

A cluster of person-to-person transmission cases caused by SFTS virus in Penglai, China

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Abstract

An emerging infectious disease, severe fever with thrombocytopenia syndrome (SFTS), was identified to be associated with a novel SFTS virus (SFTSV). Transmission of the disease among humans has been described, but clinical impact factors and transmission mechanisms still need further study. An outbreak of person-to-person transmission of SFTS in a cluster of nine patients that occurred in an SFTS endemic area, Penglai County, Shandong province, China, was investigated. We found that the onset date of all eight secondary SFTS patients ranged from 7 to 13 days after exposure to the corpse of the index patient, and clinical incubation time was mostly focused on 9–10 days ($n = 6$). The two dead patients, including the index patient and one secondary infected patient, presented unusually high levels of viral load ($6 \times 10^{8-9}$ copies/mL), low levels of platelets count ($<55 \times 10^9/L$), and significant increase of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and creatine kinase values in the second week, and died on day 10 or 11 after disease onset. Genetic sequencing revealed 100% homology among virus strains isolated from the index patient and five secondary patients. Risk factors assessment of the person-to-person transmission revealed that the major exposure factor was blood contact without personal protection equipment. Information from this study provided solid references of SFTS incubation time, clinical and laboratory parameters related to SFTS severity and outcome, and biosafety issues for preventing person-to-person transmission or nosocomial infection of SFTSV.

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Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by SFTS virus (SFTSV), a newly identified pathogenic member of the *Phlebovirus* species

in the family *Bunyaviridae* [1,2]. The major clinical signs and symptoms of SFTS include high fever, gastrointestinal symptoms, thrombocytopenia, leukocytopenia, and multi-organ dysfunction with an average case-fatality rate of 10–16%. SFTS has been reported in at least 17 provinces in the central, eastern, and northeastern regions of the People's Republic of China. Similar disease has also been reported in the United States, South Korea, and Japan, showing the risk of disease spread [3–6]. SFTS has become a severe threat to public health.

Ticks of the species *Haemaphysalis longicornis* were supposed to be the vector of SFTSV infection to humans [1], as well as sheep, cattle, and dogs, which, with relatively high infection rates of SFTSV, may act as amplifying hosts for the virus during

the epidemic season [7]; most patients reported none or unclear tick-bite or animal hosts-contact history before their onset of the disease [8]. The infection cases of SFTSV commonly showed a sporadic distribution, and clustered cases occur less frequently [9]. Transmission of SFTS among humans has been described [10–12], but clinical impact factors and transmission mechanisms still need to be more studied.

From the end of August to the middle of September, there was an outbreak of a cluster of nine suspected SFTS cases in Penglai County, Shandong province, an endemic area of SFTS in China, with two deaths, including the index patient and one secondary patient. Here we report the results of the investigation into this cluster of SFTS patients.

Methods

Patients and sample collection

During August 25–September 17, 2013, a cluster of nine SFTS-suspected patients was identified in Penglai County, Shandong province. A 66-year old female farmer, who first had onset of illness on August 25, was admitted to a local village clinic, then transferred to municipal hospital A on August 30, and hospital B on September 2, and she died on September 4. The patient was defined as the index patient. The other eight cases in the cluster were: Case 1, a 62-year old woman who was the sister-in-law of the index patient; Case 2, the index patient's 41-year-old younger son; Cases 3 and 4, both village residents; Cases 5 and 6, the index patient's brother and sister-in-law; Case 7, a 71-year old female neighbour of the index patient; and Case 8, the index patient's husband. All eight of these patients had onset of the disease from September 11 to September 17, and a series of serum samples were collected from the diagnosis of the disease and during the course of the illness.

Epidemiologic investigations

Besides the eight SFTS-suspected patients with similar clinical presentations, all contacts of the index patient included 87 hospital physicians and nurses; five patients who were staying in the same sickroom with the index patient; and 26 persons from the village who visited the room where the corpse was kept and participated in the funeral of the index patient. All of these individuals were interviewed by telephone or in person with the use of a standard questionnaire. The questionnaire requested the following information: general information such as age, gender, and profession; exposure to ticks and wild animals; contacts with the index patient—e.g. when, where, and how they had contact; exposure to the index patient's skin, blood, and respiratory secretions; whether they washed their hands after exposure. Healthcare workers were asked about their use of personal

protective equipment (PPE), such as mask, gloves, and gown. Medical records of all nine SFTS patients were reviewed for the time of onset and progression of the illness.

Laboratory analysis

Specimens from the nine clustered patients were subjected to both nucleic acid and serological laboratory test. The viral RNAs were tested by real-time reverse transcriptase polymerase chain reaction as described previously [13], and the quantities of viral RNA copies in the samples were calculated. Virus isolation, gene sequencing, and specific IgM and IgG antibody detection by ELISA were also performed as previously described [14].

