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First molecular detection of *Leishmania major* DNA within *Meriones persicus* in new focus of cutaneous leishmaniasis in Lorestan Province, West of Iran

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Abstract

In order to determine of vector(s) and reservoir of disease, a study was conducted in some rural areas of Lorestan Province (new focus of disease), west of Iran in 2015-16.

A total of 1202 sand flies were collected and identified as *Phlebotomus papatasi*, *P. sergenti*, *P. alexandri*, *P. mongolensis*, *Sergentomyia dentata*, *S. sintoni*, *S. clydei*, *S. tiberiadis* and *S. squamipleuris*. *Phlebotomus papatasi* was the dominant species in rodents burrow. Employing ITS1 PCR technique followed by HaeIII enzyme on 350 females of sand flies, only 1 out of 195 *P. papatasi* (0.5%), was found positive to *Leishmania major*.

In this study 14 rodents were captured using Sherman live traps and identified. They were *Meriones persicus* (85.7%) and *M. libycus* (14.3%). Among the collected rodents only 1 out of *M. persicus* (8.3%) was infected with *L. major*. This was the first report on infection of *M. persicus* to *L. major* in the west of Iran.

According to results of present study it seems the *P. papatasi* and *M. persicus* are playing as the main vector and reservoir in transmission of cutaneous leishmaniasis to human in west of Iran.

Keywords: *Phlebotomus Papatasi*, *Meriones persicus*, *Leishmania major*, Iran

1. Introduction

Leishmaniasis is a parasitic disease with a wide spectrum of clinical manifestations ranging from a self-healing skin lesion to lethal form of visceral disease^[1]. Leishmaniasis occurs in 98 countries worldwide, and Zoonotic Cutaneous Leishmaniasis (ZCL) is the most common form of leishmaniasis and about one-third of cases are reported from the Americas, the Mediterranean basin, and Western Asia from the Middle East to Central Asia^[1]. Countries such as Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica and Peru, which together account for 70 to 75% of global estimated of ZCL cases^[1].

The female Phlebotomine sand fly is the only vector of Leishmaniasis, thus it is the main mode of transmission^[1]. Other modes of transmission include vertical transmission, blood transmissions, and the use of contaminated needles, which has led to an alarming rise in HIV/Visceral Leishmaniasis co-infection. Leishmaniasis is found be both anthroponotic, where human are the sole reservoirs and sole sources of infection for the vector, and zoonotic, where animal hosts are involved in the transmission cycle and act as reservoirs^[2].

The most common animal reservoirs include dogs, wild carnivores, rodents, hyrax, sloths, and opossums. It is important to note that a wide range of mammals can act as reservoirs for this disease as well^[2].

Every year, nearly 20,000 new cases of CL are reported in the country; however, the actual number of cases may be four to five times more^[3].

Among 800 identified species of sand flies in the world, only 50 species are considered as vectors of Leishmaniasis^[4].

Since 1930, a total of 50 species of sand flies have been reported in Iran^[5]. These studies also have illustrated *P. sergenti* as the vector of Anthroponitic Cutaneous Leishmaniasis (ACL), and *P. papatasi* as the vector of Zoonotic Cutaneous Leishmaniasis (ZCL)^[6]. Likewise, in some foci of the southern and north east parts of the country, *P. salehi* has been reported to be infected by *L. infantum* and *L. major*^[6,7].

Based on animal reservoir hosts there are four foci of disease in Iran [8]. The first one has been located in central and northeast of Iran, where *Rhombomys opimus* and *P. papatasi* play important roles as reservoir and vector of the disease [8]. The second foci are located in the west and southwest of Iran, where *Tatera indica* is replaced with *Rhombomys opimus* as a reservoir and *P. papatasi* as a vector [8]. Baluchistan Province, in the southeast of Iran is considered as the third focus of *L. major* and *Meriones hurrianae* has been approved as its natural reservoir host [7].

The fourth zone of ZCL has been located in the south of Iran and in this area, *M. libycus* was the primary and main reservoir host of the disease, and *R. opimus* and *T. indica* were absent [8].

