Case Report

Emerging Cutaneous Leishmaniasis Caused by *Leishmania major* in a Non-Endemic Area of Iran: A Case Report

Mehdi Mohebali[1,2], Ehsan Rikhtehgar[3,4], Mehdi Badakhshan[5,6], *Samira Firooziyan[5,6]

[1]Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Internal Medicine, Taleghani Hospital, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran
[4]Urmia Deputy for Health Affairs, Urmia University of Medical Sciences, Urmia, Iran
[5]Pathogens and Vectors Research Center, Cellular and Molecular Medicine Research Institute, Urmia University of Medical Sciences, Urmia, Iran
[6]Department of Medical Entomology and Vector Control, School of Public Health, Urmia University of Medical Sciences, Urmia, Iran

***Corresponding author:** Dr Samira Firooziyan, E-mail: samirafirooziyan@gmail.com

(Received 05 Nov 2025; accepted 29 Dec 2025)

Abstract

Background: Leishmaniasis is a neglected tropical disease transmitted by vectors, ranking among the top 10 infectious diseases globally in terms of morbidity and mortality. The cutaneous form (CL) is the most common and is endemic in 19 of Iran's 31 provinces. In non-endemic regions, however, both physician familiarity and patient encounters with the disease are rare.

Methods: This study reports a case of CL in a nine-year-old boy from Sardasht County, West Azerbaijan Province, a non-endemic area for CL in Iran and with no history of travel to endemic areas. The patient presented with purulent wounds on his hands and feet that did not respond to broad-spectrum antibiotics. Diagnosis was confirmed by identifying the vacuolated amastigote forms of *Leishmania* parasites through Giemsa staining. For species identification, DNA was extracted from the slide scraping according to the manufacturer's instructions (Bioneer, Korea). The species of the *Leishmania* parasite was determined using PCR-RFLP, Fast Digest BsuR1 enzyme, ITS1 and Kinetoplast genes.

Results: The parasite was identified as *Leishmania major*. Treatment with Meglumine antimoniate and cryotherapy, in accordance with the Iranian CL surveillance guideline, led to full recovery after 2 months, with no relapse at 9 months post-treatment. Extensive local screening found no additional cases, indicating a likely sporadic infection.

Conclusion: This case highlights the need for rapid and accurate diagnosis, effective treatment, and ongoing surveillance to prevent the emergence of leishmaniasis in non-endemic regions. More attention must be paid to this disease by the healthcare system and physicians, even in non-endemic areas.

Keywords: Cutaneous leishmaniasis; *Leishmania major*; Non-endemic area; Iran

Introduction

Leishmaniasis is caused by *Leishmania* parasites transmitted through infected female sand fly bites (1). The three main forms of leishmaniasis are cutaneous leishmaniasis (CL), visceral leishmaniasis (VL) and mucocutaneous leishmaniasis (MCL), with CL being the most prevalent (2). Over 1 billion people are at risk, with an estimated 1 million new cases of CL occurring globally each year (3). Cutaneous leishmaniasis is highly prevalent in the Eastern Mediterranean, accounting for over 80% of global cases. Several Middle Eastern countries,

http://jad.tums.ac.ir
Published Online: December 30, 2025

such as Iran, Saudi Arabia, Syria, Iraq and Yemen, are highly endemic due to zoonotic transmission cycles (4). In Iran, despite ongoing public health efforts, CL remains a significant burden and its geographical distribution is expanding (5). However, CL remains largely neglected in non-endemic areas, where limited physician experience often delays diagnosis and treatment (5, 6). This neglect is compounded by insufficient awareness and reporting outside traditional endemic foci, which can allow emerging transmission to go unnoticed until clinical cases appear (6). Herein, we present a case of autochthonous CL acquired in a non-endemic area of Iran, in a patient with no travel history to recognized endemic regions. This report underscores the potential for the emergence of CL as a new public health threat in previously unaffected populations. It further highlights the crucial need for enhanced surveillance, improved diagnostic awareness, and proactive control measures, even in regions traditionally considered non-endemic.

Case Report

In May 2025, a previously healthy nine-year-old boy from Sardasht County (36°09′19″N 45°28′48″E) in northwest Iran, presented with multiple purulent wounds, ranging from 5 to 10 cm in size, on his hands and feet (Fig. 1). Physical examination and laboratory tests revealed no systemic symptoms, underlying conditions, allergies, or infections. His condition did not respond to broad-spectrum antibiotics. Sardasht County is a non-endemic region for leishmaniasis that borders Iraq, a country where leishmaniasis is prevalent (7). Notably, the boy had no travel history to any endemic areas. However, a patient's history revealed he had visited a rodent-infested rural house near his home the previous summer, where he recalled being bitten by insects.

