Coinfection of SFTSV Genotype A with Rickettsia among Farmers in Northeast

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September 21, 2020

Abstract

Severe fever with thrombocytopenia syndrome (SFTS), caused by the SFTS virus (SFTSV), is a new acute infectious disease transmitted by ticks. Rickettsiosis is caused by Rickettsia spp. carried by ticks. Here, we conducted case analyses and molecular biological diagnosis of some patients with suspected SFTS in Liaoning Province in 2019 to identify rates of SFTSV and Rickettsia coinfection. Molecular diagnostic results confirmed SFTSV infection in 5 out of 17 suspected SFTS patients. Two strains of genotype A SFTSV were successfully isolated from patient blood. Ticks collected from the patient's locations had high positivity rates for SFTSV, which was highly similar to SFTSV from patients. Antibody testing of 7 patient serum samples revealed all samples to be positive for Rickettsia antibodies. These results indicate coinfection of SFTSV with Rickettsia that may complicate disease presentation and diagnosis. Therefore, coinfection of tick-borne disease should be considered for correct diagnosis and treatment.

1 Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is a new acute infectious disease caused by the severe fever with thrombocytopenia syndrome virus (SFTSV) (Yu et al., 2011)belonging to a a novel phlebovirus (SFTS virus, SFTSV) in the family *Phenuiviridae* of the order *Bunyavirales* (Li et al., 2019). It was first reported in 2009 in the rural areas of the Hubei and Henan Provinces in Central China, and the population is generally susceptible. Since then, the incidence of SFTS has been successively reported in more than 10 provinces and cities in China(Zhang et al., 2013), and its epidemic area has a tendency to spread. The disease is also widely distributed in South Korea (Denic et al., 2011; Yun et al., 2016) and Japan(Takahashi et al., 2014), and cases of SFTS have also been reported in the United States (McMullan et al., 2012; Muehlenbachs et al., 2014). The main clinical manifestations of the disease are high fever, anorexia, muscle pain, chills, lymphadenopathy, leukopenia, thrombocytopenia, and multiple organ failure (Sun et al., 2019). Severe cases can lead to death.

SFTSV is an arbovirus mainly carried by ticks(Liu et al., 2012). The SFTSV virus genome is composed of three independent gene segments: L segment (6,368 bp; encodes the RNA-dependent RNA polymerase), M segment (3,378 bp; encodes the membrane protein precursor), and the ambisense S segment (1,744 bp; encodes the non-structural S protein and nucleocapsid protein)(Sun et al., 2014).

Rickettsiosis is a zoonotic disease mainly spread by ticks and is considered an important global emerging zoonotic disease (Parola, Paddock, & Raoult, 2005). Humans are infected by the bite of ticks that carry rickettsial bacteria. Due to human factors such as lifestyle changes and population rates, and to environmental factors such as climate change, people have become more exposed to ticks. Rickettsiosis in humans usually refer to infections caused by bacterial species belonging to the genus *Rickettsia* and*Orientia* . It can

be classified into typhus, spotted fever, and tsutsugamushi disease(Adem, 2019). Clinically, human serum rickettsia antibodies are used to determine whether the human body is infected with*Rickettsia* . The typical clinical features of these diseases include: fever, fatigue, anorexia, nausea, headache, and rash, and the bite of the tick has eschar formation (Liu et al., 2016). These symptoms usually appear within 1-2 weeks after infection(Liu et al., 2016). The clinical manifestations of typhus and SFTS are similar, thereby complicating diagnosis.

In 2019, our laboratory carried out clinical analysis, laboratory diagnosis, and traceability studies of SFTSV in suspected cases in the Infectious Disease Hospital of Shenyang City to characterize SFTSV in patients in Northeast China. Molecular diagnosis and antibody testing for *Rickettsia* were also performed to determine the rates of*Rickettsia* coinfection with other tick-borne diseases, especially with SFTSV. The results we present here provide insights into the characteristics of SFTSV in Northeast China. Further, our findings can guide clinicians in the diagnosis of tick-borne diseases.

