Abstract

Background

Yanbian is located at the junction between China, Russia, and North Korea. We aimed to determine the species distribution and pathogens carried by ticks in Yanbian.

Methods

A total of 2673 unattached ticks were collected from eight counties and cities in Yanbian and classified morphologically. Candidatus Rickettsia tarasevichiae (CRT), spotted fever group Rickettsia (SFGR), severe fever thrombocytopenia syndrome virus (SFTSV), Theileria, and other pathogens were detected using polymerase chain reaction (PCR) and real-time quantitative polymerase chain reaction followed by phylogenetic and genotypic analyses.

Results

According to the morphological classification, the main tick species in Yanbian were Haemaphysalis longicornis, Ixodes persulcatus, Dermacentor silvarum, Haemaphysalis japonica, and Haemaphysalis concinna. Candidatus Rickettsia tarasevichiae, spotted fever group Rickettsia, severe fever thrombocytopenia syndrome virus, and Theileria orientalis were detected in H. longicornis, Candidatus Rickettsia tarasevichiae, spotted fever group Rickettsia, and severe fever thrombocytopenia syndrome virus were detected in I. persulcatus, H. japonica, and D. silvarum, but only severe fever thrombocytopenia syndrome virus was detected in H. concinna. Mixed infection with Candidatus Rickettsia tarasevichiae and severe fever thrombocytopenia syndrome virus was found in I. persulcatus and H. japonica. The gene sequences of all tested pathogens exhibited 95.7%–100% homology with sequences registered in GenBank. Phylogenetic analysis showed that different spotted fever group Rickettsia and severe fever thrombocytopenia syndrome virus genotypes were closely related to the Korean strains. We provide the first evidence for the presence of the spotted fever group Rickettsia genotypes of Candidatus Rickettsia longicornii, ompA, ompB, sca4, and rrs, in Haemaphysalis longicornis in Yanbian.

Conclusions

These results provide epidemiological data to support the prevention and control of ticks and tick-borne diseases in the border areas of China, North Korea, and Russia.

Background

Ticks are arthropod disease vectors widely found in nature, which provide reservoirs for zoonotic diseases [1, 2]. Ticks include the families Ixodidae, Argasidae, and Nuttalliellidae [3, 4]. A tick bite penetrates the skin of the host, occasionally resulting in systemic diseases caused by dermatitis, ulcers, or secondary infection. The presence of large numbers of ticks on livestock not only damage the affected area, but also result in anemia and reduce the development, yield, and quality of the livestock, possibly even resulting in death. Increasing studies have clarified the ability of ticks to carry and transmit a variety of disease-causing pathogens [5], including viruses, protozoa, and bacteria [6]. Ticks and tick-borne diseases do great harm to human health and animal husbandry, and have become an important public health problem worldwide[7, 8].

China covers a vast area and includes regions with dramatically different natural conditions. There are obvious differences in the distributions of ticks between the south and the north of the country, and tick-borne diseases are sometimes reported. Yanbian is located on the border of China with Russia and Korea, in the Changbai Mountains. It is largely covered in forest and has a mid-temperate humid monsoon climate. It is rich in biological resources and provides suitable conditions for tick growth and reproduction. However, the species distribution of ticks and tick-borne pathogens in the border area of China, Russia, and North Korea are not clear. We therefore systematically examined the species distribution and pathogens carried by ticks in Yanbian to provide a scientific basis to support the prevention and control of ticks and tick-borne diseases in Yanbian.

Materials And Methods

Sample collection

A total of 2673 unattached adult ticks were collected from Hunchun, Tumen, Yanji, Dunhua, Helong, Longjing, Wangqing, and Antu in Yanbian, China, on sunny mornings from April to August 2019, using the flag-laying method. The ticks were stored live in water in the refrigerator at 4°C. The date and place of collection was recorded for each sample. All experimental procedures involving animals were conducted according to the Ethical Principles of Animal Research issued by Yanbian University.

Tick classification

The collected ticks were classified morphologically based on the ‘Classification and Identification of Important Medical Insects of China’ [9] and ‘Ticks of Japan, Korea, and the Ryukyu Islands’ [10].

