Survey of tick species and molecular detection of selected tick-borne pathogens in Yanbian, China

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Abstract – Ticks and tick-borne diseases pose a significant threat to public health. In this study, we aimed to determine the tick species distribution and pathogens carried by ticks in Yanbian, China. A total of 2673 questing ticks were collected from eight counties and cities in Yanbian and were morphologically identified. The presence of Candidatus Rickettsia tarasevichiae (CRT), spotted fever group Rickettsia (SFG), severe fever thrombocytopenia syndrome virus (SFTSV), Theileria, and other pathogens was confirmed using polymerase chain reaction (PCR) and real-time quantitative PCR assays, followed by phylogenetic and genotypic analyses. According to the morphological identification, the tick species in Yanbian consisted of Haemaphysalis longicornis, Ixodes persulcatus, Dermacentor silvarum, H. japonica, and H. concinna. In H. longicornis, CRT, SFGR, SFTSV and Theileria orientalis were detected, while CRT, SFGR, and SFTSV were detected in I. persulcatus, H. japonica, and D. silvarum. Only SFTSV was detected in H. concinna. Mixed infection with CRT and SFTSV was observed in I. persulcatus and H. japonica. The gene sequences of all tested pathogens exhibited 95.7%–100% identity with the corresponding sequences deposited in GenBank. Phylogenetic analysis showed that different SFGR and SFTSV genotypes were closely related to the Korean strains. This study is the first to describe the genetic diversity of SFGR Candidatus Rickettsia longicornis in H. longicornis in Yanbian, China, using the ompA, ompB, sca4, and rrs genes. 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.

Key words: Yanbian, Tick, Rickettsia, Severe fever thrombocytopenia syndrome virus, Theileria.

Résumé – Enquête sur les espèces de tiques et détection moléculaire de certains agents pathogènes transmis par les tiques à Yanbian, en Chine. Les tiques et les maladies transmises par les tiques constituent une menace importante pour la santé publique. Dans cette étude, nous avons cherché à déterminer la distribution des espèces et les agents pathogènes portés par les tiques à Yanbian, en Chine. Un total de 2 673 tiques errantes ont été collectées dans huit comtés et villes de Yanbian et identifiées morphologiquement. La présence de Candidatus Rickettsia tarasevichiae (CRT), de Rickettsia du groupe de la fièvre boutonneuse (SFGR), du virus du syndrome de la fièvre thrombocytopenique sévère (SFTSV), de Theileria et d’autres agents pathogènes a été confirmée à l’aide d’une réaction en chaîne par polymérase (PCR) et de PCR quantitative en temps réel, suivies par des analyses phylogénétiques et génotypiques. Selon leur identification morphologique, les espèces de tiques à Yanbian se composaient de Haemaphysalis longicornis, Ixodes persulcatus, Dermacentor silvarum, H. japonica et H. concinna. Chez H. longicornis, CRT, SFGR, SFTSV et Theileria orientalis ont été détectés, tandis que CRT, SFGR et SFTSV ont été détectés chez I. persulcatus, H. japonica et D. silvarum. Seul le SFTSV a été détecté chez H. concinna. Une infection mixte par CRT et SFTSV a été observée chez I. persulcatus et H. japonica. Les séquences des gènes de tous les agents pathogènes testés présentaient une identité de 95.7 % à 100 % avec les séquences correspondantes déposées dans GenBank. L’analyse phylogénétique a montré que différents génotypes SFGR et SFTSV étaient étroitement liés aux souches coréennes. Cette étude est la première à décrire la diversité génétique de SFGR Candidatus Rickettsia longicornii chez H. longicornis à Yanbian, en Chine, en utilisant les...
Introduction

Ticks act as vectors for many pathogens and are widespread in nature [16, 25]. Ticks include the families Ixodidae, Argasidae, Deinocrotonidae, and Nuttalliellidae [2, 17]. A tick bite penetrates the skin of the host, which can result in dermatitis, ulcers, or secondary infection. The presence of large numbers of ticks in livestock not only damages the affected area, but also results in anemia and reduced development, yield, and quality of the livestock; sometimes, infestation even results in death. More and more studies have clarified the ability of ticks to carry and transmit a variety of disease-causing pathogens [18], including viruses, protozoa, and bacteria [24]. Ticks and tick-borne diseases cause substantial harm to human health and animal husbandry, and have become an important public health problem worldwide [14, 19].

