DOI: 10.5433/1679-0359.2018v39n1p211

# Risk factors associated to canine visceral leishmaniasis in Uruguaiana city, Brazil

# Fatores de risco associados à leishmaniose visceral canina no município de Uruguaiana-RS

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## Abstract

The present study aimed to evaluate factors associated with canine visceral leishmaniasis (CVL) in areas with higher seroprevalence of it in Uruguaiana city, Rio Grande do Sul state, Brazil, during the year of 2012. A semi-structured questionnaire with socioeconomic and environmental questions was applied in *Leishmania* endemic regions of the city. The survey data were analyzed by multivariate statistics and the associations between them were presented as odds ratio within a 95% confidence limit. The relationships between the studied variables showed no statistically significant difference between the dwellings with positive and negative dogs. However, when analyzing the odds ratio, the presence of green areas larger than 10m² turned the house into an area of potential risk for CVL (OR= 2.53). There was no difference among the socioeconomic variables education and income, though the groups with lower education and income showed a higher seroprevalence of CVL. The city of Uruguaiana is already located within the CVL broadcasting area and is taken as an endemic region of the disease, with seropositive dogs in all neighborhoods. Thus, the determinants for an increased *Leishmania* sp. infection could not be indicated, even so, the maintenance of green areas around the households represented a risk factor for being a potential vector shelter.

**Key words:** Neglected disease. Epidemiology. Environment. Socioeconomic factors.

## Resumo

O objetivo da presente pesquisa foi avaliar potenciais fatores de risco associados à ocorrência de leishmaniose visceral canina (LVC) em áreas de maior prevalência no município de Uruguaiana/RS, no ano de 2012. Foram aplicados questionários semiestruturados com questões socioeconômicas e ambientais em bairros endêmicos para LVC no município de Uruguaiana. Os dados foram analisados por meio de análise multivariada e as associações entre as variáveis foram expressas em *odds ratio* com intervalos de confiança de 95%. As relações entre as variáveis estudadas não apresentaram diferença estatística significativa quando se comparou residências com cães positivos àquelas com cães negativos. Entretanto, a presença de área verde maior que 10m² apresentou-se como um potencial fator de risco (OR= 2,53) para LVC. Não houve diferença significativa entre as variáveis socioeconômicas, escolaridade e renda embora, nos grupos de menor escolaridade e de menor renda familiar, tenha havido

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Received: Apr. 27, 2017 - Approved: Oct. 10, 2017

maior soro prevalência para LVC. O município de Uruguaiana-RS já pertence à área de transmissão de LVC tornando-se uma região endêmica da doença, com presença de cães soropositivos em todos os bairros. Desta forma, não foi possível apontar os determinantes para o aumento da infecção por *Leishmania* sp, embora a manutenção de áreas verdes peridomiciliares tenha representado um fator de risco devido à conservação do vetor nesses locais.
**Palavras-chave:** Doença negligenciada. Epidemiologia. Ambiente. Fatores socioeconômicos.

## Introduction

Visceral leishmaniasis, a zoonotic disease caused by protozoa of the genus *Leishmania*, is considered a chronic, serious, and potentially fatal disease to humans if not treated properly. It is transmitted during the blood meal feeding of female phlebotomine sandflies, among which *Lutzomyia longipalpis* is the most important in Brazil; notwithstanding, other arthropods have been reported as potential vectors of this disease (COUTINHO et al., 2005; REGUERA et al., 2016).

The tropical location of Brazil favors the adaptation of arthropod species, providing human and canine exposure to a large number of diseases, including leishmaniasis (DANTAS-TORRES, 2009).

Initially known as restricted to the rural areas of the northeastern Brazil, with a transmission cycle between wild animals, since the 1980s, this disease has gradually expanded to other regions with rapid urbanization, given the high adaptability of the vector to different environmental conditions (MAIA-ELKHOURY et al., 2008; WERNECK, 2010).

During the transmission cycle, dogs are the main domestic reservoir and their role is considered essential to the increase of the disease in endemic regions (SAVANI et al., 2003; SANTOS et al., 2005; SILVA et al., 2005; REGUERA et al., 2016). Uruguaiana city, in the Brazilian southern state of Rio Grande do Sul, have notifications of positive canine cases and the vector presence since 2009, being classified by the State Center for Health Surveillance as a transmission area of canine visceral leishmaniasis (CVL).

