

American visceral leishmaniasis dissociated from *Lutzomyia longipalpis* (Diptera, Psychodidae) in the State of Espírito Santo, Brazil

Leishmaniose visceral americana dissociada da presença de *Lutzomyia longipalpis* (Diptera, Psychodidae) no Estado do Espírito Santo, Brasil

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Abstract

The occurrence of American visceral leishmaniasis in the State of Espírito Santo, Brazil has always been associated with the presence of the Lutzomyia longipalpis vector. The geographic distribution of this vector in this state is related to the presence of specific geoclimatic characteristics, such as a dry climate, low elevations (< 450m), steep slopes and rocky outcrops. The occurrence of human autochthonous cases of American visceral leishmaniasis in municipalities without these geoclimatic characteristics justifies the present study and our main goal was to test the association between the occurrence of American visceral leishmaniasis and the presence of the Lu. longipalpis. Sand flies were captured monthly from July 2006 to August 2007 using Shannon and CDC traps in two municipalities with records of autochthonous American visceral leishmaniasis and one with no record. We captured 13,112 sand flies, but no Lu. longipalpis was found. The absence of Lu. longipalpis and the possible role of another American visceral leishmaniasis vector in these localities were discussed.

Psychodidae; Vector Ecology; Leishmaniasis

Introduction

American visceral leishmaniasis is a zoonotic disease caused by the protozoan *Leishmania infantum chagasi* (Cunha & Chagas, 1937) (Kinetoplastida, Trypanosomatidae). Its transmission occurs as a result of the biting of female phlebotomine sand flies infected with the protozoan ¹.

Classic epidemiological studies indicate a strong association between *L. i. chagasi* and the sand fly *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera, Psychodidae) which is pointed out as the main vector of the disease. This correlation is based on the following factors: the occurrence of human and canine American visceral leishmaniasis cases associated with the geographic and ecological distribution of this sand fly species; finding of *Lu. longipalpis* naturally infected with *L. i. chagasi*; experimental infection of *Lu. longipalpis* with *L. i. chagasi*; attraction of *Lu. longipalpis* to *L. i. chagasi* reservoirs (dogs and foxes, mainly) and experimental transmission of *L. i. chagasi* by infected *Lu. longipalpis* ^{1,2,3,4,5,6,7,8,9}.

Some authors support the participation of other sand fly species in the transmission of American visceral leishmaniasis ¹⁰, based on their coincidental distribution with the disease. There are also those authors that defend the participation of other arthropods in American visceral leishmaniasis transmission, based on findings of natural infection in fleas ¹¹ and ticks ¹². However, none of these authors confirmed the capacity of

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these arthropods to transmit the disease through biting.

In Brazil, the first American visceral leishmaniasis cases were registered by Penna¹³ who found Leishmanias in 41 of 47,000 *post-mortem* viscerotomy exams. This author registered disease cases in the states of Alagoas, Bahia, Ceará, Pará, Pernambuco, Piauí, Rio de Janeiro, Rio Grande do Norte and Sergipe. At present, around 3,000 new cases of American visceral leishmaniasis are registered annually in Brazil, the majority associated with rural areas. However, urbanization processes of both the disease and the *Lu. longipalpis* vector in cities such as Belo Horizonte (Minas Gerais) and Campo Grande (Mato Grosso do Sul)^{14,15} have been occurring in the last years.

In Espírito Santo state, American visceral leishmaniasis was first registered by Martins et al.¹⁶, in the municipalities of Colatina and Baixo Guandu. At present, it is distributed in 10 municipalities, all located in the Center-North of the state. In all endemic areas, disease transmission was always associated with the presence of *Lu. longipalpis*.

Using the geoprocessing tool within the Geographical Information System, Feitoza et al.¹⁷ studied the geographic and climatic variables of endemic areas in Espírito Santo. The authors concluded that potential areas for the occurrence of American visceral leishmaniasis and *Lu. longipalpis* present characteristics such as sloping relief, elevation up to 450 meters above sea level, dry climate and rocky outcrops. Those characteristics always appear in the areas with registers of autochthonous American visceral leishmaniasis human cases, which also coincide with the presence of *Lu. longipalpis*. It is important to note that, until recently, the disease used to be registered only in the municipalities localized in the Rio Doce valley, in the Central-North region of Espírito Santo state. American visceral leishmaniasis had never been registered in municipalities localized in the South portion of the state.

