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Tick species, tick-borne pathogen distribution and risk factor analysis in border areas of China, Russia and North Korea

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Introduction: Ticks are important ectoparasites of livestock. Ticks and tick-borne diseases (TBDs) cause losses to the animal husbandry industry and also present a major hidden danger to public health and safety. However, the tick species and prevalence of TBDs in border regions of China, Russia, and North Korea remain unknown. The purpose of this study was to identify the tick species and tick-borne pathogens endemic in these regions.

Methods: Morphological and molecular identification of ticks was performed by microscopy and polymerase chain reaction (PCR), and the distribution of tick species, pathogen, and risk factors of infection were analyzed.

Results: In total, 1,187 ticks were collected from the border areas of 13 localities in eight cities. Five tick species were identified: *Haemaphysalis longicornis* (39.68%), *Ixodes persulcatus* (25.36%), *Haemaphysalis japonica* (15.50%), *Dermacentor silvarum* (15.42%), and *Haemaphysalis concinna* (4.04%). There were more female than male ticks, and nymphs were the least frequently collected. *I. persulcatus* was the main species in the forest environment, while *H. longicornis* was the main species in grasslands and animal surface. Four pathogens were detected: *Rickettsia*, *Bartonella*, *Anaplasma*, and *Babesia*.

Discussion: Pathogen detection in ticks differed significantly among the environments and between Sexes. There were significant differences in the proportion of ticks infected with *Rickettsia*, *Bartonella*, *Anaplasma*, and *Babesia* among regions, species, sexes, and environments. The results of this survey of the tick species in border areas of China, Russia, and North Korea provided a scientific basis for the prevention and control of TBDs.

KEYWORDS

tick species, tick-borne diseases, risk factors, border areas of China, tick-borne pathogen distribution

1 Introduction

Ticks are important ectoparasites of livestock and can be divided into three families: Ixodidae, Argasidae, and Nuttalliellidae (1). Although most common tick species are distributed throughout various provinces and cities in China, some are unique to a certain region, which may be related to environmental differences (2). Currently, 907 species of ticks have been reported worldwide. About 120 tick species have been identified in China alone, with most (80%) being hard ticks (3).

Under normal circumstances, the body length of ticks is approximately 2–15 mm, although body size can rapidly increase while sucking blood (4). Tick development occurs as a process of incomplete metamorphosis, which can be divided into the egg, larva, nymph, and adult stages (5). Ticks can be classified based on the number of hosts and molting sites (6). In addition, ticks can readily adapt to harsh environmental conditions and some species can survive without food for long periods (7). Tick distribution is closely related to climate, soil, water, geographical environment, hosts, and other factors (8, 9). Ticks and tick-borne diseases (TBDs) not only cause harm to the animal husbandry industry, but also pose a major hidden threat to public safety and health (10). Moreover, many tick species can cause anemia and other diseases, as well as transmit various pathogens to the hosts, including *Anaplasma, Bartonella, Rickettsia, Babesia*, and Tick-borne encephalitis virus (11).

Rickettsia are small Gram-negative bacteria with an obligate intracellular life cycle circulating between mammalian hosts and hematophagous arthropod vectors in nature. *Rickettsia* are transmitted to mammalian hosts during blood feeding by infected ticks and mites (12). *Rickettsia* are categorized as belonging to the spotted fever group (SFG), typhus group (TG), transitional group (TRG), and ancestral group (AG) (13). The main clinical symptoms of *Rickettsia* infection in humans are fever, headache and nausea. Severe patients may die. Overall, the public health burden of tick-borne Rickettsioses remains significantly underestimated (14). *Anaplasma* belongs to the family *Anaplasmataceae* of order *Rickettsiales*. The genus *Anaplasma* includes *Anaplasma marginale*, *Anaplasma centrale*, *Anaplasma bovis*, *Anaplasma ovis*, and *Anaplasma phagocytophilum* transmitted by ticks. Different types of *Anaplasma* cause different clinical symptoms. *A. phagocytophilum* mainly cause fever, abortion, and decreased milk production (15). *A. marginale* has the most severe symptoms and may lead to death of livestock if not treated in time (16). *A. centrale* is the least pathogenic and is often used in vaccines (17). Today, *Anaplasma* still has effects on human and animal health at the global level. *Bartonella* species are gram-negative, and zoonotic bacteria belonging to the α2-subgroup of proteobacteria (18). It is spread to mammals mainly by blood-sucking arthropods. Cat scratch disease (CDS) is the most harmful disease to humans caused by *Bartonella*, with approximately 12,000 cases reported annually in the United States (19). Although the incidence is not high, we still need to take it seriously. *Babesia* is a protozoan parasite of the phylum Apicomplexa. Babesiosis is a worldwide tick-borne zoonosis caused by hemoprotozoan parasites of the genus *Babesia* (20). Ixodes ticks are the main vectors of *Babesia* spp. Clinical manifestations of Babesiosis are mainly related to the immune function of the host. *Babesia bovis* can have a serious impact on the livestock industry. The economic loss to China is up to 60 million dollars per year (21). Therefore, scientific prevention and control of *Babesia* is crucial for the livestock industry.

