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Molecular-phylogenetic analyses of *Babesia* and *Theileria* species from small mammals and their ticks in northern China suggest new reservoirs of bovine and equine piroplasms

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ABSTRACT

Babesia and *Theileria* species (Apicomplexa: Piroplasmida) are tick-borne protozoan parasites that can cause mild to severe infection in humans, wildlife, livestock and companion animals. To date, reports on the molecular study of piroplasms from wild living small mammals and their ticks are still limited, especially in Asia. This study encompassed an extensive survey involving 907 liver samples and 145 ixodid ticks from 16 different species of small mammals (Rodentia, Lagomorpha, Eulipotyphla). These were collected in 13 cities and counties in northern China. DNA extracts from these samples were screened for the presence of piroplasm *18S rRNA* gene. Samples that tested positive were further evaluated for other genetic markers of piroplasms, including the cox1 gene and the *ITS1-5.8S rDNA-ITS2* region. Several piroplasm species were identified, including *Babesia* sp. tavsan2, *Babesia occultans*, *Theileria* sp. Xinjiang, *Theileria equi*, and *Theileria* sp. Kalecik. Among these, *Theileria* sp. Xinjiang was shown to be the most prevalent. Importantly, *Babesia* sp. tavsan2 was identified in the tick *Rhipicephalus sanguineus* from the Yarkand hare and *Theileria* sp. Kalecik in *Hyalomma asiaticum* from the long-eared hedgehog, in line with the detection of these pathogens in tissue samples of the relevant hosts. This study further disclosed the presence of DNA from *B. occultans* and *T. equi*, typically found in cattle and horses respectively, with an additional discovery in small mammals. Moreover, *Theileria* sp. Kalecik, which was first detected in small-sized mammals, and *Babesia* sp. tavsan2, were both reported for the first time in China.

1. Introduction

The families Babesiidae and Theileriidae (Apicomplexa: Piroplasmida) include tick-borne protozoan parasites that can cause mild to severe infection in humans, livestock and companion animals, as well as wildlife. Currently, at least 100 valid *Babesia* species and 40 *Theileria* species have been reported (Antunes et al., 2017; Mans et al., 2015). These *Babesia* species infect a diverse array of mammalian hosts, with rodents being the most commonly affected, as well as several bird species (Homer et al., 2000). In addition, rodents and other small mammals support immature developmental stages of several tick species with high veterinary-medical importance (Nosek, 1972; Gray et al., 2002). Since transstadial transmission is important in the case of *Theileria* species and members of the *Babesia microti* group, rodents may provide the source of

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infection for tick larvae or nymphs which can infect other hosts in the next developmental stage, as exemplified by the zoonotic *B. microti* (Gray et al., 2002).

Thus, it is not surprising that small mammals, such as lagomorphs, rodents, and insectivores have been identified as reservoirs of several piroplasms, playing a crucial role in their transmission and maintenance of these pathogens in nature. For instance, in the USA, white-footed mice and eastern cottontail rabbits were shown to be infected with the zoonotic *B. microti* and the potentially zoonotic *Babesia* sp. MO1, (Fritzen et al., 2014). In China, great gerbils have been found to harbor an unclassified *Theileria* species ("*Theileria* sp. Xinjiang") (Ji et al., 2021), while *Theileria annulata* was identified from European rabbits in Pakistan (Ramzan et al., 2022). Furthermore, North African hedgehogs are carriers of *Theileria youngi* (Balti et al., 2021). Additionally, various *Babesia* and *Theileria* species have been detected in ixodid ticks collected from small mammals. In particular, *B. microti* was identified in *Rhipicephalus turanicus* ticks from white-breasted hedgehogs (Mumcuoglu et al., 2022), and *T. equi* confirmed in both *Thrichomys fosteri* (Rodentia, Echimyidae) and their ticks (*Amblyomma parvum*) (De Sousa et al., 2018).

Xinjiang Uygur Autonomous Region (XUAR) and Inner Mongolia Autonomous Region (IMAR), located in northern China, rank as the largest and second-largest provinces in China, covering areas of 166.5 and 118.3 million square kilometers, respectively. The temperate continental climate and diverse landscapes, such as grasslands, oases, semi-deserts, and deserts, provide rich habitats for a variety of mammals and ticks (Yang and Xing, 1998). These regions are home to over 630 species of terrestrial mammals and at least 55 tick species (Yu et al., 1997; Zhang, 1997; Wu et al., 2024; Gao et al., 1978).

