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Entomological survey of phlebotomine sand flies (Diptera: Psychodidae) in a focus of visceral leishmaniasis in central Iran

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Abstract

Background & objectives: Visceral leishmaniasis (VL) is a major vector-borne disease in Iran. A focus of VL is present in Shahreza county, Isfahan province, central Iran. The main objective of this study was to determine the probable vectors in this area.

Methods: Sand flies were collected biweekly using sticky paper traps, CDC light-traps, and aspirators from outdoors as well as indoors. All female sand flies were dissected and identified. Promastigotes were inoculated to hamsters and detected by Nested PCR. Approximately 7528 sand flies representing 12 species were collected from April 2003 to October 2004. *Phlebotomus (Phlebotomus) papatasi* Scopoli, *Phlebotomus (Larroussious) major* Annandale and *Sergentomyia (Sergentomyia) sintoni* Pringle were the predominant species. Two percent of *P. major* was found with natural promastigote infections.

Interpretation & conclusion: This is the first report of natural promastigote infection in *P. major* in central Iran. The activity of *P. major* started from April and ended in October with a peak in September. The parasites were identified as *Leishmania infantum* using standard PCR. *P. major* is a possible vector of leishmaniasis and is susceptible to DDT in this area.

Key words Iran – *Leishmania infantum* – leishmaniasis vectors – *Phlebotomus (Larroussious) major* – Shahreza –Visceral leishmaniasis

Introduction

Sand flies (Diptera: Psychodidae and Phlebotominae) belonging to genera *Phlebotomus* and *Lutzomyia*, are the vectors of the leishmaniasis, in the old and new world, respectively. About 30 species of sand flies are proven vectors of at least 20 *Leishmania* species.

Visceral leishmaniasis (VL) or kala-azar is a severe and often fatal disease in the Mediterranean littoral, Central Asia, Africa, South and Central America[1]. This form of disease appears as an opportunistic disease associated with the HIV infection and in other immune suppressed patients[2]. An estimated 200 million people are at risk of VL and 500,000 of new human kala-azar cases are registered annually[3]. The disease is one of the most important vector-borne diseases in Mediterranean Basin and in Iran. In addition to dogs, wild carnivores such as jackals and foxes, which are reported as infected carriers of *Leishmania*, are also considered as the animal reservoirs of kala-azar in this area[4]. There are four main endemic foci of VL in Iran including Ardabil, East

Azerrbaijan (Northwest), Fars and Bushehr (South) provinces[5,6]. During recent years, the number of VL cases has gradually increased in Shahreza county (unpublished data). Since malaria was endemic in Isfahan province so DDT spraying, 75% water dispersible powders, 2 g/m^2 was done one round per year during 1953–58. This area entered the consolidation phase after 1958, and indoor residual spraying was stopped, but insecticides were used in agriculture as well as domestic sprays in human dwellings.

Considering the importance of the vector(s) in the transmission of the VL cycle, an entomological survey was carried out to study the sand fly fauna, species composition, population density, monthly prevalence of sand flies, number of generations and leishmanial infection rate of sand flies. The susceptibility level of vectors to DDT in the Shahreza area of Iran was also conducted. There was no classical study performed on the vectors of VL in this region and the present study provides the basic information.

Material & Methods

Study area: The investigations were carried out from April 2003 to October 2004, in Shahreza county (31° 59' N, 51°50' E, 1423 m above mean sea level), 80 km from the Isfahan province, central Iran. The Zardkooh mountain chain runs from northwest to southeast of the city. The total population of the county was 163,318 in 2003. The climate condition was very hot (up to 40°C) in the summer and cold –7°C during the winter. The mean maximum and minimum monthly temperatures were 38°C in August and –6.8°C in December, respectively. The total annual rainfall was 236.5 mm. The minimum mean monthly relative humidity was 16% (August) and the maximum was 63% (January). Fig. 1 shows the monthly changes of the temperature and rainfall during the study time. People are mainly engaged in production of wheat and vegetables and domestic animal breeding, especially cow keepers. Furthermore, most houses have at least one dog.

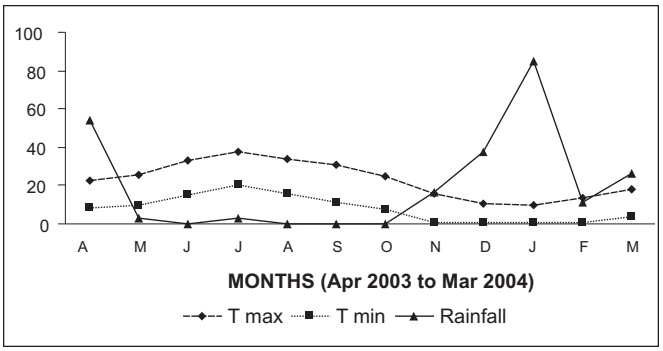

Fig. 1: Variation of the monthly temperature (maximum and minimum in ºC) and rainfall (mm) in the study area

Sand fly collection: Sand flies were collected biweekly using sticky paper traps, CDC light traps and aspirators from outdoors (cow shelters, dog shelters and holes in rocks and caves) as well as indoors (stable and living rooms). Sticky paper traps (15 × 20 cm) installed after sunset were collected before sunrise in 20 catching sites. Collection by aspirators was made within houses from 0600 to 0800 hrs. To keep safe from wind damage, light-traps were placed in a confined location, and were systematically mounted at 1900 hrs and taken down at 0500 hrs the next day.

