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Detection of *Theileria* and *Babesia* sp. in Ixodid Ticks from Qinghai Province, Northwestern China

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Abstract: The present study was conducted to investigate tick species and tick-transmitted *Theileria* and *Babesia* species in Qinghai Province, Northwestern China. A total of 357 ticks, belonging to 2 *Ixodid* genera, *Haemaphysalis qinghaiensis* (167; 46.8%) and *Dermacentor nuttalli* (190; 53.2%) were collected from the domestic animals during May and June 2010. The ticks were separated into 78 tick pools according to their species and examined for *Theileria* and *Babesia* species by a nested PCR targeting *18S rRNA* gene. Of the 25 *H. qinghaiensis* pools, 2 (8.0%) were tested positive for Theileria. Only 1 (1.9%) was tested positive for Babesia in 53 *D. nuttalli* pools. The sequence and phylogenetic analyses showed that Babesia and Theileria detected in this study were grouped into *B. caballi* and *T. luwenshuni*, respectively implying the potential existence of theileriosis and babesiosis in animals in Qinghai Province.

Key words: *Haemaphysalis qinghaiensis*, *Dermacentor nuttalli*, theileria, babesia, Qinghai Province, nested PCR

INTRODUCTION

The piroplasms including the genera *Babesia* and *Theileria* are tick-transmitted protozoa that comprise species highly pathogenic for ruminants, horses, pigs, dogs, cats and humans (Walker, 1990; Zintl *et al*., 2003). The infection is usually characterized by fever, anemia and haemoglubinuria and in severe cases can lead to death. Several *Babesia* species including *B. bigemina*, *B. bovis*, *B. major*, *B. motasi*, *B. orientalis*, *B. ovata*, *B. caballi*, *B. divergens* and *B. microti* have been reported in China. *B. bovis* and *B. bigemina* which are transmitted by *Rhipichephalis microplus* are regarded as the major causative agents of bovine babesiosis (Yin *et al*., 1997). *B. major* and *B. ovata* whose vector is *Haemaphysalis punctata* and *H. longiconis*, respectively, are the large *Babesia* species infective to cattle in China (Liu *et al*., 2008). *B. orientalis* causes water buffalo babesiosis in central and south China (He *et al*., 2012). *B. caballi* leads to equine babesiosis which is transovarially transmitted by *Dermacentor nuttalli* (Yin *et al*., 1997). *B. motasi* and *B. ovis* are the causative agents of ovine babesiosis (Wang *et al*., 2013). *B. divergens*, first identified in anemic patients in Shandong Province in 2011 and *B. microti*, isolated from *Ixodes persulcatus* in Heilongjiang Province in 2008 can cause human babesiosis (Qi *et al*., 2011; Sun *et al*., 2008b).

Three bovine *Theileria* species including *T. annulata*, *T. mutans* and the *T. buffeli*/*T. sergenti*/*T. orientalis* group have been considered most economically important (He *et al*., 2012). Recently, five ovine *Theileria* species have been identified in China including *T. luwenshuni*, *T. ovis*, *T. separata*, *T. lestoquardi* and *T. uilenbergi* (Niu *et al*., 2009).

The present study was conducted to investigate tick species and tick-transmitted *Theileria* and *Babesia* species in Qinghai Province, Northwestern China using a nested PCR targeting the 18S *rRNA* gene.

MATERIALS AND METHODS

Study site: Qinghai Province is located in Northwestern China and lies on the Northeastern Qinghai-Tibet Plateau

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where the average elevation is over 3,000 m above sea level and the mean annual temperature is approximately -5-8°C with January temperatures ranging from -18-7°C and July temperatures ranging from 15-21°C. Significant rainfall occurs mainly in Summer while precipitation is very low in Winter and Spring. Qinghai has an area of 720,000 km^2 including 38,600 km^2 of grassland. The livestock industry is one of the primary industries in Qinghai Province with about 15 million Tibetan sheep and 1.1 million yaks (*Bos grunniens*), the specific species with hypoxia tolerance and cold-resistant ability in Qinghai-Tibet Plateau (Liu *et al*., 2010, 2011).