In terms of incidence, TBDs are the most serious vector-borne diseases in the animal husbandry and veterinary fields, and the second most common human vector-borne diseases after mosquito-borne diseases (22). Tick species and the prevalence of TBDs in border areas of China, Russia, and North Korea remain unknown. Therefore, the aim of this study was to identify the tick species and pathogens in border areas of China, Russia and North Korea, and to analyze potential risk factors, so as to provide a scientific basis for the prevention and control of TBDs.

2 Materials and methods

2.1 Collection of tick samples

Free ticks were collected using the cloth flag method. The collection sites consisted of grasslands and forests with lush vegetation close to a water source. When sampling, the gauze was laid flat on the grass and moved slowly by hand with a stick. At regular intervals, a magnifying glass and tweezers were used to transfer ticks from the gauze to a 15 mL centrifuge tube and relative information was recorded. Farms and villages were randomly selected. After obtaining the consent of farmers, the surfaces of livestock (cattle and sheep) were checked for the presence of ticks at the preferred attachment sites, such as behind the ear, perineum, and lower abdomen. During collection, the head of the tick was clamped with elbow tweezers and the mouthparts were gently rotated and pulled out perpendicular to the body surface. The samples were then placed into a labeled plain 15 mL centrifuge tube.

2.2 Morphological identification of ticks

Adult ticks with relatively complete morphology of different species were selected and washed three times with sterile water to remove dust from the surface of the tick and soaked in phosphate-buffered saline (23). The morphological structures of different species of male and female ticks were observed with a stereomicroscope. Images were captured and stored following appropriate taxonomical keys (24).

2.3 Tick DNA extraction

All ticks were used to extract DNA, and each tick was tagged individually. The ticks were ground to powder in liquid nitrogen. Tick DNA was extracted using a tissue Genomic DNA Extraction Kit (Tiangen Biotech (Beijing) Co., Ltd., Beijing, China), in accordance with the manufacturer's instructions, and stored at-20°C until further use.

2.4 Detection of tick-borne pathogens

Species-specific primers were used to amplify the 16S ribosomal DNA (*16SrDNA*) gene of tick (45), outer membrane protein-A (*ompA*) gene of *Rickettsia* (41), citrate synthase (*gltA*) gene of *Bartonella* (42), 16S ribosomal RNA (*16SrRNA*) gene of *Anaplasma* (40), chaperonin-containing t-complex polypeptide 1 (*CCTeta*) gene of *Babesia* (37), and major piroplasm surface protein (*MPSP*) gene of *Theileria sinensis* and *Theileria* orientalis (43, 44). The primers used in this study are listed in Table 1. The PCR reaction was conducted with a 25-μL reaction volume comprising

TABLE 1 PCR primers for ticks and pathogens.

Pathogen	The name of the gene	Primer sequences (5′-3′)	Fragment size (bp)
Anaplasma	*16SrRNA*	F-TACCTCTGTGTTGTAGCTAACGC R-CTTGCGACATTGCAACCTATTGT	426 (40)
Rickettsia	*ompA*	F-ATGGCGAATATTTCTCCAAAA R-AGTGCAGCATTCGCTCCCCCT	530 (41)
Bartonella	*gltA*	F-GGGGACCAGCTCATGGTGG R-AATGCAAAAAGAACAGTAAACA	356 (42)
Babesia	*CCTeta*	F-GCCCGCAGGTCATCATAAAGT R-CATTTTGTGCCAGCGTTTTG	1,008 (37)
T. sinensis	*MPSP*	F-CACTGCTATGTTGTCCAAGAGATATT R-AATGCGCCTAAAGATAGTAGAAAAC	887 (43)
T. orientalis	*MPSP*	F-CTTTGCCTAGGATACTTCCT R-ACGGCAAGTGGTGAGAACT	776 (44)
Tick	*16SrDNA*	F-CTGCTCAATGATTTTTTAAATTGGGTGG R-CCGGTCTGAACTCAGATCAAGT	460 (45)

1 μL of each primer (10 pmol), 3 μL of template DNA (50–60 ng/μl), 2 μL of deoxynucleotide triphosphates (Takara Biotechnology (Dalian) Co., Ltd., Dalian, China), 2.5 μL of 10× Ex *Taq* buffer, 0.25 μL of Ex *Taq*, and 15.25 μL of distilled water. The PCR reaction conditions are presented in Table 2.

2.5 Sequencing and phylogenetic analyses

All samples verified as positive by agarose gel electrophoresis were sent to Shanghai Shenggong Biotechnology Company for Sanger sequencing. Newly obtained sequences were compared with the National Center for Biotechnology Information database[1] using the Basic Local Alignment Search Tool[2] and related sequences were retrieved from the GenBank database.[3] Selection of representative samples for use ClustalW software[4] multiple sequence alignment. Phylogenetic trees were constructed using the Maximum Likelihood method with a Tamura 3-parameter model and bootstrapping of 1,000 replicates to calculate the evolutionary relationship using Molecular Evolutionary Genetics Analysis software (25).[5]

2.6 Risk factor analysis

Prism9 software (GraphPad Software, LLC, San Diego, CA, United States) was used for statistical analysis of tick-borne pathogen infections under different conditions. A Fisher score algorithm was used to select the optimal model. Univariate logistic regression was used to identify potential risk factors. A probability (p) value <0.05 was considered statistically significant. The odds ratio (OR) and 95% confidence interval (CI) were calculated to explore the correlation between the prevalence of pathogens and different factors. Ref represents the reference value for each set of data. Relevant data were expressed with reference to Zhao et al. (26).

