

# Molecular identification of *Theileria equi*, *Babesia caballi*, and *Rickettsia* in adult ticks from North of Xinjiang, China

Yang Zhang<sup>1,2</sup>  | Xiuxiu Wen<sup>1</sup> | Peipei Xiao<sup>1</sup> | Xinli Fan<sup>1</sup> | Min Li<sup>1</sup> | Bayin Chahan<sup>1</sup>

<sup>1</sup> Department of Animal Parasitological, Xinjiang Agricultural University School of Veterinary, Uygur, XinJiang, China

<sup>2</sup> MOE Joint International Research Laboratory of Animal Health and Food Safety, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, China

## Correspondence

Yang Zhang, Department of Animal Parasitological, Xinjiang Agricultural University School of Veterinary, Uygur, XinJiang 830052, China.

Email: [1319575971@qq.com](mailto:1319575971@qq.com)

Bayin Chahan, Department of Animal Parasitological, Xinjiang Agricultural University School of Veterinary, Uygur, XinJiang 830052, China. Email: [bynch@hotmail.com](mailto:bynch@hotmail.com)

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**Background:** Ticks in Xinjiang distribute widely and account for one third of China. Ticks can carry and transmit bacteria, virus, and parasite. However, the research of tick-borne pathogens in Xinjiang is rather little.

**Objective:** To understand the situation of hard tick carry *Theileria equi*, *Babesia caballi* and *Rickettsia* spp. of Zhaosu and Altay in Xinjiang.

**Methods:** In this study, 119 tick samples were obtained from horses in Xinjiang, China. Ticks were identified morphologically to determine species and PCR was used to investigate the situation of pathogens by hard ticks.

**Results:** One hundred and seven belong to *Dermacentor marginatus*, five belong to *D. niveus*, and seven belong to *D. silvarum*. *Theileria equi* and *Babesia caballi* were detected in one tick and 18 ticks, respectively. However, the carrying rate of *Rickettsia* spp. was 51.26% (61/119). Among these, the mixed carriage rate of *T. equi* and *Rickettsia* spp. was 0.8% (1/119). The mixed carriage rate of *B. caballi* and *Rickettsia* spp. was 10.1% (12/119).

**Conclusion:** Our results revealed that hard tick can carry not only haemoparasite but also many important zoonotic pathogens in Xinjiang, and this situation was worth heeding.

## KEYWORDS

*Babesia caballi*, China, hard tick, *Rickettsia*, *Theileria equi*, Xinjiang province

## 1 | INTRODUCTION

Infectious diseases are the second most common cause of death worldwide, followed by cardiovascular diseases. Many arthropod vectors are known to transmit infection by 'vector-borne diseases' (René-Martellet et al., 2017). Both ticks and tick-borne diseases have affected the animal and human health worldwide (De et al., 2012). Globally, ticks are considered the second most crucial disease vectors after mosquitoes (de la Fuente et al., 2008). There are a large number of microorganisms that use ticks as a reservoir host, for example, virus, bac-

teria, spirochete, rickettsia, mycoplasma, chlamydia, protozoan, and nematode (Wu et al., 2013). Most of them are natural reservoirs and cause zoonosis such as forest encephalitis, Lyme disease, haemorrhagic fever, Q fever, typhus fever, pestis, tularaemia, and brucellosis (Aktas et al., 2015, 2005; Iqbal et al., 2013). Among them, *rickettsia* was also an important pathogen that was transmitted by hard ticks and caused zoonosis (Walker et al., 1998). For prevention against tick-borne disease, there is a need to develop control strategies and identify pathogens that attack the geographical area by using ticks as a vector (Aktas, 2014).

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Equine piroplasmiasis is a tick-borne protozoan disease caused by the haemoprotozoan parasites *Theileria equi* and *Babesia caballi* (Salim et al., 2013; Sant et al., 2016; Sezayi et al., 2018). The clinical signs of acute *T. equi* infection are haemolysis that causes anaemia. However, *B. caballi* caused anaemia in horses but reported a few cases of acute deaths. Therefore, the failure of multiple organ dysfunction is directly correlated with both systemic formation of microthrombi and disseminated intravascular coagulation (Wise et al., 2013). The sub-clinical infection related explicitly to the horse-racing industry where the geographical performance of healthy horses was considered to help the spread of equine piroplasmiasis or think that sub-clinical infection negatively affected the animal's activity (Rampersad et al., 2003).