2 Materials and Methods

2.1 Specimen source

Blood sample: A total of 17 cases of suspected SFTS were reported from May 2019 to October 2019 in Shenyang Infectious Disease Hospital. Serum was collected from the patients and stored at -80 °C. human samples were reviewed and approved by the ethics committee of China CDC.

Tick samples: free ticks in patients' farmlands, forests, and other environments in nearby counties and cities were collected. Liquid nitrogen milling, and centrifugation were performed to prepare tick suspensions. Total nucleic acid extraction was performed as in the following method, and reverse screening for virus isolation was performed.

2.2 Data collection

After numbering all suspected SFTS patients, the patient's epidemiological history, systemic symptoms, neurological symptoms, respiratory symptoms, etc. are collected and classified by consulting medical records; at the same time, WBC, PLT, AST, ALT, CK, LDH , BUN and other laboratory inspection results are recorded.

2.3 Nucleic acid extraction and reverse transcription

TransGen's EasyPure Viral DNA/RNA Kit (Cat No: ER201-01) was used, and total nucleic acid was extracted according to the kit instructions. Vazyme Biotech's HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (Cat No: R223-01) was used for reverse transcription according to the reagent instructions.

2.4 SFTSV nucleic acid detection

Using highly conserved regions in SFTSV M as target regions, specific primers were designed for PCR amplification. The primers were MF3 (5'-GATGAGATGGTCCATGCTGATTCT-3') and MR2 (5'-CTCATGGGGTGGAATGTCCTCAC-3') with a target band size of 560 bp. Amplification conditions: 94 , 5 min; 94 , 30 s, 55 30 s, 72 1 min, 35 cycles; 72 , 5 min; 30-μl system.

2.5 Virus isolation

The supernatant of Vero cells (African green monkey kidney cells) grown into a single layer was discarded, washed once with PBS, and incubated with 500 μL of patient serum for 2 h, and then discarded. Virus maintenance solution (DMEM containing 2% fetal bovine serum) was added and cultured at 37 °C under 5% CO_2, and cytopathic effects (CPEs) were observed daily. Tick suspensions that were positive SFTSV by the nucleic acid test were filtered through a 0.22-μm bacterial filter and were added to the Vero cells. Cells were collected, RNA was extracted, reverse transcription was performed, and PCR detection was performed to verify successful infection. The infected cells positive for SFTSV were stored at -80 °C until use.

2.6 Amplification of SFTSV whole genome

Previously designed primers for amplification of S (3 primer sets), M (7 primer sets), and L (13 primer sets) segments were used for whole genome sequencing. Primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The size of the target band amplified by each primer set for the S and M fragments was around 600 bp. Amplification conditions: 94 , 5 min; 35 cycles: 94 , 30 s; 55 , 30 s; 72 1 min; 72 , 5 min; 30-μL system. The size of the target band amplified by each primer set for the L fragment was about 350-650 bp. Amplification conditions: 94 , 5 min; 35 cycles: 94 , 30 s; 63 , 30 s; 72 , 1 min; 72 , 5 min; 30-μL system.

2.7 SFTSV whole genome sequencing and analysis

The PCR products were subjected to agarose gel electrophoresis, and the amplified products were sent to Sangon Biotech (Shanghai) Co., Ltd for sequencing. The sequences were spliced using the SeqMan software. GenBank was used as source of reported strain sequences, and DNASTAR and MEGA6.0 were used to conduct a comprehensive analysis of the three fragments of the SFTSV strain, to identify the virus genotype, and to analyze the genetic evolution of the strain. For paired and multiple alignments, the maximum likelihood method (ML) was used to build a phylogenetic tree based on the L, M, and S sequences. The bootstrap method with 1000 replications was used to estimate the reliability of the branches.

2.8 Molecular identification of *Rickettsia*

The amplification and sequence analysis of the 16S, *gltA* ,*ompA* , and *ompB* conserved sequences of *Rickettsia*carried by ticks were performed based on the study published by Xu et al(Xu et al., 2019).

2.9 Human serum *Rickettsia*-specific IgG antibody detection

The human Rickettsia-IgG ELISA KIT (Cat No: JL48935) of Jianglai Biotech Co., Ltd. was used for IgG antibody detection and the detection antibody type was *Rickettsia* IgG. Serum from healthy people and distilled water were used as negative and blank controls, respectively.

