

Relevance of epidemiological surveillance in travelers: an imported case of *Leishmania tropica* in Mexico

Edith Araceli Fernández-Figueroa^{1,2}, Sokani Sánchez-Montes², Haydee Miranda-Ortiz³, Alfredo Mendoza-Vargas³, Rocely Cervantes-Sarabia⁴, Roberto Alejandro Cárdenas-Ovando⁵, Adriana Ruiz-Remigio⁴, Ingeborg Becker^{2,4}

ABSTRACT

We report the case of a patient with cutaneous leishmaniasis who showed a rapidly progressing ulcerative lesion after traveling to multiple countries where different *Leishmania* species are endemic. Diagnosis of *Leishmania tropica*, an exotic species in Mexico was established by using serological and molecular tools.

KEYWORDS: *Leishmania tropica*. Molecular epidemiology. Local cutaneous leishmaniasis. Travel medicine.

INTRODUCTION

Human cutaneous leishmaniasis is a zoonotic emerging tropical disease caused by 20 species of flagellated protozoa of the genus *Leishmania*, generating 150,000 new human cases per year, that are distributed across 98 countries throughout the Old World and the New World¹⁻³. Most of the Old World cases are caused by *Leishmania aethiops*, *Leishmania infantum*, *Leishmania major* and *Leishmania tropica*, whereas in the New World they are mostly caused by *Leishmania amazonensis*, *Leishmania braziliensis*, *Leishmania mexicana*, *Leishmania naiffi* and *Leishmania panamensis*^{3,4}.

Many of the species cause ulcers with indurated borders, which are indistinguishable between the species, thereby making the differential diagnosis very complicated⁵⁻⁷. Yet a species-specific diagnosis is crucial since some *Leishmania* species generate visceral or mucocutaneous forms of the disease (*L. infantum* and *L. braziliensis* respectively)^{3,8}. Currently, no morphological parameters exist that permit to distinguish between *Leishmania* species^{6,7}. The increasing worldwide travel, ecotourism and displacement of migrants are affecting the distribution and incidence of patients with leishmaniasis, especially in the Middle East countries where *L. major* and *L. tropica* are the main pathogenic species⁹⁻¹¹. In Mexico, most of the cutaneous leishmaniasis cases are caused by *L. mexicana*^{12,13}. Yet, isolated reports of other species such as *L. amazonensis*, *L. braziliensis* and *L. infantum/chagasi* have also been registered¹³. The surveillance of *Leishmania* species that circulate in a region is essential to alert health authorities of the introduction of exotic species that can affect vulnerable groups and also helps to establish the degree of dissemination or flow of these species within non-endemic regions²⁻⁴.

We report the case of a patient with LCL who showed a rapidly progressing ulcerative lesion after travelling to multiple countries where different *Leishmania*

¹Instituto Nacional de Medicina Genómica, Departamento de Genómica Poblacional, Genómica Computacional e Integrativa, Ciudad de México, Mexico

²Universidad Nacional Autónoma de México, Facultad de Medicina, Unidad de Investigación en Medicina Experimental, Centro de Medicina Tropical, Ciudad de México, Mexico

³Instituto Nacional de Medicina Genómica, Unidad de Secuenciación, Ciudad de México, Mexico

⁴Universidad Nacional Autónoma de México, Facultad de Medicina, Unidad de Investigación en Medicina Experimental, Laboratorio de Inmunoparasitología, Ciudad de México, Mexico

⁵Instituto Tecnológico y de Estudios Superiores de Monterrey, Escuela de Ingeniería y Ciencias, Monterrey, Nuevo León, Mexico

Correspondence to: Ingeborg Becker
Hospital General de México, Dr. Balmis 148,
Col. Doctores, 06726, Ciudad de Mexico,
Mexico
Tel: +52 555623 2674

Email: becker@unam.mx

Received: 19 December 2019

Accepted: 22 April 2020

species are endemic. Diagnosis of leishmaniasis was established serologically and by smears, yet PCR enabled us to establish that the patient was infected by *Leishmania tropica*, an exotic species in Mexico. We highlight the importance of including a species-specific diagnosis by PCR to establish the *Leishmania* species and the possible infection site in travellers exposed to multiple species.

