Isolation and molecular identification of cutaneous leishmaniasis in humans and dogs in middle Euphrates, Iraq

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Abstract

In Iraq, one of the endemic illnesses is cutaneous Leishmaniasis. This study aimed to characterize local isolates of *Leishmania tropica* and *Leishmania major* molecularly and determine how closely related they were to reference isolates from nearby nations. A total of 140 and 60 skin lesion samples were collected from patients and dogs, respectively, from September 2021 to March 2022; molecular methods carried out to achieve the prevalence of cutaneous Leishmaniasis in humans and dogs, Nested PCR was done using the kDNA gene for phylogenetic analyses. The overall prevalence of cutaneous Leishmaniasis was 35% and 88.33% in humans and dogs, respectively; the findings showed the total prevalence of *Leishmania major* significant in dogs was 71.69% compared to *Leishmania tropica* was 28.35% with significant differences. Fifteen positive samples (Ten human and five dogs) were sequencing to Gen-bank database for phylogenetic analyses, which detected that seven of local isolates skin lesion human samples belongs to *Leishmania major* isolates IQ Kut isolates, Iraq and three isolates belongs to *Leishmania tropica* isolates IQ3, Iraq. Four of the local isolates skin lesion dog samples belong to *Leishmania major* isolates IQ Kut, Iraq, and one isolate belong to *Leishmania tropica* isolates IQ-7 Iraq. Determining many *Leishmania major* in humans and dogs indicates that dogs are key parasite reservoirs and significant zoonotic contributes to disease transmission to humans in the Middle Euphrates, Iraq.

Introduction

Intracellular flagellate protozoa of the *Leishmania* genus causes Leishmaniasis, the vector-borne illness (1). All *Leishmania* species are spread to people and other animals by bites from infected insects, sandflies (*Phlebotominae*) (2,3). There are three basic types of Leishmaniasis: cutaneous (CL), mucocutaneous (ML), and visceral (VL) (4). Worldwide, CL is the most prevalent kind of Leishmaniasis. More than 350 million humans are at risk of contracting CL, which has been detected in 98 countries (5,6). Worldwide travel has increased the frequency of leishmaniasis cases in non-native nations. Anthropogenic Leishmaniasis and zoonotic Leishmaniasis are the two types

of cutaneous Leishmaniasis, and they are both brought on by *L. tropica* and *L. major* subsequently (4,7). Unusual CL forms like ocular Leishmaniasis and uncommon forms with many nodular, ulcerative lesions that had distribution to the face, trunk, and limbs, as well as ulcers on the lower lip and around the penis, are occasionally caused by *L. major* (8,9). The infection caused by *L. tropica* is sometimes referred to as the Delhi boil, Baghdad boil, or oriental sore; it is recognized by the appearance of a skin lesion varying in size from a little pimple to a huge sore, usually found on the face, hands and legs (10). The amastigote and promastigote are the two types of the parasite *Leishmania* that exist; amastigote is situated in vertebral (intracellular) hosts, whereas promastigote is present in vector (extracellular) in addition

to in cultural media (11). The prevalence of cutaneous Leishmaniasis in Iraq has been the subject of several investigations Al Samarai and AlObaidi (12) found that the infection rate was 57%. Salah Al-Din had 468 instances in other places (13).

Leishmaniasis management programs in areas with endemic Leishmaniasis require epidemiological investigations, accurate parasite species identification, and research into the genetic diversity of the parasites. Despite the disease's endemicity, few studies in Iraq focus on phylogenetic analysis and molecular characterization of *L. tropica* and *L. major.*

Materials and methods

Ethical approval

It was obtained from the guidance of research, Publication and Ethics of College of Medicine, University of Babylon, Iraq (No. BMS/0231/016), which complies with the relevant Iraqi legislations.

Samples collection

One hundred and forty skin lesion samples were collected from patients of both sexes and different ages from four hospitals (Al-Diwaniyah teaching, Al-Hashimih general, Al-Sad,r and Al-Kafil) from single and multiple lesions. Sixty dog skin lesion samples were randomly collected from Middle Euphrates provinces (Babylon, Al-Diwaniyah, Al-Najaf AL Ashraf, and Karbala) of both sexes and different ages during the period from 1 September 2021 to 31 March 2022 for both humans and dogs.

