Natural *Leishmania* Infection in *Meriones hurrianae* and *Tatera indica* (Rodentia: Cricetidae: Gerbillinae) in Sistan - Baluchistan Province, South – Eastern of Iran

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Abstract

**Background:** Zoonotic Cutaneous Leishmaniasis (ZCL) is a major public health problem in Iran. The disease is endemic in many rural districts of Iran, in 17 out of the 31 provinces. Rodents belong to Gerbillinae subfamily are the main reservoir hosts for ZCL in Iran. Although the disease rarely causes severe morbidity, the lesions may take several months to heal and often leave ugly scares on the exposed skin. The aim of this study was focused on natural infection of leishmania parasite in *Meriones hurrianae* and *Tatera indica* (Rodentia: Gerbillinae) populations in an endemic focus of ZCL in Iran. The results will help for a better planning of disease control measures in the area.
Methods: This descriptive study was carried out in rural areas around Chabahar county (Negour, Polan and Noubandian villages), Sistan – Baluchistan province, south – east of Iran during the spring and summer of 1997. Rodents were captured by live traps baited with walnut, tomato and cucumber, twice a month. Rodents were identified according to the national systematic key. Impression smears were prepared from the rodents’ ears and investigated microscopically for amastigotes. Smears from infected animals were injected into Balb/c mice.

Results: A total of 70 small mammals were captured and examined. Predominant among the seven species of small mammals found in the Chabahar county were *Meriones hurrianae* (40%) and *Tatera indica* (38.6%). Both *M. hurrianae* and *T. indica* were found to be infected with *Leishmania*: 5 out of 28 *M. hurrianae* (17.8%) and 1 out of 27 *T. indica* (3.7%) were positive. Parasites from infected *M. hurrianae* were injected subcutaneously into 5 Balb/c mice. Nodules and ulcers containing numerous amastigotes, appeared within 30 days. In this study, we present the first report of *Leishmania* infection from *M. hurrianae* and *T. indica* in Iran and Sistan – Baluchistan Province, respectively.

Conclusion: It seems that cutaneous leishmaniasis due to *Leishmania major* is prevalent in Chabahar county. *M. hurrianae* and *T. indica* probably play an important role as reservoir hosts in the epidemiology of zoonotic cutaneous leishmaniasis in this area.

Keywords: Leishmania Infection, *Meriones hurrianae*, *Tatera indica*, Rodentia, Sistan - Baluchistan, Iran

Introduction

Leishmanioses are categorized as parasitological diseases which have different clinical symptoms. These diseases are spread in old and new world districts and have got different epidemiological features. Different species from *Phlebotomus* and *Lutzomyia* genera are distinctive vectors for twenty species of *Leishmania*. Thirty species of sandflies are distinctive vectors of different *Leishmania* spp. (WHO 1990, Desjeux 2000).

Almost 350 million people are subjected to *Leishmania* worldwide. Leishmaniasis is endemic in 80 countries. Leishmaniasis prevalence is 14 millions cases and annual incidence rate is estimated to be two millions cases. From these 1.5 million is cutaneous leishmaniasis and 500,000 cases is visceral leishmaniasis (Paho 2004, WHO 2006).
Cutaneous leishmaniasis is endemic in most of countries situated in Mediterranean region and regarded as a very basic problem from the viewpoint of public health (Nadim 1987).

*L. major*, *L. tropica* and *L. aethiopica* are causative agents of cutaneous leishmaniasis in old world countries (Molyneux et al. 1983). Cutaneous leishmaniasis is not a very important clinical manifestation but the recovery of scars take long time (few months) and finally there will reside a badly shaped scars in faces or and other parts of the human body.

