



RESEARCH PAPER

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Detection of *Babesia canis* in the blood samples of dogs in Iran by PCR method

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Abstract

Babesiosis is a tick-transmitted disease that made happen by haemoprotozoan parasites of the genus *Babesia* and an important tick-disease of dogs and in many species of mammals, which reason light fever, progressive anemia, hemoglobinuria and noticeable splenomegaly and hepatomegaly in dogs and maybe happen death in this cases around the world. The aimed of the current study was to find the presence of the dogs *Babesia canis* in blood samples species in Iran by the polymerase chain reaction (PCR). In this study, a total of 120 blood samples were collected from the saphenous vein into tubes that contained EDTA of Chaharmahal Va Bakhtiari provinces (west of Iran) between January and June 2012. DNA was extracted and PCR were applied for *Babesia canis* then PCR products were screened. The presence of *Babesia canis* DNA were detected by PCR from 9 (7.5%) out of 120 dogs. In this comparative study found that the highest infection rate in young dogs less than two years. The results showed a low-frequency of *Babesia canis* infection in dogs in the Chaharmahal Va Bakhtiari provinces (west of Iran), Iran and shows the first molecular detection of *B. canis* in dogs from Iran and the diagnostic usefulness of PCR method.

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Introduction

Babesiosis, is a tick-transmitted disease that made happen by haemoprotozoan parasites of the genus *Babesia* and an important tick-disease of dogs (Habibi *et al.*, 2004). It has been arranged as canine *Babesia* and based on the size of its intraerythrocytic shape (Camacho *et al.*, 2005). This parasite found in a variety of mammals and in mans (Baneth *et al.*, 2004). Infections occur in domestic animals, including cattle, horse, sheep, goats, pigs and dogs (Fahrimal *et al.*, 1992; Bock *et al.*, 2004).

In dogs, was at first observed as a tropical and subtropical disease. Its vector ticks are *Rhipicephalus sanguineus* and *Dermacentor reticulatus* (Caeiro, 1999). *Babesia canis* (*B. canis*) caused to pass by the tick, *Dermacentor reticulatus* (Foldvari *et al.*, 2005). Canine babesiosis increases the veterinary matters in European countries and caused by *Babesia gibsoni* and the subspecies of *Babesia canis*. In some dogs that have an illness babesiosis did not leave the places that occur this disease but it might acquire infection just by *Ixodes ricinus* bite (Cieniuch *et al.*, 2009).

Babesia can causes light fever, progressive anemia, hemoglobinuria and noticeable splenomegaly and hepatomegaly in dogs and maybe happen death in this cases (Ikadai *et al.*, 2004). Serious anemia, thrombocytopenia azotemia are usually finding in infected dogs during the disease. There are some changes in serum protein levels like albumin, in occurring *B. canis* infections and another study showed an important decrease in alpha-2 globulin and gamma globulin (Camacho *et al.*, 2005).

This is diagnostically important to control canine babesiosis from its prognosis, virulence and the effect of the antibabesial drugs may be different in organisms (Birkenheuer *et al.*, 2004). Diagnostic the signs of Canine vector borne diseases make a challenge for veterinarian because the clinical signs of many of them may be morphologically similar and it is difficult to distinguish between *Babesia* species and may be overlap typical clinical signs (Otranto *et al.*,

2010). though many workers have noticed that canine *Babesia* diagnosed by capillary smears (Bohm *et al.*, 2006).

In acute canine babesiosis, the parasite are found on the blood smear by using light microscope but in chronic form or in cases that infected with a low-level of parasite or the problems that happened for staining the blood smear such as thin or thick blood film staining according to the Giemsa (Thammasirirak *et al.*, 2003). Same protozoans may have different microscopic shape in different hosts (Habibi *et al.*, 2004). The antierythrocyte antibodies that increase in circulatory blood and that lead to immune-mediated hemolysis makes Coom's test positive in blood smears but it can influence by stress (Ikadai *et al.*, 2004).

The most widely used diagnostic test for babesiosis, is the direct smear and serologic test in all world. PCR technique offers the high sensitivity, specificity and suitable way for detection of low infection in carriers and it is more sensitive and quick than the other diagnostic method (Thammasirirak *et al.*, 2003; Habibi *et al.*, 2004).

Although PCR is a necessary method for detection and differentiate infections, it also has provided information about treatment (O'Dwyer *et al.*, 2009). 18S rDNA gene coding rRNA of the small subunit ribosomal was amplified this species have been named *Babesia canis* but the size of this gene is different in varies spp. and controlled by 1720-1770 bp. Sequence of nucleotides same in all *Babesia* spp. (Oyamada *et al.*, 2005).

