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PROTOZOOLOGY - SHORT COMMUNICATION

Canine leishmaniasis in an endemic region, Northeastern Brazil: a comparative study with four groups of animals

Matheus Resende Oliveira[1] · **Manuel Benicio Oliveira Neto**[1] · **Taynar Lima Bezerra**[2] · **Weslania Souza Inacio da Silva**[1] · **Wandklebson Silva da Paz**[3] · **Igo Gonçalves dos Santos**[1] · **Márcio Bezerra-Santos**[3,4,5] · **Victor Fernando Santana Lima**[1,3]

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Abstract

Canine leishmaniasis (CanL) is a zoonosis caused by protozoa of the genus *Leishmania* and remains an important public health concern in tropical areas. In Brazil, domestic dogs are considered the most relevant reservoir of the parasite and one of the main targets of the disease control actions. Considering this, we aimed herein to evaluate the CanL infection in different canine groups and distribution of cases in the state of Sergipe, an endemic region in Northeastern Brazil. The evaluated 467 animals were classified into four groups: hunting ($n=50$), company ($n=64$), guard ($n=140$), and wandering ($n=213$). Samples (blood, bone marrow, conjunctival swab, and lymph node aspirate) were collected from animals in nine municipalities of Sergipe. First, all animals were submitted to general and ophthalmic clinical examination. Next, they were tested serologically by TR-DPP®, and for the presence of *Leishmania*, amastigotes in samples of bone marrow, conjunctival swab, and lymph node aspirate were diagnosed by PCR and parasitological techniques. It was observed that 34.69% (162) of the evaluated dogs were seropositive. The highest rates of positivity were found in hunting 54% (27/50; OR = 3.52; p-value = 0.001) and guard dogs 42.14% (59/140; OR = 2.18; p-value = 0.01). Otherwise, the highest percentage of symptomatic dogs was observed in wandering animals (85%; OR = 9.63; p-value < 0.0001). The distribution of case analysis showed that the highest positivity rates occurred in inland municipalities situated in arid regions. Taken together, our data demonstrate that hunting and guard dogs are among the animals most exposed and affected by clinical manifestations of CanL, mainly in the inland municipalities of Sergipe State.

Keywords *Leishmania* · Wandering dogs · Diagnosis · Epidemiology

Introduction

Leishmaniasis is considered one of the most important public health concerns in the world, especially in tropical and poor areas of Africa, Asia, and Latin America (WHO 2020). They comprise a set of parasitic diseases, of a zoonotic character and broad clinical spectrum, responsible for annually affecting approximately 700,000 to 1 million people worldwide (Torres-Guerrero et al. 2017; Sundar and Singh 2018). Clinically, they are classified as tegumentary (TL) or visceral leishmaniasis (VL), and this last one can be lethal in 90% of cases if left untreated (Sousa et al. 2015a, b; Menezes et al. 2016). Despite the involvement of wild animals (foxes, opossums, and rodents) in the transmission of *Leishmania*, domestic animals, such as the canine species, are considered the main reservoir of the parasite and source of infection in

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✉ Victor Fernando Santana Lima
victor.fslima@gmail.com

1 Department of Veterinary Medicine, Federal University of Sergipe, Rodovia Engenheiro Jorge Neto, Nossa Senhora da Glória, SE, Brazil

2 Department of Anatomy, Pathology and Clinics, Federal University of Bahia, Salvador, BA, Brazil

3 Parasitic Biology Graduate Program, Federal University of Sergipe, São Cristóvão, SE, Brazil

4 Health Science Graduate Program, Federal University of Sergipe, Aracaju, SE, Brazil

5 Department of Morphology, Federal University of Sergipe, São Cristóvão, SE, Brazil

the urban environment (Navea-Pérez et al. 2015; Silva et al. 2017a, b, c; Silva et al. 2018a, b).

