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A molecular study on *Theileria* and *Babesia* in cattle from Isfahan province, Central Iran

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Abstract Bovine theileriosis and babesiosis are important hemoprotozoal diseases of cattle in tropical and subtropical regions that lead to economic losses in these animals. From March 2009 to July 2009, 176 blood samples of Holstein and crossbred cattle without any signs of disease were prepared from Isfahan province, Central Iran. The extracted DNA from blood cells were analyzed for members of the genera *Theileria* and *Babesia* by polymerase chain reaction (PCR) using a set of primers derived from the 18s rRNA gene. 42 out of 176 blood samples (23.9 %) were positive for *Theileria* spp. and none of them was positive for *Babesia* spp.. The present study showed that *Theileria* is detectable in cattle without any sign of infection but maintained a persistant sub-clinical state in the carrier cattle, which can serve as reservoirs of infection for ticks and cause natural transmission of the disease.

Keywords *Theileria* · *Babesia* · PCR · Cattle · Central Iran

Introduction

Theileria and *Babesia* species are tick-borne haemoprotozoan parasites in animals mainly cattle and sheep in tropical and sub tropical regions (Uilenberg 1995). In Iran, diseases caused by these organisms have been recognized for more than 70 years (Rafeei 1978; Hashemi-Fesharki 1988). They cause economical loss of domestic ruminants by decreasing milk production, loosing weight and death in Iran (Anwar 1974). Ixodid ticks are vectors in the transmission of these diseases and in Iran the distribution of the haemoprotozoan parasites broadly coincided with the ixodid tick vectors distribution (Hashemi-Fesharki 1997, 1998).

There are approximately 500,000 Holstein, crossbred (Holstein with local breeds) and native cattle under industrial, semi-industrial and traditional dairy farming in Isfahan province, Central Iran. In traditional and semi-industrial farming systems cattle often suffer from abundant tick infestations and exposure to tick-borne disease. The recovered cows from acute or primary theileriosis and babesiosis, remain infected for a long period and even for the rest of their lives, so acting as reservoirs of infection for ticks and cause natural transmission of the disease (Cacci et al. 2000; Kirvar et al. 2000). Recovered cattle are the major agents of spreading the infection and due to the proximity of these cattle to the industrial dairy cattle farms; there is the possibility of disease transmission to other farms by vectors.

Molecular technique such as polymerase chain reaction (PCR) has been widely used in veterinary parasitology in recent years to identify blood protozoa (Altay et al. 2008). Bovine *Theileria* and *Babesia* have been investigated using molecular techniques and microscopy in the some part of the Iran (Fakhar et al. 2012; Ziapour et al. 2011; Hoghooghi-Rad et al. 2011; Tavassoli et al. 2011; Azizi et al. 2008).

In Isfahan province there is no available information about the carrier animals; the reasons probably being due to the traditional methods are less sensitive and specific in the detection of carrier animals. Since, identification of carrier animals is important for the assessment of infection risk, the aim of the present study was the detection of *Theileria* and *Babesia* based on PCR among apparently healthy cattle from Isfahan, Central Iran.

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Materials and methods

Collection of blood samples

From March 2009 to July 2009, 32 semi-industrial farms in Isfahan province were selected for the study based on their history of outbreak of bovine haemoprotozoan parasites. Blood samples were collected from jugular vein of 176 Holstein and crossbred cattle ranging between 1 and 9 years, randomly. 500 µl of each collected blood samples was fixed with 1 ml 96 % ethanol in 1.5 ml sterile eppendorf tubes and kept at 4 °C until DNA extraction was performed.

DNA extraction and PCR

DNA was extracted using a DNA isolation kit (Molecular Biology System Transfer, MBST, Iran) according to the manufacturer's instructions. For assessment of the *Theileria* and *Babesia* infection in samples, primers Tbs-S (5′-CACAGGGAGGTAGTGACAAG-3′) and Tbs-A (5′-AAGAATTTCACCTCTGACAG-3′) were used, which amplify simultaneously an approximately 426–430 bp and 389–402 bp fragment of the 18s rRNA gene for members of genera *Theileria* and *Babesia*, respectively (Shayan and Rahbari 2005).

Positive controls of PCR were used for *Theileria* spp. and *Babesia* spp. Distilled water was used as negative control in each PCR reaction. For analysis of the PCR product, 10 µl of each of them was subjected to electrophoresis on a 2 % agarose gel in a TBE buffer at 100 V for 45 min and then visualized under UV light after staining with ethidium bromide.

