Am. J. Trop. Med. Hyg., 103(4), 2020, pp. 1496–1501
doi:10.4269/ajtmh.20-0107

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

Evan J. Kipp,[1,2] Marcos de Almeida,[3] Paula L. Marcet,[4] Richard S. Bradbury,[3,5] Theresa K. Benedict,[3] Wuling Lin,[3,6] Ellen M. Dotson,[4] and Melinda Hergert[1,7]*

[1]*Texas Department of State Health Services, Zoonosis Control Program, Temple, Texas;* [2]*College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota;* [3]*Parasitic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Georgia;* [4]*Entomology Branch/DPDM/ Centers for Disease Control and Prevention (CDC), Atlanta, Georgia;* [5]*School of Health and Life Sciences, Federation University, Victoria, Australia;* [6]*IHRC Inc., Atlanta, Georgia;* [7]*Texas Animal Health Commission, Austin, Texas*

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.[3] 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, McIlwee 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

* Address correspondence to Melinda Hergert, Texas Animal Health Commission, Epidemiology Dept., 2105 Kramer Lane, Austin, TX 78758. E-mail: melindahergert@yahoo.com

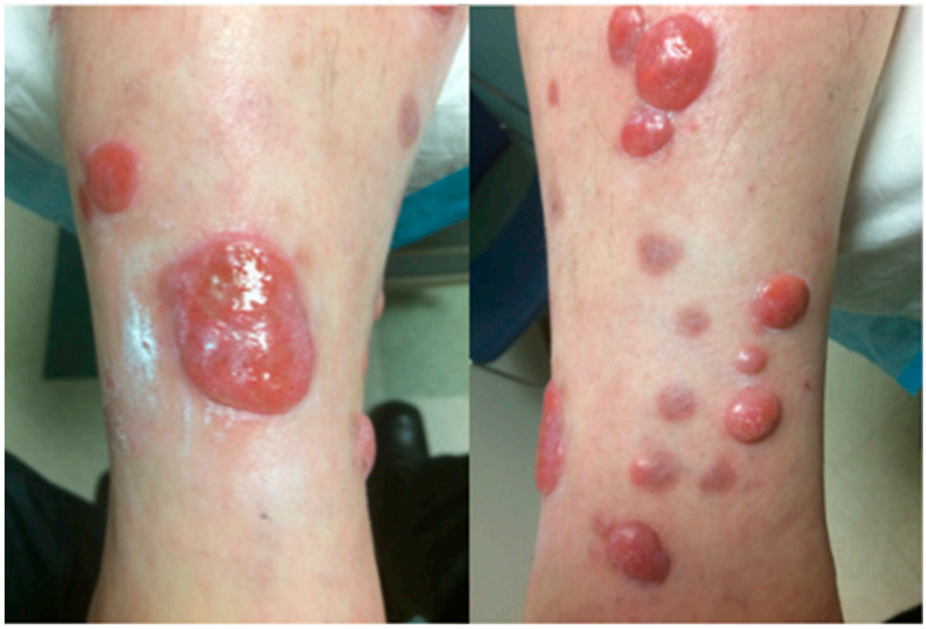

FIGURE 1. Anterior surface of case-patient's right lower extremity showing cutaneous nodules and plaques caused by *Leishmania* (*Leishmania*) *mexicana*. Largest lesion shown on the left image measures approximately 4.0 by 4.5 cm (June 2017, approximately 14 months after onset). This figure appears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

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

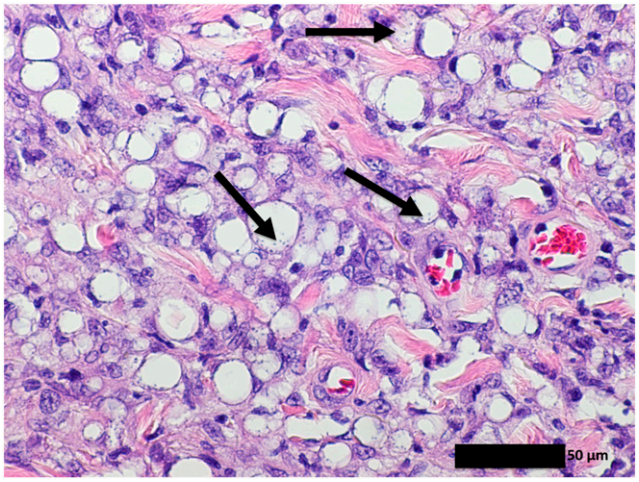

FIGURE 2. Hematoxylin and eosin–stained histologic section of clinical tissue demonstrating numerous *Leishmania* amastigotes (black arrows). Cockerell Dermatopathology, Dallas, Texas. This figure appears in color at www.ajtmh.org.