Ethical review

According to the medical research regulation of the National Health and Family Planning Commission of China, the investigation was reviewed and approved by the ethics committee of China CDC (Chinese Centers for Disease Control and Prevention), which uses international guidelines to ensure confidentiality, anonymity, and informed consent. Informed consent was obtained from all study participants.

Results

Index patient

The index patient was a 66-year-old woman. On August 25, 2013, she presented to the local village clinic with fever (38.5°C), abdominal pain, nausea, and vomiting without known reason. She was treated with ribavirin and cephalothin at the local village clinic. After 5 days, the symptoms were getting worse and she was admitted to municipal hospital A on August 30 with diagnosis of “abdominal pain of unknown origin.” Laboratory tests performed on admission revealed leukopenia (white blood cells count $2.01 \times 10^9/L$), thrombocytopenia (platelets count $96 \times 10^9/L$), elevated liver-associated enzyme levels (aspartate aminotransferase (AST) 608U/L; alanine aminotransferase (ALT) 322U/L; lactate dehydrogenase (LDH) 581U/L), and coagulopathy (activated partial thromboplastin time (APTT) 46.7 seconds). No improvement was observed after clinical treatment; her condition continued to decline. The patient was transferred to municipal hospital B on September 2 and diagnosed as a suspected SFTS case. In the evening, her condition deteriorated rapidly, with short breath and limb tremor. She was then intubated and mechanically ventilated, and received plasma and antiviral therapy. Laboratory values indicated impaired liver and kidney function (white blood cells $25.04 \times 10^9/L$, platelets $54 \times 10^9/L$, AST 2010 U/L, ALT 892 U/L, LDH 3608 U/L, APTT 95 seconds, creatine kinase (CK) 2061 U/L, CK-MB 180 U/L), and her symptoms further

developed to shock and disseminated intravascular coagulation. In the early morning of September 4, the patient was taken home with tracheal intubation and intravenous needles, and half an hour after arriving home, she died. This was at day 10 after the onset of disease, and the dead patient had history of a tick bite 1 month before.

Epidemiologic findings

Seven to 13 days after the index patient's death, eight persons who had close exposure to the index patient presented typical SFTS symptoms and were diagnosed as secondary patients. Interviews were conducted with the eight secondary clustered patients and a review of medical records reconstructed of the timeline of relevant exposures and the onset of illness in the cluster (Fig. 1, Table 1).

Case 1, the index patient's sister-in-law, took care of the dead body for 3 days and had to wipe off the blood from the corpse. During the close exposures to the index patient, she had no protection, and on September 11, she had disease onset with a severe fever of 39.5°C and died on September 20.

Case 2, the younger son of the index patient, took care of his mother when she was in the hospital and was involved in removing the tracheal tube after his mother's death. He had unprotected contacts with the index patient. On September 13, he became ill with high fever (38.5°C).

Case 3 and Case 4 were residents of the village where the index patient lived. They participated in funeral matters and had direct blood contact with the dead body. They both had onset of the disease 7 days after exposure.

Case 5 and Case 6, the index patient's brother and sister-in-law, took care of the dead body. Case 5 participated in tracheal tube removing and Case 6 changed the index patient's clothes after she died at home. They both had unprotected blood contact with the index patient and developed fevers on September 13 and 14, respectively.

Case 7 was a neighbour of the index patient who helped transport the corpse and developed a fever of 39.1°C on day 8 after exposure.

Case 8, the index patient's husband, took care of his wife when she was in the hospital, was involved in withdrawing intravenous needles, and participated in funeral matters. He had disease onset on September 18.

Clinical characteristics

All nine patients in the cluster developed a fever ranging from 38.2°C to 39.5°C, and they also had general SFTS clinical features, including gastrointestinal symptoms, thrombocytopenia, and leukocytopenia. Two deaths, including the index patient and Case 1, both had skin silt ecchymosis, limb tremor, and respiratory failure, and died of disseminated intravascular coagulation. As is shown in Table 2, laboratory tests showed high levels of liver enzymes (including ALT, AST), myocardial enzymes (including LDH, CK), blood urea nitrogen, and creatinine in the first week after disease onset, which might indicate impaired liver, heart, and kidney functions, respectively. In the surviving SFTS patients, the serum tissue enzymes reached maximal values at days 9–11 and declined thereafter, and the levels reverted to almost normal at the end of the second week. The white blood cell and platelet values also reverted to the normal range in survivors. An important factor was that for the two dead patients, parameters of ALT, AST, and LDH appeared to progressively rise and reached unusually high levels, and they had significantly elongated APTT. Consistent with the largely reduced platelet count, the index patient also showed a high level of CK value accompanied with a quite low level of fibrinogen, which represented a largely impaired coagulation function.

