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Original Research

Molecular epidemiological study on tick-borne pathogens in Qinghai Province, Northwestern China

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ABSTRACT

Recently, there has been a continuous stream of reports on emerging tick-borne pathogens affecting humans. Qinghai Province, located in the northwestern region, is one of China's major pastoral areas, providing a suitable environment for ticks' survival and transmitting tick-borne pathogens. Here, we collected 560 free-living and parasitic ticks from 11 locations in Qinghai Province using the flag-drag method or tweezers, identifying them as belonging to 4 species of ticks. The overall positivity rate for tick-borne pathogens was 51.61 %, comprising *Rickettsia* (34.64 %), *Anaplasma* (5.00 %), *Ehrlichia* (2.14 %), *Borrelia burgdorferi* sensu lato (BBSL) (7.50 %), *Babesia* (0.18 %), and *Theileria* (5.89 %). Sequencing revealed the presence of 7 species of *Rickettsia*, 4 species of *Anaplasma*, 2 species of *Ehrlichia*, 2 species of BBSL, 1 species of *Babesia,* and 3 species of *Theileria*. Among the ticks, 6.43 % were co-infected with 2 pathogens, while 0.36 % exhibited co-infection with 3 pathogens. Significant correlations ($P < 0.05$) were observed between the prevalence of tick-borne pathogens and factors including tick species, sex, developmental stages, parasitic status, and blood-feeding status. The results highlight the diverse distribution of tick-borne pathogens in Qinghai Province, posing a significant threat to both local animal husbandry and human health. It underscores the need to enhance systematic monitoring of tick-borne pathogens in the local population and livestock.
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1. Introduction

Worldwide, about 900 tick species (Parasitiformes, Ixodoidea) have been identified, capable of carrying and transmitting various pathogens such as viruses, bacteria, rickettsia, spirochete, mycoplasma, chlamydia, and protozoa, which speed up the emergence of various zoonotic diseases [1]. In China, at least 125 tick species have been recorded, including 14 soft ticks and 111 hard ticks [2]. In recent years, a total of 33 emerging and re-emerging tick-borne pathogens have been reported, including 8 species of spotted fever group *Rickettsia*, 7 species of *Anaplasma*, 6 species of *Borrelia burgdorferi* sensu lato (BBSL), 11 species of *Babesia* and 1 species of severe fever with thrombocytopenia syndrome bunyavirus (SFTSV) [3]. Additionally, there has been a continuous stream of reports on emerging tick-borne viruses affecting humans, including the Jingmen tick virus [4], Alongshan virus (ALSV) [5], Songling virus [6], and *Candidatus Ehrlichia* erythricense [7] in China. The impact of human activities on the environment is causing gradual changes in the distribution and density of ticks leading to an increasingly severe impact on human health.

The northwest region of China consists of five provinces, Xinjiang Uygur Autonomous Region, Ningxia Hui Autonomous Region, Gansu Province, Shaanxi Province, and Qinghai Province, covering a vast territory and two climatic zones (temperate and frigid). The complex and diverse geographical features, coupled with unique biological species,

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HIGHLIGHTS

Scientific question

Ticks are vectors for various pathogens, including bacteria, viruses, and protozoa, which may result in various zoonotic diseases. However, research on the prevalence and diversity of tick-borne pathogens in Qinghai Province is inadequate, and the potential public health ramifications have not been thoroughly assessed.

Evidence before this study

There have been continuous reports on emerging tick-borne pathogens in China. *Rickettsia*, ehrlichiosis, anaplasmosis, and many types of tick viruses have occurred in Northwestern China, especially in Xinjiang Uygur Autonomous Region and Gansu Province. The data on the distribution of tick-borne disease in Qinghai region are comparatively sparse.

New findings

High positivity rates for and high diversities of tick-borne pathogens were found. Sequencing revealed the presence of 7 species of *Rickettsia*, 4 species of *Anaplasma*, 2 species of *Ehrlichia*, 2 species of *Borrelia burgdorferi* sensu lato (BBSL), 1 species of *Babesia*, and 3 species of *Theileria*. A total of 6.43 % were found to be co-infected with two pathogens, while 0.36 % exhibited co-infection with three pathogens. Significant correlations ($P < 0.05$) were observed between the prevalence of tick-borne pathogens and tick species, sex, developmental stages, parasitic status, and blood-feeding status, respectively.

Significance of the study

This study highlights the high diversity and prevalence of tick-borne pathogens in Qinghai Province, posing potential risks to both local livestock and human health. It provides essential reference data for future monitoring and control efforts of tick-borne pathogens in the region.

create an ecological and biological foundation for the occurrence and prevalence of vector-borne diseases [8]. Emerging tick-borne diseases in the region include ehrlichiosis, anaplasmosis, and severe fever with thrombocytopenia syndrome [9,10], especially Tacheng tick virus 1 and Tacheng tick virus 2 in Xinjiang Uygur Autonomous Region [11,12]. Additional cases of infection with known *Rickettsia raoultii* (*R. raoultii*), *Rickettsia. aeschlimannii* [13,14], *Borrelia miyamotoi*, ALSV and BBSL in Northwestern China [15]. These instances highlight the expanding epidemic trend of various tick-borne pathogens in Northwestern China, emphasizing the ongoing need for continued monitoring and research on tick distribution and the pathogens they carry.

