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Identification of the first case of SFTSV infection in the Hunan Province of China and epidemiological surveillance in the locality

Liang Cai[a,b], Hong Zhang[b], Li-Dong Gao[b], Shi-Xiong Hu[b], Liang-Yi Xie[c], Zhi-Fei Zhan[b], Fang-Ling He[b], Fan Zhang[b], Zhi-Hong Deng[b], Qian-Lai Sun[b], Jin-Ru Wu[d], Si-Xuan Long[d], Zhong-Hui Pan[e], Wei-Min Cao[e], Jia-Hui Liu[b], Qi-Zhi Luo[a], Jie Wang[a], Yizhou Zou[a,*]

[a] School of Basic Medical Science, Central South University, Hunan Province, People's Republic of China
[b] Microbiology Laboratory, Center for Disease Control and Prevention of Hunan Province, Hunan Province, People's Republic of China
[c] Department of Clinical Examination, The People's Hospital of Hunan Province, Hunan Province, People's Republic of China
[d] Department of Infectious Diseases Control, Center for Disease Control and Prevention of Loudi, Hunan Province, People's Republic of China
[e] Department of Infectious Diseases Control, Center for Disease Control and Prevention of Xinhua County, Hunan Province, People's Republic of China

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ABSTRACT

This study reports the etiological identification, clinical diagnosis, and the results of the local epidemiological surveillance of the first case of severe fever with thrombocytopenia syndrome virus (SFTSV) infection in 2014 in Hunan Province, China. The infected patient was isolated and closely monitored. The virus is a member of the Bunyaviridae sandfly family and is characterized by real-time PCR, electron microscopy, immunofluorescence, and whole-genome sequencing. We also detected IgG and IgM antibodies against SFTSV among the local human population and domestic animals in a serological surveillance. Prevalence of SFTSV-specific antibodies was monitored in the local population for two years after the identification of the first SFTS case. Approximately 5% (4/77) of the people who had direct contact with the patient were seropositive, which is significantly higher than the seropositivity of the general local population [1.57% (44/2800), $P < 0.05$]. Furthermore, the percentage of the general population who were seropositive was higher in 2015 than in 2014 ($\chi 2 = 7.481$, $P = 0.006$). The epidemiological investigation found that the SFTSV is epidemic in goats, cattle, and chickens in Hunan Province. The risk of infection of domestic animals can be minimized by feeding in pens rather than allowing foraging.

1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is an infectious disease discovered in China in 2010 (Yu et al., 2011). The major candidate vectors of SFTSV may be ticks such as *Haemaphysalis longicornis* and *Boophilus microplus* (Yu et al., 2011; Zhang et al., 2012). SFTSV belongs to the Phlebovirus genus of the Bunyaviridae family, which is recognized as a causative pathogen of SFTS-related fatal infectious disease (Xu et al., 2011). The disease is clinically characterized by fever (≥38 °C), leucopenia and thrombocytopenia, and elevation of alanine aminotransferase, aspartate aminotransferase, and proteinuria. A small number of infected patients have severe illness and rapid disease development, and the infection can cause death due to multiple organ failure (Cui et al., 2013).

Beginning in 2012, SFTS cases have been observed in the provinces of Henan, Hubei, Anhui, Shandong, Jiangsu, Zhejiang, Jiangxi, Guangxi, Yunnan, Shanxi, and Liaoning. SFTS was also reported in Japan and South Korea in 2012 (Takahashi et al., 2014; Kim et al., 2013). From June 2009 to September 2014, 2781 confirmed cases of SFTS cases were reported in Henan, Hubei, Shandong, Liaoning, Anhui, and Jiangsu provinces; 202 of these cases were fatal (Li, 2013; Ding et al., 2013). Since 2011, active surveillance for the SFTSV-induced disease has been carried out in Hunan province but no such case was found until October 16, 2014. This manuscript describes identification and confirmation of the first case of SFTSV infection in Hunan Province and the surveillance performed on the animals and people living in the locality.

2. Materials and methods

2.1. Case finding and etiological diagnosis

On October 14, 2014, under the surveillance project of major infectious diseases, Hunan Province CDC received a blood sample from a

* Corresponding author at: School of Basic Medical Science, Central South University, No. 110, Xiangya Road, Changsha 410013, Hunan, China.
E-mail address: yizhou_zou@sina.com (Y. Zou).

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Table 1
Primer sequences for SFTSV whole-genome sequencing.

Primers	Sequences (5' to 3')	Primers	Sequences (5' to 3'))
L-1F	ACACAGAGACGCCCAGATG	S-401F	GCAATGGAAACTGGGAGA
L10F	CGCCCAGATGAACTTGGAAGTG	S-497R	GGCTTCAAGAGAATCCAATA
L-394F	CCACTAGGAGCCATAACATTG	S-526R	TCTAAGCCTTCGCTTCTCTATG
L546R	ACCCCTCCTGACGAGACTACAA	S-949F	ATTGCTGCTTACAGGTTTCT
L-587R	GCTCCTCTGCCTCATCCTG	S-1098R	ATGCTGCTGTGAACTCTGTCTT
L-1255F	GTTCAAGTGCCCGTCAAGA	S-1182F	TGCCACAAAGAGTAAGCC
L-1446R	GGACGGACTCATCTCCATAC	S-1225R	GAAGCCACGACCAAGACC
L-2070F	GGGAAACTGGATGGACCTCT	S-1718R	AAGGAAAGACGCAAAGGAGT
L-2295R	CCGTTGACTCCTCTTTGTTC	S-1727R	AAAGGAAAGACGCAAAGG
L-3081F	AAGATGATGGTGGACCTAAG	M-1F	ACACAGAGACGGCCAACAA
L-3235R	GTCTCTGTCTTTATGTAGGTTCG	M-323F	GGATGCTTGTCGTGAAGAAGG
L-3935F	ACCTTGGGCGGAAGTATG	M-508F	TCTGGTCTTCTGCCCTCA
L-4186R	GGTGAATGAACCTTCTCTGC	M-523R	AGGGCAGAAGACCAGAGC
L-4777F	CGTGAAGAAGTCAGGTGGGG	M-658R	CACACTGGTTGAACACAATGC
L-5014R	GATGACCTCTGATGGCTGTG	M-1598F	GCAACCAGGATGATGTTAGGA
L-5176F	GTGTGTGGACTGGAGTGATG	M-1769R	TTCATATTTCCGCTCCCT
L-5454R	AAGCCCCTTTCCATAATCCTGA	M-2051F	ACAGGGCTGGTTTAGGGT
L-6336R	GGAAATCCTTCTAAAACCAAC	M-2346R	GTCAGTCAAGCCAGACACCT
S-1F	ACCCCCTTCATTTGGAAAC	M-2750F	ATGTGAGTGGGTGTTATTCTTG
S-14F	CTTCATTTGGAAACCATGTCAC	M-2945R	AGTTGGCACTGCTCATCG
S-210F	GGTTGTTTGGCAACCCTA	M-3358R	CGGCCAACACTTCAACAGA