The purpose of this study is to systematically investigate the presence of Piroplasma in 16 different small mammals including rodents, lagomorphs, and shrews, as well as their ticks, in northern China through extensive molecular screening. By detecting and analyzing the 18S rRNA gene and other genetic markers such as cox1 gene and *ITS1–5.8S rDNA-ITS2* region of Piroplasma in these samples, we aim to reveal the species and genetic diversity of Piroplasma carried by small mammals and their ticks in the region, fill the gaps in current research, and provide important data support for further understanding the transmission mechanism, host specificity, and potential public health and livestock industry impacts of Piroplasma

2. Materials and methods

2.1. Study area and sample collection

From September 2021 to June 2023, 907 wild living small mammals representing 16 species were collected, and 145 ixodid ticks were removed from them, in 13 counties or cities in XUAR and IMAR (Fig. 1). The mammals were captured in live traps (30 cm ×15 cm ×15 cm wire mesh), which were placed near the entrances of occupied burrows, baited with walnut, tomato or cucumber. Each survey site included 150 traps that were checked twice a day. Each trap was removed before nightfall and replaced on the survey site the following day. All wild mammals were morphologically identified by an experienced zoologist. The rodents in Manas County were directly transported to our laboratory while the animals in Alataw City were transported to the Vector-borne Laboratory at Alataw Customs. Each

animal was weighed, Zoletil 50 (Virbac, Paris, France) was used in anesthesia by intramuscular injection, and blood-taken from heart. Small mammals, collected for plague detection, were ethically trapped by an authorized agency following legal standards and subsequently provided for research purposes. Data of sampled animals are shown in Table 1 and ticks are shown in Table 2. This table presents the detection of piroplasm (*Babesia* and *Theileria* genera) infections in samples collected from 16 species of small mammals at various locations. The data includes sampling locations, host species, tick species, the number of hosts, and the detected piroplasm species along with their infection rates. The Yarkand hare (*Lepus yarkandensis*) and five long-eared hedgehogs (*Hemiechinus auritus*) included in the study were found dead due to natural causes in a nature reserve. The wild rodents and Pallas's pika (*Ochotona pallasi*) were captured by Sherman traps (30 cm × 16 cm × 16 cm wire mesh), which were placed near the entrances of occupied burrows, baited with peanuts. Each survey site included 150 traps that were checked twice a day. Each trap was removed before nightfall and replaced on the survey site the following day (Kamranrashani et al., 2013). Liver samples of the above small mammals (n=907) were stored individually at - 80℃. Simultaneously, ticks were collected from the entire body surface of each animal and then preserved at - 20℃. All procedures performed in this study, involving wild mammals, were in accordance with the ethical standards of Animal Ethics Committee of Shihezi University (Approval No. A2022–029–01).

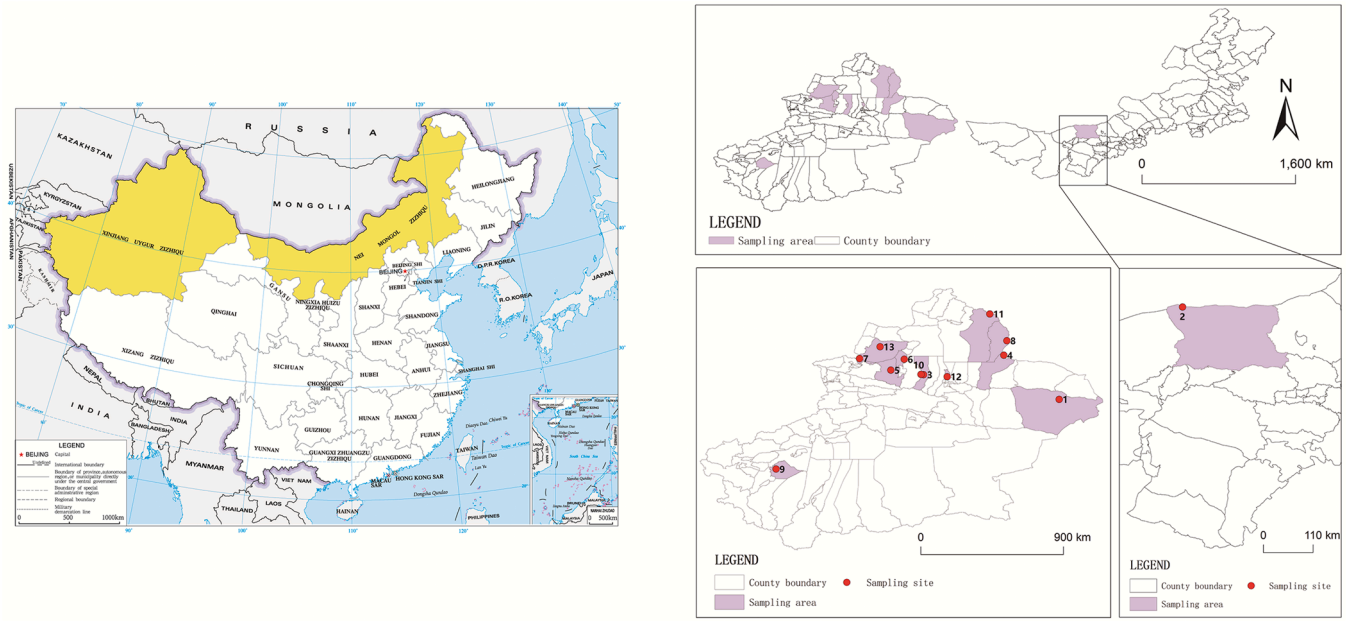

Fig. 1. The locations for capturing small mammals and their ticks in the current study.

Table 1
Data of Piroplasm Infection in Small Mammal Samples.

