Revista Contexto & Saúde
Editora Unijuí
Programa de Pós-Graduação em Atenção Integral à Saúde
ISSN 2176-7114 – v. 24, n. 48, 2024
http://dx.doi.org/10.21527/2176-7114.2024.48.14980

HOW TO CITE:
Santos JB de S, dos Santos J, Santos SB de S, Silva A de S, Campos RN de S, Dória GAA. Visceral leishmaniasis seroprevalence and associated sociocultural factors in an endemic area of Sergipe, northeastern Brazil. Rev. Contexto & Saúde, 2024;24(48):e14980

ORIGINAL ARTICLE

Visceral Leishmaniasis Seroprevalence and Associated Sociocultural Factors in an Endemic Area of Sergipe, Northeastern Brazil

Jamisson Bispo de Sousa Santos[1]; Joserlândia dos Santos[2]
Samuel Bispo de Sousa Santos[3]; Anita de Souza Silva[4]
Roseane Nunes de Santana Campos[5]; Grace Anne Azevedo Dória[6]

Highlights:
1. Identification of risk areas for cases of Visceral Leishmaniasis in dogs and humans.
2. Highlights the factors that contributed to the expansion of the disease in Lagarto-SE.
3. Providing essential information for surveillance actions.

ABSTRACT

The present study aimed to investigate the presence of anti-Leishmania antibodies in samples of domiciled dogs and associate them with epidemiological indicators in the city of Lagarto, Sergipe. The diagnosis of Canine Visceral Leishmaniasis (CVL) was carried out in 755 dogs, where the rapid two-way platform immunochromatographic test (TR-DPP) (Fiocruz/Biomanguinhos) and the serological test of Enzyme-Linked Immunosorbent Assay (ELISA) were applied. The results showed that all homes with seroreactive dogs were close to abandoned land, squares and/or green areas. There was a significant association between seroactive animals and type of shelter (p=0.008); 54.8% had an indoor shelter and 45.2% of the dogs had a shelter outside their homes. The disease is geographically distributed in 14 (73.68%) of the 19 sample areas in the municipality. Of these, five (26.31%) had cases of Visceral Leishmaniasis in dogs and humans. Eight cases of Human Visceral Leishmaniasis (HVL) were reported during the years 2017 to 2020 in the municipality. Dogs that are located in areas of greatest risk impact the maintenance of natural foci of Leishmaniasis transmission to human and animal hosts in areas endemic for Visceral Leishmaniasis. Such results are essential to assist epidemiological surveillance in the municipality of Lagarto-SE with the implementation of control measures aimed at the insect vector of the disease, the sand fly, to prevent human and canine cases.

Keywords: epidemiology; protozoa; leishmaniasis; public health; zoonotic disease.

SOROPREVALÊNCIA DE LEISHMANIOSE VISCERAL E FATORES SOCIOCULTURAIS ASSOCIADOS EM UMA ÁREA ENDÊMICA DE SERGIPE, NORDESTE DO BRASIL

RESUMO

O presente estudo teve por objetivo investigar a presença de anticorpos anti-*Leishmania* em amostras de cães domiciliados e associar com indicadores epidemiológicos no município de Lagarto, Sergipe. Foi realizado o diagnóstico de Leishmaniose Visceral Canina (LVC) em 755 cães, quando se aplicou o teste imunocromatográfico rápido de plataforma de dois caminhos (TR-DPP) (Fiocruz/Biomanguinhos), e para confirmação de casos sororreagentes o teste sorológico de Ensaio Imunoabsorvente Ligado à Enzima (Elisa). Os resultados demonstraram que todas as residências com cães sororreagentes estavam próximas a terrenos abandonados, praças e/ou áreas verdes. Houve associação entre animais sororreagentes e o tipo de abrigo (p=0,008); 54,8% apresentavam abrigo intradomiciliar e 45,2% dos cães apresentavam abrigos em áreas externa das residências. A doença está distribuída geograficamente em 14 (73,68%), das 19 áreas amostrais do município. Dessas, cinco (26,31%) apresentaram casos de Leishmaniose Visceral em cães e humanos. Foram notificados oito casos de Leishmaniose Visceral Humana (LVH) durante os anos de 2017 a 2020 no município. Os cães que estão localizados nas áreas de maior risco impactam na manutenção de focos naturais de transmissão de Leishmaniose para hospedeiros humanos e animais em áreas endêmicas para Leishmaniose Visceral. Tais resultados são essenciais para auxiliar a vigilância epidemiológica no município de Lagarto-SE, com a introdução de medidas de controle voltadas ao inseto vetor da doença, o flebotomíneo, para prevenir casos humanos e caninos.

