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Research article

Molecular identification of tick (Acari: Ixodidae) and tick-borne pathogens from Przewalski's gazelle (Procapra Przewalskii) and Tibetan sheep (Ovis aries) in Qinghai Lake National Nature Reserve, China

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ABSTRACT

The Qinghai Lake National Nature Reserve (QLNNR), renowned for its abundant natural resources and diverse ecological habitats, serves as an ideal environment for ticks, thereby increasing the risk of various tick-borne pathogens (TBPs) transmission. This study aimed to investigate the prevalence of TBPs in ticks collected from Przewalski's gazelle and Tibetan sheep within the QLNNR. A total of 313 tick samples were collected from the vicinity of Qinghai Lake. Tick species identification was conducted using both morphological and molecular biology techniques. Polymerase chain reaction (PCR) amplification was performed to detect the presence of spotted fever group (SFG) *Rickettsia*, *Coxiella burnetii*, *Anaplasma phagocytophilum*, *Babesia microti*, *Theileria* spp, *Borrelia burgdorferi*, *Brucella* spp, and *Anaplasma ovis* was performed using specific primers. Positive samples were sequenced and analyzed using BLASTn, followed by phylogenetic tree construction. The ticks collected from the Qinghai Lake area were identified as *Dermacentor nuttalli*. The overall prevalence rates of ticks carrying SFG *Rickettsia* and *C. burnetii* were 42.8 % (134/313) and 4.8 % (15/313), respectively. Three SFG *Rickettsia* species were detected, including *R. raoultii* 33.9 % (106/313), *R. slovaca* 3.8 % (12/113) and *R. sibirica* 7.7 % (24/113), with *R. raoultii* being the predominant species. The prevalence rates of SFG *Rickettsia*

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and *C. burnetii* in ticks from Tibetan sheep was 44.7 % (115/257) and 4.7 % (12/257), respectively,and in ticks from Przewalski's gazelle were 33.9 % (19/56) and 5.4 % (3/56). Furthermore, the study revealed a positive linear relationship between the abundance of Przewalski's gazelle and the number of ticks, as well as the prevalence of TBPs. The current study has identified *Dermacentor nuttalli* as the predominant tick vector species within the QLNNR region. The detection of SFG *Rickettsia* and *C. burnetii* has augmented our understanding of the epidemiological profile of ticks and TBPs in this area, thereby providing a robust theoretical foundation for the implementation of effective prevention and control strategies against TBPs.

Abbreviations

TBPs	Tick-borne pathogens
QTP	Qinghai-Tibet Plateau
QLNNR	Qinghai Lake National Nature Reserve
SFG	spotted fever group
EDTA	Ethylene Diamine Tetraacetic Acid
DNA	deoxyribonucleic acid
PCR	polymerase chain reaction
RNA	ribonucleic acid
16S rRNA	16S ribosomal ribonucleic acid
18S rRNA	18S ribosomal ribonucleic acid
dNTPs	deoxyribonucleotide triphosphate

1. Introduction

Ticks, as ectoparasitic arthropods, are known to parasitize a wide range of hosts, including humans, domestic animals, and various wildlife species. Their significance lies not only in their parasitic nature but also in their capacity to transmit a multitude of pathogens to their hosts, a role second only to that of mosquitoes [1,2]. *Dermacentor nuttalli* is primarily distributed in the southern regions of Russia and throughout Mongolia, including Selenge aimag, Tov aimag, and Dornogovi. In China, it is commonly found in the northwestern regions, including Xinjiang, Gansu, and Qinghai, and notably dominant on the Qinghai-Tibet Plateau (QTP) [3–7]. *Dermacentor nuttalli* is capable of transmitting a diverse array of pathogens, such as *Rickettsia raoultii*, *Candidatus Rickettsia tarasevichiae*, *Babesia venatorum*, and *Coxiella burnetii*, among others [8–10].

Rickettsia, a globally prevalent intracellular parasitic, gram-negative prokaryotic microorganism, is categorized into four principal groups based on interspecific evolutionary relationships: the *Rickettsia bellii* group*,* the *Rickettsia Canadensis group,* the spotted fever group (SFG) *Rickettsia,* and the typhus group *Rickettsia* (TGR) [11,12]. SFG *Rickettsia*, prevalent in ticks, can be transmitted vertically to the next generation via eggs and horizontally to host through bites, causing a spectrum of natural human-veterinary diseases with fever as the primary symptom [13]. Advancements in cultivation and molecular diagnostic technologies have confirmed the existence of at least 28 SFG *Rickettsia* species, with new species or genotypes continually being isolated from ticks [14]. In recent years, several novel tick-borne *Rickettsia* species have been identified in China, including *Candidutus Rickettsia jiaonani*, *Candidutus Rickettsia longicornii*, *Rickettsia yunnanensis*, and *Rickettsia erhaii* [15–18]. The emergence and reemergence of additional tick-borne pathogens (TBPs) also pose a significant threat to public health safety in China.

Since the large-scale outbreak of Q fever in the Netherlands from 2007 to 2010, new outbreaks of Q fever have continued to be reported globally [19]. In 2018 to 2019, a Q fever outbreak was experienced in Zhuhai City, China, with 138 patients diagnosed with Q fever, marking the first confirmed Q fever epidemic in modern Chinese cities [20]. In 2021, the outbreak of Q fever in northern Italy led to 14 tourists being infected [21]. Therefore, the prevention of Q fever was increasingly crucial. Q fever is one of the globally distributed zoonotic diseases caused by *C.burnetii* [22] *C. burnetii* can be transmitted not only through tick bites on the host but also through aerosol methods, infecting humans and animals [23,24]. The main clinical manifestations of Q fever are fever, headache, myalgia, and other flu-like symptoms. In severe cases, it can lead to complications such as chronic hepatitis, pneumonia, and myocarditis [25]. Pregnant females infected with *C. burnetii* may exhibit reproductive disorders such as abortion, stillbirths and sterility [26]. Most human infections with Q fever are associated with domestic animals, and individuals such as farmers, veterinarians, abattoir workers, and laboratory personnel are all at risk of infection [27,28].

The Qinghai Lake National Nature Reserve (QLNNR), situated in the northeast quadrant of the Qinghai-Tibetan Plateau (QTP), boasts a rich tapestry of natural resources and a diverse array of ecological species, creating an ideal habitat for ticks [29]. This sanctuary is home to the indigenous fauna of the QTP, including the Tibetan sheep, and the QTP-endemic and critically endangered Przewalski's gazelle, which is classified as a national first-level protected animal in China. Przewalski's gazelle predominantly inhabits the Bird Island, Chala Beach, and Xiaobei Lake regions in the northeast and west of Qinghai Lake, and is recognized as one of the

world's most imperiled ungulates [30]. Research by La et al. has identified the presence of parasites such as *Melophagus ovinus*, *Pediculus*, *Caenorhabditis elegans*, *Moniezia expanda*, *Cysticercus cellulosae* in the Przewalski's gazelle population of the Ganzi River area. Additionally, Wang et al. have discovered a novel species of *Eimeria* within the intestines of these gazelles [31,32]. However, the study of ectoparasites, particularly ticks, on wild Przewalski's gazelle remains relatively unexplored. Consequently, this investigation centers on the surface ticks of the Tibetan sheep and Przewalski's gazelle, indigenous to the QTP, to ascertain the tick species prevalent on these mammals within the QLNNR and to evaluate the prevalence of tick-borne pathogens (TBPs). The findings aim to provide a scientific foundation for the regional management and control of ticks and TBPs.

