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SEROLOGICAL SURVEY AND RISK FACTORS ASSOCIATED WITH VISCERAL LEISHMANIASIS IN DOGS FROM THE NORTHEAST REGION, BRAZIL

INQUÉRITO SOROLÓGICO E FATORES DE RISCO ASSOCIADOS À LEISHMANIOSE VISCERAL EM CÃES DE REGIÃO NORDESTE, BRASIL

ENCUESTA SEROLÓGICA Y FACTORES DE RIESGO ASOCIADOS A LA LEISHMANIASIS VISCERAL EN PERROS DE LA REGIÓN NORDESTE DE BRASIL

Renata Rocha da Silva1
Anita de Souza Silva2
Bárbara Regina Marques3
Gabriel de Araújo Lobão4
Geyanna Dolores Lopes Nunes5
Priscila Lima dos Santos6
Roseane Nunes de Santana Campos7

ABSTRACT

Considering the dog as the main domestic reservoir for visceral leishmaniasis, we investigated the seroprevalence of the disease in dogs and evaluated the risk factors associated with transmission in the north-eastern region of Brazil. A cross-sectional study was conducted in the municipality of Nossa Senhora da Glória, Sergipe between 2017–2018. Through the calculation for simple random samples and an expected prevalence of 5%, we obtained a total of 72 samples, and in 2016, in the anti-rabies vaccination campaign in the municipality of Nossa Senhora da Glória, 1,712 dogs were vaccinated, in 14 neighborhoods. The questionnaire applied contains information about the dog, the environment and the owner. Blood samples were collected from 195 dogs in the municipality, corresponding to 11.5% of the population estimated in the anti-rabies vaccination campaign of 1,172 dogs in the municipality in 2016. A seroprevalence of 15.4% was identified in dogs. The associated risk factors were mixed breed and male dogs, dogs having access to the street, and the presence of organic matter outside the residence. This study describes the first canine serological sample survey in the region. The municipality of Nossa Senhora da Glória -SE demonstrated a high prevalence of CVL. The percentage of seroreagents dogs was high in relation to the state capital, which is endemic for the disease. Thus, the study demonstrates the need to approach educational and preventive actions in the region.

KEYWORDS

Dogs; epidemiology. *Leishmania infantum*; public health; zoonosis.

RESUMO

Ao considerar o cão como o principal reservatório doméstico para a leishmaniose visceral, investigamos a soroprevalência da doença em cães e avaliamos os fatores de risco associados à transmissão na região nordeste do Brasil. Foi realizado um estudo transversal no município de Nossa Senhora da Glória, Sergipe entre 2017 e 2018. Por meio do cálculo para amostras aleatórias simples e prevalência esperada de 5%, obtivemos um total de 72 amostras, e em 2016, na campanha de vacinação antirrábica no município de Nossa Senhora da Glória, foram vacinados 1.712 cães, em 14 bairros. O questionário aplicado contém informações sobre o cão, o ambiente e o dono. Foram coletadas amostras de sangue de 195 cães do município, correspondendo a 11,5% da população estimada na campanha de vacinação antirrábica de 1.172 cães do município em 2016. Uma soroprevalência de 15,4% foi identificada em cães. Os fatores de risco associados foram cães mestiços e machos, cães com acesso à rua e presença de matéria orgânica fora da residência. Este estudo descreve o primeiro inquérito amostral sorológico canino na região. O município de Nossa Senhora da Glória - SE demonstrou alta prevalência de LVC. O percentual de cães sororreagentes foi elevado em relação à capital do estado, que é endêmica para a doença. Assim, o estudo demonstra a necessidade de aproximação com ações educativas e preventivas na região.

PALAVRAS-CHAVE

Cães. Epidemiologia. *Leishmania infantum*. Saúde Pública. Zoonose.