Dry form cutaneous leishmaniasis is an old endemic disease in most urban regions of Iran and wet type cutaneous leishmaniasis is endemic and prevalent in most of rural regions in seventeen out of thirty-one provinces of Iran (Akhavan et al. 2006). Isfahan province is the most important focus for rural cutaneous leishmaniasis in Iran and has a hyperendemic nature there. *Rhomobomys opimus* (great gerbile) is the main reservoir of this disease in this region (Nadim and Faghih 1968). In other high risk parts of Iran (Southern part and South-Western part) *Tatera indica* is the main reservoir host (Javadian et al. 1998). In some of the rural cutaneous leishmaniasis foci in southern parts of Iran, *Meriones libycus* is the main reservoir host. In these regions no *R. opimus* and *T. indica* could be found (Rassi et al. 2006).

Cutaneous leishmaniasis could be traced in South-Eastern of Sistan - Baluchistan province in Dashtyari region up to the main city of Chababar, Tis village and Konarak county. Chabahar is very interesting for its touristic nature to the travelers, so, a good application of control measures for cutaneous leishmaniasis will help the people coming to this place. According to above introduction and importance of research on characterizing different reservoirs of cutaneous leishmaniasis, this study was proposed to collect different reservoirs and verify them to light up more epidemiological features of leishmaniasis in the named region.

**Materials and Methods**

This research was a descriptive study and sample collection was conducted as a trans – sectional method from June 1997 up to September 1997 in three villages (Negour, Ploan and Noubandian villages) in Chabahar county, south east of Iran.

Rodent collection was done with two type of live kept trap (wooden and metal types). Region of study first was surveyed to find active colonies of rodents. Random collection of rodents was done in each 15- 20 days periods of time and in each collection twenty traps were used. Traps were positioned near active rodent places (nests). Fresh pieces of cucumber, tomatoes, broiled almonds and broiled nuts were used as baits. Active nests were selected on the basis of seeing the residues of fresh plants in the vicinity of the nest entrance, observation of fresh laid feces and very fresh soil extracted from the nest in the vicinity of the nest entrance. Traps were mounted in
the evening and were collected in the morning of the next day. After collection of rodents they have been placed in a desiccator and the rodents have been anesthetized using cotton soaked in chloroform. Ear pinas of the rodents was investigated for the appearance of induration, crusted, nodule formation and protorbence any redness, inflammation and scar formation. In any case both of ears have been washed and cleaned using ethanol 70%. Two slides were made from each ear using scraping method (Edrissian et al. 1982). Smears were fixed by methanol and the Giemsa staining was done. Smears were investigated under oil immersion by a compound microscope. When there was no Leishman body for the subjected rodent, it was kept for morphological and morphometric studies of the rodent. The rodent has been killed and its head was fixed for the study of the skull and confirmation of the rodent species. Some of the rodents were evacuated from their viscera and then, they have been kept in diluted formalin or 70% ethanol.

Results

Seventy rodents have been collected by grid trap and live catch trap during June 1997 up to September 1997. From collected rodents, 28 (40%) *Meriones hurrianae* (Fig 1), 27(38.6%) *Tatera indica* (Fig 2), 3 (4.3%) *Rattus rattus*, 4 (5.7%) *R. norvegicus*, 5 (7.1%) *Mus musculus*, 2 (2.9%) *Nezokia indica* and 1 (1.4%) *Funambulus penanty*. Ear of all collected rodents were chequed for Leishman bodies using scraping method (Edrissian et al. 1982). Microscopical study of all smears revealed from twenty eight of *M. hurrianae* 5 animals (17.8%) and from twenty seven *T. indica*, 1 animal (3.7%) were infected with amastigotes (Leishman body). These Leishman bodies have got big nucleus and distinct vacuole and kinetoplast (Fig 3), so it has been confirmed to be *Leishmania major*. Z test was used to discriminate if difference between infection in *M. hurrianae* and *T. indica* is significant or not. The result was positive. Leptomonades from the above study was injected at the basis of the tail of mouse intradermally. Some of mice have shown nodule and scare at the place of injection. Result of this study shown the feeding habits of *M. hurrianae* and *T. indica* that they depend on halophile plants like *Saueda fruticosa* and *Salsola dendroides* from family (Chenopodiaceae).

