Original Article

First molecular description of autochthonous urban cases of canine visceral leishmaniasis in the city of Belém, Pará, Brazil

Primeira descrição molecular de casos autóctones urbanos de leishmaniose visceral canina na cidade de Belém, Pará, Brasil

G. R. Galvãoᵃ ⓘ, E. C. Gonçalvesᵇ ⓘ, L. G. S. Mouraᶜ ⓘ, R. R. Virgolinoᵇ ⓘ, A. M. P. Nevesᵇ ⓘ and D. C. F. Aguiarᵇ* ⓘ

ᵃUniversidade Federal Rural da Amazônia – UFRA, Hospital Veterinário Mário Dias Teixeira, Belém, PA, Brasil
ᵇUniversidade Federal do Pará – UFPA, Instituto de Ciências Biológicas, Laboratório de Tecnologia Biomolecular, Belém, PA, Brasil
ᶜClinica CENTROPET, Belém, PA, Brasil

Abstract

Leishmaniasis is an anthropozoonosis transmitted by vectors, with dogs being the main domestic reservoirs. Brazil is one of the countries most affected by this disease, and it has been described in humans and dogs in every region in the country. In the northern region leishmaniasis cases in humans have been described in more than 100 municipalities in the State, including the capital, Belém. This study involves two cases of canine visceral leishmaniasis in which the animals developed clinical signs compatible with the disease in urban areas in Belém, the Pará state capital. The diagnosis was confirmed via polymerase chain reaction (PCR) to detect *SSUr-rDNA* and *kDNA* of *Leishmania* sp. and *Leishmania infantum*, respectively. In one of the cases the animal died and in the other the animal underwent treatment with medicines prescribed for dogs. Through this treatment, parasitemia in the second animal has been kept under control and is being monitored through molecular tests. Previously, no canine cases had been notified from urban neighborhoods in the city of Belém, but only on the island of Cotijuba, at a distance of 29 kilometers from the city. Cases of canine and human leishmaniasis have been recorded close to the capital, Belém, which has areas of conserved vegetation and where the presence of disease vectors has been described. Thus, as has been done in several other Brazilian cities, this study uses clinical and laboratory findings to confirm the presence of autochthonous cases of canine visceral leishmaniasis in the city of Belém.

Keywords: visceral leishmaniasis, dog, zoonosis, polymerase chain reaction, molecular diagnosis.

Resumo

A leishmaniose é uma antropozoonose transmitida por vetores, sendo os cães os principais reservatórios domésticos. O Brasil é um dos países mais acometidos por esta doença, sendo descrita em humanos e cães em todas as regiões do país. Na região norte casos de leishmaniose em humanos foram descritos em mais de 100 municípios do Estado, incluindo a capital, Belém. Este estudo envolve dois casos de leishmaniose visceral canina em que os animais desenvolveram sinais clínicos compatíveis com a doença em áreas urbanas de Belém, capital do estado do Pará. O diagnóstico foi confirmado pela reação da cadeia da polimerase (PCR) para detectar o *SSUr-rDNA* e *kDNA* de *Leishmania* sp e *Leishmania infantum*, respectivamente. Em um dos casos o animal veio a óbito e no outro o animal foi submetido a tratamento com medicamentos prescritos para cães. Por meio desse tratamento, a parasitemia no segundo animal foi mantida sob controle e está sendo monitorada por meio de testes moleculares. Anteriormente, nenhum caso canino havia sido notificado em bairros urbanos da cidade de Belém, apenas na ilha de Cotijuba, distante 29 quilômetros da cidade. Casos de leishmaniose canina e humana foram registrados próximo à capital, Belém, que possui áreas de vegetação conservada e onde foi descrita a presença de vetores de doenças. Assim, como tem sido registrado em várias outras cidades brasileiras, este estudo utiliza achados clínicos e laboratoriais para confirmar a presença de casos autóctones de leishmaniose visceral canina na cidade de Belém.

Palavras-chave: leishmaniose visceral, cães, zoonose, reação em cadeia da polimerase, diagnóstico molecular.

1. Introduction

Leishmaniasis is a chronic parasitic anthropozoonosis caused by several *Leishmania* species. Despite being a worldwide public health problem, it is considered a neglected disease related to poverty. Leishmania infects several types of mammals, including marsupials and canines, felines and humans (Gupta et al., 2022).

The different Leishmania species cause two main types of clinical forms, cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) (Burza et al., 2018).

