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The Prevalence of Some Epidemic Leishmania Spp. Isolated from Human Blood and Skin Samples

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Abstract

Leishmaniasis ranks as the second deadliest parasitic disease globally, following malaria. Recognized by the World Health Organization as a neglected tropical disease, leishmaniasis continues to pose a major public health challenge. This study aimed to evaluate the sensitivity and specificity of the Direct Agglutination Test as a diagnostic tool for leishmaniasis. A total of 666 dried blood samples were collected between March 7 and June 21, 2024. These included 606 samples from suspected visceral leishmaniasis cases, 63 from cutaneous leishmaniasis cases, 66 from healthy individuals as controls, and 63 from individuals with other medical conditions. The study focused on three main objectives: evaluating the Direct Agglutination Test using locally prepared Leishmania antigens (L. donovani, L. tropica, and L. major), identifying sandfly species, and monitoring their seasonal population densities. The Leishmania antigens were prepared using mercapto-ethanol and glutaraldehyde. The direct agglutination test demonstrated 100% sensitivity in confirmed cases of visceral leishmaniasis when using the L. donovani antigen, and 93% sensitivity in clinically suspected cases. For cutaneous leishmaniasis, the L. tropica antigen also achieved 100% sensitivity. In contrast, traditional diagnostic methods—such as smears and cultures—yielded lower positivity rates, at 60.16% and 69.46% respectively. Among the antigens tested, L. tropica showed the highest specificity at 96.19%, followed by L. donovani at 87.68%. Cross-reactivity with sera from individuals with other diseases was minimal. Visceral leishmaniasis was most prevalent among children under five years old, while cutaneous cases were more common in the 6–30 age group. Males were more frequently affected in both forms of the disease. Visceral leishmaniasis cases were predominantly reported in rural areas, whereas cutaneous cases were more common in urban settings. Most cutaneous lesions (96.6%) were single and primarily located on the face and upper limbs. The direct agglutination test outperformed the indirect method in sensitivity and specificity, proving its effectiveness for diagnosing leishmaniasis.

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1. Introduction

Leishmaniasis is the second most common mortality parasite disease, after malaria [1]. Human leishmaniasis is classified by the World Health Organization (WHO) as a neglected tropical disease (NTD)[2-5]. More than 12 million people have Leishmania spp. infections worldwide, and 350 million more are at risk of contracting the disease [6]. Underfunded elimination programs, little to no interest from pharmaceutical industries, and inadequate healthcare infrastructure are the main causes of leishmaniasis [7-9]. Three known forms of leishmaniasis: visceral (VL), cutaneous (CL), and mucocutaneous (MCL). CL is the most prevalent and widespread form [10]. It typically causes skin lesions on various parts of the human body that are

