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Investigation on Zoonotic Cutaneous Leishmaniasis, Southern Iran

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Abstract

Since occurrence of an epidemic of cutaneous leishmaniasis with high incidence of disease (8.9/1000) in Arsanjan county, Fars province, southern Iran, an attempt was made to investigate an epidemiological study during 2001-2002. Symptomatic investigation among 270 schoolchildren over one year of study revealed that 11.9% of population had active lesion with 28.1% scar rate. Parallel study showed that among 7968 people which comprise 1572 households, 6.18% were positive for scars and 1.2% for ulcer. In our study three species of rodents, including *Meriones libycus* (71%), *Cricetulus migratorius* (22%) and *Microtus arvalis* (6%) were captured. By employing Nested-PCR technique, 6.8% (4 of 56) of *M.libycus* were found infected with *Leishmania major.* About 83% of indoor collected sandflies comprised, *Phlebotomus papatasi.* From its density it can be concluded that this species plays an important role in *leishmania* transmission in the area. The seasonal activity of the sandflies starts at early April and ended in early November.

Keywords: *Zoonotic cutaneous leishmaniasis, Arsanjan, Fars, Iran*

Introduction

There are several reports indicating occurrence of Zoonotic Cutaneaous Leishmaniasis (ZCL) in Iran. (1). The first important focus of disease has been located in central and northeast of Iran, where *Rhombomys opimus* and *Ph.papatasi* play an important role as a reservoir and vector (2-4).The second focus of ZCL has been reported from west and south-west of Iran, where *Tatera indica* replaced with *Rh.opymus* as a reservoir and *Ph.papatasi* as a vector (5). Baluchistan province, in the south-east of Iran is considered as a third focus of ZCL. In this region *M.hurrianae* has been approved as a natural reservoir host (6).From the reported evidences it is apparent that the most rural areas of Fars province, south of Iran such as Arsanjan, Neiriz and Marvdasht cities can be considered as a ZCL foci. For example an epidemic was happened in Arsanjan in 2000. In this area the incidence of disease (passive) was reported as 8.9/1000 by Health Center, Unit of Disease Control of Fars province. This was the first report of ZCL epidemic in this region. Several factors including urbanization, environmental manipulation and displacement of the non-immune community near the rodent colonies can be assumed as the causative agent of outbreak of the disease. The aim of this study was focused on different epidemiological aspects of disease in the region. The results will help for a planning of disease control measures in the area.

Materials and Methods

Study area The investigation was carried out in Arsanjan county (26 ° 4´ N, 53° 8´ E), south of Iran. Its altitude is 1660 meter above the sea level. The total population of the county was about 37011 people in 1996. The climatic condition is very hot in summer and quite cold

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during winter. The main activities of the people are agriculture and veterinary farming.

Population studies For epidemiological studies two population groups were selected. The first group was focused only on primary schools and the second comprised all the population of the Arsanjan district. Four villages of district were selected randomly and all the children of primary schools were examined for the presence of ulcer or scars on their bodies. Four visits were paid for the students in autumn, winter, spring and summer of 2001-2002. All the important individual information including age, sex, place of lesion, number of ulcer/s or scars/s was recorded. In order to obtain a picture of the human infection rate among all age groups, a district known as Khobriz was selected. In this region a total population of 7968 people comprising 1572 households were selected and examined for presence of any symptoms as described previously for the schoolchildren. The X^2 test by employing EPI-INFO programme was used for comparing of male and female infectivity rate.

Isolation of parasite from the patients Five, 7-11 years old patient with new lesion of infection were selected and samples of lesions were inoculated subcutaneously at the base of tail of 5 Balb/C. After inoculation and after incubation period, parasites from the infected laboratory mice were transferred in to the culture in monophasic RPMI 1640 and species were identified using Nested-PCR method (7), by Dep.of Medical Parasitology, Shiraz University of Medical Sciences.The primers were:
CSB1XR (CGA GTA GCA GAA
ACT CCC GTT CA) and CSB2XF (ATT TTT CGC GAT TTT CGC AGA ACG) for the first round and LiR(TCG CAG AAC GCC CCT) and 13Z(ACT GGG GGT TGG TGT AAA ATA G) for the second.Reference strain of *L.major* (MHOM/IR/XX/LV114)and *L.tropica* (MHOM/IR/89/ARD22)were used as standards.

Collection and examination of rodents Rodents were captured by setting the Sherman live traps. Traps were baited with roasted walnut and placed in the active burrows. The traps were set up early morning and evening once a month. In order to approve the infectivity of rodents by the parasites, their ears were examined and an impression smear was taken and stained by Geimsa staining method. The presence of the parasite was checked under microscope (8). Samples from infected rodents were inoculated subcutaneously at the base of tail of Balb/c. Procedure of species identification by PCR method is described previously.

