

The first case of canine visceral leishmaniasis in the southern region of Santa Catarina, an emerging focus of visceral leishmaniasis in Brazil: regional report or reflection of the reality of a country?

O primeiro caso de leishmaniose visceral canina na região sul de Santa Catarina, foco emergente de leishmaniose visceral no Brasil: relato regional ou reflexo da realidade de um país?

El primer caso de leishmaniasis visceral canina en la región sur de Santa Catarina, foco emergente de leishmaniasis visceral en Brasil: informe regional o reflejo de la realidad de un país?

Received: 12/03/2021 | Reviewed: 12/10/2021 | Accept: 12/17/2021 | Published: 12/23/2021

Diego Soares Leote

ORCID: <https://orcid.org/0000-0002-9820-6765>
Universidade do Sul de Santa Catarina, Brazil
E-mail: dcabanha@gmail.com

Dayane Borba da Silva

ORCID: <https://orcid.org/0000-0002-1497-5859>
Universidade do Sul de Santa Catarina, Brazil
E-mail: dayanevet@hotmail.com

Paula Fassicolo Variza

ORCID: <https://orcid.org/0000-0001-9677-4438>
Universidade do Sul de Santa Catarina, Brazil
E-mail: paula_fas@hotmail.com

Joice Guilherme de Oliveira

ORCID: <https://orcid.org/0000-0001-7200-8720>
Universidade do Sul de Santa Catarina, Brazil
E-mail: joice_o@hotmail.com

Cyntia Michielin Lopes

ORCID: <https://orcid.org/0000-0003-2067-1314>
Universidade do Sul de Santa Catarina, Brazil
E-mail: cyntiamlopes01@gmail.com

Wellington Junior da Silva

ORCID: <https://orcid.org/0000-0001-9377-3800>
Universidade Federal do Rio Grande do Sul, Brazil
E-mail: wellingtonbxo@hotmail.com

Tarcísio de Freitas Milagres

ORCID: <https://orcid.org/0000-0002-7397-1612>
Universidade Federal do Rio Grande do Sul, Brazil
E-mail: tarcisiodefraitasmilagres@gmail.com

Maria Lúcia Rossetti

ORCID: <https://orcid.org/0000-0002-9672-9394>
Universidade Luterana do Brasil, Brazil
E-mail: mrossett@terra.com.br

Fernanda dos Santos Rolim

ORCID: <https://orcid.org/0000-0002-7440-7466>
Universidade do Sul de Santa Catarina, Brazil
E-mail: fernandarolim97@gmail.com

Onilda Santos da Silva

ORCID: <https://orcid.org/0000-0003-4806-8285>
Universidade Federal do Rio Grande do Sul, Brazil
E-mail: onilda.santos@gmail.com

Millena Fernandes

ORCID: <https://orcid.org/0000-0002-4641-1417>
Universidade do Sul de Santa Catarina, Brazil
E-mail: millena.bn.f@gmail.com

Josiane Somariva Prophiro

ORCID: <https://orcid.org/0000-0002-1840-1115>
Universidade do Sul de Santa Catarina, Brazil
E-mail: josiane.prophiro@hotmail.com

Abstract

Visceral leishmaniasis is among the most neglected diseases in the world and especially affects poor populations of some developing or underdeveloped countries, with human disease being a factor in the occurrence of thousands of deaths every year. Visceral leishmaniasis affect domestic dogs, main reservoirs of the *Leishmania* parasite in urban environments. Thus, the aim of this study was to analyze the prevalence of canine visceral leishmaniasis in two non-endemic municipalities located in southern Santa Catarina, as well as to identify the main risk factors for infection in dogs. A cross-sectional study was carried out with dogs living in two Zoonoses Control Centers located in the cities of Tubarão and Criciúma. Blood samples were collected and analyzed by real-time PCR for detection of *Leishmania* infection. Clinical examination and evaluation of the characteristics of the canine population were also carried out. A total of 107 dogs were evaluated, of which 19% were positive for *Leishmania*, and 85% of the positive cases were asymptomatic. In conclusion, we highlight the detection of cases of canine visceral leishmaniasis in two cities that had not yet reported cases. The high rate of positive asymptomatic animals suggests the maintenance of the parasite in the environment.

Keywords: Leishmaniasis; *Canis familiaris*; *Leishmania infantum*; Neglected diseases; Real-time PCR.

