



First reports of canine leishmaniasis in northwest Rio Grande do Sul

Page 1 a 5

[Primeiros relatos de leishmaniose canina no noroeste do Rio Grande do Sul]

P.H. Braz¹ , N.R. Silva² , J.R. Centenaro² , L. Grunitzky² , M.T. Lemes³ ,
J.C. Ferron¹ , G. Gonçalves⁴ , M.P. Campos⁴ , F.B. Figueiredo⁴ 

¹Universidade Federal da Fronteira Sul, Realeza, PR, Brasil

²Undergraduate, Instituto Federal Farroupilha, Frederico Westphalen, RS, Brasil

³Graduate, Universidade Federal da Fronteira Sul, Realeza, PR, Brasil

⁴Instituto Carlos Chagas, Fiocruz, Curitiba, PR, Brasil

ABSTRACT

This research aimed to carry out an epidemiological investigation of dogs with canine leishmaniasis in an area of Rio Grande do Sul with no history of notification of the disease. Leishmaniasis is considered an endemic zoonosis in different countries. In Brazil, it occurs mainly in the Southeast and Midwest regions, having drastically increased the number of notifications in recent years. In the state of Rio Grande do Sul, there are reports of the disease in the metropolitan region of Porto Alegre and in the border region in the west of the state, as well as in the central region. The investigation was conducted in 100 dogs from the municipalities of Frederico Westphalen and region. Whole blood was collected for immunochromatographic testing (TR DPP® Canine Visceral Leishmaniasis) and lymph node aspiration. Positives were confirmed by ELISA test and indirect immunofluorescence test with total dilution or immunohistochemistry after skin biopsy. Two animals were diagnosed with visceral leishmaniasis, considered the first reported cases of the disease in the canine population in the region. Therefore, it becomes necessary to research and control the potential presence of sandflies in the area. There is a hypothesis that the animals were contaminated in other regions of the country, remaining as a reservoir until the moment of diagnosis.

Keywords: epidemiology, zoonosis, sand fly, Lutzomyia, Leishmania.

RESUMO

Esta pesquisa objetivou realizar uma investigação de cães com leishmaniose canina, em uma área do Rio Grande do Sul, sem histórico de notificação da doença. A leishmaniose é considerada uma zoonose endêmica em diferentes países. No Brasil, ocorre principalmente nas regiões Sudeste e Centro-Oeste, tendo aumentado drasticamente o número de notificações nos últimos anos. No estado do Rio Grande do Sul, existem relatos da doença na região metropolitana de Porto Alegre e na região de fronteira no oeste do estado, bem como na região central. A investigação ocorreu em 100 cães, provenientes de municípios de Frederico Westphalen e região. Foram colhidos sangue total para realização de teste imunocromatográfico (TR DPP® Leishmaniose Visceral Canina) e punção aspirativa de linfonodo. Os positivos foram confirmados por meio do teste ELISA e do teste de imunofluorescência indireta com diluição total ou imuno-histoquímica após biópsia cutânea. Foram diagnosticados dois animais com leishmaniose visceral, sendo considerados os primeiros relatos de caso da doença canina na região, portanto torna-se necessária a pesquisa e o controle de potencial presença de flebotômios na região. Há hipótese de os animais terem sido contaminados em outras regiões do país, permanecendo como reservatório até o momento do diagnóstico.

Palavras-chave: epidemiologia, zoonose, flebotômio, Lutzomyia, Leishmania

INTRODUCTION

Leishmaniasis is a zoonosis that encompasses a series of syndromes caused by parasites of the genus *Leishmania* sp., transmitted by female sandflies of the genus *Lutzomyia* sp. (Leishmaniosis..., 2021). The main species of the etiological agent found in the Americas, including Brazil, is the *Leishmania infantum*, whose most relevant vectors are the arthropods of the species *Lutzomyia longipalpis* and *L. cruzi* (Brasil, 2020).

The epidemiology of the disease results from the interaction between parasite, vector and host. The environmental conditions in which dogs live is considered of great importance, as these animals, when raised in spaces that are open or do not have protection against sandfly vectors, are more susceptible to the infection. (Gharbi et al., 2015).

In order to assist in the diagnosis of affected animals, laboratory alterations can be found in hematological and biochemical tests due to alterations in the cardiorenal axis and liver damage caused by the disease. (Godoy et al., 2017). These laboratory findings must be associated with clinical alterations such as lymph node enlargement, skin ulceration, areas of alopecia (especially periocular), conjunctivitis, splenomegaly and hepatomegaly (Brasil, 2020), ulcerative lesions in different organs such as buccal mucosa and tongue (Braz et al., 2017; Marinho et al., 2017).