The aimed of the current study was to find the presence of the dogs *Babesia canis* in blood samples species in Iran and was to show a reliable, specific and sensitive molecular tool, the polymerase chain reaction (PCR), for the detection of *Babesia canis* in dogs, so it can diagnose very fast correct and reliable technique.

Materials and methods

Sampling and DNA extraction

In study, a total of 120 blood samples were collected from the saphenous vein into tubes that contained EDTA of Chaharmahal Va Bakhtiari provinces (west of Iran) between January and June 2012. Samples were sent to the Biotechnology Research Center of Islamic Azad University of Shahrekord Branch in a cooler with ice packs and were stored -20°C for further use.

Genomic DNA was extracted from dog's blood using DNA extraction kit (CinnaGen, Tehran, Iran) according to the protocol. The total DNA was measured at 260 nm optical density according to the method described by Sambrook and Russell (2001) (Sambrook and Russell, 2001). The extracted DNA of blood sample was kept frozen at -20°C until used.

PCR amplification

PCR was performed using oligonucleotide primers BabsiC-F: 5'-CTACCACATCTAAGGAAGGCAGC-3' complementary to the region of 18S ribosomal subunit DNA of *Babesia canis* submitted to the GenBank (accession number JX304677) and BabsiC-R: 5'-TTAAATACGAATGCCCAAC-3' (Foldvari *et al.*, 2005).

PCR was performed in a 50 µL total volume including 1 µg of template DNA, 1 µM of each primer, 2 mM

MgCl₂, 200 µM dNTP, 5 µL of 10× PCR buffer and 1 unit of *Taq* DNA polymerase (Roche Applied Science).

Amplification of PCR was performed using thermal cycle as follows: first denaturation step at 95°C for 5 min, then amplified for 30 cycles of denaturation at 94°C for 1 min, alignment at 62°C for 1 min, elongation at 72°C for 1 min and, final elongation step at 72°C for 5 min.

Analysis of PCR Products

The amplification products (10 µL) were subordinate to electrophoresis in a 1% agarose gel in 1×TBE buffer at 80 V for 30 min, stained with ethidium bromide solution, and then images were obtained under UV transillumination.

Results

One hundred-twenty dog blood specimens were checked out for attendance of *Babesia canis* DNA. The results of the prevalence of *Babesia canis* in blood samples presented in Table 1 and Table 2. Agarose gel electrophoresis of positive samples revealed a 439 bp fragment. The results of electrophoresis for blood specimen s amplification were shown in Figure 1. DNA of *Babesia canis* was found in 9 of 120 (7.5%) dog blood specimen. The results showed a low-frequency of *Babesia canis* infection in dogs in the Chaharmahal Va Bakhtiari provinces (west of Iran), Iran.

Table 1. *Babesia canis* in difference sex and age group of dogs, in Chaharmahal Va bakhtiari (west of Iran).

Age	<2years		1-5years		>5years	
	-	+	-	+	-	+
Sex						
Male	24	1	19	1	22	2
Female	16	3	17	2	13	0
Total	40	4	36	3	35	2

Babesia canis in difference age group of dogs

In this comparative study found that the highest infection rate in young dogs less than two years (4

samples), compared with dogs 1-5 years (3 samples) and above 5 years (2 samples).

Babesia canis in difference sex group of dogs

Dogs also have a greater proportion of females (9 samples) than males (4 samples) were infected.

Babesia canis in difference seasons group of dogs comparison was done on cold (2 samples) and warm seasons (7 samples). This comparison made clear the difference between season and infection rate with *Babesia canis* thus, the frequency of infection is greater in the warm seasons.

Discussion

In general, *Babesia* sp. is identified via demonstrating the organisms in blood smears under light microscope, inoculation to splenectomy or

laboratory animals and serological examination (Conrad 1991).

In recent times, molecular methods have been used for detection and identification of protozoa in different parts of the world. The genomic and extra chromosomal DNA analysis by PCR, RFLP-PCR and reverse line blot hybridization have been carried out effectively on the species (Vargas-Hernández *et al.*, 2012).

The standard technique for quantification of parasites is the microscopic examination of blood smears. Even though this is a low-cost diagnostic test, it has a low sensitivity in detecting the parasites when an animal has low parasitemia (Costa-Júnior *et al.*, 2012).

Table 2. *Babesia canis* in difference seasons and age group of dogs, in Chaharmahal Va bakhtiari (west of Iran).

Age Season	<2years		1-5years		>5years	
	-	+	-	+	-	+
Cold	18	0	15	1	18	1
Warm	22	4	21	2	17	1
Total	40	4	36	3	35	2

While the PCR is considered a highly sensitive and specific technique, its sensitivity is low when blood samples are collected from naturally asymptomatic *Babesia*-infected dogs in the chronic phase of the disease (Vargas-Hernández *et al.*, 2012). It can be explained via the fluctuation of the low parasitemia (Vargas-Hernández *et al.*, 2012).