The present investigation was conducted in some rural areas of Lorestan Province (new focus of disease), west of Iran in 2015-16. The main objectives were to determine the sand flies species responsible for transmission of *L. major* to human, as well as to determine the main host reservoir of the disease.

2. Material and Methods

2.1. Study area

This cross sectional study was done in two villages of Sarab hamam and Dokhohan in Poldokhtar county, Lorestan Province western Iran during June to November of 2015. The climate is generally sub-humid continental with winter precipitation, a lot of which falls as snow. Temperatures vary widely with the seasons and between day and night. At Khorramabad (center of Province), summer temperatures typically range from a minimum of 12 °C (54 °F) to a hot maximum of 32 °C (90 °F). In winter, they range from a minimum of -2 °C (28 °F) to a chilly maximum of 8 °C (46 °F). The population of Lorestan was estimated at 1,716,527 people in 2006.

2.2 Collection of sand flies

Using sticky papers, sand flies were collected monthly from indoors (e.g. bedroom, guest bedroom, toilet, and stable), outdoors (wall cracks and crevices) as well as animal burrows (60 papers per place) during June to October of 2015. Sticky traps were installed at sunset and recollected near sunrise. To get rid of the sticky materials, all collected sand flies were preserved in 96% ethanol alcohol.

Dissection of female's specimen was done in phosphate buffered saline (PBS) solution. After removing of terminal segments of the abdomen containing the spermatheca and the heads, they were mounted in a drop of Puri's medium for species identification by employing the key of Theodor and Mesghali [9]. The rest bodies of the sand flies were kept individually in 96% alcohol and stored at -20 °C for molecular job.

2.3 Collection of rodents

Altogether, 20 Sherman live traps were installed near active colonies of rodents adjacent to human places during September to November of 2015. The traps were baited with roasted walnut, cucumber, and tomato, and were set up early morning and evening in active burrows.

External characteristics: color, body measurements, ears, tail, feet, teeth, and cranium were used for identification of rodents [10, 11]. Parasite infection in rodents was examined microscopically by preparing an impression smear from their ears after Giemsa staining. Positive smear samples were followed for species identification by polymerase chain reaction (PCR) method is described later.

2.4 DNA amplification and PCR-RFLP

Gene All kit was used for DNA extraction of positive specimens of female sand flies as well as rodents with *Leishmania* according to kit's procedure. Polymerase chain reaction (PCR) against the ITS1 locus was performed for leishmanial infection of examined specimens employing the primers of: LITSR 5'CTGGATCATTTTCCGATG3' (Forward) and L5.8S- 5'TGATACCACTTATCGCACTT3' (Reverse) [12]. Positive PCR products was followed using HaeIII restriction Enzyme for clarifying of *leishmania* species. All PCR products were analyzed by 2–2.5% agarose gel electrophoresis, followed by gel stain and visualized under blue led transilluminator. Parasites were identified by comparison with positive controls of *L. major* and molecular weight markers. Standard DNA fragments (50 bp ladder, Fermentas) were used to permit sizing.

3. Results

In this study, a total of 14 rodents were captured and two species of rodents including *Meriones persicus* (85.7%) and *M. libycus* (14.3%) were identified. Employing PCR-RFLP, one of them (8.3%) was found positive to *L. major*. ITS1 PCR-RFLP analysis by HaeIII revealed the fragments of 210 and 140 bp for infected rodent which are characteristic of *L. major*. (Fig.1). This is the first report on infection of *M. persicus* to *L. major* in Lorestan Province west of Iran.