2. Methods

2.1. Sample collection and DNA extraction

In the current investigation, a comprehensive collection of 313 tick specimens was procured from 10 designated sampling locations within the Qinghai Lake National Nature Reserve (QLNNR: Yongfeng: 101°07'65''E, 36°91'11''N, Reshui: 100°60'85''E, 37°15'89''N, Gahai: 100°47'95''E, 37°14'20''N, Dayu: 100°83'90''E, 36°98'40''N, Rixiang: 100°81'27''E, 37°01'21''N, Dongda: 101°05'39''E, 36°88'13''N, Xicha: 100°59'12''E, 36°53'58''N, Wendu: 100°65'41''E, 36°96'17''N, Dezhou: 100°59'14''E, 36°06'19''N). From April 1st to April 14th, 2022, ticks were collected from animal surfaces. *Dermacentor silvarum* and *D. nuttalli* are morphologically similar. PCR-RFLP method was carried out to identify *D. silvarum* and *D. nuttalli* through Spe I existed in their ITS2 seqeunces. Previous studies have shown that no Spe I site in ITS2 sequence of *D. silvarum* but one in *D. nuttalli*, which suggested that PCR-RFLP based on ITS2 sequences can distinguish *D. silvarum* from *D. nuttalli*.Among these, 56 tick samples were meticulously gathered from the eight captive populations of Przewalski's gazelle by the dedicated personnel of the Qinghai Lake Wildlife Rescue and Testing Center on April 14, 2022, as depicted in Fig. 1. Owing to the 2.44 km2 expanse of the anthropogenically protected area, the indigenous traits of the wild Przewalski's gazelle populace have been largely conserved. The gender of the Przewalski's gazelle was determined through the distinctive phenotypic markers: males are characterized by a pair of ebony horns, whereas females are hornless. The acquired ticks were meticulously preserved in sterile collection vessels, hermetically sealed with breathable cotton fabric to maintain their respiratory functions, and promptly transported to the laboratory for further analysis. Prior to experimentation, the ticks underwent a thorough cleansing process with 70 % ethanol and 10 × PBS to eliminate potential environmental contaminants. Each sanitised tick was then placed in a 1.5 ml centrifuge tube and immersed in liquid nitrogen for a duration of 2 min to facilitate pulverization. Subsequently, the ticks were reduced to a fine powder using a pestle and mortar, to which protease K (manufactured by Magen, China) was added for an extensive overnight digestion. To conclude, the Hipure Insect DNA kit (also provided by Magen, China) was employed for the extraction of DNA, which was then stored at a temperature of −80 °C to ensure its integrity for subsequent use.

2.2. Identification of tick species

Ticks were identified through a combination of morphological characteristics, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques and phylogenetic analysis [33,34]. Morphological identification, mainly based on the characteristic of the anal groove, was carried out by an experienced acarologist to determine the tick species initially [8]. The peritremes of male ticks is punctate, while the peritremes of female ticks is elliptical.The PCR amplification targeted the tick's internal

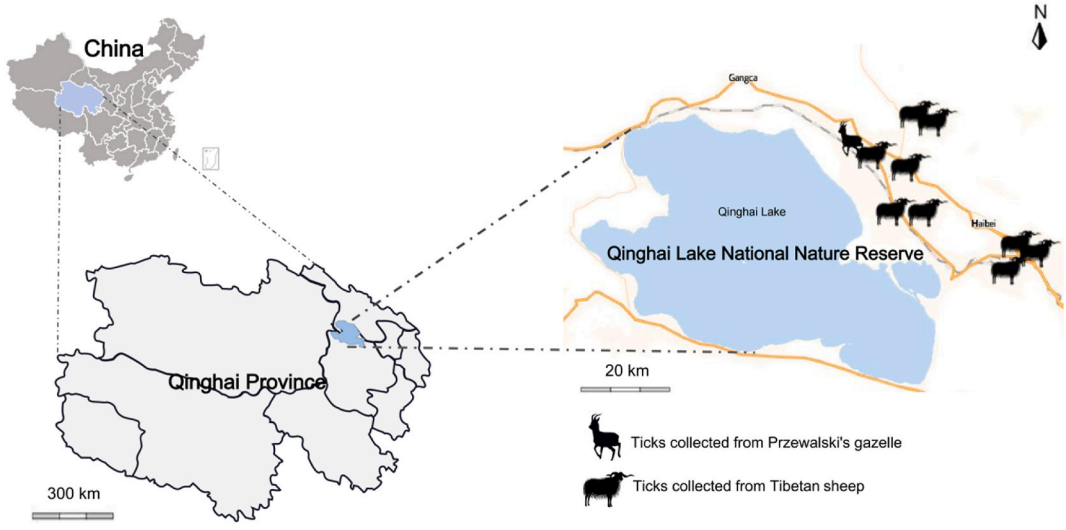

Fig. 1. Map of the Qinghai Lake National Nature Reserve the sampling sites. The Przewalski's gazelle and Tibetan sheep icon indicates the location of the sample collection in this study.

transcribed spacer 2 (ITS2) gene within a reaction volume of 10 μl, which included 1 μl of DNA template, 0.5 μl each of forward and reverse primers (100 μM), 0.1 μl of Taq polymerase (0.5 U; New England BioLab, USA), 0.2 μl of deoxyribonucleotide triphosphate (200 μM; New England BioLab, USA), 1 μl of 10 × ThermoPol Reaction Buffer (New England BioLab, USA), and 6.7 μl of double-distilled water. Subsequently, the PCR products were digested with the restriction endonuclease Spe I (TransGen, China) and separated on a 1 % agarose gel (TransGen, China), with the results visualized under ultraviolet light.

2.3. Detection of tick borne pathogens

The extracted tick DNA served as a template for PCR amplification with synthesized primers (Table 1). The PCR reaction, with a volume of 10 μl, contained 3 μl of DNA template, 0.5 μl each of forward and reverse primers (100 μM), 0.1 μl of Taq polymerase (0.5 U; New England BioLab, USA), 0.2 μl of deoxyribonucleotide triphosphate (200 μM; New England BioLab, USA), 1 μl of 10 × ThermoPol Reaction Buffer (New England BioLab, USA), and 4.7 μl of double-distilled water. The DNA samples from blood of animals infected with the respective pathogens were used as positive control and were collected and stored properly from previous studies. ddH2O was used as a negative control, and the PCR products were subsequently electrophoresed on a 1.5 % agarose gel.

2.4. Sequencing and phylogenetic analyses

The purified PCR products were obtained using the EasyPure Quick Gel Extraction Kit (manufactured by TransGen, China) and subsequently cloned into the *Escherichia coli* DH5α strain with the PMDTM 19-T Vector Cloning Kit (provided by TaKaRa, Japan). Positive clones were then selected and sent for sequencing at Sangon Biotech (Shanghai) Co., Ltd. Check for any misreading in the sequencing peak map and correct it to ensure the accuracy of the target gene fragment.The gene fragments obtained from the sequencing were spliced and trimmed using DNAStar and CmSuite8 software, and their nucleotide sequence homology was determined through GenBank BLASTn analysis (available at https://blast.ncbi.nl-m.nih.gov/Blast.cgi). Select high-quality, gene-specific, and sequence length similar sequences from GenBank. Phylogenetic trees were constructed using the neighbor-joining method and p-distance model in MEGA 11.0, with Bootstrap set to 1000, and the constructed phylogenetic trees were enhanced using tvBOT (available at https://www.chiplot.online/tvbot.html) [43].