Canine leishmaniasis is caused by intracellular protozoa of the genus *Leishmania*. In Brazil, the disease has been related to eight species of parasites, but especially *L.* (*Leishmania*) *infantum* and *L.* (*Viannia*) *braziliensis* (Rocha et al. 2015; Ferreira et al. 2018). The infection occurs mostly through the bite of female insects of the genus *Lutzomia* (Diptera: Psychodidae), including *L. longipalpis* as the main vector in the country (Santos et al. 2019; Galvis-Ovallos et al. 2020). In addition, although considered a rural endemic, the process of urbanization of leishmaniasis has been observed in Brazilian capitals and municipalities, such as the city of Aracaju-SE (Campos et al. 2017; Reis et al. 2017; Silva et al. 2017a, b, c). Factors such as adaptive capacity of sandflies, cities expanding to forest areas, and the presence of the domestic dog are mostly related to these changes in the epidemiological profile of the disease (Nunes et al. 2016; Reguera et al. 2016).

When infected, dogs usually express high cutaneous parasitism, which facilitates ingestion by sandflies and maintenance of the parasite's transmission circle (Otranto and Dantas-Torres 2013). Clinically, they may present in the most advanced stages onychoglyphosis, lymphadenomegaly, apathy, weight loss, eye damage, alopecia, and dermatitis. Otherwise, many animals can be asymptomatic, with an infection rate that can reach 80% in some places (Eguchi et al. 2017; Contreras et al. 2019). Additionally, as they are the main urban reservoirs for leishmaniasis and being some risk of transmission for humans, dogs have become the main targets of disease control actions in Brazil (Shang et al. 2011; Nunes et al. 2016).

Currently, different techniques for canine leishmaniasis (CanL) diagnosis are recommended by the Brazilian Ministry of Health (Ministério da Saúde 2016). The main screening method for CanL is the DPP Immunochromatographic Test (Dual-Path Platform, Biomanguinhos®). The immunoenzymatic assay-ELISA (EIE Canine Visceral Leishmaniasis, Biomanguinhos®) is used as a confirmatory test for seroreagent animals. Additionally, these tests can be associated with complementary tools for parasitological diagnosis, according to the stage of the disease, such as the search for amastigotes in biological samples and the molecular biology tests by polymerase chain reaction (PCR) (Ministério da Saúde 2016; Silva et al. 2016).

Notably, Brazil is the country responsible for the leishmaniasis endemic disease in the Americas (about 96% of the cases occur here) and it has historically been neglected. Regardless, surveys that assess the situation of CanL are still scarce and punctual in a few municipalities (Cordeiro et al. 2016; Santos and Sousa 2018). In addition, due to the wide territorial distribution, leishmaniasis is still worrying in Northeastern Brazil, which reinforces the need for studies that comprehend the current panorama of the disease and help in development of effective control strategies (Ribeiro et al. 2019; Lamounier et al. 2020). More importantly, Sergipe is one of the states with the highest disease positivity rate and still with related deaths. Additionally, a study conducted by Campos and colleagues Campos et al (2017) demonstrated an increasing time trend of cases of canine and human leishmaniasis in the capital Aracaju, between 2008 and 2014. Considering this, we aimed to evaluate CanL infection in four canine groups and the spatial distribution of cases in the state of Sergipe, an endemic region in Northeastern Brazil.

Material and methods

Study type and ethical aspects

An ecological time series study was conducted, using spatial analysis techniques, and assessing the seroprevalence of CanL in four different groups of dogs. The spatial analysis units consisted of municipalities from the state of Sergipe, Northeastern Brazil. The Ethics Committee on Animal Use (CEUA), from the Universidade Federal de Sergipe, approved all procedures herein performed (License number: 1003190520).

Study area

The present study was conducted in rural and urban areas of nine municipalities, located in the metropolitan/coast and inland regions of Sergipe (Supplementary Material 1). The state is divided into climatic zones semiarid and subhumid (Caatinga and Atlantic Forest), with annual rainfall index ranging from 500 to 1000 mm^3 (SEAGRI-SE, 2020). Importantly, most of the population lives in the urban area (73.52%). Regardless, Sergipe has precarious socioeconomic indicators in Brazil: the average monthly household income is R$ 1028.00 per capita; the Human Development Index (IDH) is 0.665 (below the national average), and there is lack in the basic sanitation services (IBGE, 2020). Annually, there are about 70 cases of human visceral leishmaniasis in the state of Sergipe (SINAN, 2020).

