

# Awareness of visceral leishmaniasis and its relationship to canine infection in riverside endemic areas in Northeastern Brazil

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## ABSTRACT

**Introduction:** An awareness of visceral leishmaniasis (VL) is necessary to encourage the population to participate in prevention and control in collaboration with more efficient, centrally organized health programs. The aim of this study was to evaluate the awareness of the riverside population regarding VL and the association between awareness and the prevalence of canine visceral leishmaniasis (CVL). **Methods:** In total, 71 people living in riverside areas in the City of Mossoró in State of Rio Grande do Norte participated of the study, and 71 dogs were tested for CVL by polymerase chain reaction (PCR). Association analysis of several variables related to knowledge of the riverside population regarding CVL positivity was performed, yielding odds ratios (OR) and 95% confidence intervals (CI), and significance was determined using chi-square ( $\chi^2$ ) and Fisher's exact tests. **Results:** Among individuals whose dogs tested positive for CVL, 60% did not know the cure for CVL, and these subjects were three times more likely to have a dog test positive for CVL than those who were aware the cure for CVL. Knowledge of CVL cure was the only variable that remained in the logistic model after the successive removal of variables, with an adjusted OR of 3.11 (95%CI: 1.1-8,799;  $p=0.032$ ). **Conclusions:** Insufficient awareness regarding VL in riverside areas with CVL-positive dogs was associated with increased rates of canine infection, which suggests that changes in habits and the adoption of attitudes and preventive practices may contribute to the control and prevention of this disease. This study reinforces the need to invest in better health education programs regarding VL.

**Keywords:** Visceral leishmaniasis. Risk factor. Owner. Dog. Leishmaniasis knowledge.

## INTRODUCTION

Visceral leishmaniasis (VL) is a neglected disease despite its significant risk to public health. An estimated 200,000 to 400,000 new cases of VL occur worldwide each year, and more than 90% of these new cases occur in the following six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan<sup>1</sup>. Previously, VL was restricted to rural areas; however, with urbanization, the disease has expanded to urban areas. In Brazil, VL is found in all regions, although the most cases occur in the northeastern region<sup>2</sup>. Mossoró, located in the northeastern State of Rio Grande do Norte, is at the center of a VL epidemic

and recorded 205 cases of human visceral leishmaniasis (HVL) between 2006 and 2012. During this period, 5,010 canine blood samples also tested positive for canine visceral leishmaniasis (CVL) in this city<sup>3</sup>.

Poor living conditions and poor sanitation favor human and canine infections by providing an environment that enables the survival and reproduction of the vector and facilitates transmission<sup>4-6</sup>. Previous studies have demonstrated the relationship between environmental variables and the density of vectors<sup>7,8</sup>; in particular, areas that flood during the rainy season due to their proximity to rivers are more vulnerable to VL because they have a higher insect density, particularly around homes<sup>9</sup>.

Thus, knowledge of CVL by pet owners would likely be associated with reduced canine infection with *Leishmania infantum*, as awareness of the disease would result in the population being more receptive to the use of insecticide sprays to control household sandflies<sup>10</sup>. In addition to knowledge regarding vector control measures to avoid exposure to infection from the bites of sandflies, knowledge on euthanasia, general pet care and management of the household environment is necessary<sup>11</sup>.

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It is essential to understand the knowledge, attitudes, and practices of the population with respect to VL because these factors, when satisfactory, contribute to the success of prevention and control programs through changes in behavior and more widespread participation<sup>12,13</sup>. The aim of this study was to evaluate the awareness of the riverside population of Mossoró, Rio Grande do Norte, regarding VL as well as the association between the awareness and prevalence of CVL.

## METHODS

### Definition of the area and the sample

Mossoró, located in the State of Rio Grande do Norte, Brazil, has a population of approximately 280,314 habitants. This city has an area of 2,099,333km<sup>2</sup> and is located 277km from Natal, the state capital, and 42km from the coast<sup>14</sup>. This western mesoregion has typical caatinga vegetation, and the climate is semiarid, with low and irregular rainfall and well-defined dry and rainy seasons<sup>15,16</sup>. This study included residents of the following well-defined areas along the Apodi-Mossoró River: Alto da Conceição, Ilha de Santa Luzia and Paredões. These areas were selected because they are the most affected by flooding during rainy periods, according to information obtained by the Civil Defense Department of Mossoró. The areas selected contain 477 residences. In addition, Mossoró has inadequate sanitation, hazardous environmental conditions and high rates of poverty, and these factors favor the occurrence of VL through the increased insect density during the rainy season, particularly around houses. Moreover, transmission of VL is favored by the absence of primary vegetation, the accumulation of organic material, and the presence of animals such as birds, swine and dogs<sup>8,9</sup>.

