Reviews in Medical Virology

REVIEW

Systematic review of severe fever with thrombocytopenia syndrome:virology, epidemiology, and clinical characteristics

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SUMMARY

Severe fever with thrombocytopenia syndrome (SFTS) was firstly discovered in China in 2010, followed by several reports from many other countries worldwide. SFTS virus (SFTSV) has been identified as the causative agent of the disease and has been recognized as a public health threat. This novel *Bunyavirus* belongs to the *Phlebovirus* genus in the family *Bunyaviridae*. This review also describes the different aspects of virology, pathogenesis, epidemiology, and clinical symptoms on the basis of the published article surveillance data and phylogenetic analyses of viral sequences of large, medium, and small segments retrieved from database using MEGA 5.05, SIMPLOT 3.5.1, NETWORK 4.611, and EPI information system 3.5.3 software. SFTS presents with fever, thrombocytopenia, leukocytopenia, and considerable changes in several serum biomarkers. The disease has 10~15% mortality rate, commonly because of multiorgan dysfunction. SFTSV is mainly reported in the rural areas of Central and North-Eastern China, with seasonal occurrence from May to September, mainly targeting those of ≥50 years of age. A wide range of domesticated animals, including sheep, goats, cattle, pigs, dogs, and chickens have been proven seropositive for SFTSV. Ticks, especially *Haemaphysalis longicornis*, are suspected to be the potential vector, which have a broad animal host range in the world. More studies are needed to elucidate the vector–animal–human ecological cycle, the pathogenic mechanisms in high level animal models and vaccine development. © 2013 The Authors. Reviews in Medical Virology published by John Wiley & Sons, Ltd.

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Abbreviations used
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine phosphokinase; CK-MB, creatine kinase isoenzyme; DC-SIGN, DC-specific intercellular adhesion molecule 3-grabbing non-integrin; Gc, glycoprotein C; Gn, glycoprotein N; HGA, human granulocytic anaplasmosis; LDH, lactate dehydrogenase; MOF, multiorgan dysfunction; NA, no data available; Np, nucleoprotein; NS, non-structural; Nt, nucleotide; RdRp, RNA-dependent RNA polymerase; RT-CPA, reverse transcription-cross-priming amplification; RT-LAMP, reverse transcription loop-mediated isothermal amplification; RT, reverse transcription; SFTS, severe fever with thrombocytopenia syndrome; SFTSV, severe fever with thrombocytopenia syndrome virus; VF, vertical flow.

INTRODUCTION

The mysterious agent of emerging infectious disease reported through 2009 in Central and North-Eastern China was confirmed later in 2010 as severe fever with thrombocytopenia syndrome virus (SFTSV) [1]. The common manifestations of the disease include severe fever with thrombocytopenia syndrome (SFTS), vomiting, and diarrhea, which progress to multiple organ failure with 12~30% mortality rate reported initially [1]. Surveillance data indicate that the incidence of SFTS is growing, and dissemination is expanding to at least 15 Chinese provinces in 2010~2013 [2]. Moreover, SFTS cases have been reported in other Asian and Mediterranean countries as well as in the USA [3–6].

This review is based on mining of the data reported in previous studies related to SFTSV and SFTS. This paper systematically reviews SFTSV in terms of its virology, epidemiology, and clinical characteristics, focusing on molecular evolution and epidemiology among different areas. The information described herein might improve the understanding of this novel virus and help to prevent and control it, thereby decreasing the transmission and diffusion of this novel virus worldwide.

VIROLOGY

Classification of *Bunyaviridae*

Severe fever with thrombocytopenia syndrome virus belongs to the third group of the *Phlebovirus* genus, which is a member of the *Bunyaviridae* family [1]. The family *Bunyaviridae* comprises a group of segmented, negative-strand RNA viruses that includes over 350 members classified into five genera, *Orthobunyavirus*, *Hantavirus*, *Nairovirus*, *Phlebovirus*, and *Tospovirus*[7–10]. The genus *Phlebovirus* currently includes over 70 antigenically distinct serotypes; 68 of the known serotypes are divided into two groups: *Phlebotomus fever* viruses include 55 members (transmitted by *Phlebotominae* sandflies) and the *Uukuniemi* viruses (transmitted by ticks) comprise 13 members [11,12]. Only eight species cause human disease, the *Alenquer*, *Candiru*, *Chagres*, *Naples*, *Punta Toro*, *Rift Valley fever*, *Sicilian*, and *Toscana* viruses [13,14]. SFTSV has been recognized as a novel member of the *Phlebovirus* genus because it is distantly related to both the existing *Uukuniemi* virus and *Phlebotomus fever* viruses [1,2,11].

Morphology

Severe fever with thrombocytopenia syndrome virus, such as other *Bunyaviridae*, is a spherical virion of 80–100 nm in diameter, covered by a lipid bilayer envelope of 5–7 nm in thickness, with no Ms [1,2,11,15]. The lipid bilayer envelope has spikes composed of gps, regularly arranged in genus specific arrays on the envelope exterior [1,2,16,17]. As observed by thin-section EM, the interior of the virions has a filamentous or coiled bead appearance, composed of three viral segments wrapped in the viral N in a circular genome [1,2,17,18] (Figure 1).

