

## Environmental factors associated with canine visceral leishmaniasis in an area with recent introduction of the disease in the State of Rio de Janeiro, Brazil

Fatores ambientais associados à ocorrência de leishmaniose visceral canina em uma área de recente introdução da doença no Estado do Rio de Janeiro, Brasil

Factores ambientales asociados a la ocurrencia de leishmaniosis visceral canina en un área de reciente introducción de la enfermedad en el estado de Río de Janeiro, Brasil

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doi: 10.1590/0102-311X00021117

### Abstract

*The study assessed the association between environmental characteristics obtained by remote sensing and prevalence of canine visceral leishmaniasis (CVL) in the neighborhood of Jacaré, an area with recent introduction of the disease in the municipality (county) of Niterói, Rio de Janeiro State, Brazil. This was a cross-sectional study to assess CVL prevalence, defined as a positive result in the dual path platform (DPP) rapid immunochromatographic assay, confirmed by immunoenzymatic assay (IEA). The study included 97 dogs, with 21.6% CVL prevalence. CVL prevalence was higher in dogs with contact with another dog, opossum, marmoset, or hedgehog, as well as history of culling of other dogs with CVL from the household. In the multivariate analysis, adjusted for the dog's sex and age, dogs in areas with sparse vegetation showed fivefold higher prevalence of Leishmania infantum infection compared to dogs in areas with less vegetation (OR = 5.72; 95%CI: 1.47-22.20). Meanwhile, less urbanized areas, characterized as commercial or low-income residential areas, identified by remote sensing as those with high density of gray structures, were associated with lower CVL prevalence (OR = 0.09; 95%CI: 0.01-0.92). The higher prevalence of infection in dogs living alongside wild animals and in areas with more vegetation and lower prevalence in more urbanized areas suggest a rural transmission pattern for CVL in this area.*

Visceral Leishmaniasis; Dogs; Remote Sensors

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## Introduction

Visceral leishmaniasis (VL) is an important zoonotic disease in public health and ranks ninth worldwide in burden of infectious diseases<sup>1,2</sup>. The disease is endemic in 65 countries, with an estimated incidence of 400,000 new cases and 50,000 deaths per year<sup>3</sup>.

In Brazil, the disease is caused by *Leishmania infantum*, and the principal vector is the sand fly *Lutzomyia longipalpis*, with dogs (*Canis familiaris*) as the principal reservoir in the domestic and peri-domestic environment<sup>4</sup>. In dogs, parasitism is abundant in the viscera and dermis, but infected dogs can remain without clinical signs for long periods of time, serving as a source of infection for the vector<sup>1</sup>.

For control of the canine reservoir in endemic areas, the Brazilian Ministry of Health recommends performing serological surveys and culling of seropositive dogs<sup>5</sup>.

Previously known as a disease with rural characteristics, VL has become endemic and epidemic in large Brazilian cities since the 1980s<sup>6</sup>. Disordered urban land occupation associated with migration, unplanned urban settlements, and precarious sanitation lead to substandard living conditions and environmental destruction, fostering the conditions for sand fly reproduction<sup>7,8</sup>.

The evaluation of endemic diseases from the perspective of various elements involved in the transmission cycle, such as environmental and social determinants of the disease, has been the focus of some studies employing geoprocessing techniques<sup>8,9,10,11,12</sup>. Geoprocessing tools, especially remote sensing, can help identify environmental factors associated with the occurrence of VL. The identification of these factors can support resource allocation and implementation of control measures<sup>1,11,12</sup>. However, some studies have highlighted that the use of remote sensing images for studying endemics in urban and periurban areas raises major conceptual and technical challenges<sup>12,13,14</sup>.

New areas have been identified with the occurrence of canine visceral leishmaniasis (CVL) in various municipalities (counties) in the State of Rio de Janeiro<sup>15,16,17</sup>. However, little is known about the environmental factors involved in the emergence of CVL in these areas. This article aims to evaluate the association between environmental characteristics obtained by remote sensing and the occurrence of CVL in an area with recent introduction of the disease in the neighborhood of Jacaré, Niterói.

