Epidemiological Study to Investigate a Possible Vector of Visceral Leishmaniasis in the Central Region of Iraq

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ABSTRACT
An epidemiological study in Al-Mahmmodiya (50 km south Baghdad) to investigate a possible vector of Leishmaniasis were applied. This region is considered as a foci of Leishmaniasis according to the health association statistics. CDC light traps were used to collect the insects nightly. Insects were collected by Indoor application as human dwellings and animal shelters and Outdoor application as rodents’ barrows and field trees. Sand flies were transported to the laboratory, isolated and identified according to the identification keys in Tropical Biological Researches Unit at the College of Science / University of Baghdad. Must of the collected sand flies were belonging to three species Phlebotomus papatasi Scopoli (45%), Phlebotomus alexandri Sinton (35%) and Sergentomiya baghdadis Adler & Theodor (20%). About 896 feeding females of sandflies were dissected; only 32 ones were infected with the parasites identified as Leishmania spp. five samples were cultured in parasite media to insure the type of parasite. Only the specie of Phlebotomus papatasi was containing parasites inside. The culture media was contaminated, so the attempts to get pure culture to discover the possibility of transport another type of parasite are continue. The aim of the study is to investigate a possible vector of Leishmaniasis in Al-Mahmmodiya region.

INTRODUCTION
Leishmaniasis is a parasitic disease caused by parasitic protozoa of the genus Leishmania spp. [1]. Cutaneous Leishmaniasis (CL) is known by many names as Baghdad boil, Delhi boil, Aleppo boil and Orient sore. Also Visceral Leishmaniasis (VL) is known as kala-azar or black fever [2].

Symptoms of CL may include, Breathing difficulty, Skin sores, which may become a skin ulcer that heals very slowly, Stuffy nose,
runny nose, nose bleeds, swallowing difficulty, Ulcers, wearing away (erosion) in the mouth, tongue, gums, lips, nose, and inner nose. Systemic Visceral infection in children usually begins suddenly with cough, diarrhea, fever and vomiting. Adults usually have a fever for 2 weeks to 2 months, along with symptoms such as fatigue, weakness, and appetite loss. Weakness increases as the disease gets worse [3]. Other symptoms of systemic VL may include, abdominal discomfort, fever that lasts for weeks, may come and go in cycles, night sweats, scaly, gray, dark, ashen skin, thinning hair and weight loss swelling of the spleen and liver, and anemia [4].

The most immediate form of protection to disease is preventing sand fly to bite us. We can prevent a bite by putting fine mesh netting around the bed (in areas where the disease occurs), screening windows, wearing insect repellent and wearing protective clothing. There are no vaccines or drugs that prevent Leishmaniasis [5]. The World Health Organization is providing technical and logistic support to reduce the incidence of Leishmania in Iraq through early diagnosis and response, spraying and fogging campaigns, provision of long-lasting insecticide treated bed nets, training inside and outside Iraq, entomological surveillance, rodent control activities, and increasing of community awareness [6].

The reduction in the incidence of both Malaria and Leishmaniasis was attributed to the comprehensive package of prevention and control activities conducted by the Iraqi Ministry of Health with technical and logistics support from the World Health Organization [7].

WHO has classified Leishmaniasis as emerging, uncontrolled and estimates that the disease infects two million new cases each year. The disease is widespread and may cause problems throughout the Mediterranean regions and the Middle East including Iraq [8]. There are 12 million people infected worldwide, and Leishmaniasis threatens 350 million people in 88 countries [9]. Over than 90% of Leishmaniasis is occur in Afghanistan, Algeria, Iran, Iraq, Saudi Arabia, Syria, Brazil and Peru [10]. Many researchers interested during the last century on the studies of Iraqi sand flies and their possible relations with both CL and VL in Iraq [11]. But relatively few studies have examined the temporal and geographic distribution of sand flies throughout the country [12]. The total incidence rate of cutaneous Leishmaniasis in Iraq varies from 2.3 cases per 100000 people to 45.5 cases per 100000 people. Baghdad considered as endemic area with both CL and VL [13].