*Rickettsiae* are obligate intracellular gram-negative bacteria, an agent of emerging infectious disease, especially in humans (Raoult et al., 1997). Humans are only occasional hosts for ticks and played a role in the consecutive transmission of bacteria that showed symptoms such as headache, fever, and myalgia (Minichová et al., 2017). Evidence showed 10 types of rickettsioses present in China that were confirmed by isolated pathogens from the patients and performed genetic tests including epidemic typhus, endemic typhus, tsutsugamushi disease, North Asia tick-borne spotted fever, Inner Mongolia tick-borne spotted fever, heilongjiangii tick-borne spotted fever, Q fever, human monocytic ehrlichiosis, Human Granulocytic Ehrlichiosis, and Bartonellosis (Fan, 2005). In China, the tick species that can transmit *Rickettsiae* are *Dermacentor nuttali*, *D. silvarum*, *D. sinicus*, *Haemaphysalis yehi*, *H. concinna*, *hyalomma asiaticum*, and so forth (Zhang et al., 2005).

In this research, we showed the division of tick-borne diseases present in Zhaosu and Alytai county and Xinjiang area of China. We collected hard-ticks from that area, and a polymerase chain reaction (PCR) was conducted to detect the infection of the hard-ticks with *T. equi*, *B. caballi*, and *Rickettsia* spp.

## 2 | MATERIALS AND METHODS

### 2.1 | Study areas, sampling frame, and collection of ticks

Xinjiang Uygur Autonomous Region of China covers 166 km<sup>2</sup> from 34°25'N to 48°10'N latitude and 73°40'E to 96°18'E longitudes 155 m and 8611 m altitude. On 19 May 2017, adult ticks were collected from horses in Zhaosu and Alytai and were identified morphologically to determine species. One hundred and nineteen hard ticks were collected from eight horses, 107 were *D. marginatus*, five were *D. niveus*, and seven were *D. silvarum*.

### 2.2 | DNA extraction from ticks

The tick samples were removed from 95% ethanol, blotting the ethanol of ticks, and the ticks were washed two times with 1X TE Buffer for 20 min. The ticks and liquid nitrogen were added to a mortar and ground to a fine powder. Genomic DNA was extracted from each tick

sample using the TIANamp Genomic DNA Kit (Tiangen Biotech Co., Ltd), dissolved in 70 µl of TE (Tris- EDTA) buffer, and stored at -20°C until further use.

### 2.3 | Primer synthesis and PCR

All PCR primers are listed in (Table 1). The PCR was conducted in a 25 µl volume consisting of 0.5 µl of the stored DNA templates, 1 µl of every primer (10 µM), 0.5 µl of Phanta Max Super-Fidelity DNA Polymerase (1 U/µl) (Vazyme Bio Inc.), 12.5 µl of 2X Phanta Max Buffer (Mg<sup>2+</sup> plus), 0.5 µl dNTP Mix (10 mM), and 9 µl of nuclease-free water. All DNA concentrations of all samples examined in this study were diluted to a concentration of 10 ng/µl. The PCR materials were subjected to 1% agarose gel electrophoresis, stained through ethidium bromide, and then pictured under ultraviolet light.

### 2.4 | Sequencing analysis and phylogenetic study

A suitable size of DNA pieces produced from PCR was evaluated. The generated sequences were equated to those originally recorded in the NCBI nucleotide database (<http://www.ncbi.nlm.nih.gov/nucleotide/>). Therefore, the multiple sequences were allied, and the phylogenetic tree was created by using MEGA version 6.06, which was designated the extreme likelihood test. Note that 1000 bootstrap repeats evaluated customer support.

## 3 | RESULTS

One hundred and nineteen hard ticks from horses were tested for the presence of *T. equi*, *B. caballi*, and *Rickettsia* DNA by PCR, of which the infection rate of *T. equi* was 0.84%, *B. caballi* was 15.13%, and *Rickettsia* was 51.26% (Table 2). The majority of collected *Dermacentor* ticks were *D. marginatus* 89.9% (107/119).

The nucleotide sequences of *Theileria* and *Babesia* spp. were analyzed, and they are depicted in a phylogenetic tree (Figures 1 and 2). We detected one *T. equi* from *D. niveus* (ZS-16) and three *B. caballi* from *D. marginatus* (ZS-10, ZS-12, ZS-30), 13 sequences of piroplasmiasis 18S rRNA, including *T. equi* (KP177882, KM046918, MF398476, AB733376, AY534882, KF597077, AB515310, KY464023), *T. sergenti* (FJ822144), *T. ovis* (KX989508), *B. bigemina* (KM046917), *B. gibsoni* (KC461261), and *B. caballi* (EU888904) were used in this study. For the 18S rRNA of *T. equi*, the geographical origins of the gene we chose contain Swiss, Mongolia, Spain, Kenya, Sudan, Brazil, Altai, and Ili from Xinjiang, China. A phylogenetic tree of the 18S rRNA gene detected in this study is shown in Figure 1. Our results suggest that the sequences we acquired (ZS-16), Ili strain, Altai strain, and Swiss strain, are more closely related. Compared with the 18S rRNA of different species, it is not hard to observe that the genera of *Babesia* were classified into the same cluster and the genera of *Theileria* were classified into the same cluster compared with different Rap gene of piroplasmiasis.