patient who had been admitted to the Hunan Provincial People's Hospital. The next morning, the blood sample was subjected to testing for the causative agents of hemorrhagic fever with renal syndrome (HFRS) and Dengue fever with ELISA for IgM and IgG and with quantitative real-time PCR (qRT-PCR) for viral nucleic acids; all of the results were negative. The sample was then tested for *Leptospira* (hemagglutination inhibition test), *Legionella pneumophila*, mycoplasma pneumonia, *Chlamydia pneumoniae* (by ELISA), and types A and B influenza virus, parainfluenza virus, respiratory syncytial virus, and adenovirus (by multiplex PCR) with negative results. On the following day, qRT-PCR was used to test for pathogens causative of Q fever and for *Rickettsia*, *Anaplasma,* and SFTSV. Only the test for SFTSV was positive. Immediately, the sample was tested for an SFTSV-specific antibody with ELISA, and IgM antibodies against SFTSV were detected. The epidemiological investigation was initiated as soon as the patient was diagnosed.

2.2. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from the patient serum using the QIAamp minElute Virus Spin Kit (Cat. No. 52904, Qiagen) according to the manufacturer's instructions. The eluate (5 μL) was tested for SFTSV RNA with a commercial kit (Daan Gene Bio-Tech).

2.3. Virus isolation and identification

Blood samples from the patient were centrifuged to separate the serum, and the virus was isolated and cultured with Vero and Vero E6 cells. Briefly, the Vero and Vero E6 cells were cultured in monolayers in T25 flasks After washing with Hank's solution twice, these cells were treated with 0.5 mL patient serum diluted with Hank's solution (1:10 dilution) at 37 °C for 2 h. After washing with 5 mL sterile Hank's solution twice, cells were cultured in MEM medium (Gibco) containing 2% fetal calf serum (Gibco) supplemented with 100 U/ml penicillin (Sigma), and 100 mg/mL streptomycin (Sigma) in a 5% CO_2, 37 °C incubator. The culture medium was changed every 3 days, and the cells were cultured for four consecutive passages. The culture supernatant was analyzed for viral nucleic acids.

2.4. Transmission electron microscopy (TEM)

Vero and Vero E6 cells were fixed with 2.5% glutaraldehyde for 24 h and with 2% osmium tetroxide for 2 h. The cells were subsequently subjected to the gradient dehydration with 50%, 70%, 90%, and 100% acetone (three 10-min incubations at each concentration), followed by the treatment with Epoxiaquivalentgewicht 145–160 (Epon812), dodecenylsuccinic anhydride (DDSA), methyl nadic anhydride (MNA), and dimethylaminomethyl phenol (DMP30) at 60 °C for 24 h. After embedment, the samples were cut into semi-thin sections and stained with toluidine blue. Sections of interest identified by light microscopy were cut into ultrathin (500 Å) sections on an LKB III ultramicrotome (LKB-Produkter). The sections were double stained with uranyl acetate and lead nitrate and imaged by transmission electron microscopy with a Nissan HT7700.

2.5. Immunofluorescence analysis

Human and animal host serum positive for SFTSV IgM and IgG antibodies as detected by ELISA were confirmed with immunofluorescence analysis (IFA). Briefly, the Vero cells were fixed with cold acetone to obtain the antigen sections. The serum was diluted with saline (1:50) and dropped onto these antigen sections, which were then incubated at 37 °C for 30 min. Sections were then incubated with rabbit anti-goat IgG-FITC, rabbit anti-human IgG-FITC, goat anti-human IgM-FITC, or mouse anti-human IgG-FITC (all 1:50 dilutions, Boster) at 37 °C for 30 min. After washing and sealing with 90% glycerol, the sections were observed by fluorescence microscopy (LEICA).

2.6. Whole-genome sequencing and phylogenetic analysis

Viral RNA was extracted from the culture supernatants using the RNeasy Mini Kit (Qiagen), and cDNA was synthesized from the viral RNA with the AMV Reverse Transcriptase First-strand cDNA Synthesis Kit (Promega). Primers were designed according to the published sequence of SFTSV (Table 1), and conventional PCR was used for the whole-genome sequencing of the SFTSV isolates. The sequences at the termini of viral RNA segments were determined with the RACE Kit (Invitrogen). The large (L), medium (M), and small (S) segment sequences of the SFTSV isolates were compared to the National Center for Biotechnology Information (NCBI) database through BLAST. Based on the deduced amino acid sequences coding for RNA-dependent RNA polymerase (RdRP), glycoproteins, and nucleocapsid (N) protein, phylogenetic analysis was performed using the Mega software (version 6.0) in comparison with other known phleboviruses such as the Rift Valley fever (RVF), sandfly fever, and Uukuniemi groups.

2.7. Syndromic surveillance

On October 20, 2014, researchers went to the region of the patient's home (Yuexing and Daotang Villages, Youjia Town, Xinhua County, Loudi City, Hunan Province) and collected blood samples from 77 subjects who had contact with the patient within two weeks prior to symptom onset, including the patient's husband, two sons, and one daughter. Of the subjects, 59 lived within 5 km of the Yuexing Village, whereas 18 were beyond this range. These samples were analyzed for SFTSV IgM and IgG antibodies by ELISA (Sinosbio). Serum was collected from 21 goats and three cows in the vicinity of the patient's house. Moreover, 66 ticks were collected from these animals, and 46 ticks were obtained from the tea garden where the patient had been working. On December 4, 2014, the investigators collected serum from 100 chickens and 40 goats from the Xinhua County, Loudi City to increase the surveillance. The goat, cattle, and chicken sera were analyzed for SFTSV IgM and IgG. The tick specimens were tested for SFTSV nucleic acids (Daan Gene Bio-Tech).