Genome

Comparable with the general genetic composition of *Bunyaviridae* family members, the genome of SFTSV consists of three single-stranded negative sense RNA segments [large (L), medium (M), and small (S)] [1,2,17,18]. The full-length L segment consists of 6368 bp including a single ORF between positions 17–6271 that encodes 2084 aa residues forming the RNA-dependent RNA polymerase (RdRp), which functions as the viral transcriptase/replicase [1,2,17]. The M segment is 3378 bp, forming an ORF between positions 19–3240 and
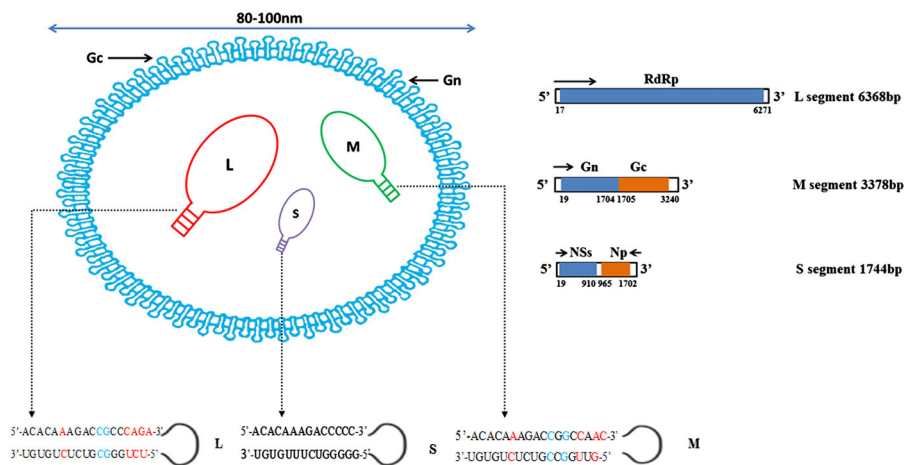

Figure 1. Schematic diagram of the structure and genome of the SFTSV [1,2]. SFTSV genome included L, M, and S segments. The antisense RNA and the encoded ORFs are represented in orange and blue boxes respectively. In L, M, and S genomic RNAs, only the first 20 Nts of both the 5′ and 3′ termini of each segment are shown. The first 15 Nts of both the 5′ and 3′ termini of each segment are paired and conserved except for the mismatches A–C/G or C–U in red color and C–C or G–G in blue color. Note: The data were sourced from the report of Li *et al*. and processed in PHOTOSHOP 7.0 [1]

encoding 1073 aa of membrane protein precursor, which matures to two envelopes, a glycoprotein N (Gn) [19–1704 nucleotide (Nt)] and a glycoprotein C (Gc) (1705–3240 Nt) [1,2]. The S segment is 1744 Nts of ambisense RNA with two oppositely ORFs separated by a 54 bp Nt intergenic region, encoding two proteins, the Np (1702–965 Nt) in antisense orientation and the NS protein (29–910 Nt) in sense orientation [1,2,18]. Np facilitates viral RNA encapsidation and is responsible for the formation of RNP complex [19]. The 5′ and 3′ termini of the L, M, and S segments possess short noncoding sequences. The 5′ noncoding regions of SFTSV are 16 Nt (L), 18 Nt (M), and 42 Nt (S); and the 3′ noncoding regions are 100 Nt (L), 141 Nt (M), and 28 Nt (S). Similar to other *Bunyaviruses*, the RNA component of which circularizes because each of the three segments having a consensus 3′-terminal Nt sequence is complementary to the 5′ terminal sequence, forming approximately 30 to 50-bp-long panhandle. The complementary 5′-termini sequences are 5′-ACA CAAAGACCGCCCAGA-3′ (L), 5′-ACACAAAGA CCGGCCAAC-3′ (M), and 5′-ACACAAAGACCC CC-3′ (S), whereas the 3′-termini complementary sequences are 3′-UGUGUCUCUGCGGGUCU-5′ (L), 3′-UGUGUCUCUGCCGGUUG-5′ (M), and 3′-UGUGUUUCUGGGGG-5′ (S) [1,2]. These sequences appear to form panhandle structures that seem likely to play a role in replication and encapsidation facilitated by binding with the viral N (Figure 1).

Cell culture

Acute blood samples in heparin anticoagulant collected within 7 days of onset of this disease are used to isolate the pathogen by inoculating into multiple cell lines of human, animal, or tick origin. Limited types of animal cell lines (Vero E6, Vero, DH82, and L929) can be infected by SFTSV [1,2]. The viral nucleic acid can be molecularly detected for 7 days after incubation in the sensitive cell lines, and the viral protein can be tested for 10 days after incubation [2]. Interestingly, DH82 cell line presents an obvious cpe after infection, in which the morphologic features of the infected DH82 cells changed from round monocytes to an elongated shape, having granular particles in the cytoplasm; however, these changes were not observed in Vero cell lines [1,2,20]. Thus, those cell lines are permissive and can be used for virus isolation and propagation [1,2,20]. It needs to further confirm that the other cell lines such animal cell line (BHK 21); human cell lines HL60, A549, 2BS; and tick cell line ISE6 are refractory to this novel virus and could be used for receptor identification and related endeavors.

Receptor

Entry into host cells is thought to occur by attachment of virions to cellular receptors utilized by *Bunyaviruses*, but the receptors for this family remain poorly characterized and different in the serotypes. *Hantaviruses* have been shown to use integrin to enter endothelial cells; it is not known whether the integrin serves as a receptor or attachment factor [21]. California serogroup of *Bunyaviruses* shared a common receptor, which is the La Crosse G1 gp [22], whereas DC-specific intercellular adhesion molecule 3-grabbing non-integrin is a *Phlebovirus* entry receptor for attachment and for promotion of entry and infection. Just like the other *Phlebovirus*, the C-type lectin DC-specific intercellular adhesion molecule 3-grabbing non-integrin was found to serve as a receptor for SFTSV, Gn/Gc-driven entry into cell lines, and dendritic cells, inducing viral spread and pathogenesis [23,24]. Furthermore, sera from convalescent SFTS patients inhibited SFTSV Gn/Gc-driven host cell entry in a dose-dependent fashion [24]. SFTSV virions and receptors binding will initiate the subsequent step of life cycle in the cell lines and human cases and animal models [24].