Detection of natural infections, age determination and identification: After recording the data and place of collection, some blood fed and gravid female sand flies were dissected and examined microscopically for promastigotes infection. For species identification, the head and the posterior part of the abdomen of each sand fly were mounted in a drop of Puries medium[7] and then they were counted and segregated by sex. The identification was made by examining the morphology of male genitalia, female spermatheca and pharynges using Theodor and Mesghali systemic identification key[8]. The gonotrophic status (unfed or fed) of all female sand flies was noted and physiological age of each female was recorded by the presence or absence of granules in the accessory glands[9].

In order to test all aspects of the promastigotes infection, we injected the parasites in hamsters. The ham-

sters were killed three to four months after inoculation to examine their liver, spleen and bone marrow for parasites. Slides of promastigote were checked for detection of *L. infantum* by Nested-PCR. DNA was extracted as described by Motazedian *et al*[10]. The dry smear was scraped with a sterile scalpel and dissolved in 200 µl buffer [50 mM Tris-HCL (pH 7.6), 1 mmol/litre EDTA, 1% Tween 20] containing 8.5 µl proteinase K solution (19 µg/µl), in a 1.5 ml tube. The tube was incubated for 2 h at 56°C before adding 200 µl phenol-chloroform: isoamyl alcohol (25:24:1 by volume). After being shaken vigorously, the tube was centrifuged at 6000×g for 10 min and then DNA was precipitated with 400 µl pure ethanol, re-suspended in 100 µl double-distilled water and stored at –20°C before being used in PCR.

The susceptibility status of predominant *Phlebotomus* species in the area to 4% DDT was determined in the field by the WHO standard method[11] during 2004. Impregnated papers were procured from WHO.

Results

During April 2003 to October 2004, 5029 males (67%) and 2499 females (33%) were collected in 7528 phlebotomine specimens. Approximately 2386 (32%) sand flies from indoor and 5142 (68%) from outdoor resting places were captured. Twelve species belonging to the genera *Phlebotomus* (78%) and *Sergentomyia* (22%) were recorded (Table 1).

Common sand flies in indoor and outdoor resting places were *P. papatasi, P. major* and *S. sintoni*. The changes in seasonal prevalence of these species are shown in Fig. 2. The population build-up of *P. papatasi, P. major* and *S. sintoni* were started in the end of April and reached to low levels in October, with a peak in September for the *P. major* and two peaks in June and September for the other species. No sand flies were captured from October 2003 until April 2004 due to severe weather conditions.

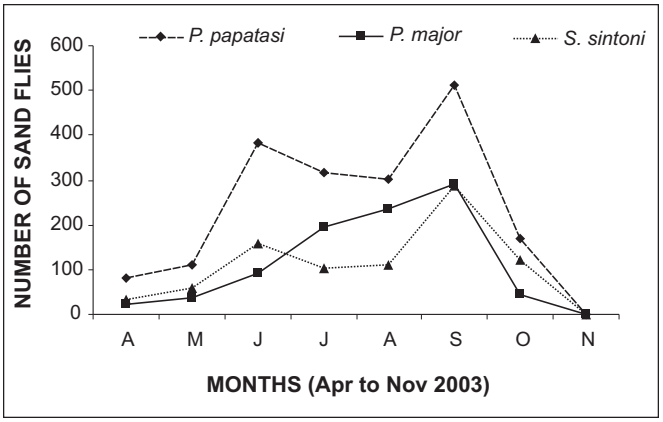

Fig. 2: Seasonal prevalence of *P. papatasi*, *P. major* and *S. sintoni* in Shahreza county, Isfahan province, Iran

Table 1. Sand fly species collected in Shahreza county and their relative abundance

Species	Male	Female	Total	Relative proportion (%)
P. (Phlebotomus) papatasi	2130	945	3075	40.85
P. (Larroussious) major	704	600	1304	17.32
P. (Paraphlebotomus) mongolensis	308	83	391	5.19
P. (Paraphlebotomus) caucasicus	182	234	416	5.53
P. (Paraphlebotomus) sergenti	181	79	260	3.45
P. keshishiani	206	52	258	3.43
P. (Synphlebotomus) ansari	161	0	161	2.14
P. (Adlerius) longiductos	11	7	18	0.24
P. (Paraphlebotomus) halpensis	11	0	11	0.14
S. (Sergentomyia) sintoni	1060	286	1346	17.87
S. (Sergentomyia) dentate	51	206	257	3.42
S. (Rondanomyis) pawlosky	24	7	31	0.42

Table 2. Results of the mortality rate of wild-caught *P. papatasi* and *P. major* females for 4% DDT in Shahreza county, Iran, 2004