Sandflies collection Sandflies were collected from indoors (bedroom, guestroom, toilet) as well as outdoors (rodent burros, wall cracks) biweekly using sticky traps. Two villages called Jafar-Abad and Kohanjan were selected and 30 sticky traps were set up during the sandflies activity period. Sandflies were rinsed from the sticky traps and mounted in a drop of Puri's medium. Species identification was conducted using a standard key (9)

Results

Human infection The prevalence of acute lesion and scar rate among 270 primary schoolchildren (118 females and 152 males) revealed the figure of 11.9 and 28.1% respectively (Table 1), among which 59.4% exhibited with one ulcer, 25% two ulcer, and the rest had more on their bodies. The faces, hands and legs were more prevalent in terms of infectivity (Fig. 1). From table 2, it can be concluded that among 7968 cases, only 1.12% had acute lesion and scar rate was calculated as 6.18%. The scar rate for people under 12 years old was measured as 9.6%. This figure for patient above 12 years old was 4.8%/. The pattern of infection on the body is presented in Fig.2. 69.4% of the cases had only one scar, 9.4% two scars and 21.2% had more. Seasonal distribution of the disease was followed from April till March. The peak of the disease was occurred in November. The acute cases were observed in all age groups and the highest

prevalence rate could be seen above 15 years old. There was no significant difference between male and female in terms of infectivity (X^2 =0.52, df =1, P<0.05). The X^2 test also showed significant difference of host age on scar (X^2= 172.82, df = 4, P<0.000001), and lesion rates (X^2=51.12, df =4, P<0.000001).

Results from inoculation of parasite from active cases into the subcutaneous of Balb/c revealed the presence of amastigotes into the nodules and ulcer of the experimentally mice after 20 days of incubation period. Among 5 Balb/c infected with parasite inoculation only 60% were positive for infection. Isolated parasites from infected mice were identified using Nested- PCR. The results of the PCR revealed the presence of *Leishmania major*.

Reservoir hosts Among all the rodents captured in the study area, 71% were identified as *M.libycus*, 22% *C.migratorius* and 6% *M.arvalis*. 6.8% (4 of 56) of *M.libycus* were found naturally infected with *Leishmania major* using PCR method.

Sand flies Sticky traps were able to collect 2808 sandflies (1199 samples from indoor and remaining from outdoor collection).

Identification of sandflies revealed the following species;*Phlebotumus (p.)papatasi* (68.37%), *P. (paraphelebotomus) sergenti* (3.95%), *P (para.)alexandri* (0.21%).*P (para.)mongolensis* (0.6%), *P (para.)andrejevi* (0.28%), *P. (larrossius) tobbi* (0.96%).

P. (larr.keshishiani (0.04%), *P. (adlerius) halepensis* (0.07%), *Sergentomyia (ser.) sintoni* (2.49%). *S. (ser.) dentata (14.7%), S. (ser.) theodori* (3.77%), and *S. (sintonius) clydei (4.56%).*Among the species identified the most common insect was *P.papatasi*. This species was largely pre-domestic and present 82.99% in indoors and 57.43% outdoors. The seasonal activity of *P.papatasi* was initiated from early April and continued until November. There were two peaks of activities of the sandflies, first occurred in early July and second in early September.

Table 1: The prevalence of active lesions and scar rate by age, among students of primary school in Arsanjan County

Age (year)	No. observed	No of scars	Percentage	No. active lesions	Percentage
7	36	9	25	3	8.3
8	51	11	21.6	6	11.6
9	59	16	27.1	6	10.2
10	70	21	30.0	10	14.3
11	54	19	35.2	7	13.0
total	270	76	28.1	32	11.9

Table 2: The prevalence of active lesion and scar rates by age among the all age groups in Khobriz district, Arsanjan County

Age (year)	No. observed	No of scars	Percentage	No. active lesion	Percentage
0-1	260	0	0	3	0.03
2-6	879	129	1.6	12	0.15
7-11	950	74	0.92	16	0.2
12-15	1107	91	1.13	18	0.24
>15	4772	198	2.47	40	0.5
total	7968	492	6.12	89	1.12