Pathogenesis

The pathogenesis of SFTSV infections is unclear as there is no fully compatible experimental animal model and cell line *in vitro* to describe it (mice do not seem to acquire severe disease). Jin *et al*. [25] claimed murine C57BL/6 mice as experimental model for SFTSV infection. In this model, viral RNA was detected in the blood and three organs (spleen, liver, and kidney) but not in the lung, intestine, heart, muscle, or brain. The main histopathological changes in the early stage (<14 days pi) were identified in the spleen, where the number of macrophages and platelets were greatly increased, and SFTSV was co-localized with platelets in cytoplasm of macrophages in that organ. The pathogenic lesions in the later stage (>14 days pi) were found in the liver and kidney, where the cells showed excessive degeneration and necrosis. In addition, Chen *et al*. [26] reported that the SFTSV

infection is lethal in newborn Kunming mice with dispersion of viral antigen and genetic material (RNA) in almost all organs, indicating a systemic infection. *In vitro*, Li further revealed that SFTSV adhered to platelets and facilitated the phagocytosis of platelets by macrophages [27]. Taken together, *in vitro* and *in vivo* tests indicate that SFTSV-induced thrombocytopenia is caused by clearance of circulating virus-bound platelets promoted by splenic macrophages, which might resemble the human SFTS disease features.

Koch's postulates in severe fever with thrombocytopenia syndrome virus

Koch's postulates were designed to establish a causal relationship between candidate microbes and diseases. The identification of SFTSV is a prime example of the rapid discovery of a truly emerging infectious disease, which has, mostly, fulfilled Koch's postulates. First, the novel virus has been found in abundance in all hosts suffering from SFTS but not found in healthy people [1]. Second, the SFTSV has been isolated from diseased cases and grown in many animal cell line cultures. Third, the cultured novel virus may cause the same disease when introduced into a healthy mouse model. Fourth, SFTS has been be reisolated from the inoculated, diseased experimental mice, and tick model and has been identified as being identical to the original specific causative agent, the identity was 99.99% [26,27].

Evolution

The evolutionary analyses described in this review were based on 43 full-length sequences of SFTSV genes downloaded from GENBANK (http://www.ncbi.nlm.nih.gov/nuccore/; NIH, Maryland,USA) sourced from different countries/areas, including 15 of L segment, 14 of M segment, and 14 of S segments (GENBANK accession numbers are provided in Tables S1, S2, and S3). Phylogenetic tree, plot similarity, and median network were performed using the MEGA 5.05 (http://www.megasoftware.net/), SIMPLOT 3.5.1 (The Johns Hopkins School of Medicine, Maryland, USA), and NETWORK 4.611 software (Fluxus Technology Ltd, England) [28–31], respectively. Phylogenetic tree showed that L, S, and M segments from all SFTSV isolates are clustered together but are almost equally distant from the *Phlebotomus fever* and *Uukuniemi* viruses. In

agreement with Zhang *et al.* [32], the phylogenies estimated from full-length sequences of SFTSV genes demonstrated four major sublineages, previously named as A, B, C, and E. Furthermore, the aa sequence similarity showed that RdRp (deduced from L gene) proteins in SFTSV have 46.3% similarity to *Rift Valley fever virus* but only shared 36.1 ~ 46.1% homology to the other *Phleboviruses* (*Uukuniemi virus*, *Naples virus*). On the contrary, RdRp from different Chinese specimens isolated from different provinces across a wide time range are closely related, sharing 96.2–98.7% aa sequence homology. Similar results are also found in reference to membrane gps (deduced from M gene) and the most highly conserved Np (deduced from S gene) of SFTSV. In general, the data from the molecular evolution and morphology indicate that SFTSV belongs to the prototype of a new member of the genus *Phlebovirus*, and all Chinese SFTS cases are caused by one genetic lineage of this novel virus. This result agrees with that reported by Yu *et al.* [1,2] (Figure 2).

The plot similarity versus position was performed by determining the degree of identity of the query sequence (L genes from Chinese strains) to a panel of reference sequences (the other groups of *Phleboviruses*) in a sliding window (windows 200 bp/step). The results showed that the homology percentage was <25%, 35%, and 0% in the position 1–2000 Nt, 2000–5500 Nt, and 5500–6500 Nt regions, respectively. In addition, the 1–2000 Nt and 5500–6500 Nt regions were highly diverse compared with the 2000–5500 Nt region (Figure 3). In agreement with the results of He and Ding [33], the query sequences from the Chinese isolates and other *Phlebovirus* reference sequences showed no crossover non-mosaic sites, which might indicate that this new virus undergoes considerable recombination and gene rearrangement processes. In addition, the similarity results provided further support for the separation of this novel SFTSV from other *Phleboviruses*.

The median-joining network analysis showed that all SFTSV isolated had no common ancestor and can be divided into four subgroups, each of sub-linkage with no regional and timeline cluster (Figure 4).