China covers a vast area and includes regions with dramatically different natural conditions. There are obvious differences in the distribution of ticks between the southern and the northern regions of the country, and tick-borne diseases occasionally occur. Yanbian is located at 41° 59′47″ – 44° 30′42″ North latitude, 127° 27′43″ – 131° 18′33″ East longitude, and is bordered by North Korea and Russia. This region, approximately covering 43,329.34 km², is largely covered in forest and has a mid-temperate humid monsoon climate. It is rich in biological resources and provides suitable conditions for the development and reproduction of ticks. However, the species distribution of ticks and tick-borne pathogens in the border area of China, Russia, and North Korea is unclear. Therefore, we 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

Ethics

All experimental procedures in animals were conducted following the Ethical Principles in Animal Research issued by Yanbian University (approval number: 20180301).

Sample collection

A total of 2673 unattached adult ticks were collected from Hunchun, Tumen, Yanji, Dunhua, Helong, Longjing, Wangjing, and Antu in Yanbian, China (Fig. 1), during sunny mornings from April to August 2019, using the dragging-flagging method. A white gauze mesh, 2 m long and 1 m wide, was completely spread out on the grass and dragged slowly over the grass to capture ticks. Ticks attached to the gauze mesh were then transferred to the collection tube by tweezers, and the related information, such as collection time and location, were marked in detail. The ticks were stored live in water in the refrigerator at 4 °C. The date and place of collection was recorded for each sample.

Tick classification and nucleic acid extraction

Pathogen detection
Table 1. Primer sequences used for the gene amplification of different pathogens.

| Pathogen gene | Primer name | Sequence (5'–3') | Anneling temperature (°C) | Fragment size (bp) | Reference |
|---------------|-------------|------------------|---------------------------|-------------------|-----------|
| CRT ompA      | Rr190.70p   | ATGGCGAAGATATTTTCCAAAA | 60                        | 346               | Jia et al. [4] |
|               | Rr190.602n  | AGTGACCATCGTCGCTCC | 58                        |                   |           |
|               | 190.70-38s1 | AAAACCG CTTTATTCACC | 59                        |                   |           |
|               | 190.602-38r1| GGCACAC AAGTCTACCTCT | 52                        |                   |           |
| CRT 17kDa     | 17K3        | GCTTTCAAAATATCTAAAAACATATA | 50                        | 395               | Jia et al. [4] |
|               | 17K5        | TGCATATCTAATCACAATCTGCCC | 52                        |                   |           |
|               | 17KD113s1   | ATTTGTCGGTACGGTGCGC | 52                        |                   |           |
|               | 17KD408r1   | CGGGCCGTGATGAAAAACGC | 52                        |                   |           |
| SFGR Candidatus Rickettsia longicornii ompA | H-LompA-F | TTCATATTTATACCTCCCATTTAATTTACATACAG | 60                        | 647               | Jiang et al. [3] |
| SFGR Candidatus Rickettsia longicornii ompB | H-LompB-F1 | GTTGCAGCTATGCGGTGCTATAACAG | 63                        |                   | Jiang et al. [3] |
| SFGR Candidatus Rickettsia longicornii sca4 | H-Lsca4-R1 | GCATCATGCGCTGTAAGACGCGC | 63                        | 885               | Jiang et al. [3] |
| SFGR Candidatus Rickettsia longicornii rrs | H-L16S-F | GCCCTTACGCTCATCTACTTTAT | 65                        | 976               | Jiang et al. [3] |
| SFTSV Small | S-F1 | ACAGAAAGACCCCCCTTATTTGG | 58                        | 588               | Liu et al. [10] |
|               | S-R1 | TGGAGGAGGGCCCATACCC | 58                        |                   | Liu et al. [10] |
| SFTSV medium | M-F1 | GATGAGATGTGCATGTGATCTCT | 58                        |                   | Liu et al. [10] |
|               | M-R1 | CTACGGGTTGGAATGTCTCCTAC | 58                        |                   | Liu et al. [10] |
| SFTSV large  | L-F1 | ACACAGAGACGCCAGATGAAAC | 60                        | 684               | Liu et al. [10] |
|               | L-R1 | GCCTCAAGCCTCTGCTACTCTTGT | 60                        |                   | Liu et al. [10] |
| T. orientalis MPSP | P1 | CATGCTATGTGTCCTCCAGAG | 53                        | 875               | Ota et al. [15] |
|               | P2 | TGTGACCTAAATGGCGGCTTA | 53                        |                   | Ota et al. [15] |
| T. sinensis MPSP | P3 | ACAGAAGGCAGCCAGATGAAAC | 53                        | 887               | Liu et al. [8] |
|               | P4 | ATGGCGCTAAAGGATGTGAGAAAC | 53                        |                   | Liu et al. [8] |