3 Results

3.1 Tick species survey

In total, 1,187 ticks, were collected from 2020 to 2021 among eight counties of border areas of China (Hunchun, Yanji, Tumen, Longjing, Dunhua, Helong, Wangqing, and Antu), Russia, and North Korea (Figure 1). Of the 1,187 ticks, 632 were female, 376 were male, and 179 were nymphs. Regarding the environments, 343 ticks were collected in forests, 351 in grasslands, and 493 on animal surfaces. According to the identification results, there were three genera and five species of ticks in border areas of China, Russia, and North Korea, which included 471 *Haemaphysalis longicornis*, 184 *Haemaphysalis japonica*, 48 *Haemaphysalis concinna*, 301 *Ixodes persulcatus*, and 183 *Dermacentor silvarum* with proportions of 39.68, 15.50, 4.04, 25.36, and 15.42%, respectively. *Haemaphysalis* accounted for 59.22%, indicating that it was the dominant tick genus in border areas of China, Russia, and North Korea.

3.2 Detection rate of TBDs

The average infection rate of *Rickettsia, Bartonella, Anaplasma*, and *Babesia* was 48.78, 22.91, 35.05, and 5.14%, respectively. Among these pathogens, four were detected in Hunchun, Wangqing,

1 https://www.ncbi.nlm.nih.gov/
2 https://blast.ncbi.nlm.nih.gov/Blast.cgi
3 https://www.ncbi.nlm.nih.gov/genbank/
4 http://www.clustal.org/
5 v.11.0; https://www.megasoftware.net/

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TABLE 2 Reaction conditions of various primers.

Primer name	Pre-denaturation temperature(°C)/time(s)	Denaturation temperature(°C)/time(s)	Annealing temperature(°C)/time(s)	Stretching temperature(°C)/time(s)	Temperature of reextension(°C)/time(s)	cycle	Storage temperature(°C)
Anaplasma	94/300	94/15	55/35	72/55	72/480	40	4
Rickettsia	95/300	95/30	50/30	72/30	72/480	35	4
Bartonella	94/300	94/30	55/30	72/30	72/420	35	4
Babesia	95/300	95/30	51.4/30	72/30	72/480	35	4
T. sinensis	94/300	94/60	56/60	72/60	72/420	35	4
T. orientalis	94/300	94/60	58/60	72/60	72/420	35	4
Tick	95/300	95/30	54/30	72/50	72/420	35	4

Tumen, Dunhua and Longjing. *Anaplasma* was not detected in Yanji. *Rickettsia* and *Bartonella* were only detected in Helong and Antu. Four pathogens were detected in *H. longicornis*, *H. japonica*, and *I. persulcatus*, while *Rickettsia*, *Bartonella*, and *Anaplasma* were found in *D. silvarum*, and *Bartonella* and *Anaplasma* were identified in *H. concinna*. *Bartonella* was confirmed in all tick species. No *T. sinensis* and *T. orientalis* were detected in this survey (Table 3).

3.3 Molecular survey of pathogens in ticks

The *Rickettsia* sequences were compared with known sequences in the GenBank database. Three genotypes of *Rickettsia* were detected in ticks in border areas of China, Russia, and North Korea: *Rickettsia raoultii*, *Candidatus rickettsia jingxinensis* and *Candidatus rickettsia tarasevichiae*. Among these, the YB-BJ-4 (PQ487798) strains were located in the same branch as isolates from India (MN537561), Sichuan province, China (MF590726), and Dandong, China (MH177456). The YB-BJ-5 (PQ487799) and YB-BJ-6 (PQ487800) strains were situated on the same branch as isolates from Siberia (MK304548), Turkey (MG920563), and Xinjiang, China (KU723511). The YB-BJ-2 (PQ487796) and YB-BJ-3 (PQ487797) strains were highly homologous and on the same branch with isolates from Harbin, China (MT019661), Mudanjiang, China (KF008247), and Hokkaido, Japan (LC379461) (Figure 2). The *Bartonella gltA* gene sequence obtained from this study (PQ487795) formed one clade with the Korea (MT362935) and Shandong province, China (KX655838) (Figure 3). Also, the *Anaplasma* sequence from this study (PQ461353) was compared with known *Anaplasma* sequences in the GenBank database. The isolates of *Anaplasma capra* from Luoyang, China (MT799937), Shanxi, China (MG869594), and Korea were located on the same branch and had the highest homology (Figure 4). Lastly, the *Babesia* sequence (PQ487801) was compared with known *Babesia ovata* sequences in the GenBank database. The isolate obtained in this study clustered with sequences from Japan (AB367928) with high homology (Figure 5).

3.4 Analysis of four pathogens under different factors

Our results suggest that regionally, ticks from four regions, Hunchun, Wangqing, Dunhua, and Longjing, may be more likely to carry *Rickettsia*; ticks from Yanji, Dunhua, and Longjing had a higher detection rate of *Bartonella*; ticks from two regions, Hunchun and Dunhua, were more likely to be infected with *Anaplasma*; and there was no significant difference in the distribution of *Babesia* across regions. When analyzed from the perspective of tick species, *H. longicornis, I. persulcatus,* and *D. silvarum* are more likely to carry *Rickettsia* and *Anaplasma*; *Bartonella* is more likely to be present in all four species of ticks except *H. longicornis*; and for the *Babesia*, *H. longicornis* is a likely potential vector. The sex of the tick is also an important factor in the prevalence of TBDs. Our study found that female ticks were more likely to carry *Rickettsia*, *Bartonella*, and *Babesia*; male ticks were more likely to carry *Anaplasma.* Finally, analyzing the collection environment we found that ticks from animal body surfaces are more likely to carry pathogens compared to the natural environment. In summary, region, tick species, sex, and collection environment may be potential risk factors for TBDs transmission (Tables 4–7).