**TABLE 1** Sequences of oligonucleotides used for target gene polymerase chain reaction (PCR) amplification

Pathogen	Target	Primer	PCR products size(bp)	Reference
<i>B. caballi</i>	Bc48	GCGACGTGACTAAGACCTTATTGG GTTCTCAATGTCAGTAGCATCCGC	451	(Li, 2016)
<i>T. equi</i>	18S	TTTGGGCTGTTTACAGTTGC CTCAAAGTAAACGTCGAGTCATG	531	(Luo, 2012)
<i>Rickettsia</i>	16S	ATCAGTACGGAATAACTTTTA TGCCTCTTGCCTTAGCTCAC	1332	(Anstead, 2013)

**TABLE 2** Results of infected *T. equi*, *B. caballi* and *Rickettsia* from different ticks

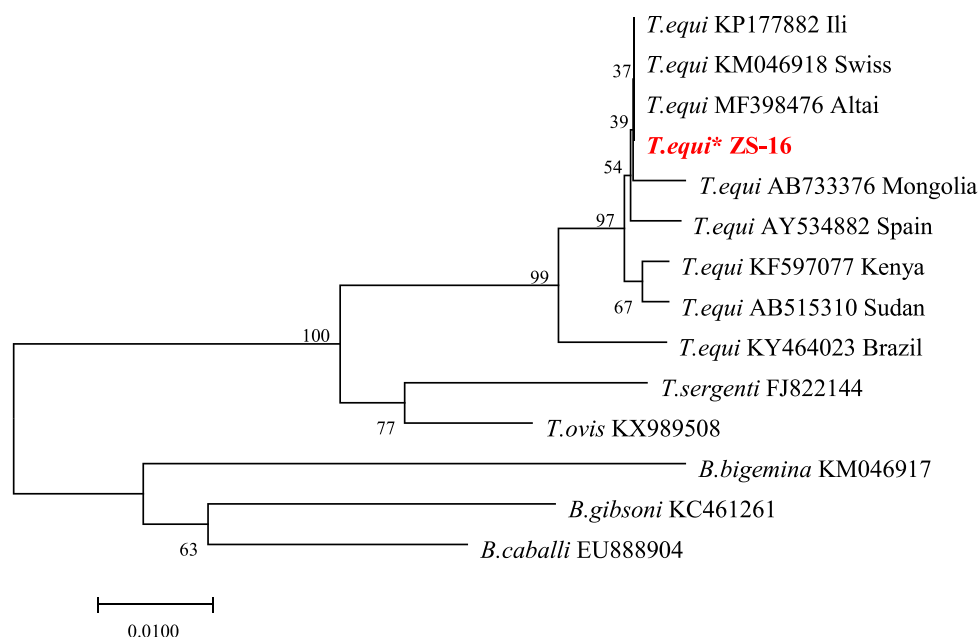
Collection area	Tick species	Number	<i>T. equi</i>	<i>B. caballi</i>	<i>Rickettsia</i>
Zhaosu	<i>D. marginatus</i>	107	1 (0.93%)	17 (15.89%)	56 (52.34%)
	<i>D. niveus</i>	2	0	0	1 (50%)
Altai	<i>D. silvarum</i>	7	0	0	3 (42.86%)
	<i>D. niveus</i>	3	0	1 (33.33%)	1 (33.33%)
Total	Hard tick	119	1 (0.84%)	18 (15.13%)	61 (51.26%)