CASE REPORT

A 35-years-old male patient, originally from Russia, attended the Dermatology Service of the General Hospital in Mexico due to two ulcerative skin lesions with a semi-dry appearance, active borders, one of which was in the lower left pre-atrial region and the other in the dorsal region of the left forearm (**Figure 1A**). The patient referred having travelled to different Middle Eastern countries (Israel, Turkey), and to the Balkan region (Armenia, Georgia) and Russia, between May and June 2019. In August of the same year, he detected red and indurated papules, leading him to seek medical attention in San Cristobal de las Casas, located in the Mexican State of Chiapas, in late August. Cryotherapy with liquid nitrogen was applied to the lesions, leading to their temporary reduction in size. After a renewed flare-up of the lesion edges, the patient was attended at the Tropical Medicine Center (UNAM/Hospital General de Mexico), where he was clinically diagnosed as having localized cutaneous leishmaniasis (LCL). For the confirmation, Giemsa-stained smears of the lesion and ELISA tests for *L. braziliensis*, *L. infantum/chagasi*, *L. major* and *L. mexicana* were carried out¹⁴. Additionally, an aspirate of the lesion was performed and the recovered material was grown in NNN medium (Novy-MacNeal-Nicolle) and in 199 Hank's culture medium (Cat. 12350039, Gibco,

Burlington, Ontario, Canada), supplemented with 10% decomplexed fetal bovine serum- FBS (Cat. 16000044, Gibco, Burlington, Ontario, Canada). The quickly growing promastigotes were used for species identification. To this end, promastigote DNA was extracted with DNeasy Blood & Tissue kit (QIAGEN Inc., Hilden, Germany) following the manufacturer's guidelines. For the PCR analysis, a 589 bp segment of the alanine aminotransferase gene (*ALAT*), a 350 bp of the heat shock protein 70 (*hsp70*) and 300 bp of the internal transcribed spacer 1 (*ITS1*)^{15,16} were amplified using 25 µL QIAGEN Master mix (QIAGEN Inc., Hilden, Germany), 1 µL (10 µM) of each primer (*ALAT*: Fwd 5'-GTGTGCATCAACCCMGGGAA-3' and Rvs 5'-CGTTCAGCTCCTCGTTCCGC-3'; *Hsp70*: Fwd 5'-GGACGAGATCGAGCGCATGGT-3' and Rvs 5'-TCCTCCGACGCCTCCTGGTTG-3'; *ITS1*: Fwd 5'-CTGGATCATTTCGATG-3' and Rvs 5'-TGATACCACTTATCGCAC-3'), 2-3 µL of extracted DNA and bi-distilled sterile water in a final volume of 50 µL. Additionally, *L. mexicana* DNA and bi-distilled sterile water were used as positive and negative controls, respectively. We followed the amplification conditions previously reported^{15,16}. The PCR products were run on a 1% agarose gel stained with Smartglow (Cat. E4500-PS, Accuris Instruments, Edison, NJ, USA). The PCR products were purified using the Agencourt AMPure XP kit (Cat. A63882, Beckman Coulter, Brea, CA, USA). Amplicons were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Cat. 4337455, Thermo Fisher, Waltham, MA, USA). The sample was purified by the BigDye XTerminator (Cat. 4376486, Thermo Fisher, Waltham, MA, USA) prior to loading on the ABI 3730xL DNA analyzer (Thermo Fisher, Waltham, MA, US). Sequencing was carried out at the Sequencing Unit of the National Institute of Genomic Medicine.