Given the above, the objective of this study was to analyze the risk factors associated with interactions between ecological, socioeconomic, and demographic conditions and the presence of CVL in endemic areas of the municipality.

## Material and Methods

A cross-sectional observational epidemiological study was carried out from April to October 2013, with the purpose of determining the prevalence and spatial distribution of CVL in Uruguaiana city for 2012.

### *Study area*

The city of Uruguaiana is located in the far west of Rio Grande do Sul state – Brazil (Latitude: –29.7495; Longitude: –57.0882 – 29º 44 ‘58 “South and 57º 5’ 18” West).

Its population is estimated to be at 125,435 thousand inhabitants, with a territorial area of 5.715.763 $Km^2$, and a population density of 21.95 inh. /$Km^2$ (IBGE, 2010). It has a subtropical climate with average summer temperature of 32°C and winter of 8°C, besides annual rainfalls of 135 mm in summer and 84 mm in winter (BURIOL et al., 2007).

Since there is no census of total canine population, inquiries were made to the Uruguaiana Municipal Health Department to verify the relationship of CVL cases reported in 2012 by the Epidemiological Surveillance sector, as well as recording the estimate of real properties (47,360) and neighborhoods (25). The five neighborhoods (20%) with the highest prevalence of CVL were selected, including the

neighborhood of Mascarenhas de Moraes, which had the first confirmed case of Human Visceral Leishmaniasis (HVL). We chose to carry out an active search in the 217 (0.45%) residences reported with CVL or suspected cases in 2012, within these neighborhoods. The exclusion criteria were the impossibility of locating or lacking address in the animal identification cards, or the absence of owners in the residences for interview, in this way, 76 residences were missed.

A total of 141 interviews were performed through a semi-structured form with socioeconomic information, according to the method applied by the Brazilian Association for Population Studies (ABEP, 2008), and environmental data based on the occurring hazardous situation as described by the Health Department and by Costa et al. (1995) and Silva et al. (2012). Interviews and evaluations of household vicinity conditions were also carried out. Dog owners or heads of households were advised on the disease prevention, besides receiving an educational booklet on leishmaniasis.

*Definition of cases and controls*

Seropositive and seronegative dogs were diagnosed through epidemiological investigations during the Visceral Leishmaniasis Control Program by the Health Department, upon spontaneous population demand in 2012. These examinations were performed obeying the recommendations of the Health Ministry, using a fast screening test – TR-DPP (Bio-Manguinhos, FIOCRUZ, Rio de Janeiro, Brazil). The reactive cases were sent to the state's central laboratory (LACEN – RS) for ELISA serological analysis (Bio-Manguinhos, FIOCRUZ, Rio de Janeiro, Brazil), according to the joint Technical Note nº 01/2011 of the Health Ministry. All reagent samples from the fast test were followed by confirmatory serology.

*Data analysis*

The data collected were initially submitted to multivariate analysis by means, considering $p \geq 0.05$, with the aid of the statistical software GraphPad Prism 5.0. The associations between the variables were expressed by odds ratio (OR) and respective confidence intervals (95% CI). The following variables were evaluated: literacy of the household heads, household income based on the minimum wage (R$ 622.00), water system supply, sewage disposal, selective garbage collection, presence of green areas in the peridomestic settings of houses near to the Uruguay River, trees and tall grass (without cutting to more than one month), backyard with accumulated rubbish and animal feces and the presence of other animals (chickens, chicks, ducks and cats).

