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EPIDEMIOLOGICAL STUDY OF CUTANEOUS LEISHMANIASIS IN DIYALA PROVINCE

Raad J. Humadi[1], Ammar T. Naser[2], Ghassan H. Jameel[2]*, Abdulwahab M. Khalifah[3], Sarah J. Abdulameer[4], Fatima Ghassan H. Jameel[5]

[1]Department of Veterinary Public Health, College of Veterinary Medicine, Diyala University, Iraq.
[2]Department of Veterinary Microbiology, College of Veterinary Medicine, Diyala University, Iraq.
[3]Department of Veterinary Microbiology, College of Veterinary Medicine, Baghdad University, Iraq.
[4]Department of Veterinary Pathology, College of Veterinary Medicine, Diyala University, Iraq.
[5]Department of Microbiology, College of Science, Diyala University, Iraq.

***Corresponding Author: Ghassan H. Jameel**
Department of Veterinary Microbiology, College of Veterinary Medicine, Diyala University, Iraq.

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ABSTRACT
Epidemiology is the study of disease occurrence in human populations. As a science, epidemiology emphasizes descriptive and analytic observation, clinical trial, behavioral intervention and the practical utility of diagnostic tests. The results were refer to the rate of Cutaneous Leishmaniasis in Diyala province according to the region and the time of infection. The disease is epidemiologically unstable, with large and unpredictable fluctuations in the number of cases. People can carry some species of Leishmaniasis for long periods without becoming ill. When we display the number of infected human, December was gave high number reach to 369 person. Baladros sector take place the high rate of infection reach to 224 person from other region in Diyala. Due to gender distribution, relatively no differences had been noted. The highest age specific active lesion prevalence was noted in the age group 5–14 (23.77%) followed by age group of 1–4 (12.73%).

KEYWORDS: Cutaneous Leishmaniasis, epidemiology, Diyala, Iraq.

INTRODUCTION
Leishmaniasis is a parasitic disease caused by haemoflagellate *Leishmania.* The disease is widespread and may cause serious health problems in communities throughout the Mediterranean regions and the Middle East, including Iraq (CDC, 2004 and Hepburn, 2003).

There are several different forms of leishmaniasis. The most common form is cutaneous leishmaniasis (CL), which causes skin sores. Visceral leishmaniasis (VL), which affects some of the body's internal organs spleen, liver and bone marrow. Leishmaniasis is endemic to Iraq, Kuwait, Iran, Afghanistan, and other places in the Middle East; and poses a health risk to service members deployed there. The sand fly season in Iraq is from April through November and peaks in September/October. While effective treatment is available, prevention remains the best option. Leishmaniasis is not the same thing as Sand fly fever which is a different disease spread by sand flies. Both VL and CL have been reported in Iraq caused by *Leishmania donovani*, *Leishmania major* and *Leishmania tropica* respectively(WHO,2003).

The genus *Leishmania* has two morphological forms in its life cycle: the amastigote within macrophages of mammalian host and promastigote in the gut of invertebrate host(Paniker,2002).[2] More than 12 million people in 88 countries are known to be infected with leishmaniasis, but the true burden remains largely hidden. Two million new cases – 1.5 million of cutaneous leishmaniasis over 90% occur in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru, 500 000 of the visceral form of the disease occur annually, but declaration of the disease is compulsory in only 32 countries and a substantial number of cases are never recorded (WHO.2005 and Ashford et al.,2002).

The aims of the present study to assess the epidemiology and to identify outbreaks of Cutaneous Leishmaniasis in Diyala governorate.

Leishmaniasis, a vector - borne disease caused by obligate intra-macrophage protozoa and characterized by diversity and complexity (Postigo, 2010). There are more than 21 species causing human infection (Singh and Sivakumar, 2003).

Leishmaniasis comprises a group of diseases caused by the genus *Leishmania* widely distributed in tropical and subtropical regions throughout the world. These diseases

transmitted by various species of Phlebotomus sand fly in Old World. *Leishmania* genus can be divided into several species complexes (Talmi-Frank et al.,2010). It occurs in three clinical forms including cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL) and visceral leishmaniasis (Rhajaoui, 2011 and Bousslimi *et al*., 2010).