Normal saline was injected into anticipated skin infection lesions (5-10 mm in diameter), drawn out again and the fluid was then maintained in the plain tube (14), transported to the Laboratory of Medical Laboratory Techniques, and stored in the refrigerator for DNA extraction.

DNA extraction

The DNA mini kit from Geneaid in the USA was used to extract genomic DNA from frozen wound skin lesion samples. Proteinase K was used for cell lysis during the extraction per the manufacturer's instructions. The extracted gDNA was then verified using a Nanodrop spectrophotometer before being kept in a refrigerator at -20C until it was utilized in PCR amplification.

PCR (polymerase chain reaction)

The nested PCR test was accomplished utilizing a specific primer for the genus leishmania's kinetoplast DNA (kDNA), which comprises the 750 bp PCR product for *L. tropica* and the 560 bp PCR product for *L. major* were amplified utilizing the external primers CSB2XF and CSB1XR and the internal primers 13Z and LiR (Table 1).

Table 1: Contain the name, type, and sequence of the primers utilized in the current study

Type	Name	Sequence	Reference
External primers	CSB2XF	5′- CGAGTAGCAGAAACTCCCGTTCA-3′	15
	CSB1XR	5′- ATTTTTCGCGATTTTCGCAGAACG-3′	
Internal primers	13Z	5′- ACTGGGGGGTTGGTGTAAAATAG-3′	15
	LiR	5′- TCGCAGAACGCCCCT-3′	

Sequence analyses

Sequencing DNA sequencing analyses were achieved utilizing Molecular Evolutionary Genetics Analyses version 6.0 (Mega 6.0), and multiple sequence alignment analyses of the partial kDNA gene depend Clustal W alignment analysis evolutionary distances Composite Likelihood manner by phylogenetic tree UPGMA approach.

Statistical analyses

Chi-square (X2) was used in statistical studies to determine whether variables were calculated utilizing the Maximum independence. Values of less than or equal to 0.05 were considered statistically significant by the IBM Statistical Package for Social Sciences (SPSS) statistical soft war, version 31.0 (16).

Results

The total infection rate of cutaneous leishmaniasis in humans and dogs according to the species and lesions shown in (Figures 1 and 2). Nested PCR showed that the overall prevalence of cutaneous Leishmaniasis in humans was 35%, while 88.33% in dogs (Table 2).

Findings showed that the total infection rate of *Leishmania major* in humans was 55.1% compared to *Leishmania tropica* which was 44.89% without significant differences P>0.05. In dogs, *Leishmania major* was 71.49%, while *Leishmania tropica* was 28.35%, with significant differences P<0.05 (Table 3).
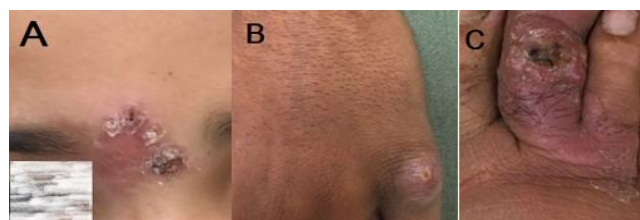

Figure 1: In Humans; A- Scaly crusted lesion in the face, B- Single scaly lesion in hand, C- Ulcerated scaly lesion on foot.

Figure 2: In Dogs, A- Scaly multiple lesions in the nose, B- Single scaly lesion in the neck, and C- multiple lesions in legs.

Table 2: Total infection rate of cutaneous Leishmaniasis in humans and dogs using nested PCR

Host	No. of samples examined	No. of +ve samples	Percentage (%)
Human	140	49	35
Dogs	60	53	88.33

Table 3: Total infection rate of cutaneous Leishmaniasis in humans and dogs according to the species

Leishmania species		No. of samples examined	No. of +ve samples	Percentage (%)	X^2	P value
Human	*L. major*		27	55.1	1.02	0.312
	L. tropica	49	22	44.89		(NS)
Dogs	*L. major*		38	71.69	19.96	<0.0001
	L. tropica	53	15	28.35		(S)

NS: No significant differences at P<0.05. S: Significant differences at P<0.05.