*Ethical considerations*

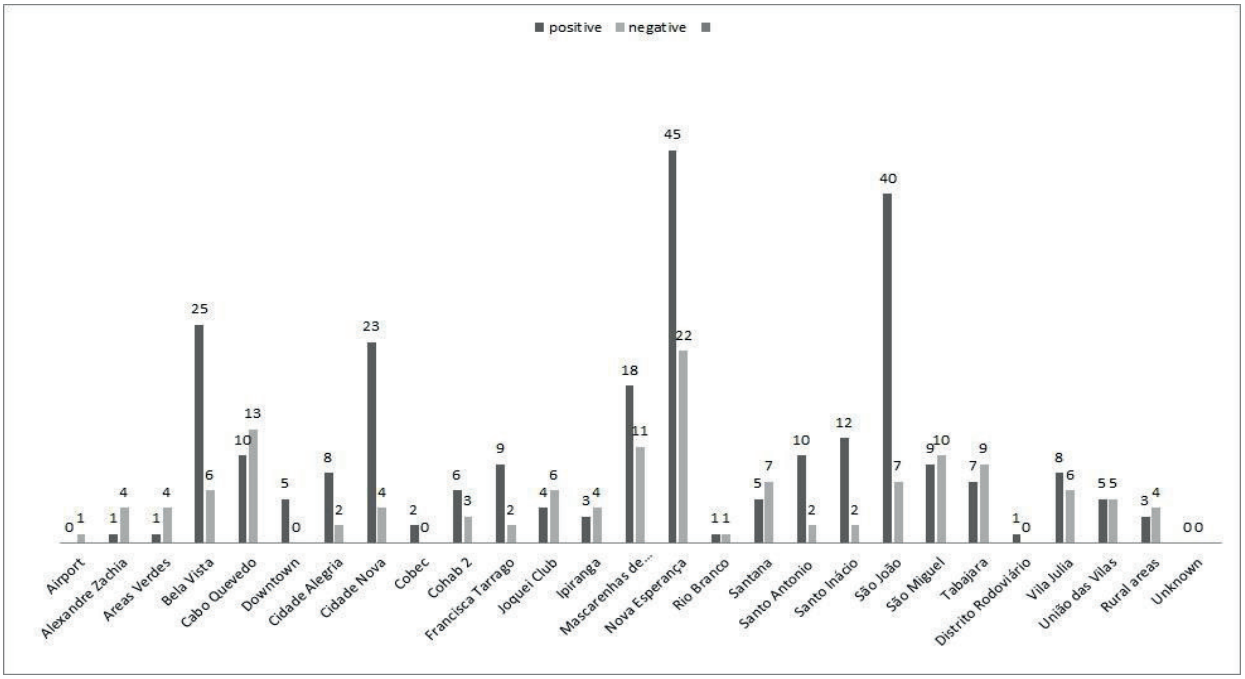
The Research Ethics Committee (CEP) of the Federal University of Pampa – UNIPAMPA approved this study, under the protocol CAAE 13390213.8.0000.5323. The researchers committed to keeping confidential any information able to identify the research subjects. The interviewees received a free and informed consent form containing information about the research, previously approved by the CEP, and only those who agreed to the terms therein participated.

## Results

In the study period, 454 suspected cases of CVL were reported. Of these, 261 (57.48%) were confirmed positive by the team of the LACEN – RS (Rio Grande do Sul – RS, Central Laboratory) through serological tests, as recommended by the Ministry of Health.

Uruguaiana city is divided into 25 neighborhoods, of which 24 had notification of CVL cases (Figure 1). Higher prevalence of CVL was observed in neighborhoods Nova Esperança, Bela Vista, Mascarenhas de Moraes, São João, and Cidade Nova (Figure 1), totaling 50% of CVL cases in this urban perimeter. These neighborhoods were selected for the questionnaire.

**Figure 1.** Epidemiological situation according to CVL notifications from the Health Surveillance Sector of Uruguaiana City in 2012.




A total of 141 interviews were conducted with dog owners between the positive (n = 116) and negative (n = 25) cases of CVL in the municipality. Among the seropositive animals, 93.9% had clinical signs such as cutaneous lesions, alopecia, onychogryphosis, weight loss, and crustal ulcer. Yet, among the negative animals, 68% presented clinical signs that could indicate CVL.

Even though the group of lower schooling, characterized by illiterate owners or incomplete primary school, had a higher number of positive dogs, no statistically significant differences were found between the studied variables (Table 1) and the presence of dogs with CVL in residences for the period. As for the educational variable, the socioeconomic characteristics (family income) seem to influence the occurrence of CVL cases since a higher prevalence of positive cases among lower-income individuals was observed.

Among the analyzed variables, the wealth of green areas around the household (> 10m$^2$) presented a higher risk for CVL (OR = 2.53; p = 0.039) when compared to residences with smaller ones. Although the presence of leafy trees, shade (OR = 1.25) and high grass (OR = 1.77) are not determinant for the occurrence of CVL, these factors might increase the chances of exposure if associated with the extent of the green area (Table 1).

