Revista da Sociedade Brasileira de Medicina Tropical 42(5):488-493, set-out, 2009

Epidemiological study on leishmaniasis in an area of environmental tourism and ecotourism, State of Mato Grosso do Sul, 2006-2007

Estudo epidemiológico das leishmanioses em área de turismo ambiental e ecoturismo, Estado de Mato Grosso do Sul, 2006-2007

Ana Rachel Oliveira de Andrade[1], Vânia Lúcia Brandão Nunes[2], Eunice Aparecida Bianchi Galati[3], Carla Cardozo Pinto de Arruda[1], Mirella Ferreira da Cunha Santos[2], Maria Elizabeth Gizi Rocca[4] and Ricardo Braga Aquino[4]

ABSTRACT

The aims of this study were to carry out a serological survey of canine leishmaniasis and identify the phlebotomine fauna in the urban area of Bonito, Mato Grosso do Sul. The serological survey was conducted on a sample of 303 dogs, by means of the indirect immunofluorescence test. Phlebotomines were captured using automated light traps. The serological survey found that 30% of the dogs were seropositive, both from the center and from all districts of the town. A total of 2,772 specimens of phlebotomines were caught and the species most found was *Lutzomyia longipalpis* (90.4%), which corroborated its role as the vector of for canine visceral leishmaniasis in the region. Phlebotomines of the species *Bichromomyia flaviscutellata* (the main vector for *Leishmania (Leishmania) amazonensis*) and *Nyssomyia whitmani* (the vector for *Leishmania (Viannia) brasiliensis*) were also caught. The findings indicate the need for continuous epidemiological surveillance, with attention towards diminishing the vector breeding sites and the transmission of these diseases in that region.

Key-words: Leishmaniasis. Canine seroepidemiological survey. Phlebotomine fauna. Bonito. Bodoquena plateau.

RESUMO

O presente trabalho teve por objetivo proceder ao levantamento sorológico para leishmanioses em cães e identificar a fauna flebotomínea da zona urbana de Bonito, Mato Grosso do Sul. O inquérito sorológico foi realizado em amostras de 303 cães com a utilização da reação de imunofluorescência indireta. As capturas de flebotomíneos realizaram-se com armadilhas automáticas luminosas. O inquérito sorológico identificou 30% cães reagentes procedentes do centro e de todos os bairros da cidade. Foram capturados 2,772 exemplares de flebotomineos, sendo a espécie mais freqüente foi *Lutzomyia longipalpis* (90.4%), o que corrobora o seu papel de vetora de leishmaniose visceral canina na região. Foram capturados, também, flebotomíneos da espécie *Bichromomyia flaviscutellata*, principal vetora da *Leishmania (Leishmania) amazonensis*, e *Nyssomyia whitmani*, vetora da *Leishmania (Viannia) braziliensis*. Os achados indicam a necessidade de uma contínua vigilância epidemiológica, atentando para a diminuição dos criadouros dos vetores e da transmissão desses agravos naquela região.

Palavras-chaves: Leishmanioses. Inquérito soroepidemiológico canino. Fauna flebotomínea. Bonito. Planalto da Bodoquena.

In humans, leishmaniases can occur in two clinical forms: American cutaneous leishmaniasis (ACL) and visceral leishmaniasis (VL)[15,20]. ACL is among the six most important infectious and parasitic diseases in Brazil, occurring throughout the country[22]. VL is a potentially life-threatening disease for humans. In Brazil, its etiology is attributed to *Leishmania (Leishmania) chagasi* and the disease presents differentiated geographical, climatic and social characteristics according to its widespread distribution involving all five Brazilian geographical regions[7].

1. Laboratory of Parasitology, Center of Biological and Health Sciences, University for the Development of the State and the Region of Pantanal, Campo Grande, MS, Brazil. 2. Laboratory of Parasitology, Center of Biological and Health Sciences, University for the Development of the State and the Region of Pantanal, Campo Grande, MS, Brazil. 3. Department of Epidemiology, Public Health Faculty, University of São Paulo, São Paulo, SP, Brazil. 4. Nucleus of Zoonoses Control of Bonito, Bonito, MS, Brazil.
Financial support: CAPES and Manoel de Barros Foundation.
Address to: Dr. Ana Rachel Oliveira de Andrade. Rua Luiz Freire Benchetrit 258, Miguel Couto, 79040-140 Campo Grande, MS.
Tel: 55 67 3306-0916
e-mail: racheloliveira_83@hotmail.com
Received in 17/02/2009
Accepted in 29/09/2009

Among the pets that may host the etiological agent for VL, dogs are considered to be the most important in epidemiological terms. They serve as a source of food for the vector, and bring the vector into contact with humans. Their high population density and their susceptibility to *Leishmania chagasi* are factors that make dogs the main domestic reservoir for VL in the Americas[20].

