

## RESEARCH ARTICLE

## Identification of *Leishmania* species isolated from cutaneous leishmaniasis using molecular techniques in some provinces of Iraq

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### Abstract

**Objectives:** To detect *Leishmania* species in selected regions in Iraq.

**Method:** The cross-sectional study was conducted from December 2021 to March 2022 at Al-Hashimiyah General Hospital in Babylon province, and Al Diwaniya Education Hospital in AL-Qadisiah province of Iraq. The sample comprised patients with lesions suggestive of cutaneous leishmaniasis, as assessed by dermatologists. Direct smear was used for parasitological examination by lesion aspiration. Molecular technique was used for the characterisation of parasite genus and species. Positive direct smear specimens were analysed by nested-polymerase chain reaction using internal transcribed spacer-1 ribosomal ribonucleic acid gene.

**Results:** Of the 120 specimens tested, 100(83.33%) tested positive; 59(59%) males and 41(41%) females. Most of the infections were leishmania major 92(92%), followed by leishmania tropica 8(8%). There were 80(80%) dry lesions and 20(20%) wet.

**Conclusions:** Cutaneous leishmaniasis were mostly of tropica and major varieties in the studied areas.

**Keywords:** Parasites, Dermatologists, Genes, rRNA, Cutaneous, *Leishmania*, Polymerase DNA.

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### Introduction

Leishmaniasis encompasses many different diseases that are all linked to different species of leishmania, each of which has its own unique set of symptoms and physical manifestations.<sup>1,2</sup> According to the spectrum of the disease, the most frequent forms are cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL), which is more generally known as 'kala-azar'.<sup>3</sup>

CL is the most common variant of the illness in the Middle East region.<sup>4</sup> In Iraq, there are two different species, leishmania (*L.*) tropica and *L. major*. Anthropic CL (ACL) is caused by *L. tropica*, whereas zoonotic CL (ZCL) is caused by *L. major*.<sup>5</sup> The epidemiology of the disease is characterised by frequent instances of large and unexpected shifts in the total number of patients. In Iraq, the rate of CL infection ranges from 2.3 to 45.5 cases per 100,000 individuals.<sup>6</sup> A leishmania infection can induce a wide variety of symptoms, and the kind of immunological reaction that arises has a significant role in determining the severity of the disease.<sup>7</sup> The basic method of microscopic

examination for CL is the Giemsa staining procedure, and it is performed on skin scrapes or tiny needle aspirates.<sup>8</sup> Using one of several different polymerase chain reaction (PCR) methods, it is feasible to diagnose parasitic infections at the genotypic level<sup>9</sup> as both the disease's clinical manifestations and its treatment outcomes can differ from one species to another. In order to accomplish this goal, a strategy that is tailored to the unique species should be utilised.<sup>10</sup> The determination of the disease's prognosis as well as a treatment approach depends largely on correctly identifying the leishmania species.<sup>11</sup>

The current study was planned to detect leishmania species in some Iraqi regions.

### Materials and Methods

The cross-sectional study was conducted from December 2021 to March 2022 at the outpatient departments (OPDs) of the Dermatology departments of Al-Hashimiyah General Hospital in Babylon province, and Al Diwaniya Education Hospital in AL-Qadisiah province of Iraq. After approval from the institutional ethics committee of the College of Medicine, University of Kufa, Iraq, the sample size was calculated by using the Glenn D. Israel formula<sup>12</sup> with 95% confidence interval (CI) and 1.62% margin of error. The prevalence was assumed to be 0.83% on the basis of an earlier research.<sup>13</sup> The sample comprised patients with lesions suggestive of CL, as assessed by dermatologists based on the patients' symptoms and parasitological evidence (Figure 1). Skin lesions were examined at the main

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laboratory of Al Diwaniya Education Hospital by aspirating samples from the edges of the lesions, which were then subjected to direct smear to see the amastigote phase (Figure 2). The samples were kept at  $-20^{\circ}\text{C}$  for molecular analysis by nested-PCR technique.

Initially, a sample of 20 microliters (aspiration) was deposited on a glass slide and was allowed to dry for 2-3 minutes. Using methanol alcohol, the slide swab was prepared for 1min, and the swab was examined using Giemsa stain and studying it under a microscope with 100X magnification to confirm the existence of the parasite.

Using the SYAN deoxyribonucleic acid (DNA) kit (Geneaid Taiwan), DNA was extracted and analysed in accordance with the manufacturer's instructions (Geneaid Taiwan). The DNA was preserved at  $-20^{\circ}\text{C}$  for further analysis.

Primers for internal transcribed spacer-1 (ITS1) (Scientific researcher. Co. Ltd. Iraq) were used to amplify a650bp product in *L. major*, and 720bp product in *L. tropica*. External primers were Leish out F GGGGTTGGTGAAAATAGGG and Leish out R TTTGAACGGGATTCTG, while internal primers were Leish out F GGGGTTGGTGAAAATAGGG and Leish out R CAGAACGCCCTACCCG.

Nested-PCR master mix was (GoTaq® Green PCR master Mix kit) was used according to the manufacturer's instructions.

All samples were verified, and Nested-PCR was performed. The overall volume of component of primary reaction was  $25\mu\text{L}$ , involving  $5\mu\text{L}$  DNA template,  $2\mu\text{L}$  of both PCR kinetoplast DNA (kDNA) primers,  $12.5\mu\text{L}$  PCR master mix and  $3.5\mu\text{L}$  PCR water. For the first round of PCR, first denaturation was done at  $95^{\circ}\text{C}$  for 5min, followed by 30 cycles of denaturation at  $95^{\circ}\text{C}$  for 30s, annealing at  $55^{\circ}\text{C}$  for 30s, extension at  $72^{\circ}\text{C}$  for 1min, and final extension at  $72^{\circ}\text{C}$  for 5min. The PCR materials from the positive controls of the first round were used in the second round with specifications similar to the first round.

