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Original Research Article

Molecular detection and species identification of *Leishmania* in phlebotomine sand flies and human cutaneous lesions in Thi-Qar Province, Iraq

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Abstract

Background: Cutaneous Leishmaniasis remains a major public health issue in Thi-Qar Province, and comprehensive molecular data on circulating *Leishmania* species and vector distribution are limited. This study provides molecular identification of *Leishmania* species in human lesion samples and naturally infected sand flies and examines their infection rates and distribution patterns. To identify sand fly species, detect *Leishmania* DNA in human and sand fly samples using molecular techniques, and determine infection rates and species diversity in Thi-Qar Province.

Materials and Methods: A total of 385 sand flies were collected using CDC light traps and sticky traps from May to October 2024. Morphological identification was performed using standard taxonomic keys. DNA was extracted from sand fly specimens and human lesion samples, and molecular detection was conducted using PCR targeting the ITS1 and kDNA regions. Gel electrophoresis was used for visualization. Statistical analysis was performed using SPSS 26.

Results: *Phlebotomus papatasi* was the predominant species (78.4%), followed by *P. sergenti* (15.3%) and P. alexandri (6.3%). *Leishmania* DNA was detected in 23.6% of sand flies. Identified species included *L. major* (76.9%), *L. tropica* (18.7%), and *L. donovani* complex (4.4%). Human lesion samples showed a 100% positivity rate for *Leishmania* DNA. Infection rates were highest in *P. papatasi* (28.4%).

Conclusion: Multiple *Leishmania* species circulate in Thi-Qar Province, with *L. major* predominating. The molecular approach demonstrated high sensitivity for detecting infections and provides essential baseline data for surveillance and vector control efforts.

Keywords: Cutaneous, Epidemiology, *Leishmania*, Leishmaniasis, Molecular detection, *Phlebotomus*, Polymerase chain reaction.

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1. Introduction

Leishmaniasis is a vector-borne disease caused by protozoan parasites of the genus *Leishmania* and transmitted through the bite of infected female phlebotomine sand flies.[1] It disproportionately affects vulnerable populations and is closely linked to factors such as poverty, malnutrition, population displacement, inadequate housing, and weakened immunity.[2] The disease presents in three major clinical forms: cutaneous, mucocutaneous, and visceral Leishmaniasis, with cutaneous Leishmaniasis being the most common worldwide.[3]

Iraq is considered an endemic region for Leishmaniasis, particularly cutaneous forms, and has experienced a notable rise in cases in recent decades due to conflict, displacement, and deteriorating living conditions.[4] Several sand fly species in Iraq act as vectors, with *Phlebotomus papatasi* recognized as the primary vector of Leishmania major.[5] This species is widely distributed across West Asia, North Africa, and Southern Europe.

Traditional detection of *Leishmania* in sand flies relies on microscopic examination and dissection, methods that require technical expertise and have limited sensitivity.[6] In recent years, molecular techniques particularly polymerase chain reaction (PCR) have significantly improved the detection and identification of *Leishmania* species due to their high sensitivity and specificity.[7]

Despite the public health importance of Leishmaniasis in Iraq, molecular studies on sand fly vectors in Thi-Qar Province remain limited.[8] Previous research in the region has addressed phenotypic and molecular characteristics of sand flies and diagnostic approaches for cutaneous Leishmaniasis, but comprehensive vector surveillance is still lacking.[9]

The present study aims to identify sand fly species circulating in Thi-Qar Province using morphological and molecular methods, determine their infection rates with *Leishmania* parasites, and assess the diversity of *Leishmania* species present.[10] This work provides essential baseline data for understanding current transmission dynamics and strengthening regional surveillance programs.

2. Materials and Methods

2.1. Research design

This study employed a descriptive cross-sectional research design integrating clinical, entomological, and molecular methodologies to investigate the occurrence and distribution of *Leishmania* species in human cutaneous lesions and phlebotomine sand fly vectors in Thi-Qar Province, southern Iraq. The design enabled concurrent assessment of human infections, sand fly species composition, and vector infection

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rates within defined temporal and ecological settings. A molecular diagnostic approach was adopted to enhance sensitivity and specificity compared with conventional microscopic methods.

