Detection of Leishmania infantum DNA in Pintomyia evansi and Lutzomyia longipalpis in Honduras

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Abstract

Background: The two most abundant sand fly species on the Honduran Pacific coast are Lutzomyia (Lutzomyia) longipalpis and Pintomyia (Pifanomyia) evansi. Both species are known vectors of Leishmania (Leishmania) infantum, the etiological agent of visceral leishmaniasis (VL) in the Americas. Although VL and non-ulcerative cutaneous leishmaniasis (NUCL) are endemic on the Pacific versant of the Central American Pacific, the latter is the most frequent manifestation of leishmaniasis there. We evaluated the circulation of Leishmania spp. in the sand fly species on El Tigre Island, an endemic area of NUCL.

Results: We collected 222 specimens of six sand fly species. Lu. longipalpis (180 specimens; 81%) and Pif. (Pi.) evansi (35 specimens; 16%) were the most abundant species. L. (L.) infantum DNA was detected in nine of the 96 specimens analyzed; seven of these specimens were identified as Lu. longipalpis, and the remaining two were Pi. evansi, with an infection rate of 9.4% and 2.7%, respectively.

Conclusion: We present the first record of L. (L.) infantum DNA in Pi. evansi from a NUCL endemic region of Central America. Our results suggest that Pi. evansi could be a secondary vector of L. (L.) infantum in the transmission cycle of leishmaniasis. The detection of natural infections of L. (L.) infantum in sand flies in this region contributes to an understanding of the epidemiology of leishmaniasis in Honduras.

Keywords: Leishmania (Leishmania) infantum, Pintomyia (Pifanomyia) evansi, Lutzomyia (Lutzomyia) longipalpis, Visceral leishmaniasis, Non-ulcerative cutaneous leishmaniasis

Background

Leishmaniasis is a vector-borne parasitic disease caused by species of the genus Leishmania (Kinetoplastida: Trypanosomatidae). Leishmania is widespread mainly in tropical and subtropical regions of 98 countries throughout Europe, Africa, Asia, and America [1]. More than 1,000 sand fly species have been identified worldwide, of which 530 species are present in the Americas [2, 3], and at least 30 species are considered Leishmania vectors [4]. In Honduras, 29 sand fly species have been reported [2, 5–7]. Members of the Lutzomyia longipalpis species complex are the main vectors of Leishmania (Leishmania) infantum; however, other sand fly species, including Lutzomyia (Lutzomyia) cruzi and Pintomyia (Pifanomyia) evansi, play a role as vectors of this parasite in some endemic areas of visceral leishmaniasis (VL) in South and Central America [8, 9]. Lu. longipalpis s.l. is the most abundant sand fly species in the area endemic for VL and non-ulcerative cutaneous leishmaniasis.
Methods

Study area and sand fly collection
This study was carried out in Amapala municipality (13°17′26.082″N, 87°39′5.543″W), Valle department, an area covering 80.7 km². The municipality comprises two islands, Zacate Grande and El Tigre, located in the Gulf of Fonseca. Sand flies were sampled for five consecutive nights in May 2018 from five localities: Las Pelonas (13°16′58.332″N, 87°36′.4.799″W), Punta Honda (13°16′21.576″N, 87°36′56.879″W), Tiguilotada (13°15′45.720″N, 87°36′57.527″W), Islitas (13°15′39.456″N, 87°37′24.276″W) and Playa Grande (13°16′4.183″N, 87°39′31.104″W). The captures were carried out from 6:00 p.m. until 6:00 a.m., using automatic CDC miniature light traps (model 512; John W. Hock, Gainesville, FL) in neighborhoods where active cases of NUCL were evident. The traps were installed in peridomestic environments, near decomposing organic matter or next to latrines. The specimens were separated and processed 1 day after capture.

Taxonomic identification of the sand fly species
The taxonomy of the phlebotomine sand flies was conducted by analyzing their morphological characteristics. In adherence to the identification procedures outlined by Young and Mejia et al. [6], the sand flies were first mounted and the specific species identified in accordance with Young and Duncan [5]. Finally, Galati et al. [13] were the primary academic resource used during the classification stage of the genera and the species.

Genomic material extraction and polymerase chain reaction
Genomic DNA was extracted from gut tissues dissected from individual female sand flies using the Chelex 100 Kit (Bio-Rad, Hercules, CA). As an internal control for the DNA extraction, the cacophony antisense (5′ TCC TTC GAC GCC TCC TGG TTG 3′) and hsp70 antisense (5′ TCC TTT CGA GCG CAT GGT 3′) were used, 5 µl of amplified DNA was added to the reaction and the mixture incubated at 37 °C for 3 h. The species profiles of each sample and reference controls were observed in 4% agarose gel. To perform the restriction of the PCR products, the restriction enzyme Hae III (Promega) was used, 5 µl of amplified DNA was added to the reaction and the mixture incubated at 37 °C for 3 h. The species profiles of each sample and reference controls were observed in 4% agarose gel subjected to electrophoresis for 3.5 h.

