

# Identifying the different genetic Species of *cutaneous leishmaniasis* for animal breeders in Wasit Governorate

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Received: 12/5/2025

Accepted: 25/5/2025

Published: 3/7/2025

**Abstract—** To diagnose the leishmania species prevalent in some regions of the central Iraq Regions of central Iraq for animal breeders, the molecular method (PCR) was used to identify two Species of disease-causing Leishmania parasites. generally, the study showed a 750-base-pair band was associated with 39 patients at a rate (17.3%) *Leishmania tropica* species., while A 560-base-pair band was recorded in 186 patients at a rate (82.6%) associated with *Leishmania major*, were infected with *Leishmania major* type in 9 Regions of for animal breeder in central Iraq. For specific studying samples in Wasit Region, the number of infected samples reached 125 samples for the years 2022-2024, divided into two parts of sample collection. However, the actual infections in the first period from October 2022 to September 2023 reached 73 infections, and the rate of infection in males for animal breeders was higher than in females (47) 64.4%, (26) 35.6%. respectively, while the results were in the second part for proud October 2023 to September 2024 (52) infections distribution by gender, male, female, "28 (53.8%), 24(46.1%" respectively. During the study period 2022-2024, at a rate of (58.4%) and (41.6%) respectively, in various populated areas in Wasit Region, where (51) cases were recorded at a rate of (40.8%) in Al-Hay District, the highest infection rate, and the lowest infection rate was in the center of Kut city, 5 cases (4%). The results also showed that the incidence of the Species of leishmaniasis infection of *Leishmania tropica* was less prevalent at 24.8%, divided into 17.6% for males and 7.2% for females for animal breeder, compared with *Leishmania major* at a rate (75.2 %) The gender ratio is 44%, 31.2% for males and females, respectively for animal breeder. Therefore, this research can be used to determine the species of parasite causing the disease for animal breeders, to reduce the risk and control the disease by preparing the

vaccine.

**Keywords** — polymerase chain reaction, *Leishmania* parasites, cutaneous and Wasit Region.

## INTRODUCTION

**L**eishmaniasis is a parasitic disease caused by protozoa and is prevalent in four continents and many regions worldwide(1,2). The World Health Organization WHO is considered one of the six most dangerous parasitic diseases affecting humans (3). Leishmaniasis is a prevalent zoonotic infectious disease worldwide and is notably endemic in Iraq, special for animal breeders, because they are in direct contact with animals. This disease has several Species, each with different clinical manifestations: cutaneous, Mucocutaneous, and visceral (4). presents a significant health challenge, particularly in Iraq's central and southern areas, including Wasit Region Especially Cutaneous leishmaniasis is also known as oriental sore Cutaneous leishmaniasis. a serious public health disease with a wide range of clinical symptoms, where Cutaneous leishmaniasis leads to various skin deformities (5). This Cutaneous leishmaniasis infection is prevalent in more than 88 countries, including Iraq. active across Iraq in favorable environmental conditions. In recent years, the animal breeders there has been an increase in cases (6), especially among impoverished communities, due to insufficient health education, the proliferation of the Insect vector (female sandflies the genus *Phlebotomus*), and inadequate prevention services, diagnostic, and treatment (7,8), Transmission occurs through bites from infected sandflies for animal breeders, with canines and rodents potentially serving as also reservoir hosts (9). Parasites live inside the macrophages of the vertebrate host in the amastigote form (*Phlebotomus* sp. Sandfly (10) Therefore, the importance of the study emerged in identifying

the most prevalent genetic type of cutaneous leishmaniasis among animal breeders in some areas of Wasit Governorate and other parts of the central governorates in Iraq

## MATERIALS AND METHODS

**Sample collection:** The study included 225 samples from people infected with leishmaniasis for animal breeders after clinical diagnosis by dermatologists in hospitals of the central region Regions in Iraq, such as Wasit, Karbala, Najaf, Babylon, Diwaniyah, Samawah, for the period from 2022 to 2024. The samples taken from the patients for animal breeder ulcer were placed in tubes of prepared culture media type Novy, MacNeal-Nicolle (NNN Medium) and incubated in the laboratory of the "College of Education for Pure Sciences - University of Wasit" at a temperature of 26°C (11). This temperature is considered optimal for the growth of the flagellate phase until DNA extraction according to Al-Mousawi, (12).