## Materials and methods

### Study design and site

An investigation was conducted in a focus of canine visceral leishmaniasis based on the index case identified in 2009 in the neighborhood of Jacaré (Figure 1). According to Ministry of Health guidelines for the evaluation of canine infection with *L. infantum*, an area was demarcated for investigation of the focus and a cross-sectional was performed in 110 dogs living around the index case, from December 2011 to March 2012<sup>5</sup>.

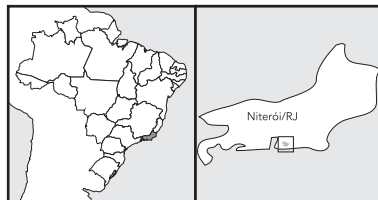
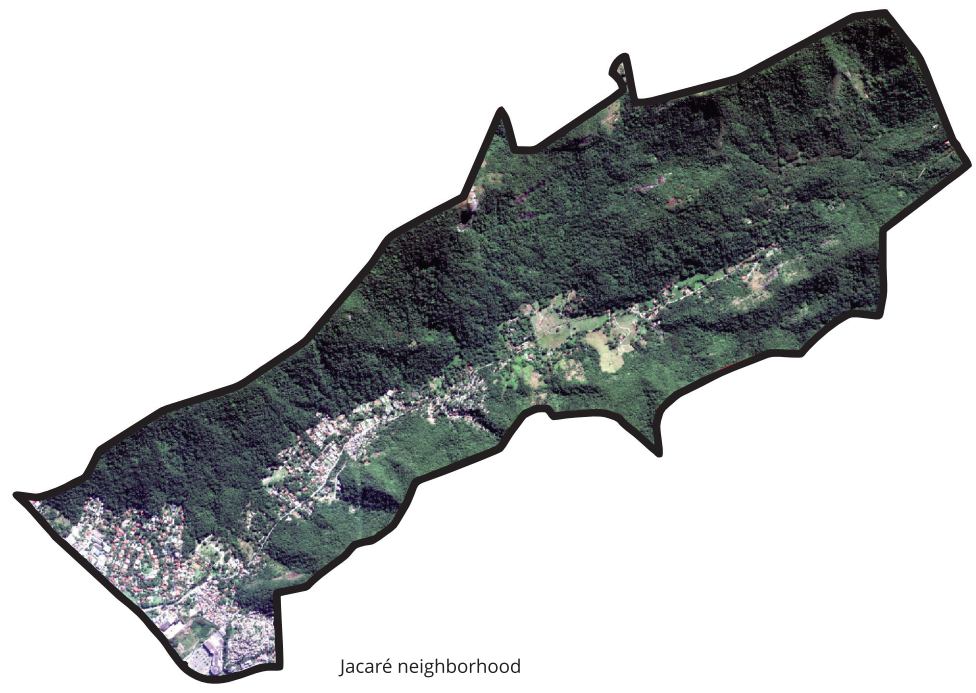
The Jacaré neighborhood borders on the neighborhoods of Piratininga, Cafubá, Cantagalo, Vila Progresso, Muriqui, Rio do Ouro, Serra Grande, and Santo Antônio<sup>18</sup>. Jacaré is located in the municipality of Niterói, located at 22°53'00" latitude south and 43°06'13" longitude west, 5 meters above sea level, with Atlantic Forest vegetation<sup>19</sup>. Since the 1960s, the area has been occupied by squatters coming from various places. The neighborhood is located in the Darcy Ribeiro Ecological Reserve, with rural characteristics and some small farmers, besides a recent increase in substandard housing clusters. Spatial occupation occurred along the neighborhood's main road, with a predominantly low-income population<sup>20</sup>. According to the 2010 Census, Jacaré has an area of 9.45km<sup>2</sup>, with 3,563 inhabitants<sup>19</sup>.

