Detection of *Babesia* infection among human, goats and sheep using microscopic and molecular methods in the city of Kuhdasht in Lorestan Province, West of Iran

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Abstract *Babesiosis* is a lethal protozoan disease, responsible for the loss of livestock in Iran and in the world. The purpose of the current study was to detect and identify *Babesia* spp. infection using microscopic and molecular methods in human, sheep and goats in Kuhdasht region, in the Lorestan Province, west of Iran. During 2013, a total of 384 blood smear samples were collected from 51 goats, 306 sheep suspected of *Babesiosis* infection and 27 humans from Kuhdasht region. The blood samples were fixed, stained and under light microscopic examined. DNA samples were extracted and amplified by polymerase chain reaction of 18S-rRNA gene. PCR and the semi-nested PCR were performed to identify to *Babesi* spp. and to differentiate genus of *Theileria* and *Babesia* spp. The results of microscopic examination indicated that a total of 47 (12.2%) samples were positive for *Babesia* spp. infection: 38 (9.9%) belonging to sheep and 9 to goats (2.3%). No *Babesia* was observed in human samples. The PCR showed a band size of 389 bp, of *Babesia* spp. and the semi-nested PCR detected *B. ovis* with a band size of 186 bp. By molecular method, 16 (4.2%) sheep and 2 (0.5%) goat blood samples were infected by *Babesia*. Totally, 18 samples (4.7%) were observed to have *Babesia*, while no infection was found in human. Thus, the results of

our study have shown sheep and goats could be vulnerable to *Babesia* spp., especially *B. ovis* in Lorestan Province, Iran. Therefore, studies on the status of the animal *Piroplasmosis* especially *Theileriosis* are recommended.

Keywords *Babesia* infection · Human · Sheep and goats · Microscopic and molecular methods

Introduction

Babesia causes *Babesiosis*, a protozoan from the *piroplasm* category, a blood parasite that is transmitted by ticks. Research has identified several species of *Babesia*, in different parts of the world, that are transmitted to cattle (*B. bovis*), humans (*B. divergens*) and sheep (*B. motasi* and *B. microti*, transmitted by the Ixodidae family of ticks and widely spread among rodents) (Bashiri Bod 1993; Askarian 1996). Researchers who studied cases in Portugal, Italy and other countries also observed the *Babesiosis* in humans (Centeno-Lima et al. 2003; Gabrielli et al. 2014). Three species that are morphologically different, *B. ovis*, *B. motasi* and *B. crassa*, affect sheep and goats severely; victims are characterized by such symptoms as fever, anemia, jaundice, emaciation, hemoglobinuria and death (Alani and Herbert 1988; Ranjbar-Bahadori et al. 2012). Delpy 1936 reported *B. ovis* in sheep for the first time. Rafiei (1966) also reported the presence of *B. motasi* in the northwest of Iran. Some researchers in Iran have characterized the *B. ovis* and *B. motasi* as the pathogenic species affecting sheep and goats, and the *B. crassa* as the non-pathogenic one (Bashiri Bod 1993; Motavalli Haghi et al. 2013). Another Iranian researcher observed that the number of geographical locations affected by the disease

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increased, so did the rate of mortality resulting from Babesiosis during 1971–1977 (Askarian 1996).

For the detection and identification of the *Babesia* and *Theileria* species microscopic examination of blood smears (Bashiri Bod 1993), serological methods (Alani and Herbert 1988) and molecular techniques have frequently been used (Tavassoli 1998; Nayel et al. 2012; Motavalli Haghi et al. 2013; Gholami et al. 2016). Particularly, PCR could be used for identification of *B. ovis* with a high degree of sensitivity (Alani and Herbert 1988; Habibi et al. 2004). Since, PCR-based molecular techniques allow discrimination of the *Babesia* and *Theileria* species at low parasitemia rates and mixed infection in domestic animals in the endemic areas (Nayel et al. 2012). Recently, PCR and semi-nested PCR were used for detection of *Babesia* and *Theileria* spp. of the 18S ribosomal ribonucleic acid (18S-rRNA) gene (Heidarpour Bami et al. 2010; Noaman 2013; Motavalli Haghi et al. 2013).

Therefore, Lorestan Province in general and Kuhdasht in particular are important livestock breeding places in Iran. Due to, the country is large and a lack of comprehensive research coupled with the application of the conventional and sometimes outdated methods of parasitology were used for laboratory diagnosis of blood parasites. On the other hand, it necessitates a paradigm shift on the part of researchers and officials to minimize and ultimately control the effects of this parasite in the Lorestan Province and Iran.