2.5. Statistical analysis

A chi-square test was performed to evaluate between the infection rate of SFG *Rickettsia* and *C.burnetii* the different hosts, with observed differences considered statistically significant when p-value <0.05 (calculator available at https://www.mathsisfun.com/data/chisquare-calculator.html).

Table 1
Primers used in this study to detect tick-borne pathogens (TBPs) infections in ticks on the Qinghai Lake National Nature Reserve.

Organism	Target gene	Primer sequence (5'~3')	Fragment (bp)	Note	References
Tick	ITS2	CGAGACTTGGTGTGAATTGCA TCCCATACACCACATTTCCCG	137–1695		[33]
SFG Rickettsia	*ompA*	GCTTTATTCACCACCTCAAC TRATCACCACCGTAAGTAAAT	209/212		[34]
	Sca4	CGATGGTAGCATTAAAAGCT CTTGCTTTTCAGCAATATCAC	624		[35]
A. phagocytophilum	16S rRNA	CACATGCAAGTCGAACGGATTATTC TTCCGTTAAGAAGGATCTAATCTCC	932	1st PCR	[36]
		AACGGATTATTCTTTATAGCTTGCT GGCAGTATTAAAAGCAGCTCCAGG	546/565	Nested PCR	
B. microti	18S rRNA	CTTAGTATAAGCTTTTATACAGC ATAGGTCAGAAACTTGAATGATACA	238	1st PCR	[37]
		GTTATAGTTTATTTGATGTTCGTTT AAGCCATGCGATTCGCTAAT	154	Nested PCR	
Theileria spp.	18S rRNA	GAAACGGCTACCACATCT AGTTTCCCCGTGTTGAGT	778	1st PCR	[38]
		TTAAACCTCTTCCAGAGT TCAGCCTTGCGACCATAC	581	Nested PCR	
B. burgdorferi	23S rRNA	AGAAGTGCTGGAGTCGA TAGTGCTCTACCTCTATTAA	261		[39]
C. burnetii	*htpB*	GCGGGTGATGGTACCACAACA GGCAATCACCAATAAGGGCCG	501	1st PCR	[40]
		TTGCTGGAATGAACCCCA TCAAGCTCCGCACTCATG	325	Nested PCR	
Brucella spp.	*omp22*	TGATGGGAGGGACCGACTA TGGTTCTTCAGGTTGTTACGC	494		[41]
F. tularensis	16S rRNA	CAGGCCTAACACATGCAAGTC GGGCGGWGTGTACAAGGC	1300		[42]

3. Results

3.1. Identification of ticks

This study utilized three methods for tick species identification: morphological identification, PCR-RFLP, and phylogenetic analysis. Preliminary morphological identification of all tick samples indicated they belonged to the species *D. nuttalli* (Fig. 2). The basis capituli is rectangular, and the scutum is widest at the peritreme, with a strong enamel color. The peritremes of male ticks is punctate, while the peritremes of female ticks is elliptical. The PCR-RFLP results further confirmed this, as the ITS2 fragment was cut into two bands with sizes of 992 bp and 275 bp, consistent with *D. nuttalli* identification (Additional file 1: Fig. S1). Thirty randomly selected tick specimens were cloned and sequenced for the ITS2 gene PCR amplification products. BLASTn analysis and phylogenetic analysis revealed extensive homology between the ITS2 gene sequence of the tick samples in this study and the published sequences of *D. nuttalli* from Russia (KF241869) and Xinjiang, China (KC203427), clustering them into the same branch (Fig. 3).

3.2. Infection rates of tick-borne pathogens

The DNA extracted from the tick genome served as a template to amplify various pathogens, including spotted fever group *Rickettsia*, *Coxiella burnetii*, *Anaplasma phagocytophilum*, *Babesia microti*, *Theileria* spp, *Borrelia burgdorferi*, *Brucella* spp, and *Anaplasma ovis*. Notably, SFG *Rickettsia* and *C. burnetii* exhibited distinct electrophoretic bands that corresponded to our expectations. In our current study, the overall prevalence of SFG *Rickettsia* was 42.8 % (134/313), with 44.7 % (115/257) of ticks from Tibetan sheep body surface and 33.9 % (19/56) from Przewalski's gazelle body surface being positive (Table 2). Subsequent sequencing of the SFG *Rickettsia*-positive samples revealed the presence of three species, with *Rickettsia raoultii* being the most prevalent at 33.9 % (106/313), followed by *Rickettsia slovaca* at 3.8 % (12/113), and *Rickettsia sibirica* at 7.7 % (24/113). Statistical analysis using the chi-square test indicated no significant correlation between the infection rate of SFG Rickettsia and different host animals ($\chi 2 = 0.3$, df = 1, p-value >0.05). The overall prevalence of *Coxiella burnetii* was 4.8 % (15/313), with 4.7 % (12/257) of ticks from Tibetan sheep and 5.4 % (3/56) from Przewalski's gazelle testing positive (Table 2). Statistical tests revealed no significant difference in the infection rate of *C.burnetii* between the two tick host species ($\chi 2 = 0.2$, df = 1, p-value >0.05). Among the 145 positive ticks, 8.3 % (12/145) were found to be co-infected with multiple pathogens. Notably, co-infection with *R. raoultii* and other pathogens was observed most frequently.

3.3. Sequencing and phylogenetic analysis of spotted fever group Rickettsia ompA gene

A total of 18 sequences of the *ompA* gene from the spotted fever group *Rickettsia* were successfully acquired and deposited in GenBank, where they were assigned accession numbers OQ253257-OQ253274 (refer to Table 3). The degree of identity among the sequences of *R. raoultii*, *R. sibirica*, and *R. slovaca* was found to be 98.6–100 %, 99.1–100 %, and 100 %, respectively. A phylogenetic tree was constructed using 13 of the uploaded SFG *Rickettsia ompA* gene sequences (Fig. 2). The phylogenetic analysis revealed that four sequences (OQ253257, OQ253259, OQ253260, and OQ253264) are closely related to *R. raoultii* (MK721057, MK307884) strains isolated from both horses and humans in Xinjiang, China. Additionally, one sequence (OQ253272) was found to be associated with *R. sibirica* (OR117584) isolated from *Rattus andamanensis* in Guangxi, China, and another sequence (OQ253274) was linked to the *R. slovaca* sequence (MN536157) isolated from yaks in Qinghai, China (Fig. 4).

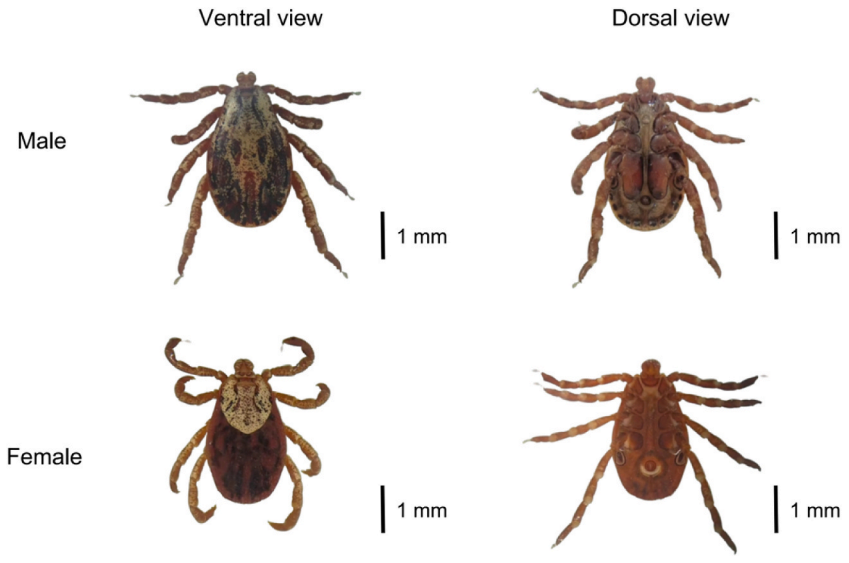

Fig. 2. The dorsal view and ventral view of *Dermacentor nuttalli*.