Resumo

A leishmaniose visceral está entre as doenças mais negligenciadas no mundo e atinge principalmente populações pobres de alguns países em desenvolvimento ou subdesenvolvidos, sendo a doença humana um fator na ocorrência de milhares de mortes a cada ano. A leishmaniose visceral afeta cães domésticos, principais reservatórios do parasita *Leishmania* em ambientes urbanos. Assim, o objetivo do presente trabalho foi analisar a prevalência de leishmaniose visceral canina em dois municípios não endêmicos localizados no sul de Santa Catarina, bem como, identificar os principais fatores de risco para infecção em cães. Foi realizado um estudo transversal com cães habitantes de dois Centros de Controle de Zoonoses localizados nas cidades de Tubarão e Criciúma. Amostras sanguíneas foram coletadas e analisadas por PCR em tempo real para detecção de infecção por *Leishmania*. Também foi realizado exame clínico e avaliação das características da população canina. Um total de 107 cães foram avaliados, destes, 19% foram positivos para *Leishmania*, sendo 85% dos casos positivos, assintomáticos. Em conclusão, destacamos a detecção de casos de leishmaniose visceral canina em duas cidades que ainda não haviam relatado casos. O alto índice de animais assintomáticos positivos sugere a manutenção do parasito no meio ambiente.

Palavras-chave: Leishmaniose; *Canis familiaris*; *Leishmania infantum*; Doenças negligenciadas; PCR em tempo real.

Resumen

La leishmaniasis visceral es una de las enfermedades más desatendidas del mundo y afecta especialmente a las poblaciones pobres de algunos países en desarrollo o subdesarrollados, siendo las enfermedades humanas un factor en la ocurrencia de miles de muertes cada año. La leishmaniasis visceral afecta a los perros domésticos, principales reservorios del parásito *Leishmania* en entornos urbanos. Así, el objetivo de este estudio fue analizar la prevalencia de leishmaniasis visceral canina en dos municipios no endémicos ubicados en el sur de Santa Catarina, así como identificar los principales factores de riesgo de infección en perros. Se realizó un estudio transversal con perros que viven en dos Centros de Control de Zoonosis ubicados en las ciudades de Tubarão y Criciúma. Se recolectaron y analizaron muestras de sangre mediante PCR en tiempo real para la detección de la infección por *Leishmania*. También se realizó examen clínico y evaluación de las características de la población canina. Se evaluaron un total de 107 perros, de los cuales el 19% fueron positivos para *Leishmania* y el 85% de los casos positivos fueron asintomáticos. En conclusión, destacamos la detección de casos de leishmaniasis visceral canina en dos ciudades que aún no habían reportado casos. La alta tasa de animales positivos asintomáticos sugiere el mantenimiento del parásito en el medio ambiente.

Palabras clave: Leishmaniasis; *Canis familiaris*; *Leishmania infantum*; Enfermedades desatendidas; PCR en tiempo real.

1. Introduction

Visceral leishmaniasis (VL) is an infectious disease, caused by *Leishmania* protozoan transmitted to humans and canids through the infecting bite of female phlebotomine sandflies (Ministério da Saúde, 2009). In Brazil and worldwide, VL is considered one of the most important parasitic protozoan diseases for public health due to its high morbidity and mortality, as well as the large number of unreported cases (Gontijo & Melo, 2004). In Brazil this disease is caused by *Leishmania infantum* and its main vector is the phlebotomine *Lutzomyia longipalpis* (Michalsky et al., 2002).

Until the 1990s, about 90% of VL cases throughout Brazil were concentrated in the Northeast Region. However, in recent years the disease has begun to expand to the North, Midwest and Southeast regions of Brazil (Werneck, 2010), with emphasis on the urbanized profile of VL recently evidenced, for example, in Minas Gerais (2020) (da Silva et al., 2020). The

Southern region recorded the lowest number of cases in the whole country (Martins-Melo et al., 2014), and until 2007 it was considered without risk of transmission to VL, when only a few imported human cases have been reported (Ministério da Saúde, 2010). However, there is an increase in the number of VL cases in the south region, as observed in the states of Rio Grande do Sul and Paraná, and even in some regions of Argentina. Until 2006, southern Brazil, Argentina and Uruguay were considered non-endemic areas. After outbreaks of VL in the city of Asunción, Paraguay, the first autochthon case of human VL in Argentina was reported in the city of Posadas, bordering Paraguay (Salomon et al., 2008; Acardi & Liotta, 2010). In 2009, the first outbreak of VL occurred in southern Brazil, was in São Borja, RS, a city bordering Argentina, 160 km from Posadas (Deboni et al., 2011). Also in Rio Grande do Sul, an epidemiological study by Riboldi et al. (2018), showed positive cases of canine leishmaniasis in three cities in the metropolitan region of Porto Alegre, concluding a prevalence of 4% (Riboldi et al., 2018). Santa Catarina, in particular, was a state considered VL-free until 2012, when Figueiredo et al. (2012) reported the first autochthone cases of canine visceral leishmaniasis (CVL) registered in Florianópolis, the capital of Santa Catarina (Figueiredo et al., 2012).