In the last five years, however, three autochthonous disease cases were registered in the municipalities of Rio Novo do Sul and Vargem Alta, in the southern region of Espírito Santo state. Using the Polymerase Chain Reaction and isoenzymes techniques, the etiologic agent isolated from two patients was identified as *L. i. chagasi*. The epidemiological investigation showed no evidence of the introduction by immigrants, and also no evidence of the migration of ill people, or their relatives, to known endemic areas. From a geographic and climatic perspective, the area where new cases occurred is different from the

pattern found in endemic areas of the Rio Doce valley, in the Central-North region of the state¹⁷. Moreover, the Southern region of Espírito Santo is isolated from the endemic areas of American visceral leishmaniasis in the Rio Doce valley by geographical and climatic barriers that limit vector dispersion. Yet, the municipalities in the northern part of Rio de Janeiro state that border the municipalities in the South of Espírito Santo state did not register the occurrence of American visceral leishmaniasis cases nor the presence of *Lu. longipalpis* (unpublished data).

Considering that *Lu. longipalpis* was never found in collections carried out in the Southern portion of Espírito Santo state, the occurrence of American visceral leishmaniasis cases in this region justified the systematic studies of the sand fly fauna for vector identification. In this sense, the objective of this study was to test the association between the occurrence of American visceral leishmaniasis and the presence of *Lu. longipalpis* in the new localities that have registered the disease.

Material and methods

The study was performed in the Central-South region of Espírito Santo state in the localities of Belém district (20°47'29" S; 40°00'09" W) and Santo Antônio district (20°50'40" S; 40°56'21" W) that belong, respectively, to the municipalities of Vargem Alta and Rio Novo do Sul, where autochthonous human American visceral leishmaniasis cases had occurred. For the sand fly fauna comparison, Joebinha district (20°41'56" S; 40°47'41" W) in the municipality of Alfredo Chaves was also included in the study as it had registered autochthonous American tegumentary leishmaniasis cases.

The three locations present similar geo-climatic characteristics, as follows: humid climate influenced by the air currents coming from the Atlantic Ocean; annual pluviometer indices higher than 1,200mm; and sloping relief, with rare rocky outcrops¹⁷. The region contains small forest patches that are the remains of the Atlantic Rain Forest which was devastated to give room to coffee crops, and latter, in part, to banana crops and extensive cattle rearing.

In the period between August 2006 and July 2007, monthly sand fly collections were performed exploring: the modified environment, represented by the domiciles in which leishmaniasis cases had occurred, including their annexes and domestic animal shelters; and the preserved environment, represented by secondary forests next to the domiciles.

The captures were performed during the three initial nocturnal hours using active search in Shannon traps with the help of the Castro suction manual capturer in the modified environment and two CDC light traps installed in the preserved environment.

The collected specimens were placed in tubes containing ethanol (70%) and taken to the laboratory, where they were assembled according to the technique proposed by Barreto & Coutinho¹⁸ and identified according to the taxonomic criteria proposed by Galati^{19,20}. The sand flies' generic names were abbreviated as proposed by Marcondes²¹.

Using the PAST program (<http://folk.uio.no/ohammer/past/>)²², the following parameters were estimated in each environment: the specific richness (S), the equitability index (J) and the Shannon diversity index (H).

Results

A total of 13,112 sand fly specimens belonging to 15 species (H = 0.59) were collected (Table 1). No instance of *Lu. longipalpis*, the main American visceral leishmaniasis vector in the New World, was captured in the three locations under study.

The only species captured in all three localities were *Nyssomyia intermedia* (Lutz & Neiva, 1912) and *Micropygomyia schreiberi* (Martins, Falcão & Silva, 1975). Other species appeared in Belém district and Joebinha district including: *Lutzomyia alencari* (Martins, Souza & Falcão, 1962), *Micropygomyia quinquefer* (Dyar, 1929), *Migonemyia migonei* (França, 1920) and *Pintomyia fischeri* (Pinto, 1926). Yet some species were captured in just one locality: *Brumptomyia nitzulescui* (Costa Lima, 1932), *Evandromyia edwardsi* (Mangabeira, 1941), *Evandromyia termiophila* (Martins, Falcão & Silva, 1964), *Nyssomyia whitmani* (Antunes & Coutinho, 1939), *Pintomyia bianchigalatae* (Andrade-Filho, Aguiar, Dias & Falcão, 1999), *Pintomyia monticola* (Costa Lima, 1932), *Pintomyia serrana* (Damasceno & Arouck, 1949) and *Psathyromyia shannoni* (Dyar, 1929) in Belém district and *Sciopemyia sordellii* (Shannon & Del Ponte, 1927) in Joebinha district.