Tick treatment

The ticks were grouped according to species and region. Each tick was washed three times in normal saline, placed in a 1.5 mL centrifuge tube, and 600L phosphate-buffered saline was added. The tick was then ground with a tissue breaker (TissueLyser II; Qiagen, GER), followed by centrifugation at 1,300 x g for 1 min, and 200 L of the supernatant was collected to extract nucleic acid.
Pathogen gene detection

Nucleic acids were extracted using a nucleic acid extraction kit (Suzhou Tianlong Science and Technology Co., Ltd., Suzhou, China). The \textit{Candidatus Rickettsia tarasevichiae} (CRT) \textit{ompA} gene and 17-kDa genes were detected as described by Jia et al. \cite{11}. The spotted fever group \textit{Rickettsia} (SFGR) \textit{Candidatus Rickettsia longicornii} \textit{ompA}, \textit{ompB}, \textit{sca4}, \textit{rrs} genes were detected as described by Jiang et al. \cite{12}, the severe fever thrombocytopenia syndrome virus (SFTSV) Small, Medium, and Large genes were detected as described by Liu et al. \cite{13}, the \textit{Theileria orientalis}MPSP gene was detected as described by Ota et al. \cite{14}, and the \textit{Theileria sinensis}MPSP gene was detected as described by Liu et al. \cite{15}. The primers for these genes are listed in Table 1.

| Pathogen gene | Primer name | Sequence (5'-3') | Annealing temperature (°C) | Fragment size (bp) | reference |
|---------------|-------------|------------------|-----------------------------|-------------------|-----------|
| CRT \textit{ompA} | Rr190.70p | ATGGCGAATATTTCTCCAAAA | 60 | 346 | Jia et al. (2013) |
| | Rr190.602n | AGTGCAGCATTGCCTCCCTT | 58 | | |
| | 190.70-38s1 | AAAACCG CTTATTCACC | | | |
| | 190.602-384r1 | GGCAAC AAGTTACCTCCT | | | |
| CRT 17 kDa | 17K3 | GCTTTCAAAATCTAAAAACCATATA | 50 | 395 | Jia et al. (2013) |
| | 17K5 | TGTCATATCAATTCACAACTTGCC | | | |
| | 17KD113s1 | ATTTGTCGTCAGGTTGCC | | | |
| | 17KD408r1 | GGGCCGGTATGAAATAGGC | | | |
| SFGR \textit{Candidatus Rickettsia longicornii} \textit{ompA} | H-LompA-F | TTTAATTTGATTTAATTTTTATTAAGGTTTACATATGGCCG | 60 | 647 | Jiang et al. (2018) |
| | H-LompA-R | GTCTTGACAGTTATTATACCTCCTCAT | | | |
| SFGR \textit{Candidatus Rickettsia longicornii} \textit{ompB} | H-LompB-F1 | GTTCAGCTATGGGTGCTGCTATACAG | 63 | 1203 | Jiang et al. (2018) |
| | H-LompB-R1 | GCACTAGCCTTTGCTAAAGTACCGT | | | |
| SFGR \textit{Candidatus Rickettsia longicornii} \textit{sca4} | H-Lsca4-F2 | AGTTCTCAGTCCAGCAAAACAC | 63 | 885 | Jiang et al. (2018) |
| | H-Lsca4-R2 | GCCTTTACCAGCTCATCTACTTT | | | |
| SFGR \textit{Candidatus Rickettsia longicornii} \textit{rrs} | H-L16S-F | TGCAAGTCGAACGGACTAATTGG | 65 | 976 | Jiang et al. (2018) |
| | H-L16S-R | AATGAGGGTTGCGCTCGTTG | | | |
| SFTSV Small | S-F1 | ACACAAAGACCCCCCTTCTATTGGA | 58 | 588 | Liu et al. (2016) |
| | S-R1 | TGAGGAGGGCCACATCCAG | | | |
| SFTSV Medium | M-F1 | GATGAGATGGTCCATGCTGATTCTTCTC | 58 | 560 | Liu et al. (2016) |
| | M-R1 | CTCTGGGTTAGAAGTGCTCCTTAC | | | |
| SFTSV Large | L-F1 | ACACAGAGACGCCAGATGAAC | 60 | 684 | Liu et al. (2016) |
| | L-R1 | GCCTCAAGCTCTTCTTCCTACTCTTCTG | | | |
| \textit{T. orientalis}MPSP | P1 | CACGCTATGTTGCTCAAGAG | 53 | 875 | Liu et al. (2010) |
| | P2 | TGAGAAGCTCATTGAGCCCTA | | | |
| \textit{T. sinensis}MPSP | P3 | CACTGCTATGTTGCTCAAGAGATATT | 56 | 887 | Liu et al. (2010) |
| | P4 | AATGCGCCTAAAGATAGTGAACAAAC | | | |

Homology and phylogenetic analyses

The PCR products of positive samples were sent to Shanghai Shenggong Co., Ltd. for sequencing. Correct gene sequences were analyzed via DNAStar and GenBank, and phylogenetic trees were constructed using the neighbor-joining method with a Kimura 2-parameter model using MEGA7 software.
Statistical analysis

Data were processed using Excel 2007 and statistical analysis was carried out using SAS8.2 software. Numerical data were expressed as a constituent ratio (%) and positive rate (%), where the constituent ratio (%) = number of each tick species in same location / total number of all tick species in same location × 100%, and the positive rate (%) = number of positive samples detected for pathogens / total number of tested samples of the same species (n) × 100%.

