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Article/Artigo

Seroprevalence and risk factors for canine visceral leishmaniasis in the endemic area of Dias D'Ávila, State of Bahia, Brazil

Soroprevalência e fatores de risco para leishmaniose visceral canina na área endêmica de Dias D'Ávila, Estado da Bahia, Brasil

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ABSTRACT

Introduction: Visceral leishmaniasis (VL) is an important zoonosis in relation to public health systems. Dogs are the main domestic reservoir. This study aimed to investigate occurrences of canine VL in Dias D'Ávila, State of Bahia, Brazil. **Methods:** The prevalence was evaluated by means of clinical and laboratory tests on a population of 312 domestic dogs from 23 localities in this municipality, using indirect immunofluorescence and immunoenzymatic assays. **Results:** Among the animals examined, 3.2% and 6.7% showed signs of VL, confirmed by indirect immunofluorescence and immunoenzymatic assays, respectively, with a distribution of 29.9% (24 dogs) in the rural zone and 4.9% (288 dogs) in the urban zone (p = 0.001). The clinical evaluation on seropositive dogs showed both asymptomatic animals (2.4%) and symptomatic animals (47.6%), along with other abnormalities (e.g. normocytic and normochromic anemia, with leukocytosis and thrombocytopenia). Observations relating to phenotypic characteristics (e.g. sex, age, breed and hair) did not present statistical significance, although high seropositivity among male, short-haired and mixed-breed dogs was observed. **Conclusions:** The findings showed that VL was a predominantly rural zoonosis and that close contact between poultry and domestic dogs significantly increased the risk of canine infection in this region.

Key-words: *Leishmania chagasi*. Visceral leishmaniasis. Dogs. Kala-azar. Seroprevalence.

RESUMO

Introdução: A leishmaniose visceral (LV) é uma importante zoonose para os sistemas de saúde pública, sendo os cães o principal reservatório doméstico. Este estudo teve como objetivo investigar a ocorrência de LV canina (LVC) em Dias D'Ávila, Estado da Bahia, Brasil. **Métodos:** A prevalência foi avaliada através de exames clínicos e laboratoriais em uma população de 312 cães domésticos de 23 localidades da cidade, utilizando-se imunofluorescência indireta e ensaio imunoenzimático. **Resultados:** Dentre os animais examinados, 3.2% e 6.7% apresentaram sinais de LV, confirmados por imunofluorescência indireta e ensaio imunoenzimático, respectivamente, com distribuição de 29.9% (24 cães) na zona rural e 4.9% (288 cães) na área urbana (p=0,001). A avaliação clínica de cães soropositivos apresentou animais assintomáticos (2,4%) e sintomáticos (47,6%), além de outras alterações (e.g., anemia normocítica e normocrômica, com leucocitose e trombocitopenia). Observações relativas a características fenotípicas (e.g. sexo, idade, raça e pelo) não apresentaram significância estatística, embora uma soropositividade alta entre machos, animais de pelo curto e de raça indefinida tenha sido observada. **Conclusões:** Os achados indicam que a LV é uma zoonose predominantemente rural e que o convívio de aves e cães domésticos aumenta significativamente o risco da infecção canina na região.

Palavras-chaves: *Leishmania chagasi*. Leishmaniose visceral. Cães. Kala-azar. Soroprevalência.

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INTRODUCTION

Canine visceral leishmaniasis (CVL) and human visceral leishmaniasis (HVL) are caused by the protozoa *Leishmania chagasi*[1]. They are transmitted to mammalian hosts through the bites of infected females of *Lutzomyia longipalpis*[2]. Dogs are the main domestic reservoir for VL[3]. VL, also known as kala-azar, is an important zoonosis in Brazil[4]. *L. chagasi* is the agent responsible for more than 2300 clinical cases of HVL in Brazil every year[2,5], and the prevalence of CVL in endemic areas has been shown to be between 1.9 and 25%[3,6].

VL is regarded as a sylvatic disease of rural areas, but its epidemiological profile has changed over time to become a major urban and suburban concern, due to the existence of favorable epidemiological conditions, such as the growth of shantytowns in urban centers, presence of domestic animal (dogs, chickens and pigs), increased vector density and human malnutrition[7,8], along with the imbalances of fauna and flora caused by deforestation[9].