Presently, research on ticks in China has achieved considerable depth. Epidemiological data surveys indicate that the highest tick records are documented in regions such as Xinjiang Uygur Autonomous Region, Gansu Province, and Shaanxi Province, while data on the distribution of tick species in Qinghai Province are comparatively sparse [16]. Owing to its high altitude, cold climate, and lack of oxygen, Qinghai Province boasts a unique natural ecosystem. The province comprises expansive grasslands, with pastureland accounting for approximately 56.2 % of its total area. It stands as one of the five major pastoral areas in China, accounting for 15 % of the national pastoral area [17]. The unique ecological environment and economic system create a conducive environment for the survival of ticks and transmission of tick-borne pathogens. In addition to the tick-borne diseases mentioned above, *Ehrlichia chaffeensis* and four *Anaplasma* spp. [18], *Bartonella schoenbuchensis*, *Anaplasma ovis* (*A. ovis*) [16], and *Rickettsia* [16,17] have also been found in Qinghai Province in recent years. As ticks depend on the blood of their hosts for survival, the transmission of pathogens through this route poses a potential threat to both livestock and humans, leading to economic losses and endangering human health.

Therefore, in this study we investigated tick-borne pathogens in Qinghai Province through field investigation and molecular epidemiological methods, furnishing a scientific foundation for devising strategies and implementing measures for the prevention and control of tick-borne diseases in the region.

2. Material and methods

2.1. Sample collection and species identification

In this study, both free-living and parasitic ticks were collected from Huangzhong District, Huangyuan County, Ledu District, Minhe Hui and Tu Autonomous County, Tu Autonomous County of Huzhu, Gangcha County, Menyuan Hui Autonomous County, Banma County, Yushu City, Zhiduo County, and Ulan County in Qinghai Province, during April to May 2019. The free-living ticks were collected by dragging with a white flannel flag (60 cm × 90 cm) over vegetation. The attached ticks were checked every 8–10 m, with a minimum collection time of 1 h at each survey site. Parasitic ticks were collected from the body surface of domesticated yaks and sheep on grassland pastures. Entomologists then identified the species under a microscope, following the guidelines outlined in *Economic Insect Fauna of China, Volume 15* [19]. Based on characteristics such as sampling locations, tick species, sex, developmental stages, and parasitic status, the ticks were organized into sample tubes and stored at −80 °C for further testing.

2.2. Genomic deoxyribonucleic acid (DNA) extraction

Genomic DNA was extracted from tick specimens using the TaKaRa MiniBEST Universal Genomic DNA Extraction Kit (Ver.5.0, Code No. 9765). The tick specimens were placed in 1.5 mL centrifuge tubes with 1 tick per tube, and 200 μL RNase free ddH_2O and 4 stainless steel beads were added to each tube. The specimens were then subjected to grinding using the grinding instrument and DNA extraction was performed following the instructions provided in the kit.

2.3. Pathogen detection

Polymerase chain reaction (PCR) was employed to detect specific genes associated with various pathogens. The *omp*A and *glt*A genes of *Rickettsia* [20], 16S ribosome ribonucleic acid (rRNA) genes of *Anaplasma* and *Ehrlichia* [21], 5S-23S rRNA gene of BBSL [22], and 18S rRNA genes of *Babesia* and *Theileria* [23] were specifically amplified. The genes targeted for detection and the primer sequences for the 6 pathogens mentioned above are shown in Table S1. PCR products were detected by agarose gel electrophoresis, and positive amplicons were sent to Beijing Tianyi Huiyuan Company for bidirectional direct sequencing. Subsequently, Main Workbench 5.5 software was utilized to splice the sequences, and the Basic Local Alignment Search Tool (BLAST) was employed to compare the homology between the spliced sequences and the reference sequences in the GenBank library. For further analysis, the spliced sequences were aligned with the reference sequences using ClustalW with default parameters in MEGA (version 11.0), and a phylogenetic tree was constructed using the neighbor-joining method with 1,000 bootstrap replications.

2.4. Statistical analysis

Statistical analysis was performed utilizing the SPSS 20.0 software. The Chi-square test or Fisher's exact probability method was used to assess the association between tick species, sex, developmental stages, parasitic status, blood-feeding status, and positive rate of tick-borne pathogens, $P < 0.05$ was considered statistically significant. The bar charts were generated using the ggplot2 package in R (version 4.4.0).

