

Serological survey of cases of canine visceral leishmaniasis and evaluation of phlebotomine fauna on Marambaia Island, municipality of Mangaratiba, Rio de Janeiro state, Brazil*

Levantamento sorológico de casos de leishmaniose visceral canina e avaliação da fauna de flebotomíneos na Ilha da Marambaia, município de Mangaratiba, estado do Rio de Janeiro, Brasil

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Abstract

The state of Rio de Janeiro is epidemiologically classified as a region of low incidence for American visceral leishmaniasis (AVL); however, endemic areas have expanded dramatically in recent years. In 2009 and 2010, autochthonous cases of AVL were reported in the municipality of Maricá and in the neighborhood of Laranjeiras in the city of Rio de Janeiro, respectively. In 2006, samples isolated in infected animals in the coastal area of the municipality of Mangaratiba were characterized as *L. (L.) chagasi*. In 2002, on Marambaia Island, located in Mangaratiba, only cases of American cutaneous leishmaniasis (ACL) were recorded. This study aimed to reassess the prevalence of CVL and identify the phlebotomine fauna on Marambaia Island, Mangaratiba, in 2012. To this end, a canine serological survey was carried out using IFA, ELISA and DPP techniques. In addition, phlebotomi were captured with the help of light traps, HP type. The census totaled 116 dogs, and 17 animals tested positive with prevalence of 14.6%. From April to November 2012, 2,524 sandfly specimens of nine different species were captured. We observed the maintenance of CVL prevalence in the region, as well as the presence of the *Lutzomyia longipalpis* vector.

Keywords: serological survey, canine visceral leishmaniasis, *Lutzomyia longipalpis*, *Leishmania (Leishmania) chagasi*.

Resumo

O estado do Rio de Janeiro é classificado epidemiologicamente como uma região de baixa incidência de leishmaniose visceral americana (LVA), no entanto, áreas endêmicas têm se expandido drasticamente nos últimos anos. Em 2009 e 2010, foram notificados casos autóctones de LVA no município de Maricá e no bairro de Laranjeiras, na cidade de Rio de Janeiro, respectivamente. Em 2006, em amostras de animais infectados no litoral do município de Mangaratiba foram isoladas e caracterizadas *L. (L.) chagasi*. Em 2002, na Ilha da Marambaia, localizada em Mangaratiba, foram registrados apenas os casos de leishmaniose tegumentar americana (LTA). Este estudo teve como objetivo avaliar a prevalência da LVC e identificar a fauna de flebotomíneos na Ilha da Marambaia, Mangaratiba, em 2012. Para este fim, um inquérito sorológico canino foi realizado utilizando as técnicas IFA, ELISA e DPP. Além disso, foram capturados flebotomos com a ajuda de armadilhas luminosas, tipo HP. O censo totalizou 116 cães e 17 animais foram positivos com a prevalência de 14,6%. Entre abril e novembro de 2012, foram capturados, nove espécies diferentes flebotomíneos, um total de 2.524 espécimes. Observou-se a manutenção da LVC prevalência na região, bem como a presença do vetor *Lutzomyia longipalpis*.

Palavras-chave: levantamento sorológico, leishmaniose visceral canina, *Lutzomyia longipalpis*, *L. (L.) chagasi*.

Introduction

Visceral leishmaniasis is a disease which occurs worldwide, and approximately 350 million people have been infected so far. In Brazil, it is a zoonosis of major public health importance. According to data from the Brazilian Ministry of Health, between 2007 and 2012, 20,353 cases of American visceral leishmaniasis (AVL) were recorded, with 1,210 deaths (SINAN, 2013).

Leishmania (Leishmania) chagasi (syn. *L. infantum*) is the etiologic agent of AVL in Brazil; this protozoan is transmitted mainly by the *Lutzomyia longipalpis* vector. In Mato Grosso do Sul state, *Lutzomyia cruzi* - a dipteran which belongs to the subfamily Phlebotominae, has been incriminated as the vector of AVL at blood-feeding time in vertebrates (Miles et al., 1999) (Santos et al., 1998).

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Didelphis marsupialis and *Lycalopex* and *Cerdocyon foxes* are the reservoirs of *L. (L.) chagasi* in forest and rural environments. In urban areas, in houses and their surroundings, the primary host is the domestic dog (*Canis familiaris*), thus a contributing factor to the maintenance of the disease cycle (Marzochi, 1994; Franca-Silva et al., 2005).

Canine visceral leishmaniasis (CVL) presents a wide spectrum of clinical manifestations, ranging from apparently healthy to severely ill dogs. This variation is conditioned to the animal's individual immunocompetence and the parasite strain inoculated (Michalick & Genaro, 2005).