In humans, infections by *Leishmania infantum* depend on clinical and environmental variables for diagnosis, with asymptomatic cases being more common. However, children under two years old more frequently present clinical symptoms of the disease. (Fraga et al., 2010). In cases in which clinical signs are found, the most commonly reported ones are fever, pale mucous membranes, and splenomegaly, which may be accompanied by low blood cell counts and inflammatory syndrome. It is important to report that in cases where there is a high population of dogs carrying the disease, there is a greater chance of transmission to humans, especially to immunocompromised populations or in children (Fraga et al., 2010).

Determining the epidemiology of leishmaniasis in different territories is considered an action of epidemiological surveillance, especially due to it being a neglected zoonosis in the Brazilian territory, despite being endemic in a large part of Brazil (Brasil, 2014). There is a lack of epidemiological data in the Northwest region of Rio Grande do Sul regarding the detection of canine leishmaniasis. This research aimed to conduct an investigation about canine leishmaniasis in an area of Rio Grande do Sul without a history of disease notification, using the serological method by using serological methods.

MATERIALS AND METHODS

The research was conducted with 100 stray dogs from three different counties in the Northwest region of Rio Grande do Sul, specifically Frederico Westphalen, Vista Alegre, and Palmitinho, representing a total population of 41,280 inhabitants in this region, with Frederico Westphalen being the largest county among the chosen ones. The animals were recruited through anti-rabies vaccination campaigns promoted by the educational institution (IFFar-FW), aimed at vaccinating dogs with adoption and/or stray histories.

All selected animals were chosen randomly, not necessarily presenting known clinical manifestations in dogs with leishmaniasis, as there is a possibility of animals being in a subclinical stage, where there are no signs of the disease.

Blood samples were collected via the jugular or cephalic vein. Prior to the procedure, trichotomy and asepsis were carried out, and subsequently, access was established using a 12x8 mm needle and a 5 mL syringe. All collected blood was stored in tubes without anticoagulant, placed in a thermal box with a temperature between 2 and 8° C, for the confirmation of diagnosis by ELISA/RIFI in DPP positive animals. The DPP test, an immunochromatographic screening test, was conducted following the manufacturer's instructions for DPP® Canine Leishmaniasis - Bio-Manguinhos/Fiocruz. The tests were donated by the Carlos Chagas Institute - Fiocruz, Curitiba (PR), for the execution of the serological survey.

Due to the gold standard diagnostic recommendation, fine-needle aspirates were performed on the popliteal lymph node using a 3 mL syringe and a 25x11mm needle. The collected material was placed on a glass slide, and a cytological smear was prepared through the squash technique. The smear was then stained with a panoptic-type dye and examined for the presence of amastigote forms of *Leishmania* sp.

For animals that tested positive in one of the previously mentioned diagnostic methods, additional tests were conducted for confirmation, and samples were sent to private laboratories. The methodology for confirming the diagnosis was carried out following the standardized procedures of each laboratory.

The survey of canine leishmaniasis cases was approved by the Ethics Committee on Animal Use (CEUA-IFFar) under protocol number 8433290419 (ID 000128).

RESULTS AND DISCUSSION

In the year 2018, according to Leishmaniasis Report No. 8 (Informe..., 2019), Brazil had an incidence of 15.8 cases of the disease per 100,000 inhabitants, with a very intense transmission rate. Among the factors contributing to the maintenance of the infection in the country, the warm climate stands out, as it favors the proliferation of the disease-carrying sandfly vector. These vectors can survive in dry environments and move to cooler and moister areas, which serve as resting sites (Gharbi *et al.*, 2015). Additionally, asymptomatic infection in dogs is considered widespread, allowing the parasite to remain present for long periods (Leishmaniosis..., 2021).

There is a high incidence of the disease throughout the country. In the state of Rio Grande do Sul, the transmission rate is considered low, since the state has an annual average incidence of only 0.02 (OPAS, 2019; Lima *et al.*, 2021). Although cases have already been reported in different regions of the state, such as in the central region, in the city of Santa Maria and Santa Cruz do Sul, in the Southwest region, in the cities of Uruguaiana, São Borja, Barra do Quaraí and Itaqui and also, in the state capital and in the metropolitan region, in the city

of Viamão, so far there are no records on the presence of the disease in any city in the Northwest region of Rio Grande do Sul (Dias *et al.*, 2022).

Among the 100 tested animals, two dogs tested positive for leishmaniasis through the DPP test. The first positive animal was an adult, non-castrated male, exhibiting clinical signs of alopecic lesions, apathy, anorexia, and lack of appetite. The dog had been residing in the city of Rodeio Bonito for three years and had a history of adoption in the state of Maranhão, where the disease is endemic and of significant public health concern (Silveira and Oliveira, 2020).

