Autochthonous *Leishmania* (*Viannia*) *lainsoni* in Dog, Rio de Janeiro State, Brazil, 2023

Isabela Cordeiro da Silva Santos, Daniel Moreira de Avelar, Luciana de Freitas Campos Miranda, Artur Augusto Velho Mendes Júnior, Lucas Keidel Oliveira, Luanna da Silva Ventura, Aline Fagundes da Silva, Fernanda Nunes Santos, Liliane de Fátima Antônio Oliveira, Rodrigo Caldas Menezes,[1] Andreza Pain Marcelino[1]

Author affiliations: Fundação Oswaldo Cruz, Rio de Janeiro, Brazil (I.C.d.S. Santos, L.d.F.C. Miranda, L.K. Oliveira, L.d.S. Ventura, A.F. da Silva, F.N. Santos, L.d.F.A. Oliveira, R.C. Menezes, A.P. Marcelino); Fundação Oswaldo Cruz, Belo Horizonte, Brazil (D.M. de Avelar); Instituto Carlos Chagas, Curitiba, Brazil (A.A.V.M. Júnior)

DOI: https://doi.org/10.3201/eid3105.241058

In Brazil, *Leishmania* (*Leishmania*) *infantum* causes canine visceral leishmaniasis; the primary vector is the *Lutzomyia longipalpis* sand fly. We describe a case of canine visceral leishmaniasis caused by *Leishmania (Viannia) lainsoni* in a dog from Barra Mansa municipality, Rio de Janeiro state. Better specificity of serologic diagnostic techniques is needed for diagnoses.

Protozoa transmitted by sand flies cause leishmaniasis, and several pathogenic species affect humans. Various clinical forms of the disease have been described, including visceral, cutaneous, and mucocutaneous leishmaniasis (*1*). *Leishmania* (*Viannia*) *lainsoni* was described in Brazil in 1987 as the causative agent of human cases of cutaneous leishmaniasis. Its vector is the *Lutzomyia ubiquitalis* sand fly (*2*). Other countries in Latin America have reported human cases of *L.* (*V.*) *lainsoni* infection. Researchers isolated the parasite from the rodent species *Cuniculus paca*, the lowland paca, in the state of Pará, Brazil, suggesting a potential wild reservoir (*3*,*4*).

This study reports the case of a dog (*Canis familiaris*) infected with *L.* (*V.*) *lainsoni* that was from the municipality of Barra Mansa, an urban area in Rio de Janeiro state with widespread visceral leishmaniasis (VL) (Appendix, https://wwwnc.cdc.gov/EID/article/31/5/24-1058-App1.pdf). The Ethics Committee on the Use of Animals–Fiocruz approved this work (license no. LW 19/20; https://www.ceua.fiocruz.br/ceuaw000.aspx).

A 5-year-old male dog of mixed breed domiciled in Barra Mansa tested positive for VL by both rapid immunochromatographic testing and enzyme immunoassay (Bio-Manguinhos, https://portal.fiocruz.br/en/unidade/immunobiological-technology-institute-biomanguinhos) during epidemiologic surveillance in 2023 and was euthanized using the recommendations of the Brazilian Ministry of Health (https://www.gov.br). The dog had not moved to other regions and had localized alopecia, crusted skin ulcers, onychogryphosis, keratoconjunctivitis, normocytic normochromic anemia, hyperproteinemia, hyperglobulinemia, hypoalbuminemia, and a low albumin:globulin ratio (Appendix). Histopathologic changes included skin with hyperkeratosis and multifocal and moderate granulomatous dermatitis, as well as lymphoid hyperplasia of the spleen. Immunohistochemistry was positive for amastigote forms of *Leishmania* in skin and spleen (Figure 1).

We performed parasitologic and PCR tests (Appendix Table 2). We used multilocus enzyme electrophoresis with 5 enzyme profiles (phosphoglucomutase, glucose-6-phosphate dehydrogenase, nucleoside hydrolase, 6-phosphogluconate dehydrogenase, and phosphoglucose isomerase) (*5*). We extracted DNA from the isolated parasite and used it for PCR restriction fragment length polymorphism analysis (*Hae*III and *Bst*UI). We sequenced the 70-kDa heat shock protein products with the same primers by Sanger sequencing (primers: BankIt2825220 and Seq1PP760383) (*6*). Those techniques identified the parasite as *L.* (*V.*) *lainsoni* in all profiles studied in the bone marrow sample (Figure 2).