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 groups of *Leishmania* parasites on basis of the amplicons' melting temperature (Tm) values, that is, G1 (*Viannia* subgenus species), G2 (*L*. (*L*.) *donovani*, *L*. (*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.

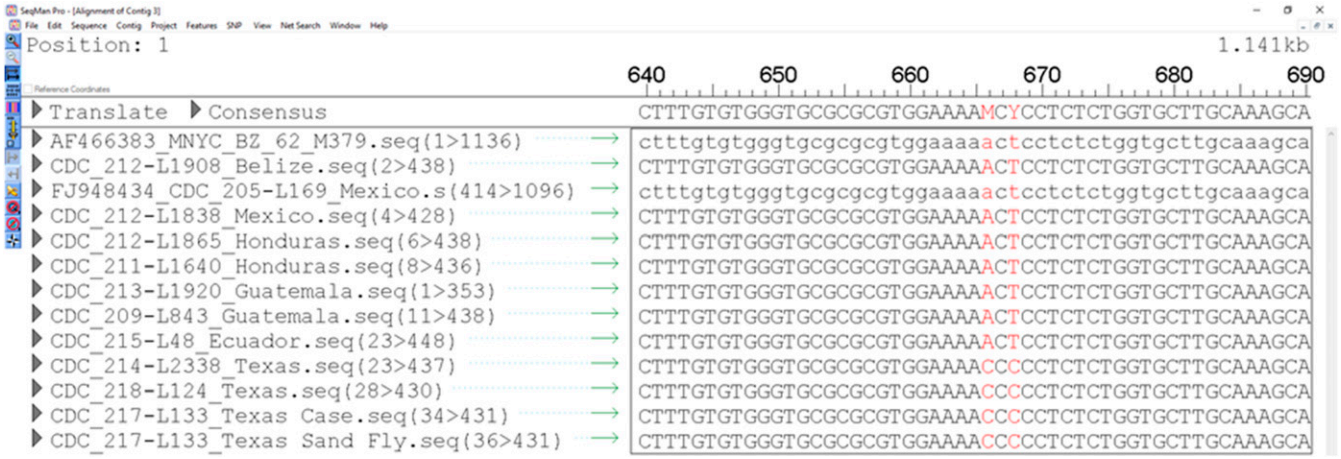

FIGURE 4. *Leishmania* (*Leishmania*) *mexicana* ITS2 diagnostic fragment sequences in isolates from Central America and Texas. This figure appears in color at www.ajtmh.org.

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 hemi-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.[9,10] 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.[20] 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 *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 *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 *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 *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 *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 *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 *Lu*. (*D*.) *anthophora* 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.

Received February 8, 2020. Accepted for publication May 26, 2020.

Published online July 6, 2020.

Disclaimer: The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the CDC.

Authors' addresses: Evan J. Kipp, Texas Department of State Health Services, Temple, TX, and College of Veterinary Medicine, University of Minnesota, St. Paul, MN, E-mail: ekipp2723@gmail.com. Marcos de Almeida and Theresa K. Benedict, Division of Parasitic Diseases and Malaria, Center for Global Health, Centers for Disease Control and Prevention, Atlanta, GA, E-mails: bnz0@cdc.gov and tbenedict@cdc.gov. Paula L. Marcet and Ellen M. Dotson, Entomology Branch/DPDM/CDC, Atlanta GA, E-mails: pvm3@cdc.gov and ebd6@cdc.gov. Richard S. Bradbury, Centers for Disease Control and Prevention, Division of Parasitic Diseases, Atlanta, GA, and School of Health and Life Sciences, Federation University, Health and Life Sciences, Victoria, Australia, E-mail: rbradbur76@gmail.com. Wuling Lin, Parasitic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA, and IHRC Inc., Atlanta, GA, E-mail: wulinglin@hotmail.com. Melinda Hergert, Texas Department of State Health Services, Zoonosis Control, Temple, TX, and Texas Animal Health Commission, Epidemiology, Austin, TX, E-mail: melindahergert@yahoo.com.

