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Original Article

Spatial analysis of the risk for canine visceral leishmaniasis in an urban area in the State of Sergipe

Análise espacial do risco para leishmaniose visceral canina em área urbana do estado de Sergipe

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Abstract

Visceral Leishmaniasis is included among the neglected tropical diseases, being directly related to conditions of social vulnerability, in urban environments, dogs act as important reservoirs. The aim of the study was to evaluate the distribution of dogs, related risk factors and identify of volatile organic compounds from infected dogs. Peripheral blood samples from 72 dogs were collected for detection using the ELISA test, in addition to hair samples for analysis by GC-MS. Of the evaluated dogs, 13 (18.05%/72) were reactive for canine VL, seven in Aracaju and six in Propriá. Factors related to vegetation, age, place where the dog stays and free access to the street, were associated with a greater chance of the dog becoming infected. Fifty-three compounds were identified from ten canine hair samples, among which 2-butoxyethanol, benzaldehyde, decane, 2-phenylacetaldehyde, nonan-1-ol, 2-phenoxyethanol, nonanoic acid, 8-heptadecene and eicosane were found in seropositive dogs for leishmaniasis. The guardian's posture has been increasingly important, requiring more attention to the dog's health and actions aimed at environmental management in an attempt to reduce cases of canine VL in the state. Even though the identified VOCs have not been associated with leishmanial infection, it is of great use for understanding canine hair substances.

Keywords: epidemiology, GC-MS, canine visceral leishmaniasis, NDVI.

Resumo

A Leishmaniose Visceral está incluída entre as doenças tropicais negligenciadas, estando diretamente relacionada às condições de vulnerabilidade social, e sendo o cão e principal hospedeiro em ambientes urbanos. O objetivo do estudo foi avaliar a presença de cães com leishmaniose visceral em municípios do estado de Sergipe, Brasil, os fatores de risco relacionados e identificar compostos orgânicos voláteis de cães infectados. Amostras de sangue periférico de 72 cães foram coletadas para a detecção por meio do teste de ELISA, além de amostras de pelos para análise por GC-MS. Dos cães avaliados, 13 (18,05%/72) foram reagentes para LV canina, sendo sete em Aracaju e seis em Propriá. Fatores relacionados a vegetação, a idade, o local de permanência do cão, o livre acesso à rua, foram associados a maior chance do cão se infectar. Foram identificados 53 compostos a partir de dez amostras de pelo canino, dentre os quais o 2-butoxietanol, benzaldeído, decano, 2-fenilacetaldeído, nonan-1-ol, 2-fenoxietanol, ácido nonanóico, 8-heptadeceno e eicosano foram encontrados em cães soropositivos para leishmaniose. A postura do tutor tem se revelado cada vez mais importante, sendo necessária mais atenção à saúde do cão e ações direcionadas a gestão ambiental na tentativa de diminuir os casos de LV canina no estado. Mesmo que os COVs identificados não tenham sido associados à infecção leishmaniótica, são de grande utilidade para a compreensão de substâncias de pelos caninos.

Palavras-chave: epidemiologia, GC-MS, leishmaniose visceral canina, NDVI.

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1. Introduction

Visceral Leishmaniasis (VL), popularly known as kala-azar, is a zoonosis caused by an intracellular protozoan of the species *Leishmania* (*Leishmania*) *infantum chagasi* Nicole (1908). *Lutzomyia longipalpis* Lutz and Neiva (1912) is considered the main vector of the etiological agent of VL in Brazil, being found in almost all geographic regions of the country, with the exception of the states of Santa Catarina and Amazonas. Transmission occurs via hematophagy of the infected vector in its main host in urban environments, the dog (Brasil, 2014; OPS, 2019).

VL is a neglected disease of great importance in public health, mainly due to its high lethality. In Brazil, this zoonosis has a wide geographic distribution, involving the North, Northeast, Midwest, Southeast and South regions, referring to the number of reported cases (Graepp-Fontoura et al., 2020). Also due to the favorable climate of the Northeast region, with changes in the pattern of precipitation and temperature, inadequate basic sanitation conditions and the advance of urbanization (Queiroz et al., 2020), it becomes the main focus of transmission with a percentage of 49,60% of human cases confirmed in the decade from 2010 to 2020, while in the same period, Sergipe confirmed 612 cases of human VL (Brasil, 2014, 2021).

Intervening factors for human and canine VL related to land use, such as agriculture, conserved vegetation, deforestation and urbanization of cities need to be considered when carrying out an epidemiological evaluation of this zoonosis. Therefore, the use of geospatial methodologies for monitoring and better understanding of diseases has become increasingly frequent. Based on these methodologies, *hotspots* can be identified and the region classified in order to observe points of influence (Araújo et al., 2022). As well as Oliveira (2011) and Campos et al. (2017) verified areas of intense transmission and the advancement of canine and human VL cases over the years (1999-2010; 2008-2014) in the capital of Sergipe.

Another tool, now to contribute to the understanding of the dynamics between the parasite, the host and the vector, are the volatile organic compounds (VOCs) released by the vertebrate host. Some studies have shown that the pathogen can interfere with the odors produced by infected hosts (Gandon, 2018) and the presence of certain compounds in infected dogs can be more attractive to sand flies (Staniek and Hamilton, 2021). Basically, because the primary mechanism of insect orientation towards hematophagy is based on odor recognition (Knols and Meijerink, 1997). The benefit of using hair is that it does not cause discomfort to the dog, is practical and requires no extensive storage methods (Rivier, 2000; Anchieta, 2016). With this, the objective of this study was to evaluate the presence of dogs infected with VL in Sergipe municipalities, the related risk factors and to identify volatile organic compounds in the hair of these animals.

2. Material and Methods

2.1. Ethical statement and area of study

All procedures involving the use of biological samples from animals comply with the rules of the Animal Use Ethics Committee and Research Ethics Committee of the Tiradentes University, and this study was approved under registration number No. 011121 and 4654181 (CAAE 36447220900005371), respectively. Free and informed consent by the tutor.