2.2. Study area

The study was conducted in Thi-Qar Province, located in southern Iraq, an area recognized as endemic for cutaneous Leishmaniasis. The province is characterized by an arid to semi-arid climate, with extremely hot summers and mild winters. These climatic conditions, together with mixed urban and rural settlements, extensive agricultural activity, and close proximity between humans and animal shelters, create favorable environments for phlebotomine sand fly breeding and disease transmission. Human sampling was carried out at the Dermatology Clinic of Thi-Qar Teaching Hospital in Nasiriyah city, while entomological sampling was conducted in multiple endemic localities across the province.

2.3. Human participants

Human sampling was conducted between November 2023 and January 2025. A total of 93 patients clinically suspected of having cutaneous Leishmaniasis were enrolled using a consecutive sampling strategy. The study population included 62 newly diagnosed, untreated patients and 31 patients who had been previously diagnosed and were receiving treatment at the time of enrollment.

Inclusion criteria were the presence of active skin lesions consistent with cutaneous Leishmaniasis and microscopic confirmation of amastigotes. Patients who declined to provide informed consent or had insufficient clinical data were excluded. Demographic and clinical information recorded for each participant included age, sex, place of residence (urban or rural), occupation, number of lesions, lesion type, and anatomical location.

2.4. Sand fly sampling

Sand fly sampling was conducted from May to October 2024, corresponding to the peak seasonal activity of phlebotomine sand flies in southern Iraq. A total of 385 sand flies were collected using purposive ecological sampling from areas reporting human cases of cutaneous Leishmaniasis. Sampling sites included human dwellings, animal shelters, agricultural fields, gardens, and peri-domestic environments to ensure representative coverage of diverse ecological niches.

The difference in sampling periods between human cases and sand flies reflects the seasonal nature of vector activity, whereas human cases present throughout the year due to prolonged incubation and lesion persistence.

2.5. Data collection and procedures

2.5.1. Clinical diagnosis and human sample collection

Clinical diagnosis was established through dermatological examination and confirmed by microscopic detection of amastigote forms following Giemsa staining of lesion smears. For molecular analysis, lesion samples were obtained under aseptic conditions. The lesion margin was disinfected with 70% ethanol, and a sterile surgical blade was used to scrape the active edge of the lesion. Exudate and dermal tissue were collected and transferred into sterile tubes containing normal saline. Samples were stored at −20°C until DNA extraction. In selected cases, additional samples were collected using a sterile dental broach inserted into the lesion to increase diagnostic yield.

2.5.2. Sand fly collection

Sand flies were collected using two standard entomological methods: CDC miniature light traps and sticky paper traps. Light traps were operated overnight from dusk to dawn, while sticky traps were placed near resting sites such as walls, cracks, animal shelters, and vegetation. Collected sand flies were carefully removed and preserved in 70% ethanol supplemented with a small amount of glycerin to maintain specimen flexibility and morphological integrity. Specimens were transported to the laboratory and stored at 4°C until further processing.

2.5.3. Morphological identification of sand flies

Sand flies were examined under a stereomicroscope and identified using standard morphological taxonomic keys. Identification was based on diagnostic features including wing venation, antennal segmentation, cibarial and pharyngeal armature, spermathecae in females, and genital structures in males. Specimens were identified to genus and species level, and sex was recorded for each specimen. Morphological identification was independently verified by experienced personnel to ensure accuracy.

2.5.4. DNA extraction

Genomic DNA was extracted from both human lesion samples and individual sand fly specimens using the Geneaid DNA Extraction Kit, following the manufacturer's instructions. For sand flies, whole specimens were homogenized prior to extraction. For human samples, lesion material was centrifuged, and the resulting pellet was used for DNA extraction. DNA was eluted in nuclease-free water and stored at −20°C until molecular analysis.

2.5.5. Molecular detection of leishmania

Molecular detection of *Leishmania* DNA was performed using polymerase chain reaction (PCR) assays targeting two genetic markers. Kinetoplast DNA (kDNA) was used for highly sensitive detection of *Leishmania*, while the internal transcribed spacer 1 (ITS1) region was used for species identification. Primer sets were selected based on previously validated protocols reported in the literature.

