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Some Ecological Aspects of Phlebotominae Sand Flies (Diptera: Psychodidae) in an Endemic Focus of Anthroponotic Cutaneous Leishmaniasis of Iran

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Abstract: Study on the Phlebotomine vectors is an interesting tool to make a better understanding of cutaneous leishmaniasis transmission dynamic which is used in prevention and control programs of the disease. This study was conducted in Isfahan city, central Iran, in 2005. Sand flies were collected with two methods, sucking tubes from indoors and sticky paper traps from indoor and outdoor resting places. A total of 3075 sand flies were collected and 10 species were identified. *Phlebotomus sergenti*, a probable vector of *Leishmania tropica*, was the predominant species through the entire period of activity. The seasonal activity of sand flies extended from late April to early October. There were two peaks in the density curve of most species. The parous rate of *P. sergenti* was high in July 92.9% and August 95.7%. The susceptibility test showed that *P. sergenti* is completely susceptible to DDT 4%. Identification of blood meals of *P. papatasi* using Enzyme Linked Immunosorbent Assay (ELISA) showed that 94.1% of sand flies fed on human and 5.9% on both human and poultry. The greatest risk of ACL transmission to human occurs probably during the second peak when parous rate and density of *P. sergenti* are high.

Key word: Sand fly, ecology, cutaneous leishmaniasis, Iran

INTRODUCTION

Leishmaniasis is parasitic disease with a wide range of clinical symptoms: cutaneous, mucocutaneous and visceral. These diseases currently threaten 350 million people in 88 countries around the world, with 1-1.5 million new case of cutaneous leishmaniasis reported annually (Desyeux, 2004).

Two *leishmania* species cause cutaneous leishmaniasis in the old world, *Leishmania major* and *L. tropica* (WHO, 2004).

Leishmaniasis control measures are impossible without correct identification of the sand flies species and their ecological aspects.

Female Phlebotomine sand flies (Diptera: Psychodidae) transmit all *leishmania* species. There are six genera in the subfamily of Phlebotominae of which only two are of medical importance, *Phlebotomus* Randani and Berte 1840 of the old world (divided into 12 subgenera) and *Lutzomyia* França, 1924 of the new world (divided into 25 subgenera and species groups). All proven vectors of leishmaniasis belong to the latter genera (Jacobson, 2003; Kellick, 1999).

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Phlebotomine sand flies are small, delicate, hairy flies (1.5-3.5 mm) with long slender and filamentous antenna. Most sand flies are either crepuscular, with peaks of activity soon after sunset and before dawn, or nocturnal in the temperate regions of the old world. Biting takes place at different times of the night according to the species. The spatial distribution and seasonal dynamics of sand flies are influenced by wind, ambient temperature, relative humidity and soil moisture. Only a few species of sand flies are endophilic; these are mostly peridomestic such as *P. papatasi* and *P. sergenti*. The majority of the sand flies prefer to bite outside, often near their breeding and resting sites (Kellick, 1999; Lane, 1987, 1993).

Most species probably have a narrow range of preferred hosts. Two genera contain anthropophagous species; *Phlebotomus* and *Lutzomyia*. Species of *Sergentomyia* feed predominantly on reptiles, as do some species of *Phlebotomus*, but the *Phlebotomus* feed mainly on mammals. Several peridomestic, mammal-feeding sand flies such as *P. papatasi* and *P. sergenti* will readily feed on poultry and chicken coops. Most species are gonotrophically concordance; taking one blood meal for maturing each egg batch. The proportion of parous females within a population indicates the epidemiological potential of the parasite transmission. The highest parous rates occur in populations towards the end of the sand fly season when sand fly infection rates are maximal and subsequent transmission is most likely. Some species in temperate regions may only have one generation per year and consequently a single peak of activity and transmission. Such species may have two or three generations per year in climatically more favorable areas (Lane, 1987).

Insecticide control of adult sand flies is only feasible where peridomestic transmission occurs in discrete and well-populated communities. Residual treatment of the walls of human dwellings depends on the availability of a suitable public health infrastructure, including adequate supplies of insecticide, spraying equipment and trained personnel. Chemical control may not be effective where sand flies are exophilic or bite away from human habitations (Lane, 1987; Alexander and Maroli, 2003).

The objectives of the present study were to determine sand flies fauna and species composition, the population density, seasonal activity and prevalence of sand flies, number of generation, susceptibility status of *P. sergenti* to DDT 4%, host preference pattern of *P. papatasi* and physiological age of three common species of sand flies (*P. sergenti*, *P. papatasi* and *P. caucasicus* group) in Isfahan city in 2005.