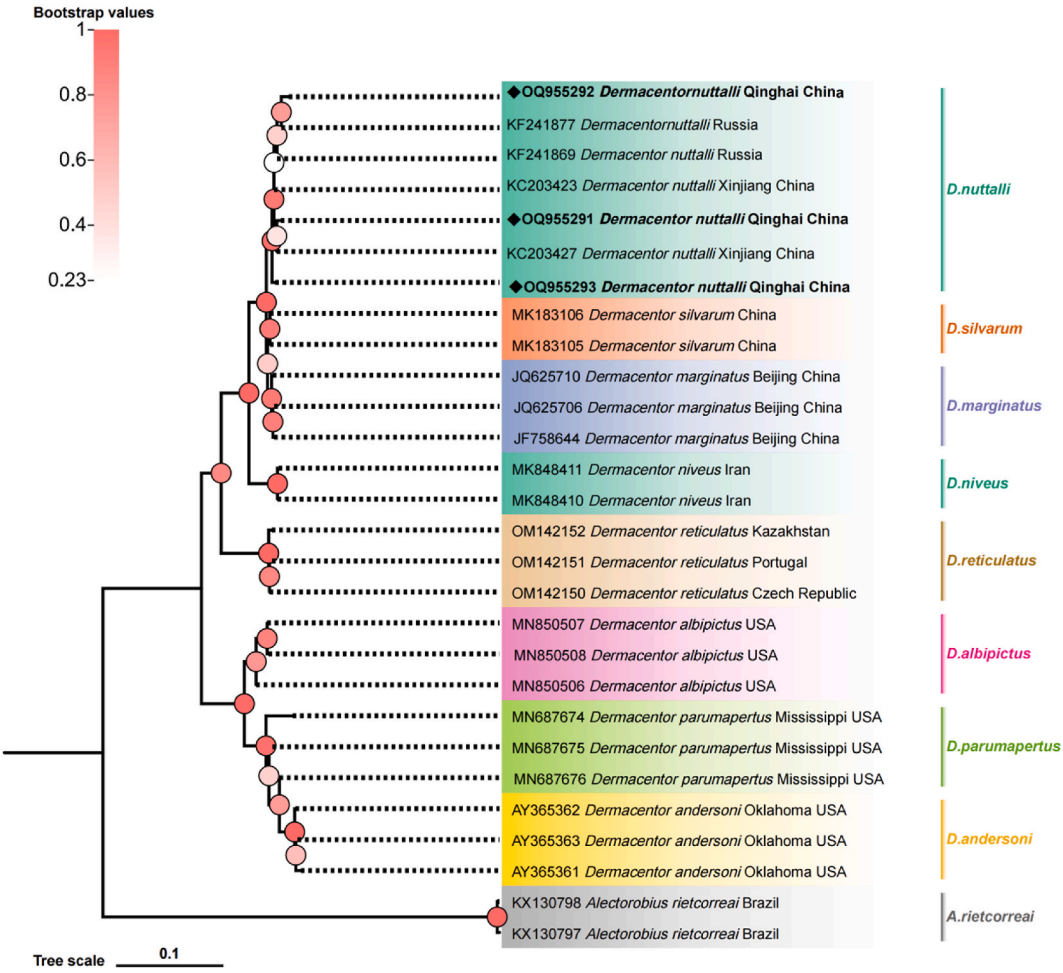

Fig. 3. Phylogenetic tree based on the internal transcribed spacer 2 gene of *Dermacentor* spp. obtained in this study. Bootstraps analysis was performedwith 1000 replicates. The solid diamond and bolded represent the sequence of this study.*Alectorobius rietcorreai* (KX130798, KX130797) was used as outgroup.

Table 2
The positive samples of spotted fever group *Rickettsia* and *Coxiella burnetii* in ticks in this study.

Organism	Number and prevalence (%) of individual ticks positive for single and co-infections		
	Przewalski's Gazelle (n = 56)	Tibetan sheep (n = 257)	Total sample (n = 313)
Single			
R. raoultii (R.rao)	13 (23.2)	81 (31.5)	94 (30.0)
R. sibirica (*R.sib*)	2 (3.6)	17 (6.6)	19 (6.1)
R. slovaca (*R.slo*)	1 (1.8)	8 (3.1)	9 (2.9)
C. burnetii (C.bur)	2 (3.6)	9 (3.5)	11 (3.5)
Double			
R. rao + *R. sib*	1 (1.8)	4 (1.6)	5 (1.6)
R. rao + *R. slo*	1 (1.8)	2 (0.8)	3 (1.0)
R. rao + *C. bur*	1 (1.8)	3 (1.2)	4 (1.3)
Total			
R. raoultii	16 (28.6)	90 (35.0)	106 (33.9)
R. sibirica	3 (5.4)	21 (8.2)	24 (7.7)
R. slovaca	2 (3.6)	10 (3.9)	12 (3.8)
SFG *Rickettsia*	19 (33.9)	115 (44.7)	134 (42.8)
C. burnetii	3 (5.4)	12 (4.7)	15 (4.8)
Co-infections	3 (5.4)	9 (3.5)	12 (3.8)

Table 3
DNA sequences of TBPs obtained in this study.

Obtained sequences				The closest Blastn match		
Organism	Target gene	Accession number	Length (bp)	Identity (%)	Pathogen isolate	Accession number (host, country)
R. raoultii	*ompA*	OQ253257	209	100	*R. raoultii*	MN450413 *Haemaphysalis longicornis* China
	ompA	OQ253258	209	100	*R. raoultii*	MN536158 yak China
	ompA	OQ253259	209	100	*R. raoultii*	MN450412 *Dermacentor nuttalli* China
	ompA	OQ253260	209	99.52	*R. raoultii*	MN450413 *Haemaphysalis longicornis* China
	ompA	OQ253261	209	99.52	*R. raoultii*	MN536158 yak China
	ompA	OQ253262	209	100	*R. raoultii*	MN394799 yak China
	ompA	OQ253263	209	99.52	*R. raoultii*	MN450413 *Haemaphysalis longicornis* China
	ompA	OQ253264	209	100	*R. raoultii*	MK307883 horse China
	ompA	OQ253265	209	99.52	*R. raoultii*	MN536158 yak China
	ompA	OQ253266	209	99.52	*R. raoultii*	MN450413 *Haemaphysalis longicornis* China
	ompA	OQ253267	209	99.52	*R. raoultii*	MN450413 *Haemaphysalis longicornis* China
	ompA	OQ253268	209	99.52	*R. raoultii*	MN450413 *Haemaphysalis longicornis* China
	ompA	OQ253269	209	99.52	*R. raoultii*	MN536158 yak China
R. sibirica	*ompA*	OQ253270	212	100	*R. sibirica*	MG811698 *Urocitellus undulatus* China
	ompA	OQ253271	212	100	*R. sibirica*	MN394806 yak China
	ompA	OQ253272	212	100	*R. sibirica*	MG598412 *Dermacentor nuttalli* China
	ompA	OQ253273	212	99.52	*R. sibirica*	MG811698 *Urocitellus undulatus* China
R. slovaca	*ompA*	OQ253274	216	100	*R. slovaca*	MN394806 yak China
R. raoultii	*Sca4*	OQ253275	624	99.04	*R. raoultii*	KP768191 *Dermacentor reticulatus* Ukraine
	Sca4	OQ253276	624	99.20	*R. raoultii*	KP768191 *Dermacentor reticulatus* Ukraine
	Sca4	OQ253277	624	99.36	*R. raoultii*	KP768191 *Dermacentor reticulatus* Ukraine
	Sca4	OQ253278	624	100	*R. raoultii*	AY331397 *Dermacentor sinicus* China
	Sca4	OQ253279	624	99.84	*R. raoultii*	CP098324 *Dermacentor silvarum* China
	Sca4	OQ253280	624	99.84	*R. raoultii*	CP098324 *Dermacentor silvarum* China
	Sca4	OQ253281	624	100	*R. raoultii*	CP098324 *Dermacentor silvarum* China
R. sibirica	*Sca4*	OQ253282	624	99.84	*R. sibirica*	AY331397 *Dermacentor sinicus* China
	Sca4	OQ253283	624	99.68	*R. sibirica*	MN394810 yak China
R. slovaca	Sca4	OQ253284	624	100	*R. slovaca*	MN581997 *Dermacentor* Pakistan
	Sca4	OQ253285	624	99.84	*R. slovaca*	MN581997 *Dermacentor* Pakistan
C. burnetii	*htpB*	OQ865125	325	99.08	*C. burnetii*	MK416231 *Hyalomma impeltatum* Tunisia
	htpB	OQ865126	325	100	*C. burnetii*	MK416231 *Hyalomma impeltatum* Tunisia
	htpB	OQ865127	325	99.69	*C. burnetii*	MK416231 *Hyalomma impeltatum* Tunisia
	htpB	OQ865128	325	99.08	*C. burnetii*	MK416231 *Hyalomma impeltatum* Tunisia