To identify the species of cutaneous Leishmaniasis, the amplified gene products from skin lesion samples from humans and dogs were subjected to nested PCR utilizing kDNA gene primers. Agarose gel runs validated the results and showed separate bands of *L. major* and *L. tropica* at 560 and 750 bp, respectively (Figure 3 and 4).
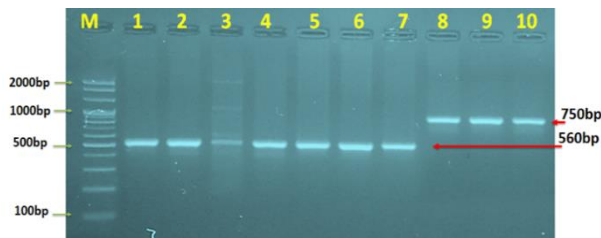

Figure 3: Image from an agarose gel electrophoresis showing the detection of kDNA by nested PCR in isolates from human skin lesion samples that are positive for Cutaneous *Leishmania*. Where M is a marker (2000-100 bp), lanes 1-7 include positive *L. major* PCR products (560 bp), and lanes 8-10 contain positive *L. tropica* PCR products (750 bp).

Genotype of *Leishmania* species in humans and dogs

Fifteen samples were positive from each (humans and dogs) after n-PCR amplification of the kDNA gene of *Leishmania* species was successfully sequenced. The genomic homologous sequencing identity ranged from 99.74-99.88% between isolates of human *L. major* (OM275415.1 to OM275421.1), and NCBI-Genebank linked *L. major* (OP046364.1) (Table 4). *L. tropica* human isolates (OM275422.1 to OM275424.1) were determined to be closely linked to NCBI- BLAST *L. tropica* (OK359041.1) noticed genomic homologous sequencing identity ranged from 99.74-99.87% (Table 5). Genomic homologous sequencing identity ranged from 99.17-99.67% between

isolates of dogs *L. major* (ON745766.1 to ON745768.1), and NCBI-Gene-bank linked *L. major* (OP046364.1). *L. tropica* dog isolates (ON745770.1) were shown to be nearly linked to NCBI -BLAST *L. tropica* (MH511156.1), which showed genomic homologous sequencing identity 99.34% (Table 6).
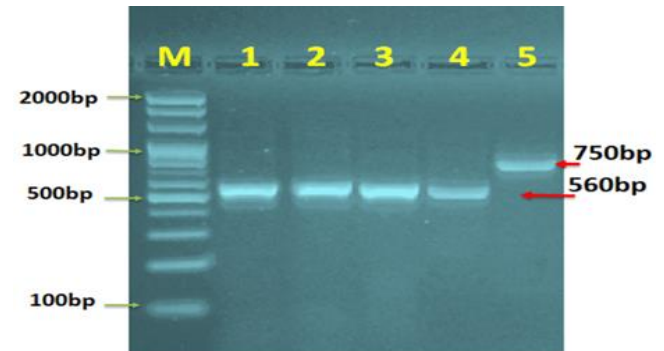

Figure 4: Image from an agarose gel electrophoresis showing the detection of kDNA by nested PCR in isolates from dog skin lesion samples that are positive for Cutaneous *Leishmania*. Where M is a marker (2000-100 bp), lanes 1-4 include positive *L. major* PCR products (560 bp), and lane (5) contain positive *L. tropica* PCR products (750 bp).

Phylogenetic analyses

Phylogenetic tree analyses depend on the kDNA gene partial sequence in *Leishmania* species local isolates and related *Leishmania* species isolates from the NCBI Gene bank. Local *L. major* human isolates OM275415.1 to OM275421.1 were shown an identity to NCBI -BLAST *L. major* isolate IQ-KUT Iraq isolates (OP046364.1) (Figure 5 and 6) and local *L. tropica* human isolates OM275422.1 to OM275424.1 were shown an identity to NCBI -BLAST *L. tropica* isolate IQ3 Iraq (OK359041.1) (Figure 7 and 8).