The natural vectors for protozoa of the genus *Leishmania* are dipterous, from the family Psychodidae, subfamily Phlebotominae. Except for some very rare autogenous species, female phlebotomines need blood from vertebrates to maintain their ovaries and mature their eggs, hence their importance in leishmaniasis transmission[25].

Like in other Brazilian states, leishmaniasis presents widespread geographical distribution in Mato Grosso do Sul (MS). In 2006, 222 cases of VL and 143 cases of ACL were confirmed. In 2007 up to March, 37 cases of ACL and 77 of VL had already been reported in 17 municipalities, and Bonito was among them[12,24]. In spite of these data, investigations on the etiology and particular features of the transmission processes of these diseases are scarce in Mato Grosso do Sul[4,16].

The above facts justified the present study, in which the aims were to carry out a serological survey on canine leishmaniasis and identify phlebotomines in the urban area of Bonito, with a view to obtaining support for planning policies to control epidemics in this region, which is an important tourist center in Brazil.

MATERIAL AND METHODS

Area of study. Bonito is located in the southwestern region of Mato Grosso do Sul, at 21°07'16"S and 56°28'55"W, in the geomorphological unit called the Bodoquena plateau. The area of the municipality area is 4,934 km^2, and the population density is 3.6 inhabitants/km^2. [10]

The region has a recognized infrastructure for ecotourism, i.e. activities developed to minimize the deleterious effects of frequent human action on nature. The municipality of Bonito is considered to be an important center for ecotourism both at national and at international level, and its main attractions are its natural landscape, rivers of crystal-clear water, waterfalls, grottos and caves.

Serological survey on canine leishmaniasis. The urban area of Bonito is divided into six zones: 1, 2, 3, 4, 5 and 6, totaling 364 street blocks. The number of blocks in each zone was surveyed, and then around 30% of the blocks were randomly selected, which resulted in a total of 108 (**Table 1**).

With the help of the Bonito Zoonosis Control Center, a canine census was carried out in all houses in the selected blocks. The number of dogs and their size, type and color were recorded for later identification.

Next, five animals per block were again randomly selected and blood was collected from them by means of puncturing the cephalic vein using a 30x8mm needle. The material was left for clot retraction for a varying length of time and then centrifuged at 2,500rpm, for five minutes, to obtain the serum. Next, the serum from each animal was divided into two portions and placed in Eppendorf tubes. After identification, these tubes were stored in a freezer for later serological assays.

To estimate the number of statistically valid samples and take into account animal refusals and losses, among other factors that could negatively interfere with the results, the number of dog samples (five) to be collected from each block was multiplied by the total number of blocks in the town, thus totaling 1,820 dogs. Taking a 95% confidence interval, margin of error of 6% and estimated prevalence of 50%, it was found that n = 233 would be enough for the present study. Nonetheless, out of the 1,820 dogs, 303 were sampled because the natural prevalence of leishmaniasis in the region was unknown.

The indirect immunofluorescence antibody test was performed by using a kit for canine leishmaniasis supplied by Bio-Manguinhos, FIOCRUZ, Rio de Janeiro. Samples that showed fluorescent promastigotes, including the flagellum, were considered positive. Those that showed parasites without fluorescence and reddish coloration were considered non-positive. Serum that was positive at a titer 1:40 was tested again in serial dilutions halving the concentration, i.e. 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1,280, in order to determine the antibody titers in the samples. Those with titers greater than or equal to than 1:80 were considered positive.

Capture and identification of phlebotomines, and analysis performed. Phlebotomines were captured fortnightly using CDC automatic light traps[14] between March 2006 and February 2007, from 6pm to 6am, irrespective of daylight saving time. The capture sites comprised 12 environments and 16 ecotopes, covering both the center and the different districts of Bonito.

In order to identify the phlebotomines, the structures of the head, chest and abdomen at a specific level were used; the male and female genitalia were enhanced[5].

TABLE 1

Blocks selected in the urban zones of Bonito, Mato Grosso do Sul, Brazil.