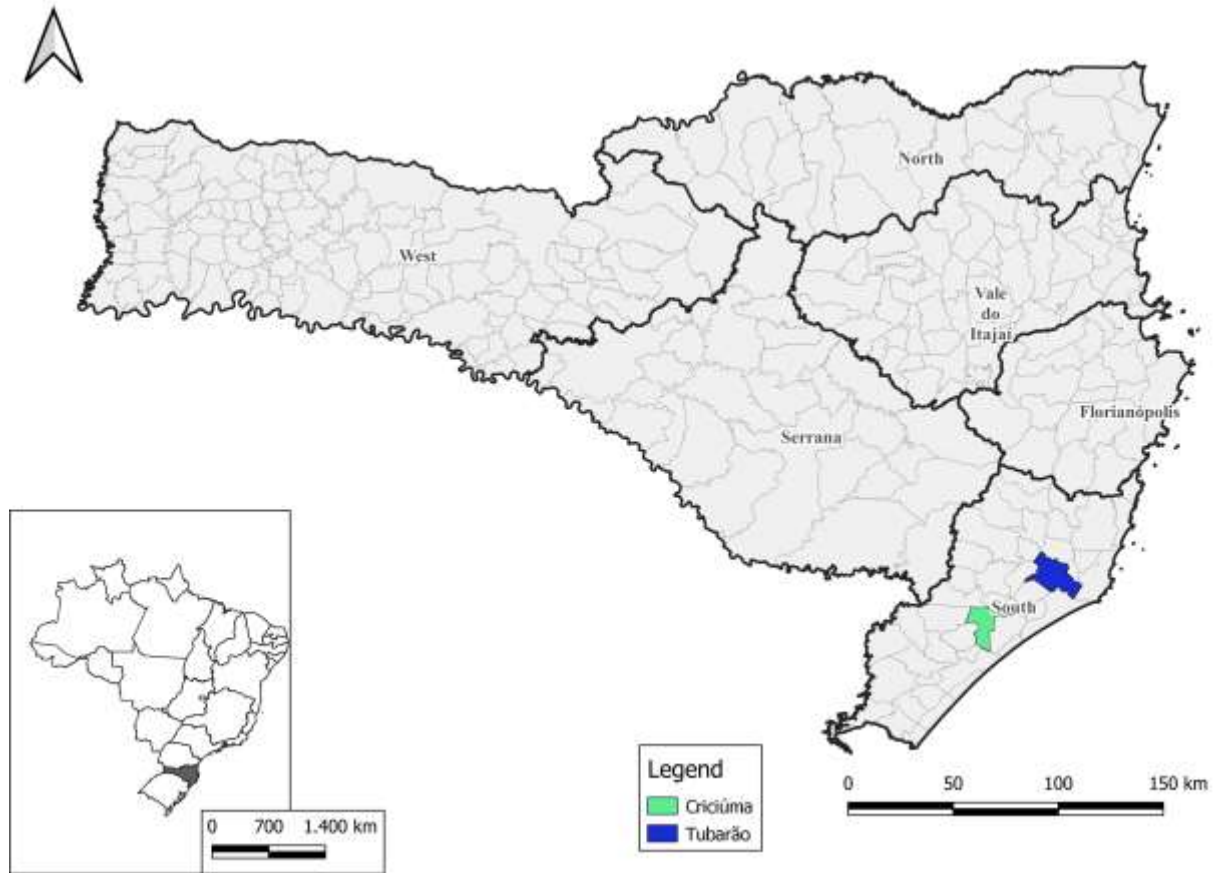
Although certain measures established for the control of the disease may reduce transmission for a long period of time, there are studies indicating that about 80% of infected dogs from endemic areas can exhibit the asymptomatic form of leishmaniasis (Dantas-Torres & Brandão-Filho, 2006). Thus, these animals act as active sources of infection and go unnoticed by current serological tests, as already described by Carvalho et al. (2018), who compared serological tests and the PCR technique, detecting *Leishmania* DNA in asymptomatic dogs that had negative results in serology. The use of molecular techniques in the search for the prevalence of CVL in non-endemic areas of VL can provide important data to Brazilian health systems and contribute to disease prevention strategies in humans, since canine infection precedes disease in humans (Marzochi et al., 2009). Thus, the aim of this study was to analyze the prevalence of canine visceral leishmaniasis in two non-endemic municipalities located in southern Santa Catarina, as well as to identify the main risk factors for infection in dogs.