PCR reactions were carried out in a final volume of 20 µL containing 5 µL PCR premix, 1 µL forward primer (10 pmol/µL), 1 µL reverse primer (10 pmol/µL), 2 µL template DNA, and 11 µL nuclease-free water. Thermal cycling conditions consisted of an initial denaturation at 94°C for 2 minutes, followed by 33 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 80 seconds, with a final extension at 72°C for 5 minutes.

Separate PCR reactions were performed for kDNA and ITS1 targets. Each PCR run included a positive control containing reference *Leishmania* DNA and a negative control containing no DNA template to monitor assay performance

and detect contamination.

2.5.6. Validation and quality control

Methodological validation was ensured through the use of standardized protocols, inclusion of appropriate positive and negative controls in all PCR assays, and repetition of selected samples to confirm reproducibility. Morphological identification of sand flies was cross-checked, and molecular results were validated by the presence of amplicons corresponding to expected fragment sizes. Strict laboratory procedures were followed to prevent cross-contamination during DNA extraction and PCR setup.

2.5.7. Gel electrophoresis

PCR products were analyzed by electrophoresis on 1.5% agarose gels prepared in 1× TAE buffer and stained with ethidium bromide. Electrophoresis was performed at 100 V for approximately 60 minutes. DNA bands were visualized under ultraviolet illumination and documented using a gel documentation system. A 100 bp DNA ladder was used as a molecular size marker to confirm amplicon sizes.

2.6. Data analysis

Data were entered into Microsoft Excel and analyzed using SPSS software version 26.0. Descriptive statistics were used to summarize demographic characteristics, sand fly species distribution, and infection rates. Infection rates were calculated as the proportion of PCR-positive sand flies relative to the total number examined. Associations between sand fly species, infection status, and collection sites were evaluated using the Chi-square test. Statistical significance was set at $p < 0.05$.

2.7. Ethical approval

The study was approved by the Institutional Ethics Committee of Thi-Qar Teaching Hospital (Approval No. IEC/TQTH/2023/019). All participants provided written informed consent prior to enrollment. Confidentiality and anonymity of participant data were strictly maintained throughout the study, and all procedures were conducted in accordance with ethical standards for research involving human participants.

3. Results

3.1. Sand fly collection and species composition

A total of 385 phlebotomine sand flies were collected between May and October 2024 from endemic localities in Thi-Qar Province. CDC miniature light traps accounted for the majority of specimens (73.8%, n = 284), while sticky paper traps yielded 26.2% (n = 101). Sand fly abundance showed clear seasonal variation, with the highest collections recorded during July and August, corresponding to peak summer temperatures.

Morphological identification revealed three *Phlebotomus species*. *Phlebotomus papatasi* was the predominant species, representing 78.4% (n = 302) of all collected specimens, followed by *P. sergenti* at 15.3% (n = 59) and *P. alexandri* at 6.3% (n = 24). Female sand flies constituted 56.4% (n = 217) of the total collection, while males accounted for 43.6% (n = 168). *P. papatasi* was detected at all sampling sites, whereas *P. sergenti* was more frequently encountered in urban residential settings.

Table 1. *Summarizes sand fly collection by trapping method, species, and sex*

Characteristic	Number Collected	Percentage (%)
By Collection Method		
CDC Light Trap	284	73.8
Sticky Trap	101	26.2
By Species		
**Phlebotomus* papatasi*	302	78.4
Phlebotomus sergenti	59	15.3
Phlebotomus alexandri	24	6.3
By Sex		
Female	217	56.4
Male	168	43.6
Total Collected	385	100.0

3.2. Molecular detection of leishmania in human samples

All 93 human lesion samples subjected to molecular analysis were positive for *Leishmania* DNA by nested PCR targeting the ITS1 region, yielding a positivity rate of 100%. This finding confirms the molecular presence of *Leishmania* in all clinically and microscopically diagnosed cases.