[1] Universidade Federal de Sergipe. Lagarto/SE, Brasil. https://orcid.org/0000-0003-3329-9654
[2] Universidade Federal de Sergipe. Nossa Senhora da Glória/SE, Brasil. https://orcid.org/0000-0002-0607-8736
[3] Universidade Federal de Sergipe. Nossa Senhora da Glória/SE, Brasil. https://orcid.org/0000-0002-6093-2390
[4] Universidade Federal de Sergipe. Lagarto/SE, Brasil. https://orcid.org/0000-0003-0478-8264
[5] Universidade Federal de Sergipe. Lagarto/SE, Brasil. https://orcid.org/0000-0001-6217-9278
[6] Universidade Federal de Sergipe. Lagarto/SE, Brasil. https://orcid.org/0000-0002-2454-6775

Palavras-chave: epidemiologia; protozooses; leishmaniose; saúde pública; zoonoses.

INTRODUCTION

Visceral leishmaniasis (VL), a serious zoonotic disease, is a threat to public health, mainly affecting children, young adults, the elderly, and immunocompromised people, with up to 95% mortality if left untreated[1]. However, this transmission depends on vectors, the availability of several different types of reservoirs, climatic seasonality, and the existence of risk groups susceptible to parasitic loads[2].

Visceral leishmaniasis has been reported as a reemerging disease, demonstrating clear epidemiological transition. Moreover, its incidence has increased in recent years in areas where it did not originally occur. However, VL cases are poorly reported, with underreporting or unknown incidence in some countries[3].

In Brazil, VL is characterized by severe endemic and epidemic conditions. It is able to be transmitted to hosts through blood-feeding by infected females of the genus *Lutzomyia; Lutzomyia longipalpis* is the species with the highest recorded number in Brazil[4]. In 2019, according to data from the Ministry of Health, Brazil had 2,471 cases of VL, of which 1,398 cases were reported to be widely distributed in the Northeast Region. The state of Sergipe had 55 new VL cases in 2019, and the fatality rate was 14.3%, which is high for Brazil. Thus, Sergipe is considered to be a state with high transmission rates and is of great epidemiological importance for studying VL incidence[5].

In the city of Lagarto, Sergipe, the main urban parasite reservoir is the numerous owned dogs (*Canis lupus familiaris*) that do not have an established epidemiological profile[3].Moreover, as the disease has higher prevalence in dogs than humans, they attract vector insects and are sources of infection. Consequently, conducting canine serological surveys turns out to be essential to identify regions with a higher risk of disease transmission[6-7]. Thus, we aimed to analyze the prevalence of *anti--leishmania antibodies* in samples from owned dogs and investigate the epidemiological indicators to identify the areas where there is a great risk of VL transmission in the city of Lagarto, Sergipe.

MATERIAL AND METHODS

Description of the study area

The study area was the city of Lagarto, south of the center of the state of Sergipe, in northeastern Brazil (Figure 1). Lagarto is the third most populous city in Sergipe, with 105,221 inhabitants. It has a tropical climate with an average temperature of 24.5 °C and an average precipitation of 1,138.1 mm. Its rainy season extends from March to August. It is subdivided into 19 health administrative areas, which are further divided into 13 urban areas and six rural areas. It is located at 10°55’02”S 37°39’00”W, at an altitude of 183 m. The total area of the city is 962.2 km² and is located 75 km away from the state capital, Aracaju.