### Molecular Study:

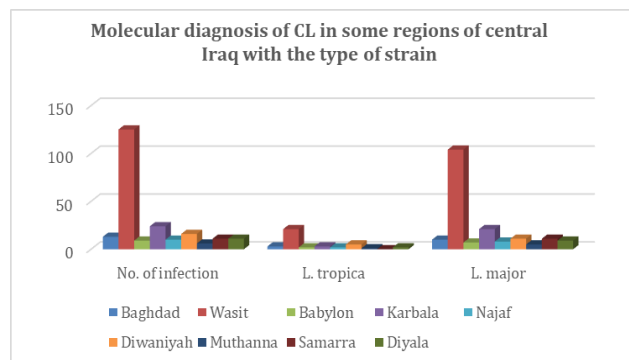
the DNA extraction from the Leishmania parasite for animal breeder samples included in the study was performed by the kit manufacturer, according to Geneaid's instructions. The step of destroying the Leishmania parasite's outer membrane was also included, as described by Falih (13). And using gel electrophoresis to determine DNA concentration by. to Sambrook and Russell method, to assess the purity and mobility of purified DNA (14).

For the purpose of selecting primers for partial detection of Leishmania  $\mu$ DNA of parasite species with a specific sequence of nitrogenous bases, the ITS region (for genetic variation between Leishmania species according to digestive restriction enzymes) was designed (CSB1xF "CGAGTAGCAGAACTCCCGTTCA") and (CSB1xR "ATTTTCGCGATTTTCGAGAACG") (15,16) after Loading of PCR product and Electrophoresis, The bands were photographed using a UV transilluminator, where the band length is 560 bp to the species *L. major*, and the band length is 750 bp to the species *Leishmania tropica*.

Statistical Analysis: using One-way analysis of variance was used in GraphPad Prism to appreciate the important differences between study values at  $P < 0.05$ . (16)

## RESULT & DISCUSSION

Detection of the Cutaneous leishmaniasis for animal breeder infection in some region of Iraq's center of the city and The results reached, as shown in Table 1& Figure 1, showed that there were 225 cases of leishmaniasis for animal breeder in hospitals in some region of the country, including 125 cases diagnosed in waist Region, which were confirmed clinically and molecular diagnosis , for the period from October 2022 to March 2024 With regard to the rest of the regions for under study, except for waist Region, which included all months of the year. the current study appeared to results after using molecular diagnosis using the PCR band 560 bp for the type *Leishmania major* and band 750 bp to the kind *Leishmania tropica*, as shown in Table 1 and Figure 1



**Figure 1.** prevalence infection for animal breeders in some regions of central Iraq with different type of strain

**Table 1.** Molecular diagnosis of Cutaneous leishmaniasis infection for animal breeders in some regions of central Iraq with the type of strain

regions	No. of infection	<i>L. tropica</i>	<i>L. major</i>
Baghdad	13	3	10
Wasit	125	21	104
Babylon	9	2	7
Karbala	24	3	21
Najaf	10	2	8
Diwaniyah	16	5	11
Muthanna	6	1	5
Samarra	11	0	11
Diyala	11	2	9
<b>Total</b>	<b>225</b>	<b>39</b>	<b>186</b>
$X^2=13$ ( $P<0.01$ )		17.3%	82.6 %

The results showed that the infection rate of *Leishmania major* was 82.6% higher than that of *Leishmania tropica* 17.3% in the study areas (urban centers for animal breeders in the central region of Iraq), indicating that this species is the major and common cause of oriental sore Baghdad boil in the central region of Iraq. many researchers have made a molecular diagnosis and confirmed the current results in the Middle East (17,18,19), separation infection by both cutaneous leishmaniasis and visceral leishmaniasis (VL) (Baghdad boil and black fever) have been recorded in Iraq, both caused by *L. Donovan*, *L. Major*, and *Leishmania tropical* (20,21).