EPIDEMIOLOGY

According to the literature and Chinese surveillance data from 2009 to 2012, we analyzed the epidemical data by EPI information software 3.5.3 (USA cdc, Atlanta Botanical, Georgia, USA) and
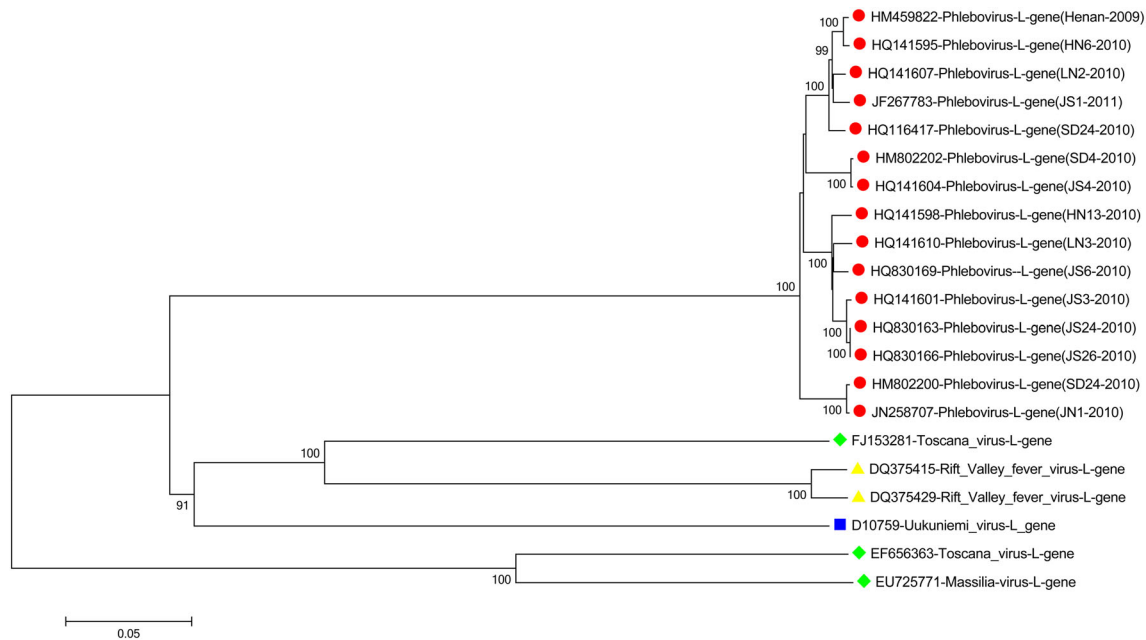
Figure 2. Phylogenetic relationship of Chinese SFTSV and other Phlebovirus strains based on sequences of L gene fragment. Evolutionary relationships of 21 taxa were inferred by the neighbor joining and maximum-likelihood methods using MEGA 5.05 software

SPSS 17.0 (SPSS Inc., Chicago, IL, USA), then summarized the epidemiological features in four ways: the disease distribution, host and vector, seroepidemical survey, and life cycle.

Disease distribution

Geographical distribution. In the past decades, *Phlebovirus* was mainly circulating in some parts of Africa and Europe, Arabian Peninsula, the regions with the highest incidences of the *phlebotomine* sandfly transmitted viruses. *Phlebovirus* has never been reported in China until SFTSV was identified in 2010. Although the SFTS have been found in some countries outside China such as Korea, Japan, and the USA since the SFTSV were identified in 2010, the major affected regions were in China. SFTS mainly occurs in the rural areas of the Eastern, Central, and North-Eastern China [1,2,34,35]. According to the national surveillance data in 2011, a total of 571 confirmed cases including 59 deaths from 13 provinces were reported to the Chinese SFTS information reporting system. The high incidence areas (>100 cases/year) were as follows: 191 in Shandong (24 deaths), 138 in Henan (four deaths), and 106 in Hubei (15 deaths); the median incidence areas (50~100 cases/year) were listed as follows: 61 in

Anhui (10 deaths); the low incidence areas (<50 cases/year) were as follows: 39 in Liaoning (four deaths), 19 in Jiangsu (two deaths), nine in Zhejiang (zero deaths), two in both Yunnan and Jiangxi (zero deaths), and one case in each of Sichuan, Shanxi, Guangxi, and city of Beijing, with no reported deaths. The mortality rate reached 10.33% nationwide although it was as high as 30% in the initial stage. In detail, provincial mortality rates were as follows: Anhui, 16.39%; Hubei, 14.15%; Shandong, 12.57%; Jiangsu, 10.53%; Liaoning, 10.26%; and Henan, 2.90% (Figure 5). In 2012, the number of confirmed cases increased slightly to 600, involved in 15 endemic provinces, but the mortality rate remained roughly 10.30% (61/600). In summary, the high rate of infection was reported among those living in hilly and wooded lands and those working in agricultural fields [1,2,36,37], which might be attributed to the underlying medical conditions, natural circumstances, exposure chances, disease detection, and laboratory testing, as well as consulting situation and medical intervention.