We visited all 477 households in the riverside areas to identify homes with dogs. However, to evaluate the awareness of the riverside population regarding VL and the association between awareness and the prevalence of CVL, only households with dogs were included in the study. The potential participants were informed of the terms and purpose of the study. Those who signed the informed consent form were interviewed, and those responsible for a dog or dogs were asked to participate in the CVL study by allowing the collection of a blood sample from the dog(s) for molecular analysis. In total, one individual was interviewed from each of 71 homes, and each residence had a dog that was tested, totaling 71 dogs.

### Awareness of visceral leishmaniasis

To evaluate the awareness of VL, semi-structured interviews that were developed specifically for this research were conducted. These interviews addressed the socioeconomic profile of the interviewees and their awareness regarding the means of transmission, the most common symptoms of the disease, the means of diagnosis and treatment, the existence of a vaccine, and the means of prevention of VL in humans (HVL) and in dogs (CVL).

### Molecular diagnosis of canine visceral leishmaniasis

Peripheral blood samples were collected from 71 dogs and sent to the *Laboratório de Imunomodulação e Protozoologia*

*do Instituto Oswaldo Cruz - Fundação Oswaldo Cruz* (Immunomodulation and Protozoology Laboratory of the Institute Oswaldo Cruz FIOCRUZ) in Rio de Janeiro, Brazil for molecular analysis by polymerase chain reaction (PCR).

DNA extraction was conducted using the phenol-chloroform method, as described by Harris et al<sup>17</sup>. PCR was performed using the following primers for species-specific amplification of a 351-bp fragment of kinetoplast DNA (kDNA) of *Leishmania infantum*: 5'-GCC CGC G(C/T)G TCA CCA CCA T - 3' and 3'-CGG CCG C(G/A)C AGT GGT GGT - 5'<sup>17</sup>.

### Statistical analysis

The data were entered on a spreadsheet and transferred to Statistical Package for Social Sciences software (version 17.0, SPSS, Inc., Chicago, IL, USA) for subsequent coding and analysis. The associations of several variables with canine positivity were tested, yielding odds ratios (OR) and 95% confidence intervals (CI), and significance was determined using chi-square ( $\chi^2$ ) and Fisher's exact tests. The latter test was used when values were to occur with an expected frequency of less than five. A logistic regression model was developed to study, simultaneously, the factors involved in the prevalence of CVL positivity. For the analysis, we used variables with p values <0.20, as well as some that did not show significant differences if they were considered important factors associated with infection. We excluded variables with CIs that showed large-scale estimates of the ORs, as well as those that presented colinearities in the logistic model. Therefore, the analysis for the final model was formed from variables with a significance level of p<0.10, which were re-evaluated by successive removal each variable. At this stage, a significance level of p<0.05 was used.

### Ethical considerations

The survey was approved by the *Comitê de Ética em Pesquisa*, (Committee of Ethics and Research) of the *Universidade do Estado do Rio Grande do Norte* (Legal Opinion Number: 71259 and CAAE: 03692212.5.0000.5294) and by the *Comissão de Ética no Uso de Animais*, Commission of Ethics for the Usage of Animals of the *Universidade Federal Rural do Semi-Árido* (Legal Opinion Number: 61/2012, Process Number 23091.002189/212-41).

## RESULTS

The PCR results showed that 39% (28/71) of the dogs tested positive for *L. infantum* DNA. Among the 71 interviewees, 79% (22/71) were female; 64% (18/71) had completed primary school; and 60% (18/71) had family incomes of more than three times the minimum wage. Age was identified as a risk factor, and this variable was used in the logistic model. Specifically, interviewees between 18 and 30 years of age or over 65 years of age had three times the risk of having a CVL-positive dog compared with those aged between 31 and 65 years (**Table 1**). Regarding transmission of the disease, among interviewees with positive dogs, 68% (19/71) did not possess knowledge of VL transmission and had twice the risk for canine positivity than those who knew how VL is transmitted (p<0.2).

Additionally, 61% (17/71) of interviewees did not have knowledge of VL prevention, and we also observed a lack of awareness of HVL symptoms and treatment. Many interviewees were not aware of a vaccine or cure for HVL, although there was better awareness of CVL symptoms.

Among those with CVL-positive dogs, 60% (15/71) did not know about cure for CVL, and these subjects had a three-fold greater risk of having CVL-positive dogs compared those who had knowledge of CVL cure. In the logistic regression analysis, only knowledge of CVL cure was kept in the logistic model after the successive removal of each variable, with an adjusted OR of 3.11 (95% CI: 1.1- 8.799,  $p=0.032$ ) (Table 2).