Results and discussion
A total of 222 sand fly specimens were collected, which were predominately males (66%) (Table 1). Six species were identified by using morphological characters. The most predominant sand fly species collected was Lu. (Lu.) longipalpis, followed by Pif. (Pif.) evansi. The other species that were captured included Micropygomyia (Micropygomyia) cayennensis cayennensis, Micropygomyia (Coromyia) Dampfomyia evansi, Dampfomyia (Coromyia) beltrani and Lutzomyia (Tricholateris) gomezi. Lu. (Lu.) longipalpis has been previously studied in the region and was incriminated as the vector of L. (L.) infantum [11]. The behavioral characteristics of this species in the study area were described by Carrasco et al. [7]. Recently, Mejia et al. [6] expounded on various aspects of sand flies’ feeding preferences within the Pacific Honduran area. These authors observed a predominance of Pif. (Pif.) evansi and Lu. (Lu.) longipalpis, but did not detect the presence of L. (L.) infantum in these species [6]. These sand flies were also predominant in other NUCL-endemic areas of Central America (i.e., Costa Rica and Nicaragua) [17, 18].
Thirty-seven of the 96 analyzed female specimens were positive for the genus *Leishmania* according to the PCR results (Fig. 1). *L. (L.) infantum* DNA was revealed in nine specimens: seven *Lu.* (*Lu.*) longipalpis and two *Pi.* (*Pi.*) evansi. The *L. (L.) infantum* infection rate was 9.4% for *Lu.* (*Lu.*) longipalpis and 2.7% for *Pi.* (*Pi.*) evansi. All of the samples analyzed produced an amplified product of 220 bp, corresponding to a *Lutzomyia* spp. constitutive gene (cacophony), which confirmed the integrity of the insect DNA preparation and the absence of PCR inhibitors [14]. We used a method for the detection of *Leishmania* spp. described in Francino et al. [15]. However, one limitation of this method is the small size of the PCR product (120 bp), which makes sequencing unlikely. Therefore, the method described by Graça et al. [16] was used to differentiate *Leishmania* species. This method amplifies a *Leishmania* 234-bp *hsp70* fragment and shows similar sensitivity to the PCR-internal transcribed spacer 1 (>70%) method used to detect *Leishmania* DNA; however, associating this 234-bp *hps70* with a RFLP protocol may give researchers the advantage of being able to distinguish this *Leishmania* species by electrophoresis [19].

Our study is the first to report the presence of *L. (L.) infantum* DNA in *Pi. evansi* females in Central America. In two studies in Colombia, in endemic areas of VL, the natural infection rate of *L. (L.) infantum* in *Pi. evansi* was found to be 0.10% [12] and 0.34% [19]. The natural infection rates of *Lu. longipalpis* were between 0.5% and 1.1% according to direct observations of dissected intestines [11, 20] and from PCR of dissected intestines [21–23]. We report a 9.4% infection rate of *L. (L.) infantum* in *Lu. longipalpis*, which is in agreement with other research [22, 23]. Although the detection of *Leishmania* DNA in sand flies does not indicate the ability of these species to transmit this parasite, we evidenced contact between *Pi. evansi* and *Lu. longipalpis* with the natural host of *L. (L.) infantum* in the study area, in the Amapala municipality.

**Table 1** Sand fly species captured, by locality, and detection of *Leishmania* spp. and *Leishmania (Leishmania) infantum*