Local *L. major* dog isolates ON745766.1 to ON745768.1 were shown an identity to NCBI -BLAST *L. major* isolate IQ-KUT kinetoplast, Iraq isolates (OP046364.1) and local *L.* *tropica* dog isolates ON745770.1 were shown an identity to NCBI -BLAST *L. tropica* isolates IQ-7 isolate Iraq (MH511156.1) (Figure 9 and 10).

Table 4: The NCBI-BLAST Homology Sequence identity (%) between local *Leishmania major* Human isolates and NCBI-BLAST isolates

Isolate No.	Gen-bank Accession number	NCBI-BLAST Homology Sequence Identity %			
		Identical Isolate	Country	Accession No.	Identity
IQ-Human No.1	OM275415.1	*L. major* isolate IQ-KUT isolate	Iraq	OP046364.1	99.87%
IQ- Human No.2	OM275416.1	*L. major* isolate IQ-KUT isolate	Iraq	OP046364.1	99.74%
IQ- Human No.3	OM275417.1	*L. major* isolate IQ-KUT isolate	Iraq	OP046364.1	99.77%
IQ- Human No.4	OM275418.1	*L. major* isolate IQ-KUT isolate	Iraq	OP046364.1	99.88%
IQ- Human No.5	OM275419.1	*L. major* isolate IQ-KUT isolate	Iraq	OP046364.1	99.78%
IQ- Human No.6	OM275420.1	*L. major* isolate IQ-KUT isolate	Iraq	OP046364.1	99.78%
IQ- Human No.7	OM275421.1	*L. major* isolate IQ-KUT isolate	Iraq	OP046364.1	99.88%

Table 5: the NCBI-BLAST Homology Sequence identity (%) between local *Leishmania tropica* Human isolates and NCBI-BLAST isolates

Isolate No.	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)			
		Identical Isolate	Country	Accession No.	Identity
IQ-Human No.1	OM275422.1	*L. tropica* isolates IQ3	Iraq	OK359041.1	99.87%
IQ- Human No.2	OM275423.1	*L. tropica* isolates IQ3	Iraq	OK359041.1	99.74%
IQ- Human No.3	OM275424.1	*L. tropica* isolates IQ3	Iraq	OK359041.1	99.77%

Table 6: the NCBI-BLAST Homology Sequence identity (%) between local *Leishmania major* and *Leishmania tropica* dog isolates and NCBI-BLAST isolates

| Isolate No.	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)			
		Identical Isolate	Country	Accession No.	Identity
IQ-dog No.1	ON745766.1	*Leishmania major* isolate IQ-KUT	Iraq	OP046364.1	99.67%
IQ- dog No.1	ON745767.1	*Leishmania major* isolate IQ-KUT	Iraq	OP046364.1	99.34%
IQ- dog No.1	ON745768.1	*Leishmania major* isolate IQ-KUT	Iraq	OP046364.1	99.17%
IQ- dog No.1	ON745768.1	*Leishmania major* isolate IQ-KUT	Iraq	OP046364.1	99.18%
IQ- dog No.1	ON745770.1	*Leishmania tropica* isolates IQ-7 isolate	Iraq	MH511156.1	99.34%
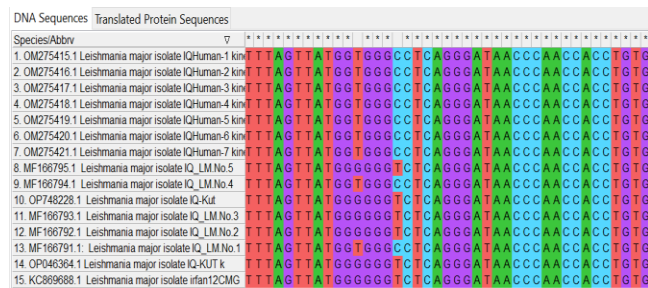
Figure 5: *Leishmania major* human isolates from the local area and NCBI-Gen-bank *L. major* isolates underwent multiple sequence alignment analysis using the Clustal W alignment tool in the MEGA 6.0 version, which revealed substitution mutations on the mitochondrial kinetoplast gene and nucleotide alignment similarity as (*).