There are two kinds of Leishmaniasis in Iraq 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. ACL is found mainly in suburban areas. VL is common in rural areas around Baghdad and about 92% of patients are at the first three years of age [14].
The traditional foci of Leishmaniasis are Diala Bridge, Medain, Sowira, Aziziya, Kahn Bane saad, Taji, Falowja, Abo-Greeb, Mahmmodiya, Latifiya, Nomnaniya, Missan, Thiqar and Alhaweja districts [15].
Human are infected via the bite of phlebotomine sandflies, which breed in forest areas, caves, or the burrows of small rodents [16].
The disease is probably transmitted among people and animals by anthropophilic species of the genus of Phlebotomus and Sergentomiya, Phlebotominae, Psychodidae and Diptera. Phlebotomine sand flies are of widespread importance in the transmission of Leishmania pathogens in Iraq [17].

Phlebotomus papatasi (Diptera: Psychodidae) is a vector of sand fly fever virus and Leishmania major, which causes CL [18]. Phlebotomus alexandri is a vector of L. donovani and is a suspected vector of L. infantum, both of which cause VL [19]. The suspicion is directed towards the anthropophilic species of Phlebotomus papatasi, P. alexendri, P. sergenti, P. clydi, Sergentomyia baghdadis, S. sintoni, S. squamipleuris and S. palestinensis. In the central region of Iraq the P. papatasi consisted about 95% of the total number of sand fly species [20].
The sand flies of Iraq were described by [21, 22 and 23]. Most recently, Abul-Hab and Ahmed reported 14 species of phlebotomine sand flies in Iraq to include six species of Phlebotomus and eight species of Sergentomyia. Of these 14 species, Phlebotomus papatasi Scopoli, Phlebotomus sergenti Parrot, and Phlebotomus alexandri Sinton are the only anthropophilic sand flies considered to be important vectors of Leishmania in Iraq [24]. P. sergenti and P. papatasi are almost certainly the primary vectors of Leishmania tropica and Leishmania major [25]. Sukkar has suggested that P. papatasi was also the primary vector of VL [26]. However, P. papatasi is a restricted vector and is incapable of transmitting any species of Leishmania other than L. major [27]. Some studies were indicating that P. alexandri should be considered a potential vector of VL in Iraq [28]. Abul-Hab and Ahmed reported that P. alexandri was the second most abundant and widely distributed species of Phlebotomus in Iraq and that because it was often associated with humans it should be considered a potential vector of Leishmaniasis [29].

P. papatasi ranges from Morocco and Spain, across the Mediterranean Basin to India and south to parts of the Sudan and Ethiopia [30]. P. papatasi is most abundant in areas with a mean minimum temperature
of 16°C and mean maximum temperature of 44°C from May to October. It can be found at elevations ranging from near sea level to over 1100 m [31]. *P. alexandri* ranges from Spain and Morocco east to the mountains in northwestern China and as far south as southern Ethiopia. This species has been recorded at elevations ranging from sea level to 1500 m above sea level [32].

Characterizing the distribution and ecology of these vector species would be valuable in better understanding the epidemiology of sand fly fever and Leishmaniasis. Researchers were developing a model of *P. papatasi* distribution in Southwest Asia based on weather and the normalized difference vegetation index (NDVI). The vectors of VL in Iraq are different from that of the Mediterranean area. This is due probably to the wide variation in Iraqi fauna caused by the geographical situation [33].

The central region of Iraq is a very wide alluvial plain; its elevation is from 36 m - 300 m.a.s.l. The climate is of a desert and semi-desert. During the last few years the dusty days increased due to dry season. The trees of Dactylifera, *Citrus siensis*, *Morus aibus*, *Pyrus cpmminus*, etc. cover the region. The main occupations of villagers are agriculture, poultry farms and catties. The aim of this study was twofold: to decide the incidence of Leishmaniasis vectors in Al-Mahmmodiya District, and to make sure the type of parasite that carried by these vectors.