Results

Tick species survey results

A total of 2673 ticks were collected, including 1373 *H. longicornis* (51.37%), 651 *Ixodes persulcatus* (24.35%), 357 *Haemaphysalis japonica* (13.36%), 140 *Dermacentor silvarum* (5.24%), and 152 *Haemaphysalis concinna* (5.68%). *H. longicornis* and *I. persulcatus* were the dominant local tick species (Table 2).

| Location | Haemaphysalis longicornis | Ixodes persulcatus | Haemaphysalis japonica | Dermacentor silvarum | Haemaphysalis concinna | Total |
|----------|--------------------------|--------------------|------------------------|----------------------|------------------------|-------|
| Hun chun | 348 (68.24) 129 (25.29) 17 (3.33) 16 (3.14) 0 (0.00) 510 | | | | | |
| Tu men   | 37 (20.22) 0 (0.00) 120 (65.57) 14 (7.65) 12 (6.56) 183 | | | | | |
| Yan ji   | 192 (48.98) 174 (44.39) 0 (0.00) 0 (0.00) 26 (6.63) 392 | | | | | |
| Dun hua  | 0 (0.00) 106 (42.74) 125 (50.40) 17 (6.85) 0 (0.00) 248 | | | | | |
| He long  | 87 (26.69) 158 (48.46) 67 (1.84) 14 (4.29) 0 (0.00) 326 | | | | | |
| Long jing| 426 (88.38) 56 (11.62) 0 (0.00) 0 (0.00) 0 (0.00) 482 | | | | | |
| Wang qing| 283 (84.73) 23 (6.89) 28 (8.38) 0 (0.00) 0 (0.00) 334 | | | | | |
| An tu    | 0 (0.00) 5 (2.53) 0 (0.00) 79 (39.90) 114 (57.58) 198 | | | | | |
| Total    | 1373 (51.37) 651 (24.35) 357 (13.36) 140 (5.24) 152 (5.68) 2673 | | | | | |

Pathogen gene detection

By detecting different tick-pathogen genes, we detected CRT, SFGR, SFTSV, and *T. orientalis* in *H. longicornis*, CRT, SFGR, and SFTSV in *I. persulcatus* and *H. japonica*, CRT and SFTSV in *D. silvarum*, and only SFTSV in *H. concinna*. Moreover, different CRT and SFGR genotypes were detected in *H. longicornis* and *H. japonica*, and different SFTSV genotypes in *H. concinna*. There was mixed infection with CRT and SFTSV in *I. persulcatus*, *H. japonica*, and *D. silvarum*. The highest incidences of CRT/SFTSV co-infection included 13 cases (2.00%) in *I. persulcatus*, five cases (1.40%) in *H. japonica*, and one case (0.71%) in *D. silvarum*. *T. orientalis* was detected in *H. longicornis*. *T. sinensis* was not detected in any ticks (Table 3).
Infection of pathogens in different tick species in Yanbian, China

