

NATURAL PROMASTIGOTE INFECTION OF *SERGENTOMYIA SINTONI*, ITS SEASONAL VARIATION AND RESERVOIR HOST IN TURKEMEN SAHARA , IRAN

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Abstract

A Six months survey (May-October 1994) was conducted, to study natural promastigote infection of *S.sintoni*, its seasonal variation and reservoir host, in Turkemen sahara, Iran.

Sixty out of 403 *S.sintoni* dissected were found infected with promastigotes. This investigation showed that the peak of promastigote infection occurs in July (18.4%) and August (28.5%). Five lizards: one *Cyrtopodion caspius* (Eichwald, 1831), two *Trapelus agilis* (Oliver 1804) and two *Eremias velox* (Pallas 1771) were captured of which *C.caspius* was infected.

Promastigotes were isolated from *S.sitoni* and *C.caspius* and both isolates were identified as *L.(Sauroleishmania) gymnodactyli* by isoenzyme characterization. This is the first report of isolation and characterization of this parasite from the vector and vertebrate host of lizard leishmaniasis in Iran.

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Introduction

Lizard leishmaniasis is widespread in Iran. Four species of lizards were found infected in the surveyed area: (8,11,16). In all areas, lizard leishmaniasis has been found accompanied by natural promastigote infection of *S.sitoni* and rarely *S.clydei*. Infection of *S.sitoni* was observed in: Mashhad (10,16), Khuzistan (4), Bakran, Shahrood (18), varamin (15), Turkemen Sahara (9). Promastigotes were found in *S.clydei* in Lotf-Abad (7).

As promastigote infection of *Sergentomyia* occurs mainly in foci of zoonotic cutaneous leishmaniasis, to understand the overall epidemiological picture of this disease in its foci, we have to study at the same time the promastigote infection not only in the Genus *Phlebotomus* but also in the Genus *Sergentomyia*, to be sure of the nature of promastigotes.

To study natural promastigote infection of *S.sitoni*, its seasonal variation as well as reservoir host, a research programme was planned in the focus of zoonotic cutaneous leishmaniasis of Turkemen Sahara (1,9). The present paper shows the findings of this study.

Materials and methods

Three study sites; Dashboroon, Daneshmand and Fadavi were chosen within 14km of Iran-Turkemenistan border line in Gonbad-Kavoos District, (37° , 40 north latitude, 54° , 49 east longitude (Fig.1).

These villages are situated on low hills and common to all sites studied were ; an altitude of 200 meters, close proximity to Atrak river bed, the presence of both burrows of *Rhombomys opimus* and lizard holes and existance of zoonotic cutaneous leishmaniasis among residents.

In this focus, observations were made on approximately 10 days intervals over a period of 6 months (May - October 1994). Three methods of trapping sand-flies were used : Aspirator, sticky paper traps and funnel-traps. Paper sheets 10 x 15cm were coated with castor oil and placed near rodent burrows, lizard holes and inside and outside of the houses, before sunset. Next morning before sunrise sticky paper traps were collected.

Funneltraps with a collection jar were used as described by Aref-saleh zadeh (15). These traps were placed on rodent burrows before sunset and were removed 2 or 3 hours after sunset.

Live sand-flies were aspirated from collection jars and picked off the sticky paper traps in a field laboratory. All alive blood fed, gravid and empty females were dissected and examined microscopically for natural promastigote infection. When promastigotes were seen, a few drops of sterile saline were added, which was then aspirated into sterile syringe and cultured in NNN medium + Liver infusion tryptose medium (LIT) with penicilline (200 IU/ml).

Dissected flies were placed in a drop of Puri's medium (17) to clear them for identification and the species were identified carefully by the morphology of the paryngeal armatures and spermatheca(19).

The lizards were captured either from the buttocks of *Rhombomys opimus* which were infected with zoonotic type of cutaneous leishmaniasis, or from lizard holes. Each lizard was killed and smears were prepared from blood taken by heart puncture. The smears were examined for leishman bodies and culture of blood was also made on NNN medium + LIT with penicillin (200 IU/ml).

Results

Sixty out of 403 *S.sintoni* females dissected were infected with flagellates (table 1). The promastigotes were observed only in the midgut in all 60 infected sand- flies.

We concluded that natural promastigote infection of *S.sintoni* in rodent burrows in the Turkemen sahara area starts in mid June and continues until mid september, 3 weeks before the end of the active season of this species. The infection rate has two peaks one in July (%18.4) and the second in August (28.5%).

In some occasions sand-flies collected by funnel traps were transferred and dissected in central laboratroy in Tehran (School of Public Health). Flagellates from one *S.sintoni* which was dissected in Tehran was cultured in NNN medium + LIT with penicilline (200 IU/ml).

On 1st. Sep. 1994, 5 lizards were collected from Dashboroon. These consisted of 3 species;

One *C.caspius* (Fig 2) (formerly called *gymnodactylus caspius*), two *T.agilis* (formerly called *Agama agilis*) and two *E.velox*.

One *C.caspius* and one *T.agilis* were killed and smears were prepared from blood taken by heart puncture. The smears were examined for leishmania bodies and the result was negative for both lizards. Culture of blood was also made. Cultures of promastigotes obtained from *S.sintoni* and blood taken by heart puncture from *C.caspius* and *T.agilis* were kept at 20-21^oc and checked after 4 days for growth of promastigotes. Bacterial or fungal contamination was not observed. Growth of promastigotes were seen in cultures from *S.sintoni* and *C.caspius*. Third passage of these cultures were sent for isoenzyme characterization to Liverpool School of Tropical Medicine, England (Dr. Motezedian). Enzyme characterization, in Liverpool School of Tropical Medicine, showed that the Dashboroon isolates from *S.sintoni* and *C.caspius* were *L.(S.) gymnodactyli*.

