کارگاه های آموزشی مرکز اطلاعات علمی جهاد دانشگاهی

کارگاه آنلاین
کاربرد نرم افزار SPSS در پژوهش

کارگاه آنلاین
اصول تنظیم قراردادها

کارگاه آنلاین
برویوزال نویسی
First Report on Isolation and Characterization of Leishmania major from Meriones hurrianae (Rodentia: Gerbillidae) of A Rural Cutaneous leishmaniasis Focus in South-Eastern Iran

Hamid Kassiri 1,*, Saied Reza Naddaf 2, Ezat-Aldin Javadian 3, Mehdi Mohebali 4

1 Department of Medical Entomology and Vector Control, School of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran
2 Department of Parasitology, Pasteur Institute of Iran, Tehran, IR Iran
3 Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran
4 Department of Medical Parasitology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran

*Corresponding author: Hamid Kassiri, School of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, IR Iran; Tel: +98-6113738269, Fax: +98-6113738282, E-mail: Hamid.Kassiri@yahoo.com.

Received: Jun 25, 2012; Revised: May 29, 2013; Accepted: Jun 11, 2013

Background: Zoonotic Cutaneous Leishmaniasis (ZCL) is an endemic health problem in many rural areas of Iran, with doubled number of incidences over the last decade. Different species of rodents serve as natural reservoir host for ZCL. The disease is considered as a major health problem in rural areas of Mirjaveh, Chabahar, and Konarak Counties of Sistan va Baluchistan Province.

Objectives: This study describes the identity of Leishmania species, isolated from Meriones hurrianae from Chabahar County using RAPD-PCR methodology.

Materials and Methods: Rodents were entrapped by live traps baited with roasted walnut, tomato, and cucumber during spring and summer. All rodents were identified based on external features including fur color, ears characteristics, tail length, hind feet, body measurements, and internal features of teeth and cranium. Giemsa-stained impressions from rodents’ ears were examined for amastigotes microscopically. The samples from infected rodents were cultured in NNN+LIT medium and then the harvested parasites at the stationary phase were subjected to DNA extraction followed by amplification with RAPD-PCR.

Results: All the 28 entrapped animals were identified as M. hurrianae. Five animals showed to harbor Leishmania parasite by microscopy. Leishmania DNA isolated from five M. hurrianae produced distinctive bands of L. major with four primers. However, the products that were amplified with primers AB1-07, 327, and 329 were stable and reproducible. This is the first report on the isolation and identification of L. major from M. hurrianae from Iran.

Conclusions: Regarding infection rate of 17.8%, M. hurrianae seems to play the major role in the maintenance and transmission of disease to humans in this area.

Keywords: Meriones hurrianae; Leishmania major; Molecular Characterization; RAPD-PCR; Reservoir Host; Iran

1. Background

Leishmaniases, most zoonotic, are complex worldwide diseases caused by more than 20 species of Leishmania belonging to the family Trypanosomatidae (order Kinetoplastida). Leishmania parasites are transmitted via the infective bites of about 30 different species of sand flies (subfamily Phlebotominae) (1, 2).

Leishmaniasis is prevalent in many tropical and subtropical areas covering about 88 countries with approximately 350 million people at risk of acquiring the infection (3). There are four main forms of leishmaniasis: Visceral Leishmaniasis (VL), Mucocutaneous Leishmaniasis (MCL), Diffuse Cutaneous Leishmaniasis (DCL), and Cutaneous Leishmaniasis (CL) (3). CL is the most common form of disease; Patient generally presents with one or several ulcer (s) or nodule (s) in the skin. Over 90% of cases of CL have been reported in Afghanistan, Iran, Iraq, Saudi Arabia, Syria, Algeria, Brazil, and Peru (4).