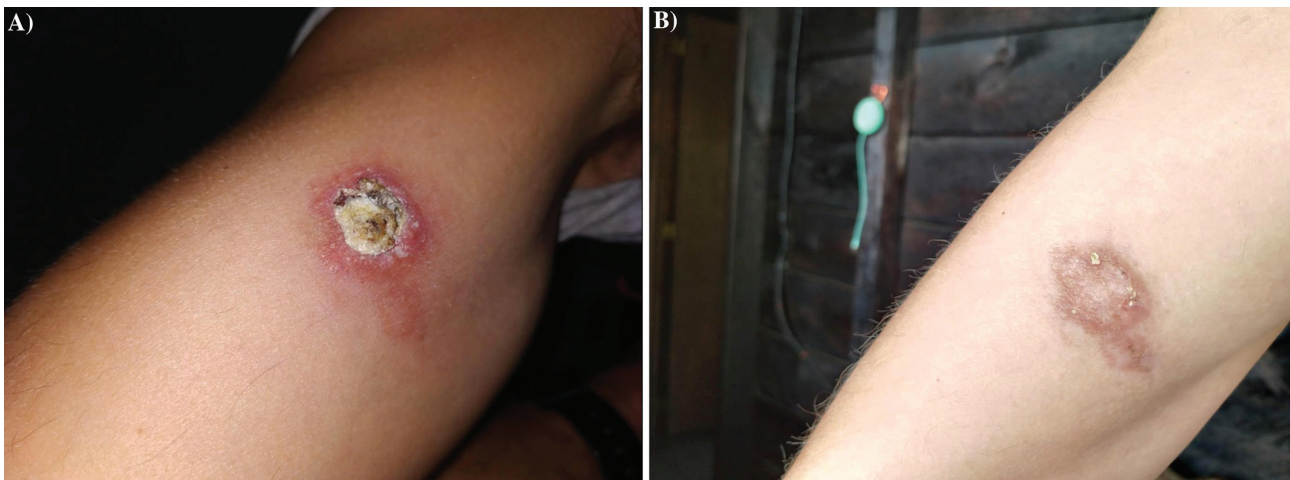


Figure 1 - Photographs of the ulcer on the forearm: A) active injury; B) injury after the glucantime treatment.

Chromatograms were visualized and a consensus sequence was generated using the Chromas software (version 5, Technelysium, South Brisbane, QLD, Australia). The consensus sequence was compared to reference sequences deposited in GenBank using the BLASTn tool (National Center for Biotechnology Information, Bethesda, MD, USA). Sequences of validated *Leishmania* species were downloaded and a global alignment was conducted using the Clustal W algorithm (Informer Technologies, Inc., Dallas, US) with the software Bioedit (Informer Technologies, Inc., Dallas, US). Finally, a Maximum Likelihood phylogenetic reconstruction was carried out using the Tamura-three parameters model with invariant sites (I) with 10,000 Bootstrap replicates in Mega 6.0. Gaps were excluded from the analysis. Sequences were deposited in GenBank under the accession numbers MT157311, MT157312, and MT140349.

The Giemsa-stained smears of the lesion showed abundant amastigotes within macrophages. The ELISA analyzed the patient’s serum revealing some degree of cross

reactivity between four *Leishmania* species: *L. braziliensis*, *L. infantum/chagasi*, *L. major* and *L. mexicana*, yet higher antibody levels were recorded for *L. major* and *L. mexicana* antigens (Table 1). The isolated parasites were used for the PCR evaluation and a successful amplification of the three genes was achieved (Figures 2A, 2B and 2C). Using the BLAST analysis, these consensus sequences were 99% (538/544 bp), 100% (323/323 bp), and 100% (277/277 bp) identical to the corresponding sequences of the *ALAT* (MH455379), *hsp70* (LN907846), and *ITS1* (FN677341), genes respectively, of *L. tropica* from Israel and Russia. For the phylogenetic analyses, we only included fragments of 540, and 320 bp of the *ALAT*, and *hsp70* genes, respectively. The Maximum Likelihood analysis confirmed that the parasite recovered from the patient corresponded to *L. tropica* (Figures 2D and 2E). The patient was treated with intralesional applications of 1 mL of glucantime per lesion at 5-day intervals with outpatient monitoring for 4 weeks. The case resolved favorably, the lesions healed adequately and the patient was discharged (Figure 1B).

Table 1 - The mean optical densities (OD) recorded in ELISA test for each *Leishmania* species.

Species	OD	Cut point	Result	Interpretation
<i>Leishmania braziliensis</i>	0.501	0.249	0.252	Borderline
<i>Leishmania infantum/chagasi</i>	0.356	0.178	0.178	Borderline
<i>Leishmania major</i>	0.435	0.191	0.244	Positive
<i>Leishmania mexicana</i>	0.548	0.204	0.344	Positive