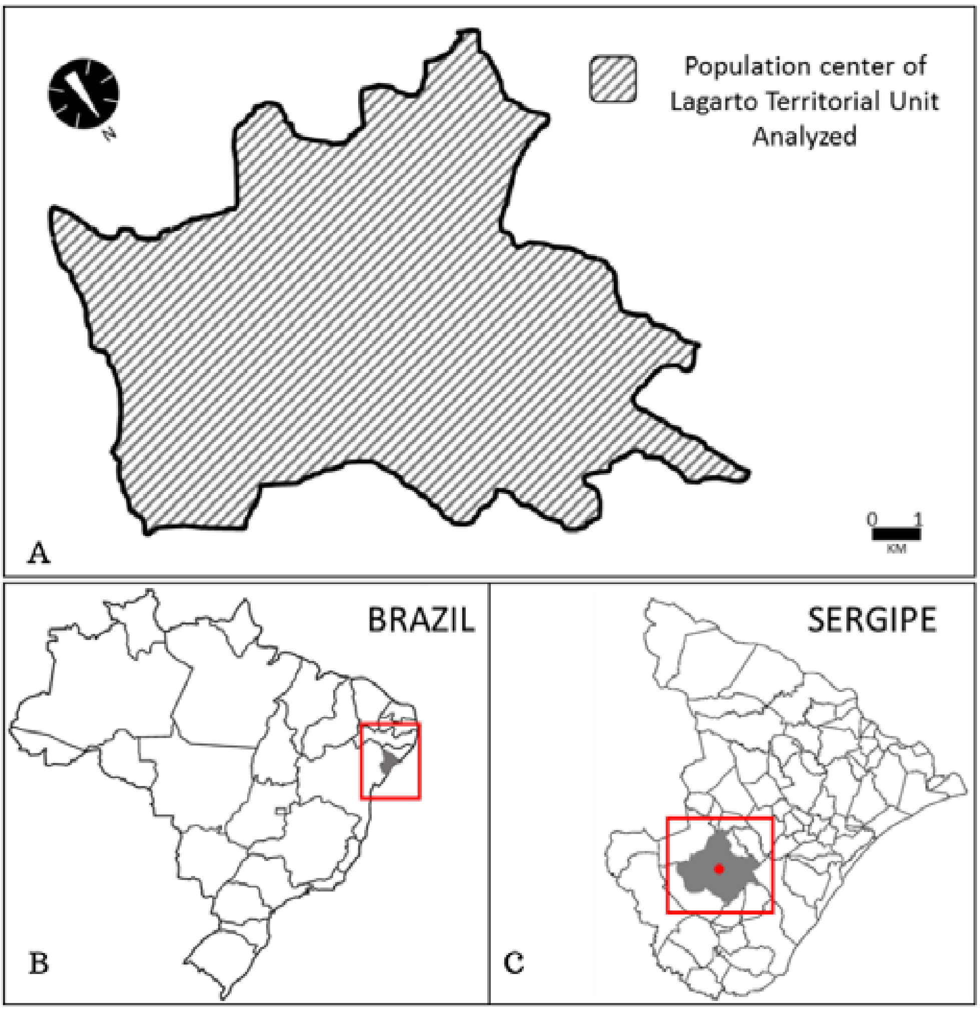

Figure 1 – Detailed view of the study area. A) Map of Lagarto-SE, highlighting the central population core. B) Map of Brazil, emphasizing the location of Lagarto-SE. C) Map of the state of Sergipe, highlighting the municipality of Lagarto and its divisions.

Source: Author himself.

Serological survey of dogs

The sample study was based on the data contained in the cadastral report of the Canine Visceral Leishmaniasis (CVL) investigation and control program, which were obtained through spontaneous demand from the inhabitants and an active search carried out by the Zoonosis Control Center of Lagarto, Sergipe in the period from 2017 to 2020, in which the formula of (8) was used. The general formula for calculating the sample size is:

$$n = \frac{N \cdot Z^2 \cdot p \cdot (1 - p)}{Z^2 \cdot p \cdot (1 - p) + e^2 \cdot (N - 1)}$$

Where:

(n) is the calculated sample size,

(N) is the total population,

(Z) is the chosen confidence level, expressed in the number of standard deviations,

(p) is the actual probability of the event (proportion of the event in the population),

(e) is the desired sampling error.