Ticks collection: Ticks were collected by directly removed from sheep or yaks using forceps to maintain the integrity of ticks. All the specimens were stored in 95% ethanol and later identified to the species level.

DNA extraction: Based on tick species and sampling sites, the collected ticks were grouped into pools and genomic DNA was extracted as earlier described (Zou *et al*., 2011).

Nested PCR: A nested PCR was conducted to detected *Theileria* and *Babesia* sp. infection in ticks. The first round reaction was carried out using the primers P1 (5'-CGGGATCCAACCTGGTTGATCCTGC-3') and P2 (5'-CCGAATTCCTTGTTACGACTTCTC-3') which are specific for a 1,700 bp fragment of the *18S rRNA* gene from *Theileria* and *Babesia* sp. (Da Silveira *et al*., 2011). The second round reaction was performed using the primers P3 (5'-GTCTTGTAATTGGAATGATG-3') and P4 (5'-TAGTCGGCATAGTTTATGGTTAGG-3') that are designed to amplify a common 460 bp fragment for Babesia and 490 bp for Theileria which were identified by aligning sequences from *Babesia* and *Theileria* sp. available at GenBank (http://www.ncbi.nlm.nih.gov). The reactions were subjected to the following cycling conditions using a Bio-Rad thermocycler: 95°C for 5 min, 30 cycles with denaturing at 94°C for 45 sec, primer annealing at 54-58°C for 30 sec and an extension step at 72°C for 100 sec or 30 sec followed by final extension at 72°C for 10 min.

Phylogenetic analyses: Phylogenetic analyses based on the *18S rRNA* genes were conducted using MEGA 4 (http://www.megasoftware.net/). *Toxoplasma gondii* (GenBank accession No. AY684849) was used as the outgroup to assess the relationship among *Babesia* and *Theileria* sp. The reliability of branches in the tree was assessed by bootstrap analysis with 1,000 replicates.

RESULTS AND DISCUSSION

A total of 357 ticks were collected from domestic animals (sheep and yaks) in Qinghai Province during May and June 2010, 2 species, *H. qinghaiensis* (167; 46.8%) and *D. nuttalli* (190; 53.2%) were identified based on morphological characters. Species and regional distribution of ticks are shown in Table 1.

The DNA samples from 78 tick pools, representing 167 *H. qinghaiensis* collected from Xining and Haidong and 190 *D. nuttalli* collected from Haidong, Hainan, Haibei, Haixi and Yushu were analyzed by a nested PCR for the presence of *Theileria* and *Babesia* sp. Of the 25 *H. qinghaiensis* pools, 2 (8.0%), collected from Xining and Haidong were tested positive for *Babesia* sp. Only 1 tick pool (1.3%), collected from Haixi was tested positive for *Theileria* sp. in the 190 *D. nuttalli* pools (Table 1).

Resulting PCR products were sequenced and compared with each other and with reference sequences. The unique sequences have been submitted to the GenBank database (KF055854 and KF055855).

The results indicated that *Babesia* detected in *H. qinghaiensis* from Qinghai Province, China belonged to *B. caballi* and Theileria found in *D. nuttalli* ticks was grouped into *T. luwenshuni* (Fig. 1).

Table 1: Results of nested PCR for detection of *Theileria* and *Babesia* sp. in Ixodid ticks in Qinghai Province, Northwestern China

				No. of nested PCR (%)	
Tick species	Location	No. of ticks (%)	No. of tick pool	Theileria	Babesia
Haemaphysalis qinghaiensis	Xining	67 (18.8)	13	1 (7.7)	0
	Haidong	100 (28.0)	12	1 (8.3)	0
	Subtotal	167 (46.8)	25	2 (8.0)	0
Dermacentor nuttalli	Haidong	11 (3.1)	2	0	0
	Hainan	84 (23.5)	29	0	0
	Haibei	9 (2.5)	5	0	0
	Haixi	61 (17.1)	14	0	1 (7.1)
	Yushu	25 (7.0)	3	0	0
	Subtotal	190 (53.2)	53	0	1 (1.9)
Total		357 (100)	78	2 (2.6)	1 (1.3)