In the epidemiology of kala-azar, the disease is considered more important in dogs than in humans. Not only is the canine disease more prevalent, but also it constitutes a reservoir because of the following factors: overlapping of human and canine habitats, frequent contact with zoophilic and anthropophilic phlebotomine species, cutaneous and visceral parasitism, high infection rates, chronic course of the disease and existence of many asymptomatic carriers capable of transmitting the disease[3,10]. Dogs may either be asymptomatic or show several clinical abnormalities. Thus, serological tests take on great importance because they demonstrate the presence of specific antibodies to *Leishmania* spp. Such tests are therefore the preferred tools in epidemiological surveys[11].

Both HVL and CVL are endemic in many regions of Brazil. They also occurs in other non-endemic regions when dogs from endemic areas are introduced into endemic-free areas, thereby contributing towards the urbanization of the disease[3,12-14]. Thus, epidemiological investigations may be necessary in order to monitor endemics and to implement strategies for their epidemiological control. The aim of the present study was to investigate canine autochthonous VL infections in Dias D'Ávila, Bahia, Brazil and to correlate the frequency of seropositive findings with possible risk factors for canine infection in this area.

METHODS

Animals

For the n sampler calculation, the formula applied was n = p(1-p) (1.96/D)2, with a 5% error level, where p = 19%. This was obtained through the arithmetic average from investigations on the epidemic carried out in other municipalities in the State of Bahia with 237 dogs. The present study used 312 domestic dogs from 23 areas in the municipality of Dias D'Ávila (18 from the urban zone and five from the rural zone). To define the animal numbers, in order to obtain proportionality, the sample was stratified according to the estimated number of dogs in each place, based on data from the anti-rabies vaccination campaign. In each place, the sample was divided into four and distributed starting from a central point, along four imaginary vectors of 250m. The size of the vector determination followed the guidance from the National Health Foundation for studies on VL foci, using the cardinal points[15]. The dogs were selected from existing houses along each vector, alternating from one side of the street to the other, and taking into consideration only one animal per house. After making the selections, an epidemiological clinical questionnaire was filled out for each animal, with data on breed, age, sex, weight, hair length and keeping and feeding habits, along with the owner's name and address and information about any pigs, chickens, hens or other domestic animals that were reared. The questionnaire also took into consideration garbage collection procedures, presence of marsupials and rodents and any occurrences of CVL and HVL. The dogs were subjected to clinical examination and blood collection. The results obtained (apparent mucous membranes, lymphadenomegaly, skin lesions, ocular alterations and onychogryphosis) were used to classify the animals as either asymptomatic (absence of clinical signs of infection, not considering isolated instances of lymphadenomegaly) or symptomatic (oligosymptomatic, i.e. moderate symptoms, with two clinical signs; and polysymptomatic, with three or more clinical signs), in accordance with the criteria suggested by Molina et al[16].

Study area and study design

The municipality of Dias D'Ávila is located in the metropolitan area of Salvador, 53km away from the state capital (**Figure 1**). It has 45,565 inhabitants and a canine population of approximately 5,695 animals, corresponding to 12.5% of the human population. A cross-sectional study was carried out using the selected sample, in order to investigate the seroprevalence of antibodies for *L. chagasi*, by means of IFI and indirect ELISA, between March and May 2002.

Blood collection

Blood samples with and without anticoagulant were collected by cephalic venipuncture. Hematological profiles were determined from whole blood collected with anticoagulant, using an automated hematological analyzer (ABX ABC Vet, ABX Diagnostics, Montpellier, France), while serum samples obtained from blood collected without anticoagulant were stored at -20 °C until required for serological analysis (IFI and ELISA).