Discussion

The total infection rate of cutaneous Leishmaniasis in humans examined by nested PCR was 35%, which was in agreement with Alsaad and Kawan (17,18), which detected 38.30% with CL in patients who had direct contact with dogs. On the other hand, lower than that detected 67.58% (18); 54.7% (19); 81.25% in Thi-Qar (20), and higher than other studies such as Amro *et al.* (21) in Libya recorded 25.96%; 15.1% in Al-Diwaniyah, 10.8% in Diyala and Salah-Edin ,13.1% in Thi-Qar ,11.2% in Baghdad (22); 25.9% in Sudi Arabia (23). The spread of the vector population is still linked to the geographic expansion and worldwide escalation of Leishmaniasis (24).
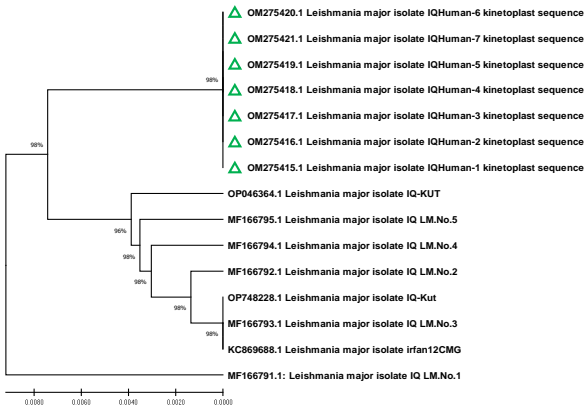
Figure 6: *Leishmania major* human isolates were analyzed for genetic relationships using a phylogenetic tree based on the partial sequence of the mitochondrial kinetoplast gene. A Phylogenetic tree was created using the Un-weighted Pairs Groups Manners with Arithmetic Means (URGMA Tree) in the Mega 6.0 Version. The local *L. major* isolates IQ-Human-1 - IQ-Human-7 isolates were shown to be nearly related to the NCBI-BLAST *L. major* isolate IQ-KUT Iraq isolate (OP046364.1) at total genomic changes of 0.008 to 0.0020 percent.

Figure 7: *Leishmania tropica* human isolates from the local area and NCBI-Genbank *L. tropica* isolates underwent multiple sequence alignment analysis utilizing the ClustalW alignment tool in MEGA 6.0 version, which revealed substitution mutations on the mitochondrial kinetoplast gene and nucleotide alignment similarity as (*).
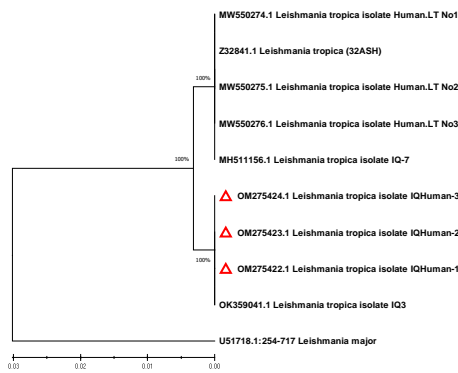

Figure 8: *Leishmania tropica* human isolates were analyzed for genetic relationships using a phylogenetic tree based on

the partial sequence of the mitochondrial kinetoplast gene. A Phylogenetic tree was created using the Un-weighted Pairs Groups Manners with Arithmetic Means (URGMA Tree) in the Mega 6.0 Version. The local *L. tropica* isolates IQ-Human-1 - IQ-Human- 3 isolates were shown to be nearly related to the NCBI-BLAST *L. tropica* isolate IQ3 Iraq isolate (OK359041.1) at total genomic changes of 0.03 to 0.01 percent.