Location	Label in Fig. 1	Host species scientific name (common name)	No. of hosts	Piroplasm species	number (%) of positive hosts
Hami City	1	*Meriones meridianus* (Midday gerbil)	2	*Theileria* sp. 1	(50)
Bayan Nur City	2	*Meriones meridianus* (Midday gerbil)	6	*Theileria* sp. 2	(33.33)
Manasi County	3	*Meriones meridianus* (Midday gerbil)	8	*Theileria* sp. 2	(25)
Qitai County	4	*Meriones meridianus* (Midday gerbil)	1	*Theileria* sp1	(100)
Huyanghe City	5	*Meriones erythrourus* (Red-tailed gerbil)	80	*Theileria* sp.	1 (1.25)
Karamay City	6	*Rhombomys opimus* (Great gerbils)	71	*Theileria* sp.	9 (12.68)
Altaw City	7	*Rhombomys opimus* (Great gerbils)	17	*Theileria* sp.	3 (17.65)
Bayan Nur City	2	*Rhombomys opimus* (Great gerbils)	5	*Theileria* sp. 1	(20)
Qitai County	4	*Rhombomys opimus* (Great gerbils)	11	*Theileria* sp.	3 (27.20)
Huyanghe City	5	*Rhombomys opimus* (Great gerbils)	37	*Theileria* sp.	5 (13.51)
Huyanghe City	5	*Meriones tamariscinus* (Tamarisk Gerbil)	1	*Theileria* sp1	(100)
Qinghe County	8	*Mus musculus* (House mouse)	8	*Theileria* sp.	1 (12.50)
Altaw City	7	*Apodemus uralensis* (Ural field mouse)	33	*Theileria* sp.	1 (3.03)
Qinghe County	8	*Citellus undulatus* (Long-tailed ground squirrel)	181	*Theileria* sp. Xinjiang *Theileria equi* *Babesia occultans*	2 (1.10) 4 (2.21) 1 (0.55)
Makit County	9	*Lepus yarkandensis* (Yarkand hare)	1	*Babesia* sp.1 tavsan2	(100)
Bayan Nur City	2	*Hemiechinus auratus* (Long-eared hedgehog)	5	*Theileria* sp. 3 Kalecik	(60)
Bayan Nur City	2	*Dipus sagitta* (Three-toed Jerboa)	1	NA	
Hami City	1	*Lepus tolai* (Tolai Hare)	2	NA	
Makit County	9	*Ondatra zibethicus* (Common Muskrat)	2	NA	
Shihezi City	10	*Rattus norvegicus* (Brown Rat)	4	NA	
Shihezi City	10	*Scarturus elater* (small five-toed jerboa)	1	NA	
Fuyun County	11	*Citellus undulatus* (Long-tailed ground squirrel)	188	NA	
Wujiaqu City	12	*Ochotona pallasi* (Pallas's pika)	83	NA	
Tuoli County	13	*Spermophilus erythrogenys* (red-cheeked ground squirrels)	159	NA	
Total			907		

2.2. DNA extraction, polymerase chain reaction (PCR) amplification, and sequencing

Each liver sample (~0.2 g) and all ticks were used to extract DNA using the TIAN amp Genomic DNA Kit (TIANGEN, Beijing, China), following the manufacturer's instructions. A 400-bp-long part of the 18S rRNA gene (*18S rRNA*) of piroplasms was amplified using different primer sets (Song et al., 2018; Wei et al., 2001). To confirm the positive PCR results, an approximately 1000-bp partial *cox1* gene and an about 900-bp partial *ITS1–5.8S rDNA-ITS2* gene of piroplasms were also amplified and sequenced (Holman et al., 2002; Schreeg et al., 2016). The primers and PCR cycling conditions are shown in **Additional file 1** (Song et al., 2018; Wei et al., 2001; Holman et al., 2002; Schreeg et al., 2016).

Ticks were identified morphologically. For molecular confirmation, a PCR was used, targeting an approximately 710-bp portion of the *cox1* gene (Folmer et al., 1994). The species of small mammals providing the above samples were identified based on morphology (e.g., body length, tail length, fur color, tooth feature and ear length) and by molecular methods targeting an approximately 1200-bp-long part of the *cox1* gene (Herbreteau et al., 2011).

The amplification products were purified using the TIANgel Midi Purification Kit, cloned into the pGEM-T Easy vector (TIANGEN, Beijing, China) and then subjected to sequencing. Obtained sequences were compared to reference sequences found in GenBank using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The method used for sequencing is Sanger.

Phylogenetic trees were constructed using the Neighbor Joining (NJ) methods in MEGA X. Bootstrap analyses (1000 replicates) were conducted to determine the relative support for clades in the consensus trees.