TABLE 3 The prevalence of tick-borne pathogens from tick samples in this study.

Location	Tick spp.	Detection of pathogen (No. positive)				
	Name	No. collected	*Rickettsia*	*Bartonella*	*Anaplasma*	*Babesia*
Hunchun	*H. longicornis*	471	227	39	206	41
	H. japonica	13	5	4	5	1
	H. concinna	8	0	7	1	0
	I. persulcatus	10	7	4	3	2
	D. silvarum	60	59	59	54	0
Wangqing	*H. japonica*	82	1	19	3	2
	I. persulcatus	151	108	10	34	5
	D. silvarum	13	13	10	10	0
Helong	*H. concinna*	40	0	15	0	0
	I. persulcatus	16	15	0	0	0
Tumen	*H. japonica*	26	0	1	2	2
	D. silvarum	43	15	9	8	0
Yanji	*H. japonica*	30	6	6	0	1
	I. persulcatus	29	4	19	0	2
Antu	*D. silvarum*	67	20	15	0	0
Dunhua	*I. persulcatus*	95	81	40	85	4
Longjing	*H. japonica*	33	18	15	5	1
Total		1,187	579	272	416	61

TABLE 4 *Rickettsia* infection under different factors.

Factors	Category	No. of samples collected	No. of positive samples	Positive rate (%)	OR	95% CI	*p*- value
Region	Hunchun	562	298	53.02	5.53	2.75–11.14	<0.01
	Wangqing	246	122	49.59	4.82	2.34–9.95	<0.01
	Helong	56	15	26.79	1.79	0.73–4.42	0.20
	Tumen	69	15	21.74	1.36	0.56–3.31	0.50
	Yanji	59	10	16.95	Ref	–	–
	Antu	67	20	29.85	2.09	0.88–4.92	0.09
	Dunhua	95	81	85.26	28.35	11.69–68.75	<0.01
	Longjing	33	18	54.55	5.88	2.24–15.44	<0.01
Species	*H. longicornis*	471	227	48.20	4.78	3.10–7.35	<0.01
	H. japonica	184	30	16.30	Ref	–	–
	H. concinna	48	0	0	–	–	–
	I. persulcatus	301	215	71.43	12.83	8.07–20.42	<0.01
	D. silvarum	183	107	58.47	7.23	4.43–11.79	<0.01
Sex	Female	632	405	64.08	4.87	3.37–7.04	<0.01
	Male	376	126	33.51	1.38	0.93–2.04	0.11
	Nymphal	179	48	26.82	Ref	–	–
Environment	Forest	343	89	25.95	Ref	–	–
	Grass	351	188	53.56	3.29	2.39–4.53	<0.01
	Body surface	493	302	61.26	4.51	3.37–6.10	<0.01

Ref, reference; 95% CI, confdence interval; OR, odds ratio.

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TABLE 5 *Bartonella* infection under different factors.

Factors	Category	No. of samples collected	No. of positive samples	Positive rate (%)	OR	95% CI	*p*- value
Region	Hunchun	562	113	20.11	1.49	0.74–2.99	0.27
	Wangqing	246	39	15.85	1.11	0.52–2.36	0.78
	Helong	56	15	26.79	2.16	0.88–5.28	0.09
	Tumen	69	10	14.49	Ref	–	–
	Yanji	59	25	42.37	4.34	1.86–10.11	<0.01
	Antu	67	15	22.39	1.70	0.70–4.12	0.24
	Dunhua	95	40	42.11	4.29	1.96–9.40	<0.01
	Longjing	33	15	45.45	4.92	1.89–12.82	<0.01
Species	*H. longicornis*	471	39	8.28	Ref	–	–
	H. japonica	184	45	24.46	3.59	2.24–5.74	<0.01
	H. concinna	48	22	45.83	9.37	4.87–18.06	<0.01
	I. persulcatus	301	73	24.25	3.55	2.33–5.40	<0.01
	D. silvarum	183	93	50.82	11.45	7.39–17.73	<0.01
Sex	Female	632	147	23.26	3.31	1.89–5.80	<0.01
	Male	376	110	29.26	4.52	2.55–8.02	<0.01
	nymphal	179	15	8.38	Ref	–	–
Environment	Forest	343	93	27.11	1.52	1.07–2.17	0.02
	Grass	351	69	19.66	Ref	–	–
	Body surface	493	110	22.31	1.17	0.84–1.65	0.35

Ref, reference; 95% CI, confdence interval; OR, odds ratio.

TABLE 6 *Anaplasma* infection under different factors.