Leishmaniasis was initially described as a rural disease. However, over the years, this vector has been adapting to peripheral and urban areas (Rangel and Vilela,, 2008). Such areas often encompass areas with preserved forests and end up becoming transition zones between urban and preserved areas, which facilitates adaptation by the vector. However, the expansion of leishmaniasis is also linked to other factors such as the presence of the main domestic reservoir (dog) (Roque and Jansen, 2014).

In Pará state, northern region of Brazil, leishmaniasis cases in humans have been described in more than 100 municipalities, including the capital, Belém, which, according to SINAN (DATASUS, 2023a, b), reported 231 cases of VL and 3,141 cases of CL in 2020.

In the Americas, dogs are mainly infected by *Leishmania infantum*, although other *Leishmania* species from these animals have also been isolated (Alvar et al., 2012, Santos et al., 2020). Dogs develop the disease at a slow rate. The initial clinical signs are skin lesions, alopecia, lymphadenopathy, ulcerative lesions, apathy, anemia, weight loss, and onychogryphosis (Dantas-Torres, 2009). However, skin lesions are generally the most frequent reason for veterinary consultation and the most common finding during physical examination (Saridomichelakis and Koutinas, 2014). Nonetheless, infected dogs as well as

humans can remain without clinical signs for an extended period (Esteva et al., 2017).
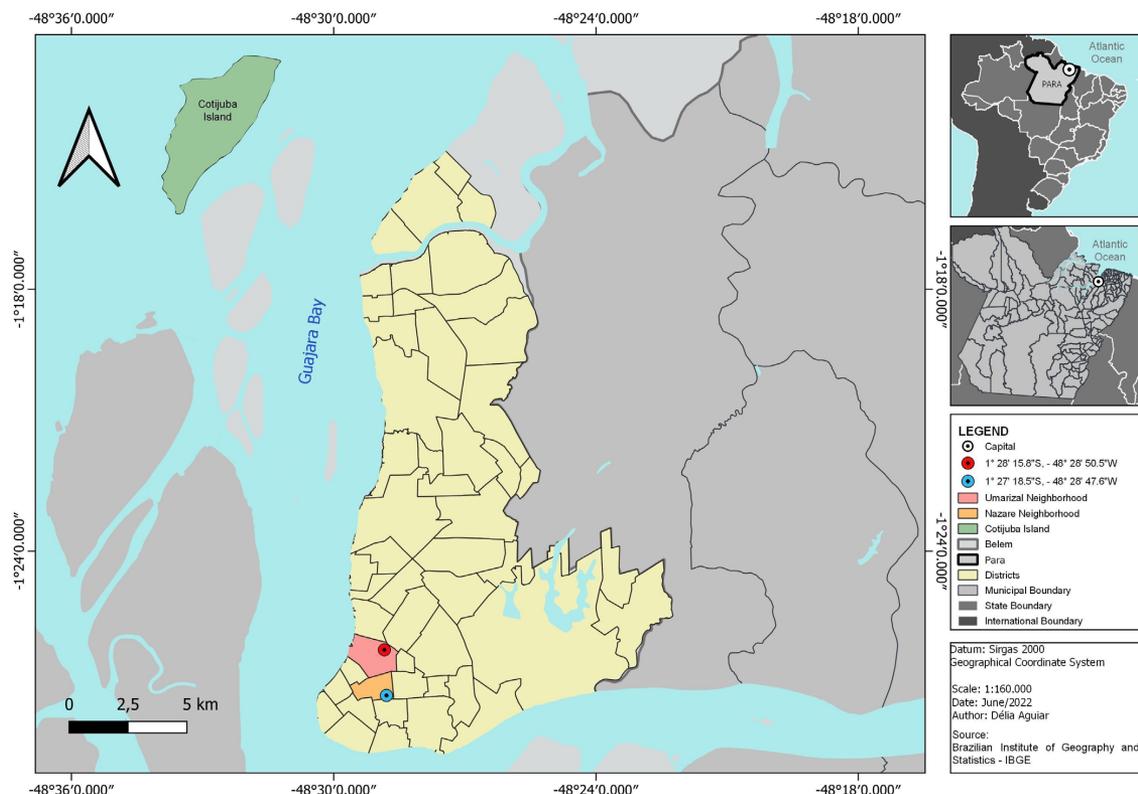
This study describes two cases of autochthonous canine visceral leishmaniasis in the city of Belém (Pará, Brazil) for the first in animals that developed clinical symptoms of the disease and were diagnosed via polymerase chain reaction (PCR).