constantly exposed to sand fly biting, such as the face, neck, arms, legs, and possibly the abdomen [11]. The most severe type of the illness, known as VL, occurs when the infection spreads to internal organs such the bone marrow, liver, and spleen [12-14]. According to Lesho et al. [15], cutaneous leishmaniasis, often known as "Oriental sore" or "Baghdad boil," is a slowly growing inflammatory skin sore. Leishmaniasis is common in Iraq. According to reports, the incidence rate of CL was 5 cases per 100,000 people annually, peaking at 45.5 cases per 100,000 in 1992. Additionally, an outbreak was looked into when 300–400 cases were reported in the province of Diwaniyah in 2008 [16]. Human leishmaniasis infections are often caused by fourteen species of Leishmania, which can present with either comparable or distinct clinical symptoms [17]. There are several species of the sandfly, which is an insect vector, the bites of female phlebotomine sandflies, carry Leishmania parasites [18]. The infection starts when the sandfly bites the host by puncturing the skin with its proboscis. The parasite is then spread by injecting tainted blood into the body of another host when the sandfly is feeding on them [18,19]. According to Ovalle-Bracho et al. [20], humans are an incidental host who contract the infection when they are bitten by a female vector while she is looking for blood. Leishmaniasis is frequently difficult to diagnose, however it can be done clinically with both direct (parasitic) and indirect (immunologic) confirmation. Microscopy, culture, and PCR through appropriate specimen collection are essential since direct evidence is preferred [21, 22]. The site of tissue collection affects VL sensitivity, with splenic biopsy producing the largest percentage of positive results [23]. Indirect immunologic tests may also be acquired because direct parasitological diagnosis by microscopy, culture, and molecular testing is not always available or confirmatory. As supplementary methods in serologic diagnosis, Western Blot, ELISA, immunofluorescence, and direct agglutination can be employed to evaluate for antibodies, typically IgG [23]. Finally, visceral leishmaniasis, cutaneous leishmaniasis can occasionally be treated with systemic medications; however, depending on the species causing the infection and the possibility of major side effects, these medications may also be applied topically or intralesional. Other treatments that have been used, either by themselves or in conjunction with medications, include cryotherapy, thermotherapy, photodynamic therapy, CO2 laser treatment, and curettage [24]. The current study sought to assess the prevalence of cutaneous leishmaniasis in the governorate of Baghdad in 2024 as well as the correlation between a few factors, including lesion site, age, and gender.

2. Materials and Methods

A total of 663 blood samples were collected from visceral leishmaniasis (506 samples), cutaneous leishmaniasis (31 samples), and other people suffering from skin and internal diseases (61 samples) and control group (65 samples) from City of Medicine Hospital, Yarmouk Teaching Hospital and Al-kindi Teaching Hospital. The study period was from March 7 to June 21, 2024. Each patient was examined clinically and parasitological and sheets were filled out on personal, clinical and laboratory data. Skin lesions were examined for size, site and number. Microscopic examination of stained smear of aspirate materials were taken from lesions' margin, fixed in acetone free methanol and stained with Giemsa stain for amastigotes or by culture on NNN diphasic medium for promastigotes [25]. The study protocol was approved by the Ethics Committee of Al-Iraqia University, Baghdad, Iraq (no. 4853) in (21/12/2023).

i. Direct Agglutination Test (DAT)

Leishmania promastigotes (L. donovani, L. tropica, and L. major) were cultured, treated with glutaraldehyde and mercapto-ethanol, stained with Coomassie Brilliant Blue, and used as antigens. Serum was eluted from dried blood spots and serially diluted in microtiter plates. DAT antigen was added, and plates were incubated at room temperature for 18–24 hours. Positive reactions were indicated by diffuse blue mats, while negative results showed compact blue dots. A titer of ≥1:3200 was considered positive for visceral leishmaniasis, and ≥1:800 for cutaneous leishmaniasis. Results were compared with microscopy and culture to assess sensitivity and specificity [26].

ii. Statistical analysis

The Statistical Package for Social Sciences, version 22, was used to do the statistical analysis. Regarding variables using a descriptive formula, the Chi-square test was used to compare them and they were expressed as numbers and percentages. Values with $p<0.05$ are considered significantly different.

3. Results and Discussion

For cutaneous leishmaniasis, the test sensitivity was 100% using the *L. tropica* antigen, with all samples testing positive. In contrast, the parasite test using smears and culture yielded positive results in 60.16% and 69.46% of cases, respectively. The highest specificity was observed with the L. *tropica* antigen (96.19%), compared to 87.68% for the *L. donovani* antigen. The test demonstrated minimal or no cross-

reactivity with sera from other diseases. The highest sensitivity for detecting visceral leishmaniasis cases was achieved using the L. donovani antigen: 100% in confirmed cases. 93% in clinical cases. 96.16% in asymptomatic carriers. Visceral leishmaniasis was most prevalent in children under five years (33.16%), with cases ranging from six months to 36 years. Cutaneous leishmaniasis predominantly affected individuals aged 6 to 30 years (66.96%), figure 1 and table 1.