2. Material and Methods

2.1 Study area

For this study, the municipalities of Tubarão (28° 28' 00" S and 49° 00' 25" O) and Criciúma (28° 40' 42" S and 49° 22' 13" O) were chosen (Figure 1), since they are the largest cities in the southern region of Santa Catarina and the only two ones with active Zoonosis Control Centers (ZCC's). Both municipalities cover approximately 537,000 km² of area and have about 323,733 inhabitants. It is estimated that there are around 80.000 dogs in the region. In relation to the urbanization, these cities have from extensive rural areas to places with high urban population density (IBGE, 2020).

Figure 1: Localization of Santa Catarina, Brazil, evidencing the municipalities where the study was conducted.



Source: Authors.

2.2 Data collection

For this study, blood of sheltered dogs from two ZCC's were evaluated for CVL. The animals were previously identified with collars containing an identification number, or by the registration number of a local non-governmental organization. Data collection regarding the evaluated animals was performed from the completion of a survey to obtain the following information: date of application, identification of the animal, municipality, race, type of pelage, age, sex, reproductive status, state and municipality of their origin, and responsible for the animal (Non-governmental organization, Caregiver, ZCC's, others). Also, current home time and location (urban/rural), animal health conditions (healthy/suspicious), being considered suspected clinical signs of leishmaniasis: dermatopathies in general, keratoconjunctivitis, progressive slimming and onicogriphosis. This questionnaire was completed through anamnesis and by clinical observation of the dogs by a veterinarian.

2.3 Samples

The samples were defined by means of a census, where all the animals' inhabitants of the sites were included. Animals with lower volume of blood collected than that necessary for analysis (5 ml) were excluded. In the collections, all animals living in places with higher concentration of dogs from each municipality were included, being healthy or with CVL symptoms, under the responsibility of the ZCC's. A 5ml of blood and a 2.5ml sample were collected from each dog and stored in a 2.6ml S-Monovette tube containing EDTA. The other sample was placed in a 2.6 ml S-Monovette tube with clot activator and stored in a refrigerator at a temperature of 2 to 8°C for a maximum of 24 hours. The contents of the tubes were centrifuged in centrifuge Fanem Excelsa Baby II at 3500 rpm for 10 minutes to obtain the serum or plasma, according to the material

collected. The material resulting from centrifugation was stored in Eppendorf tubes and packed at -20° C.

2.4 Real-time PCR amplification

For the identification of dogs positive for *Leishmania* infection, the DNA was isolated from dog serum samples (200 µl) using the commercial Nucleic Acid and Protein Purification kit (Macherey-Nagel), according to the manufacturer's instructions. The primers 13A (5'-GTG GGG GAG GGG CGT TCT-3') and 13B (5'-ATT TTA CAC CAA CCC CCA GTT-3') were used as described by Rodgers, Popper and Wirth (1990) 64 to amplify a region of 120 base pairs of *Leishmania* minicircles. Real-time PCR amplification was performed on a StepOne thermocycler™ Real Time PCR Systems (AB Applied Biosystems, Foster City) and amplified products were detected using the SYBRfluorophore® Green. The reaction was standardized in a final volume of 20 µL, containing 15 µL of Fast SYBR® Green mastermix (AB Applied Biosystems, Foster City), 10 pmol of each primer and 5 µL of DNA extracted from canine serum. Amplification conditions were: activation of the enzyme at 95°C for 20 seconds, denaturation at 95°C for 1 second and, finally, ringing and extension at 61°C for 20 seconds. For each amplification, a negative control (reaction mix) was used and purified *Leishmania* DNA was used as a positive control. All samples were made in duplicate as a form of evidence. The sample was defined as positive when it had a detectable cycle threshold (Ct) and the melting temperature (Tm) was the same as for the positive control (Rolim et al., 2016). The amplification and dissociation curves were analysed using the StepOne™equipment software.

2.5 Data processing and analysis

Pearson's chi-square method, Fisher's exact test and likelihood ratio were used for statistical analysis, according data available. All analyses were performed using the software OpenEpi 3.01 and SPSS 21.0.

2.6 Ethical principles

This study was approved by the Ethics Committee on the Use of Animals - CEUA/UNISUL to assess compliance with Normative Resolution No. 12 of September 20, 2013, under no. 16.050.2.13.IV.