In order to determine the minimum sample size based on the estimated population average of the municipality of Lagarto, with a sampling error of 4% and the Dog/Inhabitant ratio, the sample average of 1/4 inhabitants per dogs was used. This number represents the national average of inhabitants per domiciled dogs[9]. Serological analysis was performed to evaluate the prevalence of CVL and identify infected dogs from canine sampling or census using two techniques: the dual-path platform immunochromatographic rapid test (TR-DPP), performed by a team from the municipal health department, and Enzyme-Linked Immunosorbent Assay (ELISA), performed by technicians from the Central Public Health Laboratory (Lacen). The TR-DPP is recommended for screening dogs and Elisa for the diagnosis confirmation of identified as those seropositive in the immunochromatographic test[10].

Epidemiological data

The recorded epidemiological variables included health regions: the administrative geographic division used by the municipal health department, types of shelters, and the presence of apparent clinical signs; and physical characteristics: color, coat, sex, and age. Confirmed cases of human visceral leishmaniasis (HVL) in residents were obtained from the Notifiable Disease Information System (SINAN) of the Lagarto's municipal health department. CVL data were obtained from the registration data of the CVL investigation and control program of 755 owned dogs collected at the Zoonosis Control Center in the city of Lagarto between 2017 and 2020.

Data analysis

Data were analyzed descriptively and inferentially, using Statistical Package for the Social Sciences software (SPSS 25.0) and Microsoft Excel 2013. The nominal qualitative variables, absolute frequency, and percentage relative frequency were calculated for the descriptive analysis. The inferential analysis of the association between nominal qualitative variables was performed using Pearson's Chi-Square Test. A significance level of 5% was used for the inference analyses.

Spatial analysis

The epidemiological surveillance data were tabulated in Microsoft Excel 2013. Distribution maps were drafted, and spatial analyses were performed, using Autodesk Viewer software. The cartography of the city of Lagarto was provided by the geoprocessing sector of the city's municipal health department.

Ethical aspects

This research was conducted as per the guidelines laid by the Ethical Principles of Animal Experimentation of the National Council for the Control of Animal Experimentation (Concea; MCTIC, 2016). The Ethics Committee on the Use of Animals of the Federal University of Sergipe (Ceua/UFS) approved the study protocol (Ceua Nº 7403131020).

RESULTS AND DISCUSSION

The study population comprised 61% (461/755) male dogs and 38.9% (294/755) female dogs, with a mean age between 24 and 72 months; their values were close to those obtained by (11), who found a prevalence of 68.2% males and 31.3% of females. In the present study, the majority 95.8% (724/755) of the dogs had short fur, 67% (506/755) had light colored fur, and 99% were mongrels

(748/755). A percentage of 90.6% (684/755) were asymptomatic, 72.9% (551/755) slept outside their homes, 99.4% (751/755) had contact with mixed breed dogs, 99.8% (754/755) with wild animals and 46.6% (352/755) with abandoned land (Table 1).

Table 1 – Correlation between the Elisa test result and epidemiological indicators in the diagnosis of Canine Visceral Leishmaniasis