Table 2. Composition of tick species in 8 counties and cities of Yanbian.

| Location     | Haemaphysalis longicornis | Isodes persulcatus | Haemaphysalis japonica | Dermacentor silveri varum | Haemaphysalis concinna | Total |
|--------------|----------------------------|--------------------|-------------------------|-----------------------------|------------------------|-------|
|              | Quantity (%) | Constituent (%)    | Quantity (%) | Constituent (%) | Quantity (%) | Constituent (%) | Quantity (%) | Constituent (%) | Quantity (%) | Constituent (%) | Quantity (%) | Constituent (%) |
| Hunchun      | 348          | 68.24              | 129          | 25.29           | 17          | 3.33           | 16          | 3.14           | 0           | 0.00           | 0           | 0.00           | 510          | 100.00         |
| Tumen        | 37           | 20.22              | 0           | 0.00            | 120         | 65.57          | 7           | 3.72           | 0           | 0.00           | 0           | 0.00           | 183          | 100.00         |
| Yanji        | 192          | 48.98              | 174          | 44.39           | 0           | 0.00           | 120         | 65.57          | 14          | 7.65           | 12          | 6.56           | 392          | 100.00         |
| Dunhua       | 0            | 0.00               | 106          | 42.04           | 0           | 0.00           | 120         | 65.57          | 7           | 3.72           | 0           | 0.00           | 248          | 100.00         |
| Helong       | 87           | 26.69              | 158          | 48.46           | 67          | 20.55          | 120         | 65.57          | 14          | 7.65           | 0           | 0.00           | 326          | 100.00         |
| Longjing     | 426          | 88.38              | 56           | 11.62           | 0           | 0.00           | 0           | 0.00           | 0           | 0.00           | 0           | 0.00           | 482          | 100.00         |
| Wangqing     | 283          | 84.73              | 23           | 6.89            | 28          | 8.38           | 0           | 0.00           | 0           | 0.00           | 0           | 0.00           | 334          | 100.00         |
| Antu         | 0            | 0.00               | 5            | 2.53            | 0           | 0.00           | 79          | 39.90          | 114         | 57.58          | 198         | 100.00         |
| Total        | 1,373        | 51.37              | 651          | 24.35           | 357          | 13.36          | 140         | 5.24           | 152         | 5.68           | 2673        | 100.00         |

Sequence identity and phylogenetic analyses

The PCR products of the positive samples were sent to Shanghai Shenggong Co., Ltd. for sequencing. The correct gene sequences were analyzed in DNASTar and GenBank, and phylogenetic trees were constructed based on sequences obtained in this study and those previously published, using the maximum likelihood method with relative models by MEGA 7.0 software.

Statistical analysis

Data were processed using Microsoft 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 the 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 and pathogens in ticks
A total of 2673 ticks were collected, including 1373 *Haemaphysalis longicornis* (51.37%), 651 *Ixodes persulcatus* (24.35%), 357 *Haemaphysalis japonica* (13.36%), 140 *Dermacentor silvarum* (5.24%), and 152 *Haemaphysalis concinna* (5.68%) (Table 2).

