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Parasitological and Molecular Study of *Babesia microti* in Rodents of Sarab District (East Azerbaijan of Iran)

Abbas Shahbazi[1], Neda Aboualsoltani[1], Ahad Bazmani[1], Majid Khanmohammadi[2], Farkhondeh Aboualsoltani[3] and Esmail Fallah[4]*

[1]Tabriz Research Center of Infectious and Tropical Diseases.
[2]Department of Laboratory Sciences, Marand Branch, Islamic Azad University, Marand, Iran.
[3]Sajjad Private Boarding Clinic, Department of Health and Treatment, Tabriz University of Medical Sciences, Tabriz, Iran.
[4]Department of Parasitology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

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***Babesia microti*, the intraerythrocytic protozoan parasite, is the most common cause of human babesiosis all over the world and is considered one of the most important diseases of zoonotic parasitic infections. Rodents are one of the reservoirs of the disease parasite.This investigation is the first molecular study for determining rodents as reservoir hosts of Babesia microti in Iran and Sarab district.To determine if the organism is present in rodents of the region, we live trapped and sampled 100 rodents from 10 villages in the Sarab area.DNA extracts from whole blood specimens were tested by Polymerase Chain Reaction(PCR), using specific fragment for the *B.Microti*; nuclear small subunit ribosomal RNA gene (18 ss rRNA).The pear shaped intraerythrocytic babesia parasite was seen in thin blood smear study of 3 of the rodents (*Mus musculus*) and by applying PCR method, it was determined to be *B.Microti* that is pathogenic to humans. The results of this study suggest that *B.Microti* is present among rodents, particularly in rodent pets of three villages of Sarab in Iran and maybe a health risk to humans residing in the district.**

Key words: *Babesia microti*, Polymerase Chain Reaction, Rodents, Sarab, Iran.

Babesia microti a tick transmitted intra erythrocytic parasite of the genus Babesia (Apicomplexa.Piroplasmida) is a common parasite of wild rodents, which are potential reservoir hosts for human babesiosis. Often lethal for immunocompromised individuals, most cases of human babesiosis caused by *B. Microti* occur in North America[1]. However, in Europe infections with *B.Microti* in humans are getting as health problem increasingly, so that the first demonstrated case report of human babesiosis in the world was from Europe in 1957. Although *B.Microti* maybe accidentally transmitted by blood transfusion[1]. However, the normal means of transmission is via infected ixodes ticks and we know that free-living small rodents play an important role as reservoir hosts[2].The sharing of reservoir and vector (tick) hosts leads to persistence and causes frequent co infection in nature and in patients. The presence of *B.microti* in various rodent species has been documented in North America[3,4], Europe[5-7] and Asia[8,9]. The specific diagnosis of babesiosis initially is by microscopic identification of the organism in giemsa stained blood smears, but it is important to note that the prevalence of *B.microti* infections in rodents assessed by PCR is much higher than that using giemsa stained blood smears[10,11]. Because of *Babesia microti* characteristic intra erythrocytic inclusions in

* To whom all correspondence should be addressed.
Tel.: +91-9143135866;
E-mail: Fallah39@gmail.com

giemsa stained thin blood smears at some stages, these may closely resemble the ring forms of *plasmodium falciparum*, which can result in diagnostic errors.

MATERIALS AND METHODS

The study was in summer and autumn of 2011-2012. We entrapped the rodents, using live traps in residential areas, food producing factories, Sarab plains surrounding villages and storages, inside rivulets and similar places. In the laboratory at the field station in Sarab, each trapped rodent was marked and identified regarding to species level, sex and morphological characters. Then weighed and examined body for feeding tick larvae and nymphs.We took the blood samples from the tip of their tails or by heart puncture for preparing thin blood smears. The rest of blood was poured in to plastic tubes containing anti coagulant (EDTA) and reserved for molecular studies at -20°c. Briefly thin blood smears were prepared, air-dried and fixed in absolute methanol and stained with giemsa (1:10) for 20 minutes. Later, we rinsed the slides in an isotonic buffer and air-dried. Then viewed them at 1000 X magnification under a light microscope, and using immersion oil.Genomic DNA of the parasite extracted from blood using by Pak gen enzymatic kit according to the manufacturers guideline. Then extracted DNA was kept at -20°c until being used (12).When performing the test, 100 ng of extracted DNA used as template for amplification of Babesia gene. We used Primer pairs; RLB-F and RLB-R, specific to the *B.microti* small subunit ribosomal RNA genome (18 ss rRNA) (13). After amplification in a 20 µl volume, containing 20 pM primers,250 mM d NTP,3 mM $Mgcl_2$ and 5U of Taq polymerase and 10 X TBE buffer, PCR conditions started with an initial denaturation step at 95°C for 5 minutes. This followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing at 60.3°C for 40 seconds, extension at 72°C for 60 seconds and a final extension at 72°C for 7 minutes, followed by a hold step at 4°C. The PCR product analyzed in 1.5% agarose gel in 10X TBE buffer and visualized using Cinnagen safe stain, 6µ/100ml and UV illumination. The procedure of using Cinnagen safe stain, in turn caused the elimination of the carcinogenic effect of ethidium bromide.

RESULTS AND DISCUSSION

In the present study, blood samples from 100 rodents assessed, that 3 of them (3%) were positive for *Babesia microti* using parasitological method (Figure 1).

All of the 100 samples assessed by PCR using specific primers RLB-F and RLB-R. During the electrophoresis, we visualized bands about 517 bp, in 3 out of 100 samples, which is compatible to *Babesia microti* parasites (Figure 2).