MATERIALS AND METHODS

Isfahan is geographically located at 32° 38' N 51° 29' E at the foothills of the Zagros mountain range. It is situated at an altitude of 1590 m above the sea level, warm in summer and pretty cold in winter. The southern and western approaches of Isfahan are mountainous and it is bordered northward and eastward by fertile plains. Thus climate of Isfahan is varied and occasionally rainy with a precipitation average varying between 100 and 150 mm.

Sand flies were collected biweekly from indoors (bedrooms, warehouses, etc.) and outdoors (cracks in the walls, bird holes, etc) fixed locations in four quarters using 30 sticky paper traps from the beginning to the end of the active season. In the laboratory, some of the collected female sand flies were examined for detecting promastigote infection, physiological age soon after collection (Akhavan *et al.*, 2007; Zahraei-Ramazani *et al.*, 2007) while all others were washed in acetone and preserved in 70% ethanol for subsequent microscopic identification. For species identification, sand flies were mounted in Puri's medium (Smart *et al.*, 1965) and identified using the keys of Theodor and Mesghali (1964) and then they were counted and segregated by the sex.

For determining the physiological age and also promastigote infection rate, 257 female (blood fed, unfed and gravid) common sand flies were dissected (Foster *et al.*, 1970).

In order to study the host preference pattern, blood meal sand flies collected from indoor and outdoor resting places were dissected and the blood smears were sent to the Department of Medical Parasitology, School of Public Health, Medical Sciences/ University of Tehran for Enzyme Linked Immunosorbent Assay (ELISA) testing (Edrissian *et al.*, 1985).

The standard WHO test kit was used to determine the susceptibility status of *P. sergenti* against DDT 4%. The blood fed and semi gravid sand flies exposed to paper impregnated with the insecticide for 30, 45 and 60 min (Seyedi-Rashti *et al.*, 1992; WHO, 2001).

RESULTS

During this study 10 species of sand flies were collected from indoor and outdoor resting places and were identified. We found 5 species of *Phlebotomus* (*Phlebotomus sergenti*, *P. papatasi*, *P. caucasicus* group, *P. kandelakii* and *P. alexandri*) and 5 species of *Sergentomyia* (*Sergentomyia sintoni*, *S. pawlowsky*, *S. dentata*, *S. clydei* and *S. baghdadis*).

Common sand flies in indoor and outdoor resting places were *P. sergenti* and *P. papatasi*. *P. caucasicus* group was found in human dwellings as the third common sand fly (Fig. 1). The sex ratio, i.e., number of males per 100 female of *P. sergenti*, was found to be 224.55 and 391.85 in indoor and outdoor resting places, respectively.

P. sergenti, collected by sticky paper traps, constituted 49.2 and 56.4% of the total indoor and outdoor sand flies respectively (Fig. 1). The monthly activity of *P. sergenti* and *P. papatasi* in indoor resting places started to appear in late April and ended in late November. These two species had two peaks in indoors, one in early June and another in late August (Fig. 2 and 3).

Altogether, 257 female sand flies (including *P. sergenti* 16.0%, *P. papatasi* 67.7% and *P. caucasicus* 16.3%) were collected and dissected but none of them appeared to be infected.

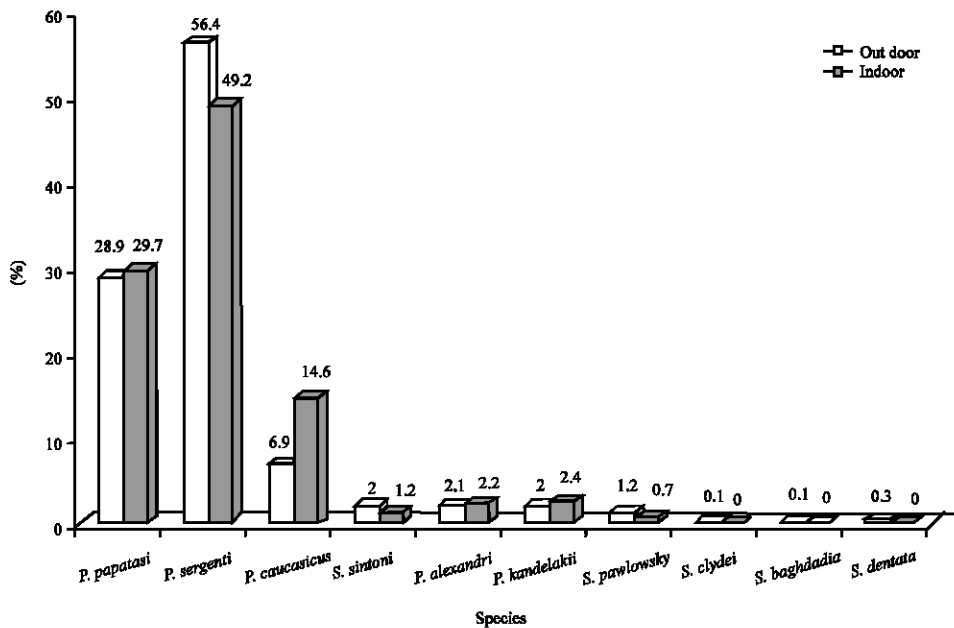


Fig. 1: The fauna and prevalence of sand flies collected from indoor and out door resting places, Isfahan city, central Iran, 2005