After screening for pathogens by targeting different 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 identified in *H. longicornis* and *H. japonica*, while different SFTSV genotypes were confirmed in *H. concinna*. Mixed infection with CRT and SFTSV in *I. persulcatus*, *H. japonica*, and *D. silvarum* were observed. The highest frequency 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*. *Theileria orientalis* was detected in *H. longicornis*, while *T. sinensis* was not detected in any ticks (Table 3).

Percent identities and phylogenetic analyses
Analysis of the sequence identity showed that the CRT *ompA* gene sequences generated from ticks in Yanbian, China and *Haemaphysalis longicornis* (MG906672) strain in China shared 100% identity with the Korean ROK-HL727 strain (MG906672). The CRT *ompA* gene sequence (MT511087) in China was in the same clade as the isolates from Korea (MG906676), Chinese Changchun (KT899081), and Chinese Dandong (MH427382). The CRT *ompB* gene sequence (MT511089) was in the same clade as the Chinese HC strain (MK620854) and exhibited a close evolutionary relationship with the Korean ROK-HL727 strain (MG906675). The CRT *ompB* gene sequence (MT511089) was in the same clade as the Chinese HC strain (MK620854) and exhibited a close evolutionary relationship with the Korean ROK-HL727 strain (MG906675). The CRT *ompB* gene sequence (MT511089) was in the same clade as the Chinese HC strain (MK620854) and exhibited a close evolutionary relationship with the Korean ROK-HL727 strain (MG906675). The CRT *ompB* gene sequence (MT511089) was in the same clade as the Chinese HC strain (MK620854) and exhibited a close evolutionary relationship with the Korean ROK-HL727 strain (MG906675).

### Table 3. Infection of pathogens in different tick species in Yanbian, China.

| Species                           | CRT | SFGR | SFTSV | SFGR + CRT | SFGR + MPSP | SFGR + SFTSV | SFGR + SFGR + CRT | SFGR + SFGR + SFTSV | SFGR + SFGR + MPSP | SFGR + SFGR + SFTSV + MPSP | MPSP |
|----------------------------------|-----|------|-------|------------|-------------|--------------|----------------------|----------------------|----------------------|------------------------|-------|
| *Haemaphysalis longicornis*      |     |      |       |            |             |              |                      |                      |                      |                        |       |
| *Ixodes persulcatus*             | 51  | 123  | 174   | 192        | 114         | 205          |                      |                      |                      |                        |       |
| *Haemaphysalis japonica*         | 10  | 38   | 21    | 39         | 24          | 27           |                      |                      |                      |                        |       |
| *Dermacentor silvarum*           | 38  | 17   | 20    | 27         | 14          | 18           |                      |                      |                      |                        |       |
| *Haemaphysalis concinna*         |     |      |       |            |             |              |                      |                      |                      |                        |       |
| Total (n = 2673)                 | 87  | 354  | 510   | 611        | 358         | 435          |                      |                      |                      |                        |       |
Korea. The *sca4* gene sequence (MT511090) was also closely related to the Korean *Candidatus R. longicornii* ROK-HL727 strain (MG906677.1) (Fig. 3). Sequence analysis showed that the four gene sequences (MT511088, MT511089, MT511090, and MT535574) shared 100%, 99.70%, 100%, and 95.70% identities, respectively with the corresponding fragments (MG906676, MG906675, MG906677, and MG906672) of SFGR *Candidatus R. longicornii* newly discovered in the Korean *H. longicornis* (ROK-HL727).

The SFTSV Large (MT517309), Medium (MT517308), and Small (MT517307) gene sequences were identified in this study. The nucleotide sequence identity data demonstrated that sequences of SFTSV obtained in this study shared more than 95% identity with most of the SFTSV gene sequences previously identified in China and South Korea. The phylogenetic analysis showed that the SFTSV Small gene sequence from ticks in Yanbian (MT517307) was in the same clade as the SFTSV gene sequence (KT890282) from Jilin ticks in China. The Medium gene sequence (MT517308) formed one clade with the Chinese JS2014-18 strain (KR230781), and the Large sequence was located in the same branch as the Chinese JS2014-18 strain (KR230761) and was closely related to SFTSV isolated from Zhejiang (KR017839) and South Korea (KY789434) (Fig. 4).