3. Results

The captured 907 small-size mammals represented 7 families, 10 genera and 16 species, including Pallas's pika (n=83), long-eared hedgehog (n=5), Yarkand hare (n=1), Tolai hare (n=2) and 13 rodent species (n=816). From these hosts, 145 ixodid ticks were collected, then identified as *Hyalomma asiaticum* (n=3, from long-eared hedgehog), *Dermacentor marginatus* (n=1, from Yarkand hare), *D. nuttalli* (n=123, from red-cheeked ground squirrels and Pallas's pika) and *Rhipicephalus sanguineus* (n=18, from Yarkand hare). Out of the 16 small mammal species, nine tested positive for piroplasms with the PCRs targeting the *18S rRNA*, *cox1*, and *ITS1–5.8S rDNA-ITS2* genes (Table 3). The average rate of infection with piroplasms was 4.63 % (42/907). *Babesia* sp. tavsan2, *B. occultans*, *Theileria* sp. Xinjiang, *T. equi* and *Theileria* sp. Kalecik were identified. The most prevalent species was *Theileria* sp. Xinjiang (78.57 %, 33/42), followed by *T. equi* (9.52 %, 4/42), *Theileria* sp. Kalecik (7.14 %, 3/42), *Babesia* sp. tavsan2 (2.38 %, 1/42) and *B.*

Table 2
Data of Piroplasm Infection in Ticks.

Location	Label in Fig. 1	Host species	Tick species	No. of ticks	tick life stages	Piroplasm species	number (%) of positive ticks
Makit County	9	Yarkand hare	*Rhipicephalus sanguineus* *Dermacentor marginatus*	18 1	Nymph	*Babesia* sp. tavsan2 NA	1 (5.56) NA
Bayan Nur City	2	Long-eared hedgehog	*Hyalomma asiaticum*	3	Nymph	*Theileria* sp. Kalecik	1 (33.43)
Wujiaqu City	12	Pallas's pika	*Dermacentor nuttalli*	50	Nymph	NA	NA
Tuoli County	13	red-cheeked ground squirrels	*Dermacentor nuttalli*	73	Nymph	NA	NA
Total				145			

Table 3
Data of Piroplasm Species Identified in Small Mammal Hosts and Ticks Including *18S rRNA* and *cox1* Genes with NCBI BLAST Maximum Identity Percentages.

Piroplasm	Host species	Tick species	*18SrRNA* (NCBI BLAST maximum identity)	*cox1* (NCBI BLAST maximum identity)
Babesia sp. tavsan2	Yarkand hare	*Rhipicephalus sanguineus*	98.35 % with *Babesia* sp. tavsan2 (MN463017)	91.00 % with *Babesia* sp. (MF078482)
Babesia occultans	Long-tailed ground squirrel	NA	99.46 % with *Babesia occultans* (LC768120)	NA
Theileria sp. Kalecik	Long-eared hedgehog	*Hyalomma asiaticum*	99.75 % with *Theileria* sp. Kalecik (MW338844)	NA
Theileria equi	Long-tailed ground squirrel	NA	99.48 % with *Theileria equi* (MT463613)	NA
Theileria equi	Long-tailed ground squirrel	NA	100 % with *Theileria equi* (MT463610)	NA
Theileria sp. Xinjiang	Midday gerbil	NA	99.74 % with *Theileria* sp. (MW338844)	79.67 % with *Theileria luwenshuni* (JQ518296)
	Great gerbils	NA	99.74 % with *Theileria* sp. (MW338844)	79.67 % with *Theileria luwenshuni* (JQ518296)
	Red-tailed gerbil	NA	99.74 % with *Theileria* sp. (MW338844)	79.67 % with *Theileria luwenshuni* (JQ518296)
	House mouse	NA	99.74 % with *Theileria* sp. (MW338844)	79.67 % with *Theileria luwenshuni* (JQ518296)
	Ural field mouse	NA	99.74 % *with* *Theileria* sp. (MW338844)	79.67 % with *Theileria luwenshuni* (JQ518296)
	Tamarisk Gerbil	NA	99.74 % *with* *Theileria* sp. (MW338844)	79.67 % with *Theileria luwenshuni* (JQ518296)
	Long-tailed ground squirrel	NA	99.23 % with *Theileria* sp. (MW338844)	NA

The same gene sequence and host are not repeatedly mentioned in the table.

occultans (2.38 %, 1/42). Splenomegaly was observed in the piroplasm-positive long-eared hedgehog. The breakdown of positive cases by family is presented in Table 1, with the highest number of positive cases observed in the Cricetidae (hamster family), where 29 of 241 individuals (12.03 %) tested positive. The Sciuridae (squirrel family) and Muridae (mouse family) had lower positive rates, with 7 of 528 (1.32 %) and 2 of 45 (4.44 %) individuals respectively testing positive for piroplasms.

Importantly, *Babesia* sp. tavsan2 in *Rh. sanguineus* from Yarkand hare and *Theileria* sp. Kalecik in *Hy. asiaticum* from long-eared hedgehog were identical with the pathogens found in their respective hosts. Furthermore, the *ITS1–5.8S rDNA-ITS2* region of *Theileria* sp. Xinjiang exhibited the highest, 93.7 % (163/174) similarity to *T. annulata*, with a sequence coverage of only 25.7 % (217/844), in part due to the scarcity and shortness of related nucleic acid sequences available in GenBank.