Despite the positive dog contributing to the dissemination of the disease as a reservoir, as it presents high levels of cutaneous parasitism - allowing the sandfly to acquire and transmit the *Leishmania* sp. parasite during the blood feeding (Van Griensven and Diro, 2019) - the dog that cohabitated with the infected animal was also tested using the DPP method and tested negative for leishmaniasis.

The second animal that tested positive for leishmaniasis through the DPP test was an elderly female who started showing cutaneous clinical signs after being adopted, presenting a non-healing surgical wound that formed sanguineous vesicles. The animal had a history of frequent travels between the cities of Frederico Westphalen (RS) and Balneário Camboriú (SC). Similarly to the first case, it is not possible to determine the exact location where the animal contracted the infection. However, Balneário Camboriú is known to be a municipality with a high frequency of dogs carrying *Leishmania*, with more than 10% of positive cases for the disease. (Heusser Júnior *et al.*, 2010).

As of the current time, one of the main vectors of leishmaniasis, *Lutzomyia longipalpis*, has not been found in the state of Rio Grande do Sul (Rêgo *et al.*, 2020). This fact is justified due to the region's temperatures, which are considered unfavorable for both the vector and the protozoan, hindering their proliferation (Lorenz *et al.*, 2017). However, other vectors with the capacity to transmit *Leishmania infantum* in Rio Grande do Sul have already been described, such

as *Pintomyia fischeri*, *Migonemyia migonei*, and *Lutzomyia gaminari* (Rêgo et al., 2020).

Of significant importance is the fact that the interstate mobility of animals favors the occurrence of the disease in Rio Grande do Sul, as its transmissibility becomes more effective, given the possibility of transitioning to different geographical locations, allowing for the dissemination of the disease to new hosts. (Thomaz-Soccol et al., 2009).

In this context, prophylactic measures are of great importance, mainly through the prevention of contact with the vector insect, using physical barriers such as fine mesh screens on windows and around kennels, and chemical barriers, such as repellents containing synthetic pyrethroids, which can be impregnated into collars to be worn by animals. Additionally, animals can be vaccinated; however, the efficacy of protection is considered low, around 68% to 71%. (Brasil, 2016; Ribeiro et al., 2018).

Although serological tests conducted for the confirmation of leishmaniasis cases can yield false-positive results, mainly due to cross-reactions with *Trypanosoma cruzi* antigens due to the close phylogenetic relationship between the parasites (Luciano et al., 2009), it is possible to consider that the results were reliable. This assertion is supported by the fact that Chagas disease, caused by *T. cruzi*, exhibited a negative incidence rate in the southern region of the country and also in the state of Maranhão until March 2021 (Brasil, 2021), regions and states from which the positive animals in the study originated.

Furthermore, the positive animals underwent a confirmatory test after the positive DPP result, considering that clinical alterations found in leishmaniasis, such as cutaneous, cardiac, and renal manifestations, cannot be considered pathognomonic for the disease. (Brasil, 2020; Luciano et al., 2009; Braz et al., 2017; Marinho et al., 2017).

Despite the absence of amastigote forms of *Leishmania* sp. in the lymph nodes of the animals in either case, confirmation was feasible through ELISA/RIFI and immunohistochemistry after cutaneous biopsy, respectively, in the first and second diagnosed animal. The tests were

conducted in private laboratories and were sent by the animal owners, with no association to the project. The results were provided by the owners to demonstrate the validation of the diagnosis in their animals.

CONCLUSION:

The seropositivity for leishmaniasis in two dogs is indicative of the need for epidemiological monitoring of the disease in the Northwest region of Rio Grande do Sul. Although the frequency of positive dogs for leishmaniasis was only 2% of the studied population, it raises concerns about the region's status as a neglected disease area due to the absence of official notification or records of the disease's occurrence in the region up to the present moment. Furthermore, it highlights the necessity to investigate the potential presence of the sandfly in this area, to confirm whether the contamination did not originate from other regions of the country.

ACKNOWLEDGMENTS

To the Foundation for Research Support of the State of Rio Grande do Sul and to the National Council for Scientific and Technological Development for the scholarships in Scientific Initiation.

REFERENCES

- BRASIL. Conselho Federal de Medicina Veterinária – CFMV. Comissão de Saúde Pública Veterinária. *Leishmaniose visceral: guia de bolso*. Brasília: CFMV, 2020. 194p.
- BRASIL. Ministério da Saúde. Secretária de Vigilância em Saúde. *Boletim Epidemiológico Doença de Chagas*. Brasília: Ministério da Saúde, 2021. 38p.
- BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. *Manual de vigilância e controle da leishmaniose visceral*. Brasília: Ministério da Saúde, 2014. 120p.
- BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis. *Manual de vigilância, prevenção e controle de zoonoses: normas técnicas e operacionais*. Brasília: Ministério da Saúde, 2016. 121p.