The MPSP gene sequence obtained from this study (MT517304) was 99.4% identical to that of the *T. orientalis* Chongqing (MG664537) isolate. The phylogenetic analysis showed that the *T. orientalis* MPSP gene sequence in this study was classified nearer the cluster of *T. sergenti* than *T. sinensis*. The *T. orientalis* MPSP gene isolate in our study was 68.2%–99.6% identical to the sequences of *T. orientalis* and *T. sergenti* cited in this study (Fig. 5).

**Figure 2.** Phylogenetic trees based on the *ompA* and 17kDa genes of CRT. The ML trees were implemented by MEGA7 with a Hasegawa–Kishino–Yano model. The numbers at the nodes represent percentage of occurrence of clades in 1000 bootstrap replications of data. The gene sequences from this study are indicated by a round shape.
Discussion

Yanbian is located at the junction of China, North Korea, and Russia, and has a long border spanning 755.2 km. Due to strengthened ecological and environmental protection in China, the species along the border have diversified gradually. Thus, the number and species of ticks are constantly changing, and their activity is increasing. 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 infection with tick-borne diseases. In this study, 2673 ticks collected from eight counties and cities in Yanbian were classified and analyzed. Among the six identified species, *Haemaphysalis longicornis* and *Ixodes persulcatus* were the dominant tick species in Yanbian. *Haemaphysalis longicornis* is widely distributed throughout Asia and the Pacific, including China, Russia, South Korea, Japan, Australia, New Zealand, and the South Pacific islands, and is known for its strong reproductive ability and environmental adaptability. While it often parasitizes medium and large wild or domestic animals, humans are accidental hosts of *H. longicornis*. Moreover, *H. longicornis* spreads a variety of pathogens that can affect wild animals and livestock, as well as human health [3].

Ticks can be infected with various pathogens, including viruses, bacteria, and spirochetes. Ticks can act as both vectors and hosts in the transmission of disease. At present, the main research into co-infections of tick-borne pathogens has been focused on *Borrelia burgdorferi*, *Babesia microti*, *Ehrlichia*, and *Anaplasma phagocytophilum* [26]. Previous studies have confirmed that one-third of patients with a CRT infection had neurological symptoms that differed from other SFGR infections [11], and were associated with a higher case-fatality rate during co-infection with SFTSV [20]. Thus, more attention should be paid to SFTSV transmission through both tick bites and close contact with infected cases [27]. In this study, we confirmed the occurrence of CRT/SFTSV co-infection in Yanbian ticks. *Ixodes persulcatus* is a common dominant tick species in Yanbian, and is especially widely distributed in Hunchun, Yanji, Helong, and other regions, resulting in a higher risk of infection with CRT and SFTSV via tick bites in these regions.

SFGR forms a long-lasting infection cycle between ticks and mammals and can also be transmitted vertically through tick eggs, making ticks the main host and vector of SFGR. In 2016, a new genotype of SFGR, *Candidatus Rickettsia longicornii ompA, ompB, sca4, and rrs* genes, were all detected in *H. longicornis*, and hosts in the transmission of disease. At present, the main research into co-infections of tick-borne pathogens has been focused on *Borrelia burgdorferi*, *Babesia microti*, *Ehrlichia*, and *Anaplasma phagocytophilum* [26]. Previous studies have confirmed that one-third of patients with a CRT infection had neurological symptoms that differed from other SFGR infections [11], and were associated with a higher case-fatality rate during co-infection with SFTSV [20]. Thus, more attention should be paid to SFTSV transmission through both tick bites and close contact with infected cases [27]. In this study, we confirmed the occurrence of CRT/SFTSV co-infection in Yanbian ticks. *Ixodes persulcatus* is a common dominant tick species in Yanbian, and is especially widely distributed in Hunchun, Yanji, Helong, and other regions, resulting in a higher risk of infection with CRT and SFTSV via tick bites in these regions.