### Data collection

The field team visited all the households, starting from the index case, until reaching the sample of 110 dogs.

**Figure 1**

WorldView 2 satellite image of the neighborhood of Jacaré, Niterói, Rio de Janeiro State, Brazil.



After explaining the study's objectives and signing the consent form, a questionnaire was completed with the owners' information on the dogs, like sex, age, and contact with other animals, and on the households (rural versus urban location), as well as previous cases of human or canine VL in the household.

Next, the dogs were restrained mechanically and gagged for drawing blood samples for the *dual path platform* (DPP) rapid immunochromatographic assay and immunoenzymatic assay (IEA), both produced by Immunobiological Technology Institute (Biomanguinhos) at the Oswaldo Cruz Foundation (Fiocruz). The DPP assay was performed in the field with whole blood. Blood samples were stored in tubes without anticoagulant and transported under refrigeration to the Immunodiagnostic Sector of the Public Health Research and Service Laboratory at the Sergio Arouca National School of Public Health (ENSP/Fiocruz), where serum was obtained for performing IEA with the IEA kit, according to manufacturer's instructions.

Dogs that tested positive dogs in the DPP assay were sedated to obtain full skin samples from the scapular region and bone marrow aspirates, according to the protocol by Abrantes et al. <sup>15</sup>. Skin samples were packed in phosphate buffer saline (PBS) solution, with the addition of antibiotics (penicillin and streptomycin) and an antifungal (fluorocytosine), and the bone marrow samples were stored directly in Novy, MacNeal, Nicole (NNN) biphasic culture, both transported under refrigeration for processing at the Laboratory for Clinical Research and Surveillance in Leishmaniasis (LapClin-VigiLeish) at the National Institute of Infectology (INI/Fiocruz) for parasitological culture based on the protocol by Silva et al. <sup>21</sup>. Isoenzymatic characterization of the isolates followed the protocol by Cupolillo et al. <sup>22</sup>.

Diagnosis of *L. infantum* infection was defined by isolation of the parasite in culture or positive concordance of DPP and IEA.

### **Geoprocessing**

Geocoding of the dogs' addresses used global positioning system (GPS). Data from the GPS signal receiver were configured to furnish the positions with flat coordinates on the projection of the Universal Transverse Mercator (UTM) system and World Geodetic System (WGS) Datum 84. Dot maps were prepared using the open-access software TerraView 4.2.2, available on the website of the Brazilian National Institute for Space Research (INPE; <http://www.dpi.inpe.br/terraview>).

Remote sensing of the Jacaré neighborhood used an image from the year 2010 in WorldView 2, a satellite sensor with high spatial resolution. The street grid from the Jacaré neighborhood was used for better definition of the urban context, acquired from the website of the Brazilian Institute of Geography and Statistics (IBGE).

A 100-meter buffer was applied around the cases, divided into 25x25 cells (625 square meters). Classification of the image, performed only in cells with dogs, aimed to extract the following characteristics pertaining to land cover: swimming pools (water), dark gray cover (characterizing commercial areas and areas with gray asbestos roofing, indicating poverty-prone urbanized areas), ceramic roof tiles (red), metal roofing, and vegetation (dense versus sparse). Classes of vegetation were discriminated by the Normalized Difference Vegetation Index (NDVI), which allowed minimizing the spectral confounding resulting from shadow effects <sup>13</sup>.

Land cover characteristics were obtained by five-stage object-oriented classification: characterization of classes, image segmentation, elaboration of the semantic network, classification, and evaluation of the results <sup>12</sup>.

Image classification used eCognition Developer 64 8.0 (<http://www.ecognition.com/>), which included two steps: multiresolution segmentation and classification with fuzzy logic and Boolean algorithms.