**Table 1** Odds Ratio between socioeconomic and environmental variables, and the occurrence of Canine Visceral Leishmaniasis.

| Characteristics | Residences with soronegative dogs (n=25) | Residences with soropositive dogs (n=116) | Odds Ratio | IC 95% | pvalue |
|---|---|---|---|---|---|
| **Head of the Family schooling level*** | | | | | |
| Level 1 – Level 2 | 03 | 20 | | | |
| Levels 3, 4, 5 e 6 | 22 | 96 | | | |
| | | | 1.5278 | 0.4168-5.5999 | 0.5225 |
| **Family income ** ** | | | | | |
| Family income inferior to R$ 622,00 | 02 | 10 | | | |
| Family income superior to R$ 622,00 | 23 | 106 | | | |
| **Piped drinking water sistem** | | | 1.0849 | 0.2226-5.2869 | 0.9197 |
| Yes | 24 | 116 | | | |
| No | 01 | 00 | | | |
| | | | 0.0701 | 0.0028-1.7724 | 0.1068 |
| **Basic Sanitation** | | | | | |
| Yes | 21 | 103 | | | |
| No | 04 | 13 | | | |
| | | | 0.6626 | 0.1966-2.2331 | 0.5067 |
| **Seletive Garbage Collection** | | | | | |
| Yes | 23 | 101 | | | |
| No | 02 | 15 | | | |
| | | | 1.7079 | 0.3649-7.9936 | 0.4966 |
| **Peridomiciliary green area** | | | | | |
| Bigger than $10m^2$ | 13 | 85 | | | |
| Absent or until $10m^2$ | 12 | 31 | | | |
| | | | 2.531 | 1.0437-6.1379 | 0.0399 |
| **Presence of hardwood trees / big tree shadow** | | | | | |
| Yes | 06 | 33 | | | |
| No | 19 | 83 | | | |
| | | | 1.259 | 0.4620-3.4312 | 0.6525 |
| **Presence of tall grass***** | | | | | |
| Yes | 01 | 08 | | | |
| No | 24 | 108 | | | |
| | | | 1.7778 | 0.2122-14.8915 | 0.5957 |
| **Residence near the Uruguay River** | | | | | |
| Yes | 08 | 34 | | | |
| No | 17 | 82 | | | |
| | | | 0.8811 | 0.3474-2.2345 | 0.7898 |
| **Presence of animal feces** | | | | | |
| Yes | 07 | 17 | | | |
| No | 18 | 99 | | | |
| | | | 0.4416 | 0.1603-1.2165 | 0.1139 |
| **Presence of peridomestic organic matter** | | | | | |
| Yes | 07 | 30 | | | |
| No | 18 | 86 | | | |
| | | | 0.897 | 0.3411-2.3590 | 0.8256 |

continue

continuation

| | | | | | |
|---|---|---|---|---|---|
| **Presence of chickens, chicks or ducks in the yard** | | | | | |
| Yes | 06 | 09 | | | |
| No | 19 | 107 | | | |
| | | | 0.2664 | 0.0850-0.8349 | 0.0232 |
| **Presence of cats in the residence** | | | | | |
| Yes | 09 | 50 | | | |
| No | 16 | 66 | | | |
| | | | 1.3468 | 0.55-3.2978 | 0.5146 |

* Level 1- illiterate, Level 2 – incomplete primary, Level 3- complete primary, Level 4- complete fundamental, Level 5 – complete high school, Level 6 – Superior Complete.
** R$ 622,00 = Brazilian minimal wage in 2012
*** Tall grass without cutting to a month.

The presence of chickens, chicks, or ducks in the surrounding areas revealed protection for the occurrence of CVL (OR = 0.266; p = 0.0232) when compared to those without these animals. Despite not significant, the presence of other animals, especially cats, tends to raise the occurrence of positive canine cases (OR = 1.34), as well as the waste collection system (OR = 1.7) (Table 1).

Neither basic sanitation nor drinking water supply was statistically significant, in spite of showing protection (OR = 0.662 and OR = 0.070, respectively) (Table 1). It should be noted that the small number of residences that did not have such conditions represents a bias in this study, which might have influenced thereof.

## Discussion

Based on our findings, we can assume that such canine disease is widespread in almost all neighborhoods of Uruguaiana, regardless of region or environmental conditions; however, prevalence is higher in some localities. This fact proves that the territorial expansion of this disease vector, an important characteristic for its epidemiology, is occurring in the Brazilian territory.

The species *Lutzomyia longipalpis*, despite widely distributed in the country, was not found in the southern region until the year 2008 (SOUZA et al., 2009; BRASIL, 2016). Conversely, it is currently disseminated and biologically adapted to the southern regions, and within the urban perimeter of the study cities. Uruguaiana had the first autochthonous case of CVL, confirmed in 2009 (DEBONI et al., 2011); however, in 2008, there was the first notification of a case in the state, consisting of an animal domiciled in the municipality of São Borja (MONTEIRO et al., 2010).