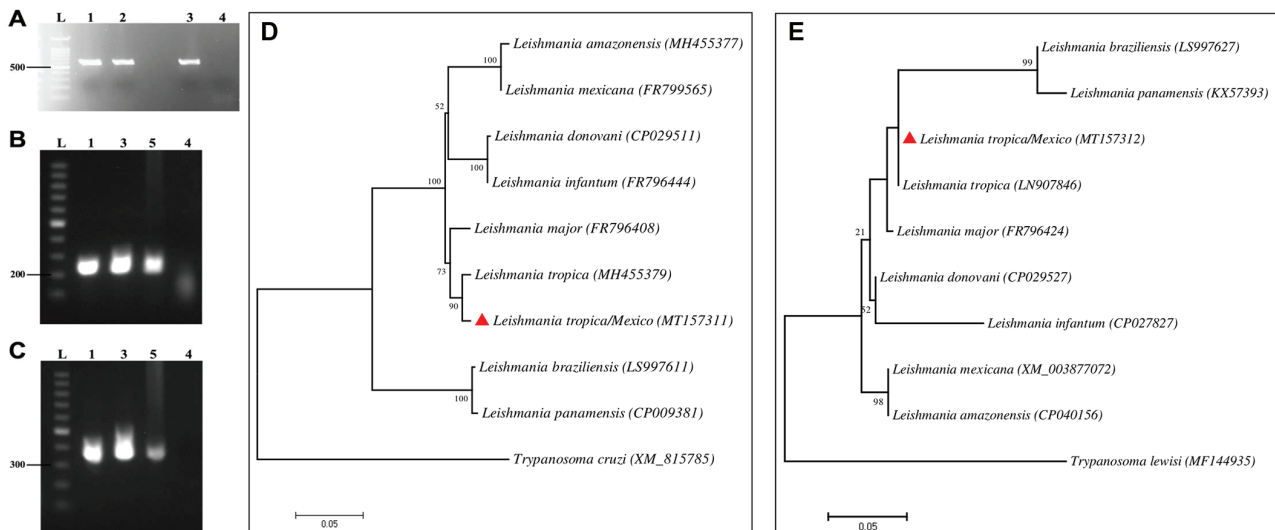


Figure 2 - Molecular identification of *Leishmania tropica*: A) *ALAT*. PCR products of parasites isolated from the patient in lines 1 and 2 (3 and 2 μ L, respectively), *Leishmania mexicana* positive control (line 3), Nuclease-free water as the negative control (line 4). L=Ladder 100 pb; B) *hsp70*-C) *ITS1*. PCR products of parasites isolated from the patient in lines 1 and 3 (3 and 2 μ L, respectively), positive control (line 5), and negative control (line 4); D) Maximum Likelihood phylogenetic trees generated using the Tamura three-parameter distance model with Invariant sites (+I) with partial sequences of the *ALAT* gene, and E) Hasegawa-Kishino-Yano with Gamma distribution (+G) of the *hsp70* gene from several members of the genus *Leishmania*. Bootstrap values greater than 50 are indicated on the nodes. The sequences generated in this study are indicated with red triangles.

DISCUSSION

The molecular epidemiology of human imported cases of leishmaniasis is lacking in Mexico^{12,13}. To the best of our knowledge, this case represents the first record of LCL caused by *Leishmania tropica* reported in Mexico. This species is registered mainly in the Middle East and Russia, representing one of the most widely distributed species causing cutaneous leishmaniasis in the Old World^{5,7,17,18}. The incidence of this pathogen has increased in the urban areas of Israel and also in regions with armed conflicts in the Middle East¹⁶. Although the patient had visited several areas where *L. tropica* is endemic, he had also visited various States of Mexico, endemic for *L. mexicana*^{1,13}. Thus, a species-specific diagnosis was mandatory. This was achieved by using molecular tools that enabled to detect the presence of *L. tropica*, an exotic microorganism in America. The rapid and correct detection of an infecting *Leishmania* species is important for establishing the correct treatment and the clinical management of the patients^{5,7}. Furthermore, the correct identification of *Leishmania* species in travelers helps alerting health authorities on the presence of a novel species within the country and enable sanitary measures to avoid the establishment and distribution of new sources of *Leishmania* transmission in non-endemic regions, where sand flies are able to transmit the parasite^{8,9}. In Middle East countries, *Phlebotomus sergenti*, is the main vector of *L. tropica*¹⁹. Although in America there are no representatives of the genus *Phlebotomus*, in Mexico 52 sand fly species have been recorded, four of them are confirmed vectors of other *Leishmania* species and whose transmission capacity of *L. tropica* is unknown¹².

Furthermore, environmental aspects such as climate change, growing urbanization, socio-economic development, tourism and migrations are changing the epidemiology of leishmaniasis⁹⁻¹¹. This highlights the need of establishing the molecular diagnostic as an essential tool for the active surveillance to avoid the emergence of outbreaks of exotic parasites.