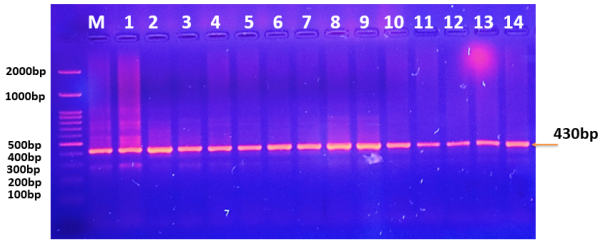

Figure 1: *PCR amplification of ITS1 region from human lesion samples. M: DNA marker (2000–100 bp); lanes 1–14: positive Leishmania bands at ~430 bp*

Figure 1 presents representative PCR amplification results from 14 randomly selected samples, demonstrating the expected ITS1 amplicon size of approximately 430 bp. The remaining samples yielded identical amplification profiles (data not shown), confirming assay consistency and reproducibility.

3.3. Molecular detection of leishmania in sand fly vectors

PCR screening of sand fly specimens targeting the ITS1 region revealed that 91 out of 385 sand flies were positive for *Leishmania* DNA, corresponding to an overall infection rate of 23.6%. Infection prevalence varied significantly among Phlebotomus species.

Phlebotomus papatasi exhibited the highest infection rate, with 86 of 302 specimens testing positive (28.5%). In contrast, *P. sergenti* showed a markedly lower infection rate (5 of 59; 8.5%), while no *Leishmania* DNA was detected in P. alexandri specimens. The difference in infection rates among species was statistically significant (Chi-square test, $p < 0.05$).

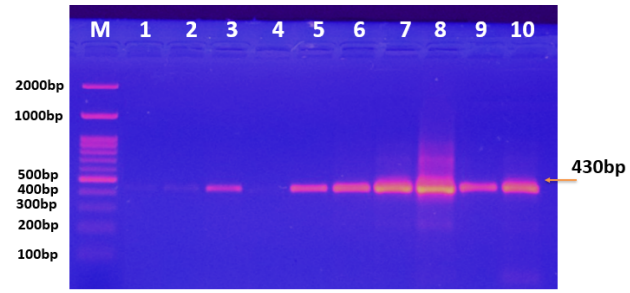

Figure 2: *PCR amplification of ITS1 region from human lesion samples. M: DNA marker (2000–100 bp); lanes 1–14: positive Leishmania bands at ~430 bp*

Figure 2 shows representative ITS1 PCR results from infected sand fly samples, illustrating amplification patterns consistent with those observed in human samples. Table 2 presents the distribution of *Leishmania* infection rates by sand fly species.

Table 2. *Distribution of Leishmania infection rates among phlebotomine sand fly species collected in Thi-Qar Province*

Sand fly species	Number examined (n)	PCR-positive (n)	Infection rate (%)
Phlebotomus papatasi	302	86	28.5
Phlebotomus sergenti	59	5	8.5
Phlebotomus alexandri	24	0	0.0
Total	385	91	23.6

Note: Infection rates were calculated as the proportion of PCR-positive specimens relative to the total number examined for each species. Differences in infection prevalence among sand fly species were statistically significant (Chi-square test, $p < 0.05$).

3.4. Molecular identification of leishmania species

Species-level identification based on ITS1 polymorphism analysis revealed the circulation of multiple *Leishmania* species within sand fly populations. Among PCR-positive sand flies, *Leishmania* major was the most frequently detected species, accounting for 76.9% (n = 70) of infections. *Leishmania tropica* was identified in 18.7% (n = 17) of infected sand flies, while the *L. donovani* complex was detected in 4.4% (n = 4).

Table 3. *Distribution of Leishmania species identified in PCR-positive phlebotomine sand fly vectors*

Leishmania species	PCR-positive sand flies (n)	Percentage (%)
Leishmania major	70	76.9
Leishmania tropica	17	18.7
Leishmania donovani complex	4	4.4
Total	91	100.0

Note: Percentages were calculated based on the total number of PCR-positive sand fly specimens (n = 91). Species identification was performed using ITS1-based molecular analysis.

L. major was predominantly associated with *P. papatasi*, whereas *L. tropica* was detected mainly in P. sergenti. The *L. donovani* complex was detected at low frequency and exclusively in *P. papatasi* specimens. Table 3 summarizes the distribution of *Leishmania* species identified in sand fly vectors.