Microscopic examination of lesion scrapings after Giemsa staining confirmed the presence of *Leishmania* parasites (Fig. 2). For species identification, DNA was extracted from the Giemsa-stained slides.

Molecular identification of the parasite was performed. Polymerase chain reaction (PCR) amplifications were performed in a 25 µL reaction volume. Each reaction mixture contained 2 µL of 10× PCR buffer, 1.2 µL dNTP mix (25 mM), 1.6 µL MgCl2 (25 mM), 1 unit of Taq DNA polymerase, 2 µL each of forward and reverse primers (10 pmol) and 1 µL of DNA template. Amplification products were compared against Iranian reference strains of *L. tropica* (MHOM/IR/02/Mash10, Accession EF653267) and *L. major* (MRHO/IR/11/GOL-2, Accession JN860745). The thermal cycling program comprised an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 94 °C for 30 seconds, annealing at 59 °C for 30 seconds and extension at 72 °C for 45 seconds, with a final extension step at 72 °C for 7 minutes.

Products were visualized on 1.2% agarose gels stained with a safe dye under UV illumination. Molecular identification of *Leishmania* species was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) targeting the internal transcribed spacer 1 (ITS1) gene. The ITS1-PCR products were digested with the Fast Digest BsuR1 (*Hae*III) enzyme to differentiate species based on their specific restriction patterns. Additionally, amplification of kinetoplast DNA (kDNA) was employed as a sensitive molecular marker to detect and confirm the presence of *Leishmania* in the clinical samples (8, 9). For RFLP analysis, 10 µL of PCR product (1 µg/µL) was incubated with 2 µL of 10× buffer and 1 µL *Hae*III restriction enzyme (10 units/µL, Fermentas) at 37 °C for 10 minutes. The enzyme cleaves at the GGCC site, generating species-specific fragment patterns. Digested products were separated on 3% agarose gels for species differentiation. The *L. major* parasite species was determined (Fig. 3).

Treatment with Meglumine antimoniate combined with cryotherapy led to complete

http://jad.tums.ac.ir
Published Online: December 30, 2025

healing after 2 months and no relapse post 9 months of follow-up. The medication was provided free of charge by the public health system. To prevent further transmission, the patient was advised to use insecticide-treated mosquito nets and to install window screens. Subsequently, a broad screening program targeting the patient's village, nearby residents, schoolchildren and the wider community identified no further cases.

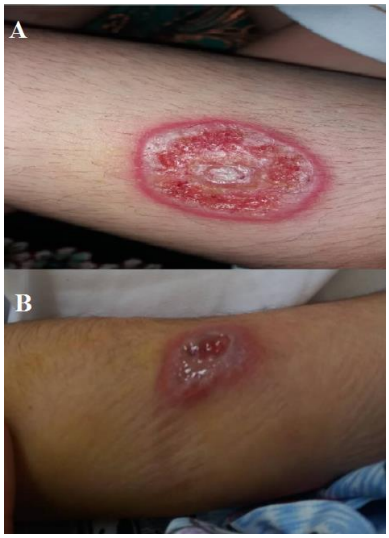

Fig. 1. Active cutaneous leishmaniasis in a non-endemic area. Photographs taken in May 2025 in Sardasht County, West Azerbaijan Province, Iran, showing ulcerative, crusted lesions on the patient's (A) foot and (B) hand, indicative of an active *Leishmania* infection

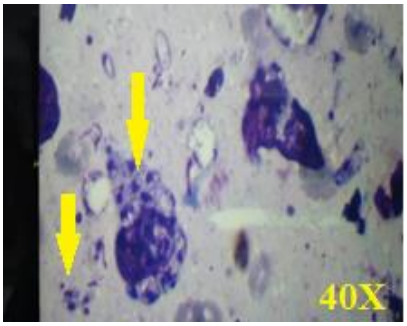

Fig. 2. Microscopic identification of *Leishmania* amastigotes. Giemsa-stained smear prepared from a dermal scraping of a cutaneous lesion from the patient in Sardasht County, Iran (May 2025). The image shows multiple amastigotes (examples indicated by arrows) within macrophage cytoplasm and outside of cells