CL is endemic in Iraq, with a high prevalence reported across most provinces. The earliest documented cases were recorded in Mosul and Baghdad [27]. Historically, Iraq possessed one of the most advanced healthcare and medical education systems in the region. However, the 2003 invasion and the subsequent wave of terrorist attacks severely damaged the country's health infrastructure. As a result, the incidence of communicable diseases, including CL, increased significantly [28]. The disease remains a major public health concern not only within Iraq but also in neighboring endemic countries such as Saudi Arabia, Iran, Turkey, and Syria [27].

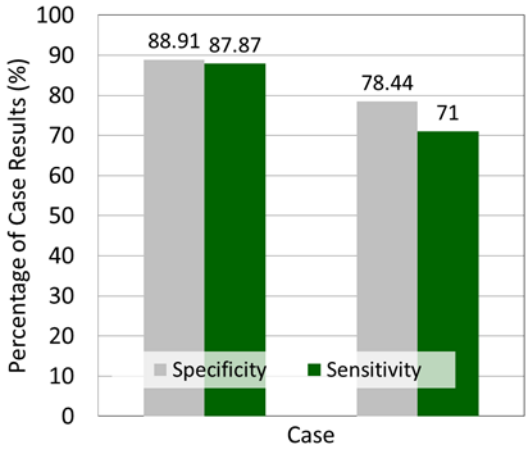

Figure 1. Sensitivity and Specificity of DAT

Table 1 shows data of the transposed Leishmaniasis.

Table 1: The data of transposed Leishmaniasis.

Titration	0	50	100	200	400	800	1600	3200	6400	12800	25600	51200
Pulmonary Tuberculosis (d)	3	7	-	-								
Pulmonary Tuberculosis (m)	-	8	2	-								
Pulmonary Tuberculosis (t)	9	1	-	-								
Scabies (d)	7	3	-	-								
Scabies (m)	6	2	2	-								
Scabies (t)	17	-	-	-								
Water Bags (d)	16	1	-	-	-	-	-	-	-	-	-	-
Water Bags (m)	15	2	-	-	5	1	-	-	-	-	-	-
Water Bags (t)	1	2	1	1	4	11	2	43	56	30	78	116
Leishmaniasis (Cutaneous) (d)	5	-	-	-	16	32	89	-	-	-	-	-
Leishmaniasis (Cutaneous) (m)	3	1	1	-	2	-	-	-	-	-	-	-
Leishmaniasis (Cutaneous) (t)	16	13	-	2	44	-	-					
Leishmaniasis (Visceral) (d)	-	7	9	8								
Leishmaniasis (Visceral) (m)	-	-	9	5								
Leishmaniasis (Visceral) (t)	22	3	8	7								
Control Group (d)	212	204	78	10								
Control Group (m)	188	120	94	60								
Control Group (t)	57	8										

Males were more affected than females in both types of leishmaniasis: Visceral leishmaniasis: 91.33% in males and 33.69% in females. Cutaneous leishmaniasis: 63.91% in males and 36.09% in females, especially in children under 5 years as in table 2. According to the current study, males are more susceptible to Leishmania spp. than females, and this difference is age-dependent. Both sexes engage in activities and behaviors that expose them to high levels of sand fly exposure. The presence of infection may be caused by age-limited immune competence, malnourishment, viral co-infection, or a lack of herd immunity, which could account for the high infection rate in children under five. As for the distribution of positive cases by gender, the infection rate was higher in males than in females. The number of infected males was 240 (59.70%), while the number of infected females was 162 (40.29%) using the indirect hemagglutination test. In contrast, using the direct hemagglutination test, the number of infected males was 265 (64.10%), while the number of infected females was 164 (35.90%).

Visceral leishmaniasis was more common in rural areas (69.16%) than in urban areas (30.99%). Conversely, cutaneous leishmaniasis cases were more concentrated in urban centers, figure 2.