- BRAZ, P.H.; SARTORETTO, M.C.; SOUZA, A.S.; SIQUEIRA, M.S. Comparação entre os valores da amplitude de distribuição de diâmetro dos eritrócitos e do volume corpuscular médio com a citometria de eritrócitos em cães com leishmaniose. *Pesqui. Vet. Bras.*, 37, p.741-784, 2017.
- DIAS, T.P.; VERSTEG, N.; JARDIM, G.C. *et al.* Leishmaniose visceral na região sul do Brasil: análise crítica frente a evolução epidemiológica. *Res. Soc. Dev.*, v.11, p.1-16, 2022.
- FRAGA, T.L.; BRUSTOLONI, Y.M.; LIMA, R.B. *et al.* Poly-merase chain reaction of peripheral blood as a tool for the diagnosis of visceral leishmaniasis in children. *Mem. Inst. Oswaldo Cruz*, v.105, p.310-313, 2010.
- GHARBI, M.; MHADHBI, M.; REJEB, A. *et al.* Leishmaniosis (*Leishmania infantum* infection) in dogs. *Rev Sci Tech.* v.34, n.2, p. 613-26, 2015
- GODOY, K.C.S.; ANTUNES, T.R.; BRAZ, P.H. *et al.* Comportamento dos marcadores bioquímicos de injúria hepática nos cães com leishmaniose visceral. *Pubvet.* v.11, p.670-675, 2017.
- HEUSSER JÚNIOR, A.; BELLATO, V.; SOUZA, A.P. *et al.* Leishmaniose tegumentar canina no município de Balneário Camboriú, Estado de Santa Catarina. *Rev. Soc. Bras. Med. Trop.* v.43, n. 6, p.713-718, 2010.
- INFORME de leishmanioses nº 8, 2019. Washington: OPAS, 2019. Available in: <<https://iris.paho.org/handle/10665.2/51738>>. Accessed in: 09/06/2023.
- LEISHMANIOSIS in manual of diagnostic tests and vaccines for terrestrial animals. Paris: OIE, 2021. (Terrestrial Manual).
- LIMA, R.G.; MENDONÇA, T.M.; MENDES, T.S.; MENEZES, M.V.C. Perfil epidemiológico da leishmaniose visceral no Brasil, no período de 2010 a 2019. *Rev. Eletrôn.Acervo Saúde*, v.13, p.1-10, 2021.
- LORENZ, C.; AZEVEDO, T.S.; VIRGINIO, F. *et al.* Impact of envi-ronmental factors on neglected emerging arboviral diseases. *Plos Negl. Trop. Dis.*, v.11, p.1-19, 2017.
- LUCIANO, R.M.; LUCHEIS, S.B.; TRONCARELLI, M.Z.; LUCIANO, D.M.; LANGONI H. Avaliação da reatividade cruzada entre antígenos de *Leishmania* spp e *Trypanosoma cruzi* na resposta sorológica de cães pela técnica de imunofluorescência indireta (RIFI). *Braz. J. Vet. Res. Anim. Sci.*, v.46, p.181-187, 2009.
- MARINHO, C.P.; SOUZA, I.M.; XAVIER, M.E.B.; DOURISBOURE, C.J.; BRAZ, P.H. Achado citopatológico de formas amastigota de *Leishmania* spp. na língua de um canino: relato de caso. *Pubvet*, v.11, p.1104-1107, 2017.
- RÊGO, F.D.; SOUZA, G.D.; MIRANDA, J.B.; PEIXOTO, L.V.; ANDRADE-FILHO, J.D. Potential vectors of *Leishmania* parasites in a recente focus of visceral leishmaniasis in neighborhoods of Porto Alegre, State of Rio Grande do Sul, Brazil. *J. Med. Entomol.*, v.57, p.1286-1292, 2020.
- RIBEIRO, R.R.; MICHALICK, M.S.M.; SILVA, M.E. *et al.* Canine Leishmani-asis: An Overview of the Current Status and Strategies for Control. *BioMed. Res. Int.*, v.2018, p.1-12, 2018.
- SILVEIRA, J.A.V.; OLIVEIRA, E.H. Leishmaniose Visceral: análise epidemiológica e temporal no Estado do Maranhão, Brasil. *Res. Soc. Dev.*, v.9, p.e838986537, 2020.
- THOMAZ-SOCCOL, V.; CASTRO, E.A.; NAVARRO, I.T. *et al.* Casos alóctones de leishmaniose visceral canina no Paraná, Brasil: implicações epidemiológicas. *Rev. Bras. Parasitol. Vet.*, v.18, p.46-51, 2009.
- VAN GRIENSVEN, J.; DIRO E. Visceral Leishmaniasis: recent advances in diagnostics and treatment regi-mens. *Infect. Dis. Clin. North Am.*, v.33, p.79-99, 2019.