Factors	Category	No. of samples collected	No. of positive samples	Positive rate (%)	OR	95% CI	*p*- value
Region	Hunchun	562	269	47.86	5.42	2.72–10.81	<0.01
	Wangqing	246	47	19.11	1.39	0.66–2.93	0.38
	Helong	56	0	0	–	–	–
	Tumen	69	10	14.49	Ref	–	–
	Yanji	59	0	0	–	–	–
	Antu	67	0	0	–	–	–
	Dunhua	95	85	89.47	50.15	19.64–128.1	<0.01
	Longjing	33	5	15.15	1.05	0.33–3.38	0.93
Species	*H. longicornis*	471	206	43.74	36.54	4.99–267.2	<0.01
	H. japonica	184	15	8.15	4.17	0.54–32.42	0.17
	H. concinna	48	1	2.08	Ref	–	–
	I. persulcatus	301	122	40.53	32.03	4.36–235.4	<0.01
	D. silvarum	183	72	39.34	30.49	4.11–226.0	<0.01
Sex	Female	632	214	33.86	1.53	1.05–2.22	0.03
	Male	376	157	41.76	2.14	1.44–3.17	<0.01
	Nymphal	179	45	25.14	Ref	–	–
Environment	Forest	343	77	22.45	Ref	–	–
	Grass	351	100	28.49	1.38	0.98–1.94	0.07
	Body surface	493	239	48.48	3.25	2.39–4.43	<0.01

Ref, reference; 95% CI, confdence interval; OR, odds ratio.

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FIGURE 1
Map of in border areas of China, Russia and North Korea. Gray ranges represent sampling areas. Black dots represent sampling points.

TABLE 7 *Babesia* infection under different factors.

Factors	Category	No. of samples collected	No. of positive samples	Positive rate (%)	OR	95%CI	*p*- value
Region	Hunchun	562	44	7.83	2.85	0.67–12.01	0.16
	Wangqing	246	7	2.85	0.98	0.20–4.84	0.98
	Helong	56	0	0	–	–	–
	Tumen	69	2	2.90	Ref	–	–
	Yanji	59	3	5.08	1.80	0.29–11.13	0.53
	Antu	67	0	0	–	–	–
	Dunhua	95	4	4.21	1.47	0.26–8.28	0.66
	Longjing	33	1	3.03	1.05	0.09–11.98	0.97
Species	*H. longicornis*	471	41	8.70	2.41	1.06–5.48	0.03
	H. japonica	184	7	3.80	Ref	–	–
	H. concinna	48	0	0	–	–	–
	I. persulcatus	301	13	4.32	1.14	0.45–2.92	0.78
	D. silvarum	183	0	0	–	–	–

(Continued)

TABLE 7 (Continued)

Factors	Category	No. of samples collected	No. of positive samples	Positive rate (%)	OR	95%CI	*p*- value
Sex	Female	632	49	7.75	2.55	1.34–4.86	<0.01
	Male	376	12	3.19	Ref	–	–
	Nymphal	179	0	0	–	–	–
Environment	Forest	343	8	2.33	1.37	0.47–4.00	0.56
	Grass	351	6	1.71	Ref	–	–
	Body surface	493	47	9.53	6.06	2.56–14.34	<0.01

Ref, reference; 95% CI, confdence interval; OR, odds ratio.