The results of phylogenetic analyses of *18S rRNA* and *cox1* genes of *Theileria* and *Babesia* species from this study reflect that (1) tick-borne *Theileria* sp. Kalecik and rodent-borne *Theileria* sp. Xinjiang clustered in the sister group containing bovine piroplasms (Fig. 2**.A-3**); (2) *T. equi* detected in rodents of this study belonged to the phylogenetic group of conspecific equine isolates (Fig. 2**.A**), and the sequences of *T. equi* clustered with genotype A (Fig. 4); (3) *B. occultans* from rodents clustered together with *B. occultans* sequences reported from cattle in GenBank (Fig. 2**.B**); and (4) *Babesia* sp. tavsan2 from Yarkand hare and its tick was most closely related to bovine *Babesia* species, the tree shows that the current genotype is closely related to tavasan2 but clearly separated with high bootstrap (Fig. 2**.B-3**).

The sequences from this study were deposited in GenBank (*Babesia 18S rRNA*: PP668148-PP668150; *Theileria 18S rRNA*: PP668142-PP668147; *Babesia cox1*: PP661517; *Theileria cox1*: PP661518; *Theileria ITS1-5.8S rDNA-ITS2*: PP664460).

4. Discussion

In this study, we detected five piroplasm species, including *Babesia* sp. tavsan2, *B. occultans*, *Theileria* sp. Xinjiang, *T. equi* and *Theileria* sp. Kalecik, by screening 907 small-size mammals and their 145 ticks in XUAR and IMAR, northern China. To the best of our knowledge, *B. occultans* and *Theileria* sp. Kalecik were for the first time detected in small-size mammals. In addition, *Babesia* sp. tavsan2 and *Theileria* sp. Kalecik were reported for the first time in China.

The results of our statistical analysis (Table 1), which show a significant difference in piroplasm prevalence between the Cricetidae, Sciuridae and Muridae ($X^2 = 42.634$, $P < 0.0001$), further support the importance of hamsters as reservoirs for these pathogens. This finding has significant implications for disease prevention and control strategies, as it underscores the need for targeted surveillance and intervention in areas where hamsters are abundant.

Previously, *Theileria* sp. Kalecik was detected in *D. marginatus*, and *Babesia* sp. tavsan2 both in the blood samples and ticks (*Rh. turanicus*) of hares in Turkey (Orkun, 2022; Orkun and Emir, 2020; Orkun and Karaer, 2017). Similarly, in this study, *Theileria* sp. Kalecik was shown to be present both in the liver samples and *Hy. asiaticum* ticks from long-eared hedgehogs, and *Babesia* sp. tavsan2 both in the liver samples and *Rh. sanguineus* ticks from Yarkand hare. The *Babesia* sp. tavsan2 genotype found in this study is closely related to the *Babesia* sp. tavsan2

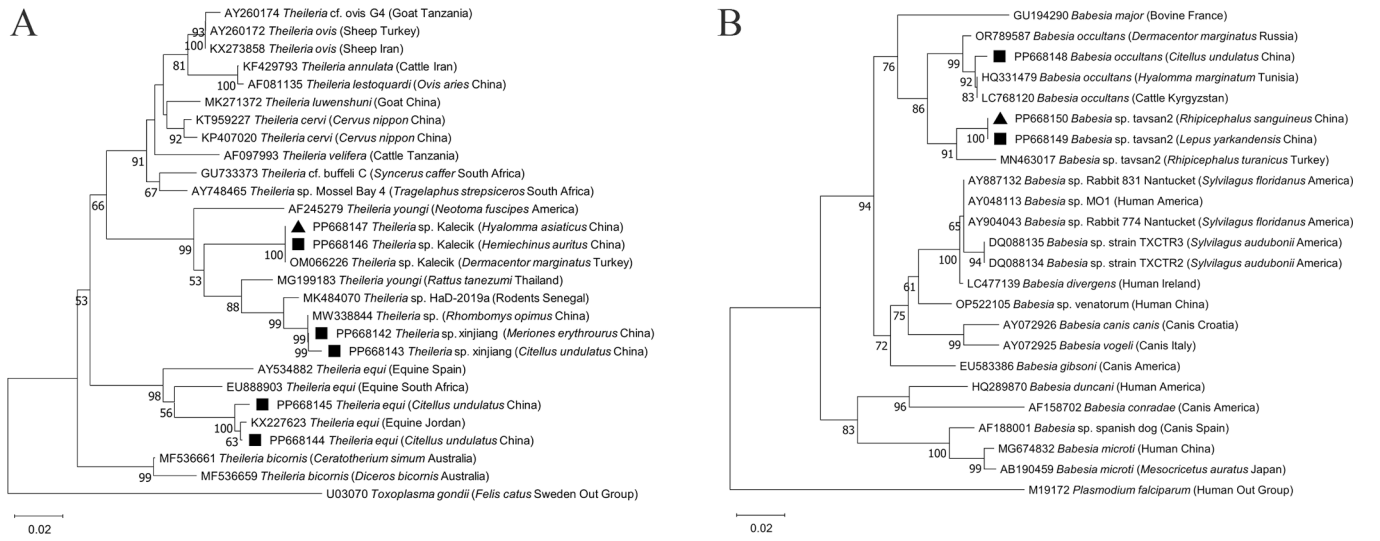

Fig. 2. Phylogenetic analysis of "piroplasms" with MEGA X. The tree was constructed with the Neighbor Joining method (NJ; bootstrap replicates: 1000). Branch lengths correlate to the number of substitutions inferred according to the scale shown. Sequences from small mammals obtained in this study are indicated by solid squares (■). Sequences from ticks obtained in this study are indicated by solid triangles (▲). A: Analysis based on the *Theileria 18S rRNA* gene fragment, Sequences of 391 bp were used in the phylogenetic tree; B: Analysis based on the *Babesia 18S rRNA* gene fragment, Sequences of 365 bp were used in the phylogenetic tree.