RESUMEN

Considerando al perro como el principal reservorio doméstico de leishmaniasis visceral, investigamos la seroprevalencia de la enfermedad en perros y evaluamos los factores de riesgo asociados a la transmisión en la región nordeste de Brasil. Se realizó un estudio transversal en el municipio de Nossa Senhora da Glória, Sergipe entre 2017 y 2018. Mediante el cálculo por muestreo aleatorio simple y una prevalencia esperada del 5%, obtuvimos un total de 72 muestras, y en 2016, en la campaña de vacunación antirrábica en el municipio de Nossa Senhora da Glória, fueron vacunados 1.712 perros, en 14 barrios. El cuestionario aplicado contiene información sobre el perro, el entorno y el dueño. Se recolectaron muestras de sangre de 195 perros del municipio, correspondientes al 11,5% de la población estimada en la campaña de vacunación antirrábica de 1.172 perros en el municipio en el año 2016. Se identificó una seroprevalencia del 15,4% en perros. Los factores de riesgo asociados fueron perros mestizos y machos, perros con acceso a la calle y presencia de materia orgánica fuera del hogar. Este estudio describe la primera encuesta muestral serológica canina en la región. El mu-

nicipio de Nossa Senhora da Glória - SE demostró una alta prevalencia de LVC. El porcentaje de perros seroreactivos fue elevado en relación a la capital del estado, donde es endémica la enfermedad. Así, el estudio demuestra la necesidad de abordar acciones educativas y preventivas en la región.

PALABRAS CLAVE

Epidemiología. *Leishmania infantum*. Perros. Salud Pública. Zoonosis.

1 INTRODUCTION

Visceral leishmaniasis (VL) is a Neglected Tropical Disease (NTD) with a higher incidence in vulnerable populations and tropical and subtropical countries, affects people who are immunosuppressed or have comorbidities, found in approximately 102 countries (PAHO, 2019).

In the Americas, there were 69,665 new cases of VL, with an annual average of 2,488 cases from 2001 to 2021 (PAHO, 2022). 90% of VL cases registered in Latin America are concentrated in Brazil (BRASIL, 2022).

In Brazil, visceral leishmaniasis (VL) is mainly caused by the protozoan *Leishmania infantum*. This protozoan is commonly transmitted by a vector, the sandfly *Lutzomyia longipalpis*. The disease transmission occurs to humans and other mammalian hosts during the blood meal of infected *L. longipalpis* females (FRANÇA-SILVA *et al.*, 2005; ABRANTES *et al.*, 2018).

The domestic dog is considered the main urban reservoir of the protozoan (SALES *et al.*, 2017). However, more than 50% of infected dogs can remain without clinical signs for a long period of time and remain a source of infection for the vector (WHO, 2010; ABRANTES *et al.*, 2018). It is estimated that for each human case, there are on average, at least 200 infected dogs with the protozoan (SALES *et al.*, 2017). The Brazilian Northeast is a region with a high prevalence of human and canine visceral leishmaniasis (ROCHA *et al.*, 2018).VL is endemic in the state of Sergipe, with most cases reported in the capital; however, the disease occurs in 75% of municipalities in Sergipe, distributed throughout all regions (SANTOS *et al.*, 2018).

In the municipality of Nossa Senhora da Glória, Sergipe, seven cases of VL in humans were reported by the Notifiable Diseases Information System between 2007 and 2022 (BRASIL, 2022) and until 2017, no canine serological survey was carried out in the region, this being the first study.

The dog is the main urban reservoir of leishmaniasis and carrying out canine serological surveys plays a fundamental role in delimiting regions with a higher risk of transmitting the disease. Carrying out canine serological surveys is essential to detect silent outbreaks of zoonoses and in delimiting regions or sectors with a higher prevalence of infectious diseases, including leishmaniasis (GRAMICCIA, 2011).

Identifying the prevalence and risk factors for leishmaniasis in dogs is crucial to develop prevention and control strategies. Thus, this study aimed to determine the seroprevalence of canine visceral

leishmaniasis (CVL) and assess the risk factors associated with disease transmission in urban and rural areas in the municipality of Nossa Senhora da Glória, Sergipe, Brazil.

2 METODOLOGY

A cross-sectional epidemiological study was developed using non-probabilistic convenience sampling. The study was conducted in the municipality of Nossa Senhora da Glória (Figure 1), located in the Northeast region of Brazil, in the west of the state of Sergipe. It is part of the Caatinga biome, with a tropical climate with an average annual temperature of 23.4 ºC and average rainfall of 763 mm, and an estimated population of 41,202 in habitants, which is equivalent to 1.8% of the total population of Sergipe (2,209,558 habitants) (IBGE, 2019; CLIMATEDATA, 2020).