3. Results

A total of 107 dog samples were analyzed, of which 77 dogs were from Tubarão (72%) and 30 from Criciúma (28%). Of these, 34 animals were classified as puppies (up to 11 months), 67 adults (from one to seven years) and six elderly (more than seven years). Also, 67 were castrated (62.6%) and 40 non-neutered (37.4%). Regarding location, 30 animals were from urban areas (28%) and 77 from rural areas (72%) (Table 1). Concerning clinical evaluation, 83 animals showed no symptoms of CVL (77.6%) and 24 were considered suspects, 19 dogs presenting only dermatopathies (79.2%). One dog was only slimming (4.2%) and another was affected by keratitis (4.2%). Three dogs had more than one associated sign, two with dermatopathies and onychogryphosis and one with dermatopathies and keratitis.

Table 1: Characteristics of the dog's population collected in the municipalities of Santa Catarina.

	Tubarão		Criciúma		Total	
	n	%	n	%	n	%
Breed						
Without	76	98,7	29	96,7	105	98,1
Pure	1	1,3	1	3,3	2	1,9
Pelage						
Small	69	89,6	28	93,3	97	90,7
Long	8	10,4	2	6,7	10	9,3
Age						
Puppy	16	20,8	18	60	34	31,8
Adult	58	75,3	9	30	67	62,6
Old	3	3,9	3	10	6	5,6
Gender						
Male	44	57,1	17	56,7	61	57
Female	33	42,9	13	43,3	46	43
Castration						
Castrated	47	61	20	66,7	67	62,6
Non-castrated	30	39	10	33,3	40	37,4
Localization						
Urban	0	0	30	100	30	28
Rural	77	100	0	0	77	72
Suspicion						
Healthy	66	85,7	17	56,7	83	77,6
Suspect	11	14,3	13	43,3	24	22,4
Signs						
Dermatopathies	9	11,7	10	33,3	19	17,8
Keratoconjunctivitis	1	1,3	0	0	1	0,9
Onychogryphosis	0	0	0	0	0	0
Slimming	0	0	1	3,3	1	0,9
Dermatopathies + Keratoconjunctivitis	1	1,3	0	0	1	0,9
Dermatopathies + Onychogryphosis	0	0	2	6,7	2	1,9

Source: Authors.

From the 107 dog samples evaluated, 20 were positive for the detection of *Leishmania* sp. DNA by qPCR, indicating a prevalence of 19%. Two dogs showed indeterminate results. Of the total number of positive dogs, 13 were from Tubarão (65%) and seven from Criciúma (35%). Analyzing the risk factors, none of the variables studied presented statistical significance (Table 2).

Table 2 - Analysis of some risk factors for *Leishmania* sp. infection in 107 dogs adopted by two Zoonosis Control Centers in Tubarão and Criciúma, Santa Catarina. (-) means negative (+) means positive and (I) indeterminate.

	(-)	%	(+)	%	(I)	%	P*
Breed							
Without	83	79	20	19	2	1,9	0,491
Pure	2	100	0	0	0	0	
Pelage							
Small	79	81,4	16	16,5	2	2,1	0,208
Long	6	60	4	40	0	0	
Age							
Puppy	27	79,4	7	20,6	0	0	0,63
Adult	54	80,6	11	16,4	2	3	
Old	4	66,7	2	33,3	0	0	
Gender							
Male	48	78,7	11	18	2	3,3	0,58
Female	37	80,4	9	19,6	0	0	
Castration							
Castrated	52	77,6	13	19,4	2	3	0,396
Non-castrated	33	82,5	7	17,5	0	0	
Localization							
Urban	23	76,7	7	23,3	0	0	0,9
Rural	62	80,5	13	16,9	2	2,6	
Suspicion							
Healthy	65	78,3	17	20,5	1	1,2	0,849
Suspect	20	83,3	3	12,5	1	4,2	
Signs							
Dermatopathies	16	84,2	2	10,5	1	5,3	
Keratoconjunctivitis	0	0	0	0	0	0	
Onychogryphosis	0	0	1	100	0	0	
Slimming	1	100	0	0	0	0	
Dermatopathies + Keratoconjunctivitis	2	100	0	0	0	0	
Dermatopathies + Onychogryphosis	0	0	0	0	1	100	

* Pearson chi-square test. Source: Authors.