In Belém district (Vargem Alta), *Ny. intermedia* was the predominant species both in the preserved and in the modified environment. In the modified ambient, it was followed by *Mg. migonei* and *Pi. fischeri*. In the preserved environment though, it was followed by *Mi. schreiberi* and *Pi. fischeri*. In Santo Antônio district (Rio Novo do Sul), individuals belonging to only two species were captured in both environment types: *Mi. schreiberi* and *Ny. intermedia*. In this location,

Mi. schreiberi was the predominant species in the modified environment and *Ny. intermedia* the predominant species in the preserved environment. In Joebinha district (Alfredo Chaves), *Ny. intermedia* was the predominant species in the two environment types, followed by *Pi. fischeri*.

The specific richness (S) and the diversity index (H) were higher in Belém district, both in the modified (S = 13; H = 0.51) and in the preserved environments (S = 7; H = 1.23), when compared with the modified (S = 2; H = 0.52) and preserved (S = 2; H = 0.50) environments in Santo Antônio district and with the modified (S = 4; H = 0.21) and preserved (S = 6; H = 1.03) environments in Joebinha district. When the two environment types were compared, the specific richness was higher for the modified environment in Belém district and for the preserved environment in Joebinha district. In Santo Antônio district, the specific richness was the same for both environment types (Table 1). The diversity index was more than twice the value in the preserved environment compared with the modified environment in Belém district and more than four times the value in Joebinha district, while in Santo Antônio district they were similar (Table 1). In Joebinha district and in Belém district the equitability index was higher for the preserved environment when compared with the modified environment, while in Santo Antônio district the index was similar in both environment types (Table 1).

Discussion

The sand flies fauna found in the two American visceral leishmaniasis foci in the Central-South portion of Espírito Santo state presents a specific composition very different from that found in other transmission areas in Espírito Santo and in Brazil^{15,23}. However, this fauna is very similar to that found in typical areas of American tegumentary leishmaniasis transmission, in which the species *Ny. intermedia*, *Mg. migonei* and *Pi. fischeri* predominate, like in the locality of Joebinha (Alfredo Chaves) and in other regions in Espírito Santo and in Brazil^{24,25,26,27}.

The absence of *Lu. longipalpis* in two areas in the South portion of Espírito Santo that registered autochthonous American visceral leishmaniasis human cases suggests a dissociation of the disease occurrence from the presence of this insect in the state. Although in agreement with the geo processing model proposed by Feitoza et al.¹⁷, this absence is unexpected due to the fact that *Lu. longipalpis* is considered the only vector of the disease in the Southeastern region of Brazil²³.

Table 1

Sand flies collected in modified and preserved environments in the municipalities of Vargem Alta, Rio Novo do Sul and Alfredo Chaves, Espírito Santo State, Brazil, between August 2006 and July 2007.

Species	Vargem Alta (Belém district)						Rio Novo do Sul (Santo Antônio district)					
	Modified environment			Preserved environment			Modified environment			Preserved environment		
	♂	♀	Subtotal	♂	♀	Subtotal	♂	♀	Subtotal	♂	♀	Subtotal
<i>Brumptomyia nitzulescui</i>	1	-	1	-	-	-	-	-	-	-	-	-
<i>Brumptomyia</i> sp.	-	-	-	-	2	2	-	-	-	-	-	-
<i>Evandromyia edwardsi</i>	1	2	3	1	3	4	-	-	-	-	-	-
<i>Evandromyia termitophila</i>	1	-	1	-	-	-	-	-	-	-	-	-
<i>Lutzomyia alencari</i>	-	1	1	-	-	-	-	-	-	-	-	-
<i>Micropygomyia quinquefer</i>	1	-	1	1	2	3	-	-	-	-	-	-
<i>Micropygomyia schreiberi</i>	8	7	15	8	12	20	33	2	35	1	-	1
<i>Migonemyia migonei</i>	1,032	147	1,179	-	2	2	-	-	-	-	-	-
<i>Nyssomyia intermedia</i>	7,799	2,170	9,969	32	21	53	7	2	9	1	3	4
<i>Nyssomyia whitmani</i>	1	-	1	-	-	-	-	-	-	-	-	-
<i>Pintomyia bianchigalatae</i>	-	3	3	-	-	-	-	-	-	-	-	-
<i>Pintomyia fischeri</i>	346	443	789	-	5	5	-	-	-	-	-	-
<i>Pintomyia monticola</i>	-	1	1	-	-	-	-	-	-	-	-	-
<i>Pintomyia serrana</i>	-	1	1	-	-	-	-	-	-	-	-	-
<i>Psathyromyia shannoni</i>	1	1	2	-	-	-	-	-	-	-	-	-
<i>Sciopemyia sordellii</i>	-	1	1	-	-	-	-	-	-	-	-	-
Total	9,193	2,774	11,967	42	47	89	40	4	44	2	3	5
Specific richness (S)			13			7			2			2
Equitability index (J)			0.21			0.63			0.73			0.72
Diversity index (H)			0.57			1.23			0.52			0.50