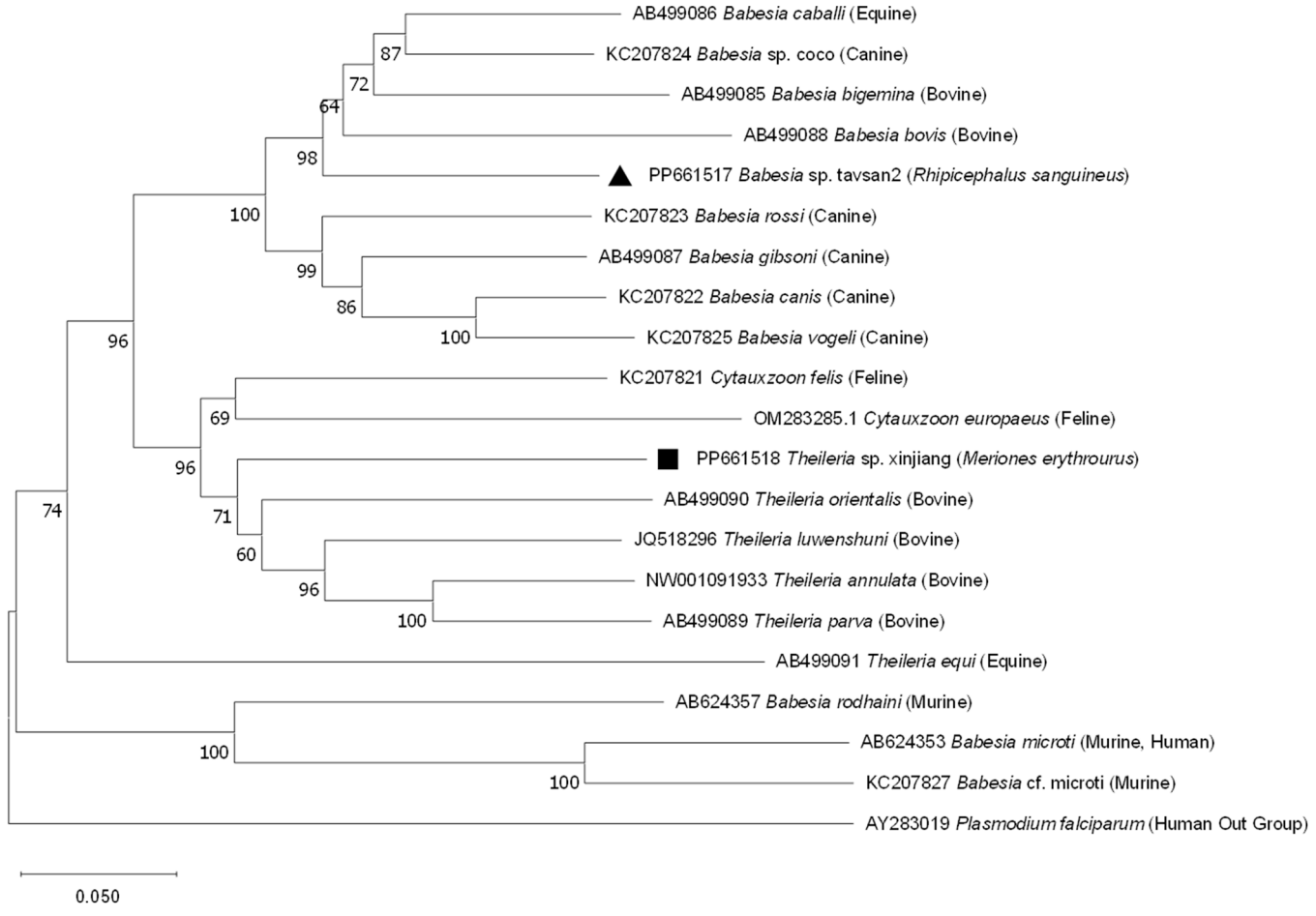

Fig. 3. Phylogenetic analysis of "piroplasms" based on the Piroplasm *cox1* gene fragment with MEGA X. Sequences of 932 bp were used to construct the phylogenetic tree with the Neighbor Joining method (NJ; bootstrap replicates: 1000). Branch lengths correlate to the number of substitutions inferred according to the scale shown. The sequence from small mammal obtained in this study is indicated by solid square (■). The sequence from small mammal obtained in this study is indicated by solid triangle (▲).