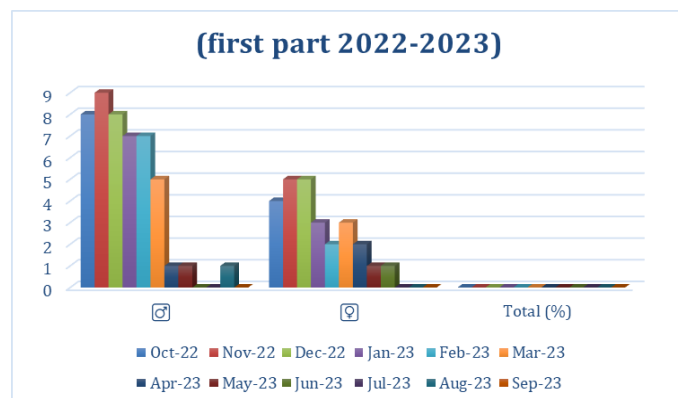
The incidence rate of cutaneous leishmaniasis for animal breeders was observed to have increased in 2020, reaching 0.96 compared to 2018, with a molecular diagnosis of 0.65 per 1000 residents of the southern regions in animal breeders of Iraq. The lowest infection rate was in Baghdad, where it was recorded at 0.05 per 1,000 people. The infection rates in the northern region varied, with 13 cases for animal breeders including 12 in the city of Sulaymaniyah (Iraqi Ministry of Health annual report) (12).

While samples were collected in Wasit Region from October 2023 to September 2024 from patients for animal breeders coming to hospitals and clinics in the study locations, the first part of the study period showed the arrival of 73 infections at a rate of 58% for animal breeders, while the second part appeared at a rate of 41.6%, equivalent to 52 patients for animal breeders.

Depending on the seasonal distribution of infection by cutaneous leishmaniasis for animal breeders, through the number of infected people coming to the hospital, the infection appeared starting in September. and then gradually increased in a significant way  $P > 0.05$  in October and November for the years (2022-2023) & (2023-2024). The highest numbers were in December, January, February and March and began to gradually decrease, with the lowest percentage reaching July and August, as shown in Table 2&3 with Figure 2&3 These results were previously confirmed by many researcher (22,23) that the difference in infection according to different months of the year depends on the effect of environmental variations such as temperature and humidity and to transporter activity, or The incidence rate depends on the activation of the transporter (23). Other factors control the spread of infection, including the difference in the duration from the incubation time (2-4 months), during which the symptoms of the disease started to appear as well as the strength of contact between the animal and its owner. (24)

**Table 2.** Distribution of *Cutaneous leishmaniasis* for animal breeders in Wasit Region (first part)

Month	♂	♀	Total (%)
October 2022	8	4	12 (16.4 %)
November 2022	9	5	14 (19.1%)
December 2022	8	5	13 (17.8%)
January 2023	7	3	10 (13.6%)
February 2023	7	2	9 (12.3%)
March 2023	5	3	8 (10.9 %)
April 2023	1	2	3 (4.1%)
May 2023	1	1	2 (2.7 %)
June 2023	0	1	1 (1.3%)
July 2023	0	0	0
August 2023	1	0	1 (1.3%)
September 2023	0	0	0
Total (%)	(47) 64.4%	(26) 35.6%	73



**Figure 2.** Distribution of *Cutaneous leishmaniasis* for animal breeders in Wasit Region (first part).

The distribution of patients for animal breeders with infection by molecular diagnosis Cutaneous leishmaniasis by region in Wasit Region for the year 2022-2023, appears as the highest infection for animal breeders' rate was recorded in the Al-Hay District 29, at a rate of 39.7%. as show table 3

**Table 3.** Distribution of patients for animal breeders with infection by *Cutaneous leishmaniasis* by region in Wasit Region for the year 2022-2023