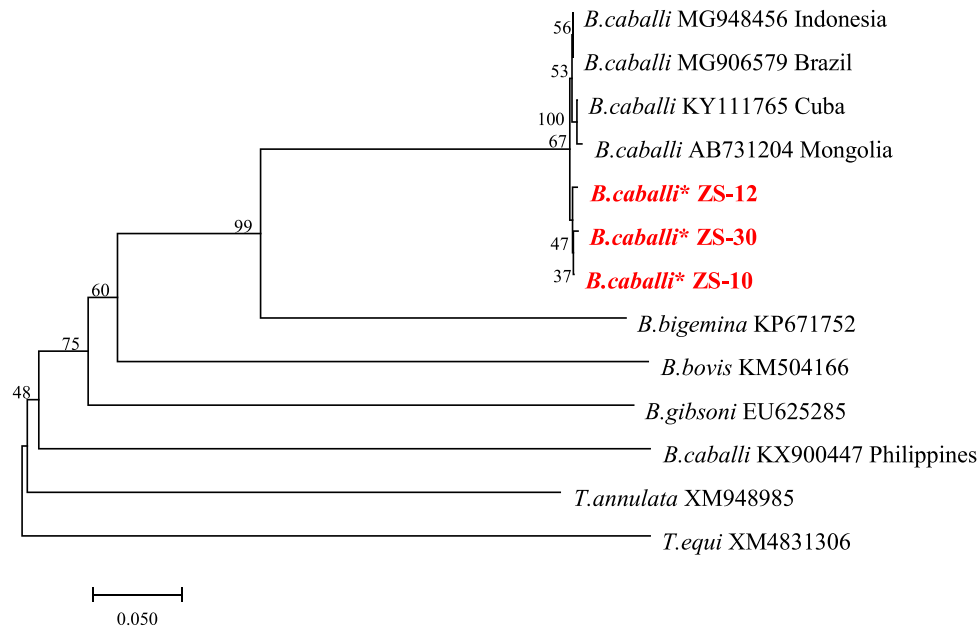
The three isolated *B. caballi* strains were also classified into the same cluster. In other countries, *B. caballi* strains and Rap gene of different species were present in the independent group (Figure 2). We detected three *Rickettsia* from *D. marginatus* (ZS-2, ZS-4, ZS-31), three sequences of *Rickettsia raoultii* from China (KY474575, MN446749) and France (NR043755), two sequences of *R. conorii* from China (MF002584) and France (NR074480), one sequence of *R. japonica* from China (MH722238), one sequence of *R. massiliae* from China (MF098399), one sequence of *R. parkeri* from the United States (KY124256), three sequences of *R. sibirica* from China (KU586293, MF098398) and Japan

(NR036848), and three sequences of *R. slovaca* from China (KJ410262, MF002588) and Pakistan (MN577235) were used in this study. Based on the phylogenetic tree, sequences of Z4, ZS-2, and ZS-31 were close to three *R. raoultii* sequences. The result could evidence that Z4, ZS-2, and ZS-31 belonged to *R. raoultii* (Figure 3).

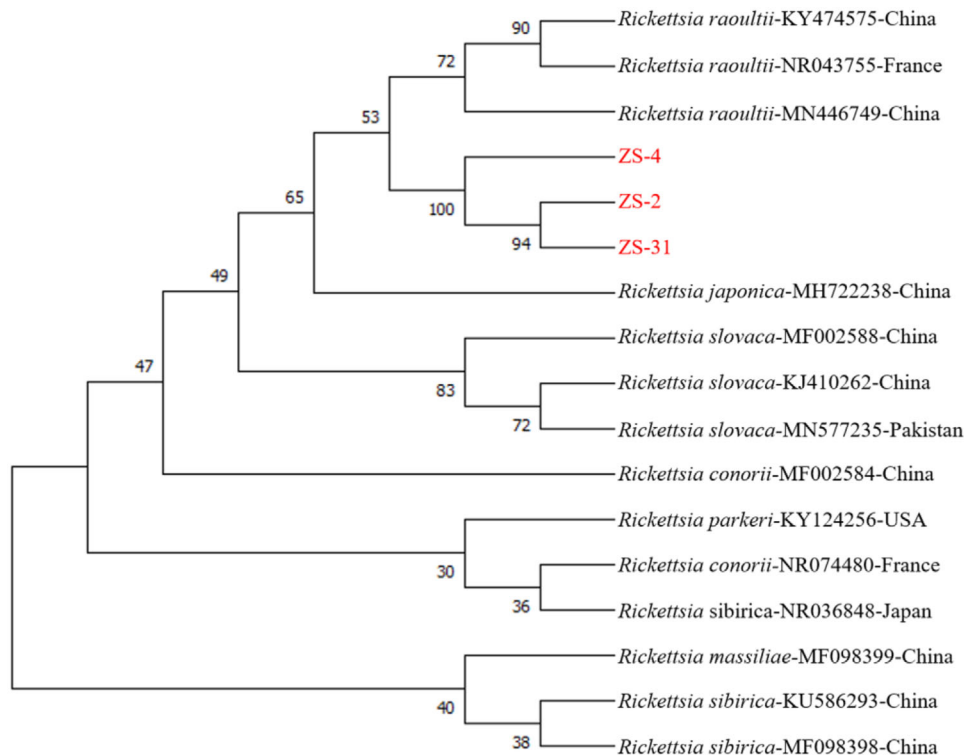
#### 4 | DISCUSSION

Three studies investigating the prevalence of apicomplexan parasites in ticks in China, using molecular methods, have previously been