Zones	Selected Blocks (30%)
Zone 1 (95 blocks)	02, 03, 10, 11, 13, 15, 19, 20, 23, 27, 30, 37, 44, 50, 53, 54, 55, 60, 61, 66, 67, 74, 75, 80, 83, 86, 90, 95 (28 blocks)
Zone 2 (67 blocks)	04, 12, 30, 15, 19, 20, 38, 23, 26, 28, 32, 37, 45, 49, 50, 54, 63, 64, 66, 11 (20 blocks)
Zone 3 (58 blocks)	03, 05, 08, 09, 22, 18, 25, 31, 32, 38, 47, 39, 30, 45, 44, 42, 35 (17 blocks)
Zone 4 (44 blocks)	04, 05, 10, 15, 16, 14, 22, 29, 33, 35, 38, 42, 28 (13 blocks)
Zone 5 (87 blocks)	02, 04, 05, 08, 14, 17, 23, 28, 31, 34, 38, 42, 46, 47, 49, 50, 54, 55, 59, 64, 70, 75, 78, 82, 84, 85 (26 blocks)
Zone 6 (13 blocks)	02, 04, 05, 09 (4 blocks)

To determine the abundance of a certain species in relation to others, at a given time and location, the Roberts and Hsi standard abundance index (SAI) was used[21]. The Williams Geometric Average[9] was used to quantify the frequency and regularity of the most abundant species. The Shannon Index (H) was used to calculate the relationship between the number of species and the number of individuals captured in a determined ecotope and Pielou's Index (J) was used to measure the proportion of the contribution of each species in the community of a given ecotope[8]. The relative frequency of the species was measured by the percentage method.

RESULTS

Dogs. Out of the 303 dogs, 91 (30%) were seropositive according to the immunofluorescence test, with titers greater than or equal to 1:80, while 202 (70%) were non-positive. The seropositive dogs were both from the center and from all the districts (**Table 2**).

Phlebotomines. According to the classification proposed by Galati[5], the phlebotomines captured belonged to four subtribes, seven genera and 13 species: Brumptomyiina: *Brumptomyia avellari*, *Brumptomyia brumpti*; Psychodopygina: *Bichromomyia flaviscutellata*, *Nyssomyia whitmani*, *Psathyromyia (Psathyromyia) shannoni*, *Psathyromyia (Forattiniella) aragaoi*, *Psathyromyia punctigeniculata*, *Psathyromyia campograndensis*; Sergentomyiina: *Micropygomyia quinquefer*; Lutzomyiina: *Evandromyia corumbaensis*, *Evandromyia (Aldamyia) lenti*, *Lutzomyia longipalpis*.

Table 3 and **Table 4** show the number of phlebotomines captured fortnightly, between March 2006 and February 2007. *Lutzomyia longipalpis* was present in all of the 12 environments within which captures were made, thus demonstrating the widespread distribution of this species within the urban area of Bonito. This species was mostly found in areas surrounding homes, near animal shelters (**Table 3** and **Table 4**). The species that was second most frequently found was *Micropigomyia quinquefer,* especially indoors. This is the species most often found in the savanna. *Evandromyia sallesi* came third in frequency and was

TABLE 2

Numbers of positive and non-positive canine samples collected between March 2006 and February 2007 in Bonito, Mato Grosso do Sul, Brazil.

Zones of Bonito	Number of samples	Positive samples	Non-positive samples	% positive	% non-positive
Zone 1	71	15	56	21.1	78.9
Zone 2	57	22	35	38.6	61.4
Zone 3	75	18	57	24.0	76.0
Zone 4	39	2	37	5.1	94.9
Zone 5	48	31	17	64.6	35.4
Zone 6	13	3	10	23.1	76.9
Total	303	91	212	30.0	70.0

TABLE 3

Phlebotomines captured fortnightly with CDC light traps, according to species, sex, ecotopes, index of species diversity by Shannon (H) and index of species equitability by Pielou (J), in 12 environments of Bonito, MS, Brazil, from March 2006 through February, 2007.