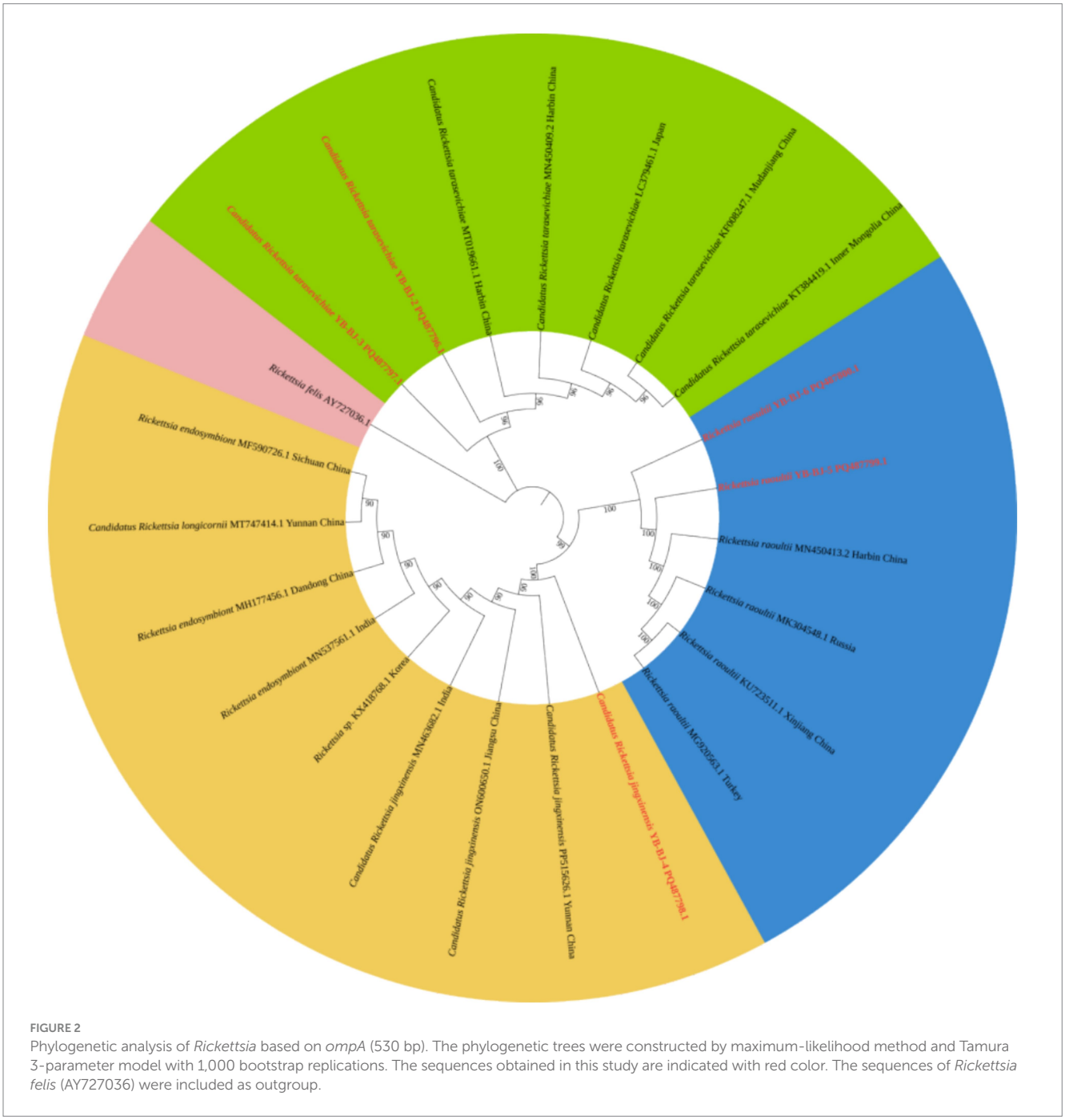

FIGURE 2
Phylogenetic analysis of *Rickettsia* based on *ompA* (530 bp). The phylogenetic trees were constructed by maximum-likelihood method and Tamura 3-parameter model with 1,000 bootstrap replications. The sequences obtained in this study are indicated with red color. The sequences of *Rickettsia felis* (AY727036) were included as outgroup.