(continues)

In an area of American visceral leishmaniasis in Pernambuco state in the Brazilian Northeastern region, Carvalho et al.¹⁰ pointed to *Mg. migonei* as a possible vector of *L. i. chagasi*, in spite of not having found females infected with *L. i. chagasi*. For identification of this insect as a vector, the authors took into account mainly their abundance in the intra and peri domiciliary environments of residences in which human cases had occurred. Yet, *Mg. migonei* presents an attraction for dogs²⁸, which can act as *L. i. chagasi* reservoirs. In this sense, the finding of this sand fly species in Belém district could explain the occurrence of American visceral leishmaniasis in this locality, but it would exclude Santo Antônio district, where the insect was not captured. Finally, *Mg. migonei* is found in many municipalities next to the American visceral leishmaniasis endemic areas, even within Espírito Santo state, where the disease was never registered. However, in the Brazilian Southeastern and Northeastern regions, *Mg. migonei* has been identified as a secondary vector of *Leishmania braziliensis*, an etio-

logic agent of American tegumentary leishmaniasis, including the finding of females naturally infected with this protozoan^{29,30}.

The finding of *Lu. alencari* in the locality of Belém district, although in low density, deserves more detailed studies to verify its possible participation in the American visceral leishmaniasis epidemiology in that locality, especially if one takes into account its phylogenetic proximity with *Lu. longipalpis*.

In Santo Antônio district, the transmission of the disease might not have occurred due to the sand flies biting since the two species found, *Mi. schreiberi* and *Ny. intermedia*, are not considered disease transmitters among human beings. The first species, *Mi. schreiberi*, possibly feeds on reptiles and it was never found biting human beings to get fed. The second species, *Ny. intermedia*, was never found naturally infected with *L. i. chagasi*, in spite of being anthropophilic, attracted by a domiciliary reservoir such as dogs and able to be experimentally infected. On the other hand, the evidence of the natural infection with *L. bra-*

Table 1 (continued)

Sand flies collected in modified and preserved environments in the municipalities of Vargem Alta, Rio Novo do Sul and Alfredo Chaves, Espírito Santo State, Brazil, between August 2006 and July 2007.

Species	Alfredo Chaves (Joebinha district)						Total		
	Modified environment			Preserved environment			♂	♀	Subtotal
	♂	♀	Subtotal	♂	♀	Subtotal			
<i>Brumptomyia nitzulescui</i>	-	-	-	-	-	-	1	-	1
<i>Brumptomyia</i> sp.	-	-	-	-	-	-	-	2	2
<i>Evandromyia edwardsi</i>	-	-	-	-	-	-	2	5	7
<i>Evandromyia termitophila</i>	-	-	-	-	-	-	1	-	1
<i>Lutzomyia alencari</i>	-	1	1	1	1	2	1	3	4
<i>Micropygomyia quinquefer</i>	-	-	-	3	3	6	5	5	10
<i>Micropygomyia schreiberi</i>	-	-	-	1	-	1	51	21	72
<i>Migonemyia migonei</i>	1	-	1	-	-	-	1,033	149	1,182
<i>Nyssomyia intermedia</i>	872	30	902	24	13	37	8,735	2,239	10,974
<i>Nyssomyia whitmani</i>	-	-	-	-	-	-	1	-	1
<i>Pintomyia bianchigalatae</i>	-	-	-	-	-	-	-	3	3
<i>Pintomyia fischeri</i>	40	9	49	4	3	7	390	460	850
<i>Pintomyia monticola</i>	-	-	-	-	-	-	-	1	1
<i>Pintomyia serrana</i>	-	-	-	-	-	-	-	1	1
<i>Psathyromyia shannoni</i>	-	-	-	-	-	-	1	1	2
<i>Sciopemyia sordellii</i>	-	-	-	-	-	-	-	1	1
Total	913	40	953	33	21	54	10,221	2,891	13,112
Specific richness (S)			4			6			15
Equitability index (J)			0.15			0,71			0.21
Diversity index (H)			0.21			1.03			0.59

ziliensis together with the insect anthropophily, the coincidental distribution with American tegumentary leishmaniasis and the attraction for reservoir animals, leads us to believe that this species could be considered the main disease transmitter in the Southeastern region of Brazil^{25,27,28}.