Three species of Leishmania parasites are etiological agents of CL in the old world: L. major, L. tropica and L. aethiopica (5). L. major and L. tropica are the causing agents for Zoonotic Cutaneous Leishmaniasis (ZCL) and Anthropo- nomic Cutaneous Leishmaniasis (ACL) are prevalent in Iran with infection prevalence ranging from 1.8% to 37.9% in different Provinces (6).

ZCL is endemic in many rural districts of Iran affecting 17 out of 31 Provinces. Various species of rodents (family: Gerbillidae) have been incriminated as natural reservoir hosts of ZCL in Iran. Rhombomys opimus is known as the primary reservoir in central, north, and north eastern Iran (7, 8). Meriones libycus plays the secondary role as...
reservoir host alongside R. opimus in central Iran (9) and is also known as primary host in Arsanjan, Neiriz, Marvdasht counties (Fars Province), Ardestan County (Isfahan Province) and Qom County (Qom Province) (10-13). In Natanz County (Isfahan Province), besides the two above mentioned species, Leishmania infection has been detected in Meriones persicus. In Damghan area (Semnan Province), north Iran, Nesokia indica, M. libycus, and R. opimus are known as main reservoir hosts (14, 15). In south, west, and south western Iran Tatera indica is the primary host along with N. indica and M. libycus as the secondary hosts (16, 17). In southeastern Iran M. hurrianae and T. indica are primary and secondary reservoir hosts for ZCL, respectively (18). There are also records for infection of Gerbillus ssp. and Rattus norvegicus to L. major in Fars Province (19, 20), and Gerbillus nanus in Jask County (Hormozgan Province) (21). One of the major problems for control of ZCL is lack of knowledge about the nature of Leishmania parasites in reservoir hosts population.

2. Objectives

This study describes isolation of Leishmania parasite from M. hurrianae in rural areas of Chabahar County, Sistan va Baluchistan Province followed by identification of the species using DNA analysis.

3. Materials and Methods

3.1. Study Area

Chabahar County is located on the shore of Oman Sea littoral in southeastern province of Sistan va Baluchistan, Iran. This County is a low landing area, with geographical coordinates of 25° 17’ North, 60° 38’ East. The climate of this area is classified as very warm desert due to its low annual precipitation. The average annual temperature and humidity are 36.4°C and 75.9%, respectively. Chabahar County covers an area of 24,729 Km², with 230,000 residents. The majority of the county’s inhabitants are ethnic Baluch, speaking the Baluchi language. This descriptive cross-sectional study was carried out in 1997 in 3 villages (Negor, Pollan and Noubandian) of Chabahar County, Dashtiyari Division of Chabahar, where CL emerged as an endemic disease.

3.2. Sample Collection

Three trained persons with the same educational and professional levels were hired for rodents catching all over the study period. The ethical principles of this research were investigated and discussed in research committee of medical entomology department and necessary modifications made, faced to be approved. The sample size was selected at the minimum accepted levels because of the ethical aspects related to the animal rights. Before killing the rodents for reducing their painful feeling, we used Ether or Chloroforme as an anesthetized agent. The sample size was chosen using below Formula:

\[
    n = \frac{Z^2 \times \sigma^2}{E^2} = 28
\]

Also, expected power was calculated 75%. Based on our research design all sampling were done using simple randomized approach. SPSS 16 and MINITAB 14 softwares were applied for statistical analysis of data.

Rodents were entrapped by live traps baited with roasted walnut, tomato, and cucumber during spring and summer. The traps were placed at sunset and collected at dawn. All rodents were identified based on external features including fur color, ears characteristics, tail length, hind feet, body measurements, and internal features of teeth and cranium.

In this research, infection of the rodents observed through microscopic and PCR (Polymerase Chain Reaction) method. Impression smears were taken from rodent’s ears, stained with Giemsa, and examined microscopically for amastigotes. The samples from infected rodents were cultured in Novy-MacNeal-Nicolle (NNN) medium, liver infusion broth tryptose (LIT) and then checked twice a week for promastigotes. The positive cultures were then transferred to RPMI 1640 medium supplemented with 10% heat inactivated fetal calf serum. The parasites were harvested at the stationary phase and kept at -20°C until used.