The tracking areas for the dogs were the municipalities of Aracaju and Propriá, located in the State of Sergipe. The capital of Sergipe, Aracaju, has a population estimate of 672,614 inhabitants distributed in a territorial area of 182,163 km^2 (IBGE, 2021a). The canine census in 2008 carried out by the Zoonoses Control Center corresponded to 7.69% of the population of Aracaju, which means 51,724 dogs. The areas of origin of the animals were the Expansion Zone with characteristics of rural-urban transition (Areia Branca village) and central neighborhoods (Jabotiana, Inácio Barbosa and Palestine, Farolândia and Treze de Julho).

The municipality of Propriá, located in eastern Sergipe, has a population density of 3,140.65 inhabitants /km^2 (IBGE, 2021b). The animals came from the Centro de Controle de Zoonoses (CCZ) and Conjunto Maria do Carmo.

2.2. Study design

Companion, breeding and stray dogs were selected by non-probabilistic convenience in the VL endemic areas under study. Adult dogs (over 6 months old) of both sexes were included and pregnant and lactating females were not included. Dogs were considered symptomatic when they showed two or more compatible symptoms and from an endemic area (Brasil, 2014).

Peripheral blood was collected from the dogs and sent to the Central Public Health Laboratory (LACEN/SE) to perform the Indirect Immunoenzymatic Assay (ELISA) test. Canine hair samples (+/- 130mg) were obtained by cutting close to the dog's body surface, using individual disposable scalpel blades. The hairs were wrapped immediately after extraction in aluminum foil and then packed in zip lock plastic bags and stored at 4 ºC until the chromatographic analyzes of VOCs were carried out.

2.3. Assessment of canine Ehrlichia canis infection

The immunochromatographic test for ehrlichiosis was performed using the Alere Ehrlichiosis Ac Test Kit, which uses 10µL of whole blood, serum or plasma in 2 drops of buffer (50 mM Tris-HCL buffer 'pH 8.5', Sodium azide, TritonX-100) The result was observed after 20 minutes. the test has a sensitivity of 98.2% and a specificity of 100%.

2.4. Geospatial analysis

The dog´s residences were georeferenced using the Timestamp Camera application (Susamp Infotech, Surat, India). The maps were created using the Qgis 3.28.2 software.

CBERS4A satellite images from 2022, extracted from the website of the National Institute for Space Research (INPE). The calculation to obtain the NDVI is based on the difference between Near Infrared (NIR) and Red (Red), by dividing the sum of them.

With the NDVI, you can observe land use to determine areas that have been deforested and vegetation cover in order to better understand information about vegetation

(Huang et al., 2021). As a result, the NDVI was performed in the present study and classified as follows: metallic roof in red (NDVI ≤ -0.10), ceramic roof in orange (0.00 ≤ NDVI ≤ 0.19), exposed soil in gray (0.2 ≤ NDVI ≤ 0.39) (NDVI ≤ 0.20),creeping or sparse vegetation in light green (0.40 ≤ NDVI ≤ 0.59), dense vegetation in dark green (0.60 ≤ NDVI ≥ 1.0), based on the classification by Abrantes et al. (2018) (Figure 1).

From this classification, the vegetated part was extracted to calculate the vegetation area within the buffer of each analyzed neighborhood. The Jabotiana neighborhood was divided into two areas, Largo da Aparecida and Juscelino Kubitschek, due to distance between the analyzed dogs and a precarious basic sanitation factor, with open sewage in Largo da Aparecida.

From the NDVI classification, the vegetated area was extracted using the semi-automatic plugin classification - postprocessing - classification to vector. The midpoint was calculated from the triangulation of the location of each dog, in neighborhoods where the dogs were not in the same household, with the aid of the GPS TrackMaker Pro ® software (Geo Studio Technology).

A radius of 300 meters was established based on the midpoint around the residences (area = 282,361.338 m ^{2}), since this is approximately the distance that the sand fly can reach, and the dogs in this study do not have free access to the street, remaining most of the time in the residential area. Then, the total area and the plant area within the established 300 meters were calculated and classified by collection neighborhood.

2.5. Clinical and breeding conditions assessment

Characteristics were observed related to the type of breeding, feeding, raising other animals, environment in which they spend more time, use of antiparasitic collar and referral to the veterinarian, type of breed, free access to the street, age, gender, level of education and income owner's family, knowledge about leishmaniasis and the importance of the dog in the transmission of the disease.

Clinical evaluation followed observation of the following external clinical signs: whether the dog was vaccinated for leishmaniasis, coloration of mucous membranes, palpable lymph nodes, ophthalmic findings, ears and muzzle. In the