2.8. Epidemiological investigation

Under the approval of the Hunan CDC Ethics Committee, Xinhua County was selected for SFTS surveillance from 2014 to 2015. Sera from healthy subjects from several towns in Xinhua County were analyzed for SFTSV antibodies. With the patient's village as the surveillance center, blood samples were collected from 1400 healthy subjects from Caojia, Xihe, Luguan, and Sangzi towns in 2014 (400 subjects 0–20 years old, 400 subjects 21–40 years old, 400 subjects 41–60 years old, and 200 subjects greater than 60 years old). In 2015, samples were collected from 1400 healthy subjects from Menggong, Wentang, Tianmen, and Wentian (400 subjects 0–20 years old, 400 subjects 21–40 years old, 400 subjects 41–60 years old, and 200 subjects greater than 60 years old). Serum was separated from blood in the Xinhua County CDC, and samples were transported on dry ice to the Hunan Provincial CDC for IgG antibody analysis.

3. Results

3.1. Clinical manifestation and treatment history of the patient

The 59-year-old female patient was a farmer with no history of related illnesses who has two sons and one daughter. From September 25, 2014 to the end of the month, she harvested tea seeds and chestnuts in the mountains. On October 3, she felt an unbearable itching on her foot with sarcosome-like swelling, which was later suspected to be caused by a tick bite. On October 4, she felt malaise and soreness and self-medicated with dipyrone but did not get better. On October 5, when the symptoms became more serious, she was admitted to the People's Hospital of Xinhua County, where she received anti-cold and antipyretic treatments and was discharged. On October 6, her fever was 38.9 °C, and she received antipyretic and fluid infusion treatments in the out-patient clinic of the Second People's Hospital of Huaihua City. The pain was eased but the fever persisted. In the afternoon of October 6, her fever reached 40 °C, and she was again admitted to the People's Hospital of Xinhua County. Upon admission, her leukocyte count was 2.56×10^9 cells/L and her platelet count was 24×10^9 cells/L. No improvement was observed after symptomatic treatments. On October 11, the patient's condition worsened. Headache, bleeding gums, nausea, vomiting, hematemesis, abdominal pain, bleeding in mouth and nose, tarry stools, scattered pinpoint hemorrhagic spots on the abdominal and thigh skin with no fading under pressure, eye conjunctival edema with no lymph node swelling, and pain were reported. On the same day, the patient was transferred to the emergency department of the Hunan Provincial People's Hospital. Upon admission, her fever was 39.5 °C, her white blood cell count was 3.59×10^9 cells/L, and her platelet count was 28×10^9 cells/L (Fig. 1). After anti-infection and anti-viral treatments, the patient's condition did not significantly improve, and she was transferred to the ICU on October 13 (Fig. 2). After diagnosis of SFTSV on October 15 and successful symptomatic treatment, the patient recovered and was discharged from the hospital on October 30.

3.2. Immunology, morphology, and molecular analyses leading to SFTSV diagnosis

IFA demonstrated that the patient's serum had antibodies against SFTSV antigen (Fig. 3). Under transmission electron microscopy, typical morphological characteristics of a Bunyaviridae virus were observed; spherical particles with a diameters of 80–100 nm and lipid envelopes with 5–10 nm polypeptide processes on the surface were detected, and enlarged nucleoli, nuclear condensation, and cytoplasmic edema were seen (Fig. 4). In nucleic acid extracted from the patient's serum, we detected SFTSV by qRT-PCR with a cycle threshold (Ct) value of 26. The Vero cells culture supernatant was detected by qRT-PCR, and the Ct value was 15.

3.3. Virus isolated from the infected patient was SFTSV

Like other members of the Bunyaviridae family, the SFTSV HNXH strain isolated from the patient's serum was an enveloped RNA virus with three single-stranded RNA genomes, consisting of large (L), medium (M), and small (S) segments. The L and M segments are of negative polarity. The L segment contains 6260 nucleotides with one open reading frame encoding the RdRP; the GC content is 48.53% and the predicted RdRP is composed of 2084 amino acids. The M segment contains 3341 nucleotides with one open reading frame encoding the 1073-amino acid precursor of glycoproteins Gn and Gc; the GC content is 49.45%. The S segment of SFTSV contains 1689 nucleotides with a GC content of 48.79%. In this segment, the nucleocapsid (N) protein is encoded in the antisense orientation and the nonstructural protein (NSs) is in sense orientation, separated by a 54-base pair intergenic region. The 5′ and 3′ termini of the L, M, and S segments possess short noncoding sequences. The 5′ noncoding regions of SFTSV strain are 16 nucleotides (nts) for L, 18 nts for M, and 42 nts for S. The 3′ noncoding regions are 100, 141, and 28 nts for L, M, and S, respectively. The sequences of L, M, and S were submitted to the GenBank as an HNXH strain and were given accession numbers KT254588, KT254589, and KT254590, respectively.

Phylogenetic and molecular evolutionary analyses were performed with MEGA version 6 using SFTSV sequences available in GenBank; representative sequences from phleboviruses were included. Phylogenetic analysis of the L, M, and S segments of the SFTSV complete sequences showed that the HNXH strain belongs to the Phlebovirus genus and the Bunyaviridae family. Further analysis of deduced amino acid sequences of the glycoprotein, RdRp, and N proteins of SFTSV strain isolated from the subject described here showed that the isolate was clustered with other known phleboviruses but was almost equally distant from the toroviruses, viruses that cause Rift Valley fever virus, sandfly fever Naples virus, sandfly fever Sicilian virus, and Uukuniemi viruses (Fig. 5).