Loading PCR materials in 1.5% for 1 hour, an agarose gel containing 3L of ethidium bromide and an electrical current of 100v, 80mA were observed.

## Results

Of the 120 specimens tested, 100(83.33%) tested positive; 59(59%) males and 41(41%) females. There were 80(80%) dry lesions and 20(20%) wet ones (Table).

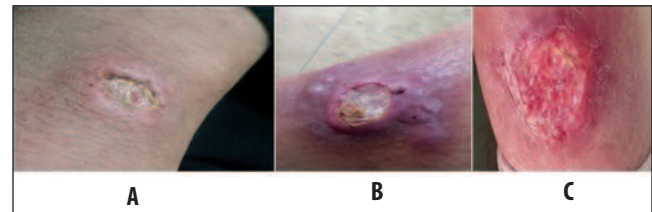
On the basis of agarose gel electrophoresis, 92(92%) infections were *L. major*, and 8(8%) *L. tropica* (Figure 3).

*Leishmania* species from skin lesion samples showed some positive samples at 350bp product size (Figure 4).

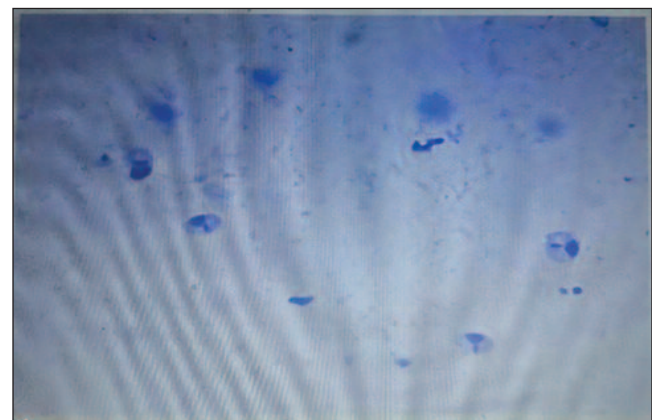
**Table:** CL cases and types of lesions registered in different provinces of Iraq in 2021-22.

Type of lesion	n (%)
Dry CL lesion	80 (80)
Wet CL lesion	20 (20)
Total	100 (100)

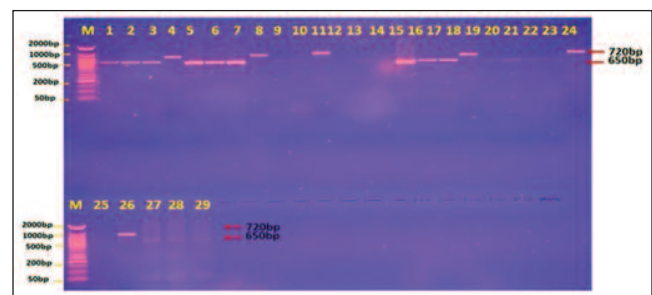
CL: Cutaneous leishmaniasis.



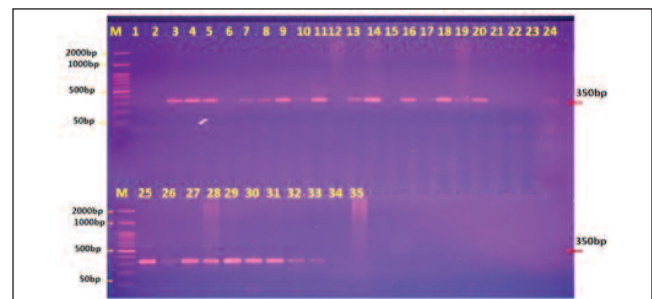
**Figure 1:** Patients with cutaneous leishmaniasis (CL).



**Figure 2:** Direct skin smear (Giemsa stain 100X) showing amastigotes in lesion aspiration.



**Figure 3:** Agarose gel electrophoresis.



**Figure 4:** Some polymerase chain reaction (PCR) products from agarose gel electrophoresis.

## Discussion

Traditionally, microscopic inspection and in vitro cultivation are used to diagnose CL. These traditional approaches show a significantly large number of alive or morphologically complete parasites, which is a challenge, particularly in the chronic phase of CL. The molecular technique, on the other hand, is both sensitive and specific.<sup>14</sup> The current study established a well-documented genus-specific PCR in order to detect leishmania species in clinical cutaneous samples. It subsequently compared the technique with the traditional methods. Giemsa-stained smear PCR-based testing appeared to be reasonably sensitive and specific for detecting leishmania parasites in chronic lesions. There is little doubt that PCR can assist in making a more accurate diagnosis of CL in such circumstances. According to the DNA bands (Figure 3), 8 samples were *L. tropica* isolates with band sizes of 720bp, and 92(93.3%) were *L. major* isolates with band sizes of 650bp. This suggests that CL foci of *L. major* and *L. tropica* can coexist in the same area at least under certain conditions. The matter, however, needs further exploration. The current findings are consistent with some earlier reports<sup>15-17</sup> but contrasting findings have also been reported.<sup>18,19</sup>

The distribution of wet and dry types of CL in the current study are consistent with some studies<sup>20,21</sup> while some studies have reported different findings.<sup>7,22</sup>

On the basis of the current findings, use of nested-PCR to identify leishmania species is recommended.

## Conclusions

*L. major* and *L. tropica* were found to be the two most common causes of CL in the studied Iraqi regions. Besides, PCR was found to be a reliable tool for diagnosing and identifying leishmania species.

**Disclaimer:** None.

**Conflict of Interest:** None

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