First, The concentration of the standard product is 100, 50, 25, 12.5, 6.25, 0 ng/mL in sequence, and 50 μL for each well. Next, Add sample diluent 40 μL on the Enzyme labeling plate first, and then add the sample to be tested 10 μL (the final dilution of the sample is 5 times). Add 100 μL enzyme reagents to each well, except for blank wells. The samples were incubated at 37 for 1 hour and then washed with washing solution,repeat 5 times,pat dry. Add color developer A 50 μL to each well, and then add color developer B 50 μL. Gently shake and mix well, and develop color at 37 in the dark for 15 minutes. Add stop solution 50 μL to each well to stop the reaction. 450nm wavelength measurement of absorbance of each hole, OD was measured after zero-adjusting the blank control reaction.

3 Results

3.1 Epidemiological information

A total of 17 patients with suspected SFTS were admitted to the clinic (Table 1). Through molecular diagnostic tests, 5 patients were confirmed to have SFTSV infection. There were 2 males and 3 females, with an average age of 51 years. All of them are farmers, mainly farming in forest areas, and have history of animal contact (rats) or tick bites.

3.2 Clinical manifestations

Clinical examination revealed that SFTS patients developed fever, gastrointestinal symptoms, and neurological symptoms, which mostly included fever, headache, dizziness, body aches, nausea, vomiting, and fatigue. Among the symptoms, fever (17/17), muscle pain (17/17), and headache (16/17) were the most frequent symptoms of SFTS. Clinical manifestations in confirmed and suspected SFTS cases were similar, showing that clinical manifestations alone cannot be used for diagnosis of SFTS (Table 1).

3.3 Laboratory test results

The 5 patients diagnosed with SFTS mainly showed decreased white blood cell counts (WBC; 4/5), platelet counts (PLT; 5/5), alanine aminotransferase (ALT; 5/5), and aspartate aminotransferase (AST; 4/5), blood

urea nitrogen (BUN; 2/5), creatine kinase (CK; 4/5), and increased lactate dehydrogenase (LDH; 5/5). All 5 patients with SFTS had thrombocytopenia, but other patients also had cytopenia (7/12) (Table 1).

3.4 SFTSV and *Rickettsia* nucleic acid detection and virus isolation results

From the 5 confirmed cases of SFTSV, 2 SFTSV strains were successfully isolated and named LNHDD2019-9 (from Dandong City, Liaoning Province) and LNHDG2019-6 (from Donggang City, Liaoning Province). No*Rickettsia* nucleic acid was detected in the serum or blood cells of the 17 patients suspected of SFTSV.

A total of 492 tick samples were collected from the patient's residence and surrounding areas (including Dandong City and, under its jurisdiction, Fengcheng City, Donggang City; Kuandian Manchu Autonomous County; and Zhuanghe City under the jurisdiciton of Dalian,which are all Changbai Mountain forest areas in the border area between China and North Korea). Environmental free tick samples (samples not collected in Beipiao City) were all identified by molecular biology. Among them, 12 samples were positive for SFTSV nucleic acids, with a positivity rate of 2.4%. Two SFTSV strains were successfully isolated from tick samples and were named LNTEDG2019-6 (collected from Erdaogou, Dandong City, Liaoning Province) and LNTYJ2019-6 (collected from Yanji City, Jilin Province) (Figure 1).

3.5 Amplification results of SFTSV whole genome sequence

The L, M, and S fragments of four SFTSV strains were amplified separately, and the corresponding fragments were spliced to assemble the whole genome sequence. The sequences of SFTSV (LNHDD2019-9/Human/China/2019 in the evolution tree) S, M, and L fragments isolated from patient NO. 1 in Dandong has been uploaded to NCBI with accession numbers MT232960, MT232961, and MT232962, respectively.

3.6 Systematic evolutionary analysis of SFTSV strain

Full-length sequences of 49 SFTSV isolates from China, Japan, and South Korea from 2010 to 2016 were selected to cover the six known (A-F) genotypes of SFTSV. The maximum likelihood method was used for phylogenetic analysis. The results are shown in Figures 2A,2B,2C. The results show that four SFTSV isolates in this study all clustered in genotype A (marked with in the figure), and no other genotypes were detected.