ETHICAL APPROVAL

The current report was approved by the Ethics and Research Committee of the Medical Faculty of the UNAM (*Universidad Nacional Autónoma de México*), FMED/CI/JMO/129/2017). The patient signed a written consent for the publication of the case.

FUNDING

This work was supported by grants from the CONACyT

221405, PAPIIT IN211418 and PAPIIT IN209314.

REFERENCES

1. Pigott DM, Bhatt S, Golding N, Duda KA, Battle KE, Brady OJ, et al. Global distribution maps of the leishmaniasis. *eLife*. 2014;3:e02851.
2. Akhoundi M, Kuhls K, Cannet A, Votýpka J, Marty P, Delaunay P, et al. Historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and sandflies. *PLoS Negl Trop Dis*. 2016;10:e0004349.
3. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One*. 2012;7:e35671.
4. Ashford RW. The leishmaniasis as emerging and reemerging zoonoses. *Int J Parasitol*. 2000;30:1269-81.
5. de Vries HJ, Reedijk SH, Schallig HD. Cutaneous leishmaniasis: recent developments in diagnosis and management. *Am J Clin Dermatol*. 2015;16:99-109.
6. Scorza BM, Carvalho EM, Wilson ME. Cutaneous manifestations of human and murine leishmaniasis. *Int J Mol Sci*. 2017;18:E1296.
7. Van der Auwera G, Dujardin JC. Species typing in dermal leishmaniasis. *Clin Microbiol Rev*. 2015;28:265-94.
8. Solomon M, Sahar N, Pavlotzky F, Barzilay A, Jaffe CL, Nasereddin A, et al. Mucosal leishmaniasis in travelers with *Leishmania braziliensis* complex returning to Israel. *Emerg Infect Dis*. 2019;25:642-8.
9. Hodiamont CJ, Kager PA, Bart A, de Vries HJ, van Thiel PP, Leenstra T, et al. Species-directed therapy for leishmaniasis in returning travellers: a comprehensive guide. *PLoS Negl Trop Dis*. 2014;8:e2832.
10. Carvalho BM, Maximo M, Costa WA, Santana AL, Costa SM, Costa Rego TA, et al. Leishmaniasis transmission in an ecotourism area: potential vectors in Ilha Grande, Rio de Janeiro State, Brazil. *Parasit Vectors*. 2013;6:325.
11. Rehman K, Walochnik J, Mischlinger J, Alassil B, Allan R, Ramharter M. Leishmaniasis in Northern Syria during civil war. *Emerg Infect Dis*. 2018;24:1973-81.
12. González C, Rebollar-Téllez EA, Ibáñez-Bernal S, Becker-Fauser I, Martínez-Meyer E, Peterson A, et al. Current knowledge of *Leishmania* vectors in Mexico: how geographic distributions of species relate to transmission areas. *Am J Trop Med Hyg*. 2011;85:839-46.
13. Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, Arenas R. Leishmaniasis: a review. *F1000Res*. 2017;6:750.
14. Escalona-Montañón AR, Pérez-Montfort R, Cabrera N, Mondragón-Flores R, Vélez-Ramírez DE, Gómez-Sandoval JN, et al. Proteín phosphatase PP2C in the flagellum of *Leishmania major*: cloning and characterization. *Parasitol Open*. 2017;3:e15.

15. Marco JD, Barroso PA, Locatelli FM, Cajal SP, Hoyos CL, Nevot MC, et al. Multilocus sequence typing approach for a broader range of species of *Leishmania* genus: describing parasite diversity in Argentina. *Infect Genet Evol.* 2015;30:308-17.
16. Graça GC, Volpini AC, Romero GA, Oliveira Neto MP, Hueb M, Porrozzini 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. Uchida S, Oiso N, Sanjoba C, Matsumoto Y, Yanagihara S, Annoura T, et al. Cutaneous leishmaniasis caused by *Leishmania tropica* in Israel. *J Dermatol.* 2018;45:e240-1.
18. Ghatee MA, Taylor WR, Karamian M. The geographical distribution of cutaneous leishmaniasis causative agents in Iran and its neighboring countries, a review. *Front Public Health.* 2020;8:11.
19. Jancarova M, Hlavacova J, Volf P. The development of *Leishmania tropica* in sand flies (Diptera: Psychodidae): a comparison of colonies differing in geographical origin and a gregarine coinfection. *J Med Entomol.* 2015;52:1378-80.