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Short Paper

A study on *Leishmania* infection rate among *Phlebotomus spp*. collected from Abardejh district, Iran

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Summary

Cutaneous leishmaniasis is a prevalent tropical parasitic disease in the Old World. The causative agents are Leishmanial parasites, which cause various forms of cutaneous leishmaniasis. The infection is commonly limited in immuno-competent individuals, but it can progress to a chronic and ulcerative disease in immuno-compromised patients. The reservoirs are dogs and rodents and the vectors are different species of sandflies. In the present study, we investigated the prevalence rate of *Leishmania* infection among *Phlebotomus* mosquitoes collected from Abardejh district, Iran. Abardejh is located next to Varamin city in southeast of Tehran having a tropical ecosystem at its eastern border. Tamarisk trees and rodents have provided a suitable condition for sandfly activity. The sandflies were collected by funnel trap from rodent burrows and transferred to the Department of Parasitology, Pasteur Institute of Iran. The sampling was carried out during spring and summer (2002) with ten-day intervals. The collected sandflies were identified using discriminative morphologic features before parasitological culture on NNN medium. Analyses of the data revealed a high prevalence rate of infection among the sandflies in this region ($P<0.01$). The maximum activities of *Phlebotomus* were in the months of June and July. Three species of sanflies were found in rodent burrow: *P. papatasi*, *P. sergenti*, and *P. caucasicus*. The results of blood-fed *Phlebotomus* culture showed that 22.07% of blood-fed females of *P. papatasi* and 8% of blood-fed females of *P. sergenti* were infected with leptomonads ($P<0.05$). This could be an important issue because human and agricultural environments are located closely to this district. Therefore, use of insecticides and environmental sanitation seems to be required to prevent the transmission of infection from sandflies to human.

Key words: *Leishmania*, *Phlebotomus*, Iran

Introduction

Cutaneous leishmaniasis is a polymorphic disease of skin and mucous membranes caused by an intracellular protozoan. Zoonotic cutaneous leishmaniasis (ZCL) is a major health problem in many rural areas of Iran (Yaghoobi and Akhavan, 1999). According to the report of Killick-Kendrick (1990) and Parvizi *et al*., (2005), *Phlebotomus papatas*i is a well-known vector of *Leishmania major*, the causative agent of cutaneous leishmaniasis in Iran (Nadim and Javadian, 1976).

Different species of *Phlebotomus* exhibit remarkable anthropophagic behaviour in pre-domestic and domestic habitats and is widespread throughout the country at both rural and urban areas.

These species of *Phlebotomus* sandflies are found in houses, domestic animal shelters, natural caves and rodent burrows of the reservoir hosts. Even a number of gerbils, *Rhombomys opimus*, have been found infected with *L. major* (Service Mick, 2000).

Adequate knowledge on the population of *P. papatasi* will be useful for planning measures to prevent transmission of *Leishmania*. These measures include the identification of sandfly population which unlikely to be replaced by immigration

following control by insecticides or habitat alteration. Since (1966), the Iranian health authorities have focused on bringing the ZCL under control in infected foci by various methods, such as house spraying with DDT, using the DDT powder in rodent burrows and killing the reservoir hosts (Nadim and Amini, 1970; Seydi-Rashti and Nadim, 1975).

The main objective of this study was to understand the epidemiology of sandfly in rodent's habitat and detection of *Leishmania* of the vectors in this area. In this study, we investigated the prevalence of *Phlebotomus* populations in Abardejh district and the rate of *Leishmania* infection among *Phlebotomus* spp. collected from Abradejh.

Materials and Methods

The study was conducted from May to September (2002) in Abardejh, situated in the eastern of Varamin city, 110 km southeast of Tehran. The area has a warm and dry climate with a very hot summer and a cold winter and covered by Tamarisk trees. During this study the mean ± SD monthly temperatures was 44.1 ± 3°C; the total annual rainfall was 81 mm. The monthly relative humidity varied between 19 and 64%. In the early May, maps of the Abardejh village and around it were prepared and a number of rodent holes in the ground were selected in an area around 1000 m^2 wide. The funnels were placed on rodent burrows one hour before and after sunset. The collected sandflies were transferred to the Department of Parasitology of Pasteur Institute of Iran. The sampling was repeated every 10 days during May to September and their densities were recorded. All collected sandflies were identified by morphologic methods, the taxonomical characters of sandflies (structure of head, male genitalia, female spermatheca) were studied and some structures (labrum, antenna, coxite, style and palpal segments) were measured according to the Theodor and Mesghali morphological key method (Theodor and Mesghali, 1964).

The blood-fed females *Phlebotomus* were selected and immersed in 70% alcohol for 5 min to remove the surface bacteria. It was then rinsed with distilled water. All the specimens were transferred to NNN culture media and their stomach crushed with a sterile needle in the liquid phase of 3 N medium culture containing 200 IU penicillin per ml. The cultures were incubated at 24°C for three days. The specimens were then investigated by using divert light microscope. Two-tenth ml of suspension of each liquid media (containing approximately 10^6 parasites/ml) was injected subcutaneously into the base of tail of two BALB/c mice to confirm the identification of the cultured parasite. Data were statistically analysed using of Student's t-test where appropriate.

Results

Eight thousand two hundred and thirty adult sandflies from rodent burrows were collected and identified during May and September.

Population group of sandflies were statistically compared depending on sandfly species. A significant difference was observed between different time of sampling ($P<0.01$) using t-test.

This study showed that, the *Phlebotomus* species with the highest frequency occurred during the June and July, were very active in this area.

Three species of sandflies were found in rodent burrows: *P. papatasi* (75.2%), *P. sergenti* (23.45%), and *P. caucasicus* (1.35%) (Table 1).