After visual interpretation of each image for definition of thematic classes, the satellite image was segmented by a multiresolution algorithm, in which objects were created on different scales according to shape, color, and homogeneity <sup>11</sup>.

## Statistical analysis

Tabulations were used to describe the following characteristics: prevalence of *L. infantum* infection, dog's sex and age, proximity of household to forest, degree of dog's confinement (indoors, access to yard, or access to street or free-roaming), contact with other animals, specifically dogs, cats, opossums, marmosets, and hedgehogs, and history of CVL in the household.

Bivariate analyses used Fisher's exact test to evaluate differences in the prevalence of *L. infantum* infection according to the characteristics of the dogs and households.

Environmental variables were categorized using the cutoff with the best predictive power, identified by classification trees as follows: minimum NDVI ( $\leq -0.85 / > -0.85$ ); medium NDVI ( $\leq 0.63 / > 0.63$ ); maximum NDVI ( $\leq 0.96 / > 0.96$ ); dense vegetation ( $\leq 67 / > 67.5$ ); ceramic tile roofing (%) ( $\leq 3.0 / > 3.0$ ); metal roofing (%) ( $\leq 1.75 / > 1.75$ ); gray cover (%) ( $\leq 5.0 / > 5.0$ ); sparse vegetation (%) ( $\leq 42.5 / > 42.5$ ).

Associations between environmental variables and canine infection were expressed as odds ratios (OR) with 95% confidence intervals (95%CI), obtained by simple logistic regression and adjusted regression according to dog's sex and age.

## Ethical aspects

The project was approved by the Institutional Review Board on Use of Animals (CEUA/Fiocruz), license LW-47-12.

## Results

A total of 49 households were investigated where there were reports of six dogs with CVL and absence of human cases of VL before the survey. In the 110 dogs examined in the canine survey, 97 were included in the analyses, due to lack of 13 georeferenced points.

As for the characteristics of the 97 dogs, 54.1% were males, 61.1% were one to seven years old, 91.3% lived in forest areas, 60% had their access limited to the yard, and 90.2% lived alongside other animals, 82.6% of which were other dogs.

Prevalence of *L. infantum* infection was 21.6%. Positive rates for DPP, IEA, and parasitological culture were 26.3%, 30.9%, and 16.6%, respectively. Of the 21 dogs that tested positive, four showed isolation of *L. infantum* in culture.

Characteristics of the dogs' rearing environment were obtained from the owners of 92 dogs. There was a higher CVL rate in dogs with contact with another dog, opossum, marmoset, or hedgehog, as well as with history of culling of other dogs with CVL from the household (Table 1).

Figure 2 shows the pattern of dense and sparse vegetation in the analytical units (squares).

The proportions of classes of squares were 0.01% (swimming pool), 3.5% (gray cover), 1.1% (ceramic tile roofing), 0.9% (metal roofing), 69.7% (dense vegetation), and 24.5% (sparse vegetation).

Dogs living in areas with more sparse vegetation showed more than fivefold higher prevalence of *L. infantum* infection than those living in areas with less vegetation (OR = 5.72; 95%CI: 1.47-22.20). Meanwhile, more urbanized areas, characterized as commercial or low-income residential, identified by remote sensing as those with high density of gray structures, were associated with lower prevalence of CVL (OR = 0.09; 95%CI: 0.01-0.92) (Table 2).

## Discussion

CVL prevalence in this study (21.65%) was similar to rates in Rio de Janeiro State both in areas of recent introduction (25.2% in Itaipuaçu, Maricá; 18.1% on Ilha de Marambaia, Rio de Janeiro) and in areas classified as endemic (25% in Barra de Guaratiba, Rio de Janeiro)<sup>15,23,24</sup>, notwithstanding differences in methodologies. Prevalence rates can vary due to the diagnostic test used and the way dogs were recruited for the study<sup>25</sup>, but the similar results in different studies indicate high endemicity of CVL in different regions of the State of Rio de Janeiro.