Figure 1 – Sudy area: municipality of Nossa Senhora da Glória – Sergipe, Brazil
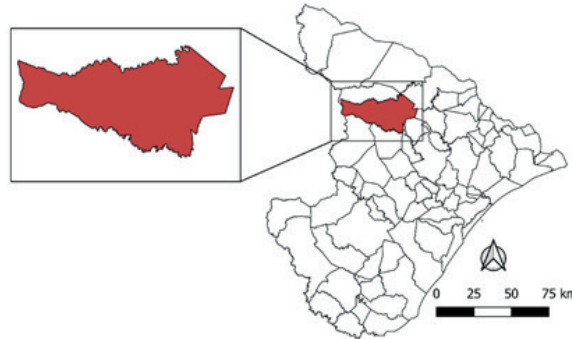
Source: Authors.

The calculation of the minimum sample size was determined by the formula for simple random samples (THRUSFIELD, 2007). The parameters adopted for the calculation were: 95% confidence level, estimated prevalence of 0.05 and error of 0.05. where N is the number of samples; p, expected prevalence; and d margin of error. As there is no data on visceral leishmaniasis in dogs available in the municipality of Nossa Senhora da Glória - SE, the expected prevalence (p) was determined according to what was observed in Aracaju, a municipality endemic for canine visceral leishmaniasis and between 2012 and 2014 5% to 10% of dogs tested for visceral leishmaniasis reacted to serological tests (CAMPOS *et al.*, 2017).

Through the calculation for simple random samples and an expected prevalence of 5%, we obtained a total of 72 samples, and in 2016, in the anti-rabies vaccination campaign in the municipality of Nossa Senhora da Glória, 1,712 dogs were vaccinated, in 14 neighborhoods. The dog's guardians signed the Free and Informed Consent Term, voluntarily allowing the physical examination and blood collection of their animal to be performed.

The dog's guardians signed the Free and Informed Consent Term, voluntarily allowing the physical examination and blood collection of their animal to be performed. Blood samples were collected from domesticated dogs of both sexes, with or without a defined breed and aged over four months. The sampling took place during the municipality's annual rabies vaccination campaign in 2017 in urban areas and 2018 in rural areas.

An average of 3 mL of blood was collected by external venipuncture or cephalic venipuncture and packed into previously labelled anticoagulant test tubes. The serum samples were analyzed at the Laboratory of the Veterinary Medicine Nucleus at the Federal University of Sergipe/Campus Sertão. The samples were centrifuged at 2000 xg for 5 min to obtain the serum, which was aliquoted into microtubes and stored at 20°C until serology was performed. The detection of antibodies to *Leishmania* was performed using the DPP® Immunochromatographic Test (Dual Path Platform, Biomanguinhos - Fiocruz, Brazil), according to the manufacturer's recommendations.

The questionnaire applied was adapted from the study by Galvão (2016) who diagnosed the epidemiological situation of canine VL in Rio Verde - GO. Each dog guardian provided information on the neighborhood in which they lived, their education level, and average income, and characteristics of the dog such as animal gender, age, breed, type of creation and feed type commercial or home-prepared, degree of confinement, and contact with other animals.

The data obtained from the epidemiological questionnaires was tabulated and analyzed in two stages for risk factors: univariate and multivariate analysis. Initially, descriptive analysis with the Chi-squared or Fisher Exact Test were applied to all variables. Univariate analysis using simple logistic regression was then performed with two groups of animals, seropositive and seronegative, which were compared for all the analyzed variables. Variables with a p-value ≤ 0.2 in the univariate analysis were selected for multivariate analysis using multiple logistic regression (BURSAC *et al.*, 2008).

The odds ratio (OR) and the corresponding 95% confidence interval (CI) were calculated for both the univariate and multiple logistic regressions. The level of significance adopted was 5%, and all analyses were performed using BioEstat 5.0 (Instituto Mamirauá, Brazil).

This study was submitted to the Animal Research Ethics Committee of the Federal University of Sergipe and approved under protocols 44/2017 and 41/2018 and to the Human Research Ethics Committee under CAAE 45526621.2.0000.5546 and opinion number 4.946.482.

3 RESULTS

Blood samples were collected from 195 dogs in the municipality, corresponding to 11.5% of the population estimated in the anti-rabies vaccination campaign of 1,172 dogs in the municipality in 2016. Thus, 60% (117/195) were from the urban area and 40% (78/195) from the rural area. A seroprevalence of 15.4% (30/195) was identified in dogs for VL, 11.1% (13/117) from urban and 21.8% (17/78) rural areas; however, there was no statistical difference between the two areas ($p = 0.06$).