According to the AVL control manual (Brasil, 2006), one of the measures of disease control is epidemiologic surveillance, which is conducted through serological surveys of the canine population, removal of seropositive dogs, and entomological surveys.

The state of Rio de Janeiro is epidemiologically classified as a region of low incidence of AVL (Brasil, 2013); however, endemic areas have expanded dramatically in recent years. In 2009 and 2010, autochthonous cases of AVL were reported in the municipality of Maricá (de Paula et al., 2009) and in the neighborhood of Laranjeiras in the city of Rio de Janeiro (Figueiredo et al., 2010), respectively. In 2006, samples isolated in infected animals in the municipality of Mangaratiba were characterized as *L. (L.) chagasi*. In 2002, on Marambaia Island, located in Mangaratiba, only cases of American cutaneous leishmaniasis (ACL) were recorded. Although no human cases have been reported in this area, it is important to conduct a new assessment so that the circulation of the disease can be confirmed, and canine prevalence and maintenance of vector presence can be reassessed. For this reason, this study aims to evaluate the seroprevalence of dogs and the presence of phlebotomine on Marambaia Island, Mangaratiba, state of Rio de Janeiro.

Materials and methods

Area of Study

Marambaia Island is located in the municipality of Mangaratiba, southern Rio de Janeiro state, Brazil (Figure 1). The island is administered by the Brazilian Army; it receives military from several regions of the country and is inhabited by 103 families, with a total of 352 inhabitants, distributed over an area of 1.6 hectares. The island presents tropical humid climate; its vegetation comprises one of the last preserves of the Atlantic Rain Forest in southeastern Brazil, large areas of sandbanks (including beaches and dunes) and mangrove swamps, with associated ecosystems (Conde et al 2005; Mattos, 2005).

Marambaia Island's population is distributed in nine beaches: Pescaria Velha, Caetana, Cutuca, do José, Grande, Suja, João Manoel, Caju and Sítio, as well as in a forested area known as Buraco Quente.

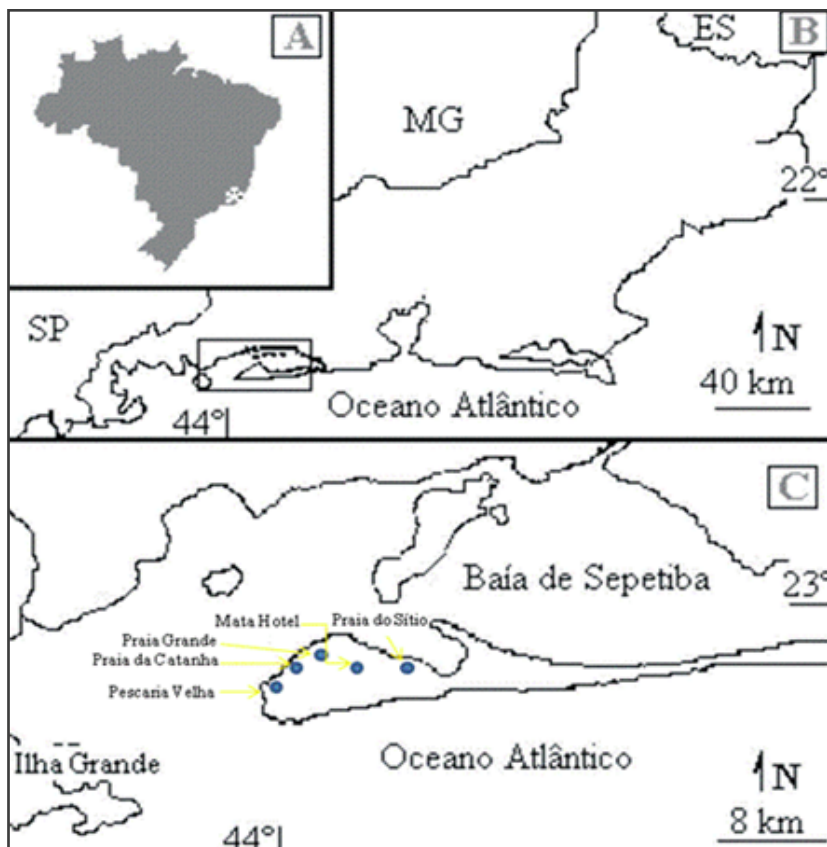


Figure 1: Map of the state of Rio de Janeiro with Marambaia Island, municipality of Mangaratiba, in highlight. Provided by Barbosa MB.

Target population. In 2012, a canine census was conducted in the study area, totaling 116 animals (including stray dogs) distributed in the ten previously mentioned locations. The animals were submitted to blood sample collection by puncture of the external jugular or cephalic veins so that serological tests could be performed. After the samples were collected, the dogs were electronically identified with microchips, Animall Tag manufactured, placed in the withers area, thus avoiding information bias, given the fact that dogs in this region move about intensely.