**MATERIAL AND METHODS**

**STUDY AREA**

One station (Al- Mahmmodiya about 50 km. south of Baghdad) was selected in this endemic area according to the following:

1. The high number of reported cases of Visceral Leishmaniasis in the three last years.
2. The high density of sand flies.
3. The possible canine Leishmaniasis and high number of species of rodents.
4. To use the data of earlier studies which had been conducted in the area as a baseline data.
5. The station is lying in Al-Rasheed district in an area called Abo-Hdlan village which has many cases of the Leishmaniasis.

**COLLECTION AND EXPERIMENTS**

Two CDC light traps were used in collecting of sand flies (model 512, John W. Hock Company, Gainesville, FL).

1. Collection of sand flies was begun in July continuing to November 2001.
2. Collection of sand flies was twice in week at 32 times along the study.
3. Sand flies were collected in two ways Indoor and Outdoor applied.
4. Collection of sand flies was from different of sand flies habitats, as human dwellings, animal shelters, near rodent's barrows and on the field trees.
5. CDC light traps were provided with collection cups with suitable standard mesh with moisture cotton piece.
6. Sand flies were transported to the laboratory handling not to direct exposure to sunlight to stay alive.
7. Sand flies were anaesthetized by cigarette fog.
8. Sand flies were identified by microscope to isolate males by genitalia while spermatheca, ciborium and pharynx was performed the female's identical [35].
9. Collected Sand flies were dissected at the next days.
10. The gut was exposed to search of flagellate. When parasite presented inoculation take place in the media. The females were kept alive with 0.5 ml. Gentamicin (or ampicillin) 500 u/ml, and 0.6 % Na Cl. Smears of midgut of feeding sand flies were done with sterile slides washed in 95% methanol alcohol, then fixed with 95% formaldehyde before staining with Giemsa stain; this simple technique offered the isolation of *Leishmania sp.* from the infected females.

**STATISTICAL ANALYSIS**

T-test was used to compare means of:
- Indoor and outdoor methods
- Types of sand fly species
- Collected places
- Months of collection

**RESULTS AND DISCUSSION**

Big number of collected sand flies in this study in total 11201 during 2 traps nights, with 90% of traps containing sand flies. Whereas most other recent studies are collecting less than 5,000 sand flies [22, 23, and 26]. CDC light traps that used in this study are a suitable method to collect the sand flies. Also light traps have been commonly used for the collection of sand flies. While other studies were used different collecting methods as sticky paper [36]. We had reported many new cases of Visceral Leishmania in this focus.

Females collected sand flies of all species were more than 70% while males of them were less than 30% and of these sand flies, 8% contained visible blood in their guts. The total numbers of collected females’ sand flies were 7948 and of males was 3253. Number of *P. papatasi* males were 1572 represent 30% and of females was 3665 represented 70% of
all species collected numbers. Number of *P. alexandri* was 1026 represented 29% and of females was 2512 represented 71% of all species collected numbers. Numbers of S. baghdadis were 655 represented 27% and of females was 1771 represented 73% of all species collected numbers. The study showed significant between abundance of different species (*t*=4.568 sig. 0.04). All 896 sand flies dissected females were *P. papatasi* because no one feeding female of the other two species. Although big numbers of sand flies were collected (11201) there is less number of feeding female (896) so it is an effect on the positive cases of parasites present (only 32). Five samples of positive cases of parasite present were cultured in parasite media to make sure the type of parasite which seems to be *L. donovani* (Table1). So that we can say it's possible to announce that these parasites are *L. donovani*. Sukker was isolated *Leishmania Sp.* from 4 infected *P. papatasi*. In Al-Nommaniya district in Wasit province, in addition to this finding one infected female of *P. papatasi* [18]. Also investigators thought that *P. papatasi* find to be the vector of visceral and cutaneous Leishmaniasis in Iraq [19, 37, and 38]. Unfortunately the culture was contaminated. The study was recorded that the type *P. papatasi* is a vector of Leishmaniasis. Many other investigators think that *Sergentomiya baghdadis* is also suspected as a possible vector of both visceral and cutaneous Leishmaniasis in Iraq [26].