Animals and sampling

From November to December 2020, a total of 467 dogs from different groups (company ($n = 64$); guard ($n = 140$); hunting ($n = 50$); and wandering ($n = 213$)), of both genders and different ages, were sampled. The animals were selected, randomly and by non-probabilistic convenience, obtaining the largest number of sample/dog. The sample characterization is shown in Table 1. Initially, animals were physically

Table 1 Characteristics of the animals of the study, according to the group of dogs, from the municipalities in the state of Sergipe, Northeastern Brazil

Variables	Company (n = 64)	Wandering (n = 213)	Guard (n = 140)	Hunting (n = 50)	Total (n = 467)
Male, n (%)	26 (40.63)	52 (24.41)	74 (52.86)	31 (62)	183 (39.51)
Age, mean ± SD	4.34 ± 3.12	3.71 ± 3.43	3.67 ± 3.14	3.65 ± 2.52	3.84 ± 3.05
Symptomatic, n (%)	21 (37.5)	59 (29.7)	37 (26.43)	13 (26)	130 (27.83)

SD, standard deviation

evaluated, and all data recorded in clinical charts. Afterwards, biological samples were obtained. We collected blood samples from all assessed animals (n = 467). Additionally, conjunctival swab from both eyes of dogs with ophthalmic manifestations was collected. Fine needle aspiration of lymph nodes in those animals presenting enlargement of this organ was performed. Finally, bone marrow aspiration was performed in dogs with at least three clinical alterations compatible with CL and reagents for anti-*Leishmania* antibodies in immunochromatographic test Dual-Path Platform TR-DPP® (Biomanguinhos®, Rio de Janeiro, RJ, BR).

Laboratory analysis

All blood samples obtained were stored in test tube containing ethylenediaminetetraacetic acid (EDTA) and maintained at 6 °C. We proceeded with the serological analysis using immunochromatographic test Dual-Path Platform TR-DPP® (Biomanguinhos®, Rio de Janeiro, RJ, BR). This test detects IgG anti-*Leishmania* antibodies through recombinant antigens K28 and Protein A conjugated to colloidal gold and adsorbed into nitrocellulose membrane.

Next, microscopic slides were prepared using the material obtained from the conjunctival swab, bone marrow aspirate, or lymph node. Each slide was stained using the rapid method panoptic (Laborclin), and they were analyzed by two professionals using optical microscopy (40× and 100×).

Lastly, DNA was extracted from bone marrow samples using a commercial kit (Kit Go Taq® Green Master Mix, Promega, Madison, WI, USA) according to the manufacturer's instructions. The amplification of fragment of 447 bp of the *L. infantum* was performed using the primers MC1: 5′-GTTAGCCGATGGTGGTCTTG-3′and MC2: 5′-CAC CCATTTTTCCGATTTTG-3′ (Cortes et al. 2004). The amplifications were analyzed after 1% agarose gel electrophoresis in UV transilluminator.

Statistical analysis

Data were statistically analyzed for absolute and relative frequencies. The chi-square test with Yates's correction was used to compare differences between groups. The significance level was set at 5% and the differences between the groups were considered statistically significant when a p-value < 0.05 was obtained. All analyses were performed using the software GraphPad Prism version 8.0.1.

Spatial analysis

To map the distribution of positive dogs, we first obtained the digital cartographic mesh (in shapefile format) of Sergipe State, segmented by municipalities, in the Geographic Projection System latitude/longitude. We obtained this mesh from the databases of the Brazilian Institute of Geography and Statistics (IBGE, 2020).

Posteriorly, maps representing positive cases of CanL were constructed. For that, we used the positivity rate considering the group of dogs: company, hunter, guard, wandering, and all of them together (total). Thereafter, the results were represented on thematic maps stratified according to the following parameters of positivity for CanL: low (0.02 to 7.22%); medium (7.23 to 16.29%); high (16.3 to 29.31%); intense (29.32 to 51.88%); and very intense (51.89 to 100%) (PAHO/WHO, 2017). Herein, we used the QGis software, version 3.4.11 (QGIS Development Team; Open Source Geospatial Foundation Project) for generating the choroplethic maps.