Discussion

Leishmania in reptiles have been studied early in the Century. Intensive studies, both on lizards and sand-flies, also transmission of strains of leishmania by sand-flies and on their serological relationships, have been carried out in recent years by a number of scientists. Soviet investigators have reported *S.arpakelensis* as a vector of lizard leishmaniasis, but it seems that it is synonymous with *S.sintoni*.

Hodukin et al. (3) after studying strains of leishmanias from 3 species of lizards in central Asia stated that lizards' promastigotes are not pathogenic for mammals and man. Scurenkova (Cited by Belova) (2) held the same opinion and even suggested the reptile promastigotes belong to an independent genus. But, it seems that it is better to consider these parasites as an independent subgenus i.e. *Leishmania (sauroleishmania)*, which comprises several species (13,14,5,12). Dergaceva reported that, *S.sintoni* as a vector of human leishmaniasis, is of no practical significance, (cited by Lewis)(6).

Nadim et al (10) mentioned that; *S.sintoni* seems to play no part in the transmission of mammalian leishmaniasis in north-eastern Iran, and they also stated leptomonads found in *S.sintoni* and lizards are probably of the same origin, but the nature of promastigotes were not identified.

In our present study , by isoenzyme characterization we isolated *L.(S) gymnodactyli* from *S.sintoni* and *C.caspicus* in Turkemen Sahara and this is the first report of isolation and characterization of *L.(S.) gymnodactyli* from *S.sintoni* and *C.caspicus* from Turkemen Sahara in Iran, which shows the nature of the parasite both in lizards and in sand-flies of the Genus *Sergentomyia*.

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Table 1- Natural promastigote infection of *S.sintoni* in rodent burrows, by dates of capture in Turkemen Sahara, Iran May-October 1994.

Date	No. Collected Per 10 Traps	No. dissected	No. infected	% infected
12 May	4	0	0	0
23 May	16.8	22	0	0
2 Jan	27.7	14	0	0
16 June	57.4	13	1	7.6
2 July	37.8	15	1	6.6
11 July	19.5	25	2	8
21 July	46.7	38	7	18.4
28 July	80.7	33	3	9
4 Aug	110.5	19	0	0
6 Aug	72.6	20	0	0
18 Aug	260.3	42	12	28.5
25 Aug	321.7	53	13	24.5
1 Sep	464	23	6	26
8 Sep	92.9	25	3	12
17 Sep	137.4	23	3	13
22 Sep	136.9	21	5	23.8
29 Sep	18.9	16	4	25
7 Oct	0.7	1	0	0
21 Oct	0	0	0	0

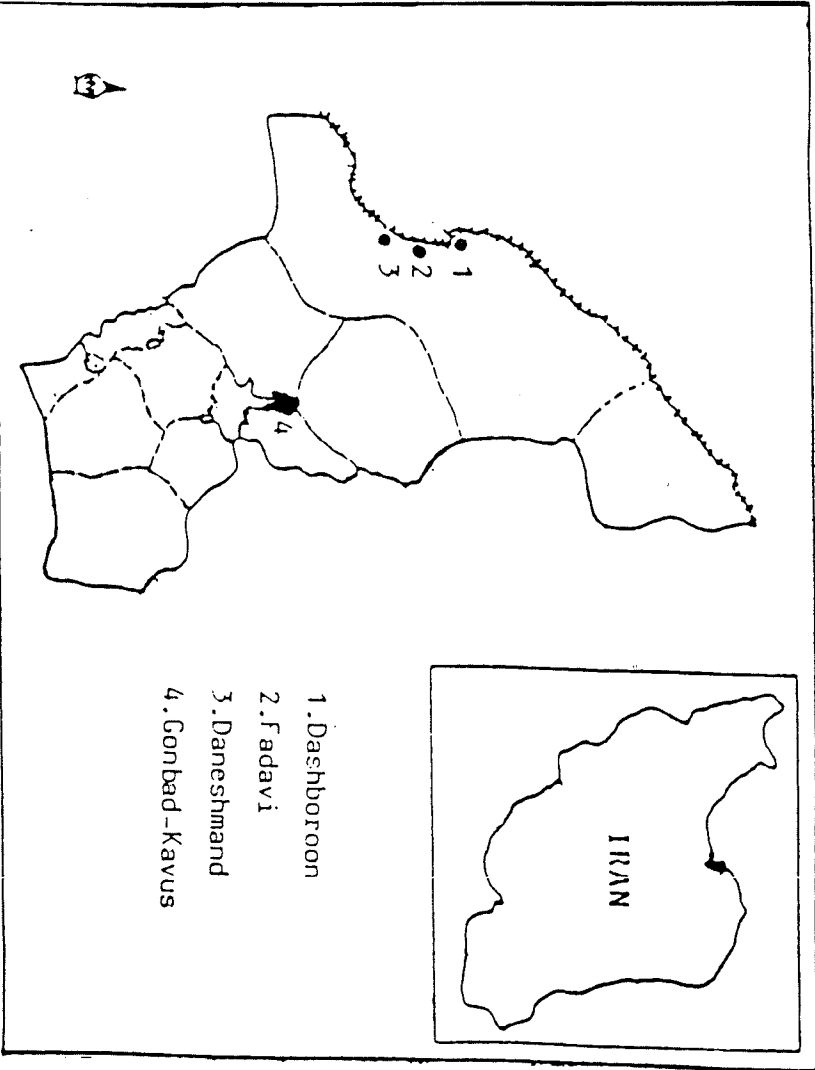


Fig 1- Map Showing Location of Study



Fig 2 - *Cyrtopodion Caspius*

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