Esses resultados sugerem que não há evidência da circulação de *L. infantum* em Palotina.

Palavras-chave: *Leishmania infantum*, flebotomíneos, sorologia, vetores.

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Introduction

Visceral leishmaniasis (VL) is an infection caused by *Leishmania infantum* and is a serious public health problem in many countries. This parasite can infect dogs, causing canine visceral leishmaniasis (CVL), a systemic disease that presents with different clinical signs such as dermatitis, lymphadenomegaly, muscular atrophy, and renal failure (Nascimento et al., 2020). In humans, the disease manifests in a chronic and systemic form, with common clinical signs such as fever and progressive weight loss, in addition to changes such as lymphadenopathy, hepato- and splenomegaly, and edema (Figueiredo et al., 2018). According to data from the Notifiable Diseases Information System (SINAN), from 2001 to 2018, 45,029 human cases of the disease were confirmed in Brazil, of which only 64 occurred in the state of Paraná (SINAN, 2020).

Leishmania infantum in Brazil is transmitted by *Lutzomyia* species *L. longipalpis* and *L.cruzi. Migonemyia migonei* was the species most found in endemic areas in a study carried out in Pernambuco and 4% were infected with the parasite *L. infantum* suggesting that the vector may be related to transmission (Carvalho et al., 2010). However, in Rio Grande do Sul, Rêgo et al. (2019) found that neither of these species transmitted *L. infantum*, and suggesting that *Pintomyia fischeri* may be an *L. infantum* vector.

In many parts of the world including Central Asia, Caucasian countries, part of the Middle East, Central America, and some countries in South America, use euthanasia of positive dogs as an aspect of the strategy to reduce cases of VL in humans, since they are considered the main urban reservoir of the parasite. However, according to Dantas-Torres et al. (2019), this strategy is futile for controlling the transmission of this zoonosis to humans.

In Paraná, *L. infantum* was isolated for the first time in 2012 in dogs that had never left the municipality of Foz do Iguaçu, demonstrating the autochthony of these CVL cases (Dias et al., 2019). The first autochthonous human case in Foz do Iguaçu was registered in 2015. The patient was a 28-year-old man who lived in the municipality and had no travel history in the 12 months prior to diagnosis. An epidemiological study conducted at the residence of the case revealed the presence of *L. longipalpis* (males and a female) in the patient's home, as well as serologically positive dogs in the neighborhood (Trench et al., 2016). According to the SINAN, a total of 19 human VL cases were recorded between 2015 and 2018 (SINAN, 2020).

Diagnosis in dogs is critical because, in addition to the importance of detecting and eliminating canine cases, canine infections usually precede human infections. In 2011, the Ministry of Health replaced the tests for diagnosing the disease in dogs, the rapid test based on Dual Path Platform (DPP®) Canine Visceral Leishmaniasis - Bio-Manguinhos test. A study by Fraga et al. (2016), compared the previous methods with the current ones, and showed that the current method has high specificity (98%), showing a reduction in false positives. This methodology was also evaluated by Figueiredo et al. (2018), who analyzed 1,446 serum samples from asymptomatic, symptomatic, and oligosymptomatic dogs and found that the methodology has better sensitivity and specificity values; however, for asymptomatic animals, the sensitivity is lower but still superior to the previous tests.

According to the Ministry of Health's Visceral Leishmaniasis Control and Surveillance Manual, the municipality of Palotina can be classified as a silent, vulnerable, and non-receptive area. It is silent because the municipality has no record of VL cases in both dogs and humans; it is vulnerable, as it is part of the same road axis as municipalities with known cases of the disease such as Foz do Iguaçu; and it is considered a non-receptive area (Brasil, 2014) because of the apparent absence of *L. longipalpis* and/or *L. cruzi* in the municipality. The present study aimed to identify the seroprevalence of canine infection, undertake captures and identify sand flies and investigate the occurrence of human VL cases in this locality.

Material and Methods

Study design

This cross-sectional study carried out in the municipality of Palotina, state of Paraná, addressed the diagnosis of canine visceral leishmaniasis (CVL) through sampling of dogs, a survey of human VL cases from the municipality's Epidemiological Surveillance records, and an entomological survey of vector occurrence. The survey of human cases covered the period 2010 to 2020 and the sampling and testing of infected dogs and survey of vectors was conducted for a year from 2017 to 2018.

Palotina (24° 16' 54" S, 53° 50' 25" W) (Figure 1), with an estimated population of 31,846 inhabitants in 2019, is located in the western region of Paraná (IBGE, 2020), and has a humid subtropical climate characterized by hot and rainy summers and no clearly defined dry season by Köppen's classification: Cfa; (IAT, 2006). This climate is conducive to sand flies development which occurs optimally in the sheltered, dark, and humid environment created by decomposing organic matter.