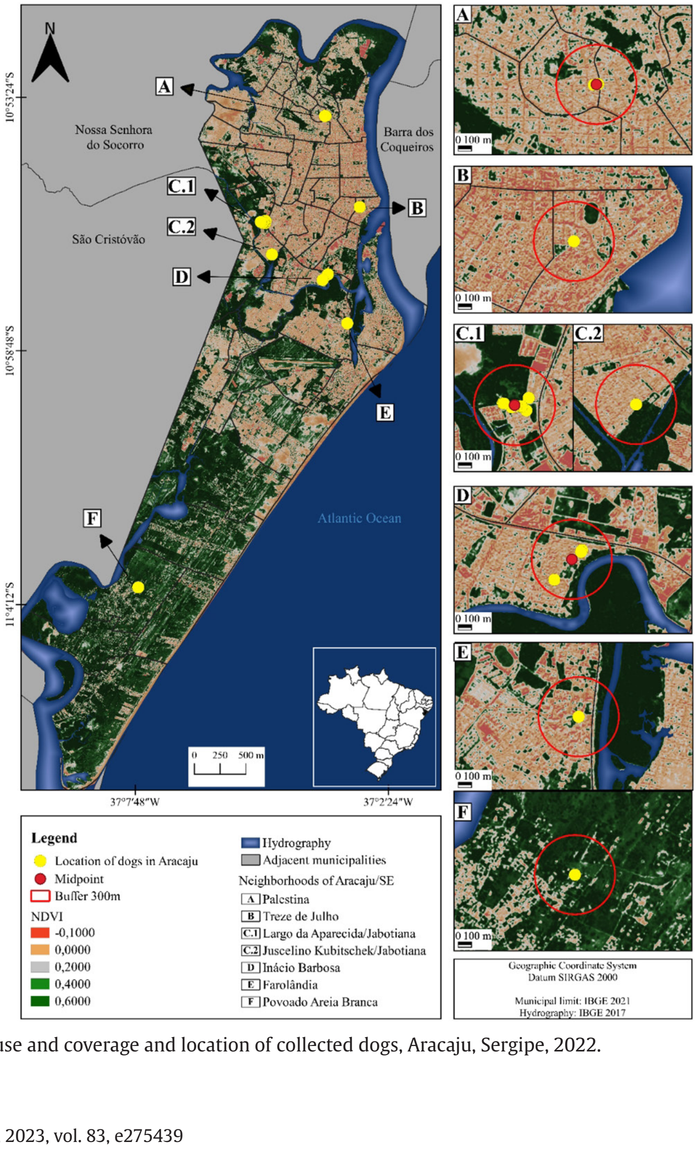

Figure 1. Classes of land use and coverage and location of collected dogs, Aracaju, Sergipe, 2022.

skin and appendages, dermatopathies, alopecia, seborrhea, erythema, pustules and onychogryphosis were considered, and the presence of ectoparasites was also observed.

2.6. Extraction and identification of Volatile Organic Compounds (VOC) from canine hair

The amount of 130 mg of fur/animal were sent to the Laboratory of Parasitology at the São Paulo State University - UNESP, Araraquara and to the Federal Institute of Education, Science and Technology of São Paulo (IFSP) – Campus Matão for the analysis of Volatile Organic Compounds by the technique Extraction, Solid Phase Micro Extraction (SPME). The hair mass of each animal was placed individually in glass *vials* (20 mL) duly sealed. Then the *vials* with hair samples were incubated in a water bath at 90°C with a polydimethylsiloxane / divinylbenzene coated fiber (PDMS / DVB, 24 Ga Pink, 65 µM, Supelco, Bellefonte) inserted into the septum of each *vial* for 18 minutes for VOC adsorption, according to the methodology described by Magalhães-Júnior et al. (2014).

Subsequently, the fiber was retracted and after each extraction, the fiber was inserted for three minutes into the gas chromatograph injector coupled to a mass spectrometer - GC-MS (Shimadzu GC-MS QP2010 Plus) at 240 ºC for VOC desorption in *splitless* mode under carrier gas flow (He 0.7 mL min^{-1}) and separated on the RTx-5MS capillary chromatography column (30 mx 0.25 mm; 0.25 µm – fused silica 5% dipheny l/95% dimethyl polysiloxane).

The temperature of the chromatographic column was programmed as follows for the analysis of VOCs present in the samples: 40 °C for 3 minutes; 40-130 °C at 2 °C min^{-1}; 130 °C for 15 minutes; 130-245 °C at 2 °C min^{-1}; 245 °C for 4 minutes (124.5 minutes total). The ion source and interface temperatures were set to 240 °C: the electron ionization impact energy was 70 eV and the spectral sweep frequency was 40 to 400 m/ z. each of the peaks of the chromatograms, the identification of the VOCs was carried out with the help of the *software 's own spectra libraries* (NIST 08, NIST 98v101, Wiley MS 229 and FFNSC 1.3), considering similarity above 75%, and also by calculating the retention index (RI) of each compound, made from the retention times of a commercial mixture of *n-alkanes* (C8-C20–Sigma-Aldrich®) analyzed under the same chromatographic conditions as the samples. The retention index (RI) of each compound was calculated according to the Van den equation Dool and Kratz. The calculated RI were compared with data from the literature using the databases of National Institute of Standards and Technology (NIST, 2023) and The LRI & odour Database on the Web (LRI, 2023).

2.7. Data analysis

To evaluate the relationship between vegetated areas and the number of infected dogs per territorial unit in each neighborhood, the G test with Williams correction was used. The evaluation of the association between the studied variables and the ELISA result was processed via the G test for the variables of feeding, educational level, family income, ectoparasites, type of rearing and *Odds Ratio* (OR) for the others, establishing a reference variable in the evaluated categories. All tests were used in the BioEstat v. 5.0 (Mamirauá Foundation), with a significance level of 5%.

Multivariate Principal Component Analysis (PCA) was performed to observe the VOC grouping trends in relation to the positivity of dogs for canine VL, adopting infected dogs as the active variable and uninfected dogs as a supplementary variable, using the Statistica 7.0 Software (StatSoft Inc.)

3. Results and Discussion

3.1. Spatial evaluation of canine infection for Leishmania spp.

A total of 72 dogs were sampled and categorized into three groups, consisting of companion, breeding and stray animals, from endemic areas for canine VL (Aracaju and Propriá) in the state of Sergipe.

The results from ELISA have shown 13 positive dogs (18.05%) for canine VL, seven in the municipality of Aracaju and six in Propriá (Table 1).

Largo da Aparecida and Povoado Areia Branca, which are the most vegetated regions and with the greatest crowding of dogs, are areas more susceptible to infection and need greater attention to canine health (Table 1). However, even though areas A and B do not present a large part of vegetation, the infection was diagnosed (Figure 2). This result shows that even the small fragments of vegetation around the dwellings is sufficient for the sand fly to complete the life cycle and consequently cause an increase in the incidence of human and canine VL (Aparicio and Bitencourt, 2004). The sand flies species *Evandromyia lenti* and *Lutzomyia longipalpis* were the most collected species in this municipality (Andrade et al., 2023).