Cutaneous leishmaniasis is the most common form of leishmaniasis, with about 1.5 million new cases every year worldwide. The surveys demonstrate that cases of leishmaniasis are increasing worldwide, mainly due to environmental changes. Irregular buildings in towns and collection of domestic garbage in suburbs, plus migration of susceptible populations could also be causative factors. Poverty and malnutrition play important roles in increased morbidity of the disease (Khan and Muneeb,2005).

Leishmania major, *L. tropica* and sometimes *L. infantum* are the causative agents of cutaneous leishmaniasis in Old World. Most of the cases of CL occur in Afghanistan, Algeria, Saudi Arabia, Brazil, Iran, Iraq, Syria and Sudan (Rasheed, 2004).

In Iraq, two species are present: *L. tropica*, the agent of anthroponotic cutaneous leishmaniasis (ACL), and *L. major*, the agent of zoonotic cutaneous leishmaniasis (ZCL). Both ACL and ZCL were reported as causative agents of leishmaniasis in Iraq, but ACL is found mainly in Suburban areas (Postigo, 2010).

Depending on the species causing the disease, different preventive measures are taken against leishmaniasis (Sharma and Singh, 2008). All *Leishmania* species have the same morphology; hence, they are not distinguishable microscopically (Mahmoodi *et al*., 2010).

Cutaneous leishmaniasis in Iraq

In all areas of Iraq there had also been cases of cutaneous leishmaniasis. The course of the disease is much more gentle than that of kala-azar (VL). In 2001 there were 625 cases of cutaneous leishmaniasis, 955 cases in 2000 and as many as 8779 cases in the peak year 1992 (45 cases for every 100 thousand citizens). Cases of cutaneous leishmaniasis caused by *L. tropica* mostly occur in the suburbs of big cities (Baghdad, Mosul) among large conglomerations of people where the sanitary conditions are unsatisfactory. Incidences caused by *L. major* are much more common; they appear primarily in rural areas, especially in the northern and southern provinces of the country (Korzeniewski 2005 and WHO,2003).

Rahi,(2013) was reported in previous study that CL was significantly associated with illiteracy and farmers as an occupation which is usually more common in rural population (67.4%).

Rahi *et al*., (2014) showed that 23(43%) of CL patients were had single lesion and 31(57%) had multiple lesions, most of them 41 (57%) in arm. The highest incidence of disease 36(67%) was observed in rural areas and the lowest incidence rate 18(33%) was in urban areas.

There are an estimated 12 million cases worldwide and there are about 1.5 million new cases of cutaneous leishmaniasis each year, of which over 90% occur in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru (Ashford *et al*., 1992). Old World disease primarily is caused by *Leishmania tropica* in urban areas and *Leishmania major* in dry desert areas (Markle and Makhoul, 2004).

In Iraq, two species are present: *L tropica*, the agent of anthroponotic cutaneous leishmaniasis (ACL), and *L. major*, the agent of zoonotic cutaneous leishmaniasis (ZCL). Both ACL and ZCL were reported as causative agents of leishmaniasis in Iraq, but ACL is found mainly in suburban areas (WHO, 2003).

The disease is epidemiologically unstable, with large and unpredictable fluctuations in the number of cases. The total incidence rate of cutaneous leishmaniasis in Iraq varies from 2.3 / 100000 to 45.5 / 100000 (WHO, 2003).

In Basrah, only 41 cases were recorded during the period 1971- 1984 (Sukker, 1984) and l47 cases for the period of 1993-1997 (Al- Edan, 2001). According to the data of CDC Surveillance Unit/ Primary Health Care Department/ Basrah, the number of cases that were reported in 2004 and 2005 was 608; about 210 (34.5%) of them were from Al-Qurna district (Jafer,2005).

The clinical diagnosis of visceral leishmaniasis is complex because it's clinical features are shared with other commonly occurring diseases, such as malaria, typhoid fever and tuberculosis; many of these diseases can be present along with visceral leishmaniasis as co-infection (Sundar and Rai, 2002). The visualization of the amastigote form of the parasite by microscopic examination of aspirates from lymph nodes, bone marrow or spleen is the classical confirmatory test for VL. Although the specificity is high, the sensitivity of microscopy varies, being higher for spleen (93-99%) than for bone marrow (53-86%) or lymph node (53-65%) aspirates (Chappuis *et al*, 2007).