Figure 9: *Leishmania major* and *Leishmania tropica* IQ- dog isolates from the local area and NCBI-Genbank *L.major* and *L. tropica* genotypes isolates underwent multiple sequence alignment analyses utilizing the Clustal W alignment tool in MEGA 6.0 version, which revealed substitution mutations on the mitochondrial kinetoplast gene and nucleotide alignment similarity as (*).
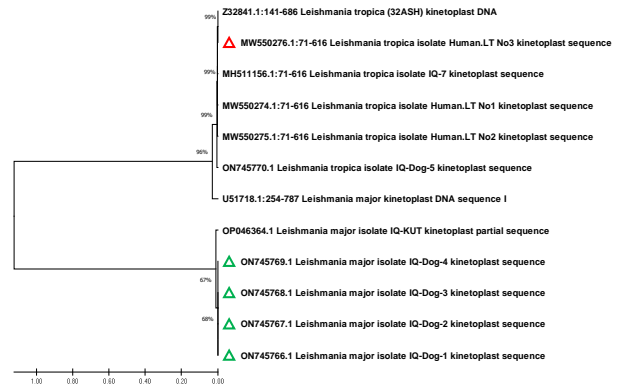

Figure 10: *Leishmania major* and *Leishmania tropica* IQ-dog isolates were analyzed for genetic relationships utilizing a phylogenetic tree based on the mitochondrial kinetoplast gene partial sequence. A Phylogenetic tree was created using the Un-weighted Pairs Groups Manners with Arithmetic Means (URGMA Tree) in the Mega 6.0 Version. The local *L. major* isolates IQ-dog -1-IQ dog-4 and *L. tropica* isolates were shown to be nearly relation to the NCBI-BLAST *L. major* isolate IQ-KUT kinetoplast, Iraq isolates (OP046364.1) and the *L. tropica* isolate IQ-dog isolate was shown to be nearly relation to NCBI-BLAST *L. tropica* isolate IQ-7 Iraq isolate (MH511156.1) at total genomic changes of 1 to 0.0020 percent.

Due to sand flies low in pursuit of prey, environmental and sociological conditions, and people's sleeping patterns all impact the spread of the disease. The province's inhabitants frequently experience power outages, which causes them to sleep outside their homes or on their roofs. During summer, high temperatures and high humidity are considered favorable conditions that significantly increase the reproduction of insects and their contact with human skin. The prevalence of Leishmaniasis in rural areas is higher than in urban as a result of both the rural areas' underdeveloped social customs, culture, and lifestyles, which are probably to blame for the absence of hygienic education and the existence of animals living nearby or inside of people's homes. In addition to facilitating the distribution of infection, rodents, bulk dogs, tankers, and insects in rural parts of the province significantly contribute to the disease's reservoir hosts. In addition, vast plantations and ranches in villages are ideal habitats for insects containing sand flies, which play a role in disease transmission (25). Cutaneous leishmaniasis was determined in rural regions more than in urban regions. This finding is compatible with other studies, such as Al-Difaie (14) and Al-Samarai *et al.* (26) in Al-Qadisiya governorate, Al-Nahhas and Kaldas (27) in Syria, Mehdi *et al.* (28) in Iran, Fahriye *et al.* (29) in Turkey, Khudhur (30) in Iraq. However, different research was detected Al-Hucheimi (31) and Al-Atabi (32).

Findings showed that prevalence in dogs was 88.33%, it was similar to those recorded 80.9% in Nineveh province, Iraq (33), and higher than Alsaad and Kawan (17) recorded 27.71% in dogs 1.6% (23). The canine family in this study is considered a key parasite reservoir and contributes significantly to the spread of the disease. The bulk and distribution of dogs play a big part in the infection's propagation. This occurs due to insufficient of health and municipal services and a decline in public health across the provinces (25). Dogs were exposed to sandflies more than once, and since dogs are usually damp, they provide an ideal habitat for the insect vector to grow and reproduce. As a result, illness developed in the dogs. This may be due to repeated infections, an overabundance of sandflies, and a lack of treatment and follow-up in the area, which led to frequent infection (34). The most frequent association between patients and the reservoir host (dogs) was direct contact, whereas indirect contact was reported in 28.211% of cases and related to stray or neighborhood dogs located close to residences. 33.486% of those with CL reported having no contact with dogs. Due to their outbreaks and the prevalence of the sand fly, patients who owned or lived near domestic dogs had a high infection risk. Some owned one dog, two, three, or even fifteen dogs (17).

Prevalence of cutaneous *Leishmania* in humans and dogs according to the species; among 140 skin lesion patient samples, numerically, the highest rate recorded with *L. major* was 55.1% compare to the lowest with *L. tropica* 44.89%.