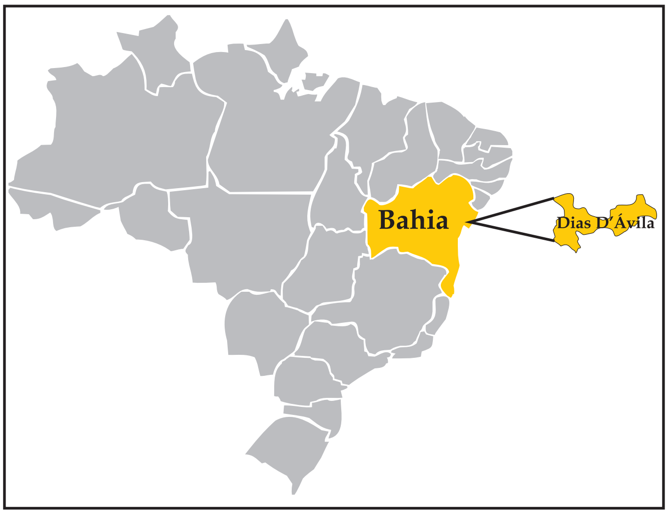

FIGURE 1 - Map of Brazil showing the location of the study area. The municipality of Dias D'Ávila is located in eastern Bahia, in the coastal region.

Indirect immunofluorescence test

The indirect immunofluorescence (IFI) reactions were performed using the canine leishmaniasis IFI kit (FIOCRUZ/Biomanguinhos, Brazil). The serum samples were screened at a dilution of 1:40, and positive and negative control samples were included on the slides. The positive samples were subjected to titration.

Indirect enzymatic immunoassay

The *L. chagasi* promastigote antigen was evaluated by means of ELISA, in accordance with the standardized protocol of ENSP, FIOCRUZ, Rio de Janeiro (the reference laboratory for leishmaniasis diagnosis), with some modifications. Briefly, ELISA plates were coated with 100µl (1µg/well) of antigen diluted in 0.06 M carbonate buffer (pH 9.6). After incubation (16h, 4°C), the plates were washed three times with PBS (pH 7.2), blocked (2h, 4°C) with PBS containing 1% non-fat milk and 0.05% Tween 20 and washed again. Serum samples, diluted 1:80 with PBS-T, were added to plate wells and incubated (1h, 37°C). After washing, the wells were incubated (1 h, 37°C) with peroxidase-labeled anti-dog IgG (Sigma Chem. Co., USA), diluted 1/60,000 with PBS-T. After washing the wells three times, 100µl of chromogenic solution (6mg O-phenylenediamine, 15ml of 0.1M citrate phosphate buffer, pH 5.1, and 10µl of H_2O_2) were added per well and incubated for color development. The optical density (OD) was measured at 490 nm in an ELISA reader (Stat Fax®, USA). The cutoff value was determined as the mean OD of the negative control serum plus two standard deviations. Positive samples were repeated for confirmation.

Statistical analysis

The data were correlated with the frequency of seropositive findings from ELISA and were analyzed using the chi-square test. For the biochemical determination analysis, Student's distribution for comparison of averages was applied. Both tests were performed using the SPSS statistical analysis software, with a significance level of $p < 0.05$.

Ethics

All animal experimentation was conducted in accordance with the guidelines for care of the Brazilian College of Animal Experimentation (COBEA).

RESULTS

In the serological analyses performed in this study, 312 canine serum samples were used. Ten were found to be positive using IFI and 21 using ELISA, thus respectively indicating seroprevalence of 3.2% with confidence interval (CI) of 3.2 ± 1.9 and 6.7% with CI 6.7 ± 2.8. Based on the data obtained using ELISA, the seroprevalence was 4.9% ± 2.5 in the urban zone and 29.9% ± 18 in the rural zone, which was statistically significant (χ^2 = 20.84; p = 0.0001), while it was absent in 10 of the 23 study localities. In the other localities, the prevalence ranged from 5 to 50% (**Table 1**).

TABLE 1 - Frequency of CVL-seropositive animals, as determined using ELISA, in villages in the municipality of Dias D'Ávila, Bahia, Brazil.

