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Short communication

The first molecular evidence of severe fever with thrombocytopenia syndrome virus in ticks in Jilin, Northeastern China

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ABSTRACT

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne zoonosis. The aim of this study was to investigate SFTS virus (SFTSV) infections in ticks in Northeastern China. A total of 6427 ticks, including 5450 *Haemaphysalis longicornis*, 463 *Dermacentor silvarum*, 351 *Dermacentor nuttalli*, and 163 *Ixodes persulcatus*, were sampled in the Liaoning, Jilin, and Heilongjiang Provinces of Northeastern China. Viral megagenomic analysis of the ticks revealed 25 contigs targeting the M and L segments of the SFTSV genome. *H. longicornis* collected from Jinxing, Jilin Province, were analyzed by RT-PCR, which showed positive results for SFTSV, with a minimum prevalence of 3.0%. The full-length sequence of the S, M, and L segments of the SFTSV were obtained, and phylogenetic analysis showed that the virus strain found in Jilin formed a monophyletic cluster with the SFTSV strains from Jiangsu, suggesting that SFTSV in the Jilin Province may have spread from the Jiangsu Province. These findings are the first to demonstrate molecular evidence of SFTSV in ticks in the Jilin Province of Northeastern China and indicate the need for measures to prevent and control SFTS.

1. Introduction

The severe fever with thrombocytopenia syndrome virus (SFTSV), which causes the severe fever with thrombocytopenia syndrome (SFTS), is a newly identified phlebovirus in the family Bunyaviridae (Xu et al., 2011; Yu et al., 2011). The clinical symptoms of SFTS often include fever, leukocytopenia, and thrombocytopenia, and the average case-fatality rate is approximately 8% (Liu et al., 2014). Since it was first identified in Central China in 2009, SFTS has been discovered in more than ten provinces of China (Liu et al., 2014). Additionally, the disease has been reported in South Korea and Japan (Kim et al., 2013; Takahashi et al., 2014), and the Hartland virus, which is genetically similar to SFTSV, has been found in the United States of America (McMullan et al., 2012).

SFTS is considered an emerging tick-borne zoonosis with the major at-risk population being farmers living in wooded and hilly areas (Yu et al., 2011). SFTSV has been found in the *Haemaphysalis longicornis* tick in endemic areas, and infection rates range from 2.2% to 5.4% (Yu et al., 2011; Wang et al., 2015). SFTSV has also been discovered in *Rhipicephalus microplus*, although at a lower prevalence rate than that of *H. longicornis* (Liu et al., 2014). The wide distribution of these ticks and the high mortality of people infected with the disease indicate that SFTS poses a severe public health threat in China and other countries.

There are large areas of coniferous forest, coniferous and broad-leaved mixed forest and meadow steppe in the Liaoning, Jilin, and Heilongjiang Provinces of Northeastern China, which are suitable habitats for tick survival. Several tick-borne zoonoses, such as babesiosis, rickettsiosis, Lyme disease, anaplasmosis, and tick-borne encephalitis, have been detected in Northeastern China (Fang et al., 2015; Lu et al., 2008). Cases of SFTSV in humans have been reported in Liaoning Province, where SFTSV has been detected in 5.0% of *H. longicornis* (Liu et al., 2013). In the present study, a viral megagenomic analysis of ticks from Northeastern China revealed the existence of SFTSV in *H. longicornis* in Jilin Province for the first time. The presence of this virus poses a threat to public health.

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2. Materials and methods

2.1. Tick collection

Ticks were collected from animals and vegetation in the provinces of Liaoning, Jilin, and Heilongjiang in Northeastern China between May and July, 2013. All ticks were morphologically identified to the species level using the standard guidelines (Chen et al., 2010). Information on the collected ticks is given in supplementary Table S1.

2.2. Viral megagenomic sequencing

RNA extraction, Solexa sequencing and data analysis were conducted as previously described (Gong et al., 2015). Briefly, 3410 ticks were divided into 9 groups (range: 163–512; average: 379 ticks per group; supplementary Table S1) and homogenized in an SM buffer (50 mM Tris, 10 mM $MgSO_4$, 0.1 M NaCl, pH7.5). Viral RNA extraction, Solexa sequencing, and data analysis were performed as previously described elsewhere (He et al., 2013).

2.3. RT-PCR

Viral megagenomic results were confirmed by RT-PCR and amplifying the partial S gene of the SFTSV with the primers SP1: 5′- GCAGCAGCTCAATTTGACT-3′ and SP2: 5′-CTTCAGCCACTTCACCCGAAC-3′. To obtain the full-length sequence of the SFTSV, the primers used to amplify the complete S, M, and L segments were designed according to the genome sequences of SFTSV isolates (Table 1). The PCR reaction used 1 μL of genomic cDNA as a template in a 25-μL reaction mixture that contained 2.5 μL of 10× PCR reaction buffer, 1.6 μL of 2.5 mM dNTP mixture, 0.2 U Taq polymerase (TaKaRa), and 1 μL of each primer (10 μM). The reaction was carried out at 94 °C for 5 min followed by 35 cycles of denaturation for 40 s at 94 °C, annealing for 30–60 s at 52–56 °C, extension for 60–90 s at 72 °C, and a final incubation for 10 min at 72 °C. All of the PCR products were analyzed using 1% agarose gel electrophoresis and were then sequenced.

2.4. Phylogenetic analysis

Phylogenetic analyses were conducted using the MEGA 5 software (http://www.megasoftware.net/). The neighbor-joining method was employed to construct a phylogenetic tree. The reliability of the branches of the tree was assessed using bootstrap analysis with 1000 replicates.