3.7 Analysis of nucleotide and amino acid similarity of SFTSV strain

The two SFTSV strains isolated from patients and the L, S segment sequence of SFTSV isolated from ticks in Dandong City, Liaoning Province and Yanji City, Jilin Province were 100% identical, while the M segment sequence similarity was 99%, which shows that the main source of transmission of SFTS in patients in the Liaoning Province are the ticks distributed in local or nearby areas.

Comparison of the four SFTSV strains with each genotype sequence revealed that it was the closest in nucleotide sequence and corresponding amino acid sequence to genotype A SFTSV (Table 2). The sequence similarities of the isolated L, M, and S nucleotides (and deduced amino acids) with other genetic A-type strains are between 95.95-100 (98.90-100), 97.12-100 (98.32-100), 95.08-100 (99.59-100)%, respectively (Table 3-5). The nucleotide sequence similarity is above 95%, and the corresponding amino acid similarity is above 98%, which further suggest that the four SFTSV strains are genotype-A strains.

3.8 Rickettsia molecular identification

The 16S, *gltA* , *ompA* , and *ompB* conserved sequences carried by tick samples collected in this study were analyzed separately and found to be highly consistent with literature (Xu et al., 2019) (sequence similarities were >99%). Our results confirm that "Dandong-type *Rickettsia* ," which has the closest genetic sequence to *Rickettsia* in Heilongjiang, followed by*Rickettsia* in Japan, were carried by the ticks. These results show that "Dandong-type *Rickettsia* " is widely distributed in the Changbai Mountain forest area.

3.9 Rickettsia-specific IgG antibody detection

Seven serum samples were randomly selected for ELISA detection for*Rickettsia* -specific IgG detection, including three for confirmed SFTS patient's serum (Dandong NO. 6; Donggang NO. 1; Fengcheng NO. 16); 4 patients with negative for SFTSV serum (Fengcheng NO. 7; Kaiyuan NO. 4; Haicheng NO. 14; Kuandian NO. 17), The results are shown in Table 1. Results show that sera from all 7 patients carried *Rickettsia* IgG (100% positivity rate).

4 Discussion

Tick-borne diseases are the main cause of vector-borne diseases and have broad global distribution. They are mainly natural epidemic diseases, most common in forests, bushes, and semi-desert grasslands(Wu, Na, Wei, Zhu, & Peng, 2013). There are at least 7 genera and 104 species of ticks in China. Northeast China has a mountainous terrain, has abundant biological resources, has geographical complexity, and has high species diversity, which provide an ecological and biological basis for the survival and reproduction of ticks. In this study, we found that the ticks distributed in the forest area of Northeast China are dominated by the long-horned blood ticks. Most patients affected by SFTS live in mountainous, hilly, or dense jungle areas and have a history of working outdoors. More than 80% of the cases are farmers, and there is history of field work, livestock contact, etc., and a few patients have history of tick bites. Among all the 17 suspected SFTS patients in this study, all cases occurred from the end of March to the end of June, and 94% of the cases (16/17) were from Changbai Mountain and other mountainous areas, forests, and hills in Northeast China, It is highly consistent with the active season and regional distribution of ticks in Liaoning Province.

SFTSV strains have been isolated from ticks in South Korea, Japan, and other regions sharing borders with or adjacent to China(Park et al., 2014; Yasukawa, 2008; Yun et al., 2015). Reports from the East Asian region show that the average mortality rate of SFTSV varies greatly, from 5.3% to 16.2% in China, 20% in Japan, and 23.3% in South Korea. Among the six known A-F genotypes of SFTSV(Fu et al., 2016), genotype B has the highest morbidity and mortality rates, which are significantly higher than those of the other genotypes. The incidence of the F genotype is lower than that of the B genotype, but its mortality rate is also higher, while the A genotype has the lowest mortality rate(Yun et al., 2020). The most common genotypes in China are genotypes A, D, and F, and the mortality rate is relatively low, while the most common genotype in Japan and South Korea is B. Therefore, a comparison of viral genotypes and mortality indicates that the differences in reported case mortality may be related to the differences in the distribution of SFTSV genotypes in different countries. The number of clinical cases infected with certain SFTSV genotypes (especially reassortant genotypes) is small, which prevents the determination of the association between genotype and case mortality.