3. Results

3.1. Tick species and distribution

A total of 560 ticks (166 free-living ticks and 394 parasitic ticks) were collected across 11 counties (cities and districts) in Qinghai Province. A total of 394 adult ticks (228 females and 166 males), 122 nymph ticks, and 44 larva ticks were collected, belonging to 4 species, 3 genera, and 1 family. Specifically, there were 428 (76.43 %) *Haemaphysalis qinghaiensis* (*H. qinghaiensis*), 78 (13.93 %) *Dermacentor nuttalli* (*D. nuttalli*), 47 (8.39 %) *Dermacentor abaensis* (*D. abaensis*), and 7 (1.25 %) *Ixodes ovatus* (*I. ovatus*). The *H. qinghaiensis* exhibited the widest distribution across 6 regions. *D. nuttalli* was distributed in Zhiduo County, Gangcha County, and Minhe Hui and Tu Autonomous County. *D. abaensis* was distributed in Yushu City and Ulan County, while *I. ovatus* had the least distribution being present only in Huangzhong District (Fig. 1A).

3.2. Pathogen detection

The results of tick-borne pathogen detection are shown in Table 1. These 6 genera of pathogens were all identified in *H. qinghaiensis*, with different prevalences ranging from 0.23 % to 19.86 %. Both *Rickettsia* and *Anaplasma* were detected in all four tick species with the overall prevalence of 34.64 % and 5.00 %, respectively. *Ehrlichia,* BBSL and *Babesia* were only found in *H. qinghaiensis* with the prevalence of 2.14 %, 7.50 %, and 0.18 % respectively.

Through correlation sequence comparisons between BLAST and GenBank, 7 known *Rickettsia*, 4 *Anaplasma*, 2 *Ehrlichia*, 2 BBSL, 1 *Babesia*, and 3 *Theileria* species were identified in the samples collected from *H. qinghaiensis*, *D. nuttalli*, *D. abaensis*, and *I. ovatus* in Qinghai Province. *R. raoultii* exhibited the broadest distribution and was detected in all 4 species of ticks (Fig. 1B).

3.3. Phylogenetic analysis

As shown in Fig. 2A, the phylogenetic analysis based on *gltA* gene of *Rickettsia* indicated that *H. qinghaiensis* from Huangyuan County, Ledu District, Tu Autonomous County of Huzhu, and Banma County in Qinghai Province were infected with the same strain of *Rickettsia principis* (AY578114) identified in *Haemaphysalis japonica* in Russia. It suggests that these *Rickettsia* strains may have spread widely in these regions. Additionally, *Rickettsia* sp. from agent *H. qinghaiensis* in Huangzhong and Huzhu, together with an uncultured *Rickettsia* sp. clone F107-2 (KT921893) in Gansu Province, located on the same phylogenetic branch. The phylogenetic tree also illustrates the presence of *R. raoultii* distributed among 4 ticks and across 7 different sampling sites, indicating its widespread distribution and ecological adaptability in the region. Additionally, the identification of *Rickettsia massiliae*, *Rickettsia barbariae*, and *Rickettsia marmionii* further highlights the diversity of *Rickettsia* in Qinghai Province and its public health implications.

In the 16S rRNA gene analysis of *Anaplasma* and *Ehrlichia*, *A. ovis* was detected in 3 tick species other than *H. qinghaiensis*, across 4 distinct sampling sites, indicating its broad host diversity and ecological adaptability. 2 distinct strains of *Anaplasma capra* (*A.capra*) were identified in ticks from Huangzhong and Huzhu, while *A. bovis* was detected in *H. qinghaiensis* ticks from Banma, Huangzhong, and Huzhu, indicating that ticks may serve as important vectors for the transmission of this pathogen among local livestock and possess a strong ability for geographic dispersal. Moreover, 2 different *Ehrlichia* sp. strains were found in *H. qinghaiensis* across 4 sampling sites, suggesting broad dispersal potential. *Ehrlichia* sp. HZ6-19 showed 99.34 % similarity to the EH727 strain (AY309970) reported in *Haemaphysalis* from Japan. Two *Ehrlichia* strains were placed on distinct branches of the phylogenetic tree and temporarily named *Ehrlichia* sp. 1 and *Ehrlichia* sp. 2, with this differentiation likely associated with ecological adaptation and selective pressures in different hosts or geographic regions, hinting at independent evolutionary pathways. (Fig. 2B).

Examining the 18S rRNA gene of *Babesia* and *Theileria*, a new potential *Babesia* sequence was detected in *H. qinghaiensis* from Huangzhong, which has a similarity of only 93.20 %, 92.99 %, and 92.99 % with the 3 *Babesia bigemina* (*Ba. bigemina*) (FJ426361, OK314932 and MH194393) sequences, respectively, and it is located on a separate branch on the phylogenetic tree. Furthermore, 2 distinct *Theileria* sp. were detected in *H. qinghaiensis* from Huzhu and Ledu, identified as *Theileria* sp. HU212 and *Theileria* sp. LD188. These findings were consistent with the previously reported *Theileria* sp. OT3 isolate LY65 (MG930118) in China. Four species of piroplasmid were confirmed in our study (Fig. 2C).