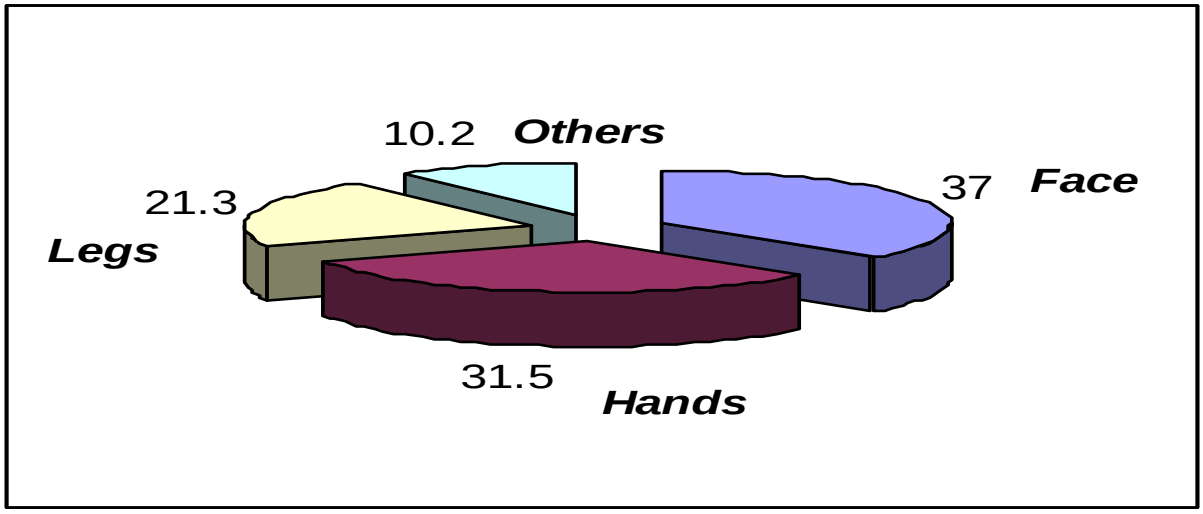

Fig.1: Percentage of acute lesion in different parts of body (school children**)**

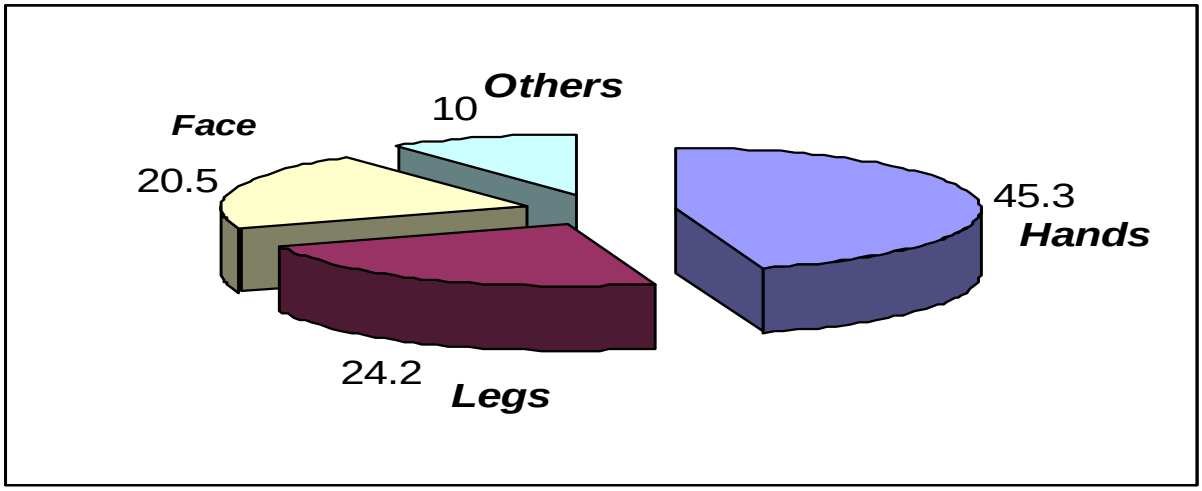

Fig.2: Percentage of acute lesion in different parts of body (all age groups)

Discussion

This study revealed that Arsanjan country, south of Iran can be considered as a new focus of leishmaniasis due to *L.major*.The disease is spread nearly all over the Fars province. *L.major* was isolated and identified from active cases as well as gerbil, *M. libycus*. The *L.major* is known as a causative agent of ZCL in other parts of Iran. The parasite also isolated from sandfly, *P.papatasi* and rodents, *R.opimus*, *Tatera indica*, *Nesokia indica* and *M.libycus* (1, 3, 10). We found that *M.libycus* live in the region with high density and infected with *L.major*. This rodent can act as secondary reservoir even the presence of *R.opimus* and *Tatera indica*. According to report of Yaghoobi-Ershadi et al (1), *M.lybicus has* been indicated as a main reservoir of disease in Badrood, Isfahan province, central Iran. From our results it can be assumed that due to absence of other reported rodents reservoir and high prevalence of *M.lybicus*, the species can play an important role in maintaining of the disease in the nature. From the entomological survey we found a high density of *P.papatasi* both indoor and outdoor. In conclusion it

should be emphasized that presence of *M.libycus* and *P.papatasi,* and close association of human dwelling with rodent burrows create a very efficient cycle of the disease transmission in the region. Several socioeconomic and ecological factors can play role in the occurrence of epidemic in this region; urbanization, construction of the building in adjacent to the rodent nests, displacement of non-immune people and presence of transmission agents.

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