The study is recorded three species of sand fly *Phlebotomus alexandri* Sinton (35%), *Phlebotomus papatasi* Scopoli (45%), and *Sergentomiya baghdadis* (20%). Fewer numbers of *Sergentomiya baghdadis* species were identified but there are no feeding females among them. The specie of *S. baghdadis* is not dissected or cultured because there are no feeding flies. The study showed that the type *P. papatasi* was containing the parasite while the other types *Phlebotomus alexandri* and *Sergentomiya baghdadis* were not contained parasites. The dominated species was *Phlebotomus papatasi* because it can be found with a very high density in the open land (not cultivated) [30]. Collection of sand flies was applied in two ways indoor and outdoor methods. The study was showed significant between applying of different collection ways (*t*=9.517 sig. 0.06). Four different habitats were chosen to collect the sand flies there are human dwellings, animal shelters, rodent barrows and field trees. Sand flies were abundances at indoor way more than outdoor way. Number of sand flies at the indoor way was 6189 representing 55.2% of the total number while that number at outdoor was 5012 represent 44.8% of the total number. Generally, all numbers of types of collecting sand flies were indoor way more than outdoor. *P. papatasi* number at the indoor way was 2775 sand
flies represent 53% while at the outdoor way it was 2462 sand flies represent 47% of the total numbers. P. alexandri number at the indoor way was 1938 sand flies represent 54.7% while at the outdoor way it was 1600 sand flies represent 45.3% of the total number. S. baghdadis number at the indoor way was 1476 sand flies represent 60.8% while at the outdoor way it was 950 sand flies represent 39.2% of the total number. Present of light at the indoor habitats which attracts the insects so that it contain sand flies more than the outdoor habitats (Table 2).

Animal shelters sand flies habitats were containing the highest of collecting numbers of sand flies which were 3138 in mean of 1064 that represent 28% of all sand flies collected. Number of sand flies collected from human dwellings habitats was 3050 in mean of 1016.6 which represent 27.3% of all collections. Number of sand flies collected from rodent barrows habitats was 2751 in mean of 917 which represent 24.5% of all collection. Number of sand flies collected from field trees habitats was 2262 in mean of 745 which represent 20.2% of all collections. Small variables between numbers of species that collected from different habitats (table 3). The study was showed signification between different habitats (t=3.361 sig. 0.04).

Period of the collection was a long four months beginning from July to November. Number of P. papatasi was increased from 1012 in July to 2033 in October. Number of P. alexandri was increase from 802 in July to 1007 in October. But the number of P. papatasi was decreased from 619 in July to 595 in October (Figure 1). The study was showed significant between months of collection (t=9.945 sig. 0.002). The habits of feeding (anthropophilic species) and wide distribution of P. papatasi in Iraq make this species as a suspected vector of infantile Leishmaniasis. Studies on the nocturnal activity of sand flies indicated that they were most active early in the evening during the cooler months, whereas they were more active in the middle of the night during the hotter months. P. alexandri was more abundant earlier in the season (April and May) than P. papatasi, whereas P. papatasi predominated later in the season (August and September). Factors evaluated include species diversity and temporal (daily and seasonal) and geographic distribution of the sand flies. In addition, the abundance of sand flies in the indoor and the outdoor habitats was observed. Many studies found that the sand fly numbers were increased with months low in April, rose through May, were highest from mid-June to early September, and dropped rapidly in late September and October [31].