**Table 1**

Prevalence of canine visceral leishmaniasis (CVL) according to characteristics of dogs' rearing environment. Jacaré neighborhood, Niterói, Rio de Janeiro State, Brazil, 2011-2012.

Characteristics of dogs' rearing environment	n	%	p-value
Location of household			
Rural	8	25.0	1.000
Forest	84	22.6	
Dog's mobility			
Indoors	7	14.2	0.734
Access to yard	54	22.2	
Access to street	29	27.5	
Contact with other animals			
No	9	0.0	0.112
Yes	83	25.3	
Contact with other dogs			
No	16	0.0	0.018
Yes	76	27.3	
Contact with cats			
No	70	27.1	0.090
Yes	22	9.0	
Contact with opossums			
No	90	21.1	0.050
Yes	2	100.0	
Contact with marmosets			
No	90	21.1	0.050
Yes	2	100.0	
Contact with hedgehogs			
No	90	21.1	0.050
Yes	2	100.0	
Previously culled dog with CVL			
No	86	18.6	0.002
Yes	6	83.3	

The geographic expansion of CVL has been recorded in other states of Brazil, attributed to various factors such as difficulties in eliminating reservoirs, epidemiological diversity of the affected regions, high financial costs of sustaining control measures, the vector's high capacity to adapt to the peridomestic, and insufficient control measures <sup>26</sup>.

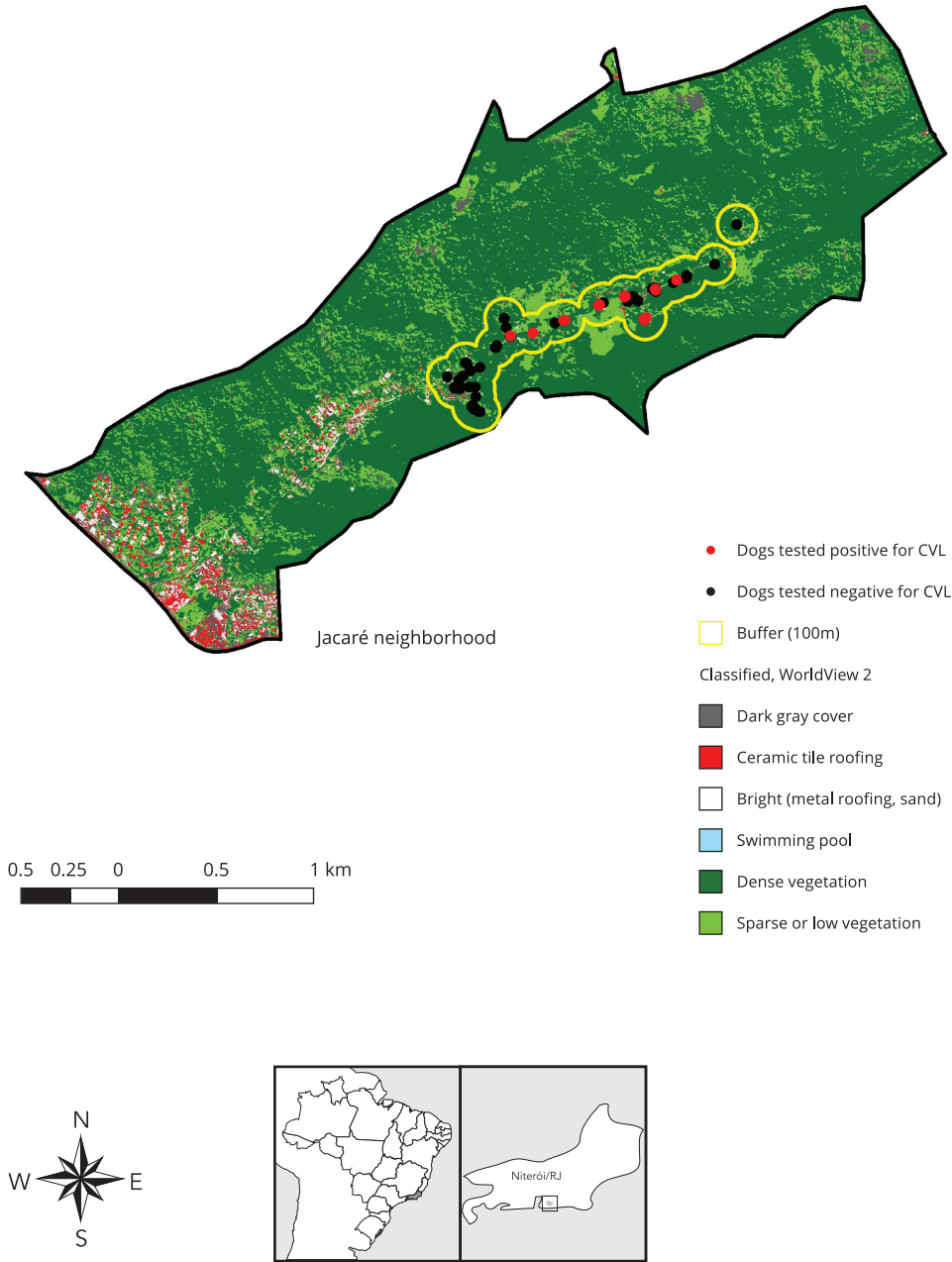
New cases of CVL have been reported in various regions of the State of Rio de Janeiro <sup>17,27,28,29,30</sup>, which indicates a possible change in the disease's behavior, allowing the emergence of outbreaks in urban areas, as has occurred in other Brazilian states <sup>31</sup>. Environmental changes associated with migratory movements and the urbanization process are possible explanations for the urbanization of VL, a disease originally limited to rural areas and which since began to occur endemically and epidemically in large Brazilian cities <sup>5,32</sup>.