| Species                  | CRT ompA (pos. rate %) | CRT 17 kDa (pos. rate %) | SFGR Candidatus Rickettsia longicornii ompA (pos. rate %) | SFGR Candidatus Rickettsia longicornii ompB (pos. rate %) | SFGR Candidatus Rickettsia longicornii sca4 (pos. rate %) | SFGR Candidatus Rickettsia longicornii rrss (pos. rate %) | SFTSV Small (pos. rate %) | SFTSV Medium (pos. rate %) | SFTSV Large (pos. rate %) | CRT + SFTSV (pos. rate %) |
|-------------------------|------------------------|---------------------------|----------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|--------------------------|---------------------------|--------------------------|--------------------------|
| Haemaphysalis longicornis (n = 1373) | 22(1.60)               | 23(1.68)                  | 323(23.53)                                               | 261(19.01)                                               | 287(20.90)                                               | 364(26.51)                                               | 16(1.17)                               | 13(0.95)                               | 15(1.09)                               | 0                       |
| Ixodes persulcatus (n = 651)          | 71(10.91)              | 57(8.76)                  | 7(1.08)                                                  | 5(0.77)                                                  | 5(0.77)                                                  | 6(0.92)                                                  | 22(3.38)                               | 17(2.61)                               | 26(3.99)                               | 13(2.00)                              |
| Haemaphysalis japonica (n = 357)       | 26(7.28)               | 25(7.00)                  | 8(2.48)                                                  | 6(1.68)                                                  | 7(1.96)                                                  | 7(1.96)                                                  | 38(10.46)                              | 23(6.44)                               | 27(7.56)                               | 5(1.40)                                |
| Dermacentor silvarum (n = 140)        | 28(20.00)              | 49(35.00)                 | 0                                                        | 0                                                        | 0                                                        | 0                                                        | 44(31.43)                              | 26(18.57)                              | 37(26.43)                              | 1(0.71)                                |
| Haemaphysalis concinna (n = 152)       | 0                      | 0                         | 0                                                        | 0                                                        | 0                                                        | 0                                                        | 17(11.18)                              | 11(7.24)                               | 19(12.50)                              | 0                       |
| Total (n = 2673)                     | 147(5.50)              | 154(5.76)                 | 338(12.64)                                               | 272(10.18)                                               | 299(11.19)                                               | 377(14.10)                                               | 137(5.13)                              | 90(3.37)                               | 124(4.64)                              | 19(0.71)                               |

### Sequence and phylogenetic analyses of pathogen genes

Analysis of the sequence homology between the CRT gene sequence obtained from I. persulcatus and the GenBank sequence showed that the CRT ompA gene sequence of the ticks in Yanbian shared 100% homology with the Henan Xinyang strain (KX365196.1), Northeast China strain (KT899079.1), Heilongjiang Mudanjiang strain (JX996053). The nucleotide sequence homologies of the 17kDa gene between Henan Xinyang (KX365195.1) and the Heilongjiang Mudanjiang (KT259906) and Jilin (KT384433) strains were 99.7% and 100%, respectively. Phylogenetic analysis showed that the CRT ompA gene of Yanbian strain YB02 (MT511087) clustered with the Henan Xinyang (KX365196.1) and Heilongjiang strains (JX996053). The 17kDa gene (MT511086) clustered with the Japanese (LC379446.1) and Heilongjiang (JX996052.1) strains (Fig. 1).

From the samples positive for SFGR Candidatus Rickettsia longicornii, four gene sequences of ompA, ompB, sca4 and rrss were obtained by PCR amplification. Sequence homology analysis showed that the four gene sequences (MT511088, MT511089, MT511090, and MT535574) had 100%, 99.70%, 100%, and 95.70% homology, respectively, with the corresponding fragments (MG906676, MG906675, MG906677, and MG906672) of SFGR Candidatus Rickettsia longicornii newly discovered in the Korean H. longicornis sample (ROK-HL727). Phylogenetic analysis showed that the sequence of Candidatus Rickettsia longicornii ompA (MT511088) was located on the same branch as the Korean ROK-HL727 strain (MG906676.1), Chinese Changchun Candidatus Rickettsia jingxinensis H6 strain (KT899081.1), and Chinese Dandong 19070 strain (MH427382.1). The ompB (MT511089) gene sequence was in the same clade as the Chinese HC strain (MK620854.1), and exhibited a close evolutionary relationship with the Korean Candidatus Rickettsia longicornii ROK-HL727 strain (MG906675.1). The rrs (MT535574) gene sequence was located in the same branch as the Candidatus Rickettsia jingxinensis-related sequence (MH500204) found in H. longicornis in China and the Candidatus Rickettsia longicornii ROK-HL727 strain (MG906672.1) in Korea. The sca4 (MT511090) gene sequence was also closely related to the Korean Candidatus Rickettsia longicornii ROK-HL727 strain (MG906677.1) (Fig. 2).

The SFTSV Large (MT517309), Medium (MT517308), and Small (MT517307) gene sequences showed 98%–99% homology with the SFTSV gene sequences identified in China and South Korea according to homology analysis. Phylogenetic analysis showed that the SFTSV Small gene sequence from ticks in Yanbian was in the same clade as the SFTSV gene sequence (KT890282.1) of Jilin ticks in China. The Medium gene sequence was located in the same branch as the Chinese JS2014-18 strain (KR230781.1), and the Small sequence was located in the same branch as the Chinese JS2014-18 strain (KR230761.1) and was closely related to SFTSV isolated from Zhejiang and South Korea (Fig. 3).