Like other species of the subgenus *Viannia*, *L.* (*V.*) *lainsoni* can cause ulcerative or nodular dermal lesions in humans (*4*). The clinical signs found in this infected dog included onychogryphosis and skin alterations. Development of skin lesions can lead to hematogenous dissemination and parasitemia of internal organs, as observed in this case, and visceral involvement of lymph nodes and spleen (*7*). The positive results of serologic tests show flaws in the specificity of the techniques because those tests were validated for detecting dogs with canine VL caused by *L.* (*Leishmania*) *infantum*. Hematocrit values less than the reference range, along with a slight increase in total protein, are expected in chronic diseases. We observed no changes in renal function markers. The host–parasite interaction has been extensively studied in dogs infected with *L.* (*L.*) *infantum*; however,

[1]These authors contributed equally to this article.

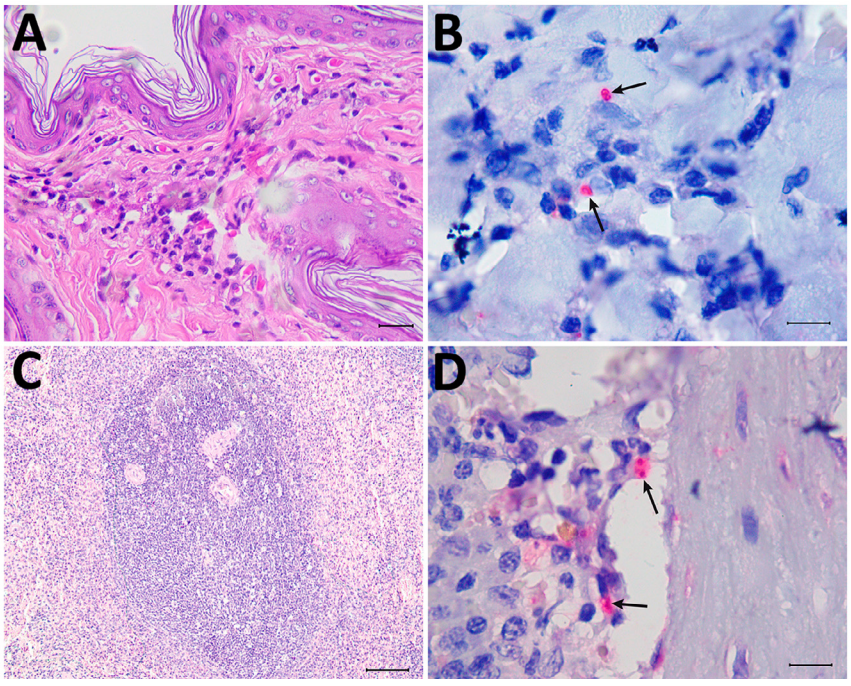

Figure 1. Histologic sections from autochthonous *Leishmania* (*Viannia*) *lainsoni* in dog (*Canis familiaris*), Rio de Janeiro state, Brazil, 2023. A, B) Skin of the examined dog: hyperkeratosis and moderate granulomatous infiltrate in the dermis are composed mainly of macrophages, with a smaller number of plasma cells and lymphocytes (A) and red-stained amastigotes in the cytoplasm of macrophages (arrows) (B). C, D) Spleen of the examined dog: lymphoid hyperplasia (C) and red-stained amastigotes in the cytoplasm of macrophages in the parenchyma (arrows) (D). A, C) Hematoxylin-eosin stain; B, D) immunohistochemistry. Scale bars indicate 10 μm.