3.5. Molecular confirmation of sand fly identity

To validate morphological identification, a subset of 16 sand fly specimens representing different species and collection sites was subjected to PCR amplification of the mitochondrial cytochrome b (Cytb) gene. All tested specimens produced the expected 359 bp amplicon, confirming their taxonomic identity as phlebotomine sand flies.

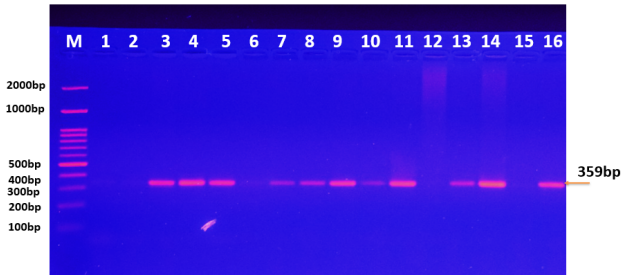

Figure 3: *PCR amplification of mitochondrial Cytb gene in sand fly samples. M: DNA marker; lanes 1–16: positive bands at ~359 bp*

Figure 3 presents representative Cytb amplification results. Molecular identification showed complete concordance (100%) with morphological classification, supporting the reliability of the morphological identification methods used in this study.

4. Discussion

This study provides the first comprehensive molecular investigation of *Leishmania* parasites in phlebotomine sand flies from Thi-Qar Province, adding critical evidence to the understanding of cutaneous Leishmaniasis transmission in southern Iraq.[11] The detection of three *Phlebotomus* species *P. papatasi*, *P. sergenti*, and *P. alexandri* and the identification of multiple *Leishmania* species highlight the complexity of vector–parasite interactions in the region.[12] The predominance of P. papatasi and the high infection rate detected using molecular diagnostic tools support its role as the primary vector of *L. major* in the area.[13] These results confirm the presence of diverse *Leishmania* species circulating in local sand fly populations and emphasize the importance of molecular methods in improving detection sensitivity and specificity.

4.1. Comparison with faunistic and epidemiological studies

The identification of three Phlebotomus species in this study aligns with previous faunistic research conducted in Iraq and neighbouring countries. *P. papatasi*, widely distributed across Western Asia, North Africa, and Southern Europe, is historically recognized as the principal vector for *L. major*.[14] Its predominance in our collections (78.4%) is consistent with its ecological adaptation to arid and semi-arid environments, where cutaneous Leishmaniasis is prevalent.[15] Our findings corroborate earlier studies documenting the species' abundance and vectorial capacity in similar ecological zones.

Seasonal variation in sand fly populations observed in this study peaking in July and August reflects known patterns

of sand fly activity influenced by climatic conditions.[16] Warm temperatures and increased humidity during summer reportedly provide optimal conditions for sand fly breeding and development.[17] Understanding these seasonal trends allows health authorities to anticipate peak transmission periods and plan targeted vector control interventions accordingly.

4.2. Molecular detection and infection rates

The overall infection rate of 23.6% among sand flies in this study is notably higher than infection rates reported in several Middle Eastern studies, including those from Iran.[18] However, direct comparisons should be approached with caution due to the variability in collection methods, ecological differences, and diagnostic techniques employed across studies.[19] Nevertheless, the elevated infection rate may suggest heightened transmission activity within Thi-Qar Province during the study period.

The significantly higher infection prevalence in *P. papatasi* (28.5%) compared to other species supports its role as the primary vector in the region.[20] In contrast, the lower infection rate observed in *P. sergenti* (8.5%) may reflect reduced vector competence or limited exposure to infected reservoir hosts.[21] Together, these results provide insight into the varying degrees of vector importance among sand fly species and highlight the ecological differences in parasite transmission cycles.

4.3. Detection of multiple leishmania species

The molecular detection of three *Leishmania* species *L. major*, *L. tropica*, and the *L. donovani* complex demonstrates the presence of diverse parasite strains circulating within Thi-Qar Province [22]. The predominance of *L. major* in infected sand flies (76.9%) is consistent with its typical association with P. papatasi and rodent reservoirs. *L. major* is widely reported in zoonotic cutaneous Leishmaniasis foci across the Middle East and North Africa, further supporting the locality's epidemiological profile.