Population distribution

On the basis of the data of 649 confirmed cases and 31 deaths, retrieved from the national surveillance data with age range of 2~87 years (average
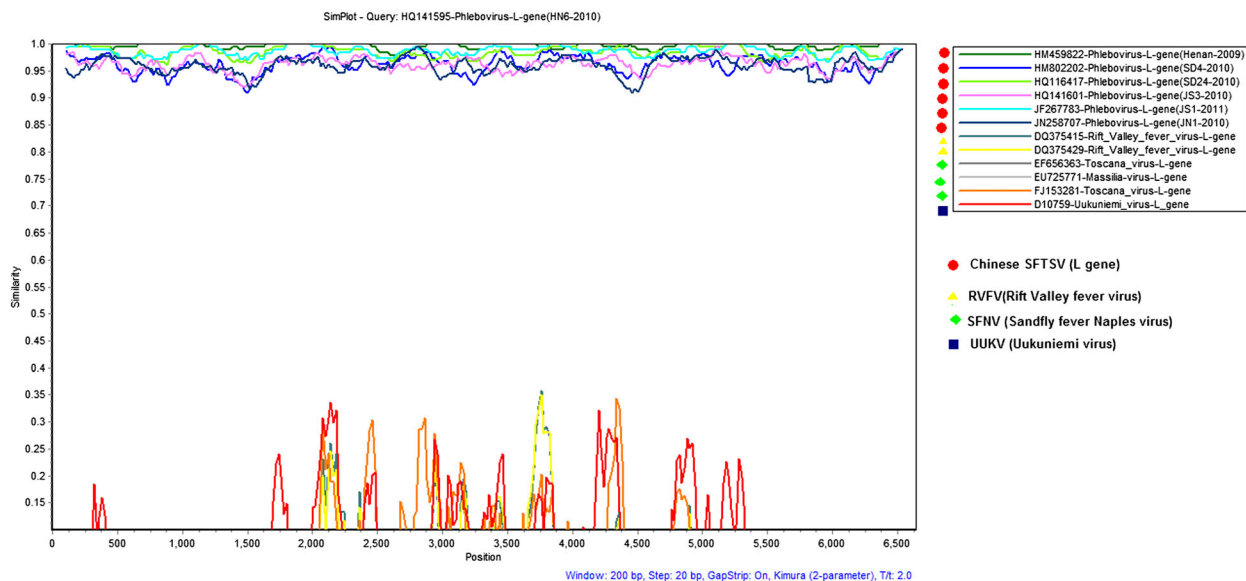
Figure 3. Similarity plot analysis of Chinese SFTSV strains and the other Phlebovirus species, with a window size of 200 bp and a step size of 20 bp. The plot were done using SIMPLOT 3.5.1 software (an interactive 32-bit software program) based on the sequence homology versus position of Chinese SFTSV strains to a panel of other Phleboviruses in database
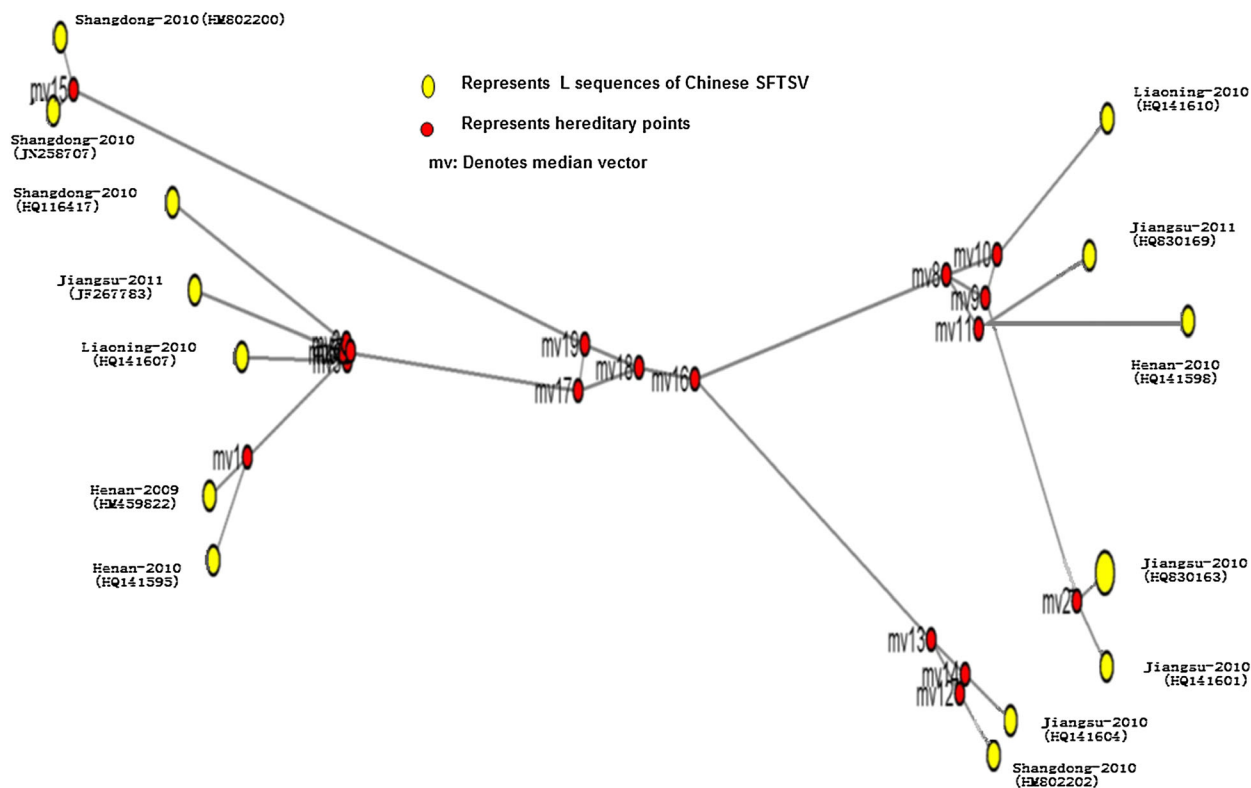

Figure 4. Median-joining network depicting the relationship of Chinese SFTSV strains and other Phlebovirus species in database. The common ancestor was inferred based on network of L gene mutations using the NETWORK 4.611 software reported by Bandelt et al. [68]

Figure 5. Geographic distribution of 571 SFTS confirmed cases and 59 deaths from Chinese SFTS surveillance in 2011, as processed in EPI information 3.5 software

57.6 years), 92.14% (598/649) of the reported cases were older than 35 years of age (35 ~ 80 years), whereas those younger than 30 years of age accounted for 3.70%, and those older than 80 years were only 2.47% (16/649). Farmers were the major population, accounted for 89.22% of infected cases. Overall, 31 deaths occurred in those aged 42–78 years (the average was 63 years old). Farmers accounted for 87.10% (27/31) of the total deaths (Figure 6). In addition, analysis of data showed that there was no obvious difference in regards of sex between confirmed cases (1:1.02) and deaths (1:1.07) of male to female participants ($p > 0.05$), which concord with previous reports from different areas [1,2,27].