Results

Study in the Northeast area observed that 34.69% (n = 162/467) of the positive dogs were positive in the screening test for anti-*Leishmania* antibodies (TR-DPP®) (Table 2). Amastigotes of *Leishmania* spp. were observed in 29.55% of bone marrow samples, in 25% of the of lymph node aspirates, and in 6.55% of the conjunctival swab of samples obtained from dogs reactive to CanL. In addition, *L. infantum* DNA was detected in 22.73% of dog samples submitted to PCR. Interestingly, a higher percentage of positives animal in males (47.54% vs. 26.41%; OR = 2.52; p-value < 0.0001) was observed, but no difference was seen comparing animals from urban and rural areas (34.68% and 34.69%, respectively; p-value > 0.99).

Thereafter, to compare the positivity for CanL considering the categories of dogs evaluated in the study, the chi-square statistical test was applied and observed a significant difference between them (p-value = 0.0004).

Table 2 Percentage of positivity for anti-*Leishmania* antibodies and *Leishmania* sp. according to the laboratory analysis technique, in samples of dogs from the state of Sergipe, Northeastern Brazil

Laboratory analysis technique	Positive *n* (%)	Negative *n* (%)
Serology (TR-DPP®)	162 (34.69)	305 (65.31)
Amastigotes in bone module	13 (29.55)	31 (70.45)
Amastigotes in lymph node	11 (25)	33 (75)
Amastigotes in optical conjunctiva	3 (6.25)	45 (93.75)
PCR (DNA for *L. infantum*)	10 (22.73)	34 (77.27)

n, number of sample; *PCR*, polymerase chain reaction; *DNA*, deoxyribonucleic acid

Table 3 Comparative analysis of dogs positive for anti-*Leishmania* antibodies and *Leishmania* sp. according to the group of animals evaluated, from the state of Sergipe, Northeastern Brazil

Group of dogs	Positive *n* (%)	Negative *n* (%)	Odds ratio	95% CI	*p*-value
Company*	16 (25)	48 (75)	1.00	–	–
Wandering	60 (28.17)	153 (71.83)	1.17	0.62 to 2.28	0.74
Guard	59 (42.14)	81 (57.86)	2.18	1.15 to 4.13	0.01
Hunting	27 (54)	23 (46)	3.52	1.6 to 7.65	0.001

n, number of samples

*Considering that the company group had the lowest percentage of positivity, it was used as a base group for the statistical analysis by chi-square test

Herewith, the group that presented the lowest percentage of positivity (company = 25%) was used as a parameter for comparison with the others (Table 3). Interestingly, hunting dogs were the ones that had the highest odds ratio for CanL (54%; OR = 3.52; *p*-value = 0.001), along with guard dogs (42.14%; OR = 2.18; *p*-value = 0.01). Surprisingly, no differences were observed between company and wandering groups (28.17%; OR = 1.17; *p*-value = 0.74).

Among the positive animals in one or more diagnostic tests, we identified symptoms for CanL in 56.18% of them. Furthermore, the presentation of symptoms between the groups and observed statistical difference was compared (*p*-value < 0.0001). Considering that the hunting group had the lowest percentage of symptomatic animals (37.04%), we used it as a comparison parameter (Table 4). Statistical differences when comparing with the guard (38.98%; OR = 1.08; *p*-value = 0.99) and company groups were not found (43.75%; OR = 1.32; *p*-value = 0.75). On the other hand, a significant difference when compared to the wandering group was observed (85%; OR = 9.63; *p*-value < 0.0001).