REFERENCES

1. Herwaldt BL, 1999. Leishmaniasis. *Lancet 354:* 1191–1199.
2. Desjeux P, 2004. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis 27:* 305–318.
3. Reithinger R, Dujardin J-C, Louzir H, Pirmez C, Alexander B, Brooker S, 2007. Cutaneous leishmaniasis. *Lancet Infect Dis 7:* 581–596.
4. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, de Boer M, 2012. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One 7:* e35671.
5. Bailey MS, Lockwood DNJ, 2007. Cutaneous leishmaniasis. *Clin Dermatol 25:* 203–211.
6. Velasco O, Savarino SJ, Walton BC, Gam AA, Neva AA, 1989. Diffuse cutaneous leishmaniasis in Mexico. *Am J Trop Med Hyg 41:* 280–288.
7. McHugh CP, Melby PC, LaFon SG, 1996. Leishmaniasis in Texas: epidemiology and clinical aspects of human cases. *Am J Trop Med Hyg 55:* 547–555.
8. Wright NA, Davis LE, Aftergut KS, Parrish CA, Cockerell CJ, 2008. Cutaneous leishmaniasis in Texas: a northern spread of endemic areas. *J Am Acad Dermatol 58:* 650–652.
9. Clarke CF, Bradley KK, Wright JH, Glowicz J, 2013. Case report: emergence of autochthonous cutaneous leishmaniasis in northeastern Texas and southeastern Oklahoma. *Am J Trop Med Hyg 88:* 157–161.
10. McIlwee BE, Weis SE, Hosler GA, 2018. Incidence of endemic human cutaneous leishmaniasis in the United States. *JAMA Dermatol 154:* 1032–1039.
11. González C, Wang O, Strutz SE, González-Salazar C, Sánchez-Cordero V, Sarkar S, 2010. Climate change and risk of leishmaniasis in North America: predictions from ecological niche models of vector and reservoir species. *PLoS Negl Trop Dis 4:* e585.
12. Claborn DM, Rowton ED, Lawyer PG, Brown GC, Keep LW, 2009. Species diversity and relative abundance of phlebotomine sand flies (Diptera: Psychodidae) on three army installations in the southern United States and susceptibility of a domestic sand fly to infection with old world *Leishmania* major. *Mil Med 174:* 1203–1208.
13. McHugh CP, Ostrander BF, Raymond RW, Kerr SF, 2001. Population dynamics of sand flies (Diptera: Psychodidae) at two foci of leishmaniasis in Texas. *J Med Entomol 38:* 268–277.
14. Rodríguez-Rojas JJ, Rodríguez-Moreno Á, Berzunza-Cruz M, Gutiérrez-Granados G, Becker I, Sánchez-Cordero V, Stephens CR, Fernández-Salas I, Rebollar-Téllez EA, 2017. Ecology of phlebotomine sandflies and putative reservoir hosts of leishmaniasis in a border area in Northeastern Mexico: implications for the risk of transmission of *Leishmania mexicana* in Mexico and the USA. *Parasite 24:* 33.
15. Kerr SF, McHugh CP, Dronen NO, 1995. Leishmaniasis in Texas: prevalence and seasonal transmission of *Leishmania mexicana* in *Neotoma micropus*. *Am J Trop Med Hyg 53:* 73–77.
16. McHugh CP, Grogl M, Kreutzer RD, 1993. Isolation of *Leishmania mexicana* (Kinetoplastida: Trypanosomatidae) from *Lutzomyia anthophora* (Diptera: Psychodidae) collected in Texas. *J Med Entomol 30:* 631–633.
17. Raymond RW, McHugh CP, Witt LR, Kerr SF, 2003. Temporal and spatial distribution of *Leishmania mexicana* infections in a population of *Neotoma micropus*. *Mem Inst Oswaldo Cruz 98:* 171–180.
18. McHugh CP, Thies ML, Melby PC, Yantis LD, Raymond RW, Villegas MD, Kerr SF, 2003. Short report: a disseminated infection of *Leishmania mexicana* in an eastern woodrat,