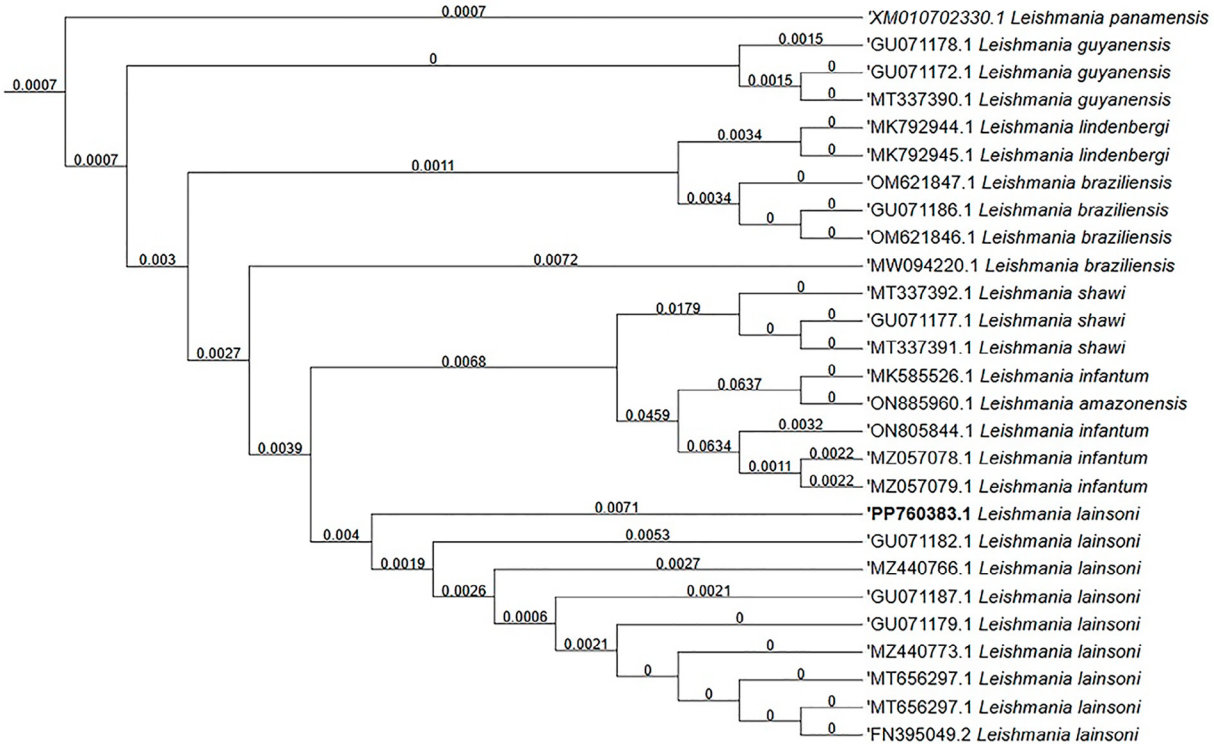

Figure 2. Evolutionary analysis of autochthonous *Leishmania* (*Viannia*) *lainsoni* in dog (*Canis familiaris*), Rio de Janeiro state, Brazil, 2023. Bold text indicates isolate from this study. Evolutionary history was inferred by using the maximum-likelihood method and Kimura 2-parameter model. The bootstrap consensus tree inferred from 1,000 replicates represents evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in <50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the BioNJ (https://bionj.org) method to a matrix of pairwise distances estimated by using the maximum composite likelihood approach. This analysis involved 33 nucleotide sequences. There were a total of 463 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (https://www.megasoftware.net). MEGA used the first position for each codon in the construction of the phylogenetic tree. GenBank accession numbers are shown.

little is known about that interaction in dogs infected with other *Leishmania* species.

In Latin America, *L.* (*V.*) *lainsoni* is found in tropical and sub-Andean regions with different climatic conditions. Its presence in other countries highlights the high dispersal capacity of the parasite and potential involvement of unidentified mammalian host vectors. Barra Mansa has a crucial migratory flow because it is located on the banks of the Paraíba do Sul River and influences the Médio Paraíba region and southern part of the south-central region of Rio de Janeiro State.

The dog lived in an area surrounded by natural and abundantly wooded areas. A large portion of the Hemlock Forest is located in Barra Mansa, and *C. paca* rodents are part of the local fauna and could serve as reservoirs of *L.* (*V.*) *lainsoni* in that area (*8*).

Few entomologic surveys have been conducted in Barra Mansa, and only *Lu. sallesi* and *Lu. longipalpis* sand flies were confirmed, limiting the conclusions of this study (*9*). Although the *Lu. ubiquitalis* sand fly is considered the primary vector of *L.* (*V.*) *lainsoni* in Brazil, other species such as *Lu. nuneztovari anglesi* and *Lu. velascoi* sand flies in Bolivia have been reported (*10*). Therefore, identification of a dog infected with *L.* (*V.*) *lainsoni* in Barra Mansa may be linked to transmission by other yet undocumented sand fly species in that municipality. The dog did not have a history of moving to other locations. We consider environmental changes caused by humans in the region, as well as local wildlife and migratory flows, as possible causes of infection. This study raises several questions. Is a new and yet unknown disease cycle being established locally? What is the risk for the disease becoming endemic in the population? Will the cycle persist? Also, could other regions in Brazil or elsewhere face similar risks of emerging *Leishmania* species infecting dogs? Further epidemiologic investigations and taxonomic characterization studies are essential and should be continuously supported. Efforts to create clearer specificity in serologic diagnostic techniques are also needed.