Concerning the main clinical manifestations in positive animals, alopecia (29.01%), onychogryphosis (22.22%), exfoliative dermatitis (20.37%), lymphadenomegaly (14.81%), and ear tip lesions (12.26%) were observed. Additionally, the identified eye disorders were compatible with canine eye leishmaniasis in 9.88% (*n* = 16) of dogs. Mainly, periocular alopecia (6.25%), blepharitis (12.5%), conjunctivitis (81.25%), keratitis (37.5%), eye discharge (68.75%), and corneal ulcer (6, 25%) were observed.

Analysis of case distribution data showed a very intense positivity rate of CanL in inland municipalities of the state. In addition, municipalities in the metropolitan area, including the capital Aracaju, had an intense positivity rate (Fig. 1). Maps in Fig. 2A–D show the spatial distribution of the positivity rate of CanL according to the groups of dogs evaluated. Inland municipalities with very intense positivity rate for CanL in the group of hunting dogs were identified (Fig. 2B). Likewise, guard and wandering dogs showed very intense and intense rates for CanL in inland municipalities and in the metropolitan area, respectively (Fig. 2C, D).

Discussion

In this study, the groups of dogs most exposed to *Leishmania* infection in endemic areas of the state of Sergipe, Northeastern Brazil, were identified. The positivity of animals observed here was higher than those obtained in

Table 4 Comparative analysis of dogs presenting signs and symptoms for CanL, according to the group of animals evaluated, from the state of Sergipe, Northeastern Brazil

Group of dogs (*n* CanL +)	Clinical classification		Odds ratio	95% CI	*p*-value
	Symptomatic *n* (%)	Asymptomatic *n* (%)			
Hunting* (*n* = 27)	10 (37.04)	17 (62.96)	1.00	–	–
Guard (*n* = 59)	23 (38.98)	36 (61.02)	1.08	0.44 to 2.71	0.99
Company (*n* = 16)	7 (43.75)	9 (56.25)	1.32	0.36 to 4.62	0.75
Wandering (*n* = 60)	51 (85)	9 (15)	9.63	3.31 to 25.29	< 0.0001

n, number of samples; *CL+*, positive for canine leishmaniasis; *CI*, confidence interval

*Considering that the hunting group had the lowest percentage of animals presenting signs and symptoms, it was used as a base group for the statistical analysis by chi-square test