Neotoma floridana, collected in Texas. *Am J Trop Med Hyg 69*: 470–472.
19. Kerr SF, McHugh CP, Merkelz R, 1999. Short report: a focus of *Leishmania mexicana* near Tucson, Arizona. *Am J Trop Med Hyg 61:* 378–379.
20. Endris RG, Young DG, Butler JF, 1984. The laboratory biology of the sand fly *Lutzomyia anthophora* (Diptera: Psychodidae). *J Med Entomol 21:* 656–664.
21. Endris RG, Perkins PV, Young DG, Johnson RN, 1982. Techniques for laboratory rearing of sand flies (Diptera: Psychodidae). *Mosq News 42:* 400–407.
22. Perkins PV, Endris RG, Young DG, 1987. Experimental transmission of *Leishmania mexicana* by a North American sand fly, *Lutzomyia anthophora* (Diptera: Psychodidae)1. *J Med Entomol 24:* 243–247.
23. Lawyer PG, Young DG, Butler JF, Akin DE, 1987. Development of *Leishmania mexicana* in *Lutzomyia diabolica* and *Lutzomyia shannoni* (Diptera: Psychodidae). *J Med Entomol 24:* 347–355.
24. Lawyer PG, Young DG, 1987. Experimental transmission of *Leishmania mexicana* to hamsters by bites of phlobotomine sand flies (Diptera: Psyhcodidae) from the United States. *J Med Entomol 24:* 458–462.
25. De Almeida ME, Koru O, Steurer F, Herwaldt BL, 2017. Detection and differentiation of Leishmania spp. in clinical specimens by use of a SYBR green-based real-time PCR assay. *J Clin Microbiol 55:* 281–290.
26. De Almeida ME, Steurer FJ, Koru O, Herwaldt BL, Pieniazek NJ, Da Silva AJ, 2011. Identification of Leishmania spp. by molecular amplification and DNA sequencing analysis of a fragment of rRNA internal transcribed spacer 2. *J Clin Microbiol 49:* 3143–3149.
27. Young DG, Perkins PV, 1984. Phlebotomine sand flies of North America (Diptera: Psychodidae) [*Lutzomyia*]. *Mosq News 44:* 263–304.
28. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R, 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol 3:* 294–299.
29. Roellig DM, Gomez-Puerta LA, Mead DG, Pinto J, Ancca-Juarez J, Calderon M, Bern C, Gilman RH, Cama VA; Chagas Disease Workgroup in Arequipa, 2013. Hemi-nested PCR and RFLP methodologies for identifying blood meals of the Chagas disease vector, *Triatoma infestans*. *PLoS One 8:* e74713.
30. Hashiguchi Y, Gomez EL, Kato H, Martini LR, Velez LN, Uezato H, 2016. Diffuse and disseminated cutaneous leishmaniasis: clinical cases experienced in Ecuador and a brief review. *Trop Med Health 44:* 1–9.
31. Calvopina M, Gomez EA, Sindermann H, Cooper PJ, Hashiguchi Y, 2006. Relapse of new world diffuse cutaneous leishmaniasis caused by *Leishmania* (*Leishmania*) *mexicana* after miltefosine treatment. *Am J Trop Med Hyg 75:* 1074–1077.
32. Zerpa O, Ulrich M, Blanco B, Polegre M, Avila A, Matos N, Mendoza I, Pratlong F, Ravel C, Convit J, 2007. Diffuse cutaneous leishmaniasis responds to miltefosine but then relapses. *Br J Dermatol 156:* 1328–1335.
33. Mauldin MR, Haynie ML, Hanson JD, Baker RJ, Bradley RD, 2014. Multilocus characterization of a woodrat (genus *Neotoma*) hybrid zone. *J Hered 105:* 466–476.
34. Texas Parks and Wildlife Department, 2020. *Texas Ecoregions*. Available at: https://tpwd.texas.gov/education/hunter-education/online-course/wildlife-conservation/texas-ecoregions. Accessed December 31, 2019.
35. Mchugh CP, 1991. Distributional records for some North American sand flies, *Lutzomyia* (Diptera: Psychodidae). *Entomol News 102:* 192–194.
36. Fernández-Figueroa EA, Imaz-Rosshandler I, Castillo-Fernández JE, Miranda-Ortíz H, Fernández-López JC, Becker I, Rangel-Escareño C, 2016. Down-regulation of TLR and JAK/STAT pathway genes is associated with diffuse cutaneous leishmaniasis: a gene expression analysis in NK Cells from patients infected with *Leishmania mexicana*. *PLoS Negl Trop Dis 10:* 1–17.