The characteristics obtained through the epidemiological questionnaire are shown in Table 1. None of the owners with seronegative animals had completed higher education; therefore, the education variable was not analyzed using simple logistic regression.

Table 1 – Descriptive analysis related to dogs, environment and guardian variables, associated with the occurrence of canine visceral leishmaniasis in the urban and rural areas of the municipality of Nossa Senhora da Glória, Sergipe

Variables	Absolute frequency (n)	Relative frequency (%)	p-value
Animal-related variables			
Breed			
Mixed breed	28	93.3	0.04*
Defined breed	2	6.7	
Genre			
Male	25	83.3	0.004*
Female	5	16.7	
Sterilized			
Yes	2	6.7	0.63
No	28	93.3	
Domiciliated animal			
Yes	8	26.6	0.002*
No	22	73.4	
Only dog food			
Yes	1	3.3	0.05*
No	29	96.7	
Contact with other animals			
Yes	30	100.0	<0.0001*
No	-	-	
Environment-related variables			
Housing área			
Urban	13	43.3	0.06
Rural	17	56.7	

Variables	Absolute frequency (n)	Relative frequency (%)	p-value
Environmental cleaning			
No	13	43.4	0.12
Yes	17	56.7	
House backyard			
With organic matter	26	86.6	0.006*
Without organic matter	4	13.4	
Inhabited street			
Yes	15	50.0	0.06
No	15	50.0	
Nearby land with rubble			
Yes	11	36.6	0.67
No	19	63.4	
Tutor-related variables			
Education			
Illiterate	5	16.6	
Incomplete elementar	16	53.3	
Complete elementar	2	6.6	0.65
Incomplete secundar	3	10.0	
Complete secundar	1	3.5	
Complete higher education	3	10.0	
Family income			
Less than 2 minimum wages	25	83.3	0.78
Above 2 minimum wages	5	16.6	

* Significant predictor (<0.05).
Source: Research data.

In this study, results of the simple logistics regression analysis showed that mixed breed dogs (OR = 4.0; CI = 0.97–17.79) and male dogs (OR = 4.0; CI = 1.48–11.14) were more likely to be seropositive for *L. infantum* (Table 2).

Table 2 – Univariate and multivariate analysis related to dogs, environment and guardian variables, associated with the occurrence of canine visceral leishmaniasis in the urban and rural areas of the municipality of Nossa Senhora da Glória, Sergipe

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	p-value	OR	95% CI	p-value
Animal-related variables						
Breed						
Mixed breed	4.0	0.97 - 17.79	0.06	1.22	0.22 - 6.81	0.81
Defined breed						
Genre						
Male	4.0	1.48 - 11.14	0.006*	3.17	1.11 - 9.04	0.03*
Female						
Sterilized						
Yes	1.6	0.32 - 8.16	0.56			
No						
Domiciliated animal						
Yes	3.92	2.65 - 9.33	0.002*	2.40	0.80 - 7.25	0.12
No						
Only dog food						
Yes	5.92	0.77 - 45.33	0.08	2.30	0.25 - 21.68	0.46
No						
Contact with other animals						
Yes	NC	0.00 -	0.65			
No						
Environment-related variables						
Housing área						
Urban	1.60	0.73 - 3.52	0.23			
Rural						

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	p-value	OR	95% CI	p-value
Environmental cleaning						
No	0.55	0.25 - 1.22	0.14	1.53	0.58 - 4.01	0.38
Yes						
House backyard						
With organic matter	4.11	1.37 - 12.35	0.001*	1.73	0.45 - 6.64	0.42
Without organic matter						
Inhabited street						
Yes	0.47	0.22 - 1.04	0.06	0.71	0.30 - 1.71	0.45
No						
Nearby land with rubble						
Yes	1.19	0.53 - 2.68	0.67			
No						
Tutor-related variables						
Family income						
Less than 2 minimum wages	1.17	0.41 - 3.37	0.76			
Above 2 minimum wages						

OR: Odds ratio; CI: Confidence interval; NC: Not calculated (NC).
* Significant predictor (<0.05).
Source: Research data.