Zone	Village	Tested using ELISA positive/number	Positive serum samples (%)
Urban	Santa Helena	1/20	5
Urban	Imbassaí	0/40	0
Urban	Varginha	0/16	0
Urban	Cristo Rei	1/12	8.3
Urban	Isaura	3/16	18.8
Urban	Jardim Garcia D'Ávila	1/4	25
Urban	Garcia D'Ávila	4/16	25
Urban	Centro	0/20	0
Urban	Campo Alegre	1/16	6.3
Urban	Santa Terezinha	1/16	6.3
Urban	Parque Capuame	0/4	0
Urban	Parque Dias D'Ávila	0/4	0
Urban	Urbis	0/28	0
Urban	Parque Petrópolis	1/8	12.5
Urban	Jardim Alvorada	1/12	8.3
Urban	Genaro	0/32	0
Urban	Bosque Dias D'Ávila	0/4	0
Urban	Nova Dias D'Ávila	0/20	0
Rural	Emboacica	2/4	50
Rural	Boa Vista de Santa Helena	1/4	25
Rural	Biribeira	2/4	50
Rural	Jardim Futurama	0/4	0
Rural	Leandrinho	2/8	25
Total		**21/312**	**6.7**

IFI-specific IgG titers ranged from 1:40 to 1:640 with the following distribution: 30% at 1:40, 40% at 1:80, 20% at 1:320 and 10% at 1:640. Although there were no statistically significant differences among the IFI titers, ELISA findings and clinical classifications of the animals, higher titers were observed in symptomatic dogs using IFI (1:320 and 1:640), which was not detected using ELISA.

From analysis on risk factors, chicken rearing (p = 0.003) was statistically significant (**Table 2**), while the presence of pigs and other animals, garbage collection, presence of marsupials and rodents and occurrences of HVL/CVL did not show statistical significance (p > 0.05) (**Table 3**).

The clinical symptoms described among the seropositive dogs showed that asymptomatic animals predominated (52.4%), followed by polysymptomatic animals (38.1%) and oligosymptomatic animals (9.5%). Comparing the serological data with clinical

TABLE 2 - Visceral leishmaniasis seropositivity of dogs according to whether chickens were reared or not.

Risk factors	p/t	FSP	CI
Rearing chickens/hens	15/127	11.81	9 – 14.6
Not rearing chickens/hens	6/185	3.24	0.64 – 5.8

p/t: number positive/number tested, **FSP:** % frequency of seropositivity, **CI:** confidence interval, χ^2 = 8.806, p = 0.003.

TABLE 3 - Visceral leishmaniasis seropositivity of dogs according to their degree of confinement.

Risk factors	p/t	FSP	CI
Free to roam	11/82	13.1	5.9 - 20.9
Limited to the backyard	10/230	4.34	3.0 - 5.6

p/t: number positive/number tested, **FSP:** % frequency of seropositivity, **CI:** confidence interval, χ^2 = 7.916, p = 0.005.

manifestations, there was a statistical difference (χ^2 = 4.026; p = 0.045). The rate of seropositive ELISA findings was 4.9% (CI 3.5-6.3) for asymptomatic animals, whereas among the symptomatic (oligosymptomatic and polysymptomatic) animals, this value was 11.24% (CI 11.24 ± 6.56). The results relating to frequency of clinical symptoms and signs among the oligosymptomatic and symptomatic animals are shown in **Table 4**. Among these, lymphadenomegaly, alopecia, onychogryphosis and exfoliative dermatitis were the clinical signs that were most pronounced. Onychogryphosis was the only statistically significant symptom (χ^2 = 10.222; p = 0.001). Although no statistical significance was established, regarding the distribution of phenotypic characteristics among the dogs studied (gender, age, breed and hair length), high rates of seropositivity among male animals (52.3%), short-haired animals (76%), mixed-breed dogs (90%) and adult dogs of 1 to 6 years of age (71.4%).

In 66% of the seropositive animals, no abnormalities in the hematological profile were observed. Morphological assessment of erythrocytes, leukocytes and platelets revealed that 23.8% presented normocytic and normochromic anemia, 4.7% presented microcytic hypochromic anemia and 4.7% presented normocytic hypochromic anemia. Regarding total while blood cells, 16 (71.6%) animals showed no abnormalities, five (31.3%) presented neutrophilic leukocytosis and four (80%) of them were symptomatic. The platelet counts were normal in 12 (57.1%) dogs, while nine animals showed thrombocytopenia. Four of the latter (44.4%) were symptomatic. However, none of these abnormalities were statistically significant.