2. Material and Methods

2.1. Study area, Dogs and sample collection

The study was approved by the Animal Use Ethics Committee (AUEC) of the Universidade Federal do Pará (UFPA) with protocol number 20220601.

The first canine case involved a male dog of no defined breed, living in the Umarizal neighborhood in Belém (Figure 1). On the block where this dog lived most houses had wooded backyards, essentially forming a small forest fragment (1°26'13.20"S 48°28'51.18"W). The second canine case occurred in June 2020, with a dog of the Pug breed, castrated, age one year and nine months, acquired as a puppy and born and raised in Belém. The guardians lived in the Nazaré neighborhood at a distance of only 600 meters (1°27'18.36"S 48°28'.47.18"W) from the Museu Emilio Goeldi Zoobotanical Park (1°27'17.04"S 48°28'37.70"W), which includes a preserved forest area with several animals living inside the park, including mammals (Figure 1).

Figure 1. Location of two cases of autochthonous canine leishmaniasis in the city of Belém, Para (Brazil).

Blood samples (5mL) were collected by venipuncture using dry and EDTA-containing tubes for PCR analysis. The nasal and lesion secretions were obtained using sterile swabs on the mucosa. In the first canine case, only the blood sample was sent for molecular evaluation, while in the second case, the veterinarian sent blood, lesion, nasal and secretion samples. The lesion and secretion samples were collected using a sterile cotton swab (Neolab®) and frozen until DNA was extracted.

2.2. Extraction of DNA and Molecular detection

For the molecular diagnosis, DNA from the blood samples was extracted using the AxyPrep ™ Blood Genomic DNA Miniprep Kit (Axygen) commercial kit, according to the manufacturer's recommendations. The extraction technique with NaCl (Abrão et al., 2005) was used to extract the DNA from lesions and nasal secretion (swab) with a final volume of 20 μL. After extraction, molecular tests were performed out through PCR targeting the small subunit ribosomal rRNA (*SSU rRNA*) gene (Uliana et al., 1994; Savani et al., 2005) and extra-chromosomal DNA kinetoplastid DNA (*kDNA*) (Francino et al., 2006) specific to Leishmania sp and *Leishmania infantum*, respectively.

For detection to target the small subunit ribosomal rRNA (*SSU rRNA*) gene (*Leishmania* sp.) the first PCR was performed with primers S4 (5'-GATCCAGCT GCAGGTTCACC-3') and S12 (5'-GGTTGATTCCGTCAACGGAC-3'). Reactions were performed with a final volume of 25 μl containing 1X PCR buffer, 2 mM MgCl2, 0.2 mM dNTPs, 0.2 μM of each primer and 1 U Taq DNA polymerase. DNA was first denatured at 94ºC for 3 min and then cycled 35 times at 94ºC for 1 min, at 50ºC for 1 min, and at 72ºC for 1 min. A final extension of 7 min was performed at 72ºC as described by Uliana et al. (1994). The fragment (520 pb) produced by S4/S12 PCR was used in a nested PCR with primers S17 (5'-CCAAGCTGCC CAGTA GAAT-3') and S18 (5'-TCGGG CGGAT AAAACCC-3'), specific for the genus Leishmania (Savani et al., 2005). The reaction was undertaken under the same conditions as those described above. The S4/S12 PCR product was denatured at 94ºC for 4 min and cycled 30 times; each cycle took place at 94ºC for 1 min, at 55ºC for 1 min, and at 72ºC for 30 s. Oligonucleotides S17 and S18 produced a 490 bp fragment.

L. infantum-specific primers Leish 1 (5'-AACTTTTCTGGTC CTCCGGGTAG-3') and Leish 2(5'-ACCCCCAGTTTCCCGCC-3') were used to amplify a 120-base-pair fragment of the Leishmania kinetoplast DNA minicircle (Francino et al., 2006). PCR was conducted in a 25 ml final reaction mixture containing PCR buffer 1, 0.150 mM dNTPs, 2 mM MgCl2, 0.2 mM of each primer and 1U Taq Polymerase. The thermal cycling profile was as follows: 94 8C for 3 min, followed by 35 cycles at 94ºC for 30 s, 60ºC for 30 s and 72ºC for 30 s; with a final extension at 72ºC for 5 min.

