Spatial and seroepidemiology of canine visceral leishmaniasis in an endemic Southeast Brazilian area

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Abstract

Introduction: Canine visceral leishmaniasis (CVL) is a public health problem, and its prevalence is associated with the coexistence of vectors and reservoirs. CVL is a protozoosis caused by Leishmania infantum that is endemic in the southeast region of Brazil. Thus, vector and canine reservoir control strategies are needed to reduce its burden. This study aimed to verify the CVL seroprevalence and epidemiology in a municipality in Southeast Brazil to initiate disease control strategies.

Methods: A total of 833 dogs were subjected to Dual Path Platform (DPP) testing and enzyme-linked immunosorbent assays. For seropositive dogs, epidemiological aspects were investigated using a questionnaire and a global position system. The data were submitted to simple logistic regression, kernel estimation, and Bernoulli spatial scan statistical analysis.

Results: The overall CVL-confirmed seroprevalence was 16.08%. The 28.93% in the DPP screening test was associated with dogs maintained in backyards with trees, shade, animal and/or bird feces, and contact with other dogs and cats, with sick dogs showing the highest chances of infection (odds ratio, 2.6; 95% confidence interval, 2.38–1.98), especially in residences with elderly people. A spatial analysis identified two hotspot regions and detected two clusters in the study area.

Conclusions: Our results demonstrated that residences with elderly people and the presence of trees, shade, feces, and pet dogs and cats increased an individual’s risk of developing CVL. The major regions where preventive strategies for leishmaniasis were to be initiated in the endemic area were identified in two clusters.

Keywords: Dual Path Platform. Kernel estimation. Leishmania. Risk factors. Zoonoses.

INTRODUCTION

Visceral leishmaniasis (VL), or kala-azar, is a protozoosis caused by Leishmania infantum inoculated by the bite of an infected phlebotomine sandfly[1]. Dogs (Canis familiaris), the only known reservoirs of this parasite, are responsible for the perpetuation of VL in such areas.

Environmental and cultural conditions are associated with infection prevalence in both reservoirs and hosts; however, the ability of the vector to infect different hosts and the close contact between owners and their pet dogs can influence the risk of VL infection[2][3]. In this scenario, the propagation of VL can be established by the high prevalence of dog seropositivity to canine VL (CVL).

Although many aspects of the ecoepidemiology of VL were discovered in the past 20 years, including its association with poor living conditions, as verified in developing countries[4], the disease in humans and dogs can present high mortality rates if left untreated. The results of spatial analyses may improve public health actions for leishmaniasis since they can, for instance, be used to estimate the coverage of control measures for VL[5] and predict the disease dispersion[6].

Brazil is classified by the World Health Organization (WHO) as one of the six major countries of VL high-burden worldwide, which together account for approximately 90% of the global cases and expose 556 million people to the risk of infection[7]. In 2019, the
WHO reported that VL is endemic in 12 countries in the Americas, with 59,769 new cases reported in 2001–2017, approximately 96% (57,582) of which were reported in Brazil. The territorial dimension of the country is over 8,500,000 km², including two tropical biomes and the various fauna and flora of this climate in addition to the cultural diversity within the territories.

Therefore, the governmental strategies developed to combat leishmaniasis address the epidemiological characteristics faced by each region and can vary among states. For example, in São Paulo, the disease has been present since 1999, while in Paraná, to the best of our knowledge, there have been no reported cases in native reservoirs or humans.

Worldwide, the leishmaniasis surveillance and control program recommended by the WHO is based on case detection and treatment combined with other health education measures, as well as taking action toward vectors and reservoirs when recommended.

Despite these actions, in 2015, the Brazilian VL Control Program announced that epidemiological canine and human transmission conditions associated with the presence of Lutzomyia longipalpis could be verified in 82/645 (12.71%) São Paulo state municipalities, such as Piacatu, where the first human case of VL was recorded in 2008 in a 12-year-old child and two cases were recorded in 2010 in a 1-year-old child and a 4-year-old child. In 2017, 4,096 cases were reported through the Brazilian administrative states, with 147 (3.65%) in São Paulo state, of which 10/147 (6.80%) were fatal. In 2018, the mortality rate reached 8.76% (8/91). This framework suggests that current strategies to control the disease are insufficient and efforts should be directed toward the major regions for public health interventions against VL.