The presence of organic matter in the area surrounding the residence was also identified as a risk factor for the development of CVL, showing a significant result (OR = 4.11 CI = 1.37–12.35). In addition, results of the univariate analysis showed that dogs that had access to the street were more likely to be reactive for VL (OR = 3.92; CI = 2.65–9.33) than dogs that were confined.

Multivariate logistic regression analysis (Table 2) of *L. infantum* seroprevalence predictors was performed on all variables (breed, gender, breeding, and food types, cleanliness of the environment, presence of organic matter in the yard, and housing street) that had a p-value ≤ 0.2 in the univariate analysis. The animal gender, where male animals were more predisposed (OR = 3.17; CI = 1.11–9.04), was the only significant variable (p = 0.03) and was identified as a risk factor for VL.

4 DISCUSSION

On the American continent, Brazil is the country with the highest prevalence for VL, with case records of leishmaniasis in all Brazilian regions, especially in the Northeast. This region is considered to have a high VL risk and needs priority attention (ALMEIDA *et al.*, 2011).

The state of Sergipe is considered endemic for VL, with an average of 58 cases per year (CAMPOS *et al.*, 2017). In the municipality studied, a human case of VL was registered in the Notifiable Diseases Information System (BRASIL, 2022) during the period of the study.

The canine serological sample survey is recommended by the Ministry of Health as one of the disease transmission monitoring strategies. It is possible to assess the prevalence rates in each sector, identifying priority areas, from the survey (BRASIL, 2006). The high seroprevalence rate of dogs in the studied municipality is worrisome, demonstrating the spread of the disease both in rural and urban areas.

This is the first epidemiological study identifying the seroprevalence of dogs for CVL in the municipality of Nossa Senhora da Glória. In 2014, Aracaju, the capital of Sergipe, presented a 12.69% prevalence of *L. infantum* infection in dogs (CAMPOS *et al.*, 2017). Other studies conducted in Brazil have reported similar rates of prevalence (SALES *et al.*, 2017; ABRANTES *et al.*, 2018; ROCHA *et al.*, 2018).

In this study, it was found that male dogs are more likely to be seropositive for *L. infantum*, corroborating the findings of Campos and collaborators (2017). The greater predominance of males may be associated with a more aggressive temperament and greater territorial dominance than females. In addition, males are more often used as guard animals, staying longer outside the household and with a greater risk of contacting the vector (SALES *et al.*, 2017).

The data from this study indicate a greater number of mixed breed dogs. According to Mariga and collaborators (2021), when evaluating the prevalence and profile of dogs seroreagents for leishmaniasis in a veterinary hospital in Rio Grande do Sul, they noticed that the clinical profile presents a predisposition for animals without a defined breed.

Most of the animals are not sterilized and not domesticated, these factors increase dog overpopulation. Sterilization is an effective strategy for population control of domiciled and non-domiciled dogs, promoting improvements in public health, as it reduces the transmission of zoonoses (ALVES; HEBLING, 2020), which influences the prevalence of leishmaniasis.

In addition to the lack of dog population control, the lack of information among owners regarding diseases causes health, social and environmental problems in many countries, especially the underdeveloped ones. Often, the lack of information about the appropriate management of this animal results in the spread of zoonoses, such as VL, and other health problems (SERRÃO *et al.*, 2000).

The dog is considered the animal that coexists most closely with the man. When the fundamentals of responsible tutoring are neglected, they favor conditions for the development of zoonoses (ROCHA *et al.*, 2011).

In 2006, Borges and collaborators (2009) observed in Belo Horizonte - MG that the risk of humans being infected by leishmaniasis is greater when there is animals in their houses, also an increased risk was analyzed when there was other animals such as ducks, rodents, birds and chickens. Environ-

mental factors and human occupation of the territory are characteristics that increase the concentration of reservoirs and vectors in rural areas (MELLO *et al.*, 2008).

VL is a disease caused by multiple factors, including environmental and biological factors. The environment plays an important role in the transmission cycle of *Leishmania*, as it provides favorable places for vector reproduction (ABRANTES *et al.*, 2018). Hence, the studied municipality has favorable environmental conditions for vector reproduction, such as temperature and rainfall and the consequent maintenance of the disease transmission cycle (CLIMATEDATA, 2020).