	Property 1		House 1		Smallholding 1				Hillside savanna				Forest				Smallholding 2			
	yard		hencoop		pigpen		veranda		slope		base		edge		interior		veranda		yard	
Species Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Bicromomyia flaviscutellata	-	-	-	-	-	-	-	-	-	-	-	-	9	6	-	5	1	4	-	9
Brumptomyia avellari	-	-	-	-	-	1	-	-	-	-	-	-	6	-	3	4	-	-	-	1
Brumptomyia brumpti	-	-	-	1	-	1	-	-	-	-	-	2	9	4	6	12	2	2	-	1
Evandromyia corumbaensis	-	-	-	-	-	-	-	-	-	-	3	1	-	-	-	-	2	2	1	-
Evandromyia. lenti	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Evandromyia sallesi	-	-	1	9	-	4	1	-	-	3	1	4	-	1	-	-	2	9	1	8
Lutzomyia longipalpis	107	9	773	115	254	55	13	4	24	7	22	14	-	1	-	2	248	58	556	68
Micropigomyia quinquefer	-	-	-	-	-	-	-	-	1	9	25	38	-	1	-	-	-	-	-	1
Nyssomyia whitmani	-	-	2	1	1	1	-	-	1	-	-	-	2	2	1	1	2	1	-	1
Psathyromyia aragaoi	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2	-	-	-	-	-
Psathyromyia campograndensis	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Psathyromyia punctigeniculata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Psathyromyia shannoni	-	-	2	-	-	-	-	-	-	-	1	1	2	-	1	-	2	-	2	-
Total	107	9	778	127	255	62	14	4	26	19	52	61	29	15	13	24	259	76	560	90
Shannon (H)	0		0,11		0,14		0,21		0,85		1,12		1,70		1,50		0,43		1,0	
Pielou (J)	0		0,06		0,08		0,30		0,61		0,57		0,77		0,77		0,22		0,43	

M: male, F: female.

TABLE 4

Phlebotomines captured fortnightly with CDC light traps, according to species, sex, ecotopes, index of species diversity by Shannon (H) and index of species equitability by Pielou (J), in 12 environments of Bonito, MS, Brazil, from March 2006 through February, 2007.

	House 2 hencoop		House 3 hencoop		House 4 yard		Animal outhouse		House 5 yard		Property 2 kennel		Total		Total	
Species Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F	MF	%
Bicromomyia flaviscutellata	-	-	-	-	-	-	-	-	-	-	1	-	11	24	35	1.26
Brumptomyia avellari	-	-	-	-	-	-	-	-	-	-	-	-	9	6	15	0.54
Brumptomyia brumpti	-	1	-	-	-	-	-	-	-	-	-	-	17	24	41	1.47
Evandromyia corumbaensis	-	-	1	1	-	-	1	-	-	-	-	-	8	4	12	0.43
Evandromyia lenti	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0.03
Evandromyia sallesi	-	1	1	3	3	1	1	-	-	-	-	1	11	44	55	1.98
Lutzomyia longipalpis	69	11	10	10	14	2	40	10	4	-	1	-	2,135	369	2,504	90.40
Micropigomyia quinquefer	-	-	-	-	-	-	-	-	-	-	-	1	26	50	76	2.73
Nyssomyia whitmani	-	-	-	-	-	-	-	-	-	-	-	-	9	7	16	0.57
Psathyromyia aragaoi	-	-	-	-	-	-	-	-	-	-	-	-	3	-	3	0.10
Psathyromyia campograndensis	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	0.03
Psathyromyia punctigeniculata	-	-	-	-	-	1	-	-	-	-	-	-	-	2	2	0.07
Psathyromyia shannoni	-	-	-	-	-	-	-	-	-	-	-	-	10	1	11	0.39
Total	69	13	12	14	17	4	42	10	4	3	2	2	2,239	533	2,772	100.0
Shannon (H)	0,13		0,68		0,66		0,18		0		0,05		0,50		% male	80.77
Pielou (J)	0,11		0,61		0,60		0.16		0		0,03		0,18		% female	19.23

M: male, F: female.

found in most of the environments studied, although in small numbers. *Brumptomyia brumpti* was present indoors and in areas surrounding homes, especially at the edges of and inside in wooded areas. *Bicromomyia flaviscutellata* came fifth and was mostly found in the woods, but some individuals were also found indoors.

The diversity index for phlebotomine species ranged from H = 0 to H = 1.70. The highest values were found at the edges of wooded areas (H = 1.70), inside woods (H = 1.50), in a farmyard in zone 2 (H = 1.0) and in hillside savannah (H = 0.85). Thus, the greatest diversity was found in regions with rich vegetation, where nine out of the 13 species studied were found (**Table 3** and **Table 4**).

In the areas surrounding homes, the index ranged from H = 0 to H = 0.66, and indoors, it ranged from H = 0 to H = 0.63. The lowest indices occurred at House 5 in zone 1, with H = 0, i.e. only one species occurred, namely *Lutzomyia longipalpis.* It was noteworthy that the diversity index was influenced not only by the number of species found in a given place, but also by the total number of individuals captured.

Regarding the proportion that each species contributed in the community of a given ecotope, **Table 3** and **Table 4** show that J = 0 at House 5 in zone 1, i.e. only one species was present. Both at the edges and inside the woods, the index was high (J = 0.77), thus demonstrating that there were equal proportions among the species captured.