There is currently no vaccine or specific antiviral drug for SFTSV. Ribavirin is considered to be a potential antiviral drug for SFTS, but several retrospective studies have shown that ribavirin has not been effective in improving disease prognosis (Li et al., 2018; Liu et al., 2013). (Li et al., 2019) found that benidipine hydrochloride (a calcium channel blocker, CCB) can inhibit SFTSV infection by interfering with virus internalization and reducing viral genome replication. Further experiments showed that a large number of CCBs including nifedipine inhibited SFTSV infection as well, indicating that CCB therapy may be developed as an effective strategy for the treatment of SFTSV infection.

Our previous research showed that among the various pathogens carried by ticks distributed in the Changbai Mountain forest area in China, the*Rickettsia* positivity rate was the highest, and it was close to 10% in some areas (Qi et al., 2014). The*Rickettsia* detected in this study is consistent with the newly discovered "Dandong*Rickettsia* " genotype in 2018 and has is genetically close to*Rickettsia* Heilongjiang. This genotype belongs to the class Alphaproteobacteria, order Rickettsiales, in the spotted fever group rickettsiae of the genus *Rickettsia* . The disease caused by*Rickettsia* in Heilongjiang is called the "Far-East tick-borne Spotted Fever" (FESF) , FESF has been considered as an important emerging infectious disease in Northeast Asia for this rickettsiosis has been diagnosed in Northeast of China, east-Siberian and far-eastern regions of Russia, and Japan. *Rickettsia* infection usually manifests as fever, headache, and rash, as well as muscle pain and arthralgia. The typical symptom is eschar-like skin lesions formed by the bite of a tick or mite, and the mortality rate is low under reasonable treatment. However, rashes and eschar did not appear in

the 17 patients in this study, which may be because the skin lesions were hidden and painless, making them difficult to observe and easy to ignore. The diagnosis of rickettsial diseases can be achieved by RT-PCR or conventional PCR detection in diseased tissues (Denison, Amin, Nicholson, & Paddock, 2014). However, sampling errors may cause false negatives, and patients with insignificant skin lesions may be completely ignored. Due to similar clinical manifestations caused by different rickettsial infections, there are cross-reactions in serological methods. Pathogen culture is difficult, and the application of molecular techniques in whole blood are limited so far. Thus, rapid and reliable molecular blood detection of rickettsial diseases are still difficult to achieve. Indirect immunofluorescence assay is considered to be the gold standard, but due to its extensive cross-reactivity, this method has limited use in the determination of species within serogroups (Paris & Dumler, 2016).

In our study, based on epidemiological data, clinical symptoms, and routine blood tests, 17 patients were initially classified as suspected SFTS patients. After molecular diagnostic testing, 5 SFTS patients were confirmed to have SFTSV. To understand whether there is a mixed infection of sftsv and rickettsia in patients bitten by ticks, From the 17 patient blood samples, 7 samples were chosen for *Rickettsia* -specific IgG antibody testing, and the positivity rate was 100%. Of these 7 samples, 3 samples were from patients diagnosed with SFTS and 4 samples were from SFTS negative patients. Considering that ticks carry SFTSV and "Dandong-type *Rickettsia* ", we conclude that tick-borne SFTSV and "Dandong-type *Rickettsia* " infections are endemic in forest areas in Northeast China are more common. We hypothesize that SFTSV-"Dandong-type Rickettsia" coinfection complicates patient conditions. Therefore, we believe that in diagnosing tick-borne diseases such as SFTSV, tick-borne encephalitis, and renal hemorrhagic fever syndrome, co-infection with *Rickettsia* will have to be considered. SFTS and other related tick-borne diseases should also be screened for patients with rickettsial infection.

Acknowledgments

We thank The Sixth People's Hospital of Shenyang for Providing the patient samples. This work was supported by the National Important Scientific & Technology Project (2018ZX10101002-002). Title: The major vector-borne pathogens in the Belt and Road, Also got support from Education Department of Liaoning Province(LSNZD201606).

Conflict of Interest

The authors declare no competing interests.