Products of small subunit ribosomal *rRNA* gene (490pb) and *kDNA* (120 bp) were applied to 1.5% and 2.5% agarose gel, respectively, in Tris-Acetate-EDTA buffer, stained with Diamond Nucleic Acid Dye (Promega Corporation, USA), and later submitted to electrophoresis and visualized under ultraviolet light. An Applied Biosystems thermocycler (Model 2720) was employed for the PCR reactions.

3. Results

The molecular tests indicated that in the first and second case the animals were infected with Leishmania sp. species *L. infantum* with the *SSUr-rDNA* and *kDNA* markers. In the first case detection in the blood sample, while in the second case it was in eye, nose and lesion secretions. In the first case, although it had never been away from Belém, the animal took walks with its guardian in public squares in Belém, slept outside the house, and did not wear a repellent collar. The main clinical signs observed in the first dog case were apathy, lack of appetite, pale mucous membranes, and exfoliative dermatitis present close to the joints. Subsequently, an ulcerative lesion appeared and a medical evaluation indicated that this might be a case of a lesion caused by *Leishmania sp.*

In the second canine case the animal developed a lesion in the area close to the snout. A cytological exam of the lesion indicated only an inflammatory process. On physical examination, the animal achieved a good body score, slightly pale oral and ocular mucous membranes, normal lymph nodes, normal rectal temperature (38° C), hypotrichosis in the anterior and posterior limbs, otitis with yellowish discharge in the ears (Malassezia), pododermatitis, papules and crusts in the dorsal region, circular lesion with well-defined edges located on the upper lip that was not itchy and not painful to touch. With the blood count, cell counts were normal, and there was only a slight increase in urea and alkaline phosphatase.

In this case both markers were amplified from the DNA sample extracted from the lesion and ocular and nasal secretion, but not from the blood. The animal began treatment for the disease and began using a repellent spray. After the first month of treatment, the animal showed improvement in the dermatological picture, total regression of the lesion on the face and hair growth in areas with hypotrichosis. A new PCR was performed to detect Leishmania, which was negative in all samples tested (blood and ocular and nasal secretion). Since the negative result, treatment has been maintained continuously to control the disease.

4. Discussion

This study provides the first autochthonous cases, with clinical presentation and molecular confirmation of two cases *of L. infantum* infection in dogs in the city of Belém, Pará state, Brazil.

The capital of Belém is known as the city of mango trees. It has extensive tree-lined streets in the center of the city and two zoo-botanical parks, in addition to presenting peripheral areas with conserved vegetation and with springs that supply the city, such as Utinga Park. Studies by Ferreira et al. (2014) described the distribution of leishmaniasis vectors in several preserved areas in the municipality of Belém, including Utinga Park. The enzootic cases of visceral leishmaniasis were restricted to forests surrounding the springs and to the island of Cotijuba (Lainson, 2010). Cotijuba Island, 29 km from Belém, is part of a group of 42 islands that make up the archipelago of Belém and is separated geographically from the capital by the Guajará bay (Figure 1).

Human (HL) and canine (CL) leishmaniasis cases have already been described on the island, and epidemiological surveys carried out in the canine population showed a prevalence of 60% of LC, indicating an alarming epidemiological situation for the state and the municipality of Belém (Silveira et al., 2016).

In the case of the state of Pará, leishmaniasis is established in an endemic and epidemic fashion in several municipalities in the state. The existing literature contains studies on cases and the incidence of leishmaniasis in Pará municipalities, including the city of Colares, in northeast Pará (Figueiredo et al., 2014; Galvão, 2018), Marabá and Parauapebas in the southeastern area of the state and Tomé-Açu (Galvão, 2018; Sá, 2019; Santos et al., 2020) and Santarém, in western Pará (Senra et al., 1985). In the Belém metropolitan region, cases have been described on the island of Cotijuba (Silveira et al., 2016) and Barcarena (Silveira et al., 2012; Lima et al., 2010). Galvão (2018) recorded a frequency of 37.7% (23/49) in biological samples from dogs residing in the city of Belém, but in none of them was it possible to prove an autochthonous case. This was because all the infected dogs had traveled to other endemic cities in Pará at some point or were animals that had previously resided in other Brazilian municipalities where human and canine cases have been described.