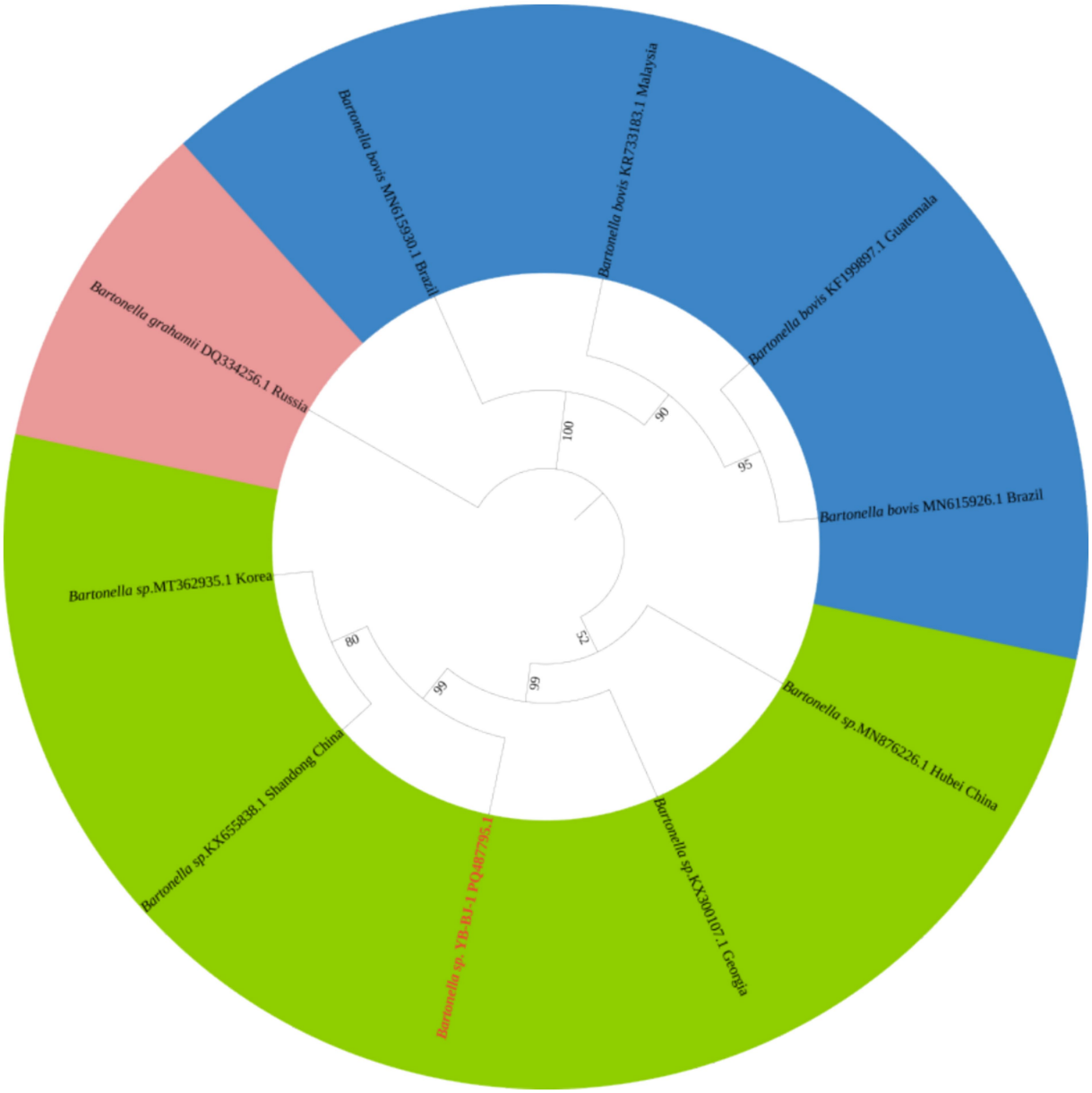

FIGURE 3
Phylogenetic analysis of *Bartonella* based on *gltA* (356 bp). The phylogenetic trees were constructed by maximum-likelihood method and Tamura 3-parameter model with 1,000 bootstrap replications. The sequences obtained in this study are indicated with red color. The sequences of *Bartonella grahamii* (DQ334256) were included as outgroup.

4 Discussion

China has a vast border with many countries. The border between China, North Korea, and Russia lies in northeast China. Because the forest hydrology resources in this area are very rich, the endemic tick species have gradually diversified. Ticks and other vectors in the border zone can freely migrate to another country through a variety of routes, which may increase the risk of tick-borne diseases. In this study, 1,187 ticks collected from eight counties and cities in border areas of China, Russia, and North Korea were classified and analyzed. Among the five identified species, *Haemaphysalis* were the dominant tick species. *H. longicornis* was the most commonly detected species in this survey. *H. longicornis*, commonly known as the New Zealand cattle tick, is found mainly in East Asia and the Pacific region (27), and more recently in the United States and other countries in the Americas. *H. longicornis* can parasitize most warm-blooded animals, including humans and domestic animals, and spread a variety of pathogens (28), thus posing major hidden dangers to public safety and the animal husbandry industry.

In this study, three types of *Rickettsia* were detected. In fact, *R. raoultii* and *C. rickettsia tarasevichiae* have been endemic along the China-Russia border areas in recent years. Related studies have shown that *R. raoultii* and *C. rickettsia tarasevichiae* were detected in *I. persulcatus* and *D. silvarum* along the China-Russia border areas in 2014 (29, 30). This is almost consistent with the results of our survey.