| Locality (sand flies; n) | Species                                      | Males (n) | Females (n) | Specimens with *Leishmania* spp. DNA (n) (%) | Females with *L. (L.) infantum* DNA (n) (%) |
|-------------------------|----------------------------------------------|-----------|-------------|---------------------------------------------|---------------------------------------------|
| Playa Grande (175)      | *Lutzomyia (Lutzomyia) longipalpis*          | 95        | 35          | 79.2 (26 (35.13))                           | 7 (9.45)                                   |
|                         | *Pintomyia (Pifanomyia) evansi*              | 10        | 24          | 20.8 (11 (14.8))                           | 2 (2.70)                                   |
| Las Pelonas (16)        | *Lutzomyia (Lutzomyia) longipalpis*          | 12        | 2           | 87.5 –                                     | –                                          |
|                         | *Micropygomyia (Micropygomyia) cayennensis cayennensis* | 1         | 1           | 12.5 –                                     | –                                          |
| Punta Honda (29)        | *Lutzomyia (Lutzomyia) longipalpis*          | 24        | 4           | 96.5 –                                     | –                                          |
|                         | *Micropygomyia (Coquillettdimaya) chiapanensis* | 0         | 1           | 3.5 –                                      | –                                          |
| Islitas (13)            | *Lutzomyia (Lutzomyia) longipalpis*          | 6         | 2           | 61.5 –                                     | –                                          |
|                         | *Dampfomyia (Coromyia) beltrani*             | 0         | 2           | 15.4 –                                     | –                                          |
|                         | *Pintomyia (Pifanomyia) evansi*              | 0         | 1           | 7.7 –                                      | –                                          |
|                         | *Micropygomyia (Micropygomyia) cayennensis cayennensis* | 0         | 1           | 7.7 –                                      | –                                          |
|                         | *Lutzomyia (Tricholateralis) gamezi*         | 0         | 1           | 7.7 –                                      | –                                          |

![Fig. 1](image1.png) Polymerase chain reaction (PCR) to determine *Leishmania* spp. infection using Leish1-Leish2 primers to target conserved DNA regions of the kinetoplast DNA from *Leishmania* spp. (120 base pairs (bp)). Lane M Molecular weight marker (100-bp DNA ladder). Lanes 1–16 Female sand fly DNA (lanes 1-10 *Lutzomyia (Lutzomyia) longipalpis*, positive female; lanes 11, 13–16 *Pintomyia (Pifanomyia) evansi*, positive female). Lane 17 PCR positive control (DNA extracted from a mixture of the male insect pool containing *Leishmania (Leishmania) infantum* DNA). Lane 18 Amplification reaction without added DNA (PCR negative control).
Considering that the vector competence of *Pi. evansi* has been previously described [12], it is probable that both *Lu. longipalpis* and *Pi. evansi* transmit *L. (L.) infantum* in southern Honduras.

**Conclusion**

We present for the first time evidence of the presence of *L. (L.) infantum* DNA in *Pi. evansi* in a NUCL endemic region of Central America. Considering the natural infection of *Lu. longipalpis* by *L. (L.) infantum*, our results indicate that *Pi. evansi* might be a secondary vector of this parasite, and might be involved in the transmission cycle of leishmaniasis. The detection of natural infections of *L. (L.) infantum* in sand flies in this region contributes to our understanding of the epidemiology of leishmaniasis in Honduras.