The detection of *L. tropica* (18.4%) in sand flies suggests active anthroponotic transmission cycles, as this species is commonly linked to urban environments and human reservoirs.[23] Its presence may indicate increasing urban transmission or movement of infected individuals into the region.

The identification of DNA belonging to the *L. donovani* complex although limited to 4.6% of infected samples raises important considerations. *L. donovani* is typically associated with visceral Leishmaniasis, a potentially fatal form of the disease.[24] While the current findings do not confirm active visceral transmission, they underscore the need for heightened surveillance, as sporadic or emerging visceral cases could occur in the region.

4.4. Vector–parasite relationships and transmission dynamics

The strong association between *P. papatasi* and *L. major* observed in this study reinforces the well-established vector–parasite compatibility [25]. Laboratory and field studies have demonstrated that *P. papatasi* is highly competent for *L. major* due to specific molecular interactions that facilitate parasite development within the sand fly midgut.[26]

Interestingly, the detection of *L. tropica* in *P. papatasi* and rare detection of *L. major* in *P. sergenti* may indicate potential shifts in vector roles or increased ecological overlap among sand fly species and parasite strains.[27] Such findings suggest possible secondary vector roles, adaptive flexibility in parasite development, or changing environmental conditions that alter vector–host interactions.[28] These observations warrant further investigation through longitudinal studies to determine whether these patterns reflect transient events or emerging epidemiological trends.

4.5. Strengths and methodological significance

A major strength of this study is the use of molecular techniques particularly PCR amplification of kDNA and ITS1 regions which offer superior sensitivity compared to traditional microscopic methods.[29] The ability to detect low-level infections is crucial for identifying early or subclinical parasite presence in vector populations. The successful amplification of the mitochondrial Cytb gene further validates the morphological identification of sand fly species and ensures accuracy in vector classification.

Advances in multiplex real-time PCR assays present promising future tools for simultaneously detecting multiple *Leishmania* species and quantifying parasite loads.[30] Such techniques could substantially enhance surveillance efficiency and improve detection of mixed infections or emerging strains.

5. Conclusions

This study provides molecular evidence of *Leishmania* circulation in phlebotomine sand flies in Thi-Qar Province, southern Iraq, and confirms Phlebotomus papatasi as the predominant vector, strongly associated with *Leishmania* major. The detection of *L. tropica* and the *L. donovani* complex indicates the coexistence of multiple *Leishmania* species and suggests the presence of both zoonotic and anthroponotic transmission cycles in the region.

PCR-based detection targeting the ITS1 and kDNA regions proved to be a sensitive and reliable approach for identifying *Leishmania* infections in both human lesions and sand fly vectors. These findings provide essential baseline data for local surveillance and highlight the value of molecular tools in supporting targeted vector control and Leishmaniasis management strategies.

6. Limitations

The study was limited to a single transmission season, which may not reflect interannual variations in sand fly abundance or infection rates. In addition, the relatively small numbers of *P. sergenti* and *P. alexandr*i constrained detailed statistical comparisons among vector species. Molecular detection by PCR does not distinguish between viable and non-viable parasites; therefore, detected infections may not directly indicate transmission potential.

As the study was confined to Thi-Qar Province, the findings may not represent the epidemiological situation in other regions of Iraq. Future studies incorporating multi-season sampling, larger vector populations, and complementary methods to assess parasite viability would

strengthen understanding of transmission dynamics at both regional and national levels.

7. Author Contributions

1. Zainab HJ Alhassona: Conceptualization, Formal analysis, Methodology, Writing – original draft.
2. Nadia K. Thamer: Formal analysis, Resources, Writing – original draft.
3. Ghazi Yaqoob Azzal: Data curation, Supervision, Visualization, Writing – original draft.

8. Source of Funding

This research received no external funding.

9. Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

10. Ethical Approval

This study was approved by the Institutional Ethics Committee of Thi-Qar Teaching Hospital under ethical approval number IEC/TQTH/2023/019. All procedures involving human participants were conducted in accordance with institutional guidelines and ethical standards. Written informed consent was obtained from all participants prior to sample collection. No animal experiments were performed in this study.

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