There is a distribution of canine VL across all regions of Aracaju, even with the presence of a positive dog in some neighborhoods (Figure 1). This permanence of infected dogs shows that in these neighborhoods there is a favorable environment for the proliferation of the vector, since they are areas that are undergoing urban development, with precarious basic sanitation and extensive vegetation, vacant land nearby and the creation of other animals, as found in this study.

The urbanization of vegetated areas, result in increased interaction between the vector insect and the canine host (Djibougou et al., 2022). This scenario promotes the decrease of the abundance of vector species in the vegetated areas (forest) (Oca-Aguilar et al., 2022) and a migration towards other microhabitats closer to the residences. As in the present study that associated transmission, the proximity of residences to vegetation ($p < 0.0001$).

The areas Areia Branca, Jabotiana and Farolândia has been identified as the most prevalent health areas for VL (>20%) in Aracaju between the years 1999 and 2010 in a previous report (Oliveira, 2011). In the health areas in which the Inácio Barbosa, Palestine and Treze de Julho neighborhoods are located, the prevalence for VL was up to 10%. The expansion of canine VL from the South region (high vegetation area) to the North (small fragments of vegetation) over the years, has spread for almost all neighborhoods in the capital of Sergipe (Oliveira, 2011),

Table 1. Distribution of dogs evaluated for leishmaniasis infection in endemic areas of the State of Sergipe, Brazil, 2023.

Region	Type of animal	Vegetation area (m²)*	Seroreagent		Total
			P	N	
Aracaju municipality					
Areia Branca Village	Breeding dog	1,814.890	2	11	13
Juscelino Kubitschek/ Jabotiana disctrict	Companion dog	933.268	0	1	1
Largo da Aparecida/ Jabotiana disctrict	Companion dog	1,195.500	1	10	12
Inácio Barbosa	Companion dog	788.460	0	3	3
Palestina		125.409	2	1	3
Farolândia	Companion dog	620.990	1	0	1
Treze de Julho	Companion dog	149.246	1	0	1
Propriá municipality					
Zoonosis Control Center	Stray dog	-	3	2	5
Maria do Carmo Village	Companion dog	-	3	30	33

*Calculated buffer vegetation area of 282,361.338m²; 300m radius.

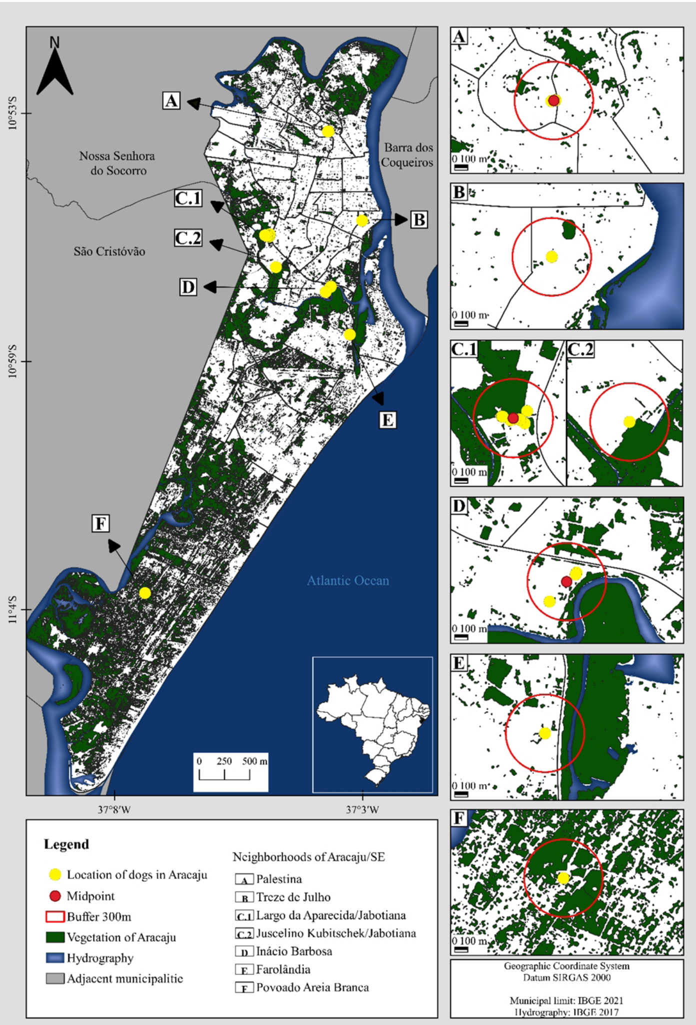

Figure 2. Vegetation cover and location of collected dogs, Aracaju, Sergipe, 2022.

demonstrating the permanence of the circulation of this infection in these areas until now.

In relation to Propriá, there are no studies on the sand fly fauna (Andrade et al., 2023) and canine infection, only reported human cases (Pantaleão et al., 2018). However, in this work, the presence of canine VL in the municipality is observed, even with low seropositivity, it is an endemic region, so there is a need for investigation to better understand the dynamics in this area.

Some dogs were co-infected with *Ehrlichia canis* Donatien & Lestoquard (1935) and *Leishmania* spp. (n=7; 9.72%). The co-infection of these microorganisms is quite common with negative consequences for the dog, by drastically compromising immunity and the development of more severe symptoms (Baxarias et al., 2018; Beasley et al., 2021). This differential diagnosis of canine VL for hemoparasites transmitted by ticks, such as ehrlichiosis and babesiosis, is of great importance given the similarity of clinical manifestations, the urbanization of vectors and the existence of cross-reactions in serological tests (Gomes and Cordeiro, 2004). In some states of Northeastern Brazil, 41.67% of the dogs were infected and with clinical aspects for *L. infantum* and *Ehrlichia canis* (Toepp et al., 2019). For this reason, pathogens can cause tissue damage through the inflammatory process and increase in amastigotes of *Leishmania* spp. (Andrade et al., 2014).