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The 'Guidelines for Experimental Animals' of the Ministry of Science and Technology (Beijing, China) were followed. all participants involved in the study provided written informed consent.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Characteristics	NO.1[a]	NO.2	NO.3	NO.4	NO.5	NO.6	NO.7
Basic Information							
Gender	Female	Male	Male	Male	Female	Male	Male
Age	47	44	54	56	76	41	58
Address	Donggang	Chang t u	Xi feng	Kaiyuan	An shan	Dandong	Fengcheng
Etiological and Molecular biology Tests							
PCR for SFTSV	Posi[b]	Neg[c]	Neg	Neg	Neg	Posi	Neg
Virus isolation	Posi	Neg	Neg	Neg	Neg	Posi	Neg
Rickettsia IgG antibody detection	Posi	NA[d]	NA	Posi	NA	Posi	Posi
Clinical symptoms							
Fever	[?]	[?]	[?]	[?]	[?]	[?]	[?]
Headache	[?]	[?]	[?]	×	[?]	[?]	[?]
Unconsciousness	semicoma	×	×	×	×	Delirium	×
Apathetic	[?]	×	×	×	×	[?]	×
Muscle joint pain	[?]	[?]	[?]	[?]	[?]	[?]	[?]
Nausea and vomiting	[?]	[?]	[?]	[?]	×	[?]	×
Lymphadenopathy	NA	×	×	×	×	[?]	×

Characteristics	NO.1[a]	NO.2	NO.3	NO.4	NO.5	NO.6	NO.7
Pharyngeal congestion	[?]	[?]	[?]	[?]	×	×	[?]
Conjunctival congestion	[?]	[?]	[?]	[?]	×	[?]	[?]
Laboratory parameters(Normal range)							
WBC(4.0-10.0×10^9/L)	5.6	7.2	2.2—	3.2—	6	1.2—	2.3—
PLT(100-300×10^9/L)	47—	27—	33—	59—	160	52—	63—
ALT(0-40U/L)	60—	156—	88—	116—	32	295—	25
AST(0-40U/L)	268—	320—	228—	175—	50—	688—	35
BUN(3.2-7.1mmol/L)	5.2	9.5—	4.74	4.59	5.02	7.4—	4.7
CK(25-200U/L)	1026—	140	356—	991—	51	4516—	204—
LDH(135-225U/L)	4032—	100000—	1976—	1209—	1293—	3673—	565—

Table 1. Basic Information, clinical symptoms, and laboratory parameters

[a] NO.1 was patient 201902845; NO.2 was patient 201903149; NO.3 was patient 201903151; NO.4 was patient 201901922; NO.5 was patient 201903976; NO.6 was patient 201904040;NO.7 was patient 201904339; NO.8 was patient 201904567; NO.9 was patient 201904687; NO.10 was patient 201904740; NO.11 was patient 201904737; NO.12 was patient 201905320;NO.13 was patient 201905311; NO.14 was patient 201905648; NO.15 was patient 201905649; NO.16 was patient 201906198; NO.17 was patient 201906189.

[b] Abbreviation of positive

[c] Abbreviation of negative

[d] No data

SFTSV strain	Source of virus	Geographical origin	Year of isolation	Genotype (L/M/S segments)	Sequence identity (%)	Sequence identity (%)	Sequence identity (%)	Sequence identity (%)	Sequence identity (%)	Sequence identity (%)
					L segments Nucleotide	L segments Amino acid	M segments Nucleotide	M segments Amino acid	S segments Nucleotide	S segments Amino acid
LNHDD2019	Human serum	Dandong City, Liaoning Province	2019-9	A/A/A	99.98	100	99.08	99.63	100	100
LNHDG2019 6	Human serum	Donggang City, Liaoning Province	2019-6	A/A/A	99.98	100	99.08	99.63	100	100
LNTEDG2019 6	*Haemaphysalis longicornis*	Liaogou, Dandong City Liaoning province	2019-6	A/A/A	99.98	100	99.08	99.63	100	100