TABLE 4 - Frequency of clinical signs of VL-seropositivity among dogs.

Clinical sign	Percentage
Lymphadenomegaly	90.0
Alopecia	80.0
Onychogryphosis	80.0
Exfoliative dermatitis	70.0
Dermatitis	60.0
Pale mucous	40.0
Ulceration	40.0
Low weight	30.0
Cachexia	30.0
Ocular abnormalities	30.0
Vomiting	10.0

DISCUSSION

It is crucial to diagnosis leishmaniasis in humans and reservoirs in order to control the disease and understand its epidemiology[4]. In Brazil, VL is considered to be endemic in 19 states, which are mostly in the northeastern region, where greater number of cases have been reported. The epidemic pattern of disease transmission is also showing changes in important urban centers such as Boa Vista, Teresina, São Luiz, Belo Horizonte and Cuiabá[2,7,17], where infected dogs represent an active reservoir for the parasite transmission because of their constant proximity with humans[7]. It is essential to diagnose CVL and HVL in order to achieve control over this zoonosis, particularly with regard to identify the epidemiological risk factors[18].

The results obtained from this work on the diagnosis of CVL add to the understanding of its seroprevalence in the endemic area of Dias D'Ávila. The data indicated serological values of 2.3 and 6.7% through testing by IFI and ELISA, respectively. These values are higher than those reported by healthcare agencies, which in 2001 reported that the prevalence was less than 1%. These data corroborate reports over similar periods from other endemic areas in northeastern Brazil[14,19]. This difference may be due to the samples that are routinely used by healthcare agencies, which are eluates of canine blood rather than serum samples. In general, these show sensitivity of less than 90%[20]. The present study was carried out from March to May, and the association between variations in climatic factors and vector density over this period suggests that these factors are potentially important for the transmission of VL in this municipality[11,21,22]. The study period coincided with the rainy season, with increased population density of the insect vector.

The prevalence of canine infection was higher in rural areas (29.9%) than in urban areas (4.9%), with p = 0.0001. This corroborates other data that showed an infection rate that was 17 times higher among dogs from rural areas than among dogs from urban areas, detected during the hot season. Similar results were observed when analyzing the prevalence in two groups of animals in rural and urban areas[23]. In contrast, other data showed the same prevalence in both rural and urban areas[13,24]. The variation in seroprevalence between these two areas is intrinsically linked to peridomestic/domestic environmental conditions for the development of infection. These conditions may include the vector, canine population, changes caused by anthropization, accumulation of organic matter, poor sanitation conditions and precarious garbage collection[4,25]. In: Dias D'Ávila CVL has a profile typical of rural disease, but its frequency is expanding to the urban area, because of the presence of large numbers of stray animals[4].

The data from this study establish a correlation between dog-rearing locations and keeping poultry in peridomestic/domestic environments. Chickens are not reservoirs, but they represent a nutrition source for phlebotomine sandflies, dogs, foxes and marsupials, which are natural reservoirs for VL transmission. In addition, domestic animals contribute towards the accumulation of organic matter, which is associated with the development of the larva of the vector[26]. The presence of chicken coops in peridomestic areas was correlated with diagnoses of high seropositivity among dogs throughout the municipality, thus demonstrating the role of chickens as promoters of high sandfly densities.

There are few studies evaluating some phenotypic characteristics (e.g. sex, age and breed) as risk factors for the incidence of CVL. The present study has revealed that characteristics such as gender and short hair correlate with high seropositivity for CVL. These results are in agreement with observations by França-Silva et al[27] in Brazil, Sideris et al[28] in Greece and Abranches et al[29] in Portugal.

The epidemiological importance of CVL was shown by the detection of a high (53.4%) frequency of asymptomatic dogs. This could be interpreted as a relevant factor for maintaining the infection in endemic areas, since several studies relating to viscero-cutaneous parasitism have shown that dogs play a role in the dissemination of VL because of the presence of the parasite in healthy skin[29]. Some authors have also demonstrated no great differences in the transmission of the parasite from the vector between symptomatic, oligosymptomatic and asymptomatic dogs[11,21].