Utilizing the 5S-23S rRNA gene of BBSL, both *Borrelia garinii* (*B. garinii*) and *Borrelia afzelii* (*B. afzelii*) were identified in *H. qinghaiensis*. *B. garinii* exhibited a similarity of 99.54 % - 100.00 % with *B. garinii* strain YN28 (GU723464) from Yunnan Province, China. Likewise, *B. afzelii* showed a similarity of 99.50 % - 100.00 % with *B.afzelii* isolate (JX888444) previously reported in humans in Heilongjiang, which enhances our understanding of the diversity of and across regions distribution of *Borrelia* and poses a potential threat to public health in Qinghai Province (Fig. 2D).

3.4. Co-infection of pathogens

Among the 560 ticks, 6.43 % (36 / 560) were infected with 2 pathogens, while 0.36 % (2 / 560) were infected with 3 pathogens. Specifically, there were 29 cases of *H. qinghaiensis*, 8 cases of *D. nuttalli* and 1 case of *I. ovatus*. *H. qinghaiensis* primarily exhibited co-infections with *Rickettsia* and *Theileria* or *Rickettsia* and BBSL. Both *D. nuttalli* and *I. ovatus* were found to be co-infected with *Rickettsia* and *Anaplasma*. In addition, 2 cases of *H. qinghaiensis* were identified with 3 pathogen infections. One involved *Rickettsia*, *Anaplasma*, and BBSL, while the others included *Rickettsia*, BBSL, and *Theileria*. Statistical data indicated that *Rickettsia* and *Theileria* co-infections were most prevalent with *H. qinghaiensis*, accounting for 11 samples, followed by *Rickettsia* and BBSL, with 9 samples. Furthermore, co-infections of *Rickettsia* and *Anaplasma* were detected across three tick species: *H. qinghaiensis*, *D. nuttalli*, and *D. abaensis*. (Table S2, Fig. 3A).

3.5. Analysis of risk factors for positivity rates of tick-borne pathogens

The characteristics of tick species, sex, developmental stages, parasitic status, and blood-feeding status were initially considered. Among different tick species, significant differences were observed in the prevalence rates of *Rickettsia*, *Anaplasma*, BBSL, and *Theileria* ($P < 0.05$, Table S3). *D. abaensis* had the highest positivity rate for *Rickettsia* (93.62 %, $P < 0.001$), followed by *D. nuttalli* (89.74 %, $P < 0.001$). Different developmental stages of ticks were associated with the positivity rates for *Rickettsia* and BBSL infections. The positivity rate for *Rickettsia* was higher in adult ticks compared to nymphs and larvae ($P < 0.001$ and $P < 0.05$, respectively). In contrast, larvae showed a higher positivity rate for BBSL compared to adults and nymphs (both $P < 0.001$) (Table S3, Fig. 3B). Only *H. qinghaiensis* was found to be infected with BBSL, while *D. nuttalli* exhibited the