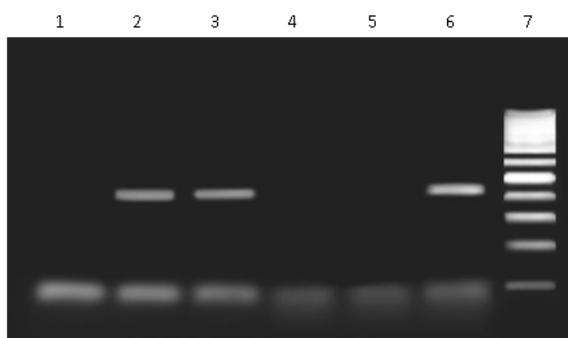


Fig. 1. Ethidium bromide-stained agarose gel electrophoresis of PCR products (439 bp) for detection of *Babesia canis* DNA in samples after PCR amplification. Agarose gel electrophoresis for identification of *Babesia canis* DNA in dogs blood

samples. Lane 1: Negative control; lanes 2, 3 and 6: Positive samples (439 bp); lanes 4 and 5: Negative samples; and 7: 100 bp DNA ladder (Fermentas, Germany).

In the present study shows, for the first time, the occurrence of and *B. canis* infections in Iranian dogs (Chaharmahal Va Bakhtiari provinces, west of Iran), confirmed by molecular methods. The molecular detection of *B. canis* in the present study was low (7.5%). The results are in agreement with earlier reports in which that *B. canis* infection has been described in other areas of Iran (Ashrafi *et al.*, 2001; Razi jalali *et al.*, 2013).

Razi jalali *et al.*, (2013), observed the stained thin blood smear that ready, displayed that 15 samples (3.7%) of 400 dogs were infected with *B.canis* and also 4 samples (2%) of 200 urban dogs appeared *B.canis* (Razi jalali *et al.*, 2013).

International, cases of canine babesiosis are on the increase with recent investigations reporting the detection of a novel large *B.canis*-like species in North America (Birkenheuer *et al.*, 2004), *B. c. vogeli* in South Africa (Matjila *et al.*, 2004), *B. c. canis* in Hungary (Foldvari *et al.*, 2005), and *B. c. vogeli* in Brazil (Passos *et al.*, 2005), *B. c. vogeli* in Australia (Martin *et al.*, 2006), *B. vogeli* in Colombia (Vargas-Hernández *et al.*, 2012), *B. c. canis* and *B. c. vogeli* in Italy (Solano-Gallego *et al.*, 2008), *B. c. canis* in Poland (Adaszek and Winiarczyk, 2008), *B. c. vogeli* in Portugal (Cardoso *et al.*, 2008) and the detection of *Babesia* and Hepatozoon infection of dogs in eastern Sudan (Oyamada *et al.*, 2005).

The percent of the identification of *Babesia canis* is different between other studies. In Iran, out of a total of 400 dogs, 3.75% (15 sample) were infected with *B.canis* in using Giemsa-stained thin blood smear for identification the parasite of the cephalic vein of dogs (Razi jalali *et al.*, 2013).

Reported of Matjila *et al* (2004), Among 297 blood samples collected from south Africa, 31 were positive for *B.canis rossi*, 13 were positive for *B.canis vogli* in using reverse line blot hybridization and 18s sequence analysis (Matjila *et al.*, 2004).

In another study, A total of 44 blood samples from dogs that showed the clinical signs of the disease 39 samples were positive (88.6%) by PCR and sequencing method (Foldvari *et al.*, 2005).

In Sudan, Among 78 dogs, 5 were infected with *B.canis rossi* and 2 others were infected with *B.c.vogeli* by single PCR and sequencing (Oyamada *et al.*, 2005). The results of Michael *et al.*, (2008) 5 (7%) blood samples from 104 dogs were infected with *B.canis vogeli* by using serology and PCR (Michael *et al.*, 2008). he study of Solano-Gallego *et al.*, (2008), using the PCR-restriction fragment length polymorphism (RFLP) in blood samples of Italian dogs showed in 103 samples of Northern Italy, 30 (29.1%) were infected with *B. canis canis* and 1 (0.9%) was infected with *B. c. vogeli* , also (16.3%) 10

from 61 samples were infected with *B. c. vogeli* in Southern Italy (Solano-Gallego *et al.*, 2008). In Poland, 20 (25.3%) from 79 blood samples were infected with *B.canis* by using PCR and nested PCR (Wele-Falęciaka *et al.*, 2009).

Conclusion

In conclusion, the present work shows the first molecular detection of *B. canis* in dogs from Iran and the diagnostic usefulness of PCR method. Future studies involving the molecular characterization of other agents in dogs and the tick species acting as vector of these agents in Iran are much-needed.

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