Discussion

Where, rodents are regarded as resources of rural type of cutaneous leishmaniasis so, identification of the infection to *Leishmania* in Chabahar focus for cutaneous leishmaniasis is very important. In this study *M. hurrianae* and *T. indica* were confirmed to be infected with *Leishmania* parasite. It is the first time that *M. hurrianae* is reported to be infected to *Leishmania* in Iran. *M. hurrianae* with 17.8% infection rate to *Leishmania* is introduced as the main reservoir host
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of cutaneous leishmaniasis in South–Eastern part (Baluchistan region) of Iran. Infection in *T. indica* to *Leishmania* is reported for the first time too, but it seems that this infection is a kind of accidental infection in the same region. *Leishmania* infection in *M. hurrianae* has been reported from North-Western part of India and *M. hurrianae* is regarded as main reservoir of rural type cutaneous leishmaniasis in that region (Peters et al. 1981 and Blancq et al. 1986). Seyedi-Rashti et al. could not confirm any *Leshmania* infection in any collected rodents in Chabahar, Baluchistan region of Iran (Seyedi-Rashti et al. 1984). In a study in country borders of Iran (Khuzestan and Ilam provinces) *Leishmania* infection in *T. indica* was found to be 12.5% in those regions (Javadian 1988). In another study in Mehran (Ilam province) there was a *Leishmania* infection in *T. indica* at 9% (Javadian et al. 1998). There was no *Leishmania* infection in *N. indica* in this study, but natural infection to *Leishmania* parasite in *N. indica* was reported from Khuzestan and Isfahan provinces (Ardehali et al. 1998 and Yaghoobi-Ershadi et al. 2001). Rab et al. has reported *Leishmania* infection in *T. indica* from Baluchistan (Pakistan) (Rab et al. 1986).

It seems that *T. indica* in Chabahar has not been very sensible to *Leishmania* parasite, or, biological features of this rodent made it to be less presented to *Leishmania* parasite transmission. Difference in the soil texture is another reason that this rodent can not dig a nest deep enough for sand flies to reproduce well and raise an increment in its population to be a useful vector.

In this study it was revealed that plant covering, soil texture and presence of many agricultural fields in Dashtiari region (Chabahar) cause high density of rodent population and rodents could make very vast colonies in this region. There was a low rate of infection in human in Chabahar in contrast to *Leshmania* infection found in *M. hurrianae*. The reason of above situation may be due to the long distance of infected rodents’ habitats from villages. Sand flies have got interrupted fly habits and can not fly for long distances, so they can not be found in very distant from their nests. Then villagers are kept safe and freed from vector bite and the rate of human infection will be low.

The best control measure is provided when plan the challenge against both vector and reservoir. It seems in this case that the distribution of reservoir is very widespread in the region; rodent control is not cost effective. There may be a chance of rodent control after destruction of all rodent colonies' nest in a range of 500 to 1000 meters then using mineral poisons like zinc phosphide or Cumarin base poisons like Klerat. Regarding vectors, indoor spraying of residual poisons can reduce acute cases of disease, but, when the nature of disease is zoonotic and outdoor sleeping habit of people of the named region in hot summer nights, leishmaniasis transmission could not be stopped. Then it is clear that vector control without reservoir control could not be useful. After, application of control measures for vector and reservoir then, human acute cases should be found and medically treated. Acute cases of human are regarded as secondary reservoirs in rural type of cutaneous leishmaniasis. Other control measures like public health education in the region and application of individual protection methods (using
repellents, scar covering, insecticides impregnated bed nets and door and windows equipped with screen are very important to be presented and applied in this region for disease control.

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Fig 1: Typic *Meriones hurrinae* collected from Baluchistan (Iran)
References


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