Considering the importance of VL in public health, this study aimed to investigate the spatial epidemiological aspects and identify the spatial and descriptive aspects associated with the risk of CVL in the Piacatu/São Paulo municipality.

**METHODS**

The Ethics Committee of FMVA School, UNESP (CEEA 2345/2014), approved the present study.

Piacatu is a municipality in the northwest region of São Paulo state (21° 35' 31" S, 50° 35' 56" W) with a population of 5,846 inhabitants and a total area of 232,488 km², of which 10% corresponds to urban areas and 90% to rural areas. The tropical climate is characterized by dry winters and rainy summers, with temperatures ranging from 18°C to ≥22°C. The region is classified as endemic for VL, with reported cases in reservoirs and humans and the presence of the vector Lutzomyia longipalpis (Figure 1).

In 2014, the Piacatu Department of Zoonotic Disease Surveillance and Control, following instructions from the Department of Epidemiological Surveillance of the Ministry of Health, published the LV Surveillance and Control Manual and initiated disease characterization in urban areas using a fragmented strategy.
Four sequential phases were implemented: A) performing a canine census in all residences within the urban areas; B) inviting the animals’ tutors to participate in dog blood collection for anti-*L. infantum* serology; C) mapping of the blocks containing residences with seropositive dogs; and D) collecting epidemiological data using a questionnaire at all residences of that positive block.

In the canine census, all residences in the urban perimeter of the municipality (Figure 2) were individually visited to verify the presence of dogs within them. A house was included if any dog was recognized by the household as being in their care, either with restricted circulation in the indoor spaces and backyard or those with free access to the street. This study was limited since it could not obtain data on the population of stray dogs within the municipality.

All residences identified in phase A with pet dogs were included in this investigation for the serum collection in phase B, resulting in 833 serosamples. Sequential data were obtained from 647 dog tutors in phase D.

The dogs were restrained manually with their tutor’s support for blood collection performed after antisepsis with Alcohol 70° GL, followed by collection via cephalic venipuncture of up to

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**FIGURE 2**: Spatial analysis of CVL in Piacatu, São Paulo, Brazil (2014). (A) Kernel estimate of dog seropositive population density by CVL. (B) Dog seronegative population density by CVL. Red indicates relatively high CVL risk, blue indicates relatively low risk. (C) Clusters (white circles with arrows head) of CVL risks inside Piacatu constructed based on high-high correlation of analyzed variables. CVL, canine visceral leishmaniasis.
5 mL of material with a Vacutainer® system in tubes with a separator gel and a clot accelerator.

After a 3–5-min rest, the flasks were sent to the Piacatu Municipal Zoonotic Control Center, where they were centrifuged for 10 minutes at 3,500 rpm for serum obtention. All serosamples were placed in sterile vials, identified, and stored at −20°C until use. The screening tests for CVL were performed with a Dual Path Platform (DPP®), and the seroreagents were tested at the Regional Brazilian Health Ministry Official Laboratory, Instituto Adolf Lutz, with enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (Biomanguinhos®).

Data were obtained from all dog tutors after they signed an informed consent form. Questions included those pertaining to the characteristics of the backyard based on vector (Lu. longipalpis) behavior and the presence of pet animals. The presence of animal feces in the backyard, trees, or shadows and the use of a repellent collar by the dog were obtained from the interviewer’s visualization. The knowledge was considered positive if at least one characteristic of prevention, transmission, and symptoms was correctly reported by the household. Other questions reported by the household included age; presence of fever, weakness, and/or discomfort; presence of children 0–12 years old in the residence; canine weight loss, onicography, alopecia, and/or wounds; and presence of stray and/or pet cats and/or dogs and/or fowl in the backyard.

The data were analyzed with simple logistic regression in which the odds ratio was obtained by exponentiation of the regression coefficient. Associations were considered significant if the probability value was lower than α = 0.05/N. Considering a 95% confidence interval and a margin of error of 16%, the estimated prevalence of CVL was 13.6–18.6%.