SFTSV strain	Source of virus	Geographical origin	Year of isolation	Genotype (L/M/S segments)	Sequence identity (%)	Sequence identity (%)	Sequence identity (%)	Sequence identity (%)	Sequence identity (%)	Sequence identity (%)
LNTYJ2019-6	*Haemaphysalis longicornis*	Yanji City, Jilin Province	2019-6	A/A/A	99.98	100	99.08	99.63	100	100

Table 2. Sequence information for SFTSV isolates in this study

Strain		1	2	3	4	5	6	7	8	9	10	11	12	
LNHDD2019-9	1		100	100	100	98.30	99.09	98.63	97.82	97.83	97.86	97.94	97.86	
LNTYJ2019-6	2	100		100	100	98.30	99.09	98.63	97.82	97.83	97.86	97.94	97.86	
LNTEDG2019-6	3	100	100		100	98.30	99.09	98.63	97.82	97.83	97.86	97.94	97.86	
LNHDG2019-6	4	100	100	100		98.30	99.09	98.63	97.82	97.83	97.86	97.94	97.86	
SFTSV	5	99.18	99.18	99.18	99.18		98.23	97.95	98.08	98.65	98.72	98.89	98.04	
JS2014-18	6	99.71	99.71	99.71	99.71	98.99		98.83	98.98	98.08	98.11	98.15	98.04	
ZJZHSH-WSH	7	99.81	99.81	99.81	99.81	98.99	99.71		99.15	97.70	97.73	97.78	97.73	
CB2	8	99.86	99.86	99.86	99.86	99.04	99.76	99.86		97.82	97.86	97.90	97.82	
JS3	9	99.47	99.47	99.47	99.47	99.04	99.47	99.47	99.52		99.69	99.61	98.47	
JS24	10	99.47	99.47	99.47	99.47	99.04	99.47	99.47	99.52	99.81		99.67	98.50	
JS2013-71	11	99.52	99.52	99.52	99.52	99.09	99.52	99.52	99.57	99.86	99.86		98.54	
LN3	12	99.57	99.57	99.57	99.57	98.94	99.47	99.57	99.62	99.52	99.52	99.57		
JS2014-Hedgehog-01	13	99.47	99.47	99.47	99.47	98.94	99.47	99.47	99.52	99.52	99.52	99.57	99.62	
HL/Adult/G2	14	99.62	99.62	99.62	99.62	98.99	99.52	99.62	99.66	99.87	99.87	99.88	99.89	
2011YSC60	15	99.52	99.52	99.52	99.52	98.90	99.42	99.52	99.57	99.47	99.47	99.52	99.57	
2012YXX1	16	99.62	99.62	99.62	99.62	98.99	99.52	99.62	99.66	99.87	99.87	99.88	99.89	
HB2016-047	17	99.62	99.62	99.62	99.62	98.99	99.52	99.62	99.66	99.87	99.87	99.88	99.89	
YSC3	18	99.66	99.66	99.66	99.66	99.04	99.47	99.57	99.62	99.52	99.52	99.57	99.52	

Table 3. Comparison (% similarity) of A-genotype SFTSV L segment nucleotide (top right) and amino acid (bottom left) sequences

Table 4. Comparison (% similarity) of A-genotype SFTSV M segment nucleotide (top right) and amino acid (bottom left) sequences

Strain		1	2	3	4	5	6	7	8	9	10	11	12	
LNHDG2019-6	1		99.97	99.97	99.94	99.06	98.74	98.30	97.82	97.91	97.97	97.88	98.03	
LNTYJ2019-6	2	100		100	99.97	99.09	98.77	98.32	97.85	97.94	98.00	97.91	98.06	
LNTEDG2019-6	3	100	100		99.97	99.09	98.77	98.32	97.85	97.94	98.00	97.91	98.06	
LNHDD2019-9	4	100	100	100		99.06	98.74	98.30	97.82	97.91	97.97	97.88	98.03	
JS2014-18	5	99.53	99.53	99.53	99.53		98.79	98.35	97.62	97.91	97.97	97.97	98.12	
CB2	6	99.53	99.53	99.53	99.53	99.72		98.56	97.38	97.74	97.79	97.74	97.88	
ZJZHSH-WSH	7	99.07	99.07	99.07	99.07	99.25	99.35		97.18	97.47	97.53	97.59	97.62	
SFTSV	8	98.88	98.88	98.88	98.88	98.79	98.79	98.32		97.79	97.85	97.85	97.88	
JS24	9	99.07	99.07	99.07	99.07	99.35	99.25	98.79	98.51		99.76	99.59	99.15	
JS3	10	99.25	99.25	99.25	99.25	99.53	99.44	98.97	98.70	99.81		99.65	99.21	
JS2013-71	11	99.25	99.25	99.25	99.25	99.53	99.44	98.97	98.70	99.63	99.81		99.15	

