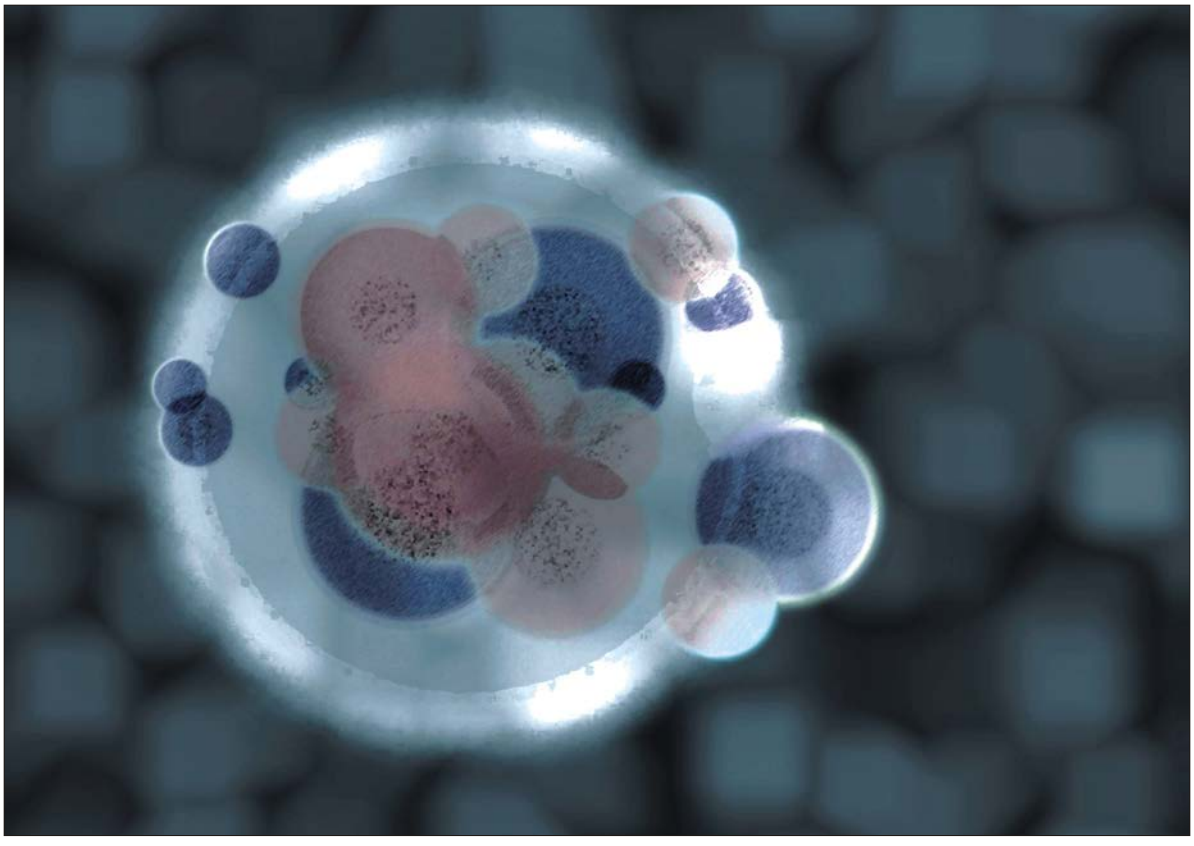

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Isolation a Lizard *Leishmania promastigote* from its Natural Host in Iran

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Abstract: This study was done to assess a lizard *Leishmania promastigote* from natural host in Iran. From different species of lizards, 144 lizards have been captured from Tehran and the provinces of Semnan, Isfahan and Golestan in Iran. Sixty one lizards have been tested by heart blood culture on NNN medium and thick and thin blood smears by light microscope. *Leishmania promastigote* forms have been detected in 3 blood smears and amastigote forms have been found in 10 cases. Then the promastigote forms of isolated parasite have been grown on NNN medium.