These results were compatible with other researches that conducted 54% *L. major* and 46% *L. tropica* in Iran (18); 49.5% *L. major* and 28.6% *L. tropica* (35); 54.7% *L. major*, 45.3% *L. tropica* (19); 98.8% *L. major*, 1.2% *L. tropica* in Iran (36) and not agree with other studies detected in Iraq (37), Flaih *et al.* (20) and in Iran Hajjaran *et al.* (38) who reached that *L. tropica* had highest prevalence than *L. major*. The nested PCR approach amplified two different sized fragments from a particular area of the kinetoplast minicircle flanked by two primer sets (CSB and LiR). According to the nested PCR results, *L. major* is predominant species over *L. tropica* in Iraqi Hosts.

Current findings were close to the study reached that according to the available epidemiological data, all forms of CL in Jordan are primarily zoonotic diseases, with *L. major* accounting for 75% of cases (39,40). *L. major* is a zoonotic infection retained by the reservoir hosts *Meriones hibiscus, Psammomys obesus,* and sand fly vector *P. papatasi*. Cases of *Leishmania tropica* are uncommon. The sand fly *P. sergenti* is thought to be the reservoir host for *L. tropica*. However, data suggests that canines or hyraxes found in all *L. tropica* foci in Jordan may also be reservoir hosts (41). For dogs, current results were in contrast with Alanazi *et al.* (23), which identified that all dogs isolated were *L. tropica*. Earlier investigations conducted in Saudi Arabia using enzymatic biochemical techniques have documented that the natural infection by *Leishmania* is *L. major* in dogs (42,43).

The results of our study were in agreement with those in Saudi Arabia and other Middle Eastern nations, showed *L. major* and *L. tropica* have been identified as agents of cutaneous Leishmaniasis according to molecular evidence of their circulation in human and dog populations from the study locations (44,45). Also, *L. major* is more common nationwide and may be found in Saudi Arabia's wide desert regions (46,47). The findings revealed that *L. major* had a higher prevalence than *L. tropica*. This was in agreement with Al-Ghabban *et al.* (48) in Diyala province, Iraq, in Iran Yadav and Shrestha (49) and Namazi *et al.* (50) which detected that *L. major* was the principal cause of CL. On the contrary, a study in Ramadi, Iraq, recorded that *L. tropica* had a higher prevalence than *L. major* (51). This could be due to the abundance of animals, notably rodents and stray dogs, which act as reservoirs and natural hosts for *L. major*. In addition, several vectors, such as sand flies, are probably major determinants of the high prevalence of human infection.

Phylogenetic analyses and sequencing: The current study showed a divergence of genotypes of cutaneous Leishmaniasis, and this may be due to different methods of transmission, choice of a specific gene for genotyping, various environmental conditions and diagnostic techniques, presence of sand fly and reservoir hosts, although noted limited diversity among the investigated local isolated comparable with other international studies and this could be due to a significant obstacle to understanding the

transmission cycles is the lack of information on the interactions between the disease, the reservoirs, and the vectors especially given that the patterns of distribution can easily change over time in particular geographic areas (52). In order to prevent epidemiology and the distribution of CL, data submitted here help close knowledge gaps. Extrachromosomal DNA, such as kinetoplast DNA, is one of the most popular techniques used to identify infections of *Leishmania* species (53). The kDNA gene has high sensitivity, and the kinetoplast contains almost 10,000 circular and convoluted kDNA minicircles per cell (54).

Previous research detected sequence analyses of kDNA in *Leishmania* evidence that five positive human samples were all *L. major* with the identity of nucleotide ranging 99.3-100% with NCBI *L. major* from Iraq (MN313423) and two positive human samples found identity 99.7-100% with NCBI *L. tropica* from Iraq (MF166799) in human in Saudi Arabia. Also, five positive dog lesion samples were the identity of nucleotide 99.33-99.80% with NCBI *L. tropica* from Iraq (MF166800, MN334661), UK (AF308689) (23). Azmi *et al.* (55) Determined that *L. tropica* is a highly heterogeneous species confirmed by little bootstrap values accomplished on the phylogenetic tree. Data showed an incidence of *L. tropica* in dogs and humans, which has been previously confirmed about the disposal of *Phlebotomus sergenti*, a suitable vector for *L. tropica* (56). All local *L. tropica* isolates noticed approximately more similar nitrogen bases sequence and genetically nearly relation to NCBI-BLAST *L. tropica* registered (K4680852.1) in Iraq (57), (AB678350.1) from patients registered in Iraq (20); (KY612611) registered in Iraq (48); all local *L. major* isolates noticed approximately more similarly nitrogen bases sequence and genetically be nearly relation to NCBI-BLAST *L. major* (MH428844.1) registered in Iraq (57), (KP773404.1) registered in Iraq (48).