### RESULTS

Anti-Leishmania spp. antibodies were observed in 28.93% (241/833) of the animals using the DPP test and confirmed in 55.6% (134/241) using ELISA. Overall, 16.08% (134/833) of the dogs were seropositive for CVL in the municipality.

Kernel analysis revealed a small difference in the dispersion of cases in the urban area of Piacatu, in which the seropositive cases showed a slight concentration in relation to the seronegative cases (Figures 1A, 1B). Two clusters were detected and considered high-high risk areas (relative risk, >6.0; p < 0.05) for CVL. Moreover, a spatial correlation was observed for seropositive dogs and environmental conditions, such as backyards with trees, birds, feces, and shade (Table 1).

### DISCUSSION

The 16.08% CVL infection verified in the Piacatu municipality according to the Brazilian Ministry of Health confirmed the transmission of *L. infantum* among reservoirs and suggested the possibility of human infection in the area.

Depending on the geographical conditions, climate, and social aspects of each affected region, seroprevalences of 4–75% have been reported in Brazilian territories; therefore, the prevalence verified in this study is within the range of those expected for endemic areas.

The risk of canine infection was associated with residences with backyards with trees, chickens, shade, animal feces, dogs and/or

### TABLE 1: Simple logistic regression and odds ratio of variables associated with the risk of canine visceral leishmaniosis infection in 647 households in Piacatu/São Paulo, from January to March 2014.

| Variable                     | OR (95% CI)         | P       |
|------------------------------|---------------------|---------|
| Feces*                       | 2.021 (1.18–3.46)   | 0.0104  |
| Trees*                       | 2.612 (1.66–4.08)   | 2.642E-05 |
| Shadow*                      | 2.389 (1.50–3.80)   | 0.0002  |
| Chickens*                    | 1.987 (1.16–3.40)   | 0.0123  |
| Elderly people*              | 1.575 (1.02–2.41)   | 0.037   |
| Dogs*                        | 3.155 (1.98–5.01)   | 1.153E-06 |
| Cats*                        | 2.830 (1.76–4.52)   | 1.440E-05 |
| Dog with clinical signs*     | 1.843 (1.18–2.86)   | 0.007   |
| Repellent collars            | 1.089 (0.44–2.66)   | 0.852   |
| Children                     | 0.816 (0.37–1.77)   | 0.607   |
| Visceral leishmaniosis definition knowledge | 0.891 (0.40–1.94) | 0.771 |
| Human symptoms               | 0.676 (0.26–1.75)   | 0.422   |
| Visceral leishmaniosis prevention knowledge | 1.209 (0.78–1.86) | 0.390 |

*Probability value lower than α = 0.05/N.
cats, and dogs with clinical signs of CVL, especially when elderly people were present at the residence.

In Brazil, one can easily find regions with both rural and urban environments that show faunal and floral diversity among geographical regions; however, the presence of abundant vegetation along sidewalks and in gardens and backyards provides conditions favorable for vector maintenance\(^1\). This mixed urban–rural characteristic may explain the association verified by this study.

Here, we observed that feces had a 2.02× greater chance of contributing to CVL prevalence than the other measured backyard characteristics. Relationships with backyard characteristics were cited as having an associated risk of VL in another Brazilian study, and studies verified that dogs sleeping in yards were more likely to be infected than those with free access to the house\(^1\).

There is a contradiction in the association between green surroundings, trees, and shade and the occurrence of CVL\(^7,17,18\). These areas are rich in the organic substrates that are required for vector reproduction, as \(Lu.\ longipalpis\) has limited dispersal capacity because they cannot fly beyond 243 m\(^3\). Thus, a close proximity to vegetated areas may be associated with the risk of CVL\(^4\). However, in endemic regions, this association is compromised because reservoir presence is common in the in-home environment and urban afforestation offers conditions for phlebotomine sandfly reproduction\(^20,21\).