The molecular and morphological characteristics of the SFTSV HNXH strain also indicate that the strain is a phlebovirus. Comparison of the amino acid sequences provided further evidence supporting the separation of SFTSV HNXH strain from other phleboviruses. The RdRP, N, Gn, and Gc proteins of the SFTSV HNXH strain are closely related to those of the SFTSV group such as the KAGBH6, HNXY-200, SD4, AH12, HN6, LN2, and HB29 strains, with identities of 94.1% to 99.7%. In contrast, identities were 29.3% to 37.0% with proteins encoded by the SFNV group (including the Massilia W strain and Toscana AR), 25.8% to 44.1% to the UUKU group (Uukuniemi slgp1 and Uukuniemi S23), 31.9% to 42.4% to the Punta Torovirus (PTV) group (PTV Adames and PTV Balliet), and 31.0% to 43.6% to the RVFV group (RVF 74HB59 and Enetbbe) and the SFSV group (sandfly Sicilian, sandfly Sicilian

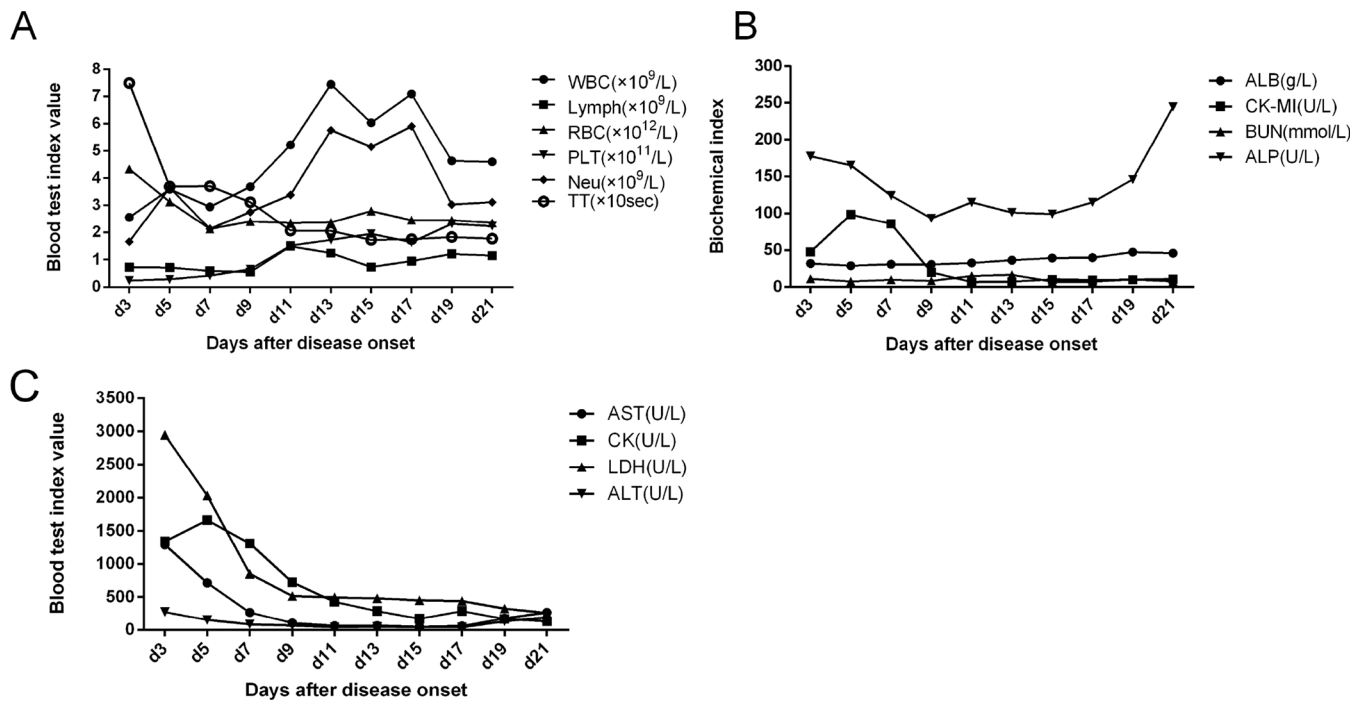

Fig. 1. Blood and biochemical test results. (A) Patient's blood test results on days 3–21 after the disease onset. (B–C) Patient's biochemical test results on days 3–21 after the disease onset.

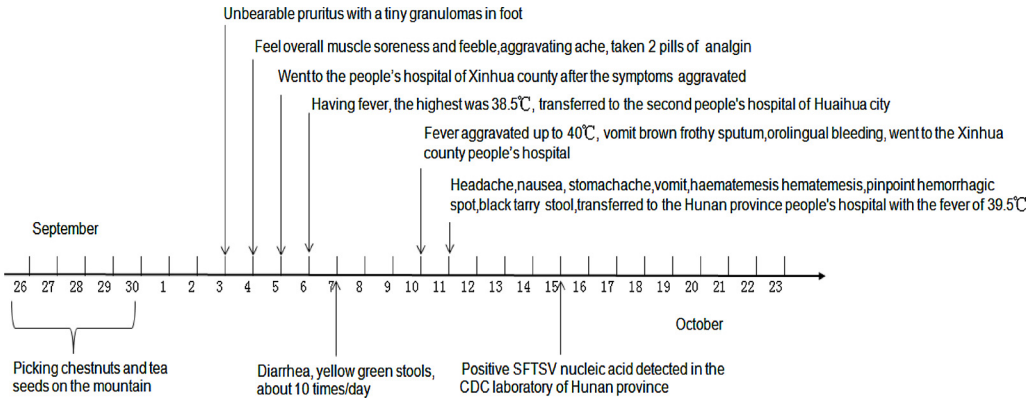

Fig. 2. Timeline of symptoms experienced by the first SFTSV-infected case in Hunan Province.

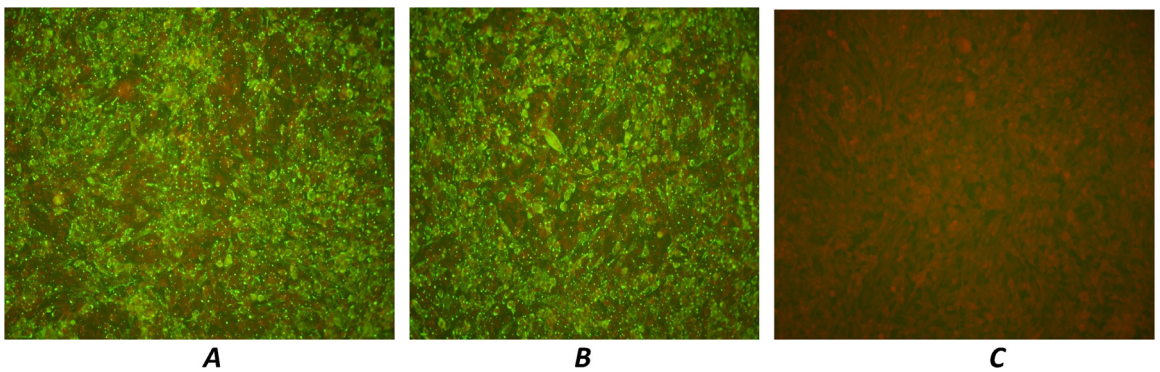

Fig. 3. IFA method detected SFTSV antibody in patient serum. (A) Vero cells infected with SFTSV, (B) Vero E6 cells infected with SFTSV, (C) Control uninfected cells.

