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BRIEF COMMUNICATION

Report of *Theileria annulata* and *Babesia canis* infections in dogs

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Abstract Piroplasmosis is a zoonotic protozoan disease transmitted by ticks. The full geographical range of canine piroplasms has been found in dogs in the Middle East, parts of Africa, North America, and Europe. Following our studies on molecular detection of piroplasmosis in the south of Iran, we found *Theileria annulata* in two herd dogs, as well as information on their 18S rRNA gene sequences. Piroplasmosis agents were detected by PCR of 280 blood samples collected from dogs in seven regions of the Shiraz suburbia in southern Iran, between November 2009 and June 2011. Two positive samples from Shiraz were infected with *T. annulata*, and one sample was infected with *Babesia canis*. PCR positive samples were further analyzed by sequence analysis. The results of this study reconfirmed that *T. annulata* are not always as host specific as accepted. This is the first report of *T. annulata* in herd dogs in southern Iran and the second report of *T. annulata* in dogs worldwide.

Keywords *Theileria annulata* · *Babesia canis* · Dogs · Iran

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Introduction

Piroplasmosis is caused by tick-borne intraerythrocytic protozoan parasites of the genus *Babesia* and is one of the most common infections of animals worldwide. In dogs, piroplasmosis was originally viewed as a tropical and subtropical disease. Its vector ticks are *Rhipicephalus sanguineus* and *Dermacentor reticulatus* (Caeiro 1992). Piroplasmosis may have a hyperacute, an acute, a chronic, or a subclinical course in dogs. The disease is characterized by fever and lethargy, and anemia is the most common clinical syndrome. The previous study described the disease in South Africa as highly virulent and causing hemolytic anemia or an acute overwhelming inflammatory response (Neer and Harrus 2006). Recently, it has gained increasing attention as an emerging zoonosis in humans. People who have had a splenectomy or who are older (more than 55 years) are especially at risk. No *Babesia* organism has, as yet, been identified that is host specific for people. As for other tick-borne zoonoses, people serve as accidental hosts for *Babesia* of animals when they are bitten by infected ticks (Neer and Harrus 2006). Traditional methods are gradually being replaced by molecular biological techniques, as morphological features by themselves are not sufficient for species differentiation. Currently, the diagnostics of piroplasmosis is based mainly on serological methods, and the immunofluorescent antibody test is most commonly used. However, even in the acute phase of the disease, seroconversion does not always occur. Clinical symptoms, because of their nonspecific feature, cannot be used to make a correct diagnosis. In this situation, other diagnostic methods are needed. The use of PCR is the most promising of these. One advantage of this method is that it allows identification of the parasite in the early stage of disease; another is the high sensitivity of this technique. PCR assays have been validated as alternative methods to achieve reliable

species-specific DNA detection of *Babesia canis* (Martin et al. 2006) with a high level of sensitivity, and they are relatively less time consuming. The most widely used diagnostic test for *Piroplasma* species in dog is the direct smear and serologic test in Iran. In the present study, molecular method has been used as a complement in the diagnosis of *Piroplasma* species in dogs in Iran.

Materials and methods

Biological samples

A total of 280 blood samples were monitored. Samples were collected in seven regions (Bidzard, Darian, Derak, Siyakhdarengun, Gharehbagh, Kaftarak, and Dinakan) of the Shiraz suburbia, between November 2009 and June 2011. Shiraz is the capital of Fars Province, which is located in the south of Iran.

DNA isolation, amplification, and sequencing

For PCR amplification of the 18S rRNA gene for *Theileria* and *Babesia*, genus-specific primers RLBF2 and RLBR2, covering the hypervariable region 4, were used as described by Gubbels et al. (1999). DNA was extracted using the REDExtract-N-Amp blood PCR kit (Sigma) following the protocol of the manufacturer. PCR was performed, amplifying a fragment on the 18S rRNA-targeted Piroplasmida (Gubbels et al. 1999). For each PCR, the reactions used positive and negative controls. Amplified PCR fragments were sequenced by automated machine (CinnaGen Co., Tehran, Iran), and sequences were confirmed with GenBank database.