There was 99.4% homology between the T. orientalis MPSP gene (MT517304) and the published GenBank entry number (MG664537.1). Phylogenetic analysis showed that the sequence of the T. orientalis MPSP (MT517304) gene in the ticks was located in the same branch as MPSP (MG664537.1) in the Chinese Chongqing strain (Fig. 4).

### Discussion

Yanbian is located at the junction of China, North Korea, and Russia, and has a long border. Strengthening ecological and environmental protection in China means that the species distribution along the border has gradually diversified; the numbers and species of ticks are thus constantly changing, and their...
activity is increasing. Ticks and other vectors in the border zone can migrate to each other using various methods, increasing the risk of tick-borne diseases. In this study, 2673 ticks collected from eight counties and cities in Yanbian were classified and analyzed. *H. longicornis* and *I. persulcatus* were the dominant tick species in Yanbian. *H. longicornis* has strong reproductive ability and environmental adaptability, and is widely distributed throughout Asia and the Pacific, including China, Russia, South Korea, Japan, Australia, New Zealand, and the South Pacific islands. It is often parasitic in medium and large wild and domestic animals, whereas humans are accidental hosts. *H. longicornis* spreads a variety of pathogens that can affect wild animals and livestock, as well as human health.

Ticks can be infected with viruses, bacteria, including *Rickettsia* and spirochetes, and other pathogens. In addition, ticks can act as both vectors and hosts in the process of infectious disease transmission. The main research into tick-borne pathogen co-infections is currently focused on *Borrelia burgdorferi*, *Babesia microti*, *Ehrlichia*, and *Anaplasma phagocytophilum* [16]. Previous studies confirmed that one-third of patients with a CRT infection had neurological symptoms that differed from other SFR infections [17], and were associated with a higher case-fatality rate when co-infected with SFTSV [18]. More attention should be paid to SFTSV transmission through both tick bites and close contact with infected cases [19]. In this study, we confirmed the existence of CRT/SFTSV co-infection in *I. persulcatus* (n = 13), *H. japonica* (n = 5), and *D. silvarum* (n = 1) in Yanbian. *I. persulcatus* is a common dominant tick species in Yanbian, and is especially widely distributed in Hunchun, Yanji, Helong, and other regions, resulting in a high risk of CRT and SFTSV infection via tick bites in these regions.

SFTSV was isolated from sheep in the SFTS epidemic area in Shandong Province, China [21]. In the current study, the SFTSV Small, Medium, and Large gene sequences were obtained by gene amplification, and homology analysis indicated 98%-99% homology with the SFTSV gene sequence found in South Korea. Phylogenetic analysis showed that the SFTSV Small, Medium, and Large gene sequences were in the same clade as isolates from Jilin and Jiangsu, respectively, and were closely related to SFTSV isolated from Zhejiang and South Korea. This may be related to the parasitism of migratory birds by ticks in the east or SFTSV transmission by migratory birds themselves during cross-sea migration. Korean researchers suggested that migratory birds may play an important role in the spread of SFTSV [22]. The above results suggest that the border area of China, North Korea, and Russia, as well as in other areas with a concentrated distribution of *H. longicornis*, to prevent cross-border transmission and an epidemic of tick-borne diseases affecting human health and animal husbandry.

**Conclusions**

*H. longicornis* and *I. persulcatus* are the dominant tick species in Yanbian, China. Four pathogens (CRT, SFGR, SFTSV and *T. orientalis*) were detected in the tick species collected in this study, and CRT/SFTSV co-infection was also identified in *I. persulcatus* and *H. japonica*. Moreover, this study provides the first evidence of the SFGR genotypes *Candidatus Rickettsia longicombi* [ompA, ompB, sca4, and rs in *H. longicornis* in Yanbian, China. Moreover, *T. orientalis* was detected in *H. longicornis*. These findings provide epidemiological data to support the prevention and control of ticks and tick-borne diseases in the border region of China, North Korea, and Russia.

**Abbreviations**

CRT: *Candidatus Rickettsia tarsievichiae*; SFGR: spotted fever group *Rickettsia*; SFTSV: severe fever thrombocytopenia syndrome virus; PCR: polymerase chain reaction.
Declarations

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Not applicable.

Declarations
Not applicable.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Availability of data and material
All data supporting the conclusions of this article are included within the article.

Competing interests
The authors declare that they have no competing interests.

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Authors contributions
JXL: Methodology, Validation, Statistical analysis the results, Writing-Original Draft.
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