The presence of organic matter in the area surrounding the residence was also identified as a risk factor for the development of CVL. The different stages of development of *Lutzomyia longipalpis*, vector of leishmaniasis, are classified as: egg, larval which has 4 stages, pupa and adult (FIOCRUZ, 2020). Sandflies eggs are deposited in places rich in organic matter (ALEXANDER, 2000; GALVÃO, 2016).

 At birth, the development of the larvae depends on the organic matter present in the soil (LEITE; WILLIAMS, 1997). If environmental conditions are not favorable, there is temporary retention of development, progressing again when favorable (TESH, 1988; GALVÃO, 2016).

To prevent VL, environmental cleaning procedures is recommended to avoid the reproduction and dissemination of the insect vector, thus it is necessary reduce the accumulation of garbage, debris and organic matter (tree leaves, feces and excreta) in the environment backyard of the residence, since they are the preferred places for the reproduction of the vector, the sandfly (FREITAS; ABIATTI, 2019).It is important to avoid twilight or nightime walks, as these are times when sandflies are most active (SCHIMMING; PINTO; SILVA, 2012).

The conditions prevailing in areas of low social vulnerability associated with the rapid and disorderly expansion of periurban areas of large cities lead to the formation of neighborhoods that lack adequate housing and basic sanitation infrastructure, favoring the proliferation of the vector and the spread of the infection (ALMEIDA *et al.*, 2011; CAMPOS *et al.*, 2017). Considering that living conditions have a direct impact on the health conditions of the population, the highest prevalence of VL in dogs occurred in peripheral neighborhoods occupied by people who were socially vulnerable (CAMPOS *et al.*, 2017; SOUZA *et al.*, 2020).

The behavior of infectious diseases, such as VL, appears as a development indicator in a specific region; its magnitude should serve as a guide for devising public policies far beyond the health sector. Intersectoral interventions of housing, sanitation, education, and other services can reduce social inequalities and produce improvements in the living conditions and health of populations (SOUZA *et al.*, 2020).

5 CONCLUSION

Considering the disquieting scenario regarding the distribution, transmission, and maintenance of the disease in dogs, and the high seroprevalence recorded in this pilot study, epidemiological surveillance services must draw attention to the region and develop intervention and control strategies, including educational and sanitary actions. Hence, these data represent an imminent risk to public health, with CVL being interconnected with cases of VL in humans.

The municipality of Nossa Senhora da Glória - SE demonstrated a high prevalence of CVL. The percentage of seroreagents dogs was high in relation to the capital Aracaju, which is endemic for the disease. Thus, this study demonstrates the need to approach educational and preventive actions in the region. With this in mind, an extension project was developed in partnership between the university and the municipal health department, entitled "The dog is not the villain: let's talk about leishmaniasis?", with the aim of educating the population about this zoonosis and preventing cases in humans and animals, and thus promoting improvements in public health.

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1 Médica Veterinária, Mestre em Ciências da Saúde. Universidade Federal de Sergipe, Aracaju, SE. E-mail: renatas2@hotmail.com

2 Médica Veterinária. Programa de Pós-Graduação em Ciências Aplicadas à Saúde, Universidade Federal de Sergipe, Campus do Sertão, Nossa Senhora da Glória, SE. E-mail: anitasilva@academico.ufs.br

3 Médica Veterinária. Universidade Federal de Sergipe, Campus do Sertão, Nossa Senhora da Glória, SE. E-mail: barbaararegina@gmail.com

4 Médico Veterinário. Universidade Federal de Sergipe, Campus do Sertão, Nossa Senhora da Glória, SE. E-mail: mv.gabriellobao@gmail.com

5 Médica Veterinária, Doutora em Ciência Animal nos Trópicos. Professora do Departamento de Medicina Veterinária, Universidade Federal de Sergipe, Campus do Sertão, Nossa Senhora da Glória, SE. E-mail: geyannadln@gmail.com

7 Biomédica, Doutora em Ciências da Saúde. Professora do Programa de Pós-Graduação em Ciências Aplicadas à Saúde, Universidade Federal de Sergipe, Aracaju, SE. E-mail: plimabio@gmail.com

8 Médica Veterinária, Doutora em Ciências da Saúde. Professora do Programa de Pós-Graduação em Ciências Aplicadas à Saúde, Universidade Federal de Sergipe, Campus do Sertão, Nossa Senhora da Glória, SE. E-mail: roseane@academico.ufs.br

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