Since no study has been carried out to determine the prevalence of the disease and identification of the species of parasites using molecular methods has not been done in this area, the present study was to detect *Babesia* protozoan species infection by using microscopic and molecular methods among human, goats and sheep in the city of Kuhdasht in the Lorestan Province, west of Iran.

Materials and methods

Collection and storage of blood samples

In current study, during 2013, a total of 384 blood smears samples were collected from 306 sheep, 51 goats and 27 individuals (the owners of animals) suspected to have Babesiosis infection, from five neighborhoods in the city of Kuhdasht, in Lorestan Province, west of Iran, each of which are considered a cluster, from the statistical point of view. 500 µL blood samples were collected and fixed with 1 mL 96% ethanol in 1.5 mL sterile Eppendorf tubes and kept at 4 °C until DNA extraction was performed. Blood smears were also prepared, fixed in methanol and stained with

Giemsa method. *Babesia* infections were identified under light microscope with high magnification (1000×). The morphological and biometrical parameters in any infected erythrocyte were considered for differential diagnosis (Almeria et al. 2001; Habela et al. 1990; Shayan and Rahbari 2005).

DNA extraction

In the present work, genomic DNA of 384 blood samples from suspected human, sheep and goats was extracted from whole blood samples using DNA extraction kit (DNA MBST, Iran), according to the manufacturer's instructions. The extracted DNA was stored at −20 °C.

PCR amplification

For amplification of an approximately 430-bp fragment of the 18S-rRNA genes of *Theileria* and *Babesia* species (Gubbels et al. 1999). PCR and semi-nested PCR of the 18S ribosomal ribonucleic acid (18S-rRNA) gene with specific primers were used for amplification of *Babesia* genomic (Table 1) (Shayan et al. 2008; Ranjbar-Bahadori et al. 2012). A total of 306 sheep, 51 goat and 27 human (ranchers) blood samples were used for DNA amplification. The difference in PCR products of the parasite is easily revealed in 1.5% agarose gel. The PCR product was performed in a total volume of 25 µL including 5 µL of DNA template, 1 X PCR buffer, 0.1 U Taq polymerase, 0.5 µL of each primer (P1/P2, 20 mM), 125 µM (Fermentas) and 1.5 mM MgCl₂ in an automatic DNA thermocycler (MWG Germany), with the following program: 5 min at 95 °C to denature double-strand DNA and 38 cycles of 45 s at 94 °C (denaturing step), 45 s at 56 °C (annealing step), 45 s at 72 °C (extension step) and final extension step at 72 °C for 10 min. After amplification, the PCR products were electrophoresed through 1.5% (w/v) Tris–Borate–EDTA (TBE) agarose gel and after staining with ethidium bromide visualize under UV light. In this study, for detection of *B. bovis*, the primers F5 and *B. ovis* R5 and primers F2 and F5 were used (Table 1). Positive controls of PCR were used for *Babesia* spp., and distilled water was used as negative control in each PCR.

Semi-nested PCR method

To confirm the specificity of the PCR product and the detection of *Babesia* species in sheep and goats, semi-nested PCR method was used. For any positive sample, 3 tested semi-nested PCRs with each pair of specific primers designed for species were carried out. Purified PCR

Table 1 Primers and produced band profile

Primers no.	Gene	Nucleotide sequence	Weight of DNA (bp)
F1	18S-rRNA gene sense	5'cacaggaggtagtgacaag3'	389–402 (<i>Babesia</i>)
R1	18S-rRNA gene antisense	5'ctgtcagaggtgaaattctt3'	426–430 (<i>Theileria</i>)
F2	Semi-nested primer for <i>B. ovis</i>	5'gtctgcgcgcggcctttgcg3'	186
F3	Semi-nested primer for <i>B. motasi</i>	5'cgcgattccgttattggag3'	205
F4	Semi-nested primer for <i>B. crassa</i>	5'gttatggcccgttggttat3'	191
F5	18S-rRNA gene sense for <i>B. bovis</i>	5'ctgtcgtaccgttggtgac3'	541
R5	18S-rRNA gene antisense for <i>B. bovis</i>	5'tcggctcctccgtgcg3'	541
F6	18S-rRNA gene sense for <i>B. microti</i>	5'cagggaggtagtgacaagaataaca 3'	67
R6	18S-rRNA gene antisense	5' ggttagattcccatcattcaat 3'	67