Acknowledgments

We thank the Municipal Health Department of Barra Mansa and the Central Laboratory of Public Health for collaboration and Adilson Benedito de Almeida and Ricardo Baptista Schmidt for processing the figures.

This study was supported by Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (grant no. CNE-E-26/201.032/2021) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil (finance code 001). R.C. Menezes is a recipient of productivity fellowships from Conselho Nacional de Desenvolvimento Cientifico e Tecnológico, Brazil (grant no. 305956/2021-3).

A.P.M., D.M.d.A., and R.C.M. conceptualized the study. I.C.d.S.S., D.M.d.A, L.d.F.C.M., A.A.V.M.J., L.K.O., L.d.S.V., A.F.d.S., F.N.S., L.d.F.A.O., R.C.M., and A.P.M. constructed the methodology. I.C.d.S.S., D.M.d.A, L.F.C.M., A.A.V.M.J., L.K.O., L.d.S.V., and F.N.S. carried out the investigation. R.C.M., A.F.d.S., L.d.F.A.O., and A.P.M. performed data analysis. A.P.M. and R.C.M. acquired funding. A.P.M. was the study administrator. R.C.M. supervised the study. I.C.d.S.S. and A.P.M. wrote the original draft. All authors contributed to the review and editing of the final manuscript.

About the Author

Ms. Santos is a PhD student at the Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil. Her research interests include the evaluation of diagnostic techniques for dogs suspected of having canine visceral leishmaniasis, using molecular techniques such as conventional PCR, quantitative PCR, and loop-mediated isothermal amplification.

References

1. World Health Organization. Leishmaniasis [cited 2024 Jul 2]. https://www.who.int/news-room/fact-sheets/detail/leishmaniasis
2. Silveira FT, Shaw JJ, Braga RR, Ishikawa E. Dermal leishmaniasis in the Amazon region of Brazil: *Leishmania (Viannaia) lainsoni* sp.n., a new parasite from the State of Pará. Mem Inst Oswaldo Cruz. 1987;82:289–91. https://doi.org/10.1590/S0074-02761987000200018
3. Silveira FT, Lainson R, Shaw JJ, Braga RR, Ishikawa EE, Souza AA. Cutaneous leishmaniasis in Amazonia: isolation of *Leishmania* (*Viannia*) *lainsoni* from the rodent *Agouti paca* (Rodentia: Dasyproctidae), in the state of Pará, Brazil [in Portuguese]. Rev Inst Med Trop São Paulo. 1991;33:18–22. https://doi.org/10.1590/S0036-46651991000100004
4. Corrêa JR, Brazil RP, Soares J. *Leishmania* (*Viannia*) *lainsoni* (Kinetoplastida: Trypanosomatidae), a divergent *Leishmania* of the *Viannia* subgenus: a mini review. Mem Inst Oswaldo Cruz. 2005;100:587–92. 10.1590/s0074-02762005000600014
5. Cupolillo E, Grimaldi G Jr, Momen H. A general classification of New World *Leishmania* using numerical zymotaxonomy. Am J Trop Med Hyg. 1994;50:296–311. https://doi.org/10.4269/ajtmh.1994.50.296
6. Graça GC, Volpini AC, Romero GAS, Oliveira Neto MP, Hueb M, Porrozzi R, et al. Development and validation of PCR-based assays for diagnosis of American cutaneous leishmaniasis and identification of the parasite species. Mem Inst Oswaldo Cruz. 2012;107:664–74. https://doi.org/10.1590/S0074-02762012000500014
7. Marquez ES, de Castro EA, Nabut LB, da Costa-Ribeiro MCV, Dela Coletta Troiano Araújo L, Poubel SB, et al. Cutaneous leishmaniosis in naturally infected dogs in Paraná, Brazil,

and the epidemiological implications of *Leishmania* (*Viannia*) *braziliensis* detection in internal organs and intact skin. Vet Parasitol. 2017;243:219–25. https://doi.org/10.1016/j.vetpar.2017.07.003
8. Brasil, Ministério do Meio Ambiente. Plano de manejo ARIE floresta da cicuta [cited 2024 Jul 16]. https://www.gov.br/icmbio/pt-br/assuntos/biodiversidade/unidade-de-conservacao/unidades-de-biomas/mata-atlantica/lista-de-ucs/arie-floresta-da-cicuta/arquivos/plano-de-manejo-arie-cicuta-oficial.pdf
9. Carvalho BM, Dias CMG, Rangel EF. Phlebotomine sand flies (Diptera, Psychodidae) from Rio de Janeiro state, Brazil: species distribution and potential vectors of leishmaniasis. Rev Bras Entomol. 2014;58:77–87. https://doi.org/10.1590/S0085-56262014000100013
10. Kato H, Cáceres AG, Mimori T, Ishimaru Y, Sayed ASM, Fujita M, et al. Use of FTA cards for direct sampling of patients' lesions in the ecological study of cutaneous leishmaniasis. J Clin Microbiol. 2010;48:3661–5. https://doi.org/10.1128/JCM.00498-10