Seasonal distribution

Data from highly affected provinces including Hubei, Henan, Jiangsu, and Zhejiang indicated that the epidemic season extended from early Summer to late Autumn with a peak of incidence through May–July [1,29,32]. On the basis of the weekly distribution across the nation, occurrence of 275 cases was reported from the 9th to 42nd week, with biphasic distribution. Since the first report on the 9th week, the cumulative cases gradually increased to the peak value at 17th–30th week with some obvious spikes, followed by short decline at 31st and 32nd weeks before burst to the second peak from the 33rd to 36th weeks (Figure 7). Seasonal occurrence may be attributed to the increased activity of the farmers such as harvesting tea, and cutting grasses, subsequently the possibility of more exposure to the potential vectors.

Host and vector

The ecological cycle of the SFTSV is not yet known, but it seems most likely that *Phleboviruses* involve arthropod vectors and mammalian hosts, including
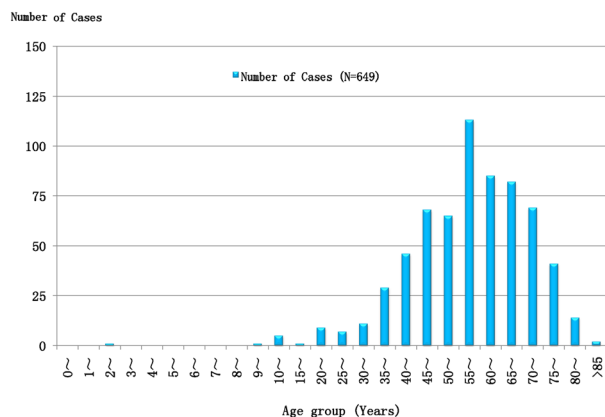
Number of Cases

Figure 6. Age distribution of 649 SFTS confirmed cases from Chinese SFTS surveillance in 2012. Note: The data were sourced from Chinese SFTS surveillance and processed in EXCEL 2010 and SPSS 17.0

cats, mice, hedgehogs, weasels, possums, and yaks. Humans appear to be an accidental host and play no essential role in the life cycle of SFTSV. Current research indicated that ticks, especially *Haemaphysalis longicornis*, were the major potential insect vector to transmit the virus to humans on the basis of the following evidence: (i) SFTSV has been detected from the ixodid ticks *H. longicornis* and *Rhipicephalus microplus* in the endemic regions [32]; (ii) the nucleic acid sequence of viruses isolated from ticks has 95~100% homology with the SFTSV isolated from patients and other mammalian hosts; (iii) about 52% of patients had a tick exposure history [36,37]; (iv) 30~70% of mammalian hosts, which

Figure 7. Weekly distribution of 275 SFTS confirmed cases in China during 2012. Note: The data were sourced from Chinese SFTS surveillance and processed in EXCEL 2010 and SPSS 17.0

likely have exposures to ticks, such as dogs and goats and cattle, had substantial antibody levels to SFTSV. On the other hand, there may be more potential vectors linked with SFTSV life cycles because 48% of patients had no tick exposure history [36,37], and the SFTSV positive rate was very low ($0.7 \sim 5.4\%$) in the tick population [1,2,37]. Viral RNA was not detected in any mosquito population so far [1,38–40], suggesting those mosquitoes, which are commonly found in endemic rural areas, are not potential vectors.

To ascertain the role of domestic animals as reservoir hosts for SFTSV, investigation of different animal species including sheep, cattle, pigs, dogs, and chickens in China showed that only a small fraction of animals (ranging from 1.7% to 5.3%) were found to carry low levels of viral RNA in their sera [37,41]. The viral isolates from the aforementioned species shared 95.4% identify with those of the patients and ticks [41].

Although rodents are known as reservoir hosts of *Bunyaviruses*, there is pronounced discrepancy on SFTSV in rodents. Results of studies on the occurrence of SFTSV in rodents indicated that SFTSV-antigen positive rate was about 4.31% (76/1762) in rats (as detected in heart, liver, spleen, lung, kidney, and brain tissue) captured from 18 Chinese provinces, as well as 11.4% (8/70) in Zhejiang province according to immunofluorescence methods [35,42]. On the other hand, Lu *et al.* could not detect SFTSV RNA in the sera of 81 rodents and 19 canines collected from Beijing [43]. In conclusion, SFTSV is a close epizootic emergency infectious disease in the hilly lands; however, there is not enough evidence to confirm which animals are reservoir hosts.
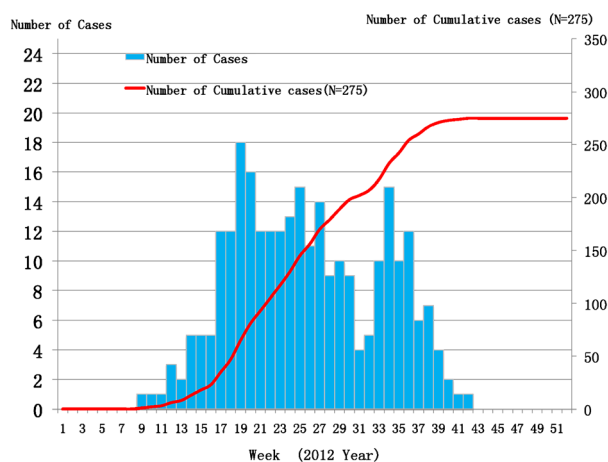
Serological epidemiology

Serological survey in patients and health population. The immune response of the patients varies according to the infection (first exposure versus reexposure) and clinical status (early, acute, convalescent phases), with no distinct pattern associated with the infection. The datasets from different geographic areas indicated that a considerable proportion of patients have showed a seroconversion to SFTSV during acute and convalescent phases of the illness [1,44], in the form of high levels of antibody titers. Of note, neutralizing antibodies to SFTSV is prone to increase [38] and persist.