Ethiopia-201, and sandfly Turkey lzmir 19). The N protein of SFTSV, the most highly conserved protein, had identities of 94.51% and 99.93% to the SFTSVs recently discovered in Japan (Japan SPL146A) and Korea (JP01-Korea-14 and JP06-Korea-14), respectively.

3.4. Investigation of potential infection sources

A total of 164 serum samples collected from potential host animals were tested for SFTSV IgM and IgG antibodies. No samples were

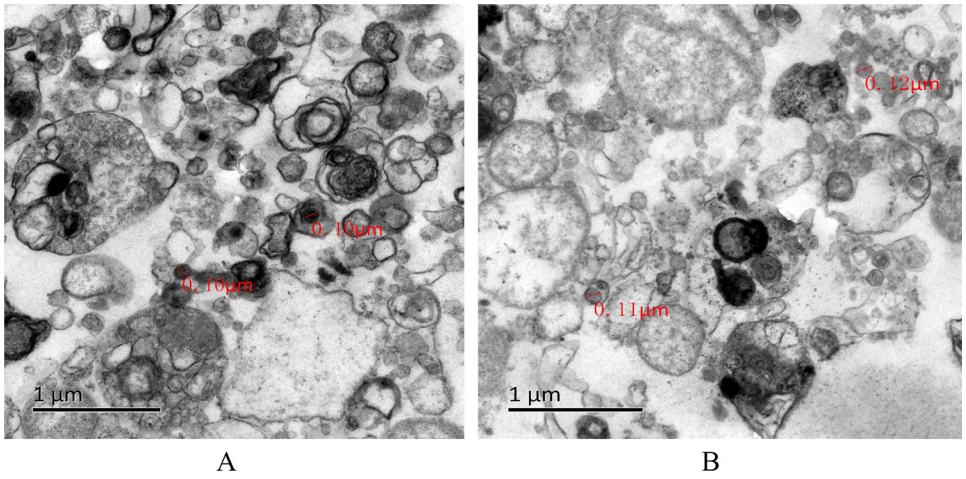

Fig. 4. Transmission electron microscopy demonstrated the presence of virus in the Vero and Vero E6 cells. Thin-section transmission electron microscopy of (A) Vero cells and (B) Vero E6 cells treated with patient serum.

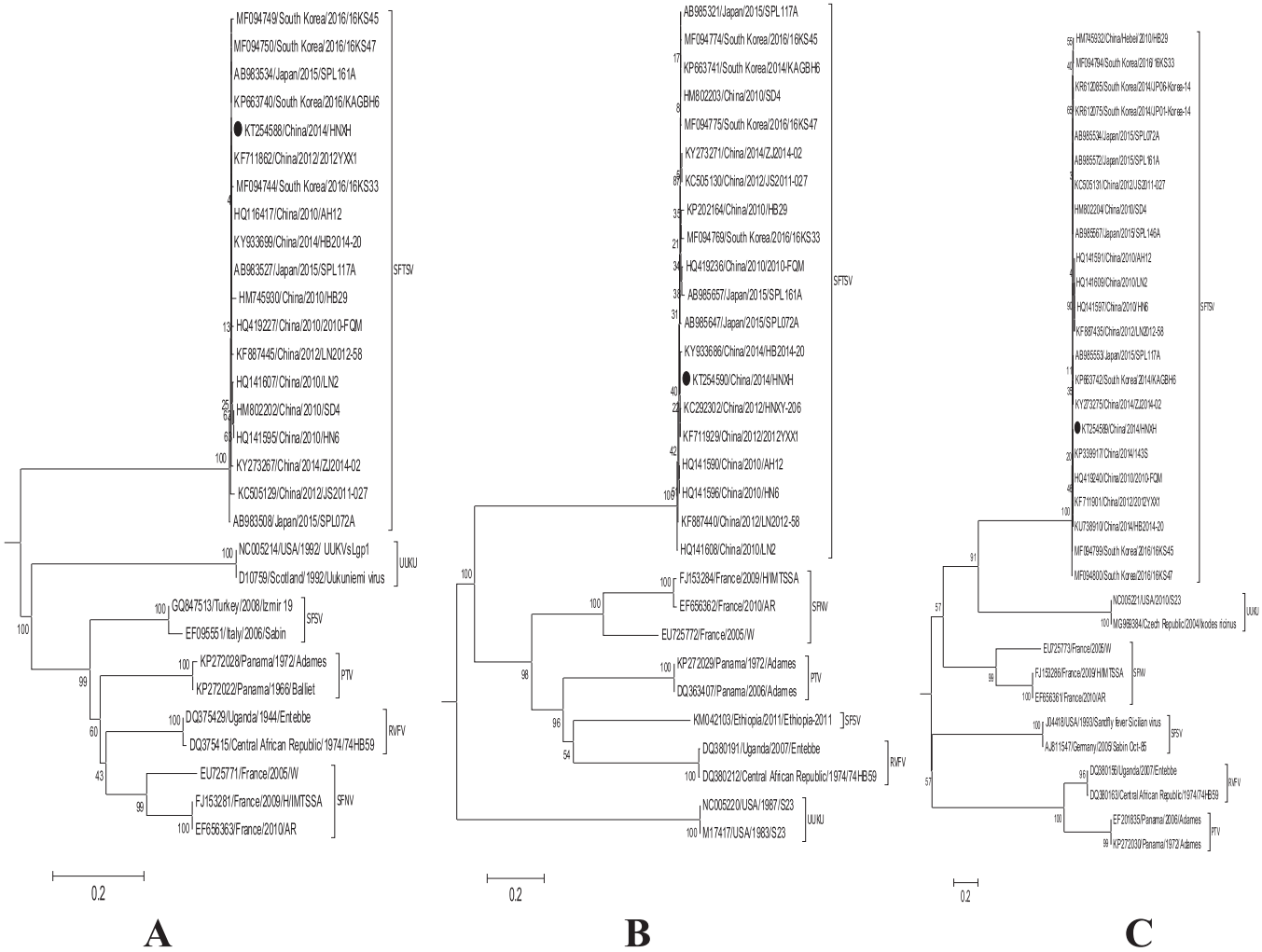

Fig. 5. Phylogenetic analysis of SFTSV and other phleboviruses. Phylogenetic and molecular evolutionary analyses were conducted using the Mega software (version 6.0) from aligned amino acid sequences of six strains of phleboviruses including the newly identified SFTSV HNXH strain. Coding regions for (A) RdRP in the L segment, (B) glycoproteins Gn and Gc in the M segment, and (C) N in the S segment were analyzed with the neighbor-joining method using the Poisson correction and complete deletion of gaps. Bootstrap testing (2000 replicates) was performed, and the bootstrap values are indicated. Sequences are identified by GenBank accession numbers and virus name (PTV, Torovirus; RVFV, Rift Valley fever virus; SFNV, sandfly fever Naples virus; SFSVS, sandfly fever Sicilian virus; UUKV, Uukuniemi virus).