Several studies in other Brazilian cities have described autochthonous cases of CL (Tolezano et al., 2007; Silva et al., 2011; Faria et al., 2017, Silva et al., 2018; Rocha et al., 2018, Pinto et al., 2021). Furthermore, although animals from Belém have been diagnosed with leishmaniasis (antibody detection) in two other studies (Campos et al., 2017, Schwanke et al., 2014) neither study indicated that the animals were born in Belém and never lived or traveled to endemic areas, as described for the animals in this study. The study by Schwanke et al. (2014) failed to amplify the *L. infantum* DNA in the samples of dogs tested, and its results are based on serological data. Thus, based on the clinical symptoms and life histories of the animals, as well as the molecular diagnosis procedures described in this study, these are the first confirmed autochthonous reports of CL caused by *L. infantum* in two neighborhoods in the city Belém, Pará (Figure 1).

Detection of canine infection is an important public health indicator since it most often precedes human infection (Silva et al., 2018). A differential diagnosis of CL is indispensable, especially in areas where there are human cases described, given that the domestic dog is considered the main reservoir of the disease in urban areas. Molecular diagnosis is advantageous over other methods because it has high specificity and sensitivity and can be applied to different types of biological samples. The widely used serological tests can fail in dogs with prepatent infections that may return to seronegativity, even in those where the immune response was not able to make the seroconversion to IgG (Coura-Vital et al., 2013).

Given that mammals (*Cerdocyon thous*) infected with *Leishmania infantum* have been found inside preserved areas in Belém (Utinga Park) and infected dogs have been found in localities close to the Capital (Ilha de Cotijuba e Barcarena). There is a need for setting up surveillance and control inside the city, as recommended by the Brazilian Ministry of Health of Brazil (Brasil, 2011, 2014), to track developments in neighborhoods with possible infections, as was done in this study, given that Belém has ideal temperature and humidity conditions for sandflies to develop. (Silveira et al., 2012). Besides our study, other researchers (Trüeb et al., 2018; Silva et al., 2022) have shown that urbanized areas present differences in sandfly richness and diversity compared to forest fragments, and have concluded that the conservation of forest areas, even as fragments in urban areas, favors the diversity of phlebotomine species. As has been done in several other Brazilian cities, this study uses clinical and laboratory findings to confirm the presence of autochthonous cases of canine visceral leishmaniasis in an urban area in the city of Belém.

References

ABRÃO, M.G., BILLERBECK, A.E.C., NISHI, M.Y., MARUI, S. and MENDONÇA, B.B.D., 2005. Standardization of DNA extraction with NaCl from oral mucosa cells: application in PROP1 gene study. *Arquivos Brasileiros de Endocrinologia & Metabologia*, vol. 49, no. 6, pp. 978-982. http://dx.doi.org/10.1590/S0004-27302005000600019. PMid:16544023.

ALVAR, J., VÉLEZ, I.D., BERN, C., HERRERO, M., DESJEUX, P., CANO, J., JANNIN, J., DEN BOER, M. and WHO LEISHMANIASIS CONTROL TEAM, 2012. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*, vol. 7, no. 5, pp. e35671. http://dx.doi.org/10.1371/journal.pone.0035671. PMid:22693548.

BRASIL. Ministério da Saúde, 2011 [viewed 14 December 2022]. *Sistema nacional de vigilância em saúde: relatório de situação: Pará* [online]. 5. ed. Brasília: Ministério da Saúde. Available from: https://bvsms.saude.gov.br/bvs/publicacoes/sistema_nacional_vigilancia_saude_pa_5ed.pdf

BRASIL. Ministério da Saúde. Departamento de vigilância em Saúde e Vigilância Epidemiológica, 2014 [viewed 14 December 2022]. *Manual de vigilância e controle da leishmaniose visceral* [online]. Brasília: Ministério da Saúde. Available from: https://bvsms.saude.gov.br/bvs/publicacoes/manual_vigilancia_controle_leishmaniose_visceral_1edicao.pdf

BURZA, S., CROFT, S.L. and BOELAERT, M., 2018. Leishmaniasis. *Lancet*, vol. 392, no. 10151, pp. 951-970. http://dx.doi.org/10.1016/S0140-6736(18)31204-2. PMid:30126638.

CAMPOS, M.P., MADEIRA, M.F., SILVA, D.A., SOLCÀ, M.S., ESPÍNDOLA, O.M., MENDES JÚNIOR, A.A.V. and FIGUEIREDO, F.B., 2017. Accuracy of quantitative polymerase chain reaction in samples of frozen and paraffin-embedded healthy skin for the diagnosis of canine visceral leishmaniasis. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, vol. 69, no. 06, pp. 1443-1450. http://dx.doi.org/10.1590/1678-4162-9053.