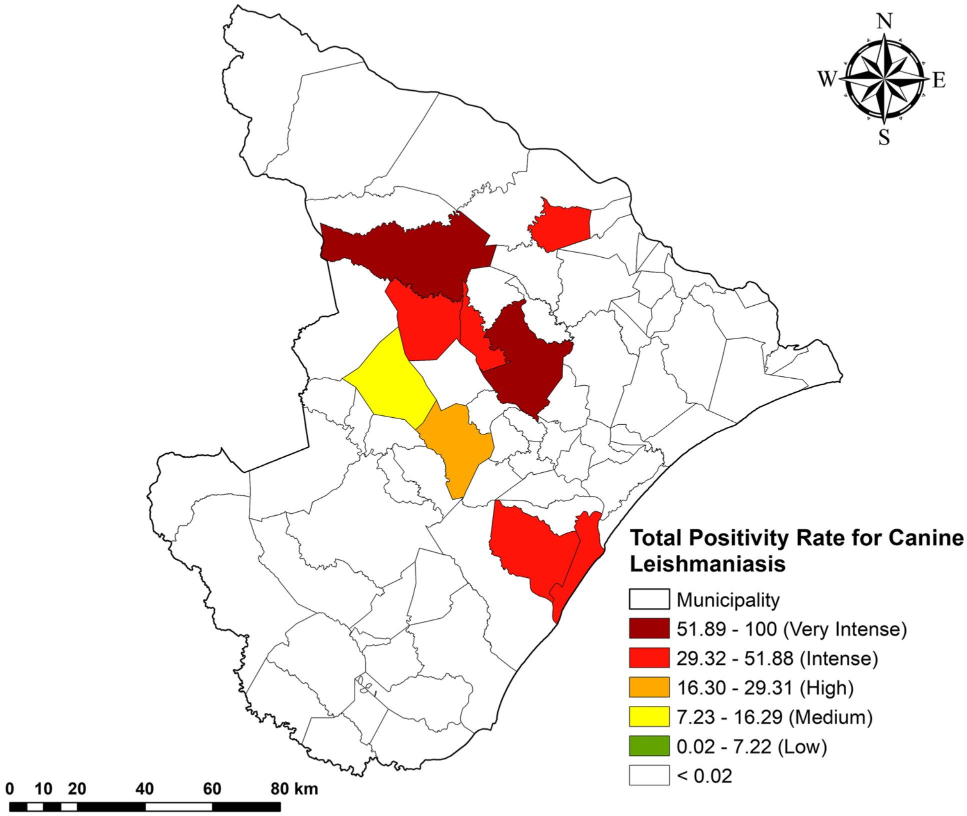

Fig. 1 Spatial distribution map according to the positivity rate of canine leishmaniasis (CL), in dog populations from municipalities in the state of Sergipe, Northeastern Brazil. Municipalities were stratified according to the parameters of positivity for CL established by the SisLeish-PAHO/WHO (2017)

epidemiological surveys carried out in other states, such as Bahia (Rodrigues et al. 2020), Mato Grosso (Carvalho et al. 2020), Minas Gerais (Castro-Júnior et al. 2014), and São Paulo (Julião et al. 2007). These results may be related to the diversity of biological samples and laboratory tests used in our study, which may allow an increase in the rates of sensitivity for the LC diagnosis (Sassaki et al. 2011; Braz et al. 2016).

Conversely, these findings corroborate a worrying and insidious scenario in relation to leishmaniasis in Sergipe. Considering that canine infection usually precedes human cases, the high number of positive dogs identified here, distributed in different regions and municipalities of the state, represents a high risk of parasite transmission to human patients and maintenance of the epidemiological circle of the disease (Campos et al. 2017).

Currently, the Brazilian Ministry of Health recommends, in epidemiological surveys, the use of Dual-Path Platform canine visceral leishmaniasis (DPP® CVL)—rapid test that uses a combination of recombinant antigens rK39, K26, and K9—for the detection of anti-*Leishmania* antibodies in animals (Grimaldi Júnior et al. 2012; Ministério da Saúde 2016). Regardless of the recommendation of more sensitive tests, such as ELISA and RIFI as confirmatory tests, it is worth noting that the sensitivity of DPP® CVL can be greater than 98%. This test is recommended for screening dogs infected with CanL, and in our study, it is demonstrated that its use, along with parasitological examination and PCR, can be highly useful for *Leishmania* sp. diagnosis in surveys (Grimaldi Júnior et al. 2012; Paltrinieri et al. 2016; Lins et al. 2018; Sales et al. 2020).

Several studies in the literature aim to assess the influence of biological factors, such as sex and age, on the positivity rate for leishmaniasis (Silva et al. 2016; Campos et al. 2017; Evaristo et al. 2020). Herein, it was observed that male dogs were mostly affected by the disease in Sergipe (OR = 2.52). According to Silva et al. (2016) and Evaristo et al. (2020), the prevalence of male dogs infected in the semiarid region of the Northeast is about 2.25 times higher than that in females, mainly in animals of 12 and 84 months of age.

Similarly, a study conducted by Campos and colleagues Campos et al (2017) in Aracaju identified a higher percentage of CanL among males. Remarkably, in the epidemiology of CanL in the Northeastern Brazil, the levels of rainfall, the occurrence of natural ecotones, and the presence of forest in the peri-urban regions are factors associated with *Leishmania* transmission and, as a result, both sexes are equally exposed (Almeida et al. 2012; Santos and Sousa 2018). Taken together, these findings demonstrate that, despite studies, the relationship between sex and susceptibility to CanL is still not fully understood, and further research is required, especially on the influence of biological factors.