Key words: *Leishmania promastigote*, lizard, Iran

INTRODUCTION

Leishmania is a protozoan parasite of kinetoplastida genus that has two forms in its life cycle: amastigote in vertebrate hosts and promastigote in invertebrate hosts and culture medium. Its amastigote form develops in reticuloendothelial system of mammalian host. Leishmaniasis is a zoonotic disease because of coetaneous and visceral lesions in human and animals, whereas some of them are anthroponotic and there is no report in birds[1], some species cause infection in reptiles[2-7]. The first report of *Leishmania promastigote* forms isolated from reptiles was published in 1915[3]. In Kenya, Heish[4] has observed a *lizard Leishmania promastigote* form in *Latastia longicaudata* heart blood culture and named it as *Leishmania adleri*. Adler[8] has injected it to the hamster spleen and after two weeks he has isolated it again. Belova[9] detected leptomonads in some species of lizards in the Turkmen SSR. Nadim *et al.*[5] have isolated Leishmania from *Agama caucasica* in Khorassan province and Manjil region in Gilan province of Iran and this parasite has been detected in *Eremias gluitata watsonata* in Khuzestan province. Seyedi *et al.*[6] have isolated lizard Leishmania promastigote forms in Turkmen Sahara and Khuzestan. Edeson *et al.*[10] and Pozio *et al.*[11] have found lizard Leishmania from *Agama stillo* in Lebanon and so from *Cyrtodactylus kotschyi* in Italy, respectively. Elwasila[12] detected *Leishmania tarentolae* from the gecko *Tarentola annularis* in the Sudan. Al Sadoon and el Bahrawy[13] found Leishmania parasite in blood of lizards in Saudi Arabia. The objective of this study was to isolation lizard leishmania from its natural host.

MATERIALS AND METHODS

Lizard capturing: Lizards have been captured by modified described methods[6,7,14]. Captured lizards have been transported to Tehran.

Blood sampling: There are some methods for blood sampling. Lizards have been washed by top water to clean their bodies which some of them anesthesised and others were anesthesised by chloroform. Lizards have been fixed in backward on surgeon tray and by a sterile bistoury a seizure was carried out on their chests to appear their hearts and blood samples have been aspirated by G25 needles. Blood samples have been divided in two sections, one section have been cultured on NNN medium and incubated in 18-22°C. Thick and thin smears were carried out by another sample section. This research was carried on by 100 magnification light microscopy studying of thick and thin gimsa stained smears (peripheral blood and visceral impression smears).

Lizard species determination: The body of each dead lizard has been ticketed by soft paper and fixed in

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formalin. Lizard species have been determined by *Razi Institute scientific team*.

RESULTS

One hundred forty four lizards (*Agama caucasica microlepis*, *Mabuya aurata*, *Eremias guttulata*, *Agama agilis* and *Phrynocephalus seatellatua*) have been captured from Shahroud in Semnan province, Isfahan province and Maraveh Tapeh in Golestan province and Abardej of Varamin in Tehran province. Sixty one heart blood samples have been cultured in NNN medium (Table 1). *Leishmania amastigote* form have been identified in 10 cases and *Leishmania promastigote* forms have been detected in 3 lizard blood smears. *Leishmania promastigote* form *from Agama caucasica microlepis* (Fig. 1) which have been captured from Shahroud have been grown on culture media (Fig. 2), whereas there have not been detected any parasite in visceral impression smears. The regions and time of capturing, number and species of examined lizards and other results have been summarized in Table 1.

Table 1: results of capturing and testing of reptiles from Iran

		Number			Results	
Regions	Time of capturing	Captured	Tested	Lizard species	Microscope	Culture
Shahroud	April	30	10	ACM	3 (am)	-
	June	35	14	ACM	1 (pr)	-
	October	13	13	ACM	1 (pr)	2
Isfahan	June	40	4	A.A(3)	2 (am)	-
	September	3	3	M.A.(1)	-	-
				AA.(1)		-
				E.G.(2)		-
Maraveh tapeh	July	22	16	AA.(2)	1 (pr)	-
				ACM (14)	4 (pro)	-
Abardej (Varamim)	September	1	1	PS	1 (pr)	-
Total		144	61		10 (am)	
					3 (pr)	2

Table abbreviation :
ACM (*Agama caucasica microlepis*) AA (*Agama agilis*)
EG (*Eremias guttulata*) PS (*Phrynocephalus seatellatus*)
Am (*Amastigote*) Pr (*Promastigote*)

Table 2: Description of isolated *Leishmania promastigote* from *Agama caucasica microlepis*

Characters	Measurement (µm)
Promastigote length	11-22
Flagellum length	11-14
Distance between kinetoplast to nucleous	6.6
Distance between kinetplast to superior end	2.2
Diameter	2.2–4.4

Description of lizard *Leishmania promastigote* form in NNN medium: There were some slender and stumpy promastigote forms and dividing parasites were observed by light microscope and so there were other forms like