COURA-VITAL, W., REIS, A.B., FAUSTO, M.A., LEAL, G.G., MARQUES, M.J., VELOSO, V.M. and CARNEIRO, M., 2013. Risk factors for seroconversion by *Leishmania infantum* in a cohort of dogs from an endemic area of Brazil. *PLoS One*, vol. 8, no. 8, pp. e71833. http://dx.doi.org/10.1371/journal.pone.0071833. PMid:23990996.

DANTAS-TORRES, F., 2009. Canine leishmaniosis in South America. *Parasites & Vectors*, vol. 2, suppl. 1, pp. S1. http://dx.doi.org/10.1186/1756-3305-2-S1-S1. PMid:19426440.

DATASUS, 2023a [viewed 14 February 2022]. *Dados epidemiológicos Sinan* [online]. Ministério da Saúde. Available from: http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sinannet/cnv/leishvpa.def

DATASUS, 2023b [viewed 14 February 2022]. *Dados epidemiológicos Sinan* [online]. Ministério da Saúde. Available from: http://tabnet.datasus.gov.br/cgi/tabcgi.exe?sinannet/cnv/ltapa.def

ESTEVA, L., VARGAS, C., VARGAS, D.E. and LEÓN, C., 2017. The role of asymptomatics and dogs on leishmaniasis propagation. *Mathematical Biosciences*, vol. 293, pp. 46-55. http://dx.doi.org/10.1016/j.mbs.2017.08.006. PMid:28864398.

FARIA, M.T., BARBOSA, F.S., TEIXEIRA-NETO, R.G., PINHEIRO, G.R.G., MANHANI, M.N., MARCELINO, A.P., BARBOSA, J.R. and FONTES, G. 2017. Autochthonous case of canine visceral Leishmaniasis in a non-endemic area in Minas Gerais, Brazil. *Pesquisa Veteterinária Brasileira*, vol. 37, no. 12, pp.1505-1508. http://dx.doi.org/10.1590/S0100-736X2017001200023.

FERREIRA, J.V.S., SANTOS, T.V., SANTOS, E.M. and GORAYEB, I.S., 2014. Phlebotomine sand flies (Diptera: Psychodidae) in forest fragments of Belem metropolitan area, Para state, Brazil, with considerations on vectors of American cutaneous leishmaniasis agents. *Revista Pan-Amazonica de Saude*, vol. 5, no. 2, pp. 29-35. http://dx.doi.org/10.5123/S2176-62232014000200004.

FIGUEIREDO, M.J.F.M., DE SOUZA, N.F., FIGUEIREDO, H.F., MENESES, A.M.C., SILVA-FILHO, E. and NASCIMENTO, G.G., 2014. Fatores de risco e classificação clínica associados à soropositividade para leishmaniose visceral canina. *Ciência Animal Brasileira*, vol. 15, no. 1, pp. 102-106. http://dx.doi.org/10.5216/cab.v15i1.25097.

FRANCINO, O., ALTET, L., SÁNCHEZ-ROBERT, E., RODRIGUEZ, A., SOLANO-GALLEGO, L., ALBEROLA, J., FERRER, L., SÁNCHEZ, A. and ROURA, X., 2006. Advantages of real-time PCR assay for diagnosis and monitoring of canine leishmaniosis. *Veterinary Parasitology*, vol. 137, no. 3-4, pp. 214-221. http://dx.doi.org/10.1016/j.vetpar.2006.01.011. PMid:16473467.

GALVÃO, G.R., 2018. *Detecção e epidemiologia molecular de Leishmania sp. em três municípios do estado do Pará*. Belém: Universidade Federal do Pará, 58 p. Dissertação de Mestrado em Biologia de Agentes Infecciosos e Parasitários.

GUPTA, A.K., DAS, S., KAMRAN, M., EJAZI, S.A. and ALI, N., 2022. The pathogenicity and virulence of Leishmania- interplay of virulence factors with host defenses. *Virulence*, vol. 13, no. 1, pp. 903-935. http://dx.doi.org/10.1080/21505594.2022.2074130. PMid:35531875.

LAINSON, R., 2010. The Neotropical Leishmania species: a brief historical review of their discovery, ecology and taxonomy. *Revista Pan-Amazônica de Saúde*, vol. 1, no. 2, pp. 13-32. http://dx.doi.org/10.5123/S2176-62232010000200002.