product was evaluated using specific primers of F2 (*B. ovis*), F3 (*B. motasi*) and F4 (*B. crassa*) (Fischer 2008; Almeria et al. 2001) (Table 1). The nucleotide sequence of these primers is completely proprietary, and each lead to the genomic proliferation and created the band only in the presence of its related protozoan. For each positive DNA sample of sheep and goats in the initial PCR, 3 tubes were taken and ingredients for PCR were prepared and poured into tubes. For amplification of DNA, these tubes were placed in thermocycler (MWG Germany). The technique was performed in a total volume of 25 μ L including 5 μ L of PCR product, one time PCR buffer, 0.1 U Taq polymerase dNTPs (each one, 1.5 mM MgCl₂ in an automated thermocycler with the following program: 5 min incubation at 95 °C to denature double-strand DNA, 35 cycles of 1 min at 94 °C, 1 min at 60 °C, 1 min at 72 °C and finally, PCR was completed with the additional extension step for 10 min). The PCR product was analyzed on 1% agarose gel and then visualized under UV light after staining with ethidium bromide.

Results

In the present study, according to the results of microscopic examination of the peripheral blood smears, *Babesia* infection was observed in 49 (12.2%) sheep and goats samples in Kouhdasht city, Lorestan Province, Iran (Table 2). Infected erythrocyte with *Babesia* parasites were found in 38 (9.9%) sheep and 9 (2.3%) goats blood smears samples, but all human blood samples were negative (Table 2).

Molecular analysis

PCR products using primers designed from the highly variable part of gene 18S-rRNA showed that *Babesia* genus can be simultaneously subtracted from each other. Amplification of the *Babesia* DNA, compared to the same part of *Theileria*, has fewer nucleotides (30 bp). PCR product with these

primers in all *Babesia* species creates a band size of 389 bp, while in *Theileria* species, the created band's size is 420 bp. For differential diagnosis of various forms of *Babesia*, semi-nested PCR method was used with specific primers of *B. ovis*, whose band size was 186 bp (Fig. 1).

Semi-nested PCR

In no case the PCR products were amplified with specific primers of *B. motasi* and *B. crassa* (Fig. 2). Results of molecular detection methods applied to 18S-rRNA gene of samples also revealed that from a total of 27 human samples, none of them were infected to *Babesia* parasites and of 306 sheep samples 16 (4.2%) were infected to *Babesia* (Table 3). According to the results of molecular methods, provincial distribution and frequency of *Babesia* infections in sheep and goats were statistically significant ($P < 0.01$) (Table 3).

Results of PCR product sequencing of sheep and goats samples which showed infection to *Babesia* by using F1 and R1 primes, indicate to it being absolutely homogeneous with *B. ovis*. Distance between nucleotides of 172–356 in sequencing is consistent with those of 622–807 in genome sequencing of *B. ovis*. Distribution and frequency of *Babesia* species detected by microscopic examination and PCR method are presented in Table 3. Of the 384 blood samples, microscopic examination revealed 47 (12.2%) positive samples, whereas 18 (4.6%) of DNA-amplified products were observed positive for two *Babesia* species (Table 4). As a result, molecular prevalence of *Babesia* spp. in the Kouhdasht city in Lorestan Province was 4.6%. These results have shown that there was a difference between the microscopic and molecular methods (Table 4).

Discussion

Babesia is a tick-borne blood protozoan parasitic disease that affects animals such as sheep, goats and cattle worldwide (Motavalli Haghi et al. 2013; Ranjbar-Bahadori

Table 2 Distribution of *Babesia* spp. infection in human, sheep and goats isolates detected from microscopy results from Kouhdasht city in Lorestan Province

Examined subjects	No. of animals	<i>Babesia</i> spp.
Human (ranchers)	27 (7%)	0 (0%)
Sheep	306 (79.7%)	38 (9.9%)
Goats	51 (13.3%)	9 (2.3%)
Total	384 (100%)	47 (12.2%)

Table 3 Distribution of *Babesia* infection in human, sheep and goats isolates detected using molecular method (PCR and DNA sequencing) from Kouhdasht city in Lorestan Province