3.2. Evaluation of environmental and clinical factors associated with canine infection by Leishmania spp.

Among the intervening factors related to canine VL, it was observed that there is a statistical difference in dogs aged between 06 months and 1 year (p=0.0207). Evaristo et al. (2020) associated this fact with the immature immune system, causing greater susceptibility to this age group. However, other authors report seroprevalence both in younger dogs (Gouvêa et al., 2016) and in older dogs (Michelin et al., 2018), expressing the lack of age predisposition or susceptibility to leishmaniasis infection (Table 2).

Mixed breed dogs (OR = 2.48) were more seropositive, as in the results of Jiang et al. (2016). This data may be related to the style of raising the animals, and is necessary to consider health care and methods of individual protection of the animal. The purebred dogs in this study used antiparasitic collars and a more hygienic environment, as previously reported, even if located in an endemic area. However, differences between races should not be disregarded, as they indicate that certain races may be resistant to leishmaniasis infection (Vasconcelos et al., 2019).

The free access of the dog to the street means that the animal has a greater chance of becoming infected than when it does not have free access (OR = 1.83) and short hair (OR = 1.15). These dogs can circulate through more prone and maintain proximity to households that raise other animals (OR = 1.35) such as chickens, cats, ducks and other dogs and spend more time outside the home (OR = 1.71), which includes the backyard of the households, where there is presence of these other mentioned animals.

The presence of facilities for commercial breeding or sheltering animals close to human residences was associated with twice the chance of leishmaniasis infection (Veloso et al., 2021), as well as the presence of annexes for raising other animals, cattle, goat, rabbit, mainly poultry (Oliveira et al., 2012; Silva et al., 2016) (Table 2).

In the present study, it was observed that, even female dogs with a higher chance of infection (OR = 1.43), there was no significant difference between the sexes, suggesting that there is no sexual predisposition to *Leishmania* infection (Veloso et al., 2021; Djibougou et al., 2022).

Some intervening factors such as the type of breeding, level of education, family income, knowing what leishmaniasis is, knowing the importance of the dog in transmitting the disease, food, use of antiparasitic collar and frequency to the veterinarian, did not show significance in the study, but they are factors that can be associated with canine infection according to the literature, requiring attention to them.

The type of breeding of the dog's results showed that stray animals were the most seropositive (60%), followed by those for breeding (15.38%) and companion animals (14.81%) (Table 2). These data corroborate Oliveira et al. (2021) who reports that stray animals are the most seropositive and symptomatic when compared to companion, guard and hunting animals and relate this positivity to the free-living habit and nutritional factors.

Tutors did not start or complete basic education (n = 43/67), resulting in a family income of less than one minimum wage (n = 34/67). When asked about what canine VL is, the tutors answered that they did not know (n = 39/67) and that they did not know the importance of the dog in the transmission of the disease (n = 48/67). They also reported that the main symptom was itching and could be transmitted by contact (Table 2).

Tutors who are illiterate or with incomplete primary education have a prevalence 0.57 times higher than tutors with complete primary education of being infected (Bernardino et al., 2020). Because they did not receive basic education in schools or information from the endemic agent or community health agent, the population is eight times more likely to develop the disease (Borges et al., 2008; Oliveira-Neto et al., 2018).

According to the clinical evaluation, 35% (n=7) of the dogs were classified as symptomatic (OR = 4.12; p = 0.0276) and 11.54% (n=6) as asymptomatic. Tick infestation was observed in 30.55% of the dogs and fleas in 2.7%, and it was observed that 20.83% of the seropositive dogs for VL did not present any of these ectoparasites (Table 3). However, when analyzing the results of the ehrlichiosis test, it was identified that of the 22 dogs with ticks, 81.82% dogs were positive for *Ehrlichia canis*. This high percentage of dogs with the tick may indicate fragility in the guardian's care for the canine's health, leaving him vulnerable to other diseases (Bernardino et al., 2020).

The most common clinical manifestations were dry or injured snout (OR = 5.46; p = 0.0149), eyeball with uveitis, ulcer and secretion (OR = 7.57; p = 0.0056), ear with otitis, lesion and desquamation (OR = 4.66; p = 0.0173), pale mucosa (OR = 4.76; p = 0.0224), lymph nodes (OR = 1.06; p = 0.5894) and skin and annex with crusted lesions,

Table 2. Distribution of intervening sociodemographic and breeding factors for Canine Visceral Leishmaniasis, Sergipe, Brazil, 2022.