Concerning the groups of dogs most affected by CanL, Almeida and colleagues Almeida et al (2012) point out that

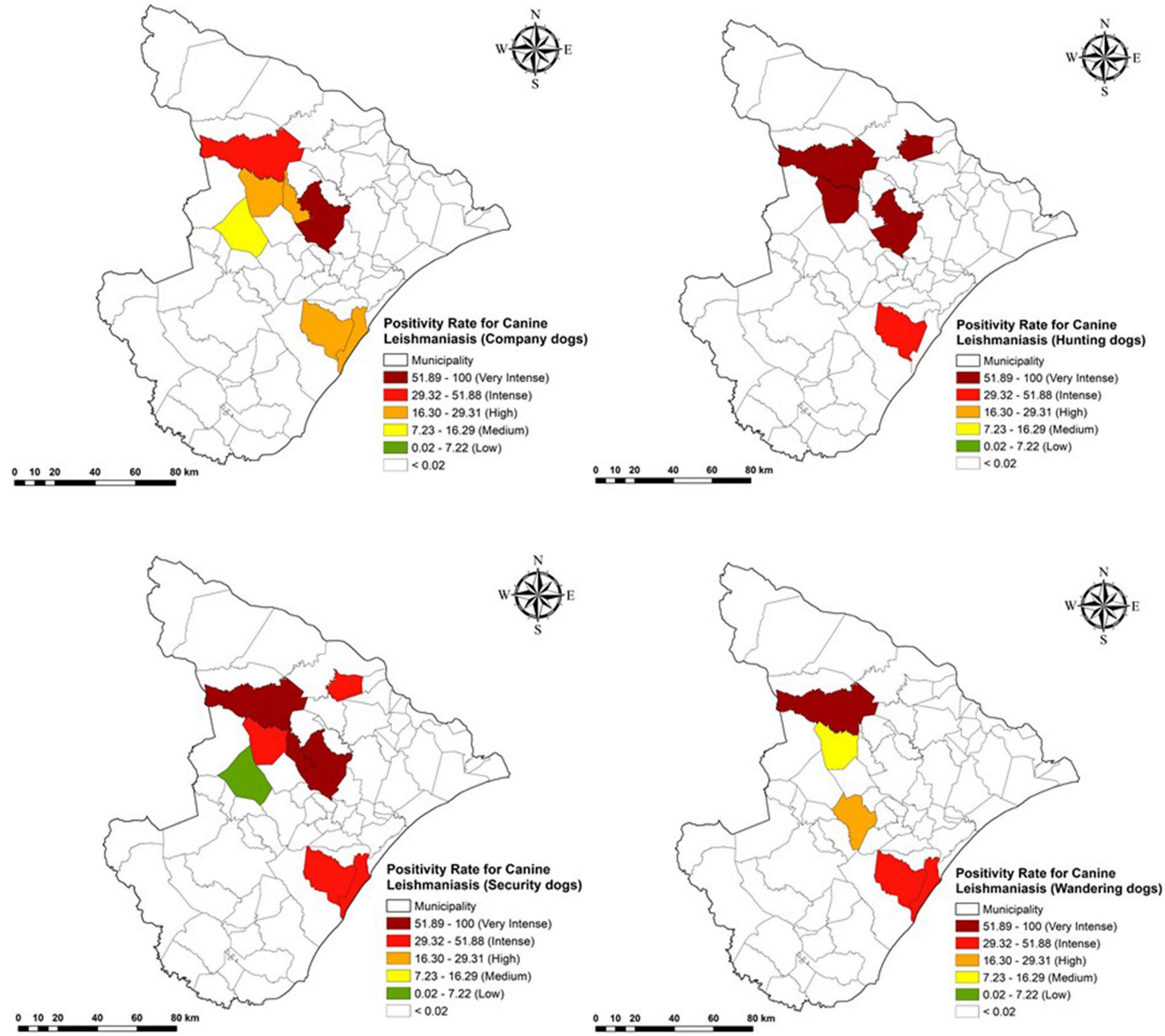

Fig. 2 Spatial distribution maps according to the positivity rate for canine leishmaniasis (CL) in different groups of dogs, from the municipalities in the state of Sergipe, Northeastern Brazil. For each map, the groups of dogs evaluated in the study were considered: **A** company; **B** hunting; **C** security/guard; and **D** wandering. Municipalities were stratified according to the parameters of positivity for CL established by the SisLeish-PAHO/WHO (2017)