The association of chickens and/or other birds with CVL-positive cases has resulted in controversial studies with no statistical significance\(^22,23\). An epidemiological review of CVL in Brazil suggested a possible route of parasite transmission between chickens and humans\(^22\). It has been verified that the continuous risk of transmission of \(L.\ infantum\) depends heavily on the chicken blood present in the peridomestic environment since \(Leishmania\) vectors show a predilection for this animal. Its DNA was detected in \(Lu.\ longipalpis\) blood present in the peridomiciliary environment since \(L.\ infantum\) chickens and humans\(^22\). It has been verified that the continuous transmission of \(L.\ infantum\) depends heavily on the chicken blood present in the peridomestic environment since \(Leishmania\) vectors show a predilection for this animal. Its DNA was detected in \(Lu.\ longipalpis\) blood present in the peridomiciliary environment since \(L.\ infantum\) chickens and humans\(^22\). It has been verified that the continuous transmission of \(L.\ infantum\) depends heavily on the chicken blood present in the peridomestic environment since \(Leishmania\) vectors show a predilection for this animal. Its DNA was detected in \(Lu.\ longipalpis\) blood present in the peridomiciliary environment since \(L.\ infantum\) chickens and humans\(^22\). It has been verified that the continuous transmission of \(L.\ infantum\) depends heavily on the chicken blood present in the peridomestic environment since \(Leishmania\) vectors show a predilection for this animal. Its DNA was detected in \(Lu.\ longipalpis\) blood present in the peridomiciliary environment since \(L.\ infantum\) chickens and humans\(^22\).

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The analysis performed in this study indicated that several factors are associated with the disease prevalence in a given region; similarly, a study conducted in India showed the spatial distribution of VL cases, making it possible to verify the association between hotspots and poverty\(^30\). Another study conducted in a rural area of Madrid where there was an outbreak of VL verified the spatial association between cases and the presence of vectors and rodents in the family Leporidae\(^31\).

The association of clusters with environmental variables suggests that, in this city, the disease behavior may be associated with the ecology of the phlebotomine sandfly; thus, it is possible that the focus of public health measures in environmental education can reduce the number of cases in dogs and, therefore, humans. These results corroborate those obtained in the city of Araçatuba, where the researchers reported transmission patterns of \(Leishmania\) of up to 45.7 m between cases, most likely related to \(Lu.\ longipalpis\) characteristics\(^6\).

After the high prevalence and spatial dispersion of CVL were verified in this study, an epidemiological task force was established focusing on health education for elderly people promoting backyard and street cleaning to remove organic waste produced by afforestation and chicken rearing.

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AUTHORS’ CONTRIBUTION

TFR: Study concept, design and acquisition of data, Analysis and interpretation of data, Drafting of the manuscript, Administrative, technical, and material support; APS and LHO:
Analysis and interpretation of data, Drafting of the manuscript, Statistical analysis, Critical revision of the manuscript for important intellectual content; **ANB:** Study concept and design, Analysis and interpretation of data, Drafting of the manuscript, Critical revision of the manuscript for important intellectual content; **ABG and KDSB:** Analysis and interpretation of data, Drafting of the manuscript, Critical revision of the manuscript for important intellectual content; **JFG:** Drafting of the manuscript, Critical revision of the manuscript for important intellectual content; **TCC:** Study supervision and contribution in all parts of the manuscript.

**CONFLICT OF INTEREST**

The authors declare no conflicts of interest.