4. Discussion

This study evaluated the prevalence of CVL in shelter dogs from two municipalities in southern Santa Catarina, using the qPCR technique to detecting the *Leishmania* DNA. The results showed that the prevalence of infection in the total number of animals evaluated (n=107) was 19% between the two municipalities studied, 16.9% in Tubarão and 23.3% in Criciúma. These regions had not yet registered any cases of the disease in dogs, although some cases have already been registered in other regions of Santa Catarina. In Florianópolis Steindel et al., (2013), evaluated dogs with suspected *Leishmania* infection between 2010 and 2011 and found positive animals with a prevalence of 1.4%. In the western region of this state, Maziero et al., (2014) also found a prevalence of 21.8% of CVL (Maziero et al., 2014).

As in Santa Catarina, in areas previously considered non-endemic for CVL in other states of Brazil (Steindel et al., 2013; Maziero et al., 2014), showed a similar situation when using the PCR technique for the testing of dogs as recorded in some municipalities of Rio de Janeiro (De Paula et al., 2009; Campos et al., 2013) and Minas Gerais (Castro et al., 2019). In the metropolitan region of Porto Alegre (RS), a non-endemic region, a prevalence of 4% was observed through real time PCR and serology, being the essential molecular method for excluding false negatives and false positives (Riboldi et al., 2018). It is very likely that CVL transmission is already occurring among dogs in other municipalities located in the south of Santa Catarina, increasing the chances of human infection (Ministério da Saúde, 2009). In relation to the tests to evaluate CVL infection, there are many variations in the sensitivity and specificity of serological methods. So, it is important to perform confirmatory tests to establish the diagnosis of CVL in non-endemic areas. However, it is important to note that even using tests with lower specificity, cases of CVL in non-endemic regions were verified, such as in Florianópolis-SC (Corrêa et al., 2010), Joinville-SC (Pacheco et al., 2013) and Poxoreo-MT (Azevedo et al., 2008).

In relation to the phlebotomine, in 2017, a survey of sandflies fauna was carried out in southern Santa Catarina, and its presence was identified (Variza et al., 2021), corroborating the high prevalence of CVL described in our results. The association between the occurrence of the parasite and the presence of the vector in the same environment favors conditions for the establishment of the disease. Therefore, it is a matter of time for the appearance of human cases, if there are no vector and canine reservoir control.

According to the Brazilian Ministry of Health (2006), dogs cohabiting urban areas have a higher rate of positivity for *Leishmania* than those from rural areas in several regions of Brazil. In Santa Catarina, this pattern seems to be confirmed. With the increased incidence of CVL in urban areas, associated with the sinantropism of some species of sandflies and the spread of reservoir hosts to anthropic environments (González et al., 2014), the State of Santa Catarina needs urgent care by public health agencies. In a negative way the population is subject to become infected and the region endemic in CVL.

Regarding the risk factors associated with dogs, there was a higher prevalence among animals older than seven years (33.3%), followed by animals up to 11 months old (20.6%). Similar results were also reported by França-Silva et al., (2003). However, advanced age does not seem to be a risk factor, since other studies suggest that younger animals pose a higher risk for CVL (Moreira et al., 2003). As in others studies (França-Silva et al., 2003; Silva et al., 2018). we also found no significant association between gender and prevalence of CVL.

From the positive animals, 85% were asymptomatic, which may have been a great reason for the dissemination and maintenance of CVL in the region. It is known that the dogs, even without symptoms, can have high parasitic load and act as a reservoir of the parasite, having an important role in the transmission through the vector (Steindel et al., 2013). Also, as reported in other study (Azevedo et al., 2008), skin lesions were the most frequently observed clinical signs in seropositive and symptomatic animals. The study presents limitations inherent to a cross-sectional approach, such as the impossibility of verifying causality. It should be considered the limitation that the form of study was carried out by census of zoonosis centers, not knowing for sure which specific location this dog belonged to, nor if the contamination occurred in the city or in the kennel itself.

5. Conclusion

In conclusion, we highlight the detection of cases of CVL in two cities that had not yet reported cases. It was not possible to define any significance among the proposed variables as risk factors for infection. However, the high rate of positive asymptomatic animals suggests the maintenance of the parasite in the environment. There are numerous socioeconomic and scientific challenges that could prevent the success of the control and prevention of this disease in all

regions. Efforts and research to understand the dynamics of CVL transmission in the region are necessary to avoid the expansion of canine cases and the consequent human infection.