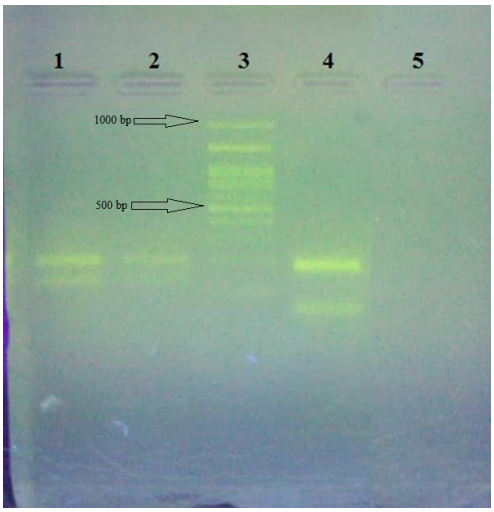

Fig. 3. Agarose gel electrophoresis for *Leishmania* species identification. PCR products from the ITS1 region were separated on a 1.5% agarose gel. Lane 1: *Leishmania major* reference strain (positive control). Lane 2: Clinical sample from a patient in Sardasht County, Iran (May 2025). Lane 3: 100 bp DNA ladder (Fermentas). Lane 4: *Leishmania tropica* reference strain. Lane 5: Negative control (no template). The patient sample in Lane 2 shows a banding pattern (approximately 220 bp and 140 bp fragments) consistent with *L. major* (Lane 1) and distinct from the *L. tropica* pattern of ~200 bp and 60 bp (Lane 4)

Discussion

Cutaneous leishmaniasis continues to pose a substantial challenge to global health, especially in endemic regions where it contributes to significant morbidity and socio-economic burden (10). While *L. major* is recognized as the primary agent of zoonotic cutaneous leishmaniasis (ZCL) in Iran (11). Therefore, the presentation of a confirmed *L. major* case in Sardasht County, a non-endemic area, represents a critical indication of possible epidemiological shifts or emerging foci of transmission. This observation resonates with global patterns whereby leishmaniasis increasingly affects new geographical zones, influenced by environmental changes, population movements and vector dynamics (12).

The molecular confirmation of *L. major* in a non-traveling patient confirms autochthonous

http://jad.tums.ac.ir
Published Online: December 30, 2025

transmission, pointing to local ecological drivers such as nearby rodent reservoirs and competent sand fly vectors. As a border region, the occurrence in Sardasht further underscores the vulnerability of areas adjacent to active endemic foci, such as Iraq, which are vulnerable to zoonotic spillover, reflecting the complex zoonotic cycle of ZCL, which can extend beyond traditionally mapped boundaries.

These findings underscore the need for enhanced epidemiological surveillance, an element often neglected in public health planning (13). Diagnostic delay in such settings remains a significant clinical challenge. As illustrated by this case, an initial misdiagnosis of a bacterial infection and subsequent ineffective antibiotic treatment can exacerbate disease progression and patient discomfort. This scenario underscores the crucial need to cultivate heightened clinical suspicion among healthcare providers practicing outside endemic zones. Furthermore, the integration of accessible diagnostic tools, from simple, cost-effective Giemsa-stained smear microscopy to more specific molecular assays, into local laboratory services is imperative. Such capacity enables the timely and accurate confirmation of CL, which is needed to initiate appropriate and prompt treatment (14).

The patient likely contracted the infection while sleeping outdoors without protection in a rural area during the sand fly season, resulting in bites on the hands and feet. This aligns with previous findings that lesions often appear on exposed body parts. The World Health Organization recognizes leishmaniasis as a neglected disease and such cases should prompt increased awareness and vigilance among physicians in both endemic and non-endemic regions (12).

The patient’s successful outcome with a combination of Meglumine antimoniate and cryotherapy confirms the standard treatment. However, reservoir management practices and vectors are essential to prevent further cases and potential outbreaks of the disease. This example confirms the efficacy of personal protective measures, notably the use of window screens and insecticide-treated nets, as viable community-level interventions for reducing transmission (15).

The detection of a single sporadic case limits widespread generalizability and the absence of more extensive entomological and reservoir host studies restricts understanding of local transmission dynamics. In addition, the short-term follow-up duration, without relapses, does not fully capture longer-term outcomes. Future research will be necessary to clarify the real extent and risk factors of CL in Sardasht and similar non-endemic regions using comprehensive epidemiological studies and patient longitudinal monitoring. This isolated case underscores the importance of continuous surveillance for leishmaniasis, even in areas considered non-endemic. The health system’s rapid response, including thorough screening of the patient's village of travel and city of residence, reflects proactive disease control. Further comprehensive surveys of sandflies and rodents in the area are warranted.