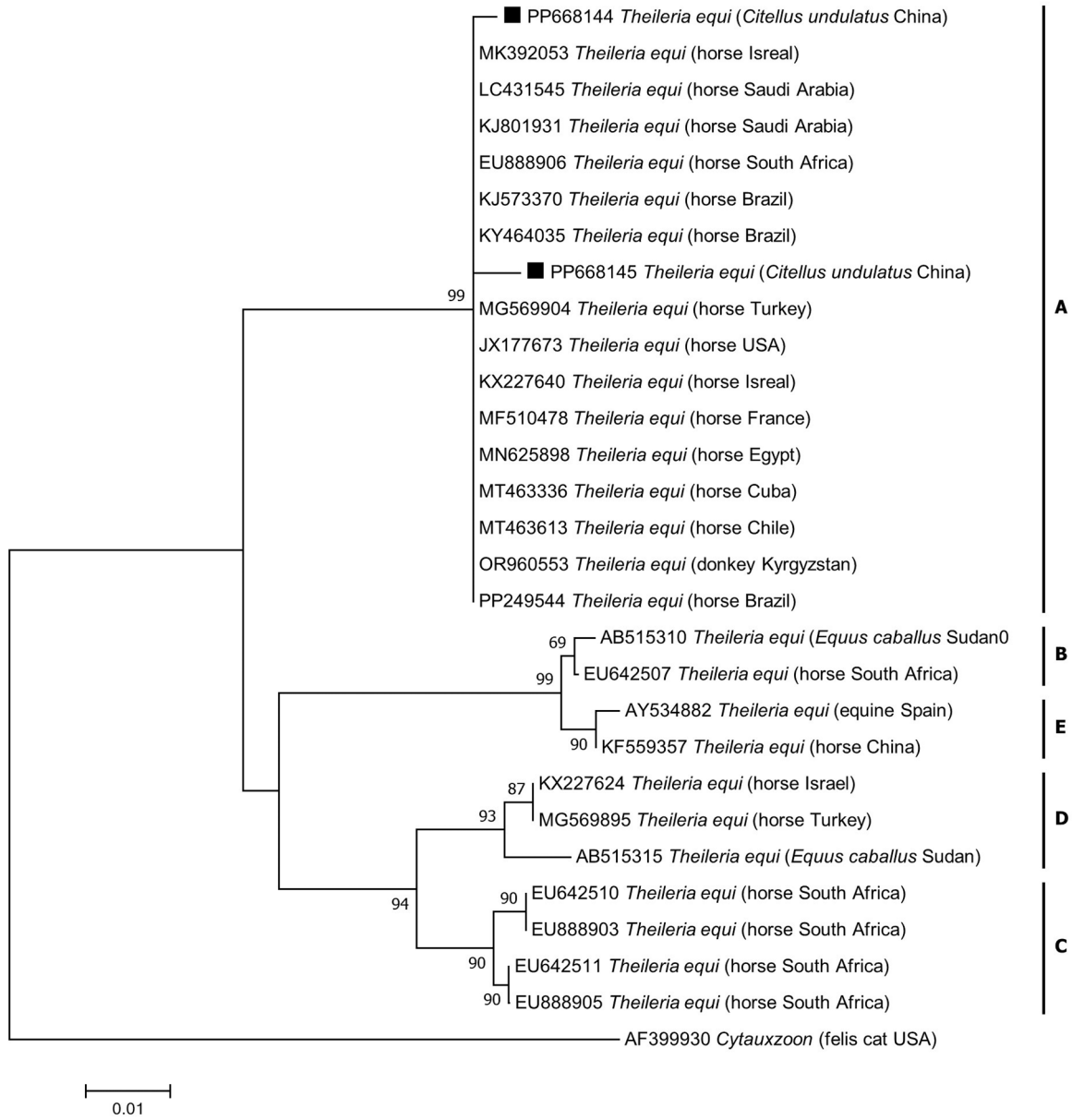

Fig. 4. Phylogenetic analysis of "*Theileria equi*" based on the Piroplasm 18 s rRNA gene fragment with MEGA X. Sequences of 386 bp were used to construct the phylogenetic tree with the Neighbor Joining method (NJ; bootstrap replicates: 1000). Branch lengths correlate to the number of substitutions inferred according to the scale shown. The sequence from small mammal obtained in this study is indicated by solid square (■).

found in Turkey, but is clearly separated from it by a high support rate, suggesting that it may be a new genotype. These findings strongly suggest that Yarkand hares and long-eared hedgehogs serve as reservoir hosts for these piroplasms. In addition, the potential role of *Hy. asiaticum* and *Rh. sanguineus* in the transmission of *Theileria* sp. Kalecik and *Babesia* sp. tavsan2, respectively, should be evaluated further.