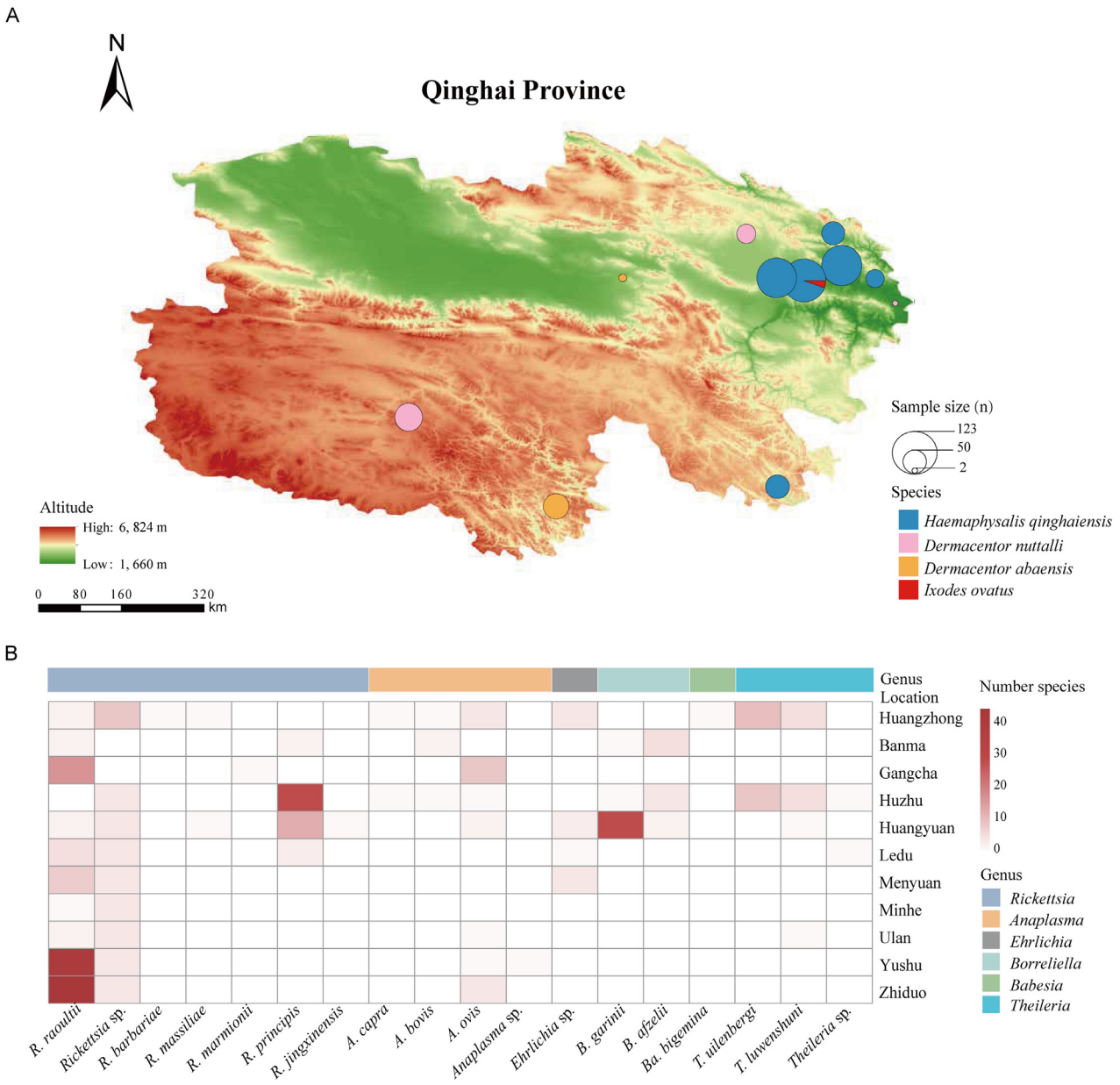

Fig. 1. Sample distribution and detected tick-borne pathogens. A) The species and size distribution of ticks collected in Qinghai Province. Circles on the map show the geographical locations in Qinghai Province where the ticks were collected (n = 560) between April and May 2019. According to the legend, the color and size of the circles represent the species and quantities of ticks collected, respectively. B) Species and numbers of tick-borne pathogens detected in ticks from Qinghai Province. Abbreviations: *R.*, *Rickettsia*; *A.*, *Anaplasma*; *B.*, *Borreliella*; *Ba.*, *Babesia*; *T.*, *Theileria.*

Table 1
Detection results of positivity rate for tick-borne pathogens.

Tick species	Number (n)	Pathogen positivity rate, % (n)					
		Rickettsia	*Anaplasma*	*Ehrlichia*	BBSL	*Babesia*	*Theileria*
H. qinghaiensis	428	19.86 (85)	2.80 (12)	2.80 (12)	9.81 (42)	0.23 (1)	7.48 (32)
D. nuttalli	78	82.05 (64)	16.67 (13)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
D. abaensis	47	93.62 (44)	4.26 (2)	0.00 (0)	0.00 (0)	0.00 (0)	2.13 (1)
I. ovatus	7	14.29 (1)	14.29 (1)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
Total	560	34.64 (194)	5.00 (28)	2.10 (12)	7.50 (42)	0.18 (1)	5.89 (33)

Abbreviations: BBSL, *Borrelia burgdorferi* sensu lato; *H. qinghaiensis, Haemaphysalis qinghaiensis*; *D. nuttalli, Dermacentor nuttalli*; *D. abaensis, Dermacentor abaensis*; *I. ovatus, Ixodes ovatus*.