Approximately 5 ml of blood were collected from each dog. The blood was then centrifuged for 10 minutes at 3000 rpm for serum separation; with a disposable sterilized pipette, the serum separated was transferred to Eppendorf microtubes and preserved at -20 °C until serologic tests started.

Serologic tests. Serologic testing included rapid immunochromatographic assay (DPP), indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA).

For qualitative serological testing, the rapid test DPP[®] Canine Visceral Leishmaniasis (Bio-Manguinhos Fiocruz) was used. This is a dual-path platform immunochromatographic test that utilizes rK28 *L. (L.) chagasi* recombinant protein as antigen to detect specific antileishmanial antibodies in dogs. The tests were conducted with samples of the canine sera, according to the manufacturer's instructions (Pattabhi et al. 2010).

ELISA was conducted following modifications of the method described in Voller's et al. (1976) using *L. (L.) chagasi* crude

antigen (MHOM/BR/74/PP75) produced according to Ribeiro et al. (2007). The sera were diluted 1:100 in duplicate. The Anti-Dog IgG peroxidase conjugated, produced in rabbit, Sigma-Aldrich manufactured, was used as conjugate. The test cut-off point was calculated based on the average reading of negative sera plus two standard deviations (Maurice, 1995).

For the IFA, logarithmic phase *L. (L.) chagasi* promastigotes (MHOM/BR/74/PP75), fixed in formalin at 2%, were used as antigen. The sera were diluted from 1:40 to lack of reactivity. The Anti-Dog IgG-FITC conjugate, developed in rabbits, Sigma-Aldrich manufactured, was used as antigen. The samples that showed reactivity as from serum dilution 1:40 were considered positive (Rebonato & Camargo, 1969).

In this study, indirect immunofluorescence assay (IFA) was used to confirm diagnosis, according to the manual provided by the Brazilian Ministry of Health (Brasil, 2006). This criterion was adopted to allow comparison with the 2009 serological survey.

Assessment of the phlebotomine fauna. HP suction light traps were placed in the houses and their surroundings, such as kennels and chicken roosts, as well as in the forest environment. The traps were set at 6 PM and removed at 7 AM the next morning (Pugedo et al, 2005). Collection occurred on a monthly basis, on three consecutive days, from April to November 2012.

Collection was performed in eleven sites: in the beaches named Pescaria Velha, Caetana, Suja, do José, Grande, Cutuca, Sítio, Caju; in the areas known as Buraco Quente and Vacaria; and at the Hotel dos Sargentos (Hotel of the Sergeants).

The specimens collected were stored in Eppendorf microtubes containing 70% alcohol. The tubes were properly identified and sent to the Miguel Alves de Souza Laboratory of Vectors - ENSP/FIOCRUZ, for clarification, mounting and species identification according to the nomenclature proposed by Galati (2003).

The statistical analysis was performed descriptively evaluating the frequency of the findings.

Results

Serology. One hundred sixteen (116) canine serum samples were analyzed: 13 (11.2%) were positive in the DPP, 45 (38.79%) in the ELISA and 17 (14.65%) in the IFA.

Twelve of these samples (10.34%) showed agreement in all serological tests performed. The seropositive dogs are distributed on the following beaches: Suja, Caetana, Grande, Pescaria Velha, José and Caju (Chart 1).

Phlebotomine Fauna. Nine phlebotomus species were found in the region: *Lutzomyia longipalpis*, *Nyssomyia intermedia*, *Migoneimyia migonei*, *Pintomyia fischeri*, *Evandromyia edwardsi*, *Micropygomyia capixaba*, *Pintomyia bianchigalatae*, *Micropygomyia schreiberi* and *Bruptomyia sp.* (Chart 2).

L. longipalpis species was found in only three beaches: Pescaria Velha, Cutuca and Caetana. *N. intermedia* species showed wide distribution, being found mainly at Suja and Pescaria Velha beaches and in the area known as Vacaria. *M. migonei* species was captured in three beaches, José, Pescaria Velha and Suja, as well as in Vacaria (Chart 3).

Collection Sites	DISTRIBUTION OF SEROPOSITIVE ANIMALS
Suja Beach	5
Caetana Beach	4
Pescaria Velha Beach	3
Grande Beach	3
Caju Beach	1
José Beach	1
Cutuca Beach	0
Sítio Beach	0
Buraco Quente	0
TOTAL	17

Chart 1: Distribution of seropositive dogs per collection site. Marambaia Island, municipality of Mangaratiba, 2012