One of the hypotheses for this vector arrival to the South would be through the border cities. *L. longipalpis* was found in several border provinces such as Misiones (Argentina), where cases of HVL and CVL were reported in years 2006 and 2007. Nevertheless, there are no cases in urban and wild areas of southern RS municipalities near those with CVL notification (SOUSA et al., 2010; DEBONI et al., 2011). In addition, Corrientes province (Argentina), contiguous to Missiones and bordered by Rio Grande do Sul state, had vector confirmation and CVL cases in 2008, in the same year as the first case was reported in Rio Grande do Sul state (SALOMON et al., 2008, 2009).

In the five neighborhoods, which comprised 50% of the CVL cases in Uruguaiana, quite a few sites with the ideal conditions for vector permanence were often encountered, which might have favored its adaptation to this municipality and to the western border of RS.

The data were gathered from two distinct regions (riverside and mainland zones). While a strip of

ciliary forest was found near the residences along the banks of the Uruguay River, the continental boroughs are far from these gallery forests. Nevertheless, there were areas (backyards and vacant lots) with a great abundance of vegetation, fruit trees, and poorly maintained sites. In both scenarios, besides the long vegetation strips, we also noticed some residences under precarious conditions of basic sanitation and unpaved streets.

Costa et al. (1995) already reported that dense afforestation in backyards might be a risk factor for domiciliation and urbanization of the vector. Leça Júnior et al. (2015) related the possibility of surrounding green areas serve as points for organic matter accumulation, mainly due to the lack of garbage collection, thus leading to the proliferation of infected canines, as well as small rodents and even the disease vector. Among the houses visited in Uruguaiana – RS, those pertaining to green areas larger than $10m^2$ had greater chances of exposure to CVL. These areas with extensive green spaces are suspected to shelter the vector, protecting it from predators, besides obtaining food and continuing the reproduction of the species. This result can be grounded by analyzing the habits of vector such as permanence and adaptation near food sources, twilight and nocturnal activity, and inhabiting shaded and humid places protected from wind and natural predators (BRASIL, 2016).

Unlike the other neighborhoods, Mascarenhas de Moraes is located near the banks of Uruguay River, which has a riparian forest along its course, thus having a particular geographical position. It is located in floodplains and areas very close to the border with Argentina. In addition, in this neighborhood, the first human case of visceral leishmaniasis was recorded in 2011, which resulted in the adoption of control measures against the vector and canine sampling (DEBONI et al., 2011). Such measures might have influenced the results regarding the effects of river proximity on CVL incidence. For this site, two distinct outcomes can be identified in relation to canine infection and, mainly, to control measures. Analyzing the epidemiological situation presented in the study, this neighborhood was among the five localities with the highest prevalence of CVL in the period, which leads us to rethink how poor was the result from the then adopted measures such as vector control and reservoir.

Endemicity in the municipality can be proven by the existence of seropositive dogs in all neighborhoods, regardless of environmental conditions, socioeconomic classes, and population knowledge. The results did not show an association of family income and head education with the disease incidence in the city of Uruguaiana. Our results diverge from other studies on risk factors associated with *Leishmania* infection; these studies have shown that increases in income and level of schooling are associated with a decreased likelihood of infection and hence disease incidence (BORGES et al., 2008; BELO et al., 2013). Thus, since a vector adapts to the environment and this area is classified as an endemic area, the entire population is at risk of exposure regardless of socioeconomic characteristics.

The information obtained from the epidemiological surveillance sector provided a panorama of the canine leishmaniasis situation in Uruguaiana during the study period. However, as they come from spontaneous demand notifications, these data may not reliably represent all of the existing positive cases, thus being a limitation.

## Conclusions

The study highlighted the endemicity of CVL in Uruguaiana through the identification of seropositive dogs in several neighborhoods in either riverside or continental locations, and for any environmental conditions, socioeconomic classes, and population educational level. The prevalence of CVL is high in Uruguaiana, and the presence of green areas larger than $10m^2$ around the households presented as a potential risk factor for CVL.

## Acknowledgments

We would like to thank the endemic and health agent of Uruguaiana Municipal Health Department for research support and the Uruguaiana community for participating in this research.

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