B. occultans was previously detected in African buffalo (*Syncerus caffer*) in Botswana (Eygelaar et al., 2015), in symptomatic cows in Iran (Noaman et al., 2021), in Sable Antelope in South Africa (Oosthuizen et al., 2008), and in cattle in South Africa, Egypt, West Africa, Poland and Kyrgyzstan (Al-Hosary et al., 2021; Van Niekerk and Zweygarth, 1996; Adjou Moumouni et al., 2021; Zhyldyz et al., 2023; Staniec et al., 2018). Its presence has also been documented in various tick species, including *Amblyomma variegatum* from cattle in Ghana (Ansah-Owusu et al., 2023), *Hy. marginatum* from cattle in south Africa (Gray and Vos, 1981) and from wild boar in Turkey (Orkun and Karaer, 2017), *Hy. marginatum* from humans in Turkey (Orkun et al., 2014), *Hyalomma* spp. from wild hare in Turkey (Orkun and Karaer, 2017), *Hy. excavatum* from one-humped camels (*Camelus dromedarius*) in Nigeria (Onyiche et al., 2020), *Hy. asiaticum* in China (Sun et al., 2019), *Hy. marginatum* and *Rh. turanicus* in Turkey (Aktas et al., 2014), and *D. marginatus* in Kazakhstan (Sang et al., 2021). Our study complements all the above findings with a rodent reservoir of *B. occultans*, i.e., it was detected in the liver of long-tailed ground squirrel (Rodentia) that is taxonomically distant from the type host, the cattle (Artiodactyla). Given the crucial supportive role of rodents in the development of tick larvae and nymphs, future surveillance of piroplasms in ticks infesting rodents should be strengthened, particularly among long-tailed ground squirrels.

Historically, *T. equi* has been reported in horses, donkeys and mules in Jordan (Qablan et al., 2013), horses in Paraguay (Ahedor et al., 2023), and donkeys and horses in Nigeria (Sunday Idoko et al., 2020). Our study revealed molecular evidence on the occurrence of *T. equi* in long-tailed ground squirrels. Apart from intrastadial transmission of this piroplasm by male ticks (Ueti et al., 2008; Scoles and Ueti, 2013), *T. equi*

can have transstadial transmission, i.e., acquired by immature stages of ticks and then inoculated into a new host by adult ticks (Zapf and Schein, 1994; Mehlhorn and Schein, 1998). Since there are tick species among the competent tick vectors of *T. equi* that typically infest rodents as larvae or nymphs, as exemplified by *Dermacentor reticulatus* (Nosek, 1972), findings of this study suggest that rodents can be a hitherto neglected group of reservoirs of this piroplasm and source of infection for ticks, necessitating further evaluation. The genotype A of *T. equi* is widespread in most countries across all continents, except Australia (Schnittger et al., 2022). It is restrictive to draw conclusions solely based on the outcomes of four positive samples, which account for approximately 1 % of the total prevalence rate. We will conduct larger-scale and more-location studies in the future to verify the role of mice as a storage host for *T. equi*.

Previously, *Theileria sergenti* Ikeda and *Theileria sergenti* Chitose were found to exhibit high polymorphism in the *ITS1–5.8S rDNA-ITS2* region, sharing only 36 % identity, indicating a level of dissimilarity comparable to that between *T. sergenti* Ikeda and the clearly divergent *Theileria mutans* (Aktas et al., 2007). In this study, a novel *Theileria* sp. Xinjiang also exhibited high genetic polymorphism in *ITS1–5.8S rDNA-ITS2*. The prevalence rate of *Theileria* sp. Xinjiang in mammals is 11.79 % (33/280). This phenomenon among *Theileria* species is known to have very important implications in diagnostic procedures (e.g., primer design) and vaccine development (Schnittger et al., 2002). Therefore, results of the present study highlight the need for further studies in this context, involving small mammals.

Previously, we found "Candidatus *Theileria xinjiangensis*" (referred to as *Theileria* sp. Xinjiang in this study) in great gerbils (*Rhombomys opimus*) (Ji et al., 2021). Detection of this novel piroplasm in further rodent species here confirms the importance of these mammals as potential hosts or reservoirs in its epidemiology. This is also supported by the phylogenetic clustering of *Theileria* sp. Xinjiang in the same clade with another small mammal-borne species, *T. youngi* (Balti et al., 2021).

5. Conclusions

Five piroplasm species, including *Babesia* sp. tavsan2, *B. occultans*, and three *Theileria spp*. were identified in small mammals and ticks using three genetic markers. *Theileria* sp. tavsan2 and *Theileria* sp. Kalecik were found in ticks and their hosts. Both *B. occultans* and *Theileria* sp. Kalecik were detected in small mammals for the first time. Further studies should explore molecular traits, reservoirs, vectors, and transmission routes of these and similar piroplasms.

Ethics approval and consent to participate

This study was approved by Animal Ethics Committee of Shihezi University (Approval No. A2022 029–01).

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CRediT authorship contribution statement

Yuan-Zhi Wang: Writing – review & editing, Methodology, Funding acquisition, Conceptualization. **Yujiang Zhang:** Methodology, Data curation. **Guoyu Zhao:** Methodology, Data curation. **Xuanchen Wu:** Resources, Methodology, Investigation. **Ente Li:** Writing – original draft, Methodology, Investigation, Conceptualization. **Chunju Zhang:** Methodology, Data curation. **Sándor Hornok:** Writing – review & editing, Conceptualization. **Meihua Yang:** Writing – review & editing, Formal analysis. **Lijuan Tang:** Writing – original draft, Validation, Investigation, Funding acquisition.

Declaration of Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability

The datasets generated during the current study are available in the GenBank repository, http://www.ncbi.nlm.nih.gov/genbank/.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetpar.2024.110304.

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