Epidemiological variables	N	P	Seroprevalence (%)	Statistical Analysis	
Educational level *					
Illiterate	7	0	0.00	G = 3.12	p = 0.3730●
Elementary School	13	3	18.75		
High school	18	2	10.00		
Complete higher education	19	5	20.83		
Family income*					
Less than 1 minimum wage	30	4	11.76	G = 4.98	p = 0.0827●
1 to 2 minimum wages	10	0	0.00		
2 to 6 minimum wages	17	6	26.09		
Do you know what Leishmaniasis is? *					
Yes	23	5	17.86	**	
No	34	5	12.82	OR=0.67 (0.17-2.60)	p = 0.4072▼
Do you know the importance of the dog in the transmission of the disease?*					
Yes	15	4	21.05	**	
No	42	6	12.50	OR=0.53 (0.13-2.16)	p = 0.2973▼
Type of creation					
Breeding	11	2	15.38	G = 1.47	p = 0.4308●
Stray	3	2	66.67		
Companion	46	8	14.81		
Food					
Commercial feed	10	3	23.08	G = 0.26	p = 0.8754●
Food prepared at home	11	2	15.38		
Mixed	38	8	17.39		
Breeding of other animals					
Yes	32	8	20.00	OR = 1.35 (0.39- 4.61)	p = 0.4352▼
No	27	5	15.63	**	
Free street access					
Yes	15	5	25.00	OR = 1.83 (0.51-6.47)	p = 0.2652▼
No	44	8	15.38	**	
Where does the dog spend most of its time?					
out of the house	45	11	19.64	OR = 1.71 (0.33-8.66)	p = 0.4046▼
inside the home	14	2	12.50	**	
Use of antiparasitic collar					
Yes	13	4	23.53	**	
No	46	9	16.36	OR = 0.63 (0.16- 2.40)	p = 0.3642▼
Type of race					
No defined race	38	9	19.15	OR = 2.48 (0.49-12.59)	p = 0.2224▼
Defined race	21	2	8.70	**	
Age					
Puppy (06 months to 1 year)	22	2	8.33	OR = 0.10 (0.01-0.70)	p = 0.0207▼
Young (2 years to 4 years)	14	3	17.65	OR = 0.25 (0.04-1.43)	p = 0.1232▼
Adult (5 years to 7 years)	6	5	45.45	**	
Elderly (over 8 years old)	17	3	15.00	OR = 0.21 (0.03-1.16)	p = 0.0787▼
Sex					
Male	23	4	14.81	**	
Female	36	9	20.00	OR = 1.43 (0.39-5.21)	p = 0.4132▼
Hair					
Short	39	9	18.75	OR = 1.15 (0.31- 4.21)	p = 0.5523▼
Medium/long	20	4	16.67	**	
Visit to the Veterinarian*					
Yes	20	4	16.67	**	
When symptoms appear	12	2	14.29	OR = 0.83 (0.13-5.25)	p= 0.6146▼
No	25	4	13.79	OR = 0.80 (0.17-3.60)	p= 0.5338▼
Vaccine - Leishmaniasis					
Yes	7	1	12.50	**	
No	52	12	18.75	OR = 1.61 (0.18-14.39)	p = 0.5558▼
Others	37	9	19.57	-	

N- Negative; P- Positive; OR: Odds Ratio (Confidence Interval). *stray dogs were removed because they did not have a guardian; n=67; **Reference category; ▼Fisher's Exact Test; ●G Test (Williams).

Table 3. Distribution of clinical signs according to Canine Visceral Leishmaniasis seropositivity of, Sergipe, Brazil, 2022.

Clinical signs by body region	N	P	Seroprevalence (%)	Statistical Analysis	
Symptomatology					
Symptomatic	13	7	35.00	OR = 4.12 (1.18- 14.44)	p = 0.0276▼
Asymptomatic	46	6	11.54	**	
Ectoparasites					
Flea	2	0	0.00	G = 1.06	p = 0.5867●
Tick	19	3	13.64		
Absent	38	10	20.83		
Snout					
Normal	51	7	12.07	**	
Changed	8	6	42.86	OR = 5.46 (1.45-20.47)	p = 0.0149▼
Eyeball					
Normal	53	7	11.67	**	
Changed	6	6	50.00	OR = 7.57 (1.90-30.06)	p = 0.0056▼
Heard					
Normal	56	9	13.85	**	
Changed	3	4	57.14	OR = 4.66 (0.89-24.40)	p = 0.0173▼
Lymph nodes*					
Normal	46	10	17.86	OR = 1.06 (0.25-4.43)	p = 0.5894▼
Changed	13	3	18.75	**	
Skin and appendages					
Normal	37	4	9.76	**	
Changed	22	9	29.03	OR = 3.78 (1.04-13.75)	p = 0.0365▼
Mucosa					
Normal	50	7	12.28	**	
Pale	9	6	40.00	OR = 4.76 (1.29-7.49)	p = 0.0224▼

N- Negative; P- Positive; OR: Odds Ratio (Confidence Interval); **Reference category; ▼Fisher's Exact Test; ●G Test (Williams).

alopecia, seborrhea and onychogryphosis (OR = 3.78; p = 0.0365) (Figure 3) (Table 3).

The characteristic symptomatology of leishmaniasis was observed in this study as well as in other studies that described dogs with lymphadenopathy, onychogryphosis, alopecia and desquamation (Pasanisi, 2020). Even though there is a difference between clinical signs and infection, it is known that symptomatic and asymptomatic dogs have the ability to transmit the disease if the vector is present (Djibougou et al., 2022).

3.3. Evaluation of Volatile Organic Compounds (VOCs) of canine hair

Among the hair samples analyzed by GC-MS, four dogs tested positive for *Leishmania* spp. by the ELISA test (A04, A13, B01 and E01). A total of 53 compounds were identified with their respective retention rates and concentration found (Table 4). These VOCs belong to six organic functions: hydrocarbon (39.62%; n= 21), aldehyde (24.52%; n= 13), alcohol (16.98%; n= 9), ketone (9.43%; n=5), ester (5.66%; n=3) and carboxylic acid (1.89%; n=1) and ether (1.89%; n=1).

It was possible to observe that the compounds 2- butoxyethanol, benzaldehyde, decane, 2-phenylacetaldehyd, nonan-1-ol, 2- phenoxyethanol, nonanoic acid, 8- heptadecene and eicosane were found only in positive dogs (highlighted in green), even though the seropositive samples were not identified at the same time. 6-methyl-5-hepten-2-one, pentadecana, 2,6,10-trimethylpentadecane, tetradecan-1-ol and methyl *n*-hexadecanoate were found in at least one negative dog (highlighted in yellow). Ten compounds were identified in all evaluated animals, seropositive and seronegative (highlighted in pink) (Table 4).

Cluster analysis was performed considering the concentration of each substance, but it did not show differentiation between positive and negative dogs. A clustering tendency is observed in two seroreactive dogs, B01 and E01. Dog B01 was found co-infected with *Ehrlichia canis* and E01 was the only dog with symptoms found, showing a higher number of identified compounds, this may be related to the inflammatory metabolic process occurred by the infections (Figure 4).