Table 2
Seropositivity of animals and people living close to the patient.

	Sample Size	Feeding method	Number IgM positive	Number IgG positive	Percentage IgG positive
Animals					
Goat	21	Backyard	0	21	100%
Cattle	3	Backyard	0	2	67%
Chicken	100	Combination	0	2	2.0%
Goat	40	Captive	0	0	0
People					
Gender					
Male	31	–	0	1	3%
Female	46	–	0	3	7%
Age					
1-40 years	12	–	0	0	0
40-50 years	13	–	0	0	0
50-70 years	33	–	0	1	3%
> 70 years	19	–	0	3	15.78%
Village location					
< 5 km from patient	59	–	0	4	7%
≥ 5 km from patient	18	–	0	0	0

positive for SFTSV IgM; however, 15.24% (25/164) of the samples were positive for SFTSV IgG. All of the 21 goats allowed to forage in areas around houses (backyard feeding) were positive for SFTSV IgG, two of the three cattle fed in backyards were positive for SFTSV IgG, and two of the 100 chickens (2.0%) were positive for SFTSV IgG. The chickens were fed by a combination of backyard and captive feeding methods. Those fed by captive feeding methods were contained in pens. All of the 40 goats were negative for SFTSV IgG, and all were fed by captive feeding (Table 2). There was a significant difference in SFTSV IgG positive rates when the three feeding methods were compared ($\chi 2 = 124.659$, $P < 0.001$). None of the 112 ticks collected carried the virus based on nucleic acid analyses.

In addition, serum samples were collected from 77 subjects living near the patient's house. The epidemiological investigation showed that all the 77 subjects had a history of poultry farming, 92% of them had worked outdoors within the past two weeks, and 61% of them were aware of the existence of the ticks in their environment. All 77 serum samples were negative for the SFTSV IgM, although 5% (4/77) were positive for the SFTSV IgG. Serum samples from the patient's husband, two sons, and daughter, who had intimate contact with the patient, tested negative for SFTSV IgM and IgG.

3.5. Longitudinal sero-epidemiological surveillance of SFTSV infection in Xinhua County

From 2014 to 2015, serum samples from 2800 healthy subjects from Xinhua County were tested for antibodies to SFTSV. The overall seropositivity was 1.57% (44/2800). In 2014, 13 of the 1400 samples tested (0.93%) were antibody-positive for SFTSV. Males were 1.6 times more likely infected than females ($\chi 2 = 0.076$, $P = 0.783$). All of the four towns surveyed had positive cases, and 84.62% of the cases were in the age range of 21 to 60 years. In 2015, 31 of 1400 samples tested (2.21%) were positive for the SFTSV antibody, and males were 2.44 times more likely than females to be infected ($\chi 2 = 1.397$, $P = 0.237$). As in 2014, positive cases were found in all four towns surveyed, and 90.32% of the cases were in the age range of 21 to 60 years. Seropositivity in healthy subjects was significantly higher in 2015 than in 2014 ($\chi 2 = 7.481$, $P = 0.006$). In the otherwise healthy subjects who tested positive for the virus over the two years, the ratio of infected males to infected females was 30:14. Of those who were seropositive, 96.64% had a history of poultry farming, 78.79% had a history of outdoor work, and 74.57% had seen ticks in their environment (Table 3).

4. Discussion

The symptoms of infection with SFTSV are similar to those of infections with *Anaplasma, Rickettsia*, leptospirosis, hemorrhagic fever with renal syndrome, and Dengue fever (Zhang et al., 2013). Although the surveillance for SFTS began in 2011 in Hunan Province, the first positive case was recognized in October 2014. Hunan Province is located in central China, south of the Yangtze River, Xinhua County is in the middle of Hunan Province. The climate and geography of this region are suitable for ticks, the intermediate host of SFTSV. The region has large stretches of forest and hilly areas where tea is grown. The epidemiological investigation reported here showed that the first SFTS patient in Hunan Province was a farmer, who had harvested tea seeds in the mountains approximately 10 days before the disease onset. The patient kept several chickens in the backyard but no other poultry or livestock; her neighbors had domestic animals such as dogs, cattle, and goats. However, the patient had not left Hunan Province in the two weeks before the disease onset. Therefore, we considered this a case of local infection.

At the early stage, the case was associated with flu-like symptoms and gastrointestinal symptoms. After admission to the hospital, the major clinical signs were non-specific and included fever, fatigue, leukopenia, thrombocytopenia, conjunctival hyperemia, proteinuria, hematuria, and lymphadenopathy. On the third day, the patient's white blood cell count dropped to 2.56×10^9 cells/L but returned to normal levels on the fifth day. Her platelet count decreased continuously from symptom onset through the ninth day and returned to normal after transfusion therapy. The prothrombin time was prolonged for nine days after disease onset. Due to limitations of local medical resources, the patient was not diagnosed with SFTSV infection until the eleventh day after symptom onset when the Hunan provincial CDC reported that the patient's serum samples had tested positive for SFTSV nucleic acid and antibodies. Despite the delay in diagnosis, the patient survived due to effective treatment of symptoms.

Infection with sandfly virus usually causes mild fever only (Gai et al., 2012a), but the risk of death is dramatically elevated when the patient has coagulopathy, disseminated intravascular coagulation, bleeding, neurological symptoms, multiple organ failure, and/or sustained platelet decline (Gai et al., 2012b). The increase of biomarkers observed in the patient described were likely indicators of the severe phase of SFTS (Cui et al., 2014). The patient had a sustained elevation of AST, CK, ALT, LDH, and CK-MI in the early stage of the disease; however, after symptomatic treatment on the eighth day in ICU, all her biomarkers except AST and LDH had returned to the normal levels.