3.4. Sequencing and phylogenetic analysis of spotted fever group Rickettsia sca4 gene

Furthermore, the study also obtained 11 sequences of the *sca4* gene from SFG *Rickettsia* (OQ253275-OQ253285). The identity among the sequences of *R. raoultii*, *R. sibirica*, and *R. slovaca* was 98.6–100 %, 99.5–100 %, and 99.8–100 %, respectively. The phylogenetic analysis of the *sca4* gene of SFG *Rickettsia* demonstrated that one sequence (OQ253277) was grouped with *R. raoultii* (KP768191) isolated from *Dermacentor reticulatus* in Ukrainian. Two sequences (OQ253282, OQ253283) were associated with *R. sibirica* (HM050295, DQ097084) isolated from *Hyalomma truncatum* in Senegal and *Homo sapiens* in France, and two sequences (OQ253284, OQ253285) were linked to the *R. slovaca* sequence (MN581997) isolated from *Dermacentor* in Pakistan (Fig. 5).

3.5. Sequencing and phylogenetic analysis of Coxiella burnetii htpB gene

In this study, a total of four *Coxiella burnetii htpB* gene sequences (OQ865125 - OQ865128) were obtained, and the identity between each sequence was 98.2–100 %, The OQ865126 sequence was clustered on the same branch as the sequence of *C. burnetii* (MK307875, EF547935, OQ872774, OQ872773, JQ346185, MH751473) isolated from horse, lion, tick, sheep, *Amblyomma variegatum*, and *Rhipicephalus evertsi* in China's Xinjiang, Italy, China's Qinghai, Nigeria, and South Africa, with a identity of 99.39–100 % (Fig. 6).

3.6. Analysis of the prevalence of ticks and TBPs in different populations of Przewalski's gazelle

This research has uncovered substantial disparities in the prevalence of ticks and tick-borne pathogens among the female and male populations of Przewalski's gazelle across eight distinct populations. A detailed examination of the collected samples indicated that the majority of ticks were retrieved from female specimens, with a significantly lower number collected from males (refer to Table 4). During the non-breeding period in April, it is observed that male Przewalski's gazelles typically lead solitary lives, while the females congregate in groups. It is only during the breeding season in December that both sexes integrate, thereby amplifying the population's size. The study has also established a positive linear correlation between the population abundance of Przewalski's gazelle and the incidence of ticks, as well as the prevalence of tick-borne pathogens (refer to Fig. 7).

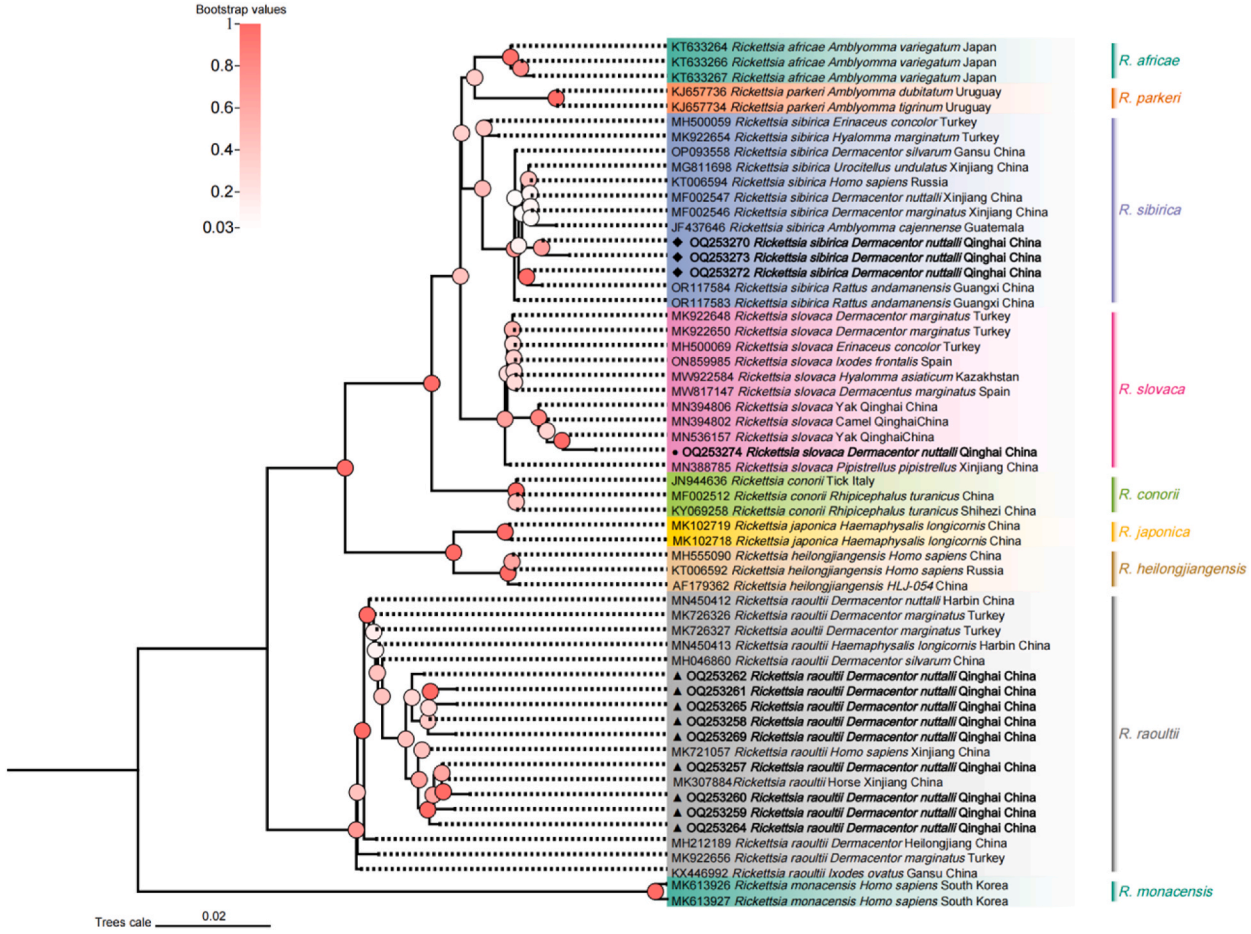

Fig. 4. Phylogenetic tree based on *ompA* particle sequences of spotted fever group *Rickettisa* obtained in this study. Bootstraps analysis was performed with 1000 replicates. The solid triangle indicates sequences from *Rickettsia raoultii*, the solid circle indicates sequences from *Rickettsia slovaca*, and the solid diamond indicate sequences from *Rickettsia sibirica.* All sequences from this study are bolded.