Acknowledgments

We thank Diogo Tavares Cardoso for the elaboration of a map for the study area.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Acardi, S. A., Liotta, D. J., Santini, M. S., Romagosa, C. M., & Salomón, O. D. (2010). Detection of *Leishmania infantum* in naturally infected *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae) and *Canis familiaris* in Misiones, Argentina: the first report of a PCR-RFLP and sequencing-based confirmation assay. *Memórias do Instituto Oswaldo Cruz*, 105, 796-799.
- Azevedo, M. Á. A. D., Dias, A. K. K., Paula, H. B. D., Perri, S. H. V., & Nunes, C. M. (2008). Avaliação da leishmaniose visceral canina em Poxoréu, Estado do Mato Grosso, Brazil. *Revista Brasileira de Parasitologia Veterinária*, 17, 123-127.
- Campos, M. P. D., Silva, D. A. D., Madeira, M. D. F., Velho, A. A. M., & Figueiredo, F. B. (2013). First autochthonous case of canine visceral leishmaniasis in Volta Redonda, Rio de Janeiro, Brazil. *Revista Brasileira de Parasitologia Veterinária*, 22, 424-426.
- Castro, J. C. D., Bueno, L. L., Milagres, T. D. F., Rêgo, F. D., Gontijo, C. M. F., Peconick, A. P., ... & Barçante, J. M. D. P. (2019). Molecular detection of *Leishmania* spp in *Lutzomyia longipalpis* in the city of Lavras, Minas Gerais, Brazil. *Brazilian Journal of Medical and Biological Research*, 52.
- Corrêa, G. L. B., Indá, F. de M. C., Ota, E. T. dos S., Almeida, E. M. de, Lacovski, R. B. (2011). Inquérito canino censitário de leishmaniose visceral no município de Florianópolis, em 2010.
- da Silva, W. J., Cardoso, D. T., Morais, M. H. F., Carneiro, M., Moraga, P., & Barbosa, D. S. (2020). Spatiotemporal patterns and integrated approach to prioritize areas for surveillance and control of visceral leishmaniasis in a large metropolitan area in Brazil. *Acta Tropica*, 211, 105615.
- Dantas-Torres, F., & Brandão-Filho, S. P. (2006). Visceral leishmaniasis in Brazil: revisiting paradigms of epidemiology and control. *Revista do Instituto de Medicina Tropical de São Paulo*, 48, 151-156.
- de Carvalho, F. L. N., de Oliveira Riboldi, E., Bello, G. L., Ramos, R. R., Barcellos, R. B., Gehlen, M., ... & Rossetti, M. L. R. (2018). Canine visceral leishmaniasis diagnosis: a comparative performance of serological and molecular tests in symptomatic and asymptomatic dogs. *Epidemiology & Infection*, 146(5), 571-576.
- Deboni, S. C., Barbosa, M., & Ramos, R. R. (2011). Leishmaniose visceral no Rio Grande do Sul. *Bol. Epidemiológico*, 13, 1-3.
- Figueiredo, F. B., de Lima Júnior, F. E. F., Tomio, J. E., Indá, F. D. M. C., Corrêa, G. L. B., & de Fátima Madeira, M. (2012). Leishmaniose Visceral Canina: Dois casos autóctones no município de Florianópolis, estado de Santa Catarina. *Acta Scientiae Veterinariae*, 40(1), 1-4.
- França-Silva, J. C., da Costa, R. T., Siqueira, A. M., Machado-Coelho, G. L., da Costa, C. A., Mayrink, W., ... & Nascimento, E. (2003). Epidemiology of canine visceral leishmaniasis in the endemic area of Montes Claros Municipality, Minas Gerais State, Brazil. *Veterinary parasitology*, 111(2-3), 161-173.
- Gontijo, C. M. F., & Melo, M. N. (2004). Leishmaniose visceral no Brasil: quadro atual, desafios e perspectivas. *Revista Brasileira de epidemiologia*, 7, 338-349.
- González, C., Paz, A., & Ferro, C. (2014). Predicted altitudinal shifts and reduced spatial distribution of *Leishmania infantum* vector species under climate change scenarios in Colombia. *Acta tropica*, 129, 83-90.
- IBGE. IBGE, Cidades e Estados [Internet]. (2020) [cited 2020 Nov 3]. Available from: <https://www.ibge.gov.br/cidades-e-estados/sc/tubarao.html>
- Martins-Melo, F. R., Lima, M. D. S., Ramos Jr, A. N., Alencar, C. H., & Heukelbach, J. (2014). Mortality and case fatality due to visceral leishmaniasis in Brazil: a nationwide analysis of epidemiology, trends and spatial patterns. *PloS one*, 9(4), e93770.
- Marzochi, M. C. D. A., Fagundes, A., Andrade, M. V. D., Souza, M. B. D., Madeira, M. D. F., Mouta-Confort, E., ... & Marzochi, K. B. F. (2009). Visceral leishmaniasis in Rio de Janeiro, Brazil: eco-epidemiological aspects and control. *Revista da Sociedade Brasileira de Medicina Tropical*, 42, 570-580.
- Maziero, N., Thomaz-Soccol, V., Steindel, M., Link, J. S., Rossini, D., Alban, S. M., & Nascimento, A. J. (2014). Rural-urban focus of canine visceral leishmaniasis in the far western region of Santa Catarina State, Brazil. *Veterinary parasitology*, 205(1-2), 92-95. <https://doi.org/10.1016/j.vetpar.2014.06.005>

