



Short communication

## Canine visceral leishmaniasis in São José de Ribamar, Maranhão State, Brazil

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### Abstract

Here, we describe the situation of canine visceral leishmaniasis in two villages of São José de Ribamar in Maranhão State/Brazil, where human cases have been registered. Blood samples of 36 household crossbred dogs from Sergio Tamer village and 43 dogs from Quinta village were collected and the serum used for serological diagnosis. An Indirect Fluorescent Antibody Test (IFAT) and enzyme-linked immunosorbent assay (ELISA) were used to detect antibodies against *Leishmania*. The clinical examination showed that 25% of the canine population of Quinta presented a poor body condition and in 39%, ectoparasites (ticks and fleas) were detected. In both tests, serology revealed that 21% (9 out of 43) of the dogs presented antibodies against *Leishmania* (55% were asymptomatic and 45% were symptomatic). In the Vila Sérgio Tamer, 25% (9 out of 36) of the dogs were seropositive for *Leishmania* (66.67% were asymptomatic and 33.33% were symptomatic), 33% presented poor body condition, and 22% have ectoparasites. The clinical signs more frequent were skin lesions. The statistical analysis showed that there was no statistical difference ( $p > 0.05$ ) between the seropositivity of the dogs from the two villages. The same was observed when the clinical signs were compared ( $p > 0.05$ ). Both villages have favorable conditions to maintain the cycle of leishmaniasis.

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### 1. Introduction

American visceral leishmaniasis (AVL) is a chronic and wasting disease characterized by the infection of the mononuclear phagocytes by *Leishmania chagasi*

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(Cunha and Chagas, 1937; Herwaldt, 1999). Although AVL occurs from Mexico to Argentina (Grimaldi et al., 1989), over 90% of the human cases come from Brazil, especially from the Northeast region of the country.

The first report of *L. chagasi* in Brazil was made by Penna (1934) during histological examination of liver specimens though post-mortem viscerotomy. Soon after, Chagas et al. (1937, 1938) observed cases of AVL in domestic dogs. Later, wild reservoirs represented by the foxes *Lycalopex vetulus* (Deane and Deane, 1954a,b) and *Cerdocyon thous* (Lainson et al., 1969; Silveira et al., 1982) were also reported.

Transmission from human to human is found in visceral leishmaniasis caused by *L. donovani* and it is prevalent in India and Sudan (Zijlstra and El-Hassan, 2001).

In this way, it is indispensable to detect infected dogs and to understand the role of asymptomatic dogs as reservoirs, as they are often undetectable upon clinical examination and a large number escape from control measures, thus contributing to the spread of leishmaniasis. Information on the geographical distribution and prevalence of canine AVL is essential to the design and implementation of appropriate control measures (Campino, 2002).

This paper describes the situation of AVL in two villages of São José de Ribamar in Maranhão State/Brazil, where human cases have been registered. Despite that leishmaniasis be a serious public health problem in Maranhão State, the seroprevalence of canine visceral leishmaniasis is underestimated.

## 2. Materials and methods

### 2.1. Description of the area

São José de Ribamar in São Luís Island, is a municipality located between 2°37'30"S and 44°07'30"L, in the north region of the Maranhão State/Brazil. It has a hot climate with annual average temperature around 26 °C. The population is approximately 107,333 inhabitants distributed in several villages. The municipality is recovered by a type of seasonally forest vegetation—Forest seasonally Perenifolia comprised mainly by babaçu palm tree

(*Orbignya speciosa*) and it also has forest Perenifolia Paludosa Maritime – Manguezal (mangrove swamp). The original vegetation was modified, due to intense human occupation in the area and nowadays “capoeiras” replaced the original vegetation although babaçu palm trees are still present (Maranhão, 2002). This work was done in Vila Sérgio Tamer and Quinta villages in which recent cases of human visceral leishmaniasis were notified. The Vila Sérgio Tamer has 586 residences and 1541 inhabitants and Quinta has 545 residences and 1435 inhabitants. The sanitary conditions are low (open sewage, no water sanitation and poor housing). In opposition to Vila Sérgio Tamer, in the last village, the residences consist mainly of small ranches.

### 2.2. Survey design

Household mongrel dogs from two villages Sérgio Tamer (24 males and 12 females) and Quinta (26 males and 17 females) were examined for clinical symptoms of AVL. Dogs were examined for poor body condition and clinical picture. The clinical signs were scored as follows: score 0 (no clinical signs), score 1 (skin lesions: ulcers, alopecia, desquamation, onychogryphosis) score 2 (visceral signs: lymph adenopathy), and score 3 (skin lesions + visceral signs).

The dogs sampled were the ones that live in streets where human cases of visceral leishmaniasis have occurred. Peripheral blood samples were collected by cephalic vein puncture and the serum was separated, stored at –20 °C until serological diagnosis. An Indirect Fluorescent Antibody Test (IFAT) and enzyme-linked immunosorbent assay (ELISA) were used to detect antibodies using a commercial kit for canine leishmaniasis provided by Biomanguinhos/FIOCRUZ. The threshold serum dilution for a positive test was 1:40 and the final dilution was 1:1280. For ELISA a sample was considered positive if the optical density was 2.6 times higher than the standard deviation of the control group.