			Elisa Reagent	 Non reactive	Total	Value	df**	p-value
BASIC SANITATION	Yes	n	19	17	36	0,031	1	0,861
		%	45,2%	47,2%	46,2%			
	Not	n	23	19	42			
		%	54,8%	52,8%	53,8%			
PRESENCE OF AREAS GREEN SQUARES	Yes	n	42	36	78	.		
		%	100,0%	100,0%	100,0%			
ABANDONED LAND	Yes	n	24	22	46	0,126	1	0,722
		%	57,1%	61,1%	59,0%			
	Not	n	18	14	32			
		%	42,9%	38,9%	41,0%			
DOMICILED	Yes	n	42	36	78	.		
		%	100,0%	100,0%	100,0%			
SEX	Female	n	13	11	24	0,001	1	0,970
		%	31,0%	30,6%	30,8%			
	Male	n	29	25	54			
		%	69,0%	69,4%	69,2%			
AGE	4-24 months	n	6	7	13	0,484	2	0,785
		%	14,3%	19,4%	16,7%			
	24-72 months	n	25	19	44			
		%	59,5%	52,8%	56,4%			
	>72 months	n	11	10	21			
		%	26,2%	27,8%	26,9%			
COAT	Long hair	n	4	2	6	0,43	1	0,512
		%	9,5%	5,6%	7,7%			
	short hair	n	38	34	72			
		%	90,5%	94,4%	92,3%			
COLORING	Clear	n	19	24	43	3,598	1	0,058
		%	45,2%	66,7%	55,1%			
	Dark	n	23	12	35			
		%	54,8%	33,3%	44,9%			
BREED	NDR*	n	40	36	76	1,759	1	0,185
		%	95,2%	100,0%	97,4%			
	Lineage animals	n	2	0	2			
		%	4,8%	0,0%	2,6%			

TYPE OF SHELTER	Intra-household	n	23	9	32	7,097	1	0,008*
		%	54,8%	25,0%	41,0%			
	Extra household	n	19	27	46			
		%	45,2%	75,0%	59,0%			
CLINICAL SIGNS	There are no obvious signs	n	22	26	48	3,224	1	0,073
		%	52,4%	72,2%	61,5%			
	Has clinical signs	n	20	10	30			
		%	47,6%	27,8%	38,5%			
DERMATOPATHIES	No clinical signs	n	22	25	47	2,357	1	0,125
		%	52,4%	69,4%	60,3%			
	With clinical signs	n	20	11	31			
		%	47,6%	30,6%	39,7%			
OMYCHOGRYPHOSIS	No clinical signs	n	28	34	62	9,173	1	**0,002***
		%	66,7%	94,4%	79,5%			
	With clinical signs	n	14	2	16			
		%	33,3%	5,6%	20,5%			
ANOREXIA	No clinical signs	n	29	34	63	8,05	1	**0,005***
		%	69,0%	94,4%	80,8%			
	With clinical signs	n	13	2	15			
		%	31,0%	5,6%	19,2%			
CONTACT WITH ANIMALS ERRANTS	Yes	n	39	36	75	2,674	1	0,102
		%	92,9%	100,0%	96,2%			
	Not	n	3	0	3			
		%	7,1%	0,0%	3,8%			
CONTACT WITH ANIMALS WILD	Yes	n	41	36	77	0,868	1	0,351
		%	97,6%	100,0%	98,7%			
	Not	n	1	0	1			
		%	2,4%	0,0%	1,3%			

*No Defined Race ** degree off freedom

More than 99% of the surveyed canine population lived in places with at least three risk factors (contact with wild animals, contact with mixed breed dogs, residence close to green areas/squares) for the disease, facilitating transmission. The increase in suitable vector reproduction areas resulted in greater epidemiological vulnerability to the disease, consistent with the findings of Scandar et al. (2011) and Menezes et al. (2016)[12-13].

Of the 755 dogs analyzed, 70.46% (532/755) were in the rural area and 29.54% (223/755) in the urban area. The results of the spatial analysis showed a wide geographic distribution of the disease, occurring in 73.68% (14/19) of the sample areas in the city. Canine and human cases were observed in 26.31% (5/19) of the sample areas. In 26.31% (5/19) of the areas of the city, there were no canine and/or human cases (Figure 2).