4. Discussion

This research delineates the collection of ticks from the body surfaces of the globally endangered Przewalski's gazelle, situated around Qinghai Lake on the Qinghai-Tibet Plateau, encompassing their identification and an epidemiological survey of tick-borne pathogens (TBPs). As global research intensifies, an increasing array of pathogens, including severe fever with thrombocytopenia syndrome virus (SFTSV), Heartland virus (HRTV), and Bourbon virus (BRBV), are being identified as tick-transmitted [1,44]. This phenomenon poses an escalating global health threat to both humans and animals. Notably, the Przewalski's gazelle population in the Qinghai Lake region has been significantly impacted by external parasitic infections, contributing to mortality and posing conservation challenges [45]. The Qinghai Lake area is recognized as a hotspot for parasitic diseases [31], with local inhabitants and grazing yaks and Tibetan sheep potentially engaging in cross-infections with Przewalski's gazelle, thereby imperiling the gazelle population, as well as human and livestock health [30]. To investigate potential disparities in tick species and TBPs harbored on the body surfaces of Przewalski's gazelle and local grazing Tibetan sheep, this study extended to collect ticks from the latter and conducted a comprehensive identification and epidemiological survey of TBPs.

In the present study, the prevalence rates of spotted fever group *Rickettsia* and *Coxiella burnetii* in ticks sampled from Tibetan sheep determined to be 44.7 % and 4.7 %, respectively. Correspondingly, the prevalence rates in ticks collected from Przewalski's gazelle were 33.9 % and 5.4 %. Overall, the quantity of ticks harvested from Tibetan sheep was greater, and the infection rate of TBPs was higher compared to those found in Przewalski's gazelle. This discrepancy is thought to be attributed to differences in host behavior and habitat use. The smaller herd size of Przewalski's gazelle, typically consisting of only two to eight individuals per group during the non-mating season [46,47]. The unique cluster behavior of Przewalski's gazelle, where the population of Proctor's gazelle has reached its maximum during the mating season, which occurs annually in November to January of the coming year, may contribute to a reduced exposure to ticks. Furthermore, the solitary behavior of females prior to giving birth further diminishes the cluster size. Previous study have shown tick abundance was positively associated with deer abundance [48]. It is hypothesized that this behavioral adaptation

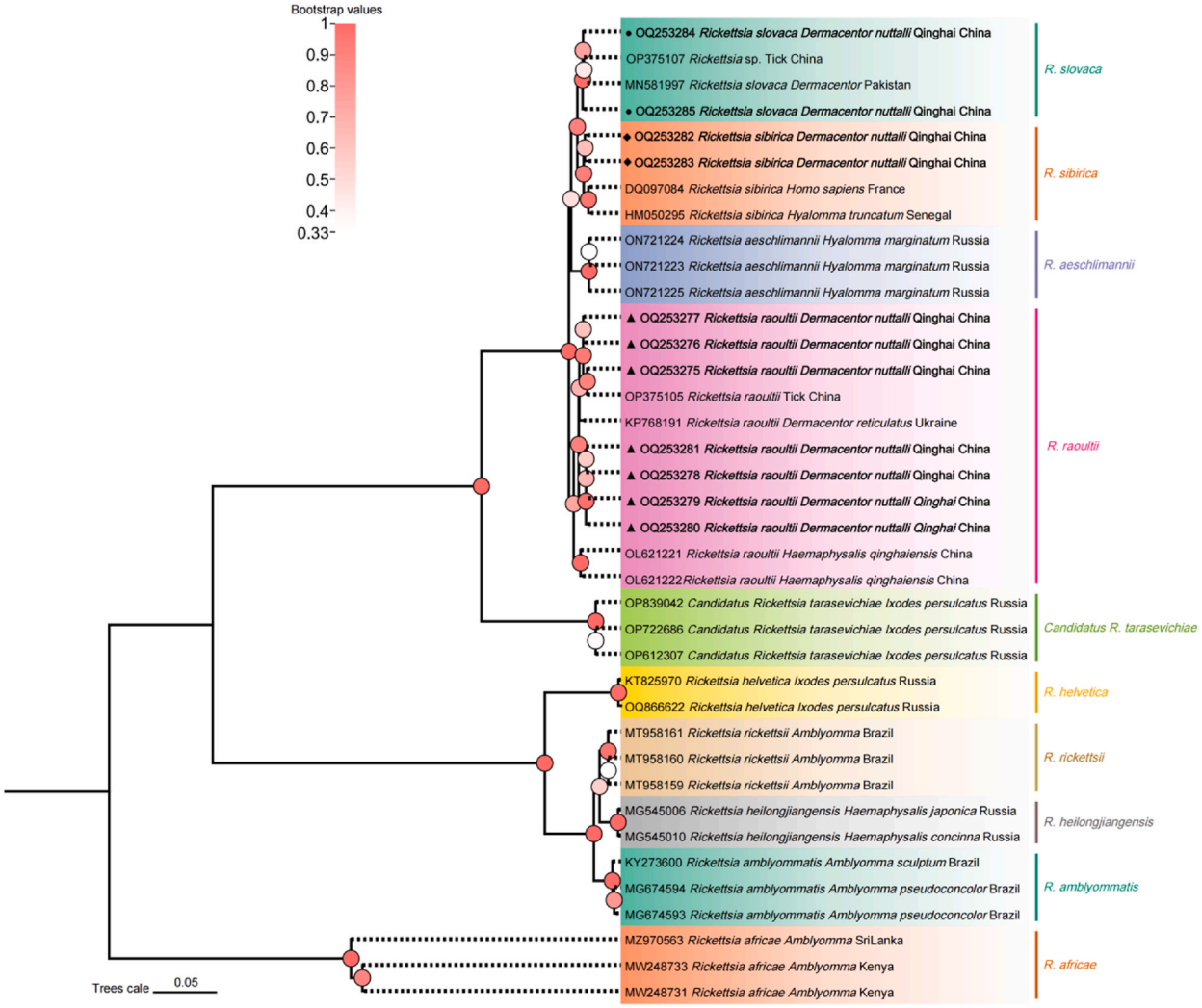

Fig. 5. Phylogenetic tree based on *sca4* particle sequences of spotted fever group *Rickettisa* obtained in this study. Bootstraps analysis was performed with 1000 replicates. The solid triangle indicates sequences from *Rickettsia raoultii*, the solid circle indicates sequences from *Rickettsia slovaca*, and the solid diamond indicate sequences from *Rickettsia sibirica*. All sequences from this study are bolded.

effectively reduces the rate of mutual infection of ticks among individuals of Przewalski's gazelle.

