An Atypical Case of Autochthonous Cutaneous Leishmaniasis Associated with Naturally Infected Phlebotomine Sand Flies in Texas, United States

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Abstract. In the United States, phlebotomine sand flies carrying Leishmania (Leishmania) mexicana are endemic along the southern border. However, relatively little is known about the enzootic and zoonotic transmission of L. (L.) mexicana within the United States, and autochthonous cases of the consequent disease are rarely reported. We investigated an atypical case of cutaneous leishmaniasis (CL) caused by L. (L.) mexicana in a patient from central Texas which did not respond to a typical antileishmanial chemotherapy. We also investigated sand fly vectors around the patient’s residence. PCR followed by DNA sequencing was used for determination of Leishmania spp., sand fly species, and host blood meal source. The L. (L.) mexicana genotype from the patient was identical to one found in a positive sand fly. Moreover, this genotype presented the same single-nucleotide polymorphisms as other historical CL cases acquired in Texas over the last 10 years, but distinct from those originating in Mexico and Central America. Three sand fly species were identified among the samples analyzed (n = 194), the majority of which were Lutzomyia (Dampfomyia) anthophora (n = 190), of which four specimens tested positive for Leishmania and two blood-fed specimens showed the presence of a human blood meal. This study highlights the complexity of clinical management of CL in a setting where the disease is infrequently encountered. The detection of human blood in Lu. (D.) anthophora is the first documentation of anthropophagy in this species. This is the first report of wild-caught, naturally infected sand flies found in association with an autochthonous case of human leishmaniasis and the specific strain of Leishmania (Leishmania) mexicana in the United States.

Human leishmaniasis is a parasitic disease caused by more than 20 species of protozoa in the genus Leishmania, subgenera Leishmania and Viannia.1–3 The parasites are transmitted through the bite of an infected female sand fly vector, primarily in tropical and subtropical regions. Among several clinical manifestations, cutaneous leishmaniasis (CL) is the most common form of the disease. Traditionally, the clinical presentation of CL is characterized by the eruption of a chronic crateriform ulcer near the sand fly bite wound; however, the disease may present with a greater diversity of dermatologic manifestations, ranging from small and localized skin lesions to large nodules covering multiple body surfaces.4–6 Management of CL is often challenging because of its variable presentation, chronic progression, and the fact that available antiprotozoal therapies are limited in efficacy and are associated with toxic side effects.6 Identification of the infecting species is not only important for clinical case management but also for epidemiological purposes.

In the United States, autochthonous cases of human CL are infrequently reported. A retrospective review identified 29 cases of autochthonous CL in the United States between 1903 and 1996, all of which occurred in the state of Texas and were either identified or suspected as caused by L. (L.) mexicana.7 Over the last two decades, cases of autochthonous CL have been reported with greater frequency, while concurrently expanding in geographic range across a larger portion of the state, ultimately extending from southern Texas into southeastern Oklahoma.8,9 Recently, Mcllwae et al.10 identified an additional group of novel cases from Texas, bringing the historical total of suspected autochthonous CL cases reported from the United States to approximately 80. It is unknown whether this increased incidence and geographical range has been due to expansion of parasite, vector, or reservoir populations; increased awareness among providers; or a combination of those factors.9–11 In an effort to capture epidemiologic and clinical data on emerging cases, the Texas Department of State Health Services made leishmaniasis a notifiable condition in 2007, requiring providers to report suspect cases.

Several species of sand flies in the genus Lutzomyia have been associated with the zoonotic/enzootic transmission of L. (L.) mexicana in North America, including Lutzomyia (Dampfomyia) anthophora, Lutzomyia (Tricholateralis) cruciata, Lutzomyia (Tricholateralis) diabolica, Lutzomyia (Psathyromyia) shannoni, and Lutzomyia (Psathyromyia) texana.12–14 The transmission dynamics of L. (L.) mexicana within the United States are incompletely understood, but enzootic maintenance of the parasite is thought to involve the sand fly Lu. (D.) anthophora and rodent reservoirs of the genus Neotoma.13,15–19 Despite serving as an enzootic vector, it is unclear whether Lu. (D.) anthophora is capable of directly transmitting L. (L.) mexicana to humans. Laboratory studies have shown that female Lu. (D.) anthophora sand flies are reluctant human biters, although they readily feed from a variety of rodent and small mammal species.20–22 Previous documentation of anthropophagy in U.S. sand flies appears limited to two species, Lu. (T.) diabolica and Lu. (P.) shannoni.23,24

Here, we report on a patient who presented with chronic nodular skin lesions caused by L. (L.) mexicana. Our public health investigation suggested that the patient’s exposure occurred on his residential farm in central Texas; therefore, a survey for phlebotomine sand fly vectors was undertaken. Both clinical samples and field-collected sand flies were sent to the U.S. CDC for detection of Leishmania spp. and
identification of vector species. The sequence of DNA fragment amplified from the clinical and vector samples were compared with \( L. (L.) \) mexicana sequences from clinical cases from Texas and from Central American countries (including Mexico) obtained by CDC during the last 10 years.