In 1988, a modified Direct Agglutination Test (DAT) was reported to be useful in the diagnosis of visceral leishmaniasis and is being used in several endemic countries (El-Harith *et al*, 1988). DAT in various studies has been shown to be (91-100%) sensitive and (72-100%) specific (Singh, 1995 and Sunder,1996). Because of the conditions prevailling in areas of endemicity, sophisticated method cannot be employed on a wider scale. There is a need for a simple, rapid and accurate test with good sensitivity and specificity which can be used without any specific expertise (Sundar and Rai,

2002). A promising ready-to-use immunochromatographic strip test is based on, a recombinant antigen rK39, which has been developed as a rapid test for use in difficult field conditions (Sundar and Rai, 2002).

The diagnosis of CL in Iraq is based on clinical signs of the disease and microscopic observation of parasites in stained skin biopsies (Rahi *et al*., 2013). Specific and sensitive molecular diagnostic tools been implemented and information about disease distribution, parasite life cycle and combining risk factors is confined (Kumar *et al*., 2007).

In an endemic area, CL is largely diagnosed by its clinical appearance. Diagnostic challenges arise when cases appear in non-endemic areas, when the clinical picture is distorted, or when any atypical variants are seen even in endemic regions (Arfan and Rahman, 2006). In addition, secondary infection or mistreatment can alter the clinical picture of CL and cause difficulty in diagnosis and delay in treatment. In such cases the diagnosis should be confirmed by examination of smears from lesions, culture, and histopathological examination (Singh and Sivakumar, 2003).

In developing countries such as Iraq, laboratory equipment and materials such as ELISA test kits or PCR technique materials are not available and dermatologists mostly have to rely on the clinical characteristics of the lesion. Giemsa- or leishman-stained smears obtained from the lesions are a rapid means of diagnosis (Ramirez *et al*.,2000). Although CL cases have been reported in Iraq, the epidemiological and clinical characteristics regarding Alhaweja district have not been well documented.

Leishmania parasite is a protozoa belonging to the order *Kinetoplastida* and the family of *Trypanosomatidae*. The genus *Leishmania* includes more than 20 species. The parasite exists in two morphological forms: the nonflagellated amastigote (3-5 mm in diameter) living intracellular in macrophages of the mammalian host, and the flagellated promastigote, living extracellular in the intestinal tract of the sandfly-vector. In the macrophages the amastigotes are able to survive and multiply within the acidic phagolysosomes of the host cells (reviewed by Alexander *et al*., 1999). After multiplication in the host cell the amastigotes are released. Subsequently other macrophages are infected and the infection spreads (Rittig and Bogdan, 2000). The parasite contains two prominent organelles, the nucleus and the kinetoplast. The kinetoplast is found in all protozoa of the order kinetoplastidae (eg. *Leishmania, Trypanosoma, Crithidia*). The transmission cycle is maintained between the vector and the reservoir. Depending on the species of *Leishmania,* the transmission is either zoonotic or anthroponotic, involving either animals or humans as reservoir. The parasite is transmitted by the bite of female sandflies of the genus *Phlebotomus* and *Lutzomyia*. During the blood meal *Leishmania* infected macrophages are ingested by the vector. In the gut of the sandfly the intracellular amastigotes develop into flagellated promastigotes at an ambient temperature of 24-28◦C. During another blood meal the mature promastigotes are inoculated into a mammalian host. Macrophages ingest the parasites, which then transform into the amastigote form.

MATERIAL AND METHODS

All cases (652) of Cutaneous Leishmaniasis notified between 1/1/2014 to 31/12/2014 were identified from central hospital of public health in the province of Diyala. Age and gender of patients was recorded in addition to the distribution of CL cases in relation of region in diyala.

RESULTS

Geographical distribution

Table 1 shown the prevalence of CL according to the regions and months(time of incidence). The high rate of infections 369/652 (56.60%), 88/652 (13.49%) and 69/652(10.58) appeared in December, January and February respectively. While the low rate of infections were recorded in May, July and September, furthermore the present study never record any case of CL at June. Also the table (1) reveals the higher percentage in Baladros (34.36%), Khanaqen (20.55%) and Baquba first sector at (18.56%), while the lower infections were recorded in Al-mansoria sector (4.14%).