The *P. papatasi* was the most prevalent type. Study on *Phlebotomus* activity showed that, they begin their activity at the early spring; during the summer, they reach the highest frequency and activity, especially in months of June and July. Their frequency is reduced to the least in September.

The monthly density of *P. papatasi* and *P. sergenti* are shown in Figs. 1 and 2. The density of *P. caucasicus* species was too low to determine the monthly density. The results of blood-fed *Phlebotomus* culture showed that 20.4% of them were infected. The results of the dissection showed that 22.07% of blood-fed females of *P. papatasi* and 8% of blood-fed females of *P. sergenti* were infected with leptomonads ($P<0.05$).

After 21 to 45 days, all infected BALB/c mice showed ulcers on the base of their tails.

Table 1: Frequency of sandfly species collected in Abardejh district

Species	Frequency		Female		Blood-fed female		Positive case in 3N media	
	No	%	No	%	No	%	No	%
P. papatasi	6188	75.2	2622	43.5	1518	56.4	335	22.07
P. sergenti	1930	23.45	718	73.2	180	25	14	8
P. caucasicus	112	1.35	45	40.17	12	26.6	---	----
Total	8230	100	3455	41.98	1710	49.49	349	20.4

Fig. 1: Monthly density of *P. papatasi* in Abardejh (rodent burrows)

Fig. 2: Monthly density of *P. sergenti* in Abardejh (rodent burrows)

Geimsa-stained slides prepared from the lesions showed *Leishmania* amastigotes (Leishman-Donovan body).

Discussion

Geographical distribution of leishmaniasis is restricted to tropical and temperate regions and natural habitat of the sandflies. Leishmaniasis is endemic in 88 countries including 16 developed and 72 developing countries in the continents of Africa, Asia, Europe and America. A total of 350 million people are at risk. Geographical distribution of leishmaniasis is limited by the distribution of the sandflies, its susceptibility to cold climates, its tendency to take blood from humans or animals only, and its capacity to support the internal development of specific species of *Leishmania*.

P. papatasi was the most abundant species in rodents' burrows. Three species, *P. papatasi* (75.2%), *P. sergenti* (23.45%), and *P. caucasicus* (1.35%) were present from May to September (2002) in Abardejh area. *P. papatasi* was the only species found to be infected with a rate of 22.07%. The density of *P. caucasicus* was very low so that the role of this species in circulation of *L. major* among the rodents seems to be doubtful in this area.

The previous studies report the highest infection rates in Turkmen Sahara (14.9%) and Badrood (37.8%) (Yaghoobi *et al*., 2000). Sandflies were collected from burrows of rodent colonies during June to October (1991) and natural infections with *Leishmania* promastigotes were monitored in *Phlebotomus* vectors from villages of Borkhar, northeast of Isfahan, central Iran, where *L. major* has been identified as the ethiological agents for ZCL and leptomonad infection rates were 8% for *P. caucasicus* and 11% for *P. papatasi* (Yaghoobi *et al*., 1995). An outbreak of ZCL, as a result of man-made changes to the environment, has been observed since (1994) in Kashan, north of Isfahan. The reported incidence was 8–15% among local inhabitants. Available data indicate that the epidemy started after an increase in the number of *Rhombomys opimus, a* rodent serves as the reservoir of ZCL in that area. Because sandfly vectors were present there, the active transmission cycle of *Leishmania* has been formed (Yaghoobi *et al*., 2000).

In Afghanistan, the incidence of endemic and sporadic anthroponotic cutaneous leishmaniasis (ACL) has dramatically increased during the decades of civil war, because of the associated deterioration of the infrastructure and migration (Reithinger *et al*., 2003).

The lack of diagnostic facilities and drugs for treatment have been among the possible contributing factors to the epidemic

of kala-azar in Sudan between (1988) and (1993). Other contributing factors were the outbreak of kala-azar and anthroponotic cutaneous leishmaniasis in Iraq after the Gulf War and subsequent economic embargo imposed in (1991). A four- to six-fold rise in the number of cases in (1991) compared to (1990), was observed in Iraq, namely 576 cases of visceral leishmaniasis and 1799 cases of cutaneous leishmaniasis in (1990) vs 3713 cases of visceral leishmaniasis and 8233 cases of cutaneous leishmaniasis in (1991). However, we can add other factors such as population movement and the destruction of health and vector-control facilities during the outbreak of leishmaniasis in Iraq and the Islamic Republic of Iran (Neouimine, 1996).

Less is known about the current distribution of the disease in neighbouring Pakistan, where it has always been widespread though considered “patchy” and no endemic. Recently, local authorities and non-government health providers have reported an increasing number of ACL cases in Afghan refugee camps which causes concern about the potential spread of the disease among the population and local Pakistani villagers (Brooker *et al*., 2004; Kolaczinski *et al*., 2004). These findings in addition to our animal model experiments indicate that *P. papatasi* is an efficient vector to human and gerbils. *P. sergenti* is the suspected vector. The close contacts between vectors, reservoirs and human have created a very complete cycle for the transmission of the disease in the villages around Abardejh.

According to the report of Brooker *et al*., (2004) *P. papatasi*, the main vector of ZCL, is susceptible to DDT, so it remains as the main candidate insecticide for sandfly control in the area by rodents’ burrows spraying. It can of course, be substituted by permethrin as suitable alternative insecticide (Mutinga, 1993).

The numbers of both gerbil reservoir and sandfly vector appear to be increased by land improvement. The close association of *P. papatasi* and rodents with human has created a very suitable cycle for the transmission of the disease in this area (Nadim and Amini, 1970).

However, since the published data are widely dispersed in the literature, a comprehensive review of existing information on the prevalence of sandfly and their infection is particularly difficult. Therefore, before planning any control measures against *Leishmania* vectors, a comprehensive study should be done in the area.

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