The entomological survey reported by Oliveira et al. <sup>16</sup>, conducted in the same area and at the same time as this study, did not detect the principal vector of CVL transmission, *Lu. longipalpis*. Even considering the inherent difficulties in the detection of leishmaniasis vectors, the finding suggests that other sand fly species found in this area, like *Lu. migonei*, may be involved in CVL transmission <sup>16,33</sup>.

There was no association between *L. infantum* infection and the dog's sex, age, or degree of confinement, corroborating other studies <sup>24,34,35,36,37</sup>. The household's proximity to forest areas also failed to show an association with *L. infantum* infection. However, a study in Cuiabá, capital of Mato

**Figura 2**

WorldView 2 satellite image of the neighborhood of Jacaré, Niterói, Rio de Janeiro State, Brazil.





**Table 2**

Odds ratios (OR) and 95% confidence intervals (95%CI) for canine visceral leishmaniasis associated with environmental characteristics. Jacaré neighborhood, Niterói, Rio de Janeiro State, Brazil, 2011-2012.

Environmental variables	OR	95%CI	p-value	OR *	95%CI *	p-value *
Minimum NDVI						
≤ -0.85	1.00	0.50-9.08	0.300	1.00	0.40-8.89	0.421
> -0.85	2.14			1.88		
Medium NDVI						
≤ 0.63	1.00	0.12-1.64	0.224	1.00	0.12-2.11	0.352
> 0.63	0.44			0.50		
Maximum NDVI						
≤ 0.96	1.00	0.18-2.31	0.517	1.00	0.88-5.98	0.807
> 0.96	0.66			0.84		
Dense vegetation (%)						
≤ 67.5	1.00	0.09-1.77	0.235	1.00	0.13-2.90	0.544
> 67.5	0.41			0.61		
Ceramic tile roofing (%)						
≤ 3.0	1.00	0.04-2.28	0.249	1.00	0.02-2.15	0.194
> 3.0	0.30			0.22		
Metal roofing (%)						
≤ 1.75	1.00	1.58-108.90	0.017	1.00	0.91-78.9	0.060
> 1.75	13.10			8.48		
Gray cover (%)						
≤ 5.00	1.00	0.01-1.06	0.058	1.00	0.01-0.92	0.042
> 5.00	0.13			0.09		
Sparse vegetation (%)						
≤ 42.5	1.00	1.77-28.40	0.006	1.00	1.47-22.2	0.012
> 42.5	7.11			5.72		

NDVI: Normalized Difference Vegetation Index.