LIMA, L.V.R., CARNEIRO, L.A., CAMPOS, M.B., CHAGAS, E.J., LAURENTI, M.D., CORBETT, C.E., LAINSON, R. and SILVEIRA, F.T., 2010. Canine visceral leishmaniasis due to *Leishmania (L.) infantum chagasi* in Amazonian Brazil: comparison of the parasite density from the skin, lymph node and visceral tissues between symptomatic and asymptomatic, seropositive dogs. *Revista do Instituto de Medicina Tropical de São Paulo*, vol. 52, no. 5, pp. 259-265. http://dx.doi.org/10.1590/S0036-46652010000500007. PMid:21049230.

PINTO, A.O., CARVALHO, D., FRIZZO, C., LOPES, K., TESSARI, G.B., CATECATI, T., DHOM-LEMOS, L.C., PASQUALI, A.K.S., QUARESMA, P.F., STOCO, P.H., GRISARD, E.C., STEINDEL, M. and WAGNER, G., 2021. First case of canine visceral leishmaniasis in the midwestern of Santa Catarina, Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 82, pp. e241162. http://dx.doi.org/10.1590/1519-6984.241162. PMid:34133561.

RANGEL, E.F. and VILELA, M.L., 2008. *Lutzomyia longipalpis* (Diptera, Psychodidae, Phlebotominae) and urbanization of visceral leishmaniasis in Brazil. *Cadernos de Saude Publica*, vol. 24, no. 12, pp. 2948-2952. http://dx.doi.org/10.1590/S0102-311X2008001200025. PMid:19082287.

ROCHA, M.A.N., MATOS-ROCHA, T.J., RIBEIRO, C.M.B. and ABREU, S.R.O., 2018. Epidemiological aspects of human and canine visceral leishmaniasis in State of Alagoas, Northeast. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 78, no. 4, pp. 609-614. http://dx.doi.org/10.1590/1519-6984.166622. PMid:29412243.

ROQUE, A.L. and JANSEN, A.M., 2014. Wild and synanthropic reservoirs of Leishmania species in the Americas. *International Journal for Parasitology. Parasites and Wildlife*, vol. 3, no. 3, pp. 251-262. http://dx.doi.org/10.1016/j.ijppaw.2014.08.004. PMid:25426421.

SÁ, G.J.L., 2019. *Epidemiologia da leishmaniose visceral canina em Parauapebas, Pará, Brasil.* Tocantins: Universidade Federal do Tocantins, 55 p. Dissertação de Mestrado em Sanidade Animal e Saúde Pública nos Trópicos.

SANTOS, F.J.A., NASCIMENTO, L.C.S., SILVA, W.B., OLIVEIRA, L.P., SANTOS, W.S., AGUIAR, D.C.F. and GARCEZ, L.M., 2020. First report of canine infection by *Leishmania* (*Viannia*) *guyanensis* in the Brazilian Amazon. *International Journal of Environmental Research and Public Health*, vol. 17, no. 22, pp. 8488. http://dx.doi.org/10.3390/ijerph17228488. PMid:33207754.

SARIDOMICHELAKIS, M.N. and KOUTINAS, A.F., 2014. Cutaneous involvement in canine leishmaniosis due to *Leishmania infantum* (syn. L. chagasi). *Veterinary Dermatology*, vol. 25, no. 2, pp. 61-71, e22. http://dx.doi.org/10.1111/vde.12105. PMid: 24461051.

SAVANI, E.S., NUNES, V.L., GALATI, E.A., CASTILHO, T.M., ARAUJO, F.S., ILHA, I.M., CAMARGO, M.C., D'AURIA, S.R. and FLOETER-WINTER, L.M., 2005. Occurrence of co-infection by *Leishmania (Leishmania) chagasi* and *Trypanosoma (Trypanozoon) evansi* in a dog in the state of Mato Grosso do Sul, Brazil. *Memorias do Instituto Oswaldo Cruz*, vol. 100, no. 7, pp. 739-741. http://dx.doi.org/10.1590/S0074-02762005000700011. PMid:16410962.

SCHWANKE, K., SILVA, A.M.M., PACHECO, A., BAHIA, M., SILVEIRA, F.T., SCOFIELD, A. and GÓES-CAVALCANTE, G., 2014. Molecular diagnosis and frequency of anti-*Leishmania infantum chagasi* antibodies in dogs in Belém/Pará, Brazil. *Pesquisa Veterinária Brasileira*, vol. 34, no. 3, pp. 255-260. http://dx.doi.org/10.1590/S0100-736X2014000300010.