Isolate	No. samples collected	<i>Babesia</i> spp.
Human (ranchers)	27 (7%)	0 (0%)
Sheep	306 (79.7%)	16 (4.2%)
Goat	51 (13.3%)	2 (0.5%)
Total	384 (100%)	18 (4.7%)

Fig. 1 Agarose gel (1 positive control of *Babesia*, 2 positive control of *Babesia ovis*, 3 *Babesia* sample, 4 *Babesia ovis* sample, 5 negative control); M 100 bp marker **Fig. 2** Amplification of extracted DNA with specific primers of *Babesia ovis* using semi-nested PCR (1 negative control sample, 2 positive control, 3, 4, 5, 6, and 7 positive blood samples of *Babesia ovis*), M 100 bp DNA marker

et al. 2012; Shayan et al. 2008). Research has confirmed the presence of *B. ovis* in different parts of Iran (Gabrielli et al. 2014; Hashemi Fesharaki and Uilenberg 1981; Razmi et al. 2002; Shayan et al. 2008; Motavalli Haghi et al. 2013) and also in the Kurdistan region of Iraq (Renneker et al. 2013). In our study, results of the molecular methods

Table 4 Distribution of *Babesia* species in sheep and goats comparing microscopy and molecular results in the Kouhdasht city in Lorestan Province

Methods	No. samples collected	<i>Babesia</i> spp.
Microscopic	384 (100%)	47 (12.2%)
Molecular	384 (100%)	18 (4.6%)

(PCR- 18s-rRNA) applied to 384 samples have shown 18 (4.7%) isolates, *B. ovis* infection from sheep and goats, in Kouhdasht region in Lorestan Province. However, 47 samples (12.2%) were distinguished as *Babesia* by microscopic examination, which is statistically significant. This difference is due to inability of the microscope to distinguish between *Babesia* and *Theileria*. Blood protozoan parasite *Babesia* is one of the most dangerous pathogenic parasites in animals such as sheep, goats and cattle especially in west, east and north of Iran and other countries in the world (Anwar 1974; Hashemi Fesharaki and Uilenberg 1981; Motavalli Haghi et al. 2013; Razmi et al. 2002; Tavassoli 1998). Babesiosis is responsible for the high economical losses in livestock and high morbidity and high mortality in ruminants. Therefore, understanding the pathogenic parasite species (*Babesia* or *Theileria*) for the healthy economy in livestock farming and early diagnosis and therapy are required (Centeno-Lima et al. 2003; Gabrielli et al. 2014; Heidarpour Bami et al. 2010; Shayan and Rahbari 2007).

Using molecular method, the results of the present study include the identification of *B. ovis* in sheep and goats samples. The presence of *B. ovis* has been reported from different parts of Iran and also the Kurdistan region of Iraq (Hashemi Fesharaki and Uilenberg 1981; Razmi et al. 2002; Shayan et al. 2008; Renneker et al. 2013). Sheep, goats and cattle are sources of infection for tick as vectors. *Babesiosis* could be considered being highly pathogenic disease in the livestock. In endemic areas, molecular methods are sensitive and specific for detection of the *Babesia* and *Theileria* species. Thus, future studies could be performed to detect the distribution of the parasite and for the identification of species in the endemic regions. Since, sheep, goats and cattle are main sources of infection

for tick vectors, latent infections of the diseases should be diagnosed on the bases of clinical findings, microscopic examination of blood smears and molecular techniques (PCR and sequencing of 18s-rRNA), especially for the low parasitaemia levels, and simultaneously *Theileria* and *Babesia* species be identified.

Detection of *Babesia ovis* using semi-nested PCR method revealed that the presence of *B. ovis* was higher than the *B. motasi* in sheep in Mazandaran and Golestan provinces. Another study (Motavalli Haghi et al. 2013) identified the *Babesia* spp. infection using microscopic and molecular methods among small ruminants in Mazandaran and Golestan provinces, northern Iran, during 2011–2012. Similar to our research, other studies conducted in various parts of Iran confirmed the presence of *B. ovis* (Ranjbar-Bahadori et al. 2012; Razmi et al. 2002, 2003). Motavalli Haghi et al. 2013 identified *Babesia* spp. infection using microscopic and molecular methods among small ruminants in Mazandaran and Golestan provinces, northern Iran, during 2011–2012. This reported that from a total of 220 blood samples (160 sheep and 60 goats), 34 cases (15.4%) showed *Babesia* infection using microscopic examination, whereas 11 cases (5%) were found positive for *Babesia* spp. using PCR. Also, two positive cases showed mixed infection with *Theileria* species. Our results confirm the findings of a study concerning distribution of *B. ovis* in Khorasan Province in northeastern Iran (Razmi et al. 2002). Molecular studies in different parts of Iran considered *B. ovis* as a main causative agent of sheep (Ranjbar-Bahadori et al. 2012; Razmi et al. 2003). Compared to the results of research studies which were done in the Kurdistan Region of Iraq (Renneker et al. 2013) (and Khorasan Province of Iran (Razmi et al. 2003), the current study shows a smaller percentage of infection due to *Babesia*. In conclusion, the results of present study showed that *Babesia ovis* infection is common in sheep and goats in Kouhdasht area.