**References**

1. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. WHO Leishmaniasis Control Team. Leishmaniasis worldwide and global estimates of its incidence. PLos ONE. 2012;7:e35677.
2. Shimabukuro PHF, De Andrade AJ, Galati EAB. Checklist of American sand flies (Diptera, Psychodidae, Phlebotominae): genera, species, and their distribution. ZooKeys. 2017;660:67–106.
3. Galati EAB, Galvis-Ovallos F, Lawyer P, Léger N, Depaquit J. An illustrated guide for characters and terminology used in descriptions of Phlebotominae (Diptera, Psychodidae). Parasite. 2017;24:26.
4. Beati L, Caceres AG, Lee JA, Munstermann LE. Systematic relationships among *Lutzomyia* sand flies (Diptera: Psychodidae) of Peru and Colombia based on the analysis of 12S and 28S ribosomal DNA sequences. Int J Parasitol. 2004;34(2):225–34.
5. Young DG, Duncan MA. Guide to the identification and geographic distribution of *Lutzomyia* sand flies in Mexico, the West Indies, Central and South America (Diptera: Psychodidae) Gainesville. Mem Am Entomol Inst. 1994;5:1–881.
6. Mejía A, Matamoros G, Fontecha G, Sosa-Ochoa W. Bionomic aspects of *Lutzomyia evansi* and *Lutzomyia longipalpis*, proven vectors of *Leishmania infantum* in an endemic area of non-ulcerative cutaneous leishmaniasis in Honduras. Parasites Vectors. 2018;11(1):15.
7. Carrasco J, Morrison A, Ponce C. Behaviour of *Lutzomyia longipalpis* in an area of southern Honduras endemic for visceral/atypical cutaneous leishmaniasis. Ann Trop Med Parasitol. 1998;92(8):869–76.
8. Lainsen R, Rangel EF. *Lutzomyia longipalpis* and the eco-epidemiology of American visceral leishmaniasis with particular reference to Brazil: a review. Mem Inst Oswaldo Cruz. 2005;100(8):811–27.
9. Chaves LF, Anez N. Nesting patterns of sand fly (Diptera: Psychodidae) species in a Neotropical semi-arid environment. Acta Trop. 2016;153:7–13.
10. Sosa-Ochoa W, Morales Cortesano X, Arguello A, Zuniga C, Hernandez J, Mejia R, et al. Ecopathology of the Leishmania cutaneous no ulcerada in Honduras. Rev Cienc Tecnol UNAH. 2014;4:115–28.
11. Ponce C, Ponce E, Morrison A, Cruz A, Kretzner R, McMahon-Pratt D, et al. Leishmania donovani chagasi: new clinical variant of cutaneous leishmaniasis in Honduras. Lancet. 1991;337(8733):67–70.
12. Travi BL, Vilez ID, Brutus L, Segura I, Jaraillo C, Montoya J. *Lutzomyia evansi*, an alternate vector of *Leishmania chagasi* in a Colombian focus of visceral leishmaniasis. Trans R Soc Trop Med Hyg. 1990;84(5):676–7.
13. Galati EAB. Morphologia e terminologia de Phlebotominae (Diptera: Psychodidae). Classificação e identificação de táxons das Américas. Vol I. Apostila da Disciplina Biologia e Identificação de Phlebotominae do Programa de Pós-Graduação em Saúde Pública. Faculdade de Saúde Pública da Universidade de São Paulo. 2019. https://www.fsp.usp.br/egalati/wpcontent/uploads/2020/02/Apostila_Vol_I_2019.pdf. Accessed 18 May 2019.
14. Pita-Pereira D, Souza GD, Zواتech A, Alves CR, Britto C, Rangel EF. First report of *Lutzomyia (Nyssomyia) neivai* (Diptera: Psychodidae: Phlebotominae) naturally infected by *Leishmania (Viannia) braziliensis* in a periurban area of south Brazil using a multiplex polymerase chain reaction assay. Am J Trop Med Hyg. 2009;80(4):593–5.
15. Francino O, Alet T, Sánchez-Robert E, Rodríguez A, Solano-Gallego L, Alberola J, et al. Advantages of real-time PCR assay for diagnosis and monitoring of canine leishmaniasis. Vet Parasitol. 2006;137:214–21.
16. Graça GC, Volpini AC, Romero GAS, Oliveira Neto MP, Huelb M, Porroozii R, et al. Development and validation of PCR-based assays for diagnosis of American cutaneous leishmaniasis and identification of the parasite species. Mem Inst Oswaldo Cruz. 2012;107:664–74.
17. Zeledón R, Murillo J, Gutiérrez H. Observaciones sobre la ecología de *Lutzomyia longipalpis* (Lutz & Neiva, 1912) y posibilidades de existencia de leishmaniasis visceral en Costa Rica. Mem Inst Oswaldo Cruz. 1984;79(4):455–9.
18. Raymond RW, McHugh CP, Kerr SF. Sand flies of Nicaragua: a checklist and reports of new collections. Mem Inst Oswaldo Cruz. 2010;105(7):889–94.
19. Travi BL, Montoya J, Gallego J, Jaramillo C, Llanos R, Velez ID. Bionomics of *Lutzomyia evansi* (Diptera: Psychodidae) vector of visceral leishmaniasis in northern Colombia. J Med Entomol. 1996;33(3):278–85.
20. Silva JGD, Werneck GL, Cruz MSP, Costa CHN, Mendonça IL. Infección natural de *Lutzomyia longipalpis* por *Leishmania* sp. en Teresina, Piauí Brasil. Cad Saúde Pública. 2007;23(7):1715–20.
21. Flórez M, Martínez JP, Gutiérrez R, Luna KP, Serrano VH, Ferro C, et al. *Lutzomyia longipalpis* (Diptera: Psychodidae) en un foco suburbano de leishmaniosis visceral en el Cañón del Chicamocha en Santander Colombia. Biomédica. 2006;26(Supl 1):109–20.

22. Michalsky EM, Guedes KS, França-Silva JC, Dias CLF, Barata RA, Dias ES. Infeccao natural de *Lutzomyia* (Lutzomyia) longipalpis (Diptera: Psychodidae) por *Leishmania infantum chagasi* em flebotomineos capturados no municipio de Janaúba, Estado de Minas Gerais Brasil. Rev Soc Bras Med Trop. 2011;44(1):58–62.

23. Mota TF, de Sousa OMF, Silva YJ, Borja LS, Leite BMM, Solcà MDS, et al. Natural infection by *Leishmania infantum* in the *Lutzomyia longipalpis* population of an endemic coastal area to visceral leishmaniasis in Brazil is not associated with bioclimatic factors. PLoS Negl Trop Dis. 2016;13:e0007626.

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