Conclusion

Nested PCR based on the kDNA gene detected non-significant differences P>0.05 according to *L. major* and *L. tropica* in humans, while there were significant differences P<0.05in dogs. Phylogenetic tree analyses determined that 7 of local isolates skin lesion human samples (OM275415.1 to OM275421.1) belong to *L. major* isolates IQ Kut isolates, Iraq (OP046364.1) and three isolates (OM275422.1 to OM275424.1) belongs to *L. tropica* isolates IQ3, Iraq (OK359041.1). Four of the local isolates skin lesion dog samples (ON745766.1 to ON745768.1) belong to *L. major* isolates IQKut, Iraq (OP046364.1), and one isolates (ON745770.1) belong to *L. tropica* isolates IQ-7 Iraq (MH511156.1). Determining the large number of *L. major* in humans and dogs indicates that dogs are key parasite reservoirs and significant zoonotic contributes to the transmission of disease to humans in the Middle Euphrates, Iraq.

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العزل والتشخيص الجزيئي لداء الليشمانيات الجلدي في الإنسان والكلاب في منطقة الفرات الأوسط، العراق

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الخلاصة

يعد داء الليشمانيا الجلدي أحد الأمراض المستوطنة في العراق. يعتبر المرض مشكلة صحية واسعة الانتشار ومرض غير قابل للشفاء. الغرض من هذه الدراسة هو توصيف العزلات المحلية لليشمانيا الاستوائية والليشمانيا الكبرى جزيئيًا وتحديد مدى ارتباطها الوثيق بمصادر العزلات للدول المجاورة. جمعت ١٤٠ و٦٠ لعينات الآفات الجلدية من المرضى والكلاب على التوالي، من مختلف الأجناس والفئات العمرية والمناطق خلال الفترة من ١ أيلول ٢٠٢١ إلى ٣١اذار ٢٠٢٢، أجريت الطرق الجزيئية لتحديد انتشار داء الليشمانيا الجلدي في الإنسان والكلاب، يعمل تفاعل البلمرة المتداخل باستخدام جين k للتحليل الجيني. حيث بلغ معدل الانتشار الكلي لليشمانيا الجلدية ٣٥% و٨٨,٣٣% في الإنسان والكلاب على التوالي، وقد أظهرت النتائج أن معدل الانتشار الكلي لليشمانيا الكبرى في الكلاب بلغ ٧١,٦٩% مقارنة مع الليشمانيا الاستوائية ٢٨,٣٥%. تم أرسال ١٥ عينة موجبة من الآفات الجلدية (١٠ إنسان و٥ كلاب) إلى قاعدة بيانات بنك الجينات لتحليل العلاقة الجينية، والتي حددت ٧ من العزلات الجلدية المحلية لعينات الأنسان تعود إلى عزلات مسجلة في محافظة واسط، العراق والتي تعود الى نوع الليشمانيا الكبرى و٣ عزلات تعود إلى عزلات الليشمانيا الاستوائية. وجدت ٤ من العزلات المحلية لعينات الآفات الجلدية للكلاب تعود لعزلات الليشمانيا الكبرى وعزلة واحدة تعود لعزلات الليشمانيا الاستوائية. إن تحديد الأعداد الكبيرة من طفيليات الليشمانيا الكبرى في الإنسان والكلاب يشير إلى أن الكلاب هي الخازن والمصدر الرئيسي للطفيليات ويساهم بشكل كبير في نقل المرض إلى الإنسان في منطقة الفرات الأوسط، العراق.

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