It is possible that fleas can participate in the transmission of American visceral leishmaniasis in Santo Antônio district since infection by *L. i. chagasi* was detected in females of *Ctenocephalides felis felis* (Bouché, 1835) by means of kDNA-PCR (unpublished data). However, the sensibility of the molecular technique can allow the detection of the parasite DNA in the blood ingested by the fleas of an infected dog and therefore it does not prove that these parasites can install themselves in the flea's digestive tract to be transmitted to other dogs or to human beings.

The fauna found both in Belém district and in Santo Antônio district were similar to those found in Joebinha district. The predominance of *Ny. intermedia* shows the potential for American tegumentary leishmaniasis transmission in these areas, but does not prove American visceral leish-

maniasis transmission in the region. Yet, the finding of *Pi. fischeri* in the localities of Belém district and Joebinha district supports the fact that the fauna has a pattern typical of that found in an American tegumentary leishmaniasis transmission area, since the species, like *Mg. migonei*, is incriminated as a secondary vector of the disease.

The fact that the species richness and diversity index are higher in Belém district may be related to the size of the forest fragment and the composition of the vegetation around the fragment in the preserved environment. In this location, the forest fragments are bigger and located next to banana trees, unlike in the other locations that contain smaller fragments that are next to cattle rearing areas. On the other hand, the higher species richness for the modified environment in Belém district can be related with the proximity of residences to the forest and with the presence of food sources for chicken and pigs. The diversity index and the equitability index, as expected, were higher in the preserved environment in Belém and Joebinha districts. Possibly, the forest

represents a system in equilibrium, with smaller variations of temperature and humidity, favoring the development of sand flies.

In conclusion, the present study dissociates the occurrence of autochthonous American visceral leishmaniasis human cases from the presence of *Lu. longipalpis*, as this insect is not found in two foci of the disease in the southern part of Espírito Santo state. Moreover, the study contributes to the sand flies fauna knowledge in the region and points out the possibility of the participation of other sand fly species, apart from *Lu. longipalpis*, in the transmission of American

visceral leishmaniasis in these locations. Considering the absence of sand flies suspected of transmitting the American visceral leishmaniasis in Santo Antônio district and the low frequency of the two species collected in these locales, it is possible that other vectors or other transmission mechanisms occur in the region. However, additional studies that reinforce this hypothesis must be developed, including the investigation of natural infection in sand flies and the investigation of possible *L. i. chagasi* vertebrate reservoirs in these localities in both domestic and sylvatic environments.

Resumo

A ocorrência de leishmaniose visceral americana no Estado do Espírito Santo, Brasil, sempre foi associada à presença do vetor Lutzomyia longipalpis. A distribuição geográfica desse vetor no estado está relacionada à ocorrência de características geoclimáticas específicas, tais como clima seco, baixas altitudes, relevo acidentado e afloramentos rochosos. A ocorrência de casos humanos autóctones de leishmaniose visceral americana em municípios sem essas características geoclimáticas justificou o presente estudo, com objetivo de testar a associação entre a ocorrência de leishmaniose visceral americana e a presença de Lu. longipalpis. Flebotomí-

neos foram capturados mensalmente de julho de 2006 a agosto de 2007 com uso de armadilhas de Shannon e CDC em dois municípios com registros de leishmaniose visceral americana autóctone e um sem registros. Foram capturados 13.112 flebotomíneos, mas nenhum Lu. longipalpis foi encontrado. A ausência de Lu. longipalpis e a possível participação de outro vetor de leishmaniose visceral americana nessas localidades foram discutidas.

Psychodidae; Ecologia de Vetores; Leishmaniose

Contributors

I. S. Pinto participated in the collection of the sand flies, species identification, data analysis and interpretation and the writing up of the article. C. B. Santos and A. L. Ferreira participated in the sand fly collection, species identification and writing up the article. G. Grimaldi Jr. writing up the article and was responsible for the *leishmania* species identification. A. Falqueto participated in the sand fly collection, writing up the article and was responsible for coordinating the study.

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