Figure 3. Signs and symptoms of collected dogs. A and E (strays) – alopecia, dehydrated, ectoparasite and skin lesions; B, C, D and F (companions) – uveitis, alopecia, skin lesions, ocular alopecia, and ocular discharge.

Despite the existence of studies identifying and evaluating the attractiveness of VOCs associated with leishmaniasis (Oliveira et al., 2008; Magalhães-Junior et al., 2014, 2019; Tavares et al., 2018), the present research is one of the few that proposed to evaluate volatile compounds in dog hair and demonstrate a promising path for identifying biochemicals, with the prospect of monitoring these substances.

From VOCs in canine hair and it was possible to differentiate the profile of dogs based on clinical characteristics and leishmaniatic infection, namely: pentadecane, hexadecane, 2-hexanone, 2-decanone, dodecanone, 2-octanone, 2-nonanone, eicosane, 2-octenal, benzaldehyde, tridecane, 2-heptenal, heneicosane, nonadecane, heptanal, 2,4-nonadienenal, pentadecane, nonanal, decanal, octadecane, heptadecane, tetradecane, hexanal and octana. The identified compounds benzaldehyde, 2-hexanone and 2,4-nonadienal showed greater potential for association with infected dogs (Oliveira et al., 2008).

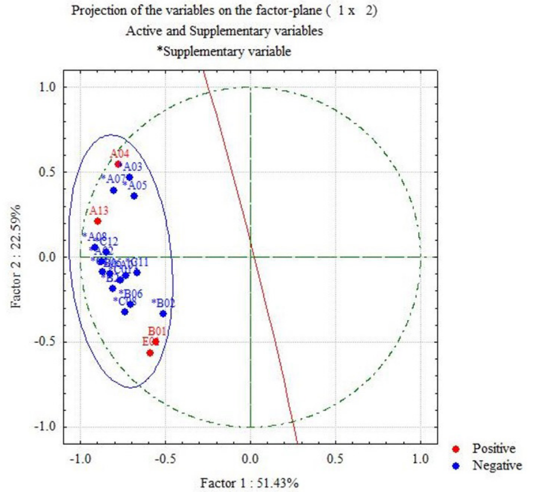

Figure 4. Principal Component Analysis of hair samples from infected and uninfected dogs.

Table 4. Identified Volatile Organic Compounds (VOC), in ascending order of retention time (tR), with their respective retention indexes (RI) calculated and obtained from the databases. Numbers indicate the relative area of the compound in the sample; a trace (-) indicates the absence of the compound in the sample.

	IR Exp	IR lit	Compound	Seroreagent									
				Negatives						Positives			
				A03	A05	A07	A08	B24	C01	A04	A13	B01	E01
1	892	880	cyclooctatetraene	-	-	0.27	0.54	-	-	-	-	**4.72**	-
2	905	902	heptanal*	-	-	0.46	1.26	-	1.34	**0.24**	**0.56**	**0.47**	-
3	905	909	2-butoxietanol	-	-	-	-	-	-	-	-	-	**3.86**
4	965	960	benzaldehyde *	-	-	-	-	-	-	-	-	**0.66**	-
5	992	987	6-metil-5-hepten-2-ona	-	-	-	-	-	0.85	-	-	-	-
6	1007	1005	octanal	-	-	-	-	1.89	-	-	**0.21**	-	**1.00**
7	1016	1000	decane*	-	-	-	-	-	-	-	-	**1.49**	-
8	1036	1029	2-etilhexan-1-ol	1.60	2.06	3.64	2.70	5.53	1.84	**3.12**	**2.44**	**39.39**	**0.82**
9	1041	1043	benzyl alcohol	6.10	2.59	3.07	4.79	3.44	1.68	**3.09**	**3.53**	**3.73**	**12.29**
10	1046	1049	2-fenilacetaldeído	-	-	-	-	-	-	-	-	-	**0.18**
11	1072	1068	1-feniletanona*	3.69	1.61	2.17	2.42	-	0.98	**2.17**	**2.35**	**2.02**	-
12	1079	1072	octan-1-ol*	-	-	0.33	0.43	1.61	1.06	**0.36**	-	**0.46**	**0.92**
13	1107	1109	nonanal*	5.70	7.47	8.18	20.70	25.89	17.96	**6.57**	**16.19**	**16.42**	**18.80**
14	1165	1163	(*E*)-non-2-enal*	0.20	0.28	0.70	0.89	1.42	2.56	-	-	-	**1.73**
15	1174	1172	nonan-1-ol	-	-	-	-	-	-	-	**0.67**	-	**0.75**
16	1183	1183	naphthalene*	2.36	-	2.40	2.48	0.61	2.55	-	**2.55**	**5.93**	**0.52**
17	1196	1191	decan-2-ona*	-	0.73	0.40	-	1.64	-	**1.13**	-	-	**0.88**
18	1200	1200	dodecane*	1.97	1.29	2.45	4.14	1.71	2.10	**3.79**	**2.66**	**1.36**	-
19	1208	1208	decanal*	1.73	2.62	1.62	2.77	2.41	10.30	**3.19**	**3.47**	**3.47**	**3.50**
20	1216	1220	2-fenoxietanol*	-	-	-	-	-	-	-	-	-	**0.24**
21	1266	1266	decan-1-ol	-	1.46	0.92	-	0.79	-	**1.48**	**0.79**	-	**0.52**
22	1284	1278	nonanoic acid	-	-	-	-	-	-	-	-	**0.52**	**1.36**
23	1292	1295	undecan-2-ona*	-	-	-	0.18	-	-	-	-	-	**0.24**
24	1300	1300	tridecane*	-	-	-	1.00	-	0.61	-	-	**1.35**	-
25	1310	1306	undecanal*	-	1.33	0.80	1.48	0.84	1.28	**1.30**	**1.27**	**1.51**	**0.79**
26	1373	1372	3-metiltridecano	-	3.00	5.35	4.59	2.55	-	-	**3.93**	-	-
27	1400	1400	tetradecane*	14.47	7.86	12.08	11.55	11.13	8.98	**17.70**	**11.06**	**3.55**	**1.21**
28	1410	1408	dodecanal*	1.40	1.92	1.05	2.02	3.61	1.30	**2.30**	**2.26**	**1.64**	-
29	1453	1455	geranylacetone	2.75	1.43	2.26	1.05	3.82	6.19	**2.48**	**2.48**	**0.48**	**1.87**
30	1478	1473	dodecan-1-ol*	2.39	1.16	0.96	1.00	11.70	-	-	-	**1.36**	**4.81**
31	1500	1500	pentadecane*	4.88	2.52	1.11	2.87	0.87	1.10	**2.77**	**2.31**	**1.40**	**3.68**
32	1512	1513	tridecanal	0.64	1.42	1.09	0.91	-	-	**1.75**	-	**0.85**	**2.50**
33	1568	1570	3-methylpentadecane*	10.67	6.26	8.75	5.91	6.18	4.42	**11.79**	**7.73**	**1.62**	**0.96**
34	1600	1600	hexadecane*	15.19	10.22	9.95	7.03	5.19	6.05	**13.33**	**8.55**	**2.65**	**2.15**
35	1617	1612	tetradecanal	1.69	1.28	2.14	0.98	-	0.56	**1.43**	**1.69**	**0.59**	**5.76**
36	1654	1649	pentadecane, 2,6,10-trimethyl-	-	5.90	-	0.98	-	-	-	-	-	-
37	1679	1676	8-heptadecene	-	-	-	-	-	-	-	-	-	**2.80**