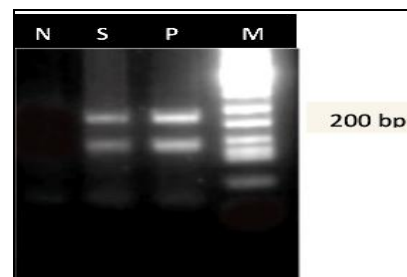


Fig 1: RFLP of ITS1 positive samples of *Meriones persicus* to *L. major* by HaeIII using 2.5% agarose gel, Sarab hamam, Lorestan Province, 2015; M:Size marker(50 bp), P: Positive control (MHOM/IR/75/ER) of *L. major*,S: positive sample of *Meriones libycus* to *L. major*, N: negative control.

In total 350 female sand flies comprising four species of *P. papatasi* (55.7%), *P. sergenti* (0.6%), *P. alexandri* (0.9%) and *Sergentomyia sintoni* (42.8%) were surveyed to find *Leishmania* parasites. Our results showed, only 1 out of *P. papatasi* (0.5%) was positive to *L. major* (Fig. 2). This is the first detection of *L. major* in *P. Papatasi* in Lorestan Province west of Iran.

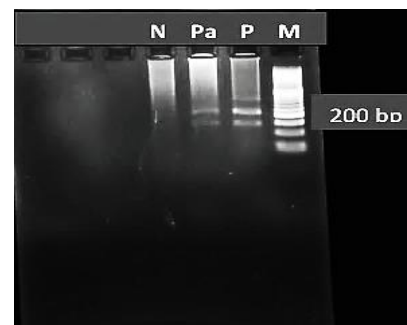


Fig 2: RFLP of ITS1 positive samples of *P. Papatasi* to *L. major* by HaeIII using 2.5% -agarose gel, Sarab hamam, Lorestan Province, 2015; N: negative control, Pa: Positive sample *P. papatasi* to *L. major*, P: positive control (MHOM/IR/75/ER) of *L. major* and M: Size marker(50bp).

4. Discussion

Due to the fact of the nature of zoonotic vector borne diseases, the clarifying of complex structure of ecological systems are very important for the effective decision of control measures [13]. In Iran, the zoonotic form of cutaneous leishmaniasis is an old as well as main public health problem. According to registered data, more than 80% of all new cases were zoonotic cutaneous leishmaniasis and the disease is endemic in 17 out of 31 Provinces in the country [14-17].

Epidemiological data along with entomo-parasitological surveying are the most important elements for planning of leishmaniasis control programs. The capacity of wild caught sandflies as the vectors of leishmania parasites to human associated with natural infection of them to metacyclic form of promastigotes and high anthrophilic index [18].

In the present study, high density of *P. papatasi* in outdoors, indoors as well as rodent burrows and its natural infection with *L. major* were the basic reasons for introducing of it as the vector in the region. *Phlebotomus papatasi* is known as a restricted vector and specifically is able to support only the development of *L. major* [19].

A few species of rodents belonging to the subfamily Gerbillinae are the main reservoir hosts of ZCL due to *L. major* in endemic areas of Iran and Middle East [20-22].

In Iran, *Leishmania major* infection of eight species of *Rhombomys opimus*, *Meriones libycus*, *Tatera indica*, *Nesokia indica*, *Meriones hurrianae*, *Meriones persicus*, *Gerbillus nanus* and *Rattus norvegicus* have been confirmed [23-29].

However, we detected the present of infection due *L. major* in *M. persicus* (8.3%), the dominant collected species, in the studied region. For the first time, the infection of this rodent with *amastigote* parasites was observed in north west of Iran and reported as a probable reservoir of ZCL in 1975 [30]. According to recent studies on animal reservoir of ZCL, nine out of 25 (36%) *M. persicus* were infected with *L. major* in Fars Province south of Iran [24]. It should be noted, bases on detection of *Leishmania infantum* in *M. persicus* it is assumed to be potential reservoir for visceral leishmaniasis in North West of Iran [30-32].

5. Conclusion

In conclusion, the result of the current study revealed the important factors present for establishment of the disease in the region. These include human activities close to *M. persicus* burrows, the presence of high density of *P. papatasi* in the rodent burrows, outdoors and indoors, and proximity of human habitat to *M. persicus* colonies, which have led to emergence of a new focus of *L. major* in the region.

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