In the current work, from 27 human blood samples (ranchers) were not observed *Babesia* infection by morphological and molecular methods. But, the human cases of *Babesiosis* were previously reported in Yugoslavia (Skrahalo and Deanovi 1957), *B. divergens* in Europe and *B. microti* in the USA (Kjemtrup and Conrad 2000). Also, human *Babesia* infection has been described in China, Egypt, Mexico, South Africa and Taiwan (Michael et al. 2001; Gorenflot et al. 1998). Also, using molecular method, the 18S-rRNA gene of piroplasms from wildlife and human cases of *Babesiosis* were found by PCR and sequenced in the western USA (Kjemtrup et al. 2000). So, there is the possibility of parasite transmission to humans in endemic areas of Iran and other countries. For this reason, the future study of *Piroplasmosis* in humans in different areas could be considered by researchers.

In conclusions, Lorestan Province in the west of Iran is an important area of animal husbandry, and detecting the animal diseases is of great urgency, particularly blood parasitic infection. However, the present study for the first time was to detected *Babesia protozoan* species infection among sheep and goats in this area of Iran. In our study, it is also demonstrated that the sheep and the goats, in the city of Kouhdasht in the Province of Lorestan, are vulnerable in terms of *Babesia* spp. infection. In this study, the prominence of the *B. ovis* species was confirmed in Lorestan Province. Thus, conducting more research with regard to the status of the animal *Babesiosis* and *Theileriosis* using parasitological and molecular methods is recommended.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

- Alani AJ, Herbert IV (1988) The pathogenesis of *Babesia motasi* (Wales) infection in sheep. *Vet Parasitol* 27:209–220
- Almeria S, Castella J, Ferrer D (2001) Bovine piroplasma in Minorca (Balearic Island, Spain): a comparison of PCR based and light microscopy detection. *Vet Parasitol* 99(3):249–259
- Anwar M (1974) Geographical distribution of blood protozoan parasites of ruminant in Iran. *Bull Off Int Epiz* 81(9–10):793–798
- Askarian M (1996) *Babesia* bodies overview of the human and animal. Dissertation, Veterinary Faculty of Tehran University [Full text in Persian]
- Bashiri Bod H (1993) Human pathogenic parasites, chapter IV: the blood, 2nd edn. Tehran University Press, Tehran, pp 145–147
- Centeno-Lima S, Do Rosario V, Parreira R, Maia AJ, Freudenthal AM, Nijhof AM, Jongejan F (2003) A fatal case of human babesiosis in Portugal: molecular and phylogenetic analysis. *Trop Med Int Health* 8:760–764
- Delpy RLP (1936) Agents en Iran dans le sang des animaux domestiques. *Bull Path Exot* 29:157–161
- Fischer M (2008) Manual of tropical veterinary parasitology. CAB Int Lond 2:16–20
- Gabrielli S, Calderini P, Cassini R, Galuppi R, Tampieri MP, Pietrobelli M, Cancrini G (2014) Human exposure to piroplasms in Central and Northern Italy. *Vet Ital* 50(1):41–47
- Gholami S, Laktarashi B, Shiadeh MM, Spotin A (2016) Genetic variability, phylogenetic evaluation and first global report of *Theileria luwenshuni*, *T. buffeli*, and *T. ovis* in sheepdogs in Iran. *Parasitol Res* 115(5):2125–2130
- Gorenflot A, Moubri K, Precigout E, Carcy B, Schetters TP (1998) Human babesiosis. *Ann Trop Med Parasitol* 92:489–501
- Gubbels JM, de Vos AP, Van der Weide M, Viseras J, Schouls LM, deVries E, Jongejan F (1999) Simultaneous detection of bovine