SPECIES	MALE	%	FEMALE	%	TOTAL	%
<i>N. intermedia</i>	1029	77,07	1016	85,5	2045	81
<i>M. migonei</i>	263	19,7	141	11,92	404	16
<i>L. longipalpis</i>	34	2,55	6	0,5	40	1,6
<i>P. fischeri</i>	4	0,3	16	1,3	20	0,8
<i>M. schreiberi</i>	4	0,3	6	0,5	10	0,4
<i>M. capixaba</i>	0	0	2	0,1	2	0,08
<i>P. bianchigalatae</i>	0	0	1	0,09	1	0,04
<i>E. edwardsi</i>	1	0,08	1	0,09	2	0,08
TOTAL	1335	100	1189	100	2524	100

Chart 2: Phlebotomine fauna on Marambaia Island, from April to November 2012

Collection Sites	Distribution of the species		
	<i>L.longipalpis</i>	<i>N. intermedia</i>	<i>M. migonei</i>
Suja Beach	Absent	Present	Present
Caetana Beach	Present	Present	Present
Pescaria Velha Beach	Present	Present	Present
Grande Beach	Absent	Present	Present
Caju Beach	Absent	Present	Absent
José Beach	Absent	Present	Present
Cutuca Beach	Present	Present	Present
Sítio Beach	Absent	Present	Present
Buraco Quente	Absent	Absent	Absent
Vacaria	Absent	Present	Present

Chart 3: Distribution of the species *L. longiplapis*, *N. intermedia* and *M. migonei* in the collection sites. Marambaia Island, municipality of Mangaratiba, 2012

Discussion

Currently, canine visceral leishmaniasis is a serious public health problem in expansion in Brazil, mainly in urban areas previously considered unaffected (Costa, 2008).

The confirmation that 17 (14.65%) dogs tested positive for CVL on Marambaia Island in 2012 reveals a region with a high prevalence of the disease. The island, which belongs to the Armed Forces, prohibits the entry and exit of animals; therefore, we suspect that the cases of CVL may be indigenous. This prevalence is close to the findings by Silva et al. (2013), who reported prevalence of 11.59% for CVL in Mangaratiba, Rio de Janeiro state. This finding corroborates Paranhos-Silva et al. (1996), who carried out a cross-sectional study in Bahia state, and found prevalence of 23.5%. In the municipality of Bom Sucesso, Minas Gerais state, prevalence of 10.8% was reported in a survey conducted in two neighborhoods (Silva & Santa Rosa, 2005).

Most dogs roam freely across the island during the day and return to their owners' houses at twilight. At this time of day, the risk of exposure to the AVL vector is high.

The study area, Marambaia Island, holds an Atlantic Rain Forest preserve. Its population is distributed in homes installed near the forest environment or even in the woods. Anthropogenic changes in the natural environment, poor sanitation, lack of sewage system and regular garbage collection, as well as the presence of chicken roosts in backyards, causes the accumulation of organic matter around the dwellings, promoting the creation of an environment favorable for sand flies. Cerbino et al (2009), in a study conducted in Teresina, Piauí state, between 1991 and 2000, found that vast vegetation and population increase are related to increased rates of the disease. Monteiro et al (2005), in a study conducted in the municipality of Montes Claros, Minas Gerais state, reported that substandard housing conditions, low socioeconomic status, and lack of basic sanitation, along with very close interaction with domestic animals, increase the risk of AVL transmission.

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The occurrence of CVL in the study area may be related to a specific characteristic of this region, with the houses are inserted in the woods, where the presence of the vector and wild reservoirs is abundant, and garbage collection is irregular with large accumulation of organic matter in the surroundings of residences.

The phlebotomine fauna on Marambaia Island was studied in 2009 by Novo et al. (2013), who reported a total of thirteen species collected in three different ecotopes: domiciliary, peridomiciliary and forest, with *N. intermedia* species found in higher density and distributed throughout the region. The presence of *L. longipalpis* species was observed, but at low density.

In our study, *N. intermedia* is still the phlebotomus species presenting the highest density, followed by *M. migonei*, *L. longipalpis* and *P. fischeri*. Such species are of great vector relevance for leishmaniasis. *L. longipalpis* was found with reduced density in only three beaches: Pescaria Velha, Cutuca and Caetana. Brandão-Filho et al (2002) conducted a study at the region of Zona da Mata, Pernambuco state, and described the presence of AVL cases with no record of *L. longipalpis*, which suggests the involvement of another vector species of the disease, such as *M. migonei*.

The low density, or even the absence of *L. longipalpis*, might explain the absence of human cases in the study area. According to Souza et al (2003), in the west part of the city of Rio de Janeiro, autochthonous CVL cases have occurred for years with no record of *L. longipalpis* presence, which can also be related to the participation of another phlebotomus species in the transmission cycle, whose feeding habits are targeted at dogs and other domestic animals.

In the present study, we observe the occurrence of CVL on Marambaia Island, and that the phlebotomine fauna has maintained the same patterns, thus contributing to greater knowledge on the dynamics of this disease in the region.

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