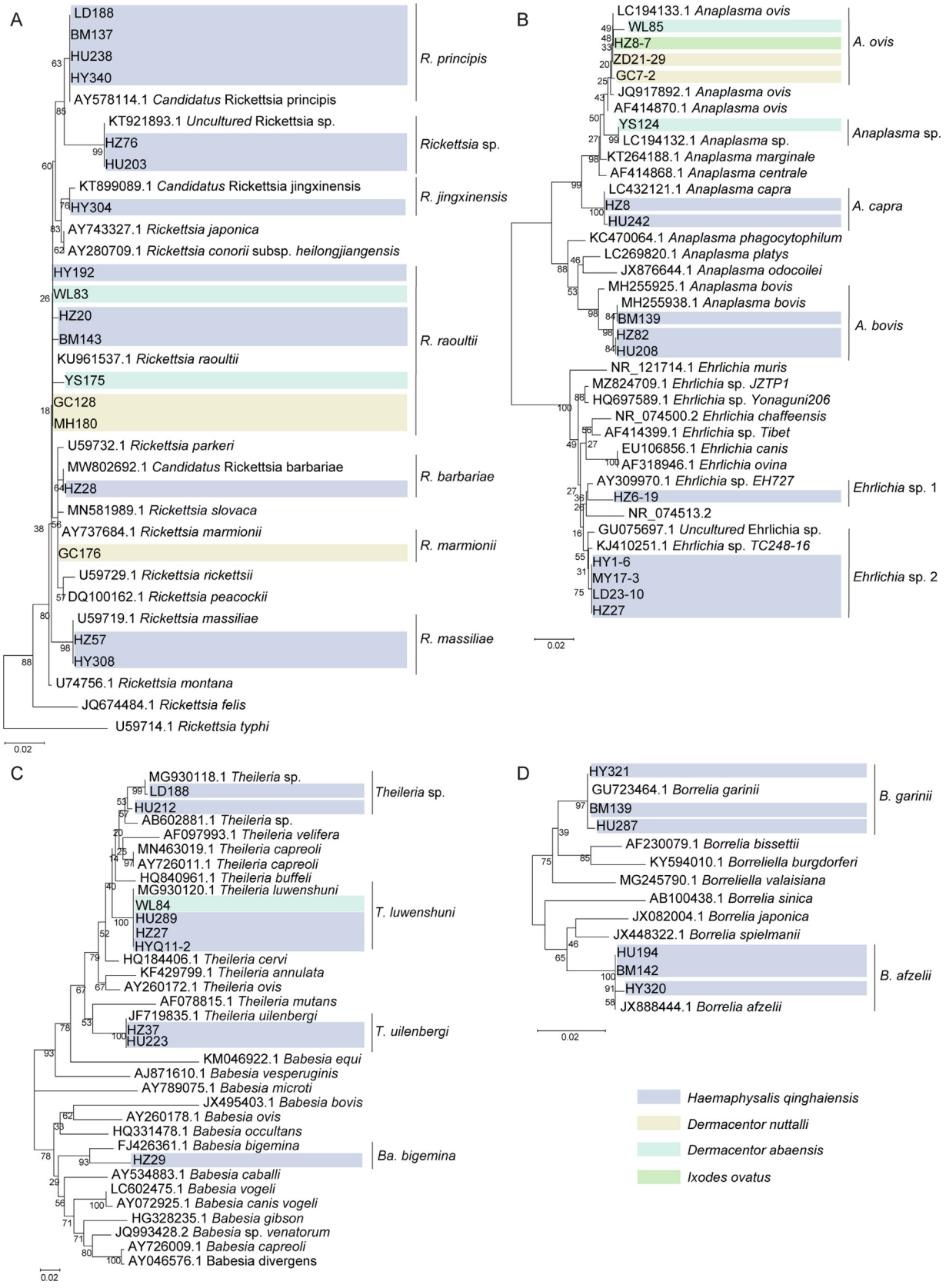

Fig. 2. Phylogenetic tree. A) Phylogenetic tree of *Rickettsia* based on *glt*A gene. B) Phylogenetic tree of *Anaplasma* and *Ehrlichia* based on 16S rRNA gene. C) Phylogenetic tree of *Theileria* and *Babesia* based on 18S rRNA gene. D) Phylogenetic tree of *Borrelia burgdorferi* sensu lato based on 5S-23S rRNA gene. They were constructed by the neighbor-joining method and the length of the branch indicates the number of nucleotide substitutions per site. The number on each branch shows the percent occurrence in 1,000 bootstrap replicates. The branch labels are marked by colors that indicate it was detected in this study and different colors represent that the sample comes from different tick species. Abbreviations: *R.*, *Rickettsia*; *A.*, *Anaplasma*; *T.*, *Theileria*; *Ba.*, *Babesia*; *B.*, *Borreliella;* sp., species.