District	♂	♀	Total (%)
Al-Hay	19	10	29 (39.7%)
Sheikh Saad	12	9	21 (28.7 %)
Al-Numaniyah	5	4	9 (12.3%)
Al-Aziziyah	5	3	8 (10.9%)
Al-Suwaira,	3	1	4 (5.4%)
Kut city	1	1	2 (2.7%)
Total	45	28	73 (58.4 %)

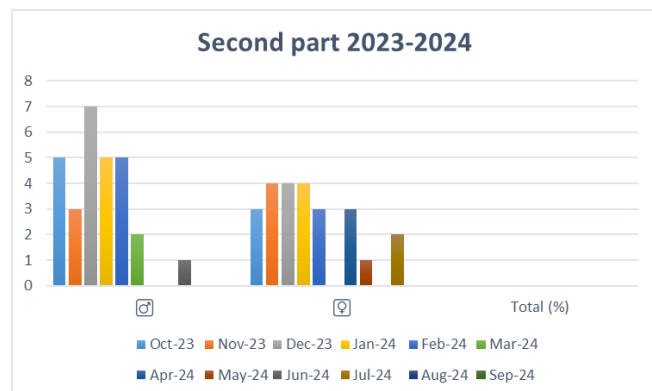
The whale the infection rate reached a high rate among males 19 for animal breeders compared to females 10 in other regions. These results agree with others (25,26). As for the total number of areas in Wasit Region, the number of males infected was higher than the number of females 45, 28 respectively. The rate of infections in the first group of the sample collection period was higher by 73 (58.4%) These results have been proven and are consistent with the results of others (27,28). These results are also similar to what was found by Ali (29). This may be due to the tendency of males to work outside the home, especially those with jobs in open areas outside cities (30,31) are more susceptible to infection than females. The results of the current study differed from a study in Turkey by Tarish (32) which indicated that females are more susceptible to skin lesions, attributing the reason for this to environmental conditions or sex differences such as hormonal differences and defenses. (4).

The infection by CL appears with the onset of autumn and during the winter months for animal breeders, as shown in Table 4. These findings are consistent with the results (25). These results may be due to sandfly activity and favorable environmental conditions, particularly about temperature (8,14) Gender also has a significant impact on infection, with males and females accounting for 53.8% to 46.1% respectively.

**Table 4.** Distribution of *Cutaneous leishmaniasis* for animal breeders in Wasit Region (second part)

Month	♂	♀	Total (%)
October 2023	5	3	8 (15.3 %)
November 2023	3	4	7 (13.4%)
December 2023	7	4	11 (21%)
January 2024	5	4	9 (17.3 %)
February 2024	5	3	8 (15.3 %)
March 2024	2	0	2 (3.8 %)
April 2024	0	3	3 (5.7 %)
May 2024	0	1	1 (1.9 %)
June 2024	1	0	1 (1.9 %)
July 2024	0	2	2 (3.8 %)
August 2024	0	0	0
September 2024	0	0	0
Total (%)	(28) 53.8%	(24) 46.1%	52

When distributing infections among region for animal breeders in Wasit Region for the period from October 2023 to September 2024 as shown in Table 5, appear as the highest infection rate was recorded in the Al-Hay District group, at a rate of 22 (42.3 %).



**Figure 3.** Distribution of *Cutaneous leishmaniasis* for animal breeders in Wasit Region (Second part)

These results are consistent with those of others (23,32, 33). On the other hand, the results of the current study indicated that the infection appears clearly in patients in the Kut city center of Wasit Region at a rate of 3 (5.7%) For the same period. These results agree with another researcher (10,34)

**Table 5:** Distribution of patients for animal breeders with infection by *Cutaneous leishmaniasis* by region in Wasit Region for the year 2023-2024

District	♂	♀	Total (%)
Al-Hay	12	10	22 (42.3 %)
Sheikh Saad	7	1	8 (15.3 %)
Al-Numaniyah	3	5	8 (15.3 %)
Al-Aziziyah	2	6	8 (15.3 %)
Al-Suwaira	3	0	3 (5.7 %)
Kut city	2	1	3 (5.7 %)
Total	29	23	52 (41.6 %)