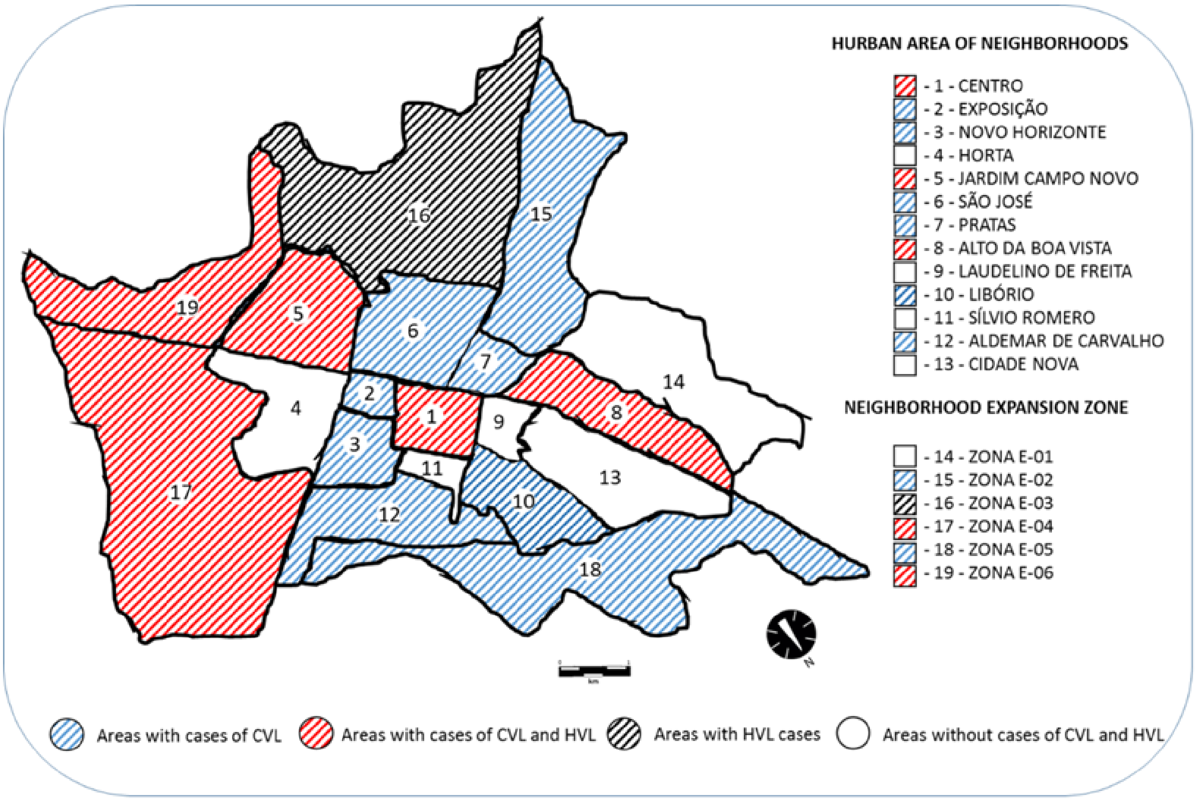

Figure 2 – Map of the municipality of Lagarto SE showing the division areas according to health regions, showing the areas that presented cases of CVL (Canine Visceral Leishmaniasis), HVL (Human Visceral Leishmaniasis). (1) Center: 4 canine cases and 1 human case; (2) Exposure: 2 canine cases; (3) Novo Horizonte: 1 canine case; (4) Vegetable garden: no cases; (5) Jardim Campo Novo: 4 canine cases, 1 human case; (6) São Jorge: 2 canine cases; (7) Silver: 5 canine cases; (8) Alto da Boa Vista: 4 canine cases; (9) Laudelino de Freitas: No cases; (10) Liborio: 2 canine cases; (11) Silvo Romero: no cases; (12) Aldemar de Carvalho: 3 canine cases; (13) Cidade Nova: No cases; (14) Zone E-01: No cases; (15) Zone E-02: 2 canine cases; (16) Zone E-03: 1 human case; (17) Zone E-04: Fourteen canine cases, 3 human cases; (18) Zone E-05: 1 canine case; (19) Zone E-06: 2 canine cases, 1 human case.

Source: Author himself.

A total of 73.1% (552/755) dogs lived with their owners in areas that did not have access to basic sanitation, but green areas were observed in all (100%) of the households. These heavy vegetation areas (roots, tree trunks, and soil organic matter) are conducive to an increased density of sand flies as they are a refuge for the development of the vector[14-15].