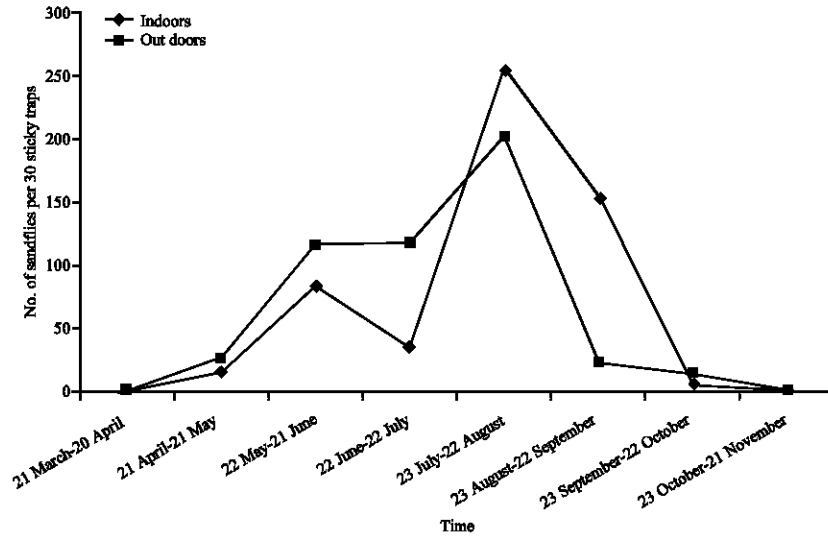


Fig. 2: Seasonal activity *P. sergenti* from indoor and out door resting places, Isfahan city, central Iran, 2005

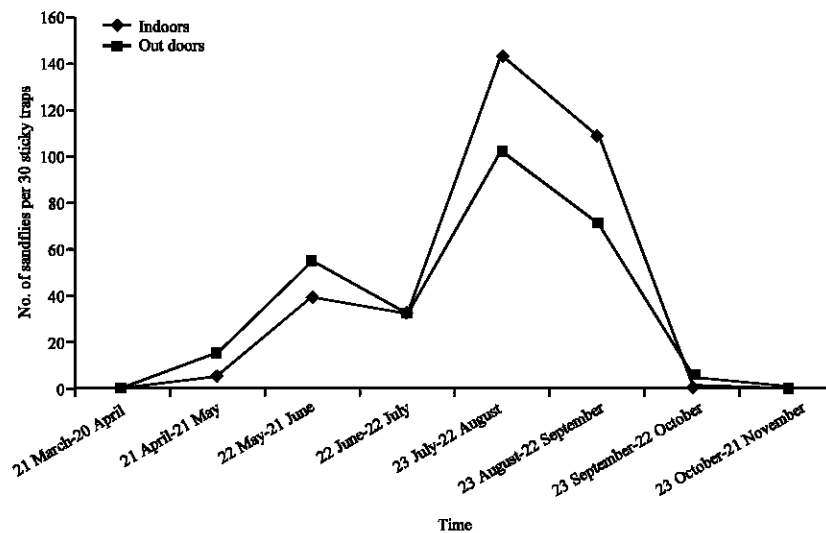


Fig. 3: Seasonal activity *P. papataasi* from indoor and out door resting places, Isfahan city, central Iran, 2005

The results of examining the accessory glands of these female sand flies showed that the highest parous rate occurred in old populations towards the end of the second peak and transmission season in late August (Table 1).

ELISA testing of 17 blood meal females of *P. papataasi* showed that human and poultry are preferred hosts of this species. The anthropophilic index was calculated to be 94.1% and double blood meals of human-poultry was observed 5.9%. Blood fed females of *P. sergenti* were few in number so the test did not achieve.

Table 1: Physiological age of three species of sand flies that dissected for promastigote infection, Isfahan city, Central Iran, 2005

Sand fly species	Infected (dissected) No.	May		June		July		August		September	
		Nuliparus	Parus	Nuliparus	Parus	Nuliparus	Parus	Nuliparus	Parus	Nuliparus	Parus
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
<i>P. papatasi</i>	0(174)	0	0	3(37.5)	5(62.5)	17(35.4)	31(64.4)	3(2.8)	106(97.2)	0	9(100)
<i>P. sergenti</i>	0(41)	0	0	1(50.0)	1(50.0)	1(7.1)	13(92.9)	1(4.3)	22(95.7)	0	2(100)
<i>P. caucasicus</i>	0(42)	4(44.4)	5(55.6)	2(33.3)	4(66.7)	0	4(100)	0	21(100)	0	2(100)

A total of 258 *P. sergenti* were exposed to papers impregnated with DDT 4% for 30, 45 and 60 min. The mortality rate of the sand fly was 100% after exposure time followed by 24 h recovery period.