Results

PCR assays to amplify *Theileria annulata* and *B. canis*

Amplification of the 18S rRNA gene of *Theileria* and *Babesia* with genus-specific primers was demonstrated by a 460–520-bp fragment (Fig. 1) and visualized in three of the 280 samples (1 %). Three positive samples were sent for sequencing. Sequencing results from the three positive samples of blood showed that two samples were infected with *T. annulata* (100 %, identities with *T. annulata*, GenBank accession numbers DQ287944.1 and GU224091.1), and one sample was infected with *B. canis* (98–99 %, identities with *B. canis*, GenBank accession numbers HQ148664.1 and AY371197.1). *T. annulata* was identified in Siakh darengoon and Kaftarak, while *B. canis* was identified in Bidzard. *T. annulata* was found in two of the 280 samples (0.7 %), in one adult male, and one adult female dog.

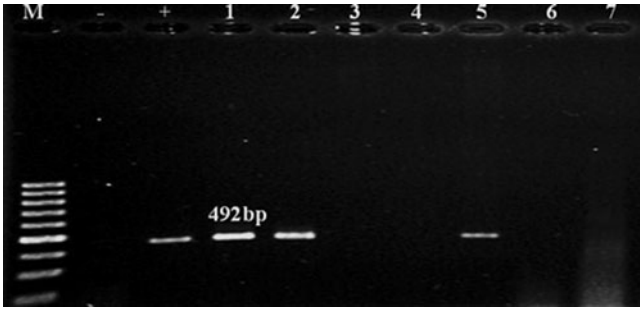

Fig. 1 *Theileria* and *Babesia* PCR with genus-specific primers RLB-F2/RLB-R2 by 1.2 % agarose gel electrophoresis. DNA ladder (100 bp) (*M*); lanes 1, 2, and 5 are positive; positive control (*plus sign*); negative control (*minus sign*)

B. canis was found in one adult male dog of ≥3 years old ($P>0.05$). *T. annulata* and *B. canis* were detected in asymptomatic dogs.

Discussion

In this study, three out of the 280 dogs were infected with piroplasmids (two *T. annulata* and one *B. canis*). The low prevalence of *B. canis* is in agreement with the scarcity of reports of these parasites in Iran based on morphological observations (Ashrafi et al. 2001). An interesting finding in our dog survey was that two asymptomatic animals were infected with *T. annulata*. This piroplasmid has been reported in symptomatic dogs for the first time in Spain (Criado et al. 2006). To our knowledge, this study presents the second report of *T. annulata* infection in dogs. Fars province, which is the locality of the collection of blood samples, has the highest level of theileriosis cases in Iran (Haddadzadeh et al. 2004). On the other hand, natural infection of sheep with *T. annulata* has been reported from Iran (Zaeemi et al. 2011). Also, infections with *Ehrlichia canis* have previously been reported in sheep in the rural suburbia surrounding Shiraz (Spitalska et al. 2005). Therefore, the fact that "exchange" of "specific" parasites was detected between sheep and dog seems to be yet another reason to believe that the infection of sheep with *E. canis* and the infection of dogs with *T. annulata* are unlikely to happen. Also, this event, in which both of the *Theileria*-infected dogs in this study were sheepdogs, strengthened the hypothesis that the origin of dog infection is sheep. A BLAST search against the GenBank database revealed 100 % identity between Spanish dog isolates sequence (accession number DQ287944.1) and *T. annulata* found in dog in Iran.

It is our belief that only the use of molecular methods with universal primers can lead to such findings. In the last few years, it has become evident that some piroplasms

parasitize accidental hosts, as shown by other authors (Criado-Fornelio et al. 2003; Criado-Fornelio et al. 2004; Nagore et al. 2004a, b). In our study, the report of *T. annulata* in dog reconfirmed that *T. annulata* is capable of infecting unspecific hosts such as dog. This study presents the second report of *T. annulata* infection in dogs worldwide and is the first report of *T. annulata* infection in asymptomatic herd dogs worldwide. Therefore, more clinical samples and data will need to be collected and analyzed to understand the importance of *T. annulata*. However, more and better designed studies into the transfer of this disease in dog are needed to reconfirm the *T. annulata* as a causative agent for dog piroplasmosis.

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