### 2.3. Statistical analysis

The chi-square test was used to compare the clinical score and serology between the two villages. The relationship was considered significant when  $p < 0.05$ .

### 3. Results

Habitations of both villages, Quinta and Vila Sérgio Tamer, have minimal infrastructure and sanitation conditions are precarious. Household crossbreed dogs are kept outdoors during the day, are not well fed, do not receive vaccines nor veterinary assistance. The clinical examination showed that 25% of the canine population of Quinta presented a poor body condition and in 39%, ectoparasites (ticks and fleas) were detected. The serology revealed that 21% (9 out of 43) of the dogs presented antibodies against *Leishmania* (55% were asymptomatic and 45% were symptomatic). In these symptomatic animals, the clinical signs more frequent were skin lesions (ulcer in the pinnula, alopecia, onychogryphosis, and dry desquamation). In the Vila Sérgio Tamer, 25% (9 out of 36) of the dogs were seropositive for *Leishmania* (66.67% were asymptomatic and 33.33% were symptomatic), 33% presented poor body condition and 22% had ectoparasites (ticks and fleas). The clinical signs more frequent were also skin lesions. The statistical analysis showed that there was no statistical difference ( $p > 0.05$ ) between the seropositivity of the dogs from the two villages. The same was observed when the clinical score 0 and 1 were compared ( $p > 0.05$ ). No animal presented clinical signs for score 2 and only one animal from Sérgio Tamer village showed signs for score 3. Serological titers by indirect immunofluorescence test ranged from 1/40 to 1/640. There was no difference between the serological tests used.

### 4. Discussion

The Vila Sérgio Tamer and Quinta could be a risk area for leishmaniasis maintenance since positive cases of human and canine leishmaniasis had been detected and the vector, *Lutzomyia longipalpis*, is commonly captured in the area according to previous entomological studies (Carvalho et al., 2000). Both villages have minimal infrastructure associated to intense urbanization in the last years. The native vegetation (moist tropical broadleaf forest) is being gradually replaced by secondary-growth forest called “capoeira”. This can contribute to the establishment of the sand fly vector in the peridomestic and intradomestic habits. Urbanization clearly appears

to be one of the major worldwide risk factors for leishmaniasis and largely contributes to the persistence of the burden of the disease especially in anthroponotic foci (Desjeux, 2002).

Since the first studies about visceral leishmaniasis, dogs have been incriminated as the most important urban reservoir of the disease (Deane, 1956; Alencar, 1961). However, the culling of infected dogs had not been enough to control the disease in endemic areas, such as, São Luís Island where canine seroprevalence remains underestimated. Previous studies showed that in other village of the same municipality, the canine seroprevalence was 64% (Melo et al., 2002). Here, it was observed that the seroprevalence was 21% (Quinta) and 25% (Vila Sérgio Tamer), although it is lesser when compared with previous study, the risk for leishmaniasis remains high. The difference in seroprevalence can be observed not only in this municipality but also in other endemic areas in Brazil as demonstrated by Alves et al. (1998) who observed a seroprevalence of 1.59% among dogs from Fortaleza/Ceará. França-Silva et al. (2003), in Montes Claros/Minas Gerais, detected 9.7%, Cabrera et al. (2003) found out 25% of seropositivity in dogs of Barra de Guaratiba/Rio de Janeiro, Guerra et al. (2004) detected 10.3% in Roraima and Cortada et al. (2004), observed a seroprevalence of 75.3% among dogs from Anastácio/Mato Grosso do Sul.

In dogs, the clinical picture of this disease is characterized by skin lesions (dry desquamation, alopecia and muzzle ulcer), hepatomegaly, splenomegaly, weight loss, generalized lymphadenopathy, ocular lesions, and onychogryphosis (Ferrer et al., 1988; Cortada et al., 2004). Here, it was also observed that in both areas, the more frequent clinical signs were skin lesions, which suggests that in dogs, *L. chagasi* or *L. infantum* primarily induces cutaneous lesions and after that it spreads to internal organs.

Our findings are in agreement with Ferrer et al. (1991) and Campino (2002) that state that the great majority of dogs infected by *Leishmania* remain asymptomatic. We found out 61% of asymptomatic dogs in both villages. The presence of latent infections in dogs is typical and important in maintaining the long-term presence of the parasite in endemic areas (Palatinik-de-Sousa et al., 2003).

In conclusion, both environmental and ecological aspects, may have contributed to the increase in VL

incidence in the municipality of São José de Ribamar since, in the last years, there were expansion of villages, the establishment of new settlements, and increased agricultural activity. These conditions have led to an increase in refuse and solid waste providing good habitats for sand fly vectors and also attracting stray dogs and wild canid reservoir hosts.

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