Exposure time (min)	% mortality after 24 h	
	P. papatasi	*P. major*
5	80.5	87
10	86.2	89.4
15	90.1	92.5
20	92.7	95.9
25	94.1	100
30	100	100
60	100	100

Because the density of the other species was very low, it was impossible to determine the monthly density. All of the *P. ansari* and *P. halpensis* were malesand all of the females of *P. caucasicus* and *P. longiductus* were found unfed. The results of the dissections showed that only five *P. major* (2% of 120 dissected females sand flies, because of the limited budget) were infected with the natural promastigote infection. Flagellates from four infected sand flies were inoculated to hamsters (each to one animal). The hamsters survived only for two and half to three months after inoculation. In addition, the amastigotes were observed in the spleen, they were identified as *L. infantum* using standard PCR.

Twenty-two series of susceptibility tests were carried out on 210 fed *P. papatasi* and 180 *P. major*. The mortality rates for 4% DDT after 5, 10, 15, 20, 25, 30 and 60 min of exposure time followed by 24 h recovery time was 100% (Table 2). We concluded that field collected *P. papatasi* and *P. major* are susceptible to DDT in this area.

Discussion

This is the first report on sand fly vectors of VL in Isfahan province, central Iran. In this study, 12 species (nine *Phlebotomus* and three *Sergentomyia*) were collected and identified in Shahreza county. Other than the county, there is a mountain side area, surrounding plains, various plants, local wind patterns and a river, which created different niches in the region.

P. papatasi, P. major and *Sergentomyia sintoni* were the most abundant species in both indoors and outdoors, but *P. major* was the only species found to be infected with a rate of 2%. This is the first report of high density (17%) of *P. major* and natural promastigote infection in the Iranian plateau. The five infected females of *P. major* were caught by CDC light-traps in houses with VL patients. Moreover, the walls and the floors of most houses are made of mud, leading to an ideal resting and breeding ground for sand flies. Prevalence of *P. major* was observed for five months with one peak in September. *P. major* has a wide distribution from Morocco to southeast China. In Iran, *P. major* infected with natural promastigote has been reported only from Fars province, southern Iran[12]. Dogan *et al*[13] reported that *P. major* was found to be abundant (36%) in VL regions in northwestern Turkey. Also Sawalha *et al*[14] found that *P. major* is a potential vector of VL caused by *L. infantum* in the Palestinian West Bank.

With no existing cases showing cutaneous leishmaniasis in the area and leishmania isolated, we believe that *P. major* is suspected to be the main vector of VL in central Iran. In the study region, most of the people have homestead dogs that can in turn attract sand flies into the homes, which leads to the transmission of the disease to humans. Furthermore, in recent years, change of employment (agriculture activities to animal husbandry) is the reason why people are taking care of their dogs within their settlements in the county's borders.

The epidemiological data on kala-azar in Iran indicate that the disease is of the Mediterranean type and *Leishmania* strains isolated from kala-azar patients have been identified as *L. infantum* by isoenzyme characterization[5]. Dogs are the principle reservoir of these parasites and play a central role in the transmis-

sion cycle to man by phlebotomine sand flies[4]. Since sand fly vectors are present in the area, the active transmission cycle of the *Leishmania* parasite could have established, resulting in an increase in the occurrence of leishmaniasis in humans.

This study showed that *P. major* and *P. papatasi* (the main vector of ZCL in Iran[15] are susceptible to DDT in Shahreza county. For the first time the existence of resistant population of *P. papatasi* to DDT, in kala-azar epidemic areas of Bihar, India, was reported by Kaul *et al*[16]. Reduction of the number of leishmaniasis cases was also postulated as a collateral benefit of the malaria intervention programme in Iran[17] although Nadim & Amini[18] had earlier concluded that it did not interrupt *Leishmania* transmission in the city of Isfahan, based on catches of sand flies using sticky traps. Field-collected specimens of *P. papatasi* from Isfahan showed greater tolerance to DDT than populations from other areas routinely treated with DDT from 1950 to 1968, even 20 yr after cessation of the antimalaria programme[19]. The outdoor insecticide spraying is an important strategic entomological activity for leishmaniasis control programmes in field conditions. Thus before planning any control measure against leishmania vectors, a study should be done in order to establish the baseline susceptibility to representative insecticides.

Several cutaneous leishmaniasis foci, such as Badrood, Borkhar & Sedeh[20] have been reported in Isfahan province where sporadic cases of VL have been found. No cases of cutaneous leishmaniasis were reported in Shahreza county in recent years. Shahreza county, located between Isfahan county (the most important focus of CL) and Fars province are recognized as the VL foci in Iran[6]. Geographical distribution of cases, risk factors and underlying disease occurrences are not systemically monitored and documented[21]. In spite of the increasing number of diagnosed cases, there is no regular record of the mortality rate from kala-azar in Iran. In conclusion, public health measures such as case detection and treatment, the control of sand flies, the conjunction elimination of infected stray dogs and health education can be effective in controlling the disease.

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