*VOC previously identified from dog hair samples (Magalhães-Junior et al., 2014). A: Breeding dogs; B and E: Companion dog; C: stray dog.

Table 4. Continued...

	IR Exp	IR lit	Compound	Seroreagent									
				Negatives						Positives			
				A03	A05	A07	A08	B24	C01	A04	A13	B01	E01
38	1679	1680	1-tetradecanol	-	-	-	-	2.51	-	-	-	-	-
39	1700	1700	heptadecane*	5.28	3.13	1.49	1.69	-	2.94	**2.47**	**1.64**	**0.76**	**3.96**
40	1710	1703	2,6,10,14-tetramethylpentadecane	-	8.14	1.19	0.98	-	-	-	**2.75**	-	-
41	1718	1715	pentadecanal	-	-	-	0.55	-	-	-	-	-	-
42	1751	1746	2-benzylideneacetone	11.94	-	-	-	-	2.82	**7.99**	-	**0.91**	-
43	1774	1770	3-methylheptadecane	-	5.32	6.52	3.13	-	-	**5.57**	**3.84**	-	**0.75**
44	1801	1800	octadecane*	3.71	3.17	2.02	1.29	0.86	3.39	**2.84**	**1.53**	**0.69**	**0.97**
45	1806	1810	2-phenoxybenzamine	-	11.39	11.91	4.97	-	-	-	**11.06**	-	-
46	1820	1821	hexadecanal	-	-	0.66	0.35	-	0.28	-	-	-	**1.15**
47	1832	1826	propan-2-yl tetradecanoate *	1.64	1.41	0.71	0.58	-	0.50	**1.14**	**0.52**	-	-
48	1869	1857	benzyl salicylate	-	3.03	2.03	1.79	-	-	-	**1.97**	-	-
49	1885	1880	hexadecanol	-	-	-	-	3.80	12.10	-	-	-	**15.09**
50	1901	1900	nonadecane*	-	-	-	-	-	0.92	-	-	-	**1.11**
51	1931	1926	methyl *n*-hexadecanoate	-	-	0.85	-	-	3.34	-	-	-	-
52	1993	1991	ethyl hexadecanoate	-	-	0.47	-	-	-	-	-	-	**0.41**
53	1999	2000	icosane	-	-	-	-	-	-	-	-	-	**2.42**

*VOC previously identified from dog hair samples (Magalhães-Junior et al., 2014). A: Breeding dogs; B and E: Companion dog; C: stray dog.

As also in the report by Magalhães-Junior et al. (2014) who related the compounds octanal, nonanal, undecane, β-hydroxyethylphenyl ether, decanal, tetradecane, nonylcyclopentane, 8-pentadecanone, heptadecane and 2-ethylhexyl-salicylate to parasitized dogs. When using some of these compounds to evaluate the attractiveness of *Lu. longipalpis* was observed that the compounds octanal, nonanal, decanal and heptadecane activated and attracted males and females of *Lu. longipalpis* (Magalhães-Junior et al., 2019).

In another study, it was possible to relate VOCs and canine VL using a Y-tube olfactometer, where the activation/attraction of the vector species to infected and non-infected dogs was observed. It is suggested that the pathogen may change the odor of its hosts to attract the vector (Gandon, 2018).

The compounds pentadecane, hexadecane, dodecane, eicosane, benzaldehyde, tridecane, nonadecane, heptanal, pentadecane, nonanal, decanal, octadecane, heptadecane, tetradecane and octanal, were related to infection, according to the mentioned works, and were present in the animals here analyzed.

Benzaldehyde was identified in this work in a positive dog, being important for the orientation of the species *Lu. longipalpis* (Dougherty et al., 1999). The other correlated compounds were found in negative dogs, and it is still not possible to establish a volatile release pattern for this disease in the present study.

4. Conclusion

There is active transmission of *Leishmania* spp. in the municipalities of Aracaju and Propriá in the state of Sergipe. Some aspects related to the presence of nearby vegetation, age, keeping dogs, free access to the street and keeping other animals can contribute to the maintenance of the leishmanianiasis transmission. Because of this, control measures aimed at peridomiciliary environmental management and care for the health of dogs can help reduce the rates of canine VL. The volatile organic compounds identified were consistent with the literature, but it was not possible to associate VOCs with infection by *Leishmania* spp.

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