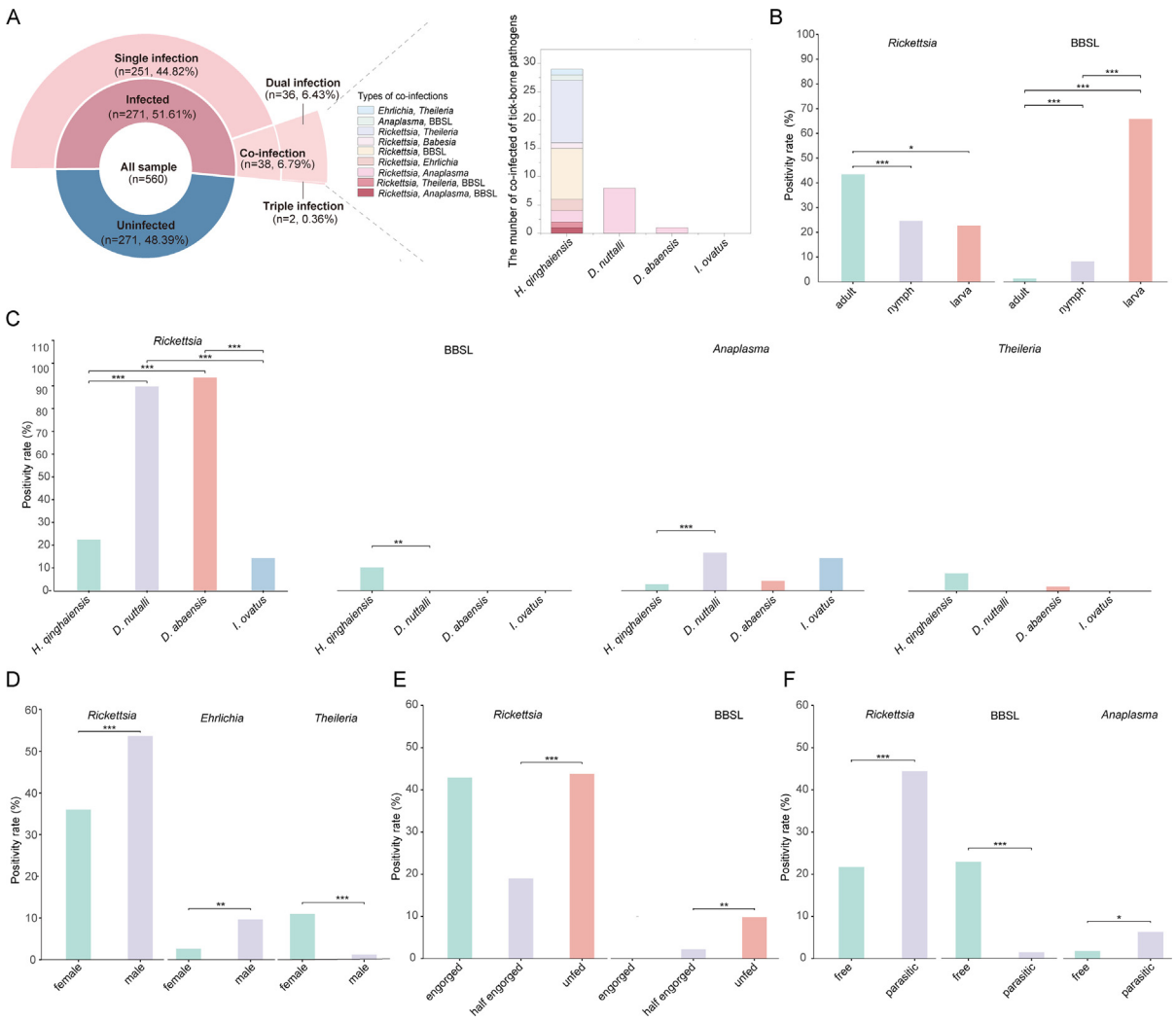

Fig. 3. Infection status of tick-borne pathogens and associated risk factors for positivity rate. A) Detection results of tick-borne pathogens. B) − F) Analysis of the correlation between positivity rates of tick-borne pathogens and developmental stages, tick species, sex, blood-feeding status, and parasitic status of ticks. The above results are based on overall significant differences assessed through the Chi-square test or Fisher's exact probability method ($P < 0.05$), followed by pairwise comparisons. Asterisks (*) indicate the levels of significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Abbreviations: BBSL, *Borrelia burgdorferi* sensu lato; *H. qinghaiensis, Haemaphysalis qinghaiensis*; *D. nuttalli, Dermacentor nuttalli; D. abaensis, Dermacentor abaensis*; *I. ovatus, Ixodes ovatus*.

highest positivity rate for *Anaplasma* (16.67 %, $P < 0.001$) (Table S3, Fig. 3C). Furthermore, male ticks had higher positivity rates for *Rickettsia* and *Ehrlichia* than female ticks ($P < 0.001$ and $P < 0.01$, respectively), while the positivity rate for *Theileria* was lower in male ticks compared to females ($P < 0.001$) (Table S3, Fig. 3D). Unfed ticks exhibited higher positivity rates for *Rickettsia* and BBSL than half engorged ticks ($P < 0.001$ and $P < 0.01$, respectively) (Table S3, Fig. 3E). Lastly, parasitic ticks had higher positivity rates for *Rickettsia* and *Anaplasma* compared to free-living ticks, but the positivity rate for BBSL was lower in parasitic ticks than in free-living ones (Table S3, Fig. 3F).