Regarding the Wasit Region, when a study of molecular study by PCR showed that cutaneous leishmaniasis was caused by two Species of Leishmania parasites for animal breeders: *Leishmania tropica* 31(24.8%) distributed, with 22 (17.6%) for males, and 9 (7.2%) for females. *Leishmania major* was 49 (75.6%), distributed as 55 (44%) infections in males and 39 (31.2%) in females, where the significant difference appeared clearly between the species as shown in the Table.6 & Figure 4

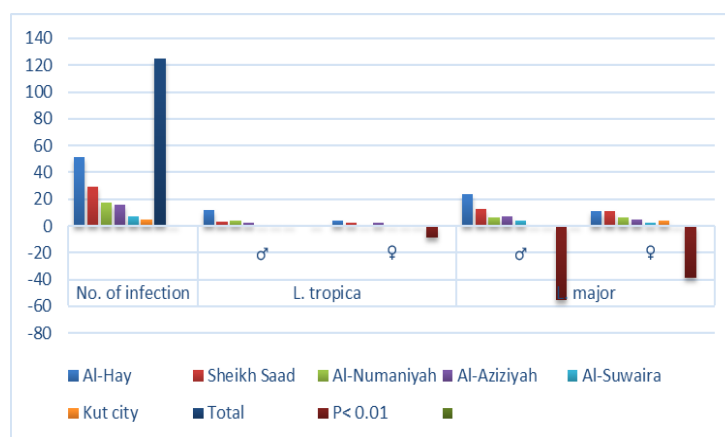
This indicates that *Leishmania major* is the main cause of the widespread cutaneous disease for animal breeders in Wasit Region. These results are consistent with those of a study that indicated the high prevalence of *Leishmania major* compared to other *Leishmania* species for animal breeders (29,35)

These results agree with Dawood (11). The cause of increased infection in remote region loss health for animal

breeders' awareness is often more prevalent in the center of large cities than in remote areas this is also due to the widespread spread of sand flies (vector host),

**Table 6 .** Distribution of Species of *Cutaneous leishmaniasis* for animal breeders' infection based on gender in Wasit Region

District	No. of infection	<i>L. tropica</i>		<i>L. major</i>	
		♂	♀	♂	♀
Al-Hay	51	12	4	24	11
Sheikh Saad	29	3	2	13	11
Al-Numaniyah	17	4	1	6	6
Al-Aziziyah	16	2	2	7	5
Al-Suwaira	7	1	0	4	2
Kut city	5	0	0	1	4
Total	125	17.6 % (22)	7.2% (9)	44% (55)	31.2% (39)
P< 0.01	$\chi^2=9.55$	24.8% (31)		75.2% (94)	



**Figure 4.** Distribution of Species of *Cutaneous leishmaniasis* for animal breeders' infection based on gender in Wasit Region

In addition to rodents in general stray cats and dogs that provide a suitable habitat, as a contributing factor in increasing the incidence (2,14) Due to the available of the transporting medium, as a Falih (13) most of the infected people raise animals at home.

Also, the Malnutrition among residents of remote areas, neglect of referring patients to health centers, or delays in detecting infections also contribute to the spread of the disease. Therefore, leishmaniasis was recently classified as a serious disease because the connected to attacks on people with lower immunity.

**Conclusion:** In this study, the results of the current research showed that molecular techniques are a reliable means for diagnosing and identifying *Leishmania* species for animal breeders and can be applied in epidemiological investigations. Also, the *Leishmania tropica*, *Leishmania major*- Both of the major *Leishmaniasis* species are causative agents of cutaneous leishmaniasis for animal breeders in the central Regions, especially Wasit Region, and *Leishmania majoris* one of the main species causing cutaneous leishmaniasis.



Recommendations: Modern technologies can be utilised to diagnose Leishmania parasite species using gene modification diagnosis to obtain an accurate diagnosis for animal breeders and other Zoonotic diseases of animal breeders, which can be used for treatment and reducing the risk of leishmaniasis for animal breeders, or by producing vaccines against this disease

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