**METHODS**

**The patient.** The patient is a 67-year-old Caucasian male from Caldwell County, Texas, who presented to his primary care physician in September 2016 with multiple hypertrophic papules. The lesions were first noticed in April 2016 and were confined to the anterior surface of the right leg, between the tibial tuberosity and ankle. Before onset, the patient was healthy and without known chronic medical conditions or underlying immunodeficiency. In the weeks and months preceding appearance of the lesions, the patient frequently spent time outdoors at his rural residence in central Texas and had taken several hiking trips to parks across the state. He did not recall any recent arthropod contact, although he attributed the lesions to “insect bites” when they first appeared. His international travel history was unremarkable, although he did describe travel to central Mexico approximately 10 years earlier.

Following initial presentation, the lesions grew in size and quantity, with many evolving into well-circumscribed nodules (Figure 1). No lymphadenopathy was noted, and the lesions were described as painless and non-pruritic. Histopathologic examination of punch biopsy specimens in October 2016 revealed protozoa consistent with \( Leishmania \) amastigotes (Cockerell Dermatopathology, Dallas, TX) (Figure 2). The patient returned to his primary provider for care in January 2017 and was prescribed a 42-day course of oral fluconazole (200 mg/day). In May 2017, he reported worsening of the lesions, and second punch biopsy was taken from the margin of one lesion and a fine-needle aspirate was collected from the lesional tissue. Clinical specimens were sent to the CDC for diagnostic confirmation and \( Leishmania \) identification. Portions of clinical samples were incubated at 25°C in Novy–MacNeal–Nicolle (NNN) medium agar with 10% defibrinated rabbit blood and an overlay of Roswell Park Memorial Institute medium with 15% fetal bovine serum and were cultured for 1 week.

On confirming the diagnosis of CL by PCR followed by sequencing analysis, the patient was given two consecutive 28-day courses of oral miltefosine (150 mg/day) beginning in June 2017, followed by a 28-day course of oral ketoconazole (600 mg/day) in November 2017. By the end of 2017, the lesions had not resolved, and two consecutive 42-day courses of oral

**FIGURE 1.** Anterior surface of case-patient’s right lower extremity showing cutaneous nodules and plaques caused by \( Leishmania \) (\( Leishmania \)) mexicana. Largest lesion shown on the left image measures approximately 4.0 by 4.5 cm (June 2017, approximately 14 months after onset). This figure appears in color at www.ajtmh.org.

**FIGURE 2.** Hematoxylin and eosin–stained histologic section of clinical tissue demonstrating numerous \( Leishmania \) amastigotes (black arrows), Cockerell Dermatopathology, Dallas, Texas. This figure appears in color at www.ajtmh.org.
ketoconazole (600 mg/day) were prescribed, followed by an additional 28-day course of oral miltefosine (150 mg/day) in April 2018. All therapies were well tolerated with no significant side effects; however, at the end of our follow-up period in June 2018, approximately 26 months after initial onset of symptoms, the patient’s lesions remained refractory to treatment.

Field collections. To determine if it could be a case of autochthonous transmission, sand flies were captured over two consecutive nights on the patient’s property in early August 2017 using CDC miniature light traps (John W. Hock Company, Gainesville, FL) baited with dry ice. Light traps were deployed overnight, placed within roughly 100 m of the patient’s home, and were positioned outside of an unused barn, above animal burrows, and adjacent to piles of brush (Figure 3).

MOLECULAR ANALYSIS

Detection and identification of Leishmania species by PCR and DNA sequencing. DNA extraction from clinical and cultured specimens and from 138 female sand fly samples (80 residual bodies and 58 intact sand flies) was performed using DNeasy blood and tissue kit and QIAamp DNA Micro Kit (Qiagen), respectively, following the manufacturer’s instructions. The presence of Leishmania spp. in clinical specimen, culture, and vectors was investigated using the current CDC reference leishmaniasis diagnostic approach.25,26 Extracted DNA was tested using a conventional PCR targeting Leishmania spp. rRNA-internal transcribed spacer 2 region (ITS2-PCR) followed by DNA sequence analysis for species discrimination.26 and a SYBR Green qPCR targeting the rRNA-ITS1 region (SG-ITS1-qPCR), which allows the separation of three species, with melting temperature (Tm) values, that is, G1 (Viannia subgenus species), G2 (L. donovani, L. infantum, and L. (L.) tropica), and G3 (L. (L.) amazonensis, L. (L.) mexicana, L. (L.) major, and L. (L.) aethiopica).25