Michalsky, E. M., Fortes-Dias, C. L., Pimenta, P. F., Secundino, N. F., & Dias, E. S. (2002). Assessment of PCR in the detection of *Leishmania* spp in experimentally infected individual phlebotomine sandflies (Diptera: Psychodidae: Phlebotominae). *Revista do Instituto de Medicina Tropical de São Paulo*, 44, 255-259.

Ministério da Saúde. Doenças negligenciadas: estratégias do Ministério da Saúde. *Rev Saúde Pública*. 2010; 44(1):200–2.

Ministério da Saúde. Guia de vigilância epidemiológica. (7th ed.) 2009. 816pp.

Ministério da Saúde. Manual de Vigilância e Controle da Leishmaniose Visceral. In: Série A Normas e Manuais Técnicos. Brasília; 2006. p. 1–120.

Moreira Júnior, E. D., Souza, V. M. M. D., Sreenivasan, M., Lopes, N. L., Silva, R. B. B. D., & Pontes-de-Carvalho, L. C. (2004). Peridomestic risk factors for canine leishmaniasis in urban dwellings: new findings from a prospective study in Brazil. *American Journal of Tropical Medicine and Hygiene*, v. 69, n. 4, p. 393–397.

Pacheco, A. D., Laurenti, M. D., de LIMA, V. M. F., Tomokane, T. Y., & Marcondes, M. (2013). *Leishmania* sp. infection in dogs from Florianópolis, Santa Catarina, SC, Brazil. *Braz. j. vet. res. anim. sci*, 50(3), 220-225.

Paula, C. C. D., Figueiredo, F. B., Menezes, R. C., Mouta-Confort, E., Bogio, A., & Madeira, M. D. F. (2009). Leishmaniose visceral canina em Maricá, Estado do Rio de Janeiro: relato do primeiro caso autóctone. *Revista da Sociedade Brasileira de Medicina Tropical*, 42(1), 77-78.

Riboldí, E., Carvalho, F., Romão, P. R. T., Barcellos, R. B., Bello, G. L., Ramos, R. R., ... & Dallegrove, E. (2018). Molecular method confirms canine *Leishmania* infection detected by serological methods in non-endemic area of Brazil. *The Korean journal of parasitology*, 56(1), 11.

Rolim, F., de Carvalho, F. L. N., Bello, G. L., Gehlen, M., Halon, M. L., Lemos, R. R., & Barcellos, R. B. (2016). Leishmaniose visceral canina: detecção de DNA em soro por PCR em tempo real. *Revista de Iniciação Científica da ULBRA*, (14).

Salomón, O. D., Sinagra, A., Nevot, M. C., Barberian, G., Paulin, P., Estevez, J. O., ... & Estevez, J. (2008). First visceral leishmaniasis focus in Argentina. *Memórias do Instituto Oswaldo Cruz*, 103, 109-111.

Silva, R. B. S., Porto, M. L., Barbosa, W. D. O., Souza, H. C. D., Marques, N. F. D. S. P., Azevedo, S. S., ... & Melo, M. A. D. (2018). Seroprevalence and risk factors associated with canine visceral leishmaniasis in the State of Paraíba, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, 51, 683-688.

Steindel, M., Menin, Á., Evangelista, T., Stoco, P. H., Marlow, M. A., Fleith, R. C., ... & Grisard, E. C. (2013). Outbreak of autochthonous canine visceral leishmaniasis in Santa Catarina, Brazil. *Pesquisa Veterinária Brasileira*, 33, 490-496.

Variza, P. F., Pereira, T. N., de Oliveira, J. G., Fernandes, M., de Avelar, D. M., de Freitas Milagres, T., ... & Prophiro, J. S. (2021). Notes on the sand fly fauna (Diptera: Psychodidae) in a region of Brazil. *Research, Society and Development*, 10(14), e596101422480-e596101422480.

Werneck GL. (2010). Expansão geográfica da leishmaniose visceral no Brasil. *Cad Saude Publica*. 26(4):644–5.