Of the 755 dogs, 10.3% were seropositive in the TR-DPP, and of this percentage, only 53.80% were later confirmed as seropositive by ELISA. The high prevalence of seropositive dogs in the TR-DPP was consistent with observations of Brazuna[16]; he demonstrated the interference by trypanosomatids and the members of the *Anaplasmataceae f*amily in the diagnosis of the disease, with TR-DPP false positive results being associated with the presence of parasites, such as *Babesia spp*. or *Neospora caninum*. TR-DPP detects against rK26 and rK39 and has a sensitivity of only 57.45%, asymptomatic animals 12%, symptomatic dogs 88.9% and oligosymptomatic animals 52.4% [11]. This corroborates one of the differences observed between the values of TR-DPP and Elisa in the current research.

There was an association between a seropositive Elisa test for the diagnosis of CVL and the group/year variable (p = 0.001). This finding confirms that there is an exponential growth in the cases of leishmaniasis in the city. Of the 19 analyzed areas, 12 had CVL outbreaks between 2018 and 2020. According to Campos[17], there was an increase in the percentage of dogs with CVL in the city of Aracaju in 2013 (9.21%) and 2014 (12.69%) when compared with that in 2008, with an infection rate of 4.73%; the disease was widely distributed in 75% of the capital's neighborhoods. This demonstrates that there is a relationship between an increase in infection in dogs and the geographic distribution of the parasite.

Table 1 shows that there was an association between the seropositive Elisa test for the diagnosis of CVL and the type of shelter (p = 0.008), with 45.2% of the dogs housed in outdoor shelters and 54.8% in indoor shelters. Moreover, all households were close to abandoned land, squares, and green areas, which, according to Campos[17], correlated the association between owners who have access to information and the possibility of dogs getting sick with CVL, in which case it would contribute to owners offering care to the animals, resulting in a greater probability of an increased lifespan, increasing the time of exposure to the risk of VL infection, as well as the susceptibility of the animal, which makes them remain in the environment as reservoirs of the disease for other dogs and for the human population.

Areas at risk of transmission of VL (with 33.3% [of dogs positively diagnosed with] CVL) were near places with high density vegetation, rivers, canals, and the backyards of households that had vegetation and debris present. The environment is a factor that can influence the increase in cases of VL, as it can provide favorable conditions for the reproduction of the vector insect, the sandfly, which requires organic matter for its development[18].

For most of the dogs with CVL, onychogryphosis was the main symptom, caused by bacterial, fungal, or parasitic infection; trauma; and systemic, proliferative, and immune-mediated diseases associated with CVL parasitism[19]. Thus, the onset of onychogryphosis is an important clinical sign necessitating the initial evaluation of dogs with suspected CVL[20-21]. Anorexia was observed in 31% of the dogs that presented with symptoms in this study; anorexia was triggered by pathological disorders associated with parasitism, indicating emesis, apathy, and emaciation[22.]

In the city of Lagarto, eight cases of HVL were reported between 2017 and 2020, with an average of two cases per year. However, as no cases of HVL were reported in 2019, the disease in the city was classified as having sporadic transmission[23-24]. In 2017, two autochthonous cases of HVL and no cases of CVL were identified; in 2018, three cases of HVL and 11 seropositive cases of CVL in dogs were identified; in 2019, there were no reports of cases in humans and 10 seropositive cases of CVL in dogs were reported; in 2020, there was a significant increase in the number of cases of CVL in dogs, with 21 cases of seropositive dogs and three cases of humans with HVL.

In humans, males (6 cases, 75%) were more affected than females (2 cases, 25%). The affected age groups were as follows: two children aged between 5 and 11 years old (25%) and six adult individuals, four (50%) of those between 20 and 30 years old and two (25%) between 40 and 60 years old. Four individuals (50%) had an incomplete primary school education, one individual (12.5%) had completed high school, and one individual (12.5%) had received no formal education. These factors that affected the likelihood of VL were confirmed in previous studies that showed a higher prevalence of the disease among male individuals aged between 20 and 39 years with a low level of education[15,17,25.]