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I. persulcatus, and H. japonica collected in this study. The SFGR Candidate R. longicornii gene sequences detected in our study showed high identity with the related gene sequences newly discovered in South Korea (ROK-HL727) and the sequences of ompA and ompB genes belonging to an unknown SFGR genotype found in H. longicornis from Dandong, China (border between China and North Korea). The results of our study suggested that the new SFGR Candidatus R. longicornii genotypes are widely distributed throughout the border between China and North Korea. Although there have been no reports of infections caused by the new SFGR genotype, the ompA gene sequence of Candidatus R. longicornii found in Yanbian was highly identical to an unknown species of Rickettsia detected in mouse spleen tissue in South Korea [3]. These findings indicate that the Candidatus R. longicornii identified in this study was likely to be infectious in mammalian hosts and even in humans. Therefore, it is necessary to strengthen the surveillance for the SFGR Candidatus R. longicornii infection in ticks and relevant hosts in the border of China, North Korea, and Russia, as well as other areas with a concentrated distribution of H. longicornis, in order to prevent cross-border transmission and an epidemic of tick-borne diseases affecting human health and animal husbandry [3].

SFTS is a novel infectious disease that was first discovered in China [5, 6, 12]. SFTSV was first isolated from H. longicornis in Korea [22]. The SFTSV Small, Medium, and Large gene sequence analysis indicated that sequences from the present study had more than 95% identity with the SFTSV gene sequences found in South Korea. Phylogenetic analysis showed that the SFTSV Small, Medium, and Large gene sequences were in the same clade as the isolates from Jilin and Jiangsu, and were closely related to SFTSV isolated from Zhejiang and South Korea. This may be related to the tick-parasitized migratory birds from the eastern part of China or the transmission by migratory birds infected with SFTSV. A previous study suggested that migratory birds may play an important role in the spread of SFTSV [23]. The abovementioned results suggest that the border area of China, North Korea, and Russia may be a key region for tick-borne SFTSV, and should be considered in the prevention and control of imported infectious diseases.

Theileria orientalis is a protozoan parasite that infects cattle and buffalo and is generally transmitted by ticks of the genus Haemaphysalis [7]. Theileria sinensis named by Chinese scholars was originally isolated from naturally infected cattle by Bai et al. in Gansu Province [1]. Theileria orientalis is transmitted by H. longicornis, H. concinna, and H. japonica ticks. However, the tick species differ among regions; for example, H. japonica is the main vector of Oriental Taylor disease in Russia, followed by H. concinna. Haemaphysalis concinna is also the main vector in Korea, whereas H. longicornis is the main vector in China and Japan. In this investigation, we...
detected *T. orientalis* in *H. longicornis* but failed to detect *T. sinensis* in any ticks. The non-detection of *T. sinensis* may be attributed to the relatively smaller collection area and small numbers of its vector, *H. japonica*.

In this study, some regional endemic tick-borne pathogens were detected, such as CRT, SFGR, SFTSV, and *Theileria*, but whether there are other tick-borne pathogens remains to be studied. The phylogenetic analysis of tick-borne pathogens was mainly described and analyzed using the epidemic strains in China and the strains in Korea adjacent to Yanbian. The results of our study determined the epidemic trend of tick-borne pathogens in the border zone among China, Russia, and North Korea.

**Conclusions**

*Haemaphysalis 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 R. longicornii* ompA, ompB, sca4, and rrs in *H. longicornis* in Yanbian, China. In addition, *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.

**Author Contributions**

JXL: Methodology, validation, conducted the statistical analysis of the results, wrote the original draft of the manuscript. SZ: Validation, conducted the statistical analysis of the results. WFL: Investigation, recorded the sample data. SWZ: Edited and revised the manuscript. ZW: Investigation, recorded
the sample data. HL: Investigation, recorded the sample data. BYY: Investigation, recorded the sample data. ZZ: Investigation, recorded the sample data. JLL: Investigation, recorded the sample data. LJH: Conceptualization, manuscript writing-review and editing, funding acquisition.

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**Conflicts of interest**

The authors declare that they have no competing interests.

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