Conclusion

Given the occurrence of autochthonous cutaneous leishmaniasis in a previously non-endemic area, it is recommended that further comprehensive studies be undertaken. Investigation of local sand fly populations, animal reservoir hosts and parasite species, along with detailed clinical evaluation of suspected cases, will be essential to elucidate the epidemiological characteristics of the disease in this region. This highlights the importance of enhanced clinical suspicion and integrated surveillance in historically non-endemic locations. Strengthening surveillance systems and multisectoral collaboration are critical for an effective response to this emerging public health challenge.

http://jad.tums.ac.ir
Published Online: December 30, 2025

Acknowledgements

The authors thank Dr. Homa Hajjaran at the School of Public Health, TUMS, for *Leishmania* species identification, as well as Hamideh Ghasemi (microscopist) and the health workers of Urmia and Sardasht for their contributions.

Ethical consideration

This study was approved by the Ethics Committee of Urmia University of Medical Sciences (IR.UMSU.REC.1404.265) and conducted in accordance with institutional ethical standards and the principles of the Declaration of Helsinki, where applicable.

Conflict of interest statement

The authors declare there is no conflict of interest.

Generative AI statement

The authors declare that they have used Grammarly AI to improve readability and proofreading. After using this tool/service, the author has reviewed and edited the content.

References

1. World Health Organization (2023) Leishmaniasis. Switzerland, Geneva, Available at: https://www.who.int/news-room/factsheets/detail/leishmaniasis.
2. Lysenko AJ (1971) Distribution of leishmaniasis in the Old World. Bull World Health Organ. 44(4): 515–520.
3. World Health Organization (2025) Routine health information system and health facility data for neglected tropical diseases: general guidance for national programme managers and data clerks. WHO, Geneva.
4. Tabbabi A (2019) Review of leishmaniasis in the Middle East and North Africa. Afr Health Sci. 19(1): 1329–1337.
5. Sarfraz A, Sarfraz Z, Liaqat M, Khan MH, Abdul Jabbar HA, Abdullah M (2024) Leishmaniasis–still a diagnostic challenge: an individual participant data systematic review. Trop Doctor. 54(4): 352–358.
6. Sharifi I, Khosravi A, Aflatoonian MR, Salarkia E, Bamorovat M, Karamoozian A, Nekoei Moghadam M, Sharifi F, Aghaei Afshar A, Agha Kuchak Afshari S, Gharachorloo F, Shirzadi MR, Amiri B, Zainali M, Doosti S, Zamani O, Gouya MM (2023) Cutaneous leishmaniasis situation analysis in the Islamic Republic of Iran in preparation for an elimination plan. Front Public Health. 11: 1091709.
7. Mancy A, Awad KM, Hafedh Z (2024) Cutaneous leishmaniasis: the frequency according to geographical distribution in Al-Ramadi, Iraq. Our Dermatol Online. 15(2): 111–115.
8. Schönian G, Nasereddin A, Dinse N, Schweynoch C, Schallig HDFH, Presber W, Jaffe CL (2003) PCR diagnosis and characterization of *Leishmania* in local and imported clinical samples. Diagn Microbiol Infect Dis. 47(1): 349–358.
9. Hajjaran H, Mohebali M, Mamishi S, Vasigheh F, Oshaghi MA, Naddaf SR, Teimouri A, Edrissian GH, Zarei Z (2013) Molecular identification and polymorphism determination of cutaneous and visceral leishmaniasis agents isolated from human and animal hosts in Iran. Biomed Res Int. 2013: 789326.
10. Cosma C, Maia C, Khan N, Infantino M, Del Riccio M (2024) Leishmaniasis in humans and animals: a One Health approach for surveillance, prevention and control in a changing world. Trop Med Infect Dis. 9(11): 258.
11. Nasiri Z, Kalantari M, Mohammadi J, Daliri S, Mehrabani D, Azizi K (2022) Cutaneous leishmaniasis in Iran: a review

of epidemiological aspects, with emphasis on molecular findings. Parasite. 29: 47.
12. World Health Organization (2023) Global report on neglected tropical diseases 2023. WHO, Geneva.
13. Badakhshan M, Yaghoobi-Ershadi MR, Moin-Vaziri V, Charrel R, Hanafi-Bojd AA, Rezaei F, Akhavan AA, Rassi Y, Oshaghi MA (2018) Spatial distribution of phlebotomine sand flies (Diptera: Psychodidae) as phlebovirus vectors in different areas of Iran. J Med Entomol. 55 (4): 846–854.
14. Poloni A, Giacomelli A, Corbellino M, Grande R, Nebuloni M, Rizzardini G, Ridolfo AL, Antinori S (2023) Delayed diagnosis among patients with cutaneous and mucocutaneous leishmaniasis. Travel Med Infect Dis. 55: 102637.
15. Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, Jannin J, den Boer M, WHO Leishmaniasis Control Team (2012) Leishmaniasis worldwide and global estimates of its incidence. PLoS One. 7(5): e35671.