**REFERENCES**

1. World Health Organization (WHO). World Leishmaniasis Report 2017. Geneva:WHO;2013.
2. Rangell O, Oliveira SS, Françall AC, Ciavarolol RM, Henriques LF. Leishmaniose visceral no estado de São Paulo: Tendência geral da letalidade entre 1992 a 2013 e o risco de óbitos por estratificação epidemiológica dos municípios e regionais de vigilância epidemiológica entre 2011 a 2013. BEPA. 2015;12(143):1-8.
3. Abrantes TR, Werneck GL, Almeida AS, Figueirredo FB. Environmental factors associated with canine visceral leishmaniasis in an area with recent introduction of the disease in State of Rio de Janeiro. Brazil. Cad S Publ. 2018;34(1):e0021117. doi: 10.1590/0102-311x0021117.
4. Belo VS, Struchiner CJ, Werneck GL, Barbosa DS, Oliveira RB, Neto RGT et al. A systematic Review and meta-analysis of the factors associated with *Leishmania infantum* infection in dogs in Brazil. Vet Parasitol. 2013;195(1-2):1-13. doi: 10.1016/j.vetpar.2013.03.010.
5. Ministério da Saúde (MS). Secretaria de Vigilância em Saúde. Situação epidemiológica das zoonoses de interesse para a saúde pública. Bol Eletr Epidemiol. 2010;10(2):1-17.
6. Costa DNCC, Bermudi PMM, Rodas LAC, Nunes CM, Hiramoto RM, Tolezano JE, et al. Human visceral leishmaniasis and relationship with vector and canine control measures. Rev S Publ. 2018;52(92):1-11. https://doi.org/10.11606/S1518-8787.2018052000381
7. Sevá, AP, Mao L, Galvis-Ovallos, Lima JMT, Valle D. Risk analysis and prediction of visceral leishmaniasis dispersion in dogs in Brazil. Vet Parasitol. 2018;259:80-84.
8. Word Health Organization/Pan American Health Organization (WHO/PAHO). Leishmaniasis: Epidemiological Report of the Americas [internet]. 2019;7 [update 2019 March 1; cited 2019 Aug 26] Available from: https://www.who.int/leishmaniasis/resources/who_paho Era 2019/en/.
9. Instituto Brasileiro de Geografia: IBGE apresenta nova área territorial brasileira.8.515.767,499 km². 2012 [internet]2012 [update 2012 nov 27; cited 2019 Aug 26] Available from: https://www.ibge.gov.br/agencia-sala-de-imprensa/2013-agencia-de-noticias/releases/14318-asi-ibge-apresenta-nova-area-territorial-brasileira-8515767499-km3.
10. Bermudi PMM, Guirado MM, Rodas LAC, Dibo MR, Chiaravalloti-Neto F. Spatio-temporal analysis of the occurrence of human visceral leishmaniasis in Araçatuba, State of São Paulo, Brazil. Rev Soc Bras Med Trop 2018;51(4):452-460. doi: 10.1590/0037-8682-0505-2017
11. Benitez AN, Martins FDC, Mareze M, Nino BSL, Caldart ET, Ferreira FP, et al. Spatial and simultaneous seroepidemiology of anti- *Leishmania* spp. antibodies in dog owners and their dogs from randomly selected households in a major city of southern Brazil. Prev Vet Med. 2018;154:47-53. doi: 10.1016/j.prevetmed.2018.02.011
12. Dantas-Torres F, Brandão-filho SP. Visceral leishmaniasis in Brazil: revisiting paradigm of epidemiology and control. Rev Inst Med Trop. 2006;48(3):151-6. doi: 10.1590/S0036-46520060000300007.
13. Sant’Anna MRV, Nascimento A, Alexander B, Dilger E, Cavalcante RR, Díaz-Albiter HM, et al. Chicken blood provides a suitable meal for the sand fly *Lutzomyia longipalpis* and does not inhibit Leishmania development in the gut. Parasit Vectors. 2010;3(3):1-11.
14. Ferreira TS, Timbó RV, Minuzzo-Souza TTC, Rocha DA, Neiva M, de Albuquerque Ribeiro J, et al. High molecular prevalence of Leishmania in phlebotomine sand flies fed on chicken blood in Brazil. Vet Parasitol. 2018;259:80-4.
15. Silveira F T, Carneiro L A, Ramos PK, Chagas, EJ, Lima LV, Campos MB, et al. A cross-sectional study of canine *Leishmania (L.) infantum chagasi* infection in Amazonian Brazil ratifies a higher prevalence of specific IgG-antibody response than delayed-type hypersensitivity in symptomatic and asymptomatic dogs. Parasitol Res. 2012;111(4):1513-22.
16. Coura-Vital W, Marques MJ, Veloso VM, Roatt BM, Aguiar-Soares RDO, Reis LES, et al. Prevalence and factors associated with *Leishmania infantum* infection of dogs from an urban area of Brazil as identified by molecular methods. PLoS Negl Trop Dis. 2011;5(8):e1291. doi: 10.1371/journal.pntd.0001291.
17. R core Team RA. Language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. [Internet]. 2018 [30 mar. 2018]; Available from: <https://www.r-project.org/>.
18. Kulldorff M, Nagarwalla N. Spatial disease clusters: Detection and Inference. Statistics in medicine, 1995;14:799-810. doi: 10.1002/sim.4870140809.
19. Spada JCP, Silva DT, Martins KRRM, Rodas LAC, Alves ML, Occurrence of *Lutzomyia longipalpis* (Phlebotominae) and canine visceral leishmaniasis in a rural area of Ilha Solteira, SP, Brazil. Braz J Vet Parasitol. 2014;23(4):456–62.
20. Oliveira AM, López RVM, Dibo MR, Rodas LAC, Guirado MM. Dispersion of *Lutzomyia longipalpis* and canine visceral leishmaniasis in São Paulo State, Brazil: identification of associated factors through survival analysis. Parasit Vectors. 2018;11(1):503.
21. Michelin AF, Maciel MOS, Okajima M, Nunes CM, Perri SHV, et al. Factors associated with positivity for canine visceral leishmaniasis in an endemic area in Brazil. Vet Parasitol. 2018;12:13-16.
22. Lainson R, Rangel EF. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis, with particular reference to Brazil: a review. Mem Inst Oswaldo Cruz. 2005;100(8):811-27. doi: 10.1590/ S0074-02762005000800001.
23. Instituto Brasileiro de Geografia e Estatística [Internet]. Piacatu – SP – IBGE Cidades 2018]. Available from: https://cidades.ibge.gov.br/brasil/sp/programa/piaucu/panorama.
24. Ferreira TS, Timbó RV, Minuzzo-Souza TTC, Rocha DA, Neiva M, Ribeiro JA, et al. High molecular prevalence of Leishmania in phlebotomine sand flies fed on chicken blood in Brazil. Vet Parasitol. 2018 Aug 15;259:80-84. doi: 10.1016/j.vetpar.2018.07.004. Epub 2018 Jul 7.
25. Birighente KBS, Cutolo AA, Motaic G, Meira-Strejevitch CS, Pereira-Chioccola VLP. Molecular detection of *Leishmania (Leishmania)* *infantum* in phlebotomine sandflies from a visceral leishmaniasis endemic area in northwestern of São Paulo State, Brazil. Acta Tropic. 2018;181:1-5.
26. Brianti E, Napoli E, Gagliolo G, Falsone L, Gannezio S, Solari BF, et al. Field Evaluation of Two Different Treatment Approaches and Their
Ability to Control Fleas and Prevent Canine Leishmaniosis in a Highly Endemic Area. PLoS Negl Trop Dis. 2016;10(9):e0004987. doi:10.1371/journal.pntd.0004987