Furthermore, reports of SFTSV-specific antibodies in healthy populations are sparse and

inconsistent. Whereas some studies failed to detect SFTSV-specific antibodies in healthy individuals from both endemic areas and nonendemic areas [1], other studies indicated SFTSV antibody positive rate 0.8 ~ 3.8% [37,38,44–46]. This discrepancy may be attributed to the season of sample collection, age and sex of the subjects, previous exposure or low level of infection, and/or detection method utilized in individual studies.

Serological survey in animals. Although molecular methods could detect viral RNA in the small fraction (1.7–5.3%) of samples from domesticated animals (goat, cattle, dog, and pig), poultry (chicken, duck, etc.), and rodents (rat, etc.), serological assays indicated the presence SFTSV-specific antibodies in high numbers of animals. In a study of small size (<500 samples), 47.7% of domesticated animals were positive for SFTSV antibodies, of which 36.7 ~ 83% of goat, 31.82 ~ 80% of cattle, 50.00% of hedgehogs, 6.40 ~ 55% of dogs, 2.00 ~ 6.00% of pigs, and 0.98 ~ 2.00% of chickens were positive according to the report from Hubei, Jiangsu, and Shandong province [37,38,44,45]. Serology survey of more than 3000 domestic animals showed that approximately 70% of sheep, 60% of cattle, 38% of dogs, 3% of pigs, and 47% of chickens were SFTSV antibody positive. Another large size investigation of 1454 rodents indicated that 3.03% of rats were SFTSV antibody positive in the main epidemic areas, of which *Apodemus agrarius* and *Rattus norvegicus* accounted for 52.27% and 20.45% among the positive rats, respectively [37]. Serological findings indicated controversy on the data from different regions, but there were some common features: the incidence of livestock infection was significantly higher than the incidence in poultry, humans, and rats. Therefore, it suggested that livestock plays an important role during the cycle of this novel virus transmission to human.

Transmission model. Until now, human exposure to ticks is one of the most suspected transmission routes of the virus, but some studies have provided evidence that SFTSV can be transmitted from person-to-person. The concept of person-to-person transmission was confirmed through several reports, where the secondary patients are likely to be infected with SFTSV via direct contact with blood of index patients [47], forming confined family and/or contact clusters [48–52]. Individuals in close contact with primarily patients but not exposed to index blood did not contract the illness. Complete genomic sequencing indicated that the viral strains isolated from the secondary patients were virtually identical to the sequence of the virus isolated from the index case [49,50]. Notably, the viral RNA could be detected in throat, urine, and fecal specimens of a substantial proportion of patients, including all fatal cases [53], throwing light on the potential role of body fluids and excreta of SFTSV patients for person-to-person transmission, posing a great challenge to the patients' companions and health care staff.

CLINICAL CHARACTERISTICS

Clinical symptoms

The clinical course of the SFTS patients could be divided into three major stages starting with fever, followed by multiorgan dysfunction and the convalescent stage. The incubation period ranges between 1 and 2 weeks, leading to fever stage, which ranges from day 1 to day 7 post-onset of illness. This stage is characterized, both in survivors and fatal cases, by flu-like symptoms, thrombocytopenia and leukocytopenia, and slight increase in serum biomarkers alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine phosphokinase, and creatine kinase isoenzyme. These symptoms may resolve after 1 week in survivors, and serum enzymes begin to decline toward normal levels and show signs of recovery after 2 weeks of the disease onset. Later, some cases moved to the multiorgan dysfunction stage, the serum viral load decreased in nonfatal cases but still remained high in fatal cases, while the platelet counts reverted to a normal value in nonfatal cases but continued to decline in fatal cases. The biomarker began to decline at approximately day 9 or day 11 in nonfatal cases but progressively increased in fatal cases. In the third stage (over 14 days), nonfatal cases could recover from the disease with most clinical parameters converting to normal. In contrast, the fatal cases showed the serum enzymes increasing accompanied with the main complication including then multiple organ failure, coagulation, shock, and acute respiratory distress syndrome, and so on. [27,54,55].

Review of clinical symptoms and signs reported in previous studies [1,2,36,55] for surviving and fatal cases, respectively, indicated fever (78.28%, 100.00%), anorexia (63.35%, 64.00%), fatigue (62.44%, 55.00%), body sores [52.71%, no data available (NA)], nausea (52.49%, 45.00%), vomiting (45.93%, 36.00%), diarrhea (45.70%, 27.00%), myalgia (44.12%, 18.00%), chills (41.63%, NA), abdominal pain (40.05%, 36.00%), coarse breathing sounds (38.46%, NA), dizziness (37.10%, NA), headache (36.43%, NA), enlargement of lymph nodes (34.16%, 18.00%), cough (28.73%, 18.00%), confusion (22.00%, 36.00%), sputum production (20.14%, NA), arthralgia (19.91%, NA), throat congestion (12.00%, 18.00%), conjunctive congestion (10.00%, NA), apathy (9.00%, 9.00%), petechiae (7.00%, 27.00%), coma (6.00%, 27.00%), slurred speech (6.00%, 9.00%), and skin rash (4.30%, NA) (Figure 8).