4. Discussion

Our study highlights the diverse distribution of tick-borne pathogens in Qinghai Province, Northwestern China. Previous studies on these areas have reported that *D. nuttalli* harbours *A. ovis* [24], *D. abaensis* harbours *R. raoultii* and *H. qinghaiensis* harbours *R. raoultii* [17], *A. capra* [25], *Ehrlichia* [26], *Ba. bigemina* [27] and *Theileria uilenbergi* [28]. These findings are consistent with the results of our study, and we report a larger spectrum of pathogens harboured in ticks in Qinghai Province. Qinghai Province provides an environment benefit to the growth, reproduction, and habitat of ticks. The unique geographical location of Qinghai Province makes it an ideal living environment for specific tick species. In the current study, it was observed that *H. qinghaiensis* emerged as the most abundant and widely distributed tick species, which is consistent with the study by Y. Gao [29]. In contrast, the number and distribution range of *D. nuttalli*, *D. abaensis,* and *I. ovatus* in this study were relatively limited, possibly influenced by the local natural environment and selection of sampling sites.

With increasing urbanization and global climate change, the prevalence of tick-borne diseases is expanding, and new tick-borne diseases are emerging [4–7]. Meanwhile, the rapid onset, high mortality, and nonspecific symptoms of tick-borne diseases pose challenges for primary medical staff, leading to potential misdiagnosis and missed diagnosis of these conditions [30], such as anaplasmosis, Rickettsiosis, Babesiosis and so on, which have no specific symptoms. In this study, *Rickettsia* was detected in 194 out of 560 tick samples, yielding a total positivity rate of 34.64 %. This was the highest positivity rate among

the 6 pathogens investigated in this study, with *H. qinghaiensis*, *D. nuttalli*, *D. abaensis,* and *I. ovatus* all exhibiting positivity rates for *Rickettsia*. The widespread distribution of *Rickettsia* across tick species suggests a high risk of transmission and disease in local animal hosts and humans.

Moreover, we identified co-infections of multiple pathogens in our tick samples. It is worth noting that *H. qinghaiensis* exhibited mixed infections involving three pathogens. In previous studies, co-infections of *Rickettsia* and *Anaplasma* were reported in *Ixodes ricinus* (*I. ricinus*) [31] and a female patient [32]. In addition, the co-infection of *A. ovis* and *Rickettsia* in *D. nuttalli* in the border area of China and Russia [33], which was consistent with the type of co-infections identified in *D. nuttalli* in this study. Besides the co-infection of *D. nuttalli* with *Anaplasma* and *Rickettsia*, there were no reports of co-infection of multiple pathogens in *H. qinghaiensis*, *D. nuttalli,* and *I. ovatus* until now. The phenomenon of co-infection observed in this study provides a theoretical basis for future research and the mechanisms underlying pathogen interaction and pathogenicity in co-infections warrant further study.

The significant different prevalence of pathogens belonging to four genera among different tick species reflects the varying capacities of these tick species to carry and transmit pathogens. Notably, BBSL is exclusively carried by *H. qinghaiensis*, with higher infection rate in larvae than those in other developmental stages ($P < 0.001$). Previous studies have indicated the potential for transovarial transmission of BBSL in *I. ricinus* [34] and *I. persulcatus*[35]. Therefore, it suggests that BBSL may also be transmitted transovarially through *H. qinghaiensis*.

The infection rate of *Rickettsia* in adult ticks is higher than in the other two developmental stages ($P < 0.001$ and $P < 0.01$), and the infection rates in *D. nuttalli* and *D. abaensis* are greater than that in *H. qinghaiensis* ($P < 0.001$). This may indicate differences in the transmission efficiency of these pathogens among different tick species.

Moreover, the infection of *Theileria* is also primarily distributed in *H. qinghaiensis*, with a higher infection rate in females compared to males ($P < 0.001$). This may be attributed that the infection rate of *Theileria* in livestock is relatively high in these pastoral areas [36]. Among the collected *H. qinghaiensis*, there were 158 female ticks and 104 male ticks. Since male ticks do not feed on blood, the infection rate of *Theileria* in females is higher than in males.

This study has three limitations. Firstly, our study is a cross-sectional epidemiological investigation, not a longitudinal study. We were unable to carry out an analysis of seasonal dynamic changes for ticks. Secondly, our study didn't screen for any viruses among the ticks investigated. As most new tick-borne viruses were mainly discovered through high-throughput sequencing, limited research and understanding of their distribution characteristics, and there are few reports on the discovery of tick-borne viruses in Qinghai Province, it is difficult to select which certain viruses should be tested one by one using PCR. Finally, there is some sampling bias. The large uninhabited areas in the southwest and the Gobi Desert in the northwest were not chosen as sampling sites.

5. Conclusions

In conclusion, the diverse distribution of tick-borne pathogens in Qinghai Province, and the phenomenon of co-infection pose a significant threat to both local animal husbandry and human health. Therefore, enhancing systematic monitoring of tick-borne pathogens affecting residents and domestic animals in the region is crucial.

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Conflcit of interest statement

The authors declare that there are no conflicts of interest.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bsheal.2024.11.005.

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