Regarding clinical manifestations and seropositivity rates, 11% of the symptomatic animals were positive, compared with 4.9% of the asymptomatic animals. This suggests that in Dias D'Ávila, the clinical signs evaluated may indicate that the animals have acquired the infection. The clinical symptom profile indicated greater frequency of lymphadenomegaly, followed by onychogryphosis and skin abnormalities, which is consistent with profiles reported from other endemic areas[30]. Moreover, in the current study, the presence of onychogryphosis was statistically significant. It was detected in 80% of the seropositive symptomatic dogs, thus suggesting high likelihood of CVL in this municipality. In addition, the hematological abnormalities were mostly in animals that had some clinical manifestations.

In conclusion, the epidemiological profile of CVL in the municipality of Dias D' Ávila indicated that the seroprevalence of anti-*L. chagasi* antibodies in dogs was spread out heterogeneously across many localities in the region. It was often characteristically rural, although it was expanding into the urban area, because of the presence of high number of dogs in domestic and peridomestic localities. It was also evident that the clinical symptoms, especially onychogryphosis, indicated high likelihood of canine infection in this municipality. In addition, high numbers of domestic animals near households may be considered to be a risk factor for VL transmission.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Baneth G, Koutinas AF, Solano-Gallego L, Bourdeau P, Ferrer P. Canine leishmaniasis - new concepts and insights on an expanding zoonosis: part one. Trends Parasitol 2008; 24:324-330.
2. Costa CHN. Characterization and speculations on the urbanization of visceral leishmaniasis in Brazil. Cad Saude Publica 2008; 24:2959-2963.
3. Dantas-Torres F, Brito ME, Brandao-Filho SP. Seroepidemiological survey on canine leishmaniasis among dogs from an urban area of Brazil. Vet Parasitol 2006; 140:54-60.

4. Gramiccia M, Gradoni L. The current status of zoonotic leishmaniasis and approaches to disease control. Int J Parasitol 2005; 35:1169-1180.

5. Silveira FT, Lainson R, Pereira EA, Souza AAA, Campos MB, Chagas EJ, et al. A longitudinal study on the transmission dynamics of human *Leishmania* (*Leishmania*) *infatum chagasi* infection in Amazonian Brazil, with special reference to its prevalence and incidence. Parasitol Res 2009; 104:559-567.

6. Nascimento ELT, Martins DR, Monteiro GR, Barbosa JD, Ximenes MFFM, Maciel BL, et al. Forum: geographic spread and urbanization of visceral leishmaniasis in Brazil. Postscript: new challenges in the epidemiology of *Leishmania chagasi* infection. Cad Saude Publica 2008; 24:2964-2967.

7. Arias JR, Monteiro PS, Zicker F. The reemergence of visceral leishmaniasis in Brazil. Emerg Infect Dis 1996; 2:145-146.

8. Maciel BL, Lacerda HG, Queiroz JW, Galvão J, Pontes NN, Dimenstein R, et al. Association of nutritional status with the response to infection with *Leishmania chagasi*. Am J Trop Med Hyg 2008; 79:591-598.

9. Ready PD. Leishmaniasis emergence and climate change. Rev Sci Tech 2008; 27:399-412.

10. Guimarães KS, Batista ZS, Dias EL, Guerra RMSNC, Costa ADC, Oliveira AS, et al. Canine visceral leishmaniasis in São José de Ribamar, Maranhão State, Brazil. Vet Parasitol 2005; 131:305-309.

11. Reithinger R, Davies CR. Canine leishmaniasis: novel strategies for control. Trends Parasitol 2002; 18:289-290.

12. Almeida ABPF, Faria RP, Pimentel MFA, Dahroug MAA, Turbino NCMR, Sousa VRF. Seroepidemiological survey of canine leishmaniasis in endemic areas of Cuiabá, State of Mato Grosso. Rev Soc Bras Med Trop 2009; 42:156-159.

13. Cortada VM, Doval MEC, Souza-Lima MAA, Oshiro ET, Meneses CRV, Abreu-Silva AL, et al. Canine visceral leishmaniosis in Anastácio, Mato Grosso State, Brazil. Vet Res Commun 2004; 28:365-374.