3. Results and discussion

In this study, we collected 6427 ticks from the Liaoning, Jilin and Heilongjiang Provinces and found four tick species with the most abundant being *H. longicornis* (84.8%), followed by *D. silvarum* (7.2%), *D. nuttalli* (5.5%), and *I. persulcatus* (2.5%). Of these ticks, 3410 were divided into 9 groups according to the sampling sites and tick species (Supplementary Table S1), and homogenized in an SM buffer (50 mM Tris, 10 mM $MgSO_4$, 0.1 M NaCl, pH 7.5) for viral megagenomic analysis (Gong et al., 2015). Among the sequences annotated as mammalian viruses, 25 contigs were found to target the M (n = 9) and L (n = 16) segments of the SFTSV genome (Supplementary Tables 2 and 3).

The Solexa results were confirmed by amplifying the partial fragment of the SFTSVS gene via RT-PCR. The *H. longicornis* group that was collected from cattle in Jinxing, Jilin Province (130°38′ E, 42°25′ N) was detected SFTSV-positive, while the other groups were negative. The sequences that were obtained shared 99% identity with the SFTSV strain isolated from humans in Zhejiang (GenBank access no. JX462447).

The full-length sequence of the SFTSV (Jilin, China) was then obtained via RT-PCR using primers based on the Solexa sequences or the conserved sequences of the SFTSV. The complete sequences of the S, M, and L segments of the SFTSV in Jilin, China (GenBank access nos. KT890280-KT890282) contained 1744, 3378, and 6368 nucleotides, respectively (i.e., they were similar to other SFTSVs). Sequence comparisons showed 96–99% identity with other SFTSVs at the nucleotide level and 97–99% identity at the deduced amino acid level. Phylogenetic analysis based on the nucleotide sequences of the S, M, and L segments grouped the virus with the SFTSVs strains found in Jiangsu, China (Fig. 1).

The remaining tick samples from the SFTSV-positive groups were used to determine the infection frequency via RT-PCR using primers for SP1 and SP2. In total, of the 91 tick pools with an average of 12 ticks in each pool, representing 1095 ticks from Jinxing of Jilin Province, 33 tick pools collected from cattle were detected SFTSV-positive, showing a minimum prevalence (i.e., assuming 1 positive tick/positive pool) of 3.0%.

Previous studies have indicated that the SFTSV may originate from Huaiyangshan and Jiangsu in China. The virus appeared in Huaiyangshan between 1756 and 1817, and then spread to the Jiangsu and Anhui Provinces between 1773 and 1834 (Huang et al., 2014). There are three routes by which the SFTSV can spread in China, which include the routes from Huaiyangshan to Jiangsu, from Jiangsu to Shandong, and from Jiangsu to Liaoning (Huang et al., 2014). Phylogenetic analysis indicated that the SFTSV can be divided into 5 clusters and that strains from the Liaoning Province cluster with the SFTSV strains from Jiangsu Province. Phylogenetically, the strain from Jilin in this study formed a monophyletic

Table 1
Primer sequences used to amplify the complete genome of SFTSV (Jilin, China).

Genome segment	Interest fragment	Forward (location) (5′ → 3′)	Reverse (location) (5′ → 3′)
Small	1–752	S-F1: ACACAAAGACCCCCTTCATTTGGA (1–24)	S-R1: CAGGCTCATCATCCTCATCCAAGA (752-729)
	729–1063	S-F2: TCTTGGATGAGGATGATGAGCCTG (729–752)	S-R2: GTTCGGGTGAAGTGGCTGAAG (1063-1043)
Medium	1–610	M-F1: ACACAGAGACGGCCAACAATGATGAAAGT (1–29)	M-R1: TAAAACTCTTCAGCTCCAGAAATGTC (610-585)
	601–1776	M-F2: AAGAGTTTTAGCCAAAGTGAATTCCC (601–626)	M-R2: ACATTCCTTCATATTTCCGCTCCC (1776-1753)
	1759–3378	M-F3: GGAAATATGAAGGAATGCGTCACAACT (1759–1785)	M-R3: ACACAAAGACCGGCCAACACTTCAATA (3378-3352)
Large	1–493	L-F1:ACACAGAGACGCCCAGATGG (1–20)	L-R1: GATATCAACACGCCTTGAGATTG (493-471)
	311–1524	L-F2: TTCCCCATTACCCATGATGGTT (311–332)	L-R2: TTGAGGGCATGAGTGAGCTCTGT (1524-1502)
	1502–2428	L-F3: ACAGAGCTCACTCATGCCCTCAA (1502–1524)	L-R3: GCTCCGTGAGAATTCATGCTTCTTAG (2428-2403)
	2290–3289	L-F4: AGAGGAGTCAACAGAGCTAAATGCC (2290–2314)	L-R4: GTGGAGGAGACTGGATGTAAAGTGC (3289-265)
	3265–3867	L-F5: GCACTTTACATCCAGTCTCCTCCAC (3265–3289)	L-R5: GCAAATGCAGGGTTGTCCATGAG (3867-3845)
	3848–4857	L-F6: ATGGACAACCCTGCATTCG (3848–3866)	L-R6: TCAGCTTCTAGGCTAAAACCAG (4857-4836)
	4648–5355	L-F7: ATTTGCATGGTTGAGCACAGACC (4648–4670)	L-R7: GATGCTACCCTGTTGTTGATCCCA (5355-5332)
	5092–6368	L-F8: TGAGAGGGCACAGTCAGGCAC (5092–5112)	L-R8: ACACAAAGACCGCCCAGATCTT (6368-6347)

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