In spite of spread and abundance of rodents in different areas and climates of Iran and health importance of these animals, we have access to only one study regarding *Babesia microti* in rodents, which was merely by parasitological methods. Mohebali and colleagues performed the mentioned research from September of 1994 until October of 1995 ,regarding 132 rodents of four different species in Meshkin shahr city of Ardabil. In thin blood smear of one of the rodents of *Meriones persicus*, we saw binary and ring forms of *Babesia microti* inside erythrocytes (14). In our study, regarding four different species of rodents in Sarab, including *Mus musculus*, *Meriones persicus*, *Cricetalus migratorius* and *Mesocricetus auratus* , using parasitological and molecular (PCR) methods, we observed that rodents belonging to *Mus musculus* species, had babesia infection. In

Table 1. Prevalence of Babesia microti parasite in rodents of Sarab, Iran.

Species	No.of rodentstested	No.of rodents positive forB. *microti* (%)
Mus musculus	54	3(5.5%)
Meriones Persicus	38	-
Cricetulus migratorius	7	-
Mesocricetus auratus	1	-
Total	100	3(3.0%)

addition, the genus of the isolated babesia parasites from rodents, found to be *B. microti* using molecular method of PCR. In this study of blood samples of rodents of Sarab city and surrounding villages, using PCR method, we detected *Babesia microti* in blood of 3 of the rodents of *Mus musculus* in three villages called Razlig, Hoolig and Heris.

This is the second report of babesia parasites in rodents of Iran, after the first one from Meshkin shahr. There are also few studies regarding this field in other countries. There are reports, related to human babesiosis cases from Thailand and Japan, using peripheral blood smears.

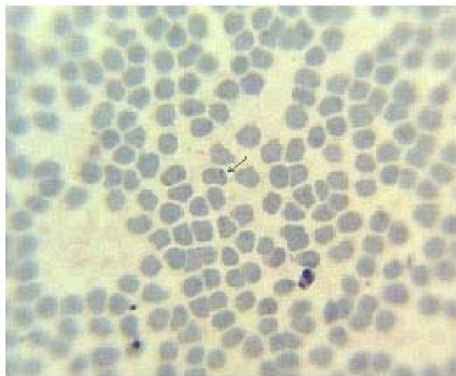

Fig. 1. Blood smears stained with giemsa from *Babesia microti* in *Mus musculus*

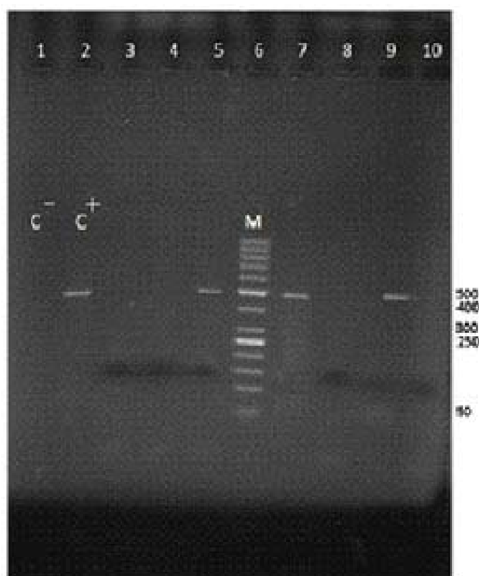

Fig. 2. DNA isolated from the blood of rodents and analysis by PCR. PCR analysis with primers Bab F2,Bab R2 specific for 18 s rRNA gene of *Babesia microti*. M-Marker

In Thailand Kuntz and Manwell found piroplasms in mice of *Bandicota inindica* and mice of *Rattus coxinga* in 1964.The first patient in Thailand had been reported in 1997[15]. In addition, Saito-ito *et al*., have reported the first human case of *Babesia microti* in 1999 in Japan, which was due to blood transfusion[15]. Karabowiac, *et al*., carried out a research on mice *Apodemus agrarius*, regarding blood parasites and their characteristics in east of Slovakia, during 1998-2005,from March to November. Using peripheral blood smears, they found Babesia and Piroplasma infections in only two regions and the intensity of infection (Pathogenesis) was not more than 0.1% (16). In a serologic and molecular study by Çiçek *et al*., during May and June of 2006-2007 in Anatolian district of Turkey, they documented presence of *Babesia microti* in tree squirrels of genus *Spermophilus xanthophrymnus*[17]. In the year 2006 in Poland, Sinski *et al*., conducted a research on ticks of *Ixodes ricinus* and wild rodents simultaneously. They used peripheral blood smear preparations and giemsa staining and applied molecular methods (Nested-PCR). They proved presence of *Babesia microti* in both ticks and wild rodents (18).In Japan a study by Okabayashi *et al*., on blood samples of 97 entrapped mice in Hokkaido of Japan from June to September,1998 using blood absorbing filter papers and molecular method of Nested-PCR, *Babesia microti* had been detected (19). In 2011, in Croatia, Beck *et al*., conducted an investigation in Croatia to assay presence of Babesia microti in 120 wild rodents. They used PCR technique and concluded that two species of *A. flavicollis* and *M. glareolus* ,were *Babesia microti* infected, whiles two other species named *Apodemus sylvaticus* and *Apodemus agrarius* were free of parasites (20).Regarding above studies in different countries of the world, presence of Babesia microti in rodents has been documented which is compatible with our study. Therefore, according to our findings, we should consider the role of rodents as reservoir hosts for babesia, in controlling programs and plans.

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