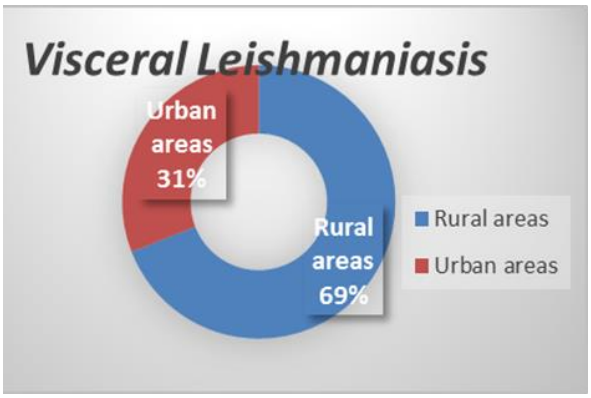

Figure 2. The geographical pattern for Visceral leishmaniasis

Visceral leishmaniasis peaked in February (90.99%), while cutaneous leishmaniasis peaked in January (69.60%) and February (66.96%). Single lesions accounted for 96.6% of cases, while multiple lesions represented 3.99%. Most lesions were located on the face (60.16%) and upper limbs (39.10%). This test showed lower sensitivity and specificity compared to the direct agglutination test: Visceral leishmaniasis: Sensitivity 61%, specificity 69.60% (L. donovani antigen). Cutaneous leishmaniasis: Sensitivity 16.6%, specificity 66.36% (L. tropica antigen), figure 3.

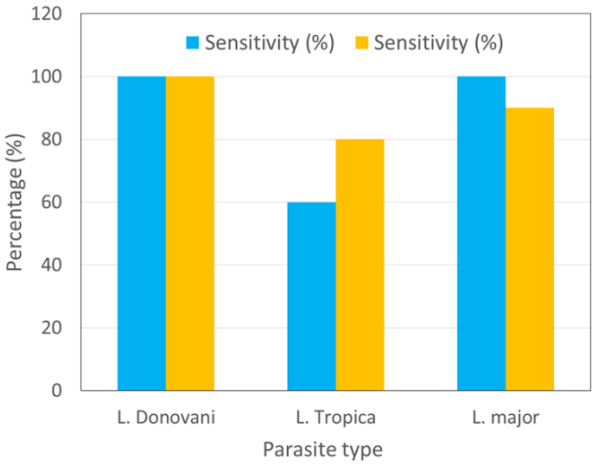

Figure 3. Sensitivity and Specificity of DAT and in DAT

There was seasonal variation in Leishmania infection. This might be connected to the sand fly's activation during the cold season, particularly in the ruler area close to water and lack of public health awareness. Figure 4, 5.