Table 4 shows the standard abundance index. The most abundant species was *Lutzomyia longipalpis* with SAI = 0.90. Thus, on this scale, with a value close to one, this finding indicated maximum abundance.

The correlation between the mean temperature and rainfall in the region over the period analyzed and the frequency and regularity of the most abundant species collected (*Lutzomyia longipalpis*) was investigated (**Figure 1**). This species was reported throughout the year, with the highest incidence in the

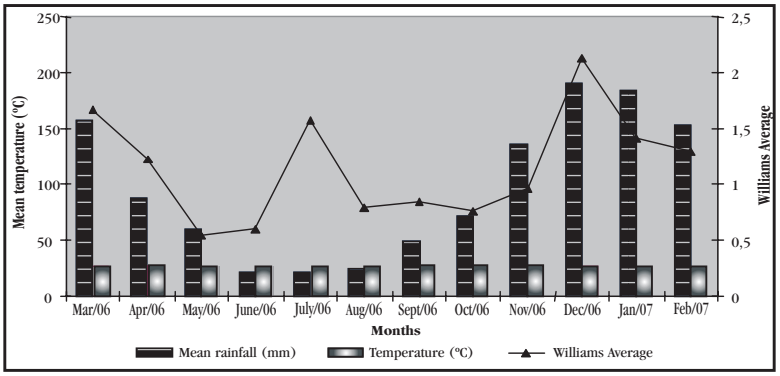

FIGURE 1

Mean monthly temperature (°C), monthly rainfall (mm) and monthly frequency (Williams Average) of *Lutzomyia longipalpis* captured in 16 ecotopes of Bonito, MS, Brazil, from March 2006 through February, 2007.

rainiest months: March, November and December 2006 and January and February 2007. However, in July 2006, there was a peak in the number of species, without any relationship to the rainy season.

DISCUSSION

In this study, serum samples with titers greater than or equal to 1:80 were regarded as positive. This limit has also been used by other researchers[3 13 26] for the Bio-Manguinhos immunofluorescence kit, in relation to *Leishmania (Leishmania) chagasi*.

Using this limit of 1:80, the number of cross-reactions with other parasites is lower[26]. Although the serological cross-reactivity between *Leishmania sp* and *Trypanosoma sp* is well defined, little is known about the influence of other microorganisms that infect dogs, such as Babesia *canis, Erlichia canis* and *Plasmodium sp.* Thus, the specificity of the immunofluorescence test is influenced by infections caused by different parasite species[2 11].

In the state of Mato Grosso do Sul, the presence of *Lutzomyia longipalpis* was reported in the municipalities of Anastácio and Aquidauana and in the state capital, Campo Grande, where VL is transmitted by both *Lutzomyia longipalpis* and Lutzomyia *cruzi*[18]. In Bonito, the frequency of *Lutzomyia longipapis* was 90.4%, especially indoors and in areas surrounding homes, all year round. This species is thus believed to be the vector for VL in that region.

The presence of *Bicromomyia flaviscutelatta*, i.e. the main vector for *Leishmania (Leishmania) amazonensis,* indoors and in areas surrounding homes, is highly significant. This demonstrates gradual adaptation of this species to the human environment, in contrast with its previous restriction to wooded areas, as suggested by Oliveira et al[18] and Nunes et al[17] in studies conducted in Campo Grande, Mato Grosso do Sul, and in a studied carried out in Mato Grosso[27]. In Mato Grosso do Sul, *Leishmania amazonensis* has been reported to affect dogs, cats and humans[4 22 28].

Nyssomyia whitmani, i.e. the vector for *Leishmania (Viannia) braziliensis*, was the fifth most abundant species. It was present both indoors and in areas surrounding homes, which shows that it has become adapted to human-modified environments, as seen in other Brazilian regions[1] and in other studies in Mato Grosso do Sul[5 17 19 27].

The increasing environmental changes are bringing ACL and VL vectors closer to humans[4 17 19]. Capture of these insects in the Bonito region, in addition to findings of dogs that are seropositive for leishmaniasis, shows that these parasitic diseases are still being introduced into urban areas, thereby giving rise to new foci of these diseases. The results from the present study point towards a need for epidemiological surveillance to follow up the human occupation of the environment that is taking place in the Bonito region, especially because of its importance as a tourist center.

There also needs to be special training for tourist guides and for healthcare and educational workers, in order to make the community aware of the importance of environmental preservation and of the ways to avoid proliferation of phlebotomines, with the aim of decreasing leishmaniasis transmission in this region.

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