Table 3
SFTSV IgG antibody detection in healthy subjects in Xinhua County, Hunan Province.

	2014			2015		
	Number of samples	Number seropositive	Percentage positive	Number of samples	Number seropositive	Percentage positive
Sex						
Male	809	8	0.99%	850	22	2.73%
Female	591	5	0.85%	550	9	1.64%
Age						
1-20 years	400	2	0.50%	400	2	0.50%
21-40 years	400	5	1.25%	400	20	5.0%
41-60 years	400	6	1.50%	400	8	2.0%
> 61 years	200	0	0	200	1	0.5%
Towns						
Caojia	352	3	0.85%	–	–	–
Xihe	348	1	0.29%	–	–	–
Luguan	328	5	1.52%	–	–	–
Sangzi	372	4	1.08%	–	–	–
Menggong	–	–	–	358	2	0.56%
Werntang	–	–	–	342	3	0.88%
Tianmen	–	–	–	339	11	3.24%
Tianwen	–	–	–	361	15	4.16%

The virus was isolated from the patient's serum after culture with Vero and Vero E6 cells. Infection caused no significant cytopathic changes over four passages; however, SFTSV nucleic acid was detected in the cell culture supernatant. Analysis of the cells by TEM revealed particles with the typical morphology of a bunyavirus. The isolated virus was used to prepare antigen sections, and the corresponding SFTSV antibodies in the human and goat serum were detected by indirect immunofluorescence with the FITC-labeled IgM and IgG secondary antibodies. IFA was consistent with the results from ELISA. Genome sequencing and phylogenetic analysis showed that the SFTS HNXH isolate belongs to the same branch as SFTSV strains isolated in Hubei, Shandong, and Anhui Provinces. The molecular characteristics of the virus isolated were typical of the sandfly virus. SFTS HNXH had 94.1% to 99.7% sequence identity with domestic SFTSV isolates. However, it was distant from other sandfly viruses based on our evolutionary analysis. The molecular findings indicate that the SFTS HNXH isolated from Hunan Province were essentially the same as other isolates from China.

After the occurrence of disease, animals and people living near the patient were tested. Of the 164 animal sera tested, all were negative for IgM, and 15.24% were positive for IgG antibody. More importantly, all 21 goats fed in backyards were positive for the SFTS IgG antibody, whereas none of the 40 goats fed with the captive feeding method were infected. Therefore, this suggests lower likelihood for SFTSV to be found in pens. The seropositive rate in the cattle serum, all fed in backyards, was 67% and that for chickens was 2.0% (all chickens were fed using a combination of a backyard and captive feeding). These results indicate that the feeding methods significantly affect infection of potential host animals with SFTSV. The risk of SFTSV infection for the animals fed in backyards was much higher than the captive-fed animals. Several studies from China have shown that almost 47.7% of domestic animals are positive for SFTSV antibody (Jiao et al., 2012; Ding et al., 2014; Zhang et al., 2011). The following are the seropositive rates of the potential host animals reported in Shandong Province: 75%–95% for goats, 57% for cattle, 53% for dogs, and 36% for chickens (Jiao et al., 2012; Ding et al., 2014). In Jiangsu province, 1% of chickens, 5% of pigs, 6% of dogs, 32% of cattle, and 57% of goats sampled were positive for SFTSV (Zhang et al., 2011). In Hubei province, 55%, 36.7%, and 80% of dogs, goats, and cattle, respectively, were seropositive (Liu et al., 2012). In Minnesota in the USA, 11% of goats, 13% of sheep, 16% of cattle, 12% of white-tailed deer, and 18% of elk had antibodies against the SFTSV nucleoprotein (Xing et al., 2013). These data suggest that infection is widespread.

There is evidence from a study conducted in China that the person-to-person transmission of SFTSV is likely through direct blood contact when the patient has a high viremia (Cui et al., 2013; Bao et al., 2011). The 77 subjects who had been in contact with the patient (including her husband, two sons, and one daughter) were negative for SFTSV IgM. Within a distance of 5 km of the patient's home, there were four subjects positive for SFTSV IgG, but none of the patient's family tested positive. Our epidemiological investigation showed that all four subjects were villagers over 50 years old with a history of outdoor labor and feeding goats, pigs, cattle, and/or chicken. These subjects had no obvious clinical manifestations and no medical treatments within the past two years that would suggest presence of SFTSV infection. As only SFTSV IgG antibody-positive subjects were identified, it could not be determined whether person-to-person transmission had occurred. It remains uncertain how or when the patient was infected, although she did report itching and sarcosome-like swelling on her foot two days before she fell ill. Perhaps due to the limited sample size, none of the 112 collected ticks were positive for SFTSV. Monitoring of ticks is being increased in the area.

Data from the serum epidemiological surveillance in China indicate that approximately 1.0% to 3.8% of the population in hilly regions are positive for the SFTSV antibody (Liu et al., 2014). Since 2010, samples from most of the suspected SFTS cases in Hunan Province had been subjected to testing for viral nucleic acids, but seroepidemiological surveys in the healthy population have not been carried out. In 2014, the towns around the patient's village, including Caojia, Xihe, Luguan, and Sangxi, were chosen as surveillance points, and 1400 subjects were tested for SFTSV antibodies using ELISA. Overall, 0.93% of those sampled were seropositive. In 2015, the surveillance area was expanded, with Menggong, Wentang, Tianmen, and Wentian residents included, and another 1400 subjects were tested: 2.21% were seropositive. The overall percentage of seropositivity in the two-year period was 1.57%, which was lower than that in healthy subjects from the towns near the patient's home (5%). The SFTSV antibody-positive subjects were mainly laborers from rural areas, indicating the existence of SFTSV latent infections in several regions in Xinhua County, but the dates of infection could not be inferred. The overall seropositive rate in the healthy subjects in 2015 was higher than in 2014; further surveillance is needed to monitor whether SFTSV infection is spreading.