Sand fly species identification and blood meal analysis. Sand fly species determination (n = 205) was undertaken through morphological and/or molecular identification. Sand flies were examined individually under a stereomicroscope, and 80 individual specimens were dissected by separating the head, one wing, and the terminal segments of the abdomen, which included the genitalia. The removed parts were examined under a microscope and identified following the keys of Young and Perkins.27

Molecular ID was carried out in 153 specimens targeting a 650–700 bp DNA fragment of the mitochondrial cytochrome c oxidase subunit I (COI-barcode) with primers HCO and LCO.28 For the species not yet available in GenBank, reference sequences were established by morphological validation of the haplotype detected. Blood meal source of six blood-engorged female sand flies was investigated using a hemi-Nested PCR amplification targeting a 450-bp region of the 16S rRNA gene.29

PCR product sequencing. PCR products were subjected to direct DNA Sanger sequencing method. Cycle sequencing reactions were prepared with the BigDye Terminator v. 1 or v. 3.1 kits (Applied Biosystems, Waltham, MA) in both forward and reverse directions, using an ABI 3500 or ABI 3130XL ABI Prism Genetic Analyzer (Applied Biosystems) automated sequencer. Sequences were assembled and reconciled using Lasergene Seqman Pro (DNASTAR, INC., Madison, WI). Comparison analyses for species identification were performed using Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information, Washington, DC). The criteria for species identification were similarities of 98% or higher.

RESULTS

Leishmania identification. The ITS2-PCR was prepared using clinical specimen, and culture DNA yielded fragments of approximately 420 bp; the consensus sequences showed 99% similarity with L. (L.) mexicana when compared with the GenBank database (accession numbers: FJ948434 and AF466383). The ITS2 sequences obtained in this study were compared with other L. (L.) mexicana sequences from 123 clinical CL cases in which infection was confirmed over the last 10 years at the CDC. Of these cases, 38 originated in Texas, 78 were from Mexico and Central America, and 7 were of unknown origin. Sequencing of ITS2 fragments identified two possible distinct L. (L.) mexicana genotypes associated with cases of Texas or Central American origin. Using accession number FJ948434 as reference, single-nucleotide polymorphisms (SNPs) C647 and C649 were detected in 95% (36/38) of Texas cases (including this study’s case), whereas SNPs A647 and T649 were detected in 97% (76/78) of isolates from Central American cases (Figure 4). Among four discordant instances, one patient from Texas, infected with SNPs A647 and T649, was apparently infected in Mexico. We could not resolve the other three cases because details of the travel histories were not available. The infection with L. (L.) mexicana was also presumptively identified on the basis of Tm values (81.5°C) of fragments amplified using SYBR Green qPCR. Leishmania spp. promastigotes were observed in the NNN culture after 1 week of incubation.

Molecular analysis of DNA aliquots from 138 female sand flies yielded four insects positive by the SG-ITS1-qPCR test, with an average Tm of 81.75°C, corresponding to group G3. One positive insect was also positive by ITS2-cPCR with a consensus sequence identical to the clinical case sequence (genotype C647/C640). The other three cases in

![Figure 3. Representative placement of a CDC light trap at the field collection site in Caldwell County, Texas (August 2017). This figure appears in color at www.ajtmh.org.](image-url)
which ITS2-PCR amplification failed, the SG-ITS1-qPCR yielded average Ct values of > 37, indicating low parasite load.

Sand fly species identification and blood meal analysis.

The sand flies were identified as females (n = 160), males (n = 44), and non-determinate (n = 1) by morphology. Eighty samples were dissected and identified through morphological analyses. Lutzomyia COI DNA barcoding yielded PCR products from 153 samples. By either molecular analysis, morphology, or both methods, three species were identified: Lu. (P.) shannoni (n = 1), Lu. (P.) texana (n = 3), and Lu. (D.) anthophora (n = 188). Only Lu. (P.) shannoni sequences were available in GenBank. Barcode sequences for Lu. (P.) texana (1 haplotype) and Lu. (D.) anthophora (64 haplotypes) were submitted to GenBank (accession numbers: MK952614 and MK952615–MK952678). The condition of two specimens did not permit morphological identification. The low quality/quantity of DNA template was the most probable reason for the PCR failure on the other 11 samples.

Blood meals were evidenced in six Lu. (D.) anthophora sand flies by the insect’s body engorgement. The semi-nested PCR yielded the amplification of two samples. The sequencing data of these amplicons were compared with the GenBank database and corresponded to human DNA.

DISCUSSION

In this study, we report a rare autochthonous case of CL caused by L. (L.) mexicana in a 67-year-old man from central Texas. The course of illness experienced by the patient was atypical in comparison to most cases of CL caused by L. (L.) mexicana, including previously reported autochthonous cases from the United States. Whereas a large majority of indigenous CL cases from Texas presented with a solitary, often-ulcerated lesion on the head, neck, or upper extremities (where sand fly bite wounds are most likely to occur), the patient reported here developed numerous nodular lesions across his lower extremity.8,10 When interviewed, the patient stated that he always wore long, heavy pants while outdoors, and it remains possible that he was inoculated with the parasite at an anatomic location distant from where the lesions ultimately developed.