Address for correspondence: Andreza Pain Marcelino, Laboratório de Pesquisa Clínica e Vigilância em leishmanioses, Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz, 4365 Brasil Ave, Manguinhos, Rio de Janeiro 21040-900, Brazil; email: andreza.marcelino@ini.fiocruz.br

Unexpected Zoonotic and Hybrid Schistosome Egg Excretion Patterns, Malawi, 2024

Angus M. O'Ferrall, Sekeleghe A. Kayuni, Lucas J. Cunningham, Peter Makaula, John Archer, Adam P. Roberts, Janelisa Musaya,[1] J. Russell Stothard[1]

Author affiliations: Liverpool School of Tropical Medicine, Liverpool, UK (A.M. O'Ferrall, S.A. Kayuni, L.J. Cunningham, J. Archer, A.P. Roberts, J.R. Stothard); Malawi-Liverpool-Wellcome Trust Clinical Research Programme, Queen Elizabeth Central Hospital, Blantyre, Malawi (S.A. Kayuni, P. Makaula, J. Musaya); School of Medicine and Oral Health, Kamuzu University of Health Sciences, Blantyre (S.A. Kayuni, J. Musaya); Wolfson Wellcome Biomedical Laboratories, Natural History Museum, London, UK (J. Archer)

DOI: https://doi.org/10.3201/eid3105.241757

[1]These senior authors contributed equally to this article.

Two exemplary cases of mixed urogenital and intestinal schistosomiasis in Malawi show hybridizations of *Schistosoma mattheei* with *S. haematobium* and *S. mansoni*, indicating newly emerging genetic diversity. Complex egg excretion patterns in feces expose current diagnostic gaps and alert to future sampling needs for effective surveillance of zoonotic schistosomiasis.

Schistosomiasis is a waterborne, parasitic disease transmitted by several species of *Bulinus* and *Biomphalaria*, two distinct freshwater snail genera common across sub-Saharan Africa (*1*). In sub-Saharan Africa, *Schistosoma haematobium* is the predominant cause of urogenital schistosomiasis, and *S. mansoni* is the predominant cause of intestinal schistosomiasis (*1*). *S. haematobium* is endemic in Malawi, where infections with zoonotic and hybrid species from the *S. haematobium* group (*S. mattheei* and *S. haematobium* × *S. mattheei*) have also been detected in humans (*2*,*3*). *S. mattheei* is considered a livestock-infecting schistosome that causes intestinal disease (*4*); however, excretion of ova from humans infected with *S. mattheei* and associated *S. haematobium* group hybrids reportedly has occurred through the urogenital tract (*2*,*3*). Meanwhile, *Biomphalaria* freshwater snails were first detected along the southern shores of Lake Malawi in 2017 (*5*). Since then, autochthonous *S. mansoni* transmission and intestinal schistosomiasis outbreaks have been confirmed in Mangochi District, Malawi (*5*,*6*).

To clarify *S. haematobium* group hybridization dynamics, we conducted a longitudinal cohort study in southern Malawi. The College of Medicine Research Ethics Committee, Malawi (approval no. P.08/21/3381, https://www.ncst.mw) and the Liverpool School of Tropical Medicine Research Ethics Committee, United Kingdom (approval no. 22-028, https://www.lstmed.ac.uk/research/research-integrity/research-ethics-committee) provided ethics approval. This study also tracked *S. mansoni* prevalence in a community cohort recruited from Samama Village, Mangochi District (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/31/5/24-1757-App1.pdf), where the outbreak of intestinal schistosomiasis was initially reported (*5*,*6*).

In June 2024, we determined *S. mansoni* prevalence in Mangochi District to be 14.8% (165/1,116) using point-of-care urine circulating cathodic antigen cassette tests (POC-CCA; ICT International, https://ictinternational.com), and considered trace results positive. Those results represented the lowest reported *S. mansoni* prevalence in Samama Village since it emerged in 2017 (*5*–*7*). However, we observed numer-