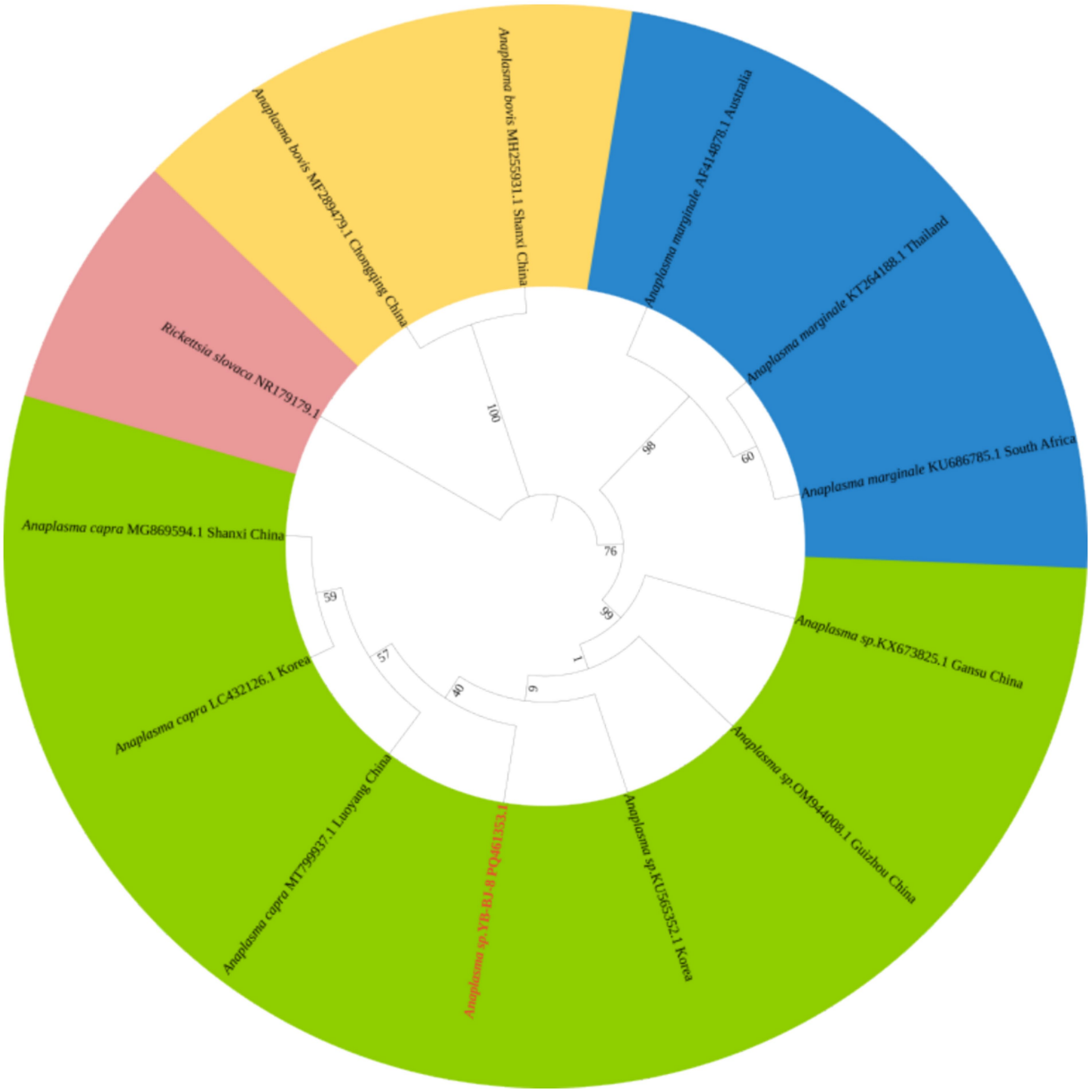

FIGURE 4
Phylogenetic analysis of *Anaplasma* based on *16SrRNA* (426 bp). The phylogenetic trees were constructed by maximum-likelihood method and Tamura 3-parameter model with 1,000 bootstrap replications. The sequences obtained in this study are indicated with red color. The sequences of *Rickettsia slovaca* (NR179179) were included as outgroup.

Notably, in previous studies, *C. rickettsia jingxinensis* have been reported mainly in Southwestern China and Korea (31, 32). The *C. rickettsia jingxinensis* detected in this survey were also consistent with the above areas in terms of their affinities. This suggests that there is potential for the spread of *C. rickettsia jingxinensis* to the border areas. *Bartonella* is widely prevalent around the world, and usually lice and fleas are considered to be the main vectors of *Bartonella* (33). Whether ticks are capable of transmitting *Bartonella* remains controversial. In 2022, some researchers from Portugal surveyed 268 ticks in Portugal and found that none of the ticks had infected *Bartonella* (34). However, a total of 272 *Bartonella* infections were detected in 1,187 ticks in our survey, indicating that ticks do have the ability to carry *Bartonella*. It remains to be investigated whether ticks can transmit *Bartonella* to their hosts through blood-sucking. *A. capra* is an emerging zoonotic tick-borne pathogen with a broad host range, including many mammals. In 2012, *A. capra* was detected in goats in China. Although current studies are not sufficient, domestic ruminants are considered the main host (35). Prior to this, *A. capra* was mainly prevalent in south-central China. *A. capra* have been reported to be detected in *H. longicornis* in Hubei Province, China, with a positivity rate of 1.32% (36). This is the first report of *A. capra* detected at the border areas of China, Russia, and North Korea. Gene sequences were in the same clade as the isolates from Luoyang and Shanxi. The positivity rate in this survey was significantly higher than in previous studies, a result that reminds us to

FIGURE 5
Phylogenetic analysis of *Babesia* based on *CCTeta* (1,008 bp). The phylogenetic trees were constructed by maximum-likelihood method and Tamura 3-parameter model with 1,000 bootstrap replications. The sequences obtained in this study are indicated with red color. The sequences of *Theileria sergenti* (AY727036) were included as outgroup.

pay close attention to the prevalence of *A. capra*. *B. ovata* is more frequently reported in Japan (37). The isolates from this investigation showed the highest homology with isolates from Japan. There are fewer reports on the epidemiology of *B. ovata*. It has been reported that 646 bovine bloods from various regions of China were positive for *B. ovata* at a rate of 1.5% (38). The positivity rate of *B. ovata* was also low in this survey. There are many reasons for this phenomenon, but of course scientific prevention and control is essential.

Our survey identified four potential risk factors that influence the prevalence of TBDs. Tick species are one of the most important factors in the prevalence of TBDs. Different species of ticks can carry different pathogens. It has been reported that *H. longicornis* can carry up to 44 pathogens. It's one of the tick species that carries the highest number of pathogens (39). Our study found similar problems. Among the ticks we collected, *H. longicornis* was the most abundant and infected with four pathogens. The relationship between region and tick species is inextricably linked, and the distribution of ticks is significantly regional. In China, tick species are more abundant in the Northwestern and Southwestern regions, TBDs epidemics are also more severe. Although the samples were collected only in the border area of Northeast China, we can see from the results that some species of ticks were detected only in specific areas. Interestingly, sex and collection environment were also found to be risk factors for TBDs, and although the exact reasons for this are unclear, this phenomenon deserves to be studied in depth.

5 Conclusion

Haemaphysalis are the dominant tick genus in border areas of China, Russia, and North Korea. Four pathogens (*Rickettsia*, *Bartonella*, *Anaplasma,* and *Babesia*) were detected in the tick species collected in this study. Based on our results, scientific exclusion of potential risk factors may provide a new idea for controlling the spread of tick-borne diseases. These findings provide epidemiological data to support the prevention and control of ticks and tick-borne diseases in the border region of China, Russia, and North Korea.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

PM: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. JS: Data curation, Writing – original draft. SZ: Data curation, Writing – review & editing. ZM: Data curation, Formal analysis, Supervision, Writing – review & editing. YM: Writing – review & editing. ZT: Supervision, Writing – review & editing. ZW: Writing – review & editing. SL: Writing – review & editing. FZ: Writing – review & editing. ML: Formal analysis, Writing – review & editing. LW: Methodology, Writing – review & editing. LJ: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of 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.

Generative AI statement

The authors declare that no Gen AI was used in the creation of this manuscript.

Publisher's note

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