3.3. DNA Extraction

The harvested parasites were washed in cold sterile PBS (pH 7.2) several times. The recovered pellet was re-suspended in 300 μL cell lysis buffer (50 mM NaCl, 50 mM EDTA, 1% SDS, and 50 mM Tris-HCl, pH 8.0) with 20 μL of 20 mg/mL proteinase K and incubated at 55°C overnight. DNA was extracted from lysate with phenol/chloroform followed by ethanol precipitation. The DNA was re-suspended in distilled water and working solutions were adjusted to 5 ng/μL in distilled water.

3.4. RAPD-PCR Analysis

The RARD-PCR assays were performed as outlined by others (14, 22). Each 25 μL reaction contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl2 , 2 mM MgCl2, 200 μM of each dNTP, 50 pmol of one of the primers (Table 1), 1 unit of Taq DNA polymerase, and 10 ng of DNA. Reactions were overlaid with 25 μL of mineral oil and amplified with a
thermocycler programed for one cycle at 94°C for 5 min followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 37°C to 38°C for 1 min, and extension at 72°C for 2 min and a final extension step at 72°C for 5 min. A negative control, containing all components except DNA, was included in all assays. Amounts of 8-10 µl of amplicons were run alongside a DNA size marker (Roche, Germany) on a Electrophoresis (1.2% agarose gel containing ethidium bromide) and visualized on a UV Transilluminator. Resulting bands were examined and photographed.

### Table 1. The Primers Used in RAPD-PCR Analysis

<table>
<thead>
<tr>
<th>No.</th>
<th>Code</th>
<th>Sequence</th>
<th>% GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ABI-07</td>
<td>GGT GAC GCA G</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>327</td>
<td>ATA CGG CGT C</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>329</td>
<td>GCG AAC CTC C</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>335</td>
<td>TGG ACC ACC C</td>
<td>70</td>
</tr>
</tbody>
</table>

### 4. Results

A total of 28 rodents were collected with live traps from three villages. All the animals were identified as *M. hurrianae* (Figure 1 and Figure 2). Microscopical examination of Giemsa-stained impression smears from rodents ears showed that five out of 28 animals were infected with amastigotes. The parasites from 5 animals were grown successfully in culture medium. DNA amplification with RAPD-PCR of DNA from 5 isolates yielded distinctive bands that were characteristic of *L. major* with four primers, but the products that were amplified with primers ABI-07, 327, and 329 were stable and reproducible in all assays (Figure 3, Figure 4 and Figure 5). This is the first report on the isolation and identification of *L. major* from this rodent species in Iran.
the results of this research, it is obvious that the ZCL foci in Chabahar County exist and this form is dominant. As L. major (dominant species), is a zoonotic parasite, then we should have special concern related to the rural areas immigrants and also rodent control in these regions. By this respect, the PCR molecular technique is a highly reliable procedure for diagnosis of Leishmania specie. In PCR method, as DNA of Leishmania species is examined, therefore its sensitivity and specificity are so high.

Acknowledgements

We gratefully acknowledge our colleagues at Iranshahr Institute of Health Research, Tehran University of Medical Sciences, and Chabahar Health Center for assisting us throughout the project.

Authors' Contribution

None declared.

Financial Disclosure

None declared.

Funding/Support

None declared.

References

2. WE C. Control of the leishmaniasis. 1990.
11. RASSI Y, GHASEMI MM, JAWADIAN E, MOTAZEDIAN H, RAFIZADEH S, AGHAIE AFSHAR A, et al. Determination of reservoir (S) and...
VECTOR (S) OF CUTANEOUS LEISHMANIASIS BY NESTED-PCR IN MARVDASHT DISTRICT, FARSI PROVINCE, SOUTHERN IRAN. J KERMAN UNIVER MED SCI. 2007;14(2):334-9.


Kassiri H et al.