## DISCUSSION

### Factors associated with the population

The education level of the interviewees with CVL-positive dogs was not associated with an increased risk of infection. However, in research conducted in Belo Horizonte, in the State of Minas Gerais, Brazil, the authors observed a correlation between a low educational level and an increased risk of VL; in particular, individuals who had never attended school had an eight-fold higher risk of infection<sup>13</sup>. In our study, there was a three-fold increased risk among interviewees with CVL-positive dogs, who were aged between 18 and 30 years or over 65 years, and canine infection was associated with less access to information regarding diseases such as VL and their preventive

measures. This association may be due to restriction within domestic environments with inadequate health education.

### Factors associated with transmission and prevention

Most of the interviewees had only vague knowledge of the mechanics of VL transmission. The interviewees were generally aware that transmission occurs through an insect bite; however, they failed to identify the sand fly as a vector. Similar results were observed in a survey conducted in Divinópolis, State of Minas Gerais, Brazil, in which approximately one-half of the population interviewed was unaware of how VL was transmitted, and only 29% knew of the necessity for an insect bite<sup>18</sup>.

The knowledge of the riverside population participating in this study regarding the mode of LV transmission showed an inverse relationship with canine infection. Lack of knowledge regarding the transmission of this disease influenced the occurrence of CVL in these areas, which may imply a lack of preventive measures. Such a lack of awareness regarding the transmission of VL and information regarding the vector responsible for the disease emphasizes the importance of disseminating transmission information, including the biological and ecological aspects of the vectors, to allow the community to participate in preventative measures<sup>19</sup>.

Previous studies performed in Mossoró reported the presence of the VL vector in different areas of the city throughout the year<sup>7</sup>. The riverside areas in this study included favorable conditions for sand flies and dogs infected with *L. infantum*; thus, we assumed that this population is vulnerable to VL, as confirmed by previous surveys<sup>20,21</sup>.

**TABLE 1 - Results of the association between canine visceral leishmaniasis and several variables regarding the population of riverside areas in Mossoró, State of Rio Grande do Norte, 2014.**

Variable	Canine visceral leishmaniasis		OR	95% CI	P value
	positive	negative			
	n	%	n		
Gender					
male	6	21.4	9	1.03	0.322 - 3.299
female	22	78.6	34	1	
Age					
between 18 and 30 years	8	28.6	6	3.022	0.891 - 10.24
>65 years	5	17.8	3	3.778	0.798 - 17.887
31 to 54 years	15	53.6	34	1	-
Family income					
≤ minimum wage	10	35.7	14	1,151	0.422 - 3.135
> minimum wage	18	64.3	29	1	
Education					
≥ elementary school	18	64.3	28	0.9	0.329 - 2.458
middle or high school	10	35.7	14	1	

OR: odds ratio; 95% CI: confidence interval 95%.

**TABLE 2 - Results of the association between canine visceral leishmaniasis and several variables regarding the knowledge of the population of riverside areas in Mossoró, State of Rio Grande do Norte, 2014.**

Variable	Canine visceral leishmaniasis			OR	95% CI	P value
	positive		negative			
	n	%	n			
<b>Aware of transmission</b>						
yes	9	32.2	21	1	0.7462 - 5.442	0.164
no	19	67.8	22	2.015		
<b>Aware of human visceral leishmaniasis</b>						
yes	27	96.4	39	2.769	0.293 - 26.158	0.642
no	1	3.6	4	1		
<b>Aware of the human symptoms<sup>¥</sup></b>						
yes	8	29.6	8	1.632	0.525 - 5.072	0.395
no	19	70.4	31	1		
<b>There is a cure for humans<sup>¥</sup></b>						
yes	14	51.8	21	0.923	0.345 - 2.467	0.873
no	13	48.2	18	1		
<b>There is a vaccine for humans<sup>¥</sup></b>						
no	3	11.1	7	0.571	0.134 - 2.442	0.508
don't know	24	88.9	32	1		
<b>Aware of canine visceral leishmaniasis</b>						
yes	25	89.3	40	0.625	0.117 - 3.342	0.674
no	3	10.7	3	1		
<b>Aware of the canine symptoms<sup>¥</sup></b>						
yes	19	76.0	34	0.559	0.158 - 1.976	0.513
no	6	24.0	6	1		
<b>There is a cure for dogs<sup>¥</sup></b>						
no	10	40.0	27	1	1.103 - 8.801	0.029
don't know	15	60.0	13	3.115		
<b>There is a treatment for dogs<sup>¥</sup></b>						
don't know	12	48.0	18	1.128	0.414 - 3.072	0.813
euthanasia	13	52.0	22	1		
<b>There is a canine vaccine<sup>¥</sup></b>						
yes	11	44.0	19	0.868	0.318 - 2.371	0.783
no	14	56.0	21	1		
<b>Preventive measure</b>						
don't know	17	60.7	24	1	-	-
focused on the human and canine population	0	0.0	2	0.28	0.012-6.206	0.66
environmental handling and focused on the canine population	2	7.1	2	1.41	0.18-11.04	0.841
environmental handling and focused on the human population	0	0.0	1	0.466	0.017-12.15	0.844
focused on the canine population	4	14.3	11	0.513	0.139-1.88	0.366
environmental handling	5	17.9	3	2.353	0.4941-11.21	0.4397