14. Queiroz PVS, Monteiro GRG, Macedo VPS, Rocha MAC, Batista LMM, Queiroz JW, et al. Canine visceral leishmaniasis in urban and rural areas of Northeast Brazil. Res Vet Sci 2009; 86:267-273.

15. Ministério da Saúde. Fundação Nacional de Saúde. Informe Epidemiológico do Sistema Único de Saúde. Brasília, DF. MS/FNS 1999; 7:86-87.

16. Molina R, Amela C, Nieto J, San-Andrés M, González F, Castillo JA, et al. Infectivity of dogs naturally infected with *Leishmania infantum* to colonized *Phlebotomus perniciosus*. Trans R Soc Trop Med Hyg 1994; 88:491-493.

17. Tasca KI, Buzetti WA, Tenorio MS, Paulan SC, Lima FL, Queiroz NM, et al. Parasitological, immunohistochemical and histopathological study for *Leishmania chagasi* detection in splenic tissues of dogs with visceral leishmaniasis. Rev Bras Parasitol Vet 2009; 18:27-33.

18. Moreira-Jr ED, Souza VMM, Sreenivasan M, Lopes NL, Barreto RB, Carvalho LP. Peridomestic risk factors for canine leishmaniasis in urban dwellings: new findings from a prospective study in Brazil. Am J Trop Med Hyg 2003; 69:393-397.

19. Paranhos-Silva M, Freitas LAR, Santos WC, Grimaldi-Jr G, Pontes-de-Carvalho LC, Oliveira-dos-Santos AJO. A cross-sectional serodiagnostic survey of canine leishmaniasis due to *Leishmania chagasi*. Am J Trop Med Hyg 1996; 55:39-44.

20. Costa CA, Genaro O, Lana M, Magalhães PA, Dias M, Michalick MS, et al. Canine visceral leishmaniasis: evaluation of the serologic method used in epidemiologic studies. Rev Soc Bras Med Trop 1991; 24:21-25.

21. Bavia ME, Carneiro DD, Gurgel HC, Madureira-Filho C, Barbosa MG. Remote sensing and geographic information systems and risk of American visceral leishmaniasis in Bahia, Brazil. Parassitologia 2005; 47:165-169.

22. Sherlock IA. Ecological interactions of visceral leishmaniasis in the State of Bahia, Brazil. Mem Inst Oswaldo Cruz 1996; 91:671-683.

23. Courtenay O, Macdonald DW, Lainson R, Shaw JJ, Dye C. Epidemiology of canine leishmaniasis: a comparative serological study of dogs and foxes in Amazon Brazil. Parasitology 1994; 109:273-279.

24. Zaffaroni E, Rubaudo L, Lanfranchi P, Mignone W. Epidemiological patterns of canine leishmaniosis in Western Liguria (Italy). Vet Parasitol 1999; 81:11-19.

25. Camargo-Neves VL, Brasil MT. American leishmaniasis in the state of São Paulo: epidemiological status in 2001-2002. Rev Soc Bras Med Trop 2003; 36(suppl):30-35.

26. Alexander B, Carvalho RL, McCallum H, Pereira MH. Role of the domestic chicken (*Gallus gallus*) in the epidemiology of urban visceral leishmaniasis in Brazil. Emerg Infect Dis 2002; 8:1480-1485.

27. França-Silva JC, Costa RT, Siqueira AM, Machado-Coelho GLL, Costa CA, Mayrink W, et al. Epidemiology of canine visceral leishmaniosis in the endemic area of Montes Claros Municipality, Minas Gerais State, Brazil. Vet Parasitol 2003; 111:161-173.

28. Sideris V, Karagouni E, Papadopoulou G, Garifallou A, Dotsika E. Canine visceral leishmaniasis in the greater Athens area, Greece. Parasite 1996; 3:125-130.

29. Abranches P, Santos-Gomes GM, Rachamin N, Campino L, Schnur LF, Jaffe CLL. An experimental model for canine leishmaniasis. Parasite Immunol 1991; 13:537-550.

30. Abranches P, Campino L, Santos-Gomes GM. Leishmaniose canina, novos conceitos de epidemiologia e imunopatologia e seus reflexos no controle da leishmaniose visceral humana. Acta Med Port 1998; 11:871-875.