\* Adjusted for dog's sex and age.

Grosso State, identified as the principal risk factors for canine infection the confinement in the peridomiciliary environment and households' proximity to forest and dense vegetation <sup>36</sup>. This fact may be associated with greater exposure to the vector, increasing the odds of canine infection in the area. The lack of identification of *Lu. longipalpis* in this study may be related to the difference in these results.

The association of *L. infantum* infection with history of CVL in the household was considered a factor for the increase in prevalence of infection, corroborating findings in Teresina, Piauí State, Brazil <sup>38</sup>. This suggests that removing and sacrificing infected dogs as a control strategy is ineffective for interrupting leishmaniasis transmission, given the high rate of replacement of culled dogs with new and susceptible dogs <sup>39</sup>.

The presence of domestic and wild animals was associated with higher prevalence of infection. This suggests that the presence of these animals favors maintenance of the transmission cycle for canine infection, due to the animals' attraction of sand flies, as described in other studies <sup>24,38,40</sup>.

Despite the existence of low-income settlements and substandard clusters in the study area, the spatial distribution of *L. infantum* infection only occurred in the rural area of the neighborhood, along the main road, in less clustered houses with better building materials. These findings contradict others showing the proximity of households with dogs to slum areas as a risk factor for *Leishmania* infection <sup>9</sup>. However, one should not underestimate the risk that the disease will expand throughout the neighborhood, since the local environment shows favorable conditions for the vector's development and adaptation and maintenance of the leishmaniasis transmission cycle. Factors such as temperature,



relative humidity, and precipitation can influence the sand fly's population density. Environmental changes due to disordered human occupation and encroachment on forest areas allow the vectors to approach the domicile and peridomicile, and the leishmaniasis cycles occur in this modified environment<sup>41,42</sup>.

The prevalence rates for *L. infantum* infection were higher in areas with sparse vegetation. Meanwhile, there was no statistically significant association between dense vegetation and *L. infantum* infection. The fact that no association was found with the presence of dense vegetation or proximity to it, which various studies of human and canine VL have highlighted<sup>11,43,44</sup>, may be due to the fact that this type of land cover was highly abundant, covering at least 40% of the area in the squares. Thus, all the squares were under the influence of favorable conditions for the vector's existence due to the presence and abundance of dense vegetation. Sparse vegetation was less frequent (24.5%), with squares with a small area covered by this class of plant cover (10%), and others with much higher cover (55%), a variety that favors the identification of associations when they actually exist.

Despite numerous uncertainties on the factors related to patterns in the occurrence and spread of visceral leishmaniasis, the use of GIS and remote sensing proved useful for identifying environmental characteristics that can be used to define areas at increased risk of CVL and thus back the implementation of surveillance and control strategies for human VL. The higher prevalence of infection in dogs living in contact with wild animals and in areas with more plant cover, combined with the lower prevalence in urbanized areas, indicates a rural pattern of CVL transmission in this area.

## Contributors

T. R. Abrantes was responsible for the study conception, clinical evaluation, collection of samples in the dogs, interpretation of results, and writing of the article. G. L. Werneck was responsible for the statistical analysis, interpretation of the results, and revision of the article. A. S. Almeida was responsible for processing the satellite images, interpretation of the results, and revision of the article. F. B. Figueiredo was responsible for the study conception and revision of the article.