**FIGURE 1** Phylogenetic tree based on nucleotide sequences of the 18S rRNA genes of *Theileria* and *Babesia* spp. detected in this study along with reference sequences. This tree was constructed using the neighbour joining method in MEGA version 6.06



**FIGURE 2** Phylogenetic tree based on nucleotide sequences of the RAP genes of *Theileria* and *Babesia* spp. detected in this study along with reference sequences. This tree was constructed using the neighbour joining method in MEGA version 6.06



**FIGURE 3** Phylogenetic tree based on nucleotide sequences of the 16S rRNA genes of *Rickettsia* spp. detected in this study along with reference sequences. This tree was constructed using the neighbour joining method in MEGA version 6.06

published (Aodungerile et al., 2015; Tuersong et al., 2018; Yi et al., 2014). Yi et al. (2014) tested 303 individual ticks belonging to *Rhipicephalus sanguineus* and *D. nuttalli* collected from Guangzhou province and Xinjiang province. The positive rate of *B. caballi* was 5.9% (18/303).

Aodungerile et al. (2015) also collected 347 *D. nuttalli* from Inner Mongolia and Xinjiang. In their study, the positive rate of *B. caballi* from Inner Mongolia was 8.16% (12/147), and Xinjiang was 13.5% (27/200). Tuersong et al. (2018) collected 181 *D. niveus* and found that the carrier

rate of *T. equi* was 7.2% (13/181). These three surveys collected samples in Xinjiang, meaning that Xinjiang is the endemic of equine piroplasmiasis. However, the above three studies just focused on one of the pathogens of equine piroplasmiasis but still not have been a comprehensive study of the *T. equi*, *B. caballi* and *Rickettsia* spp.

As the early report, equine piroplasmiasis was transmitted by the genera of *Boophilus*, *Hyalomma*, *Dermacentor*, and *Rhipicephalus* (Alhassan et al., 2007). *B. microplus*, *H. uralense*, *R. evertsi mimeticus*, and *R. pulchellus* can transmit *T. equi*, *D. ralbipictus*, *D. nitens*, *D. silvarum*, *D. reticulatus*, and *H. truncatum*. *H. volgense* can transmit *B. caballi*. *T. equi* and *B. caballi* can be transmitted by *D. marginatus*, *D. nuttallii*, *D. pictus*, *D. variabilis*, *H. anatolicum*, *H. marginatum*, *H. dromedarii*, *R. bursa*, *R. evertsi evertsi*, and *R. sanguineus* (Rothschild, 2013). In this scenario, we studied that *D. marginatus* could carry *T. equi*, *B. caballi*, and *Rickettsia*; *D. silvarum* could carry *Rickettsia*; *D. niveus* could carry *B. caballi* and *Rickettsia*. The research conducted by Tiersong showed that *D. niveus* could also carry *T. equi*. Unfortunately, we did not detect the gene of *T. equi* from *D. niveus*, this might be due to the small numbers of *D. niveus*.

In conclusion, this is the report describing the presence of *T. equi*, *B. caballi*, and *Rickettsia* in ticks in Xinjiang, China. Interestingly, our results indicated that *D. niveus* could also carry *B. caballi*. Our results also suggest that *Rickettsia* was more prevalent in Xinjiang than previously thought. Although we could not confirm that these ticks are biological vectors of equine piroplasmiasis and *Rickettsia*, a high infection rate of equine piroplasmiasis in Xinjiang and the presence of *Rickettsia* show we should find out ways to prevent tick-borne disease as soon as possible.

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#### AUTHOR CONTRIBUTION

Yang Zhang: Conceptualization, Investigation, Writing-original draft, Writing-review & editing; Xiu Wen: Data curation, Software; Pei Xiao: Investigation; Xinli Fan: Investigation; Min Li: Investigation; Bayin Chahan: CRediT contribution not specified.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### ETHICS STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. The experiments conducted in this research were according to the Laboratory Animal-Guideline for ethical review of animal welfare (GB/T 35892-2018).

#### DATA AVAILABILITY STATEMENT

The raw/processed data required to reproduce these findings cannot be shared at this time.

#### PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.613>.

#### ORCID

Yang Zhang  <https://orcid.org/0000-0001-5810-4775>

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