SENRA, M.S., PIMENTEL, P.S. and DE SOUZA, P.E., 1985. Visceral leishmaniasis in Santarem/PA: general aspects of the control, serological survey in dogs and treatment of the human cases. *Revista Brasileira Malariologia e Doenças Tropicais*, vol. 37, pp. 47-59. PMid:3843132.

SILVA, A.S., FRANÇA, R.T., RICHINI-PEREIRA, V.B., LANGONI, H., MONTEIRO, S.G. and LOPES, S.T.A., 2011. First autochthonous case of canine visceral leishmaniasis in the Center of Rio Grande do Sul State, Brazil do Sul State, Brazil. *Acta Scientiae Veterinariae*, vol. 39, no. 2, pp. 968-970. http://dx.doi.org/10.1590/0037-8682-0143-2017. PMid:30304285.

SILVA, B.Q.D., AFONSO, M.M.D.S., FREIRE, L.J.M., SANTANA, A.L.F., PEREIRA-COLAVITE, A. and RANGEL, E.F., 2022. Ecological aspects of the Phlebotominae Fauna (Diptera: Psychodidae) among forest fragments and built areas in an endemic Area of American Visceral Leishmaniasis in João Pessoa, Paraíba, Brazil. *Insects*, vol. 13, no. 12, pp. 1156-1169. http://dx.doi.org/10.3390/insects13121156. PMid:36555066.

SILVA, C.J., MATTOS, C.B., FELIPIN, K.P., SILVA, H.P.J., CANTANHÊDE, L.M., PORROZZI, R., MEDEIROS, J.F. and FERREIRA, R.G.M., 2018. First autochthonous case of canine visceral leishmaniasis in Rondônia, Brazil, a region with no history of visceral leishmaniasis. *Revista da Sociedade Brasileira de Medicina Tropical*, vol. 51, no. 5, pp. 712-715. http://dx.doi.org/10.1590/0037-8682-0143-2017. PMid:30304285.

SILVEIRA, F.T., CARNEIRO, L.A., RAMOS, P.K.S., CHAGAS, E.J., LIMA, L.V.R., CAMPOS, M.B., LAURENTI, M.D., GOMES, C.M.C. and CORBETT, C.E.P., 2012. A cross-sectional study on 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. *Parasitology Research*, vol. 111, no. 4, pp. 1513-1522. http://dx.doi.org/10.1007/s00436-012-2989-4. PMid:22706905.

SILVEIRA, F.T., LIMA, L.Y.R., SANTOS, T.V., RAMOS, P.K.S. and CAMPOS, M.B., 2016. Reviewing the trajectory of American visceral leishmaniasis in Brazilian Amazon: from Evandro Chagas to the current days. *Revista Pan-Amazônica de Saúde*, vol. 7, no. spe., pp. 15-22. http://dx.doi.org/10.5123/S2176-62232016000500003.

TOLEZANO, J.E., ULIANA, S.R., TANIGUCHI, H.H., ARAÚJO, M.F., BARBOSA, J.A., BARBOSA, J.E., FLOETER-WINTER, L.M. and SHAW, J.J., 2007. The first records of *Leishmania* (*Leishmania*) *amazonensis* in dogs (*Canis familiaris*) diagnosed clinically as having canine visceral leishmaniasis from Araçatuba County, São Paulo State, Brazil. *Veterinary Parasitology*, vol. 149, no. 3-4, pp. 280-284. http://dx.doi.org/10.1016/j.vetpar.2007.07.008. PMid:17720321.

TRÜEB, I., PORTELA, R.D., FRANKE, C.R., CARNEIRO, I.O., RIBEIRO JUNIOR, G.J., SOARES, R.P. and BARROUIN-MELO, S.M., 2018. *Trypanosoma cruzi* and *Leishmania* sp. infection in wildlife from urban rainforest fragments in northeast Brazil. *Journal of Wildlife Diseases*, vol. 54, no. 1, pp. 76-84. http://dx.doi.org/10.7589/2017-01-017. PMid:28977769.

ULIANA, S.R., NELSON, K., BEVERLEY, S.M., CAMARGO, E.P. and FLOETER-WINTER, L.M., 1994. Discrimination amongst Leishmania by polymerase chain reaction and hybridization with small subunit ribosomal DNA derived oligonucleotides. *The Journal of Eukaryotic Microbiology*, vol. 41, no. 4, pp. 324-330. http://dx.doi.org/10.1111/j.1550-7408.1994.tb06085.x. PMid:8087103.