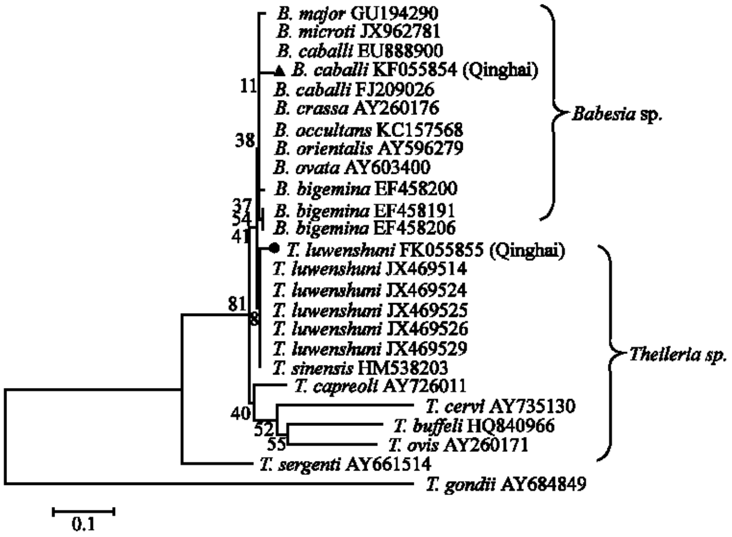

Fig. 1: Phylogenetic relationship of *Theileria* and *Babesia* sp. detected in Ixodid ticks from Qinghai Province, Northwestern China. Distance analysis of the 18S rDNA sequences was conducted using MEGA 4. Numbers on branches are percent bootstrap values (>50) using 1,000 replicates. The dot indicates *T. luwenshuni* detected in *H. qinghaiensis* and the triangle indicates *B. caballi* found in *D. nuttalli*

B. caballi causes equine babesiosis. In China, the seroprevalence of *B. caballi* infection in horses was 24.3% in Xinjing (Xuan *et al*., 2002) and 32% in Jilin Province (Xu *et al*., 2003). Additionally, *B. caballi* infection in donkeys was reported 38.7% in Western Xinjiang (Chahan *et al*., 2006). *D. nuttalli* is the vector for this parasite (Yin *et al*., 1997). Other tick species such as *D. silvarum*, *Rhipicephalus haemaphysaloides*, *D. niveu*, *D. sinicus* may be vectors of *B. caballi* but it need to be experimentally verified (Yin *et al*., 1997). In the present study, *B. caballi* was found in *D. nuttalli* in Qinghai Province, suggesting that *D. nuttalli* may play an important role in maintaining endemicity of equine babesiosis in the studies regions.

Of the five ovine *Theileria* species, *T. luwenshuni* and *T. uilenbergi*, the newly identified *Theileria* sp. (China 1 and 2), pose a major constraint to the development of sheep and goat husbandry in Northwestern China (Yin *et al*., 2008). *T. luwenshuni* has been reported in Jilin, Gansu and Zhejiang Provinces (Cao *et al*., 2013; Sun *et al*., 2008a). In Gansu Province, *H. qinghaiensis* is the vector of the parasite with the natural infection rate of 6.6% (Sun *et al*., 2008a; Yin *et al*., 2007). In this study, *T. luwenshuni* was also detected in *H. qinghaiensis* in Qinghai Province, showing the potential threat of such hemaprotozoas to the health of livestock in these areas.

Though only a few samples were detected positive, the results of this survey indicate that the studied area is under threat from *T. luwenshuni* and *B. caballi*. To the knowledge this is the first report on *Piroplasma* species in ticks in Qinghai Province which is important for a better understanding of the epidemiology of theileriosis and babesiosis in China.

CONCLUSION

Two tick species, including *H. qinghaiensis* and *D. nuttalli* were found in Qinghai Province, Northwestern China and they were tested positive for *T. luwenshuni* (8.0%) and *B. caballi* (1.9%), respectively. The findings show the potential existence of theileriosis and babesiosis in animals in Qinghai Province.

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