27. Rocha AVVO, Moreno BFS, Cabral AD, Louzeiro NM, Miranda LM, Santos VMBD, et al. Diagnosis and epidemiology of *Leishmania infantum* in domestic cats in an endemic area of the Amazon region, Brazil. Vet Parasitol. 2019 Sep;273:80-85. doi: 10.1016/j.vetpar.2019.08.007. Epub 2019 Aug 15.

28. Mallmann DG, Galindo Neto NM, Sousa JC, Vasconcelos, EMR. Health education as the main alternative to promote the health of the elderly. Ciência & Saúde Coletiva. 2015;20(6):1763-72. DOI: 10.1590/1413-81232015206.02382014

29. Dantas-Torres, F. Current epidemiological status of visceral leishmaniasis in Northeastern Brazil. Rev S Publ. 2006;40(3):537-41. doi: 10.1590/S0034-89102006000300024.

30. Galvis-Ovallos F, Casanova C, Sevá AP, Galati EAB. Ecological parameters of the (s)-9-methylgermacrene-B population of the *Lutzomyia longipalpis* complex in a visceral leishmaniasis area in São Paulo State, Brazil. Parasit Vectors. 2017;10(1):269. doi: 10.1186/s13071-017-2211-8.

31. Bulstra CA, La Rutte EA, Malaviya P, Hasker EC, Coffeng LE. Visceral leishmaniasis: Spatiotemporal heterogeneity and drivers underlying the hotspots in Muzaffarpur, Bihar, India. PLOS Negl Trop Dis. 2018;6(12):1-21.