CONCLUSION
We think that the number of dissected sand flies is not enough to investigate the parasites and this type of study must continue for a long
time, use a big number of traps and collect sand flies from many foci of Leishmaniasis. The isolation of parasites from the vector in large numbers and in traditional focus of Visceral Leishmaniasis in central Iraq is important. Only *P. papatasi* was containing the parasite in a recent study so it is the possible vector of Leishmaniasis in study area while the other types *P. alexandri* and *S. baghdadis* were not contained parasites. The type of the parasite which found at this study was *L. donovani*.

**ACKNOWLEDGMENT**

We are grateful to Dr. Shihab Ahmed Salman for helping of identification of sand flies and parasites in this study.

Table 1: showing the numbers of males, females, dissected feeding females, present of parasites and results of parasite culture.

<table>
<thead>
<tr>
<th>Species of SF</th>
<th>Males (%)</th>
<th>Females (%)</th>
<th>Dissected Feeding female</th>
<th>Present of parasite</th>
<th>Parasites media results</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. papatasi</em></td>
<td>1572(30)</td>
<td>3665(70)</td>
<td>896</td>
<td>32</td>
<td>5+</td>
</tr>
<tr>
<td><em>P. alexandri</em></td>
<td>1026(29)</td>
<td>2512(71)</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><em>S. baghdadis</em></td>
<td>655(27)</td>
<td>1771(73)</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>3253</td>
<td>7948</td>
<td>896</td>
<td>32</td>
<td>5+</td>
</tr>
</tbody>
</table>

Table 2: showing the numbers and percentages of sand flies at indoor and outdoor application.

<table>
<thead>
<tr>
<th>Sand fly species</th>
<th>Indoor No. (%)</th>
<th>Outdoor No. (%)</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. papatasi</em></td>
<td>2775 (53)</td>
<td>2462 (47)</td>
<td>5237 (46.8)</td>
</tr>
<tr>
<td><em>P. alexandri</em></td>
<td>1938 (54.7)</td>
<td>1600 (45.3)</td>
<td>3538 (31.5)</td>
</tr>
<tr>
<td><em>S. baghdadis</em></td>
<td>1476 (60.8)</td>
<td>950 (39.2)</td>
<td>2426 (21.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>6189(55.2)</td>
<td>5012(44.8)</td>
<td>11201 (100)</td>
</tr>
</tbody>
</table>

Table 3: showing the numbers, means, and percentages of sand flies collected from different habitats.

<table>
<thead>
<tr>
<th>Traps location</th>
<th>SF No. (%)</th>
<th>Mean of SF</th>
<th>Numbers of sand flies species</th>
<th>S. baghdadis No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human dwellings</td>
<td>3050</td>
<td>1016.6</td>
<td><em>P. papatasi</em> No. (%) 1300 (42.6)</td>
<td>925 (30.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>P. alexandri</em> No. (%) 1013 (32.2)</td>
<td>825 (27.1)</td>
</tr>
<tr>
<td>Animal shelters</td>
<td>3138</td>
<td>1064</td>
<td><em>S. baghdadis</em> No. (%) 863 (31.3)</td>
<td>650 (20.8)</td>
</tr>
<tr>
<td>Rodent barrows</td>
<td>2751</td>
<td>917</td>
<td></td>
<td>613 (22.3)</td>
</tr>
<tr>
<td>Field trees</td>
<td>2262</td>
<td>745</td>
<td></td>
<td>338 (14.9)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>11201</td>
<td>3733.66</td>
<td><em>P. papatasi</em> No. (%) 5237(46.8)</td>
<td>3538(31.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>P. alexandri</em> No. (%) 3538(31.5)</td>
<td>2426(21.7)</td>
</tr>
</tbody>
</table>
Figure-1: showing the numbers of collected sand flies through the period of study.

REFERENCES