animals living outside (hunting, guard, and wandering dogs) are more accessible to sandflies and therefore more exposed to infection by *Leishmania* sp. Corroborating this, our data showed that hunting and guard dogs were mostly affected by CanL and had higher odds ratio of infection by the parasite (OR = 3.52 and 2.18, respectively), when compared to company dogs. This may be related to the environment in which these animals are kept, since many of these dogs live adjacent to the feeding and breeding sites of *Lutzomyia* females (chicken coops, pig pens, corrals, and barns). Moreover, these animals are exposed to forest areas during hunting activities, usually in twilight and post-twilight periods, coinciding with the highest activity of sandflies (Carvalho et al. 2010; Dantas-Torres et al. 2010; Gálvez et al. 2010; Píantedosi et al. 2016; Aklilu et al. 2017; Campino and Maia 2018; Silva et al. 2018a, b; Trájer et al. 2018; Lago et al. 2020).

Notably, several studies report that wandering dogs are the most affected by CanL. Nonetheless, a statistical difference here when comparing the positivity rate between wandering and company groups was not observed. On the other hand, considering the seropositive animals, wandering dogs were those mostly diagnosed with signs and symptoms (85%) and odds ratio of 9.63. This significantly higher odds ratio in this group may be related, first, to the habit of these animals roaming the streets, peripheral, and forest areas (places of exposure to sandflies). Additionally, this may be linked to nutritional factors, such as protein-calorie deficiency, which is much more common in this group. As a result, it directly affects the general health of these animals, compromises immunity, and facilitates the development of the disease, and the clinical presentation of signs and symptoms (Melo et al. 2018; Lewgoy et al. 2020; Nweze et al. 2020).

Concerning the clinical aspects, among symptomatic dogs, classic clinical alterations of leishmaniasis such as alopecia, onychogryphosis, lymphadenomegaly, and ear tip lesions were observed (Moreira et al. 2016; Contreras et al. 2019; Azevedo and Marcili 2020; Pasanisi 2020). Furthermore, the ocular form of CanL was also observed in these dogs, in which changes such as periocular alopecia, blepharitis, keratitis, and conjunctivitis are among the most reported ophthalmic and clinical manifestations are consequences of systemic impairment caused by Leishmania, which involves direct mechanisms of tissue damage due to the presence of the parasite and specific cytotoxic immune responses. Clinical manifestations are consequences of systemic impairment caused by Leishmania, which involves direct mechanisms of tissue damage due to the presence of the parasite and specific cytotoxic immune responses (Peña et al. 2008; Brito et al. 2010; Rálic and Jovanovic 2011; Pietro et al. 2016).

Importantly, our study had some limitations that deserve to be mentioned. First, the sample number was lower among guard and hunting animals. Nevertheless, this is expected, as this population of dogs is usually smaller. Second, some difficulties in accessing communities in rural areas for data collection and sampling were faced. Finally, collections due to the COVID-19 pandemic and the need for social distance had to be the limitations. For this reason, other municipalities in the state were not contemplated. In spite of this, an expressive sample number was obtained ($n = 426$) which, divided among the study groups and along with the statistical and spatial analysis, allowed to identify the categories most exposed to the infection and to map the spatial dynamics of the leishmaniasis in the state.

Conclusion

Altogether, our results showed that hunting and guard dogs are among the animals most exposed and affected by clinical manifestations of CanL, mainly in the inland municipalities of Sergipe State. This corroborates the occupational risks involved in the transmission of the parasite. Additionally, although street animals are the main targets of seroepidemiological surveys for CanL, our data clearly point to the need to routinely assess other canine groups, such as guard and hunting dogs, especially in endemic areas. Importantly, it was observed that, among positive animals, the wandering group is the one with the most clinical signs and symptoms for leishmaniasis. Thereby, this requires national public policies for the epidemiological monitoring and care of these animals. Furthermore, this study aimed to improve the diagnosis of CanL, reduce the risk of *Leishmania* transmission, and control the disease in the country.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-021-07319-0.

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