Strain		1	2	3	4	5	6	7	8	9	10	11	12	
LN3	12	99.25	99.25	99.25	99.25	99.53	99.44	98.97	98.70	99.63	99.81	99.81		
JS2014-Hedgehog-01	13	99.16	99.16	99.16	99.16	99.44	99.35	98.88	98.60	99.25	99.44	99.44	99.44	
HL/Adult/G2	14	99.16	99.16	99.16	99.16	99.44	99.35	98.88	98.79	99.35	99.53	99.53	99.53	
2012YXX1	15	99.25	99.25	99.25	99.25	99.53	99.44	98.97	98.88	99.44	99.63	99.63	99.63	
2011YPQ12	16	98.70	98.70	98.70	98.70	98.97	98.88	98.42	98.32	98.88	99.07	99.25	99.07	
HB2016-047	17	98.88	98.88	98.88	98.88	99.16	99.07	98.60	98.51	99.07	99.29	99.44	99.25	

Strain		1	2	3	4	5	6	7	8	9	10	11	12	
LNHDD2019-9	1		100	100	100	99.32	98.64	97.06	98.36	97.34	97.51	97.29	97.57	
LNTYJ2019-6	2	100		100	100	99.32	98.64	97.06	98.36	97.34	97.51	97.29	97.57	
LNTEDG2019-6	3	100	100		100	99.32	98.64	97.06	98.36	97.34	97.51	97.29	97.57	
LNHDG2019-6	4	100	100	100		99.32	98.64	97.06	98.36	97.34	97.51	97.29	97.57	
SFTSV	5	100	100	100	100		98.81	97.17	98.25	97.63	97.80	97.57	97.85	
JS2014-18	6	100	100	100	100	100		97.57	98.19	97.91	98.08	97.85	98.13	
ZJZHSH-WSH	7	100	100	100	100	100	100		97.18	96.27	96.44	96.33	96.50	
CB2	8	100	100	100	100	100	100	100		96.89	97.06	96.89	97.12	
JS3	9	99.59	99.59	99.59	99.59	99.59	99.59	99.59	99.59		99.83	99.60	99.38	
JS24	10	100	100	100	100	100	100	100	100	99.59		99.77	99.55	
JS2013-71	11	100	100	100	100	100	100	100	100	99.59	100		99.32	
LN3	12	100	100	100	100	100	100	100	100	99.59	100	100		
JS2014-Hedgehog-01	13	100	100	100	100	100	100	100	100	99.59	100	100	100	
HL/Adult/G2	14	100	100	100	100	100	100	100	100	99.59	100	100	100	
2011YSC60	15	100	100	100	100	100	100	100	100	99.59	100	100	100	
2012YXX1	16	100	100	100	100	100	100	100	100	99.59	100	100	100	
HB2016-047	17	100	100	100	100	100	100	100	100	99.59	100	100	100	
YSC3	18	100	100	100	100	100	100	100	100	99.59	100	100	100	

Table 5. Comparison (% similarity) of A-genotype S segment nucleotide (top right) and amino acid (bottom left) sequences

Figure 1. Regional distribution of reported cases and tick isolation in Liaoning Province in 2019. Areas 1-6 are where the 17 patients live; tick samples were collected in areas 1-7; SFTSV was isolated from ticks collected in areas 4 and 7.

Figure 2A. Phylogenetic analysis based on the complete nucleotide sequences of the L segment of SFTSV isolates.

Figure 2B. Phylogenetic analysis based on the complete nucleotide sequences of the M segment of SFTSV isolates.

Figure 2C. Phylogenetic analysis based on the complete nucleotide sequences of the S segment of SFTSV isolates.

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