In the present study, we observed a notable occurrence of mixed infections by two pathogens within *Dermacentor nuttalli*, as detailed in Table 2. Prior investigations have demonstrated that *D.nuttalli* is capable of harboring multiple pathogens concurrently, with the most prevalent being the dual co-infection of *R. raoultii* and the *Coxiella-like endosymbiont* (CLE), as well as the triple co-infection involving *R. raoultii*, *Anaplasma* spp., and CLE in Inner Mongolia, China [49]. The tendency for *R. raoultii* to be co-infected with other pathogen aligns with our findings, as evidenced by the discovery of mixed infections between *R. raoultii* and *R. sibirica*, *R. slovaca*, and *C. burnetii* in our study. This correlation may be attributed to the significantly higher infection rate of *R. raoultii* compared to the other three pathogens. These findings corroborate recent research indicating an upward trend in the detection rate of spotted fever group (SFG) *Rickettsia* in ticks, thereby increasing the likelihood of mixed infections with other pathogens [50–53].

Over the past quarter-century, the global prevalence of tick-borne pathogens has been on the rise, underscoring their growing significance as a public health concern [1]. To date, over 30 SFG *Rickettsia* species have been reported globally, with 21 confirmed as pathogenic [54]. Notably, *D. nuttalli* from various regions in China, including Xinjiang, Yunnan, Gansu, Shanxi, Heilongjiang, Jilin, and Inner Mongolia, have been identified as carriers of SFG *Rickettsia* species such as *R. raoultii*, *R. sibirica*, and *R. slovaca* [55–58]. Moreover, the presence of SFG *Rickettsia* in ticks has been documented in border areas between China and its neighboring countries. Specifically, *R. raoultii* and *R. sibirica* have been detected on ticks from long-tailed ground squirrels in proximity to the China-Kazakhstan border [59]. SFG *Rickettsia* has been detected on the body surfaces of wild rodents' ticks in the northwestern border region of China [60]. Additionally, *R. raoultii* and *R. sibirica* have been identified as being carried by *D.nuttalli* in neighboring countries, Mongolia and Russia [61,62]. As personnel and trade exchanges with neighboring countries intensify, the risk of transmission of TBPs

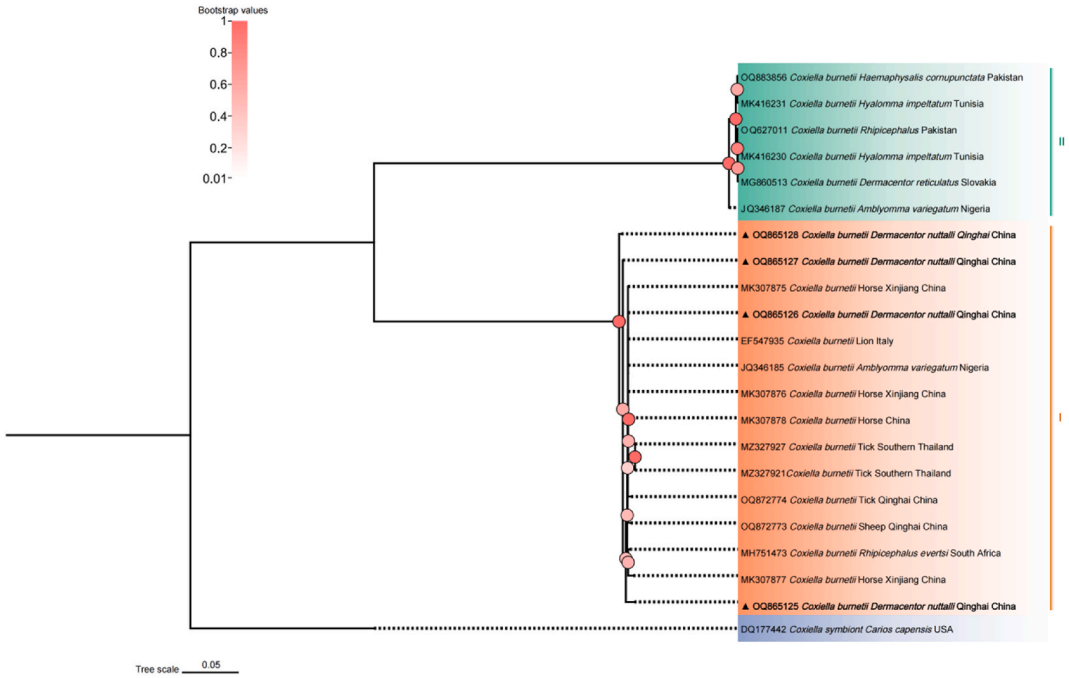

Fig. 6. Phylogenetic tree based on *htpB* particle sequences of *Coxiella burnetii* obtained in this study. Bootstraps analysis was performed with 1000 replicates. The solid triangle and bolded represent the sequence of this study. *Coxiella symbiont* (DQ177442) was used as outgroup.

Table 4
The number and prevalence of ticks and TBPs among different populations of Przewalski's Gazelle in Qinghai Lake National Nature Reserve.

Przewalski's Gazelle	Cluster type	Number	Collecting ticks	SFG *Rickettsia* prevalence (%)	*C. burnetii* prevalence (%)
Group 1	Male	1	N (0)	0 (0.0)	0 (0.0)
Group 2	Female	6	Y (10)	3 (30.0)	0 (0.0)
Group 3	Female	5	Y (13)	5 (38.5)	1 (7.7)
Group 4	Female	7	Y(11)	4 (36.4)	0 (0.0)
Group 5	Male	3	Y(6)	2 (33.3)	1 (16.7)
Group 6	Male	1	N (0)	0 (0.0)	0 (0.0)
Group 7	Male	1	N (0)	0 (0.0)	0 (0.0)
Group 8	Female	8	Y(16)	5 (31.3)	1 (6.3)

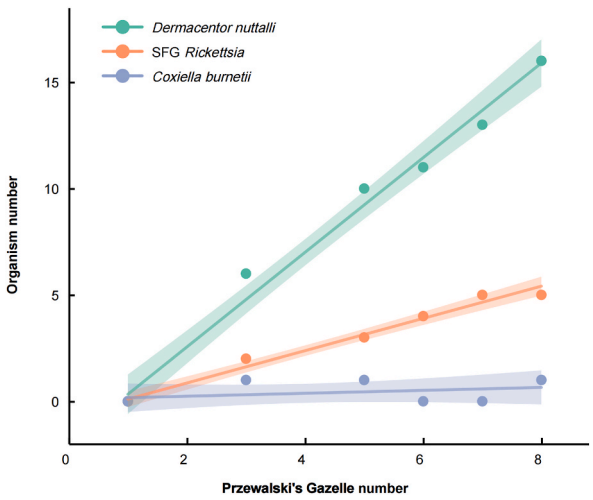

Fig. 7. The relationship between the number of individuals in the Przewalski gazelle population and the prevalence of ticks and tick borne diseases.

continues to escalate. It is noteworthy that our previous research isolated *R. raoultii* from yaks, Tibetan sheep, and *Haemaphysalis qinghaiensis* on the QTP, with respective positivity rates of 5.9 %, 0.3 %, and 54.1 % [63]. However, the positive rate of *R. raoultii* infection in *D. nuttalli* in the present study was 33.9 %, which is relatively lower compared to the infection rate in *Haemaphysalis qinghaiensis*. This discrepancy may be due to differences in environmental conditions, tick species. QTP It is evident that both *Haemaphysalis qinghaiensis* and *D. nuttalli* in the region are infected with *R. raoultii* at a relatively high prevalence. Consequently, the local livestock economy on the QTP is significantly impacted by ticks and TBPs.