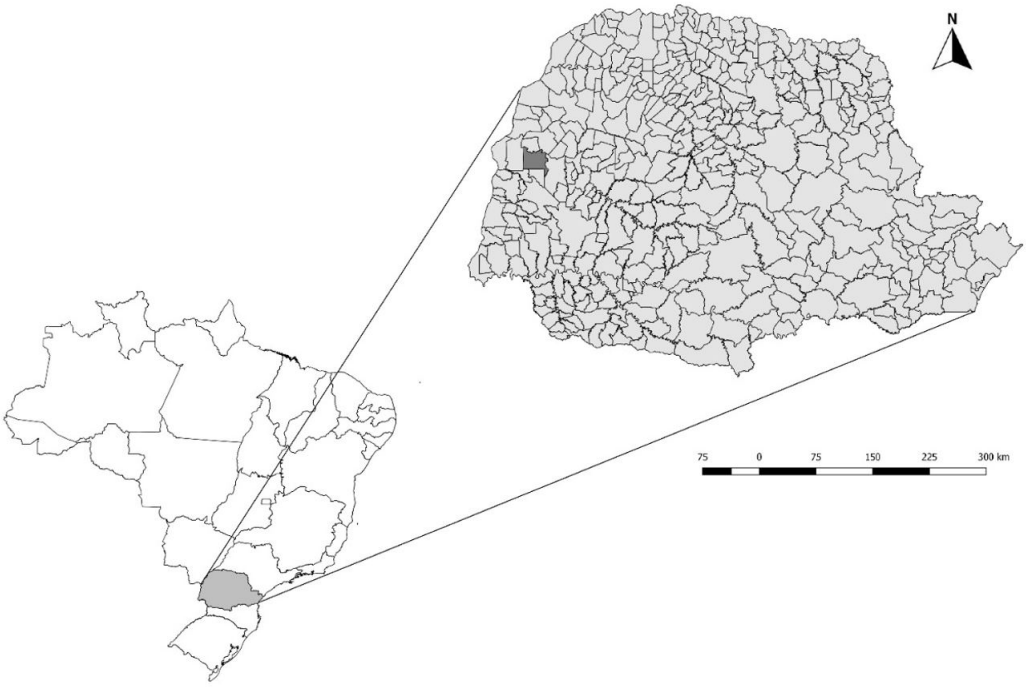

Figure 1. The location of the state of Paraná (light gray) and the municipality of Palotina, the study site (dark gray). Source: Research data. Geoprocessing was performed using the QGIS program 2.14.

Dog blood collection and processing

To calculate the sample size, an expected prevalence of 5% was considered, with an admitted error of 3.7%, confidence level of 95%, and design effect (DEFF) of 1.5. A total of 204 samples, selected by systematic random sampling, were collected in 33 census sectors. Approximately 7 mL of blood per dog was collected by venipuncture of the cephalic and jugular veins. The samples were placed in tubes without anticoagulant and centrifuged for 10 min at 1800 rpm to obtain blood serum for the serological test. Subsequently, the samples were analyzed in the Cell Biology Laboratory - Instituto Carlos Chagas / Fiocruz-PR using the rapid test based on Dual Path Platform (DPP®) Canine Visceral Leishmaniasis - Bio-Manguinhos test, which is a fast and qualitative test for the detection of anti-*Leishmania infantum* antibodies.

Collection and identification of sand flies

A total of 18 strategic locations within the urban area of the municipality were chosen because they had animals and/or environments possibly associated with the presence of *L. infantum* vectors including dogs, pigs, birds, organic matter and moisture, and forests. Sand fly specimens were collected once a month over a one-year period. The lunar cycle was taken into account, and specimens were collected during the waning and new moon phases, aiming for minimum interference of the lunar albedo on the traps. Luminous Falcon traps (hawk light traps) with white light bait were set at the sampling locations, left overnight (12 h), and collected in the early morning. All collected specimens were killed using chloroform, counted, and subjected to entomological pre-classification. The sand fly underwent a clarification process, and were entomologically identified through analysis of the copulatory apparatus of the males and the spermatheca of the females, using the identification key according to Young & Duran (1994) and Galati (2003).

Number of notified human cases

The number of notified human cases of leishmaniasis in Palotina was determined from information provided by the municipality's Epidemiological Surveillance.

Animal welfare statement

This study is following the Ethical Principles of Animal Experimentation and was approved by the Ethics Committee on the Use of Animals at the Federal University of Paraná-Setor Palotina, with protocol number 33/2015, and by the Chico Mendes Institute for Biodiversity Conservation – ICMBio/SISBIO with protocol number 67208.

The human data used were secondary (obtained from the Epidemiological Surveillance records of Palotina); therefore, approval by the Ethics Committee on Research with Humans was not needed.