The case reported herein and the detection of the SFTSV IgG antibody in residents and animals in the area surrounding the patient's home in Yuexing Village revealed the presence of SFTSV in some areas in Hunan Province. Therefore more effort must be made to monitor the

prevalence of this virus in the residents and potential animal hosts in the mountainous and hilly areas of the province. It is also of great importance to strengthen the training of medical personnel in these areas in order to avoid delays in treatment due to misdiagnosis and to prevent person-to-person transmission. Blood samples should immediately be taken from the patients with fever accompanied by significantly reduced white blood cell and platelet counts, and the samples should be promptly delivered to the CDC for SFTSV testing.

Conflict of interest

None of the authors have competing financial interests.

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References

Yu, X.J., Liang, M.F., Zhang, S.Y., Liu, Y., Li, J.D., Sun, Y.L., 2011. Fever with thrombocytopenia associated with a novel bunyavirus in china. N. Engl. J. Med. 364 (16), 1523–1532.

Xu, B.L., Liu, L.C., Huang, X.Y., Ma, H., Zhang, Y., Du, Y.H., 2011. Metagenomic analysis of fever, thrombocytopenia and leukopenia syndrome (ftls) in henan province, china: discovery of a new bunyavirus. PLoS Pathog. 7 (11), e1002369.

Cui, F., Cao, H.X., Wang, L., Zhang, S.F., Ding, S.J., Yu, X.J., 2013. Clinical and epidemiological study on severe fever with thrombocytopenia syndrome in yiyuan county, shandong province, china. Am. J. Trop. Med. Hyg. 88 (3), 510–512.

Takahashi, T., Maeda, K., Suzuki, T., Ishido, A., Shigeoka, T., Tominaga, T., 2014. The first identification and retrospective study of Severe Fever with Thrombocytopenia Syndrome in Japan. J. Infect. Dis. 209 (6), 816–827.

Kim, K.H., Yi, J., Kim, G., Choi, S.J., Jun, K.I., Kim, N.H., 2013. Severe fever with thrombocytopenia syndrome, South Korea, 2012. Emerging Infect. Dis. 19 (11), 1892–1894.

Li, D., 2013. A highly pathogenic new bunyavirus emerged in China. Emerg. Microbes Infect. 2 (1), e1.

Ding, F., Zhang, W., Wang, L., Hu, W., Soares, M.R., Sun, H., 2013. Epidemiologic features of severe fever with thrombocytopenia syndrome in China, 2011–2012. Clin. Infect. Dis. 56 (11), 1682–1683.

Zhang, X., Liu, Y., Zhao, L., Li, B., Yu, H., Wen, H., Yu, X.J., 2013. An emerging hemorrhagic fever in China caused by a novel bunyavirus SFTSV. Sci. China 56 (8), 697–700.

Gai, Z., Liang, M., Zhang, Y., Zhang, S., Jin, C., Wang, S.W., 2012a. Person-to-person transmission of severe fever with thrombocytopenia syndrome bunyavirus through blood contact. Clin. Infect. Dis. 54 (2), 249–252.

Gai, Z.T., Zhang, Y., Liang, M.F., Jin, C., Zhang, S., Zhu, C.B., Li, C., Li, D.X., 2012b. Clinical progress and risk factors for death in severe fever with thrombocytopenia syndrome patients. J. Infect. Dis. 206 (7), 1095–1102.

Cui, N., Bao, X.L., Yang, Z.D., Lu, Q.B., Hu, C.Y., Wang, L.Y., 2014. Clinical progression and predictors of death in patients with severe fever with thrombocytopenia syndrome in China. J. Clin. Virol. 59 (1), 12–17.

Jiao, Y., Zeng, X., Guo, X., Qi, X., Zhang, X., Shi, Z., Zhou, M., Bao, C., Zhang, W., Xu, Y., Wang, H., 2012. Preparation and evaluation of recombinant severe fever with thrombocytopenia syndrome virus nucleocapsid protein for detection of total antibodies in human and animal sera by double-antigen sandwich enzyme-linked immunosorbent assay. J. Clin. Microbiol. 50 (2), 372–377.

Ding, S., Yin, H., Xu, X., Liu, G., Jiang, S., Wang, W., Han, X., Liu, J., Niu, G., Zhang, X., Yu, X.J., Wang, X., 2014. A cross-sectional survey of severe fever with thrombocytopenia syndrome virus infection of domestic animals in Laizhou City, Shandong Province, China. Jpn. J. Infect. Dis. 67 (1), 1–4.

Zhang, W.S., Zeng, X.Y., Zhou, M.H., Jiao, Y.J., Wen, T., Guo, X.L., 2011. Epidemiology of severe fever with thrombocytopenia syndrome bunyavirus in Jiangsu province, China. Dis Surveill. 09, 676–678 (In Chinese).

Liu, L., Guan, X.H., Xing, X.S., Sheng, X.F., Xu, J.Q., Yue, J.L., Huo, X.X., Zhan, F.X., 2012. Epidemiologic analysis on severe fever with thrombocytopenia syndrome in Hubei province. Zhonghua Liu Xing Bing Xue Za Zhi 33 (2), 168–172 (In Chinese).

Xing, Z., Schefers, J., Schwabenlander, M., Jiao, Y., Liang, M., Qi, X., 2013. Novel bunyavirus in domestic and captive farmed animals, Minnesota, USA. Emerg. Infect Dis. 19 (9), 1487–1489.

Bao, C.J., Guo, X.L., Qi, X., Hu, J.L., Zhou, M.H., Varma, J.K., 2011. A family cluster of infections by a newly recognized bunyavirus in eastern China, 2007: further evidence of person-to-person transmission. Clin. Infect. Dis. 53 (12), 1208–1214.

Liu, Q., He, B., Huang, S.Y., Wei, F., Zhu, X.Q., 2014. Severe fever with thrombocytopenia syndrome, an emerging tick-borne zoonosis. Lancet Infect. Dis. 14 (8), 763–772.

Zhang, Y.Z., Zhou, D.J., Qin, X.C., Tian, J.H., Xiong, Y., Wang, J.B., Chen, X.P., Gao, D.Y., He, Y.W., Jin, D., Sun, Q., Guo, W.P., Wang, W., Yu, B., Li, J., Dai, Y.A., Li, W., Peng, J.S., Zhang, G.B., Zhang, S., Chen, X.M., Wang, Y., Li, M.H., Lu, X., Ye, C., de Jong, M.D., Xu, J., 2012. The ecology, genetic diversity, and phylogeny of Huaiyangshan virus in China. J. Virol. 86, 2864–2868.