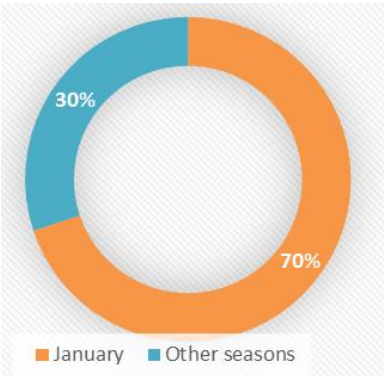

Figure 4. Seasonal distribution for Cutaneous Leishmaniasis

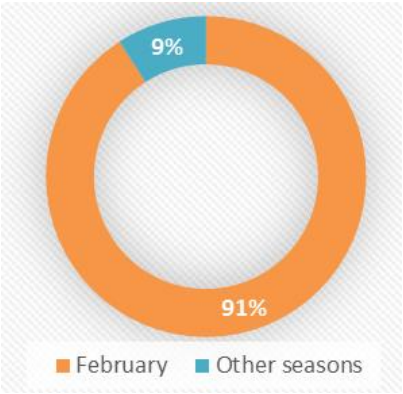

Figure 5. Seasonal distribution for Visceral Leishmaniasis

In a study conducted in Sudan with 50 blood donors, aimed to ascertain the prevalence of visceral leishmaniasis among healthy, asymptomatic donors at the Al-Gadarif Teaching Hospital. They also evaluated the application of molecular techniques for diagnosing leishmaniasis in blood donors, but still unclear why none of the 50 donors' DNA exhibited a positive test result for parasitic infection; but the high sensitivity of the SSU-PCR in detection of leishmania DNA and the adequate sample size go in favor of the validity of the result [29]. A local study in Al-Ramadi, a three-year descriptive study on cutaneous leishmaniasis was carried out between 2019 and 2021 included 532 individuals were afflicted with cutaneous leishmaniasis. The greatest number of afflicted patients in 2020 comprised infections mostly surfaced in the winter months. Males were more affected by cutaneous leishmaniasis than females, and the disease primarily affected those under the age of twenty [30]. Another study in al Ramadi city in Iraq included 120 suspected cases of cutaneous leishmaniasis, they found cutaneous leishmaniasis affects both sexes, with a small male predominance. The condition is more common in rural areas and in younger age groups. With the highest rates in January and the lowest in October, there is a notable seasonal variation in the disease's prevalence [31]. These findings align with our current study, which similarly observed a higher prevalence among males and younger individuals, as well as a greater burden of visceral cases in rural settings. By further assessing the diagnostic efficiency of DAT and identifying species-specific patterns using local antigens, our research builds on these regional observations and provides enhanced diagnostic insights to support targeted public health interventions in endemic areas. A study in Babylon included 143 cases of CL conduced to evaluate the epidemiological situation of CL. male infection was more infected than female and the age group of 5-14 years was found to have the highest recorded CL cases [32].

Table 2: Number of subjects examined and positive cases of infection with Leishmania species in the field study using direct agglutination test using antigens, distributed according to gender and age.

Antigen type		DAT L. tropica								DAT L. major					
age	total number of examinees	Males			Females			Total		Males		Females		Total	
		Examined	Injured	%	Examined	Injured	%	Injured	%	Injured	%	Injured	%	Injured	%
<1	82	43	-	-	39	-	-	-	-	-	-	-	-	-	-
1-2	112	67	-	-	45	-	-	-	-	-	-	-	-	-	-
< 2-3	167	82	-	-	85	1	1.17	1	0.59	-	-	-	-	-	-
< 3-4	136	73	1	1.36	63	1	1.58	2	1.47	-	-	-	-	-	-
< 5-6	121	61	-	-	60	1	1.66	1	0.82	-	-	-	-	-	-
< 5-10	130	57	10	17.54	73	4	5.47	14	10.76	1	1.75	-	-	1	0.76
< 10-20	104	43	7	16.27	61	7	11.47	14	13.46	8	18.60	4	6.55	12	11.53
<20-30	92	32	8	25	60	5	8.33	13	14.13	8	25	3	5	11	11.95
<30-40	136	62	12	19.35	74	3	4.05	15	11.02	10	16.12	8	10.81	18	13.23
<40-50	122	72	3	4.16	50	2	4	5	4.09	-	-	-	-	-	-
<50-60	107	47	-	-	60	1	1.66	1	0.93	-	-	-	-	-	-
<60-70	152	87	-	-	75	-	-	-	-	-	-	-	-	-	-
<70-80	78	32	-	-	46	-	-	-	-	-	-	-	-	-	-
>80	57	29	1	3.44	28	-	-	1	1.75	-	-	-	-	-	-
Total	1596	787	42	5.33	809	25	3.09	67	4.19	27	3.43	15	1.85	42	2.63

5. Conclusions

The direct agglutination test outperformed the indirect method in sensitivity and specificity, proving its effectiveness for diagnosing leishmaniasis.

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Conflicts of Interest: The authors declare no conflict of interest

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