Diagnostic methods

Just like most *Phlebovirus* infections, the diagnosis of SFTSV is based on viral nucleic acid tests and serological detection. In general, viral nucleic acids can be tested in the acute phase serum of patients. One-step TaqMan real-time assays were applied widely in amplifying L, M, and S of SFTSV segments. The molecular techniques improved the detection of SFTSV. For example, Yang *et al.* [56] and Xu *et al.* [57] established a modified, low cost, and rapid visualized one-step RT loop-mediated isothermal amplification method for the detection of RNA from the SFTSV. A specific two-tube multiplex real-time RT-PCR assay for the detection of SFTS, along with other closely related viruses revealed high sensitivity (10 copies/µL) with no cross reactivity between tested viruses indicating the high specificity of the assay [58]. Furthermore, a highly sensitive one-step real-time RT-PCR method using a minor groove binding probe was developed for detection and quantitation of SFTSV by Li *et al.* [59]. The unique assay of Cui *et al.* [60] involved a nearly instrument-free, simple molecular method that incorporates RT-cross-priming amplification coupled with a vertical flow visualization strip for rapid detection of SFTSV. The RT-cross-priming amplification coupled-vertical flow assay targets a conserved region of the M segment of the SFTSV genome and has a limit of detection of 100 copies per reaction, with no cross–reaction with other vector-borne *Bunyaviruses* and bacterial pathogens. The sensitivity and specificity of the assay were 94.1% and 100.0%, respectively. Moreover, potential detection limit of 10 viral RNA copies/µl was achieved using quantitative real-time PCR technique utilizing primer–probe sets to detect L, M, and S genes of SFTSV, with 98.6% sensitivity and over 99% specificity [61]. The improved molecular assay was 1000 times more sensitive than the conventional PCR.

Nonetheless, serology testing methods including the in-house Mac-EIA assay, indirect EIA assay, and double-antigen sandwich EIA assay have been also performed in testing virus specific IgM, IgG, and total antibodies in serum samples, respectively. Improvement of EIA system utilizing N of SFTSV was developed by Jiao *et al.* [62] with high sensitivity and specificity. Additionally, the conventional
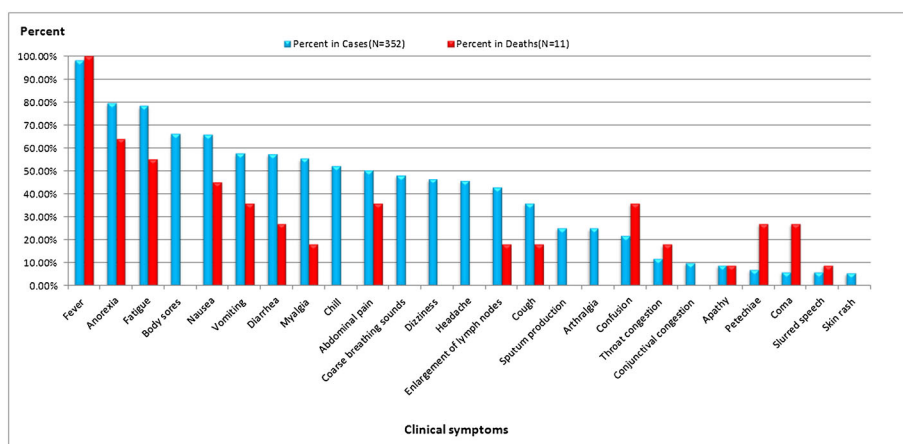

Figure 8. Clinical symptom percentages among 352 SFTS cases and 11 fatal cases from China. Note: The data were sourced from References [1], [38], and [40] and processed in EXCEL 2010 and SPSS 17.0

methods such as indirect immunofluorescence and the serum neutralization assays are still the key, however these methods are costly and need more time and well-trained personnel.

CONCLUSIONS

This review highlights the virology, epidemical, and clinical features of SFTS caused by SFTSV. SFTSV belongs to the third group of *Phlebovirus*, with transmission by the tick (especially, *H. longicornis*) and blood/body fluids contact. The fatality rate of the disease is about 10–15% because of multiple organ failure. SFTS has been reported in villagers and farmers older than 50 years of age in rural areas of North, East, and Central China from March to November with peak on May–July. Many domesticated animal, especially goats and cattle might be potential amplifying hosts.

There are still many questions that need clarification. First, other pathogens presented similar symptoms to SFTSV, such as *Anaplasma phagocytophilum*, *Ehrlichia* species, and *hemorrhagic fever virus* should be considered, because SFTSV RNA cannot be detected in many patients [63–66]. Furthermore, more studies related to pathogenesis of the disease should be performed in detail using experimental models of different species from murine to higher

nonhuman primates. Second, the SFTSV ecologic life cycle (vector–host–human) should be clarified, including potential additional vectors such as mosquitoes, acaruses, and sandflies. It also needs to be determined whether domestic animals play an important role as reservoir. Except by contaminated blood and secretions, it remains unknown whether airborne transmission by aerosols, mother-to-child transmission, or fecal–oral transmission might occur in some cases [67]. Third, the endemic areas are expanding and, without specific antiviral treatment, there is an urgent need for the production of an efficient and safe SFTSV vaccine(s) for those at high-risk populations [26] and for the most susceptible animals, which might help to prevent and control this novel disease in the world.

CONFLICT OF INTEREST
The authors have no competing interests.

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