Results

Of the 204 serologically tested dogs, none showed positive titration, corroborating previous studies in the State of Paraná where no positive animals were found, except in the city of Foz do Iguaçu. During the one-year sampling period, a total of seven sand flies were captured, two specimens of *Lutzomyia neivai* (one male and one female), one female of *Lutzomyia* spp. (very similar to *L. neivai*) and four specimens of *Brumptomyia brumpti* (two males and two females) were collected. Just *Lutzomyia neivai* is apparently involved with transmission of *L. infantum*. According to information from Palotina Epidemiological Surveillance, no cases of human VL were reported from 2010 to 2020.

Discussion

The absence of positive antibody titers in the domestic dogs sampled in this study did not match the results obtained by Thomaz-Soccol et al. (2009) who found positive titers in 92% (22/24) of tested dogs. The absence of positive animals in the present study suggests the absence of circulation of the agent and / or vectors. Another possible explanation for this difference is that, in the present study, dogs were sampled randomly (following the norms of epidemiological collection by sampling) whereas in the work of Thomaz-Soccol et al. (2009), only dogs with clinical and epidemiological suspicion of VL were tested. In addition, the serological techniques used were different. In this study, the DPP® Canine Visceral Leishmaniasis - Bio-Manguinhos test was used, whereas the indirect immunofluorescence reaction (RIFI) and enzyme-linked immunosorbent assay (ELISA) techniques were used in the Thomaz-Soccol study and may have contributed to increased detection of antibodies.

In another study, carried out by Ludwig et al. (2016) in the city of Toledo (about 50 km from Palotina), anti-*Leishmania* spp. antibodies were detected in four of 175 dogs (2.28%) using indirect immunofluorescence. These data are not corroborated by those obtained in this study, probably because the DPP is a serological test specific for detection of *L. infantum* antibodies and not for *Leishmania* spp. in general, as tested for in the Ludwig et al. (2016) study, suggesting that a different *Leishmania* species may have been detected in that study.

In 2017, a canine with mild leukopenia and anemia, extensive dermatological lesions, and onychogryphosis was treated at the Veterinary Hospital of the State University of Londrina. This animal was positive for serological, cytological, PCR, and parasitic cultures, confirming a case of CVL in the municipality. However, being a street dog, the origin of this animal was not identified. In addition, there were no other positive animals near the location where this animal was found, nor were there any *L. longipalpis* specimens in the vector collections carried out in the same area (Caldart et al., 2018). The municipality of Londrina is considered to be free as well as Palotina. However, in 2011 a dog with VL clinical signs was diagnosed in that same veterinary hospital. The animal had come of a municipality of metropolitan region of Londrina - Cambé. At that time, *Leishmannia amazonensis* was isolated, but had a suspect of coinfection with *Leishmania infantum*, hypothesis not confirmed. In addition, *L. amazonensis*, may lead to the development of clinical signs in the animal similar to those caused by *L. infantum*, making it difficult the correct diagnostic of etiological agent (Hoffmann et al., 2012). Consequently, active epidemiological surveillance must be carried out continuously because of the traffic routes along which infected animals may end up arriving at locations considered to be indene (Zinsstag et al., 2011).

The lower frequence of CVL vectors in this Palotina study may explain why there are no autochthonous cases of this zoonosis. Other studies, such as those carried out by Santos et al. (2016, 2018) on Paraná River islands, have demonstrated a predominance of *L. neivai* in this region. This predominance may be related to the capacity of this species to adapt to modified environments and forests as well as forest edges, thus enabling it to invade residences (Reinhold-Castro et al., 2013). These characteristics may have influenced the possible adaptation of *L. neivai* to the environments in the municipality of Palotina. Although until now it is not known as a vector for *L. infantum*, the DNA of the protozoan has already been isolated from *Lu. neivai*, in this sense, the species can become a potential vector of the protozoan that causes visceral leishmaniasis (Saraiva et al., 2009; Dias et al., 2013).

Souza et al. (2014), studied the urban sand fly fauna in the municipality of Timóteo (Minas Gerais) showed that annual temperature variation was the predominant environmental factor influencing the occurrence of sand fly species. The region studied by Souza et al. (2014) is autochthonous for cutaneous leishmaniasis, so it is expected to find specimens related to the transmission of the agent in high numbers. Since in Palotina there were never any autochthonous cases of canine or human visceral leishmaniasis, the low number of sandflies was expected and, therefore, it was not possible to relate the presence of the vector with the season.

Conclusion

The municipality of Palotina appears to be free of CVL as well as its main vector, *L. longipalpis*. In addition, the absence of reported cases from 2010 to 2020 suggests that the municipality can also be considered free of human VL. Despite this, active and passive surveillance measures must be carried out continuously to ensure the rapid detection of human and animal cases, as well as detection of the parasite, should it be introduced into the locality via any of the aforementioned potential dispersion routes. In this way, the indene character of the municipality may be preserved and/or the effects of the introduction of the disease, if it occurs, reduced.

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