Fig. 1: *Agama caucasica microlepis* captured from Shahroud in north of Iran

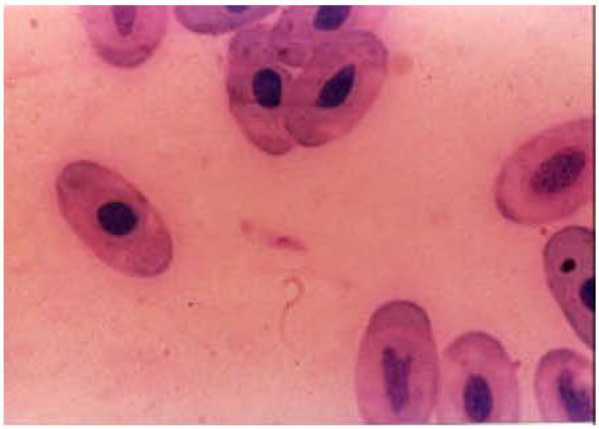

Fig. 2: *Leishmania promastigote* form in peripheral blood smear of lizard

leishman donovan bodies with small flagella. Promastigotes were measured by micrometric equipped microscope (Table 2). Its promastigote length, flagellum length, distance of kinetoplast from nucleous, distance of kinetoplast from superior end and diameter is 11-22, 11-14, 6.6, 2.2 and 2.2–4.4 µm, respectively. Parasite promastigotes were cryopreserved for further works.

DISCUSSION

There is a lizard *Leishmania promastigote* form in circulation of *Agama caucasica microlepis* in Shahroud region which had not been reported up to now. There are some species of lizard Leishmania and Adler has reported 9 species of them[2]. Each lizard Leishmania has specific characters. Hoar and Wallace[15] suggested that *Leishmania promastigote* forms are observed in NNN medium or insect as vector, whereas amastigote forms are observed in mammalian hosts. Read and Chandler[16] suggested that *Leishmania amastigote* forms are

detected in vertebrate hosts but promastigote form of *Leishmania chameleonis* parasitized epithelial cells of Chameleon digestive tract[7,16]. Telford [7] isolated *Leishmania amastigote* from *Agama agilis* and *Teratoscincus scincus* in Pakistan, who also observed *Leishmania amastigote* in lizard peripheral blood thrombocytes. There are some different lizard Leishmania species in respect of parasite establishment in host. Promastigote form of mentioned Leishmania was seen in lizard circulation. *Leishmania chameleonis*, *Leishmania henrici* and *Leishmania davidi* were seen in host bowel. Swaminath and Short found amastigote forms in Hemidaktylus lizard peripheral mononuclear cells. Frankel observed intracellular Leishmania in bowel epithelial cells of *Chameleonis vulgaris*; whereas Martini and Rioux isolated *Leishmania tarantula* amastigote from *Tarantula mauritanica* peripheral monocytes in south of France[7]. Paperna *et al.*[17] observed leishmania amastigote in erythrocytes of *Pachydactylus turneri* lizard in Sudan. Heish[4] observed different forms of *Lishmania adleri* promastigotes in NNN medium. Who reported the length, the width and the free flagella length in 6.5-21, 1.3-2.6 and 4-17 μm, respectively. Mentioned parasite in compared with *leishmania adleri* is longer. Mc Millan[18] reported *Leishmania hoogstraali* with length in 5.2-26 μm and width in 1-2.6 μm.

Tahvildar-Bideroni[19] reported infected *Sergentomya dentata* by *Leishmania promastigote* in Shahroud region. Some scientists suggest that Sergentomya feeds from lizard blood[3]. Nadim *et al.*[5] captured some *Sergentomya sintoni* from Khorassan province that according to his report, 39 were infected by *Leishmania promastigote*, but they did not observe infected Sergentomya sintoni captured from Isfahan[20]. Javadian and Mesghali[21] reported infected *Sergentomya sintoni* from Khuzestan. There is one report about lizard Transovarian transmission[3]. Lizard Leishmania was classified in Sauroleishmania genus by WHO expert committee[22], but others believed that lizard Leishmania is in trypanosome genus[23]. Gomez-Eichelmann *et al.*[24] compared lizard Leishmanias with mammalian Leishmanias at kinetoplast nucleic acid sequences, chromosomes and membrane lipids. There are some differences between lizard Leishmania and trypanosoma and they are not the same). Wallbanks *et al.*[25] suggested that *Leishmania tarantula* is Leishmania and not trypanosoma.

This is the first documented report of lizard *Leishmania promastigote* in *Agama caucasica microlepis* from Shahroud region of Iran.

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