DISCUSSION

The geographical, ecological and socio-economical characteristics play an important role in the distribution and epidemics of Cutaneous Leishmaniasis (Belen *et al.*, 2004).

P. (paraphlebotomus) sergenti has been found in anthroponotic cutaneous leishmaniasis foci, namely Iran, Taza, Morocco, Tanant, Tunisia, Urfa, Turkey, Aleppo, Syria and Kabul Afghanistan.

L. tropica was first isolated from this vector in Saudi Arabia.

P. sergenti is considered to be a natural vector, based on the isolating of *L. tropica*, epidemiological evidence and also its susceptibility to the parasite after artificial infection. It was also demonstrated that *P. sergenti* feeds naturally on humans (Svobodova and Votycka, 2003).

P. sergenti is captured in many foci of cutaneous leishmaniasis due to *L. tropica*. The presence of this sand fly in the foci of the disease is another evidence which suggest its vectorial role in transmission (Jacobson, 2003).

In anthroponotic foci of cutaneous leishmaniasis in Afghanistan, Saudi Arabia and Turkey, *P. sergenti* was shown to be highly endophilic. The degrees of its endophily and anthropophagy differ between geographical populations of *P. sergenti* and probably influence their vectorial capacity for *L. tropica* (Kravchenko *et al.*, 2004).

Knowledge on the host preferences of phlebotomine sand flies under natural conditions is essential to understand their vectorial capacity in this focus.

Observations on the host preferences of *Phlebotomus* in this study region permit us to draw a tentative conclusion that should be confirmed by more detailed studies in other urban CL foci.

The feeding habits of the *Leishmania* vectors may have relevant implications for the epidemiology of leishmaniasis in urban areas, where sand fly female, being deprived of other vertebrate hosts, particularly the larger species, may begin to bite humans and dogs more frequently.

In the phlebotomine sand flies, only females feed on blood (Lane, 1987; Kan *et al.*, 2004) and a female that wants to feed on human reservoir, searches for it by flying. Furthermore, it is considered that the migration by flight is necessary for the female sand flies not only have a blood meal but also to search for their oviposition sites.

In the present study both male and female *P. sergenti* and *P. papatasi* were captured from indoor human places, although the males of the phlebotomine sand flies do not feed on blood (Lane, 1987; Kan *et al.*, 2004). This suggests that males of these species are attracted to the humans for mating.

Three of the *Sergentomyia* species were new record for this region (Zahraei-Ramazani *et al.*, 2006). *Sergentomyia* sp. feed primarily on reptiles. They probably represent no more than an occasional minor nuisance to local residents.

The seasonal activity of sand flies in Isfahan city indicated that *P. sergenti* and *P. papatasi* have two generations in a year.

In this study, the extremely high parous rate of *P. sergenti* from indoor resting places indicates that human is either common or the almost exclusive source of the blood meals.

Human seems to be a preferred host for *P. papatasi*, the second prevalent species, in Isfahan city and the main vector of zoonotic CL in Iran (Yaghoobi-Ershadi *et al.*, 2006).

L. tropica isolated from active lesions of infected patients in this city (Farid Moaeir *et al.*, 1997). The abundance of *P. sergenti* in Isfahan city is high, also percent parous of *P. sergenti* (also *P. papatasi* and *P. caucasicus* group) especially during the second peak of seasonal activity or second generation is high. According to above data and also regarding the movement of infected people and high crowded congregation of none immune human in dwelling places, an epidemic of ACL will be probably occurred in future in Isfahan city.

In this study, *P. sergenti* was completely susceptible to DDT 4% in this region.

DDT is the cheapest insecticide available for interruption of the cycle of ACL but for reasons of environmental impact and other concerns, its use is no longer permitted in the majority of countries (Alexander and Maroli, 2003).

DDT spraying in the houses with active cases and all their neighboring houses and in all places with high density of *P. sergenti* should be applied only once during the active season of sand flies (Yaghoobi-Ershadi *et al.*, 2002).

A more effective sand fly control through residual insecticide spraying of the houses and the use of insecticide impregnated bed nets and curtains are needed, especially in this region that ACL is endemic (Moosa-kazemi *et al.*, 2007). Human individual protection, health education, active case detection and treatment are also recommended.

Further investigations are needed to clarify all ecological aspects and molecular characterizations of vector (s) and clarify the *L. tropica* transmission risk in this city by Isolating and typing leishmania strains from vectors.

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