Age and Sex distribution

Table-2 demonstrates that the highest percentage of infections in the age between 5-15 years of life (41.1%) and the males were mainly affected (23.77%). After that the percentage was decreased sharply as the age increased to record the lowest percentage of infections in the age mor than 45 years of life (4.45%) and the females were mainly affected (2.76%). In addition, there was difference in the frequency of the disease between males and females in all age groups.

Table (1): Distribution of CL cases in relation of months and some regions of Diyala

region Months	Baquba first	Baquba Second	Al-khalis	Baladros	Al-mansoria	Al-moqdadia	Khanaqen	Total number	%
January	6	3	6	17	4	2	50	88	13.49%
February	4	1	7	22	2	2	31	69	10.58
March	10	1	2	13	Non	1	1	28	4.29
April	Non	Non	Non	13	Non	Non	10	23	3.53
May	Non	Non	Non	1	Non	Non	1	2	0.31

June	Non	Non	Non	Non	Non	Non	Non	Non	0
July	4	1	Non	Non	1	Non	Non	6	0.92
August	7	Non	Non	1	1	1	Non	10	1.53
September	1	1	3	1	Non	Non	Non	6	0.92
October	8	4	3	1	3	3	Non	22	3.37
November	12	3	7	1	3	3	Non	29	4.45
December	69	47	15	154	13	30	41	369	56.60
total	121	61	43	224	27	42	134	652	
%	18.56	9.36	6.60	34.36	4.14	6.44	20.55		

Table (2): Distribution of CL cases in relation of months and gender

age / Months	Less one year		1-4 years		5-14 years		15-45 years		More than 45 years		total	%
	Male	female	Male	female	Male	female	Male	female	Male	female		
January	2	4	20	15	21	9	9	6	1	1	88	**13.49%**
February	4	2	8	5	17	11	8	9	1	4	69	**10.58**
March	2	2	0	3	10	3	1	4	2	1	28	**4.29**
April	0	0	5	0	5	3	5	5	0	0	23	**3.53**
May	0	0	0	0	0	0	1	1	0	0	2	**0.31**
June	0	0	0	0	0	0	0	0	0	0	Non	**0**
July	0	0	2	0	2	1	1	0	0	0	6	**0.92**
August	0	3	1	1	1	0	0	3	1	0	10	**1.53**
September	1	0	0	1	2	0	1	0	0	1	6	**0.92**
October	2	1	1	6	1	4	2	4	0	1	22	**3.37**
November	0	4	2	7	4	7	2	33	0	0	29	**4.45**
December	13	16	40	45	92	75	30	42	6	10	369	**56.60**
total	24	32	79	83	155	113	60	77	11	18	652	
%	3.68	4.91	12.12	12.73	23.77	17.33	9.20	11.81	1.69	2.76		

DISCUSSION

Leishmaniasis is caused by parasites from the genus *Leishmania*. A person gets leishmaniasis when bitten by an infected sand fly.

The parasite lives and multiplies inside the female sand fly. The insect is most active in humid environments during the warmer months and at night, from dusk to dawn. Domestic animals, such as dogs, can serve as reservoirs for the parasite. Transmission can occur from dog to sand fly to human(WHO,2012).

The results in table (1) were revealed the rate of cutaneous leishmaniasis in Diyala province according to the region and the time of infection. The disease is epidemiologically unstable, with large and unpredictable fluctuations in the number of cases. People can carry some species of *Leishmania* for long periods without becoming ill. When we display the number of infected human, December was gave high number reach to 369 person wherethrough poor protection from the vector of the disease and is more than other months as April, May, June and July.

Baladros sector takeplace the high rate of infection reach to 224 person from other region in Diyala.

In table (2) considering gender distribution, relatively no differences had been noted. This result did not agree with Bsrat *et al*., (2015) which refer to the high infection be in the males rather than females. The highest age specific active lesion prevalence was noted in the age group 5–14 (23.77%) followed by age group of 1–4 (12.73%).

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