Although the dermatologic presentation was atypical for L. (L.) mexicana, this case does not appear to be consistent with the rare forms of CL—diffuse cutaneous leishmaniasis (DCL) and disseminated cutaneous leishmaniasis (DSL). Although DCL and DSL can be caused by L. (L.) mexicana, may result with similar lesion types, and respond poorly to treatment, these uncommon forms are characterized by more widely dispersed lesions and are often associated with immunologic deficits in the host.6,30–32 Although the patient did have active lesions, they remained confined to his anterior lower limb and did not spread to other body surfaces. In addition, there was no indication that the patient was immunocompromised.

Three phlebotomine species (Lu. (D.) anthophora, Lu. (P.) texana, and Lu. (P.) shannoni) were collected on the patient’s property. Lutzomyia (Dampfomyia) anthophora was the most numerous, accounting for approximately 98% of sand flies identified. Lutzomyia (Dampfomyia) anthophora is also the most relevant to the transmission L. (L.) mexicana, considering its role in the enzootic maintenance of the parasite.15,17,18 We did not attempt small mammal trapping; however, the field collection site is within the known distribution of the reservoir species, Neotoma micropus and Neotoma floridana.11,33,34 Of significance is the identification of a single Lu. (P.) shannoni specimen which represents, to our knowledge, only the second collection of this species recorded in Texas.12 Lutzomyia (Psathyromyia) shannoni has a wide geographic distribution throughout the Americas but has not been reported until recently in Texas.15,35 Considering Lu. (P.) shannoni as a known anthropophagic vector of CL in the Americas, its distribution in Texas and its possible role in the transmission of L. (L.) mexicana deserve further inquiry.

Human DNA was detected in two engorged female Lu. (D.) anthophora samples, an unexpected finding given that earlier studies have proposed that Lu. (D.) anthophora is not an anthropophagic vector, preferring to feed on small mammals.39 Although human strains of L. (L.) mexicana can replicate in and are transmitted by this sand fly species under laboratory settings, it has been unclear whether Lu. (D.) anthophora is capable of directly transmitting the parasite to humans.22 Our findings suggest that Lu. (D.) anthophora may be capable of naturally serving as both an enzootic and
zoonotic vector of \( \text{L. (L.) mexicana} \); additional studies to help elucidate this are indicated.

The sequencing database of ITS2 diagnostic fragments accumulated by CDC over the last 10 years identified \( \text{L. (L.) mexicana} \) genotypes C647/C649 and A647/T649 associated with cases of Texas or Central American origin, respectively. These findings strongly suggest that the \( \text{L. (L.) mexicana} \) strain in the United States is distinct from those of Central American countries. Because progression of the disease is dependent on both the patient’s immune response and parasite factors, we are performing a comparative genome study using several \( \text{L. (L.) mexicana} \) isolates from Texas and Central America, including this case.\(^{36}\) The data will facilitate the interpretation of the association between the clinical manifestation observed in this study and parasite factors. Nevertheless, the detection of the \( \text{L. (L.) mexicana} \) genotype with SNPs C647 and C649 in both clinical and sand fly samples provides additional epidemiologic evidence that the patient’s infection was indigenously acquired in Texas.

Most of the cases of human leishmaniasis previously identified in the United States have occurred among persons who have traveled or lived abroad. However, a growing number of autochthonous CL cases have been reported in the United States in recent years. Whether this observed increase in incidence is due to expansion of vector/reservoir populations, introduction of the parasite into new foci, increased human encroachment into natural settings, or some other combination of factors remains unclear. Additional ecologic and epidemiologic studies are needed in Texas to better understand the rates of infection in vector and reservoir populations, the geographic extent of the parasite, and to characterize the risk of zoonotic transmission of the disease to humans within the United States. Healthcare providers should be aware of the possibility of autochthonous transmission in the United States and how to diagnose and treat CL.

CONCLUSION

This study highlights the complexity of clinical management of CL in a setting where the disease is infrequently encountered. This is the first report of wild-caught, naturally infected sand flies found in association with an autochthonous case of human leishmaniasis in the United States. The identification of specific SNPs in \( \text{L. (L.) mexicana} \) sequences from Texas cases suggests the existence of a new strain of this parasite well adapted in this region. The unexpected detection of human blood in \( \text{Lu. (D.) anthrophora} \) is the first documentation of anthropophagy in this species, suggesting that it plays a role in maintaining the life cycle of these parasites and facilitating the transmission to humans and other mammals.

REFERENCES