**OR:** odds ratio; **95% CI:** confidence interval 95%; <sup>¥</sup>variables with no response by some respondents.

The interviewees had very limited knowledge of the measures to prevent the disease, as observed in other surveys performed in the country<sup>18,22</sup>. Although there was no significant association with the CVL test results, these data remain relevant. Regarding the knowledge of prevention methods directed at the environment, humans or animals, measures such as proper environmental management with intra- and peri-domestic cleaning, the use of protective screens on doors and windows, repellents on the skin, insecticide sprays and insecticidal collars for dogs<sup>23</sup> could be implemented to enable the active participation of the population in the control and prevention of VL<sup>13</sup>.

#### Factors associated with the reservoir

Our results demonstrated less awareness of the symptoms of HVL compared to CVL, and similar data have been reported in surveys in Petrolina, in the State of Pernambuco, Brazil, which is another endemic VL region. There, 92% of the population interviewed could name the common symptoms of CVL<sup>5</sup>. Awareness of CVL is crucial because the disease is more common in dogs than in humans in urban areas<sup>4</sup> and because the campaigns for controlling human disease are focused on the canine reservoir<sup>20,24</sup>.

Knowledge regarding the cure of CVL was a statistically significant factor associated with canine infection in the studied riverside areas. Thus, a lack of knowledge regarding a cure for CVL likely favors the continuation of the disease in these areas and also makes it more difficult for the population to agree to euthanize infected dogs to interrupt the epidemiological chain of the disease.

It is important to consider that, in most cases, canine infection precedes human cases of infection<sup>3,4,25</sup>. The detection of *L. infantum* DNA in the canine population is significant because these infections maintain the disease cycle for the human population; thus, dogs may serve as effective sentinels in these areas.

The interviewees were not aware of a vaccine to prevent HVL, which is significant, because no such vaccine exists. There was a similar lack of awareness for the vaccine used to prevent CVL, showing that this preventive measure is not widely used (although two vaccines are available in Brazil, Leishmune® and Leishtec®). Indeed, the former vaccine has been available in the country since 2004<sup>26</sup>. The low rate of vaccination against CVL may be associated with the low income in the interviewed population.

The parasite was detected by PCR in the peripheral blood of dogs in the studied riverside areas. PCR has been performed for the diagnosis of CVL, with its potential for parasite detection showing high levels of sensitivity and specificity<sup>27</sup>, and no significant difference has been reported for samples obtained from canine peripheral blood, bone marrow, and skin fragments<sup>28</sup>.

The presence of CVL-positive dogs in Mossoró should concern local authorities because dogs are the primary domestic reservoir for human disease in urban areas<sup>29</sup>. Thus, the identification of infected dogs is important to interrupt the cycle of transmission in urban areas<sup>30</sup>. This interruption involves the euthanasia of infected dogs, according to the Surveillance Program and Visceral Leishmaniasis Control Program of the Ministry of Health in Brazil<sup>31</sup>.

The riverside populations interviewed in Mossoró have a low socioeconomic status and poor living conditions, and the low level of awareness regarding VL in this population was associated with canine infection. However, changes in habits and attitudes and the addition of preventive practices may contribute to the control and prevention of this disease. It is important to consider that a study evaluating only households with dogs could be biased, as the knowledge of the entire population is important for the prevention and control of CVL. Thus, future studies should address the knowledge of the general population.

This study reinforces the need for greater governmental investment in health education regarding VL. These programs should provide information on how to prevent transmission and subsequent outbreaks; in particular, such programs are particularly needed in areas near rivers and lakes (as in this study) to enable high-risk populations to participate actively in the prevention and control of this disease.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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