## Acknowledgments

The study was funded by the Brazilian Federal Agency for Support and Evaluation of Graduate Education (Capes) and the Rio de Janeiro State Research Foundation (FAPERJ), with the Young Scientist of Our State and Outstanding Doctoral Student Grant for Tuanne Rotti Abrantes, and by the National Council for Scientific and Technological Development (CNPq; case 475658/2013-2; Call for projects 14/2013) and for the research productivity grant, F. B. Figueiredo.

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## Resumo

Foi avaliada a associação entre características ambientais obtidas por sensoriamento remoto e a prevalência da leishmaniose visceral canina (LVC) no bairro do Jacaré, área de recente introdução da doença, no Município de Niterói, Estado do Rio de Janeiro, Brasil. Trata-se de um estudo seccional para avaliação da prevalência de LVC, definida por meio da positividade no teste imunocromatográfico rápido em dupla plataforma (dual path platform – DPP), confirmada com o ensaio imunoenzimático (EIE). Foram incluídos 97 cães com prevalência de LVC de 21,6%. Houve maior frequência de LVC em cães com a convivência com outro cão, gambá, mico e ouriço-terrestre, assim como com a história de remoção de outros cães com LVC do domicílio. Na análise multivariada, ajustada por sexo e idade do cão, cães residentes em áreas com maior cobertura de vegetação esparsa apresentaram prevalência da infecção por *Leishmania infantum* cinco vezes maior do que aqueles que residiam em áreas menos vegetadas (OR = 5,72; IC95%: 1,47-22,20). Por outro lado, áreas mais urbanizadas caracterizadas como comerciais ou residenciais carentes, identificadas pelo sensoriamento remoto como aquelas com alta densidade de estruturas cinza, estiveram associadas à menor ocorrência da LVC (OR = 0,09; IC95%: 0,01-0,92). A maior prevalência de infecção em cães convivendo com outros animais silvestres e em áreas com maior cobertura vegetal, associada com menor prevalência em áreas urbanizadas, indica um padrão rural de transmissão da LVC nessa área.

*Leishmaniose Visceral; Cães; Sensores Remotos*

## Resumen

Se evaluó la asociación entre las características ambientales obtenidas por teledetección y la prevalencia de la leishmaniosis visceral canina (LVC) en el barrio de Jacaré, área de reciente introducción de la enfermedad, en el municipio de Niterói, Estado de Río de Janeiro, Brasil. Se trata de un estudio seccional para la evaluación de la prevalencia de LVC, definida mediante la positividad en el test inmunocromatográfico rápido en una plataforma de doble vía (dual path platform – DPP), confirmada con un ensayo inmunoenzimático (EIE). Se incluyeron a 97 perros con una prevalencia de LVC de un 21,6%. Hubo una mayor frecuencia de LVC en perros que conviven con otros perros, zarigüeyas, monos y erizos terrestres, así como con el historial de retirada de otros perros con LVC del domicilio. En el análisis multivariado, ajustado por sexo y edad del perro, los perros residentes en áreas con una mayor cobertura de vegetación muy dispersa presentaron una prevalencia de infección por *Leishmania infantum* cinco veces mayor que aquellos que residían en áreas con menos vegetación (OR = 5,72; IC95%: 1,47-22,20). Por otro lado, las áreas más urbanizadas, caracterizadas como comerciales o residenciales con pocos recursos, identificadas mediante teledetección como aquellas con una alta densidad de estructuras en gris, estuvieron asociadas a una menor ocurrencia de la LVC (OR = 0,09; IC95%: 0,01-0,92). La mayor prevalencia de infección en perros, conviviendo con otros animales silvestres y en áreas con una mayor superficie vegetal, se asocia con una menor prevalencia en áreas urbanizadas, lo que indica un padrón rural de transmisión de la LVC en ese área.

*Leishmaniasis Visceral; Perros; Sensores Remotos*

Submitted on 09/Feb/2017

Final version resubmitted on 23/May/2017

Approved on 13/Jun/2017