Since the initial discovery of Q fever in Australia in 1937, reports of the disease have been on the rise both domestically and internationally, making it one of the most extensively distributed zoonotic diseases today [64]. In the border area of the Xinjiang Uygur Autonomous Region in China, the infection rate of *C. burnetii* in *D. nuttalli* was found to be 58.91 % (205/348) [9]. Moreover, *C.burnetii* was detected in ticks collected from goats (7.7 %) and sheep (31.0 %) in Pakistan [65]. Additionally, *D. nuttalli* in Mongolia, ticks in Pakistan, and *Hyalomma dromedarii* collected from camels in Egypt were found to carry SFG *Rickettsia*, with varying infection rates of 90.4 %, 5.4 %, and 57.1 %, respectively [66–68]. In this study, the infection rates of SFG *Rickettsia* and *C. burnetii* carried by *D. nuttalli* in QLNNR were 42.8 % and 4.8 %, respectively. These findings suggest the presence of similar TBPs in Mongolia, Pakistan, Egypt, Xinjiang, and Qinghai, China, all of which are located along the East Africa-West Asia migratory route. QLNNR serves as a habitat for migratory birds, and the tick-bird association contributes to the dissemination of ticks and TBPs. Although tick infection in migratory birds does not necessarily imply the establishment of a new species in a new territory, global warming may alter the boundaries of their original distribution range and increase the risk of introducing new pathogens [69]. Therefore, further investigations are necessary to better understand the role of migratory birds in the introduction and spread of TBPs in the QLNNR.

The genes predominantly employed for species identification within the *Rickettsia* genus encompass *ompA*, *Sca4*, *ompB*, 16S rRNA, and *gltA* [70]. Notably, the 5' end of the *ompA* gene exhibits high specificity and has been extensively utilized in the identification and classification of SFG *Rickettsia* genotypes or subtypes [71]. The *Sca4* gene has also been employed to effectively differentiate members of the genus *Rickettsia* [36]. In prior studies, the *ompA* and *Sca4* genes were specifically utilized to detect SFG *Rickettsia* [72–75]. In the present study, these genes were targeted to detect SGF *Rickettsia* in ticks, with the research indicating the presence of SGF *Rickettsia* in ticks collected from the body surfaces of Przewalski's gazelle and Tibetan sheep. Detected species included *R.raoultii* (33.9 %), *R. slovaca* (3.8 %), and *R.sibirica* (7.7 %), with *R. raoultii* being the predominant species. This finding aligns with previous research on Qinghai *Dermacentor nuttalli* and *Haemaphysalis qinghaiensis* in China [61,76]. For the identification of Coxiella burnetii, genes such as *IS1111*, *icd*, *scvA*, *p1*, *GroEL*, and *htpB* genes are commonly utilized [77]. The *htpB* gene, in particular, is highly conserved and considered universal across various *C. burnetii* types [78]. Consequently, it is frequently used as a target for identifying *C. burnetii*. This study successfully obtained four *C. burnetii* sequences, with one sequence (OQ865126) sharing 99.39 %–100 % homology with *C. burnetii* sequences isolated from regions including Xinjiang, Italy, Qinghai, Nigeria, and South Africa. This suggests a widespread geographical distribution of *C. burnetii* across the globe.

In summary, the research conducted has identified the presence of spotted fever group *Rickettsi*a and *Coxiella burnetii* within *Dermacentor nuttalli* collected from Przewalski's gazelle and Tibetan sheep. The study has also demonstrated a positive linear correlation between the population density of Przewalski's gazelle and the prevalence of ticks, as well as the incidence of TBPs. However, the potential impact of seasonal variations in the population size and composition of Przewalski's gazelle on the dissemination of ticks and TBPs requires further examination.

5. Conclusions

This study focuses on the prevalence of tick-borne pathogens in ticks obtained from the globally endangered Przewalski's gazelle and from Tibetan sheep residing in the Qinghai Lake area. The findings have revealed the presence of SFG *Rickettsia* (42.8 %, 134/313) and *C. burnetii* (4.8 %, 15/313) in tick vectors within the protected area. Individuals residing near Qinghai Lake, along with yaks, Tibetan sheep, and the endangered Przewalski's gazelle, are at heightened risk of tick-borne infections, posing a threat to the Przewalski's gazelle population and to human and livestock health. Furthermore, the QLNNR, serving as a habitat for migratory birds, creates favorable conditions for the propagation of ticks and TBPs. Consequently, there is an urgent need to establish a robust monitoring system within the QLNNR to gain a comprehensive understanding of the diversity of tick species and the prevalence of TBPs. Notably, the current study has been limited by the small number of body tick samples collected from Przewalski's gazelle. It is anticipated that future research will expand the scope of detection to provide a more detailed insight into the diversity of tick species and TBPs in the QLNNR, thereby enhancing the effectiveness of tick and TBP prevention and control strategies in the region.

CRediT authorship contribution statement

Qiang Chen: Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Zengkui Li:** Writing – review & editing, Supervision, Methodology. **Ming Kang:** Writing – review & editing, Supervision, Methodology. **Guangwei Hu:** Writing – review & editing, Supervision, Methodology. **Jinshan Cai:** Writing – review & editing, Supervision, Methodology. **Jing Li:** Writing – review & editing, Supervision, Methodology. **Xiaoling Han:** Writing – review & editing, Supervision, Methodology. **Changjiang Chen:** Writing – review & editing, Supervision, Methodology. **Shunfu He:** Writing – review & editing, Supervision, Methodology. **Xiaoyu Hu:** Methodology. **Yongcai He:** Visualization, Validation, Methodology, Formal analysis. **Zhongyu Li:** Supervision, Methodology. **Jiyong Chen:** Supervision, Methodology. **Pengcheng Geng:** Supervision, Methodology. **Shuo Jiang:** Methodology. **Jinghua Ma:** Methodology. **Xiao Zhang:** Methodology. **Ximei Tai:** Methodology. **Ying Li:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis.

Availability of data and materials

All data generated or analyzed during this study were included in this published.

Rickettsia raoultii OmpA data have been deposited at Genbank repository with accession numbers OQ 253257 - OQ 253269. *Rickettsia sibirica OmpA* data have been deposited at Genbank repository with accession numbers OQ 253270 - OQ 253273. *Rickettsia slovaca OmpA* data have been deposited at Genbank repository with accession numbers OQ 253274. *Rickettsia raoultii Sca4* data have been deposited at Genbank with accession numbers OQ 253275 - OQ 253281. *Rickettsia sibirica Sca4* data have been deposited at Genbank repository with accession numbers OQ 253282 - OQ 253283. *Rickettsia slovaca Sca4* data have been deposited at Genbank repository with accession numbers OQ 253284 - OQ 253285. *Dermacentor nuttalli* ITS2 data have been deposited at Genbank repository with accession numbers OQ 955291 - OQ 955293. *Coxiella burnetii htpB* data have been deposited at Genbank repository with accession numbers OQ 865125 - OQ 865128.

Ethics statement

The study was conducted in compliance with the ethical policies of the journal and the rules of the ethics committee of Qinghai University.

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Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

Not applicable.

Supplementary information

Additional file 1: Fig. S1: PCR amplification and electrophoresis results of the ITS2 gene fragment of *Dermacentor nuttalli* before and after Spe I digestion. Note: M: DL 2000 DNA Marker; A-J: The size of the ITS2 gene fragment before enzyme digestion; A-j: The size of ITS2 gene fragments after enzyme digestion; Kk: Negative control.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40205.

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