Results

After amplification of DNA samples using the Tbs-S/Tbs-A primer set for PCR and analysis these PCR products on the 2 % agarose gel electrophoresis, 42 out of 176 blood samples (23.9 %) showed bands of 426–430 bp corresponding to the hypervariable V4 region of the 18s rRNA gene of *Theileria* species and none of them was positive for *Babesia* spp. (Fig. 1).

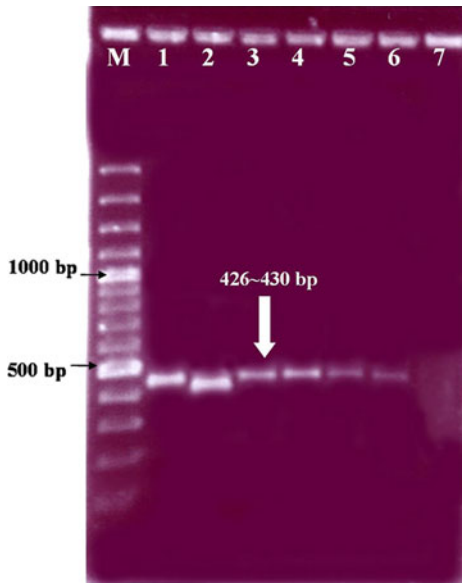

Fig. 1 DNA isolated from blood was analysed by PCR. *Theileria* spp. positive control (*lane 1*), *Babesia* spp. positive control (*lane 2*), DNA was amplified with Tbs-S/Tbs-A primer set resulting in PCR product of 426 ~ 430 bp in length (*lanes 3–6*), negative control (*lane 7*) and M (Marker 100 bp)

Discussion

The province of Isfahan covers an area of approximately 107,027 km2 and is situated in the center of Iran. The province experiences a moderate and dry climate on the whole, ranging between 40.6 and 10.6 °C on a cold day in the winter season. The average annual temperature has been recorded as 16.7 °C, and the annual rainfall on average has been reported as 116.9 mm. This type of climate with seasonal fluctuation provides a very suitable environment for development and spread of Ixodidae ticks which have the capacity to transmit haemoprotozoan parasites. *Hyalomma* is the most dominant tick genus which infests cattle in Isfahan province, Central Iran (Noaman et al. 2008).

There are approximately 300,000 Holstein and crossbred (Holstein with local breeds) cattle under semi-industrial dairy farming in Isfahan province. In semi-industrial dairy farms, the number of cattle per farm is less than 20 heads; the farm is thoroughly run by private holders whose knowledge and equipments are less advanced than industrial holders. Breeders are considerably dependent on agricultural crop residues, external feed, drugs, genetic resources, the amount of milk production, hygiene, veterinary services, tick control and nutrition program is in the lower level than industrial farms (Kamalzadeh et al. 2008). Such conditions predispose semi-industrial dairy cattle to tick infestation and transmission of haemoparasites.

The results showed that 42 of total 176 blood samples were *Theileria* positive by specific primers based on 18s rRNA gene. This report is the first detection of this agent in pure and crossbred carrier cattle under semi-industrial farming in Isfahan province. Previous studies showed that the positive infection rate of native carrier cattle was 40 and 7.5 % by PCR in Charmahal-Va-Bakhtiari and Golestan province of Iran, respectively (Azizi et al. 2008; Hoghooghi-Rad et al. 2011).

In present study none of the samples was positive for the *Babesia*. Unlike to bovine theilerosis that annually many clinical cases reported to Isfahan veterinary service, to date no clinical cases of bovine babesiosis have been reported in this region. *Ixodes ricinus* and *Boophilus annulatus* are major vectors of *Babesia* species in Iran. These ticks are only present in the Caspian region of Iran (Rahbari et al. 2007). Noaman et al. 2008 in identification of hard ticks of domestic ruminants in Isfahan province found that *Hyalomma* were most abundant ticks collected from cattle and they not found *Ixodes* and *Boophilus* ticks in this region. So, in present study presence of *Theileria* and absence of *Babesia* in samples was predictable.

In conclusion, the PCR can clearly identify the *Theileria* and *Babesia* parasites simultaneously in asymptomatic carrier cattle especially in the screening epidemiological surveys, although species-specific PCR is needed to identification of species. Further studies on identification of *Theileria* species and their vectors are necessary in this region.

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