According to the study by Guerra-Silveira and Abad-Franch, the prevalence of leishmaniasis is higher in male individuals due to hormonal issues; testosterone impacts the immune system, making men more susceptible to infection[26]. The level of education is also a factor that can influence the occurrence of zoonotic diseases, such as leishmaniasis, for example[13].

The most observed clinical manifestation in humans was fever, present in all (100%) of the clinical cases between 2017 and 2020, followed by fatigue, weight loss (75%), and hepatosplenomegaly (62.5%). Similar results were obtained in the state of Goiás, with fever being the most reported clinical manifestation, followed by fatigue (75%) and hepatosplenomegaly (66%). In this study there was one case (12.5%) of HVL-HIV co-infection observed, resulting in death.

In the state of Sergipe, VL is characterized as a versatile disease transmitted in rural and urban environments. In this study, five individuals diagnosed with VL (62.5%) were from rural areas and three (27.5%) were from urban areas. A total of 45.2% of the dogs diagnosed with CVL by ELISA were located in urban areas and 52.4% were in rural areas. The Northeast Region is characterized by both rural and urban environments, with a diverse fauna and flora, providing favorable conditions for the vector maintenance. This combination of urban and rural environments may explain the associations found in the present study[25].

The study conducted by Silva and collaborators on the serological survey and risk factors associated with VL in a region of the Northeast shows that in the municipality of Nossa Senhora da Glória – SE, there is a high prevalence of CVL, demonstrating the need for educational and preventive actions in the region focused on vector control[27].

CONCLUSION

The prevalence of canine Leishmania infections approximately doubled in the city of Lagarto between 2018 and 2020. The spatial distribution analysis identified the areas with the highest number of CVL and HVL cases. The regions in areas where the disease had expanded had at least three epidemiological indicators associated with a higher susceptibility of humans and dogs to zoonotic diseases transmitted by vectors, and these factors likely contributed to the vectorial expansion of the disease in the city. Of the 13 areas that contained dogs that were seropositive for CVL, six of them presented cases in humans, explaining the growing number of CVL cases accompanied by an increase in HVL cases.

Dogs that are located in areas of greatest risk impact the risk of maintaining natural foci of transmission of Leishmaniasis to human and animal hosts in VL endemic areas. Overall, it reveals the importance of canines on a domestic scale in developing countries for regional and global applications, providing the city with essential information for epidemiological surveillance programs and actions. Such results are essential to assist epidemiological surveillance in the municipality of Lagarto-SE with the implementation of control measures aimed at the insect vector of the disease, the sand fly, to prevent human and canine cases.

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Received: August 8, 2023
Accepted: December 6, 2023
Published: February 21, 2024

Authors' Contributions:

Jamisson Bispo de Sousa Santos: Conceptualization; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing – original draft.

Joserlândia dos Santos: Conceptualization; Methodology; Validation; Data curation; Writing – original draft.

Samuel Bispo de Sousa Santos: Conceptualization; Methodology; Validation; Data curation; Writing – original draft.

Anita de Souza Silva: Investigation, Methodology; Validation; Visualization; Writing – review & editing.

Roseane Nunes de Santana Campos: Conceptualization; Data curation; Project administration; Supervision; Validation; Visualization; Writing – review & editing.

Grace Anne Azevedo Dória: Conceptualization; Formal analysis; Data curation; Project administration; Supervision; Validation; Visualization; Writing – review & editing.

All authors have approved the final version of the text.

Conflict of interest: There are no conflicts of interest.

Financing: Does not have financing.

Corresponding Author
Anita de Souza Silva
Universidade Federal de Sergipe- Programa de Pós-Graduação em Ciências Aplicadas à Saúde
Av. Governador Marcelo Déda, 13, Centro, Lagarto/SE, Brasil.
E-mail: anitasouza581@gmail.com

Editor: Dra. Christiane de Fátima Colet
Editor-in-chief: Dra. Adriane Cristina Bernat Kolankiewicz