- Theileria* and *Babesia* species by reverse line blot hybridization. J Clin Microbiol 37:1782–1789
- Habela M, Reina D, Nieto C, Navarrete I (1990) Isolation and identification of *Babesia ovis* in Extremadura (Spain). Vet Parasitol 35:233–238
- Habibi GR, Hashemi-Fesharki R, Bordbar N (2004) Detection of *Babesia ovis* using polymerase chain reaction. Arch Razi Inst 57:1–10
- Hashemi Fesharaki R, Uilenberg G (1981) *Babesia crassan.* sp. (Sporozoa, Babesiidae) of domestic sheep in Iran. Vet Q 3(1):1–8
- Heidarpour Bami M, Khazraiiinia P, Haddadzadeh HR, Kazemi B (2010) Identification of *Theileria* species in sheep in the eastern half of Iran using nested PCR-RFLP and microscopic techniques. Iran J Vet Res 11(3):262–266
- Kjemtrup AM, Conrad PA (2000) Human babesiosis: an emerging tick-borne disease. Int J Parasitol 30(12–13):1323–1337
- Kjemtrup AM, Thomford J, Robinson T, Conrad PA (2000) Phylogenetic relationships of human and wildlife piroplasm isolates in the western United States inferred from the 18S nuclear small subunit RNA gene. Parasitology 120(5):487–493
- Michael R, Fibin MD, Eleftherios E, Mylonakis MD, Callegari L, Eric Legome M (2001) Babesiosis. J Emerg Med 20:21–24
- Motavalli Haghi SM, Fakhari M, Sharif M, Paghe A, Sharbatkhori M, Tavakoli R, Gholami SH (2013) Molecular identification of ovine *Babesia* spp. in north of Iran. Res Mol Med 1(1):35–39
- Nayel M, El-Dakhly KM, Aboulaila M, Elsify A, Hassan H, Ibrahim E, Salama A, Yanai T (2012) The use of different diagnostic tools for *Babesia* and *Theileria* parasites in cattle in Menofia, Egypt. Parasitol Res 111(3):1019–1024
- Noaman V (2013) A molecular study on *Theileria* and *Babesia* in cattle from Isfahan province, Central Iran. J Parasit Dis 37(2):208–210
- Rafiei H (1966) Veterinary and comparative protozoology. Publications of the Ministry of Science and Higher Education, pp 144–155, 649–709
- Ranjbar-Bahadori S, Eckert B, Omidian Z, Sadr Shirazi N, Shayan P (2012) *Babesia ovis* as the main causative agent of sheep Babesiosis in Iran. Parasitol Res 110:1531–1536
- Razmi GR, Naghibi A, Aslani MR, Fathirand M, Dastjerdi K (2002) An epidemiological study on ovine babesiosis in the Mashhad suburb area province of Khorasan. Vet Parasitol 18(2):109–115
- Razmi GR, Naghibim A, Aslani MR, Dastjerdi K, Hossieni H (2003) An epidemiological study on *Babesia* infection in small ruminants in Mashhad suburb, Khorasan Province, Iran. Small Rumin Res 50:39–44
- Renneker S, Abdo J, Bakheit MA, Kullmann B, Beyer D, Ahmed J, Seitzer U (2013) Coinfection of sheep with *Anaplasma*, *Theileria* and *Babesia* species in the Kurdistan Region, Iraq. Transbound Emerg Dis 60(1 2):113–118
- Shayan P, Rahbari S (2005) Simultaneous differentiation between *Theileria* spp. and *Babesia* spp. on stained blood smear using PCR. Parasitol Res 97:281–286
- Shayan P, Rahbari S (2007) Differentiation of sheep *Theileria* spp. and *Babesia* spp. by polymerase chain reaction. J Vet Res 62(2):15–20
- Shayan P, Hooshmand E, Nabian S, Rahbari S (2008) Biometrical and genetical characterization of large *Babesia ovis*. Parasitol Res 103(5):217–221
- Skrabalo Z, Deanovi Z (1957) Piroplasmosis in man: report on a case. Doc Med Geogr Trop 9(1):11–16
- Tavassoli M (1998) Seroepidemiological study of *Babesia ovis* in sheep in different climatic regions of Iran, using indirect immunofluorescent test (IEAT). Ph.D. thesis on parasitology, Faculty of Veterinary Medicine, Tehran University, Iran