Laboratory data

By real-time reverse transcriptase polymerase chain reaction assay, the SFTSV viral RNA, including L, M, and S gene

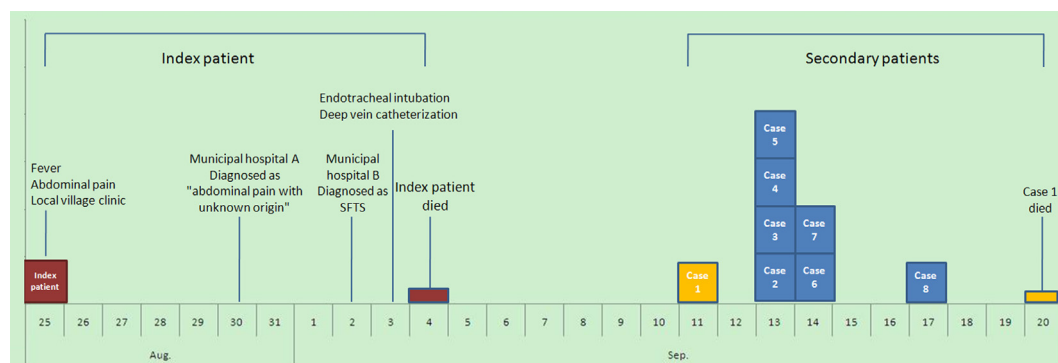


FIG. 1. Timeline of disease onset dates for a cluster of nine SFTS (severe fever with thrombocytopenia syndrome) patients. Epidemic curve shows progression of critical symptoms during the index patient's illness, and the onset of SFTS in the eight clustered patients, of which the onset date ranged from 7 to 13 days after exposure to the dead body of the index patient, and clinical incubation time was mostly focused on 9–10 days ($n = 6$).

TABLE 1. Epidemiologic features of a cluster of 9 patients with SFTS

	Index Patient	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Sex	Female	Female	Male	Male	Male	Male	Female	Female	Male
Age, years	66	62	42	58	60	71	61	71	71
Occupation	Farmer	Farmer	Farmer	Farmer	Farmer	Farmer	Farmer	Farmer	Farmer
Tick-bite history	Yes	No	No	No	No	Yes	No	No	No
Previous diseases	Uterine myoma	Healthy	Healthy	Healthy	Healthy	Healthy	Type 2 diabetes	Healthy	Healthy
Date of onset	Aug 25, 2013	Sep 11, 2013	Sep 13, 2013	Sep 13, 2013	Sep 13, 2013	Sep 13, 2013	Sep 14, 2013	Sep 14, 2013	Sep 17, 2013
Incubation time, days	NA	7	9	9	9	9	10	10	13
Length after onset, days	11	10	21	18	15	17	18	27	20
Outcome	Death	Death	Discharge	Discharge	Discharge	Discharge	Discharge	Discharge	Discharge

SFTS = severe fever with thrombocytopenia syndrome.

fragments were detected in the sera collected from the index patient and the other eight clustered patients. It is noticeable that the sera collected from the two dead patients contained relatively high levels of SFTS viral load, which reached about 6.04×10^9 copies/mL and 6.92×10^8 copies/mL, respectively. These numbers were much higher than viral copies detected in the other seven clustered patients' sera, which ranged from 10^3 to 10^4 copies/mL (Table 2). The SFTS virus-specific IgM and IgG antibodies in serum samples collected in the acute phase were demonstrated by ELISA. Elevated IgM and IgG antibodies against SFTSV showed in serum samples of all seven surviving patients. The two patients who died, however, had minimal or undetectable levels of virus-specific IgM antibodies.

We further isolated the SFTSV strains from the index patient and five of the secondary patients. Phylogenetic trees based on complete viral genomic sequences of L, M, and S segments showed that the three SFTSV isolates were clustered together, with 100% amino acid homology for all three segments, and also

closely related to other SFTSV strains. The high homology of the viral isolates from the index patient and five affected patients provided genetic evidence for the epidemic findings on the possible transmission of SFTS.

Risk factors of person-to-person transmission of SFTSV

To assess risk factors of this person-to-person transmission of SFTSV, we collected information on all 126 individuals who had exposure to the index patient from onset of the illness to cremation of the corpse. As shown in Table 3, of the total 126 exposed individuals, 41, including 28 healthcare workers, eight family members, and five villagers, reported direct blood contact with the index patient by taking care of her before or after her death; 11 of them got infected and eight developed the disease (19.5%). Twenty-five individuals reported exposure to the urine and faeces of the index patient, while only two developed the disease (8.0%). Furthermore, the protective effect of PPE was evaluated. Of the total 126 contacts, 86 were

TABLE 2. Clinical symptoms and laboratory findings of a cluster of 9 patients with SFTS

	Index Patient	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	
Clinical symptoms										
Fever	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
Nausea	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
Vomiting	Yes	No	Yes	Yes	No	No	Yes	Yes	Yes	
Myalgia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
Haematuria	Yes	No	No	No	No	No	No	No	No	
Skin silt ecchymosis	Yes	Yes	No	No	No	No	No	No	No	
Tremors	Yes	Yes	No	No	No	No	No	No	No	
Meningitis pathological character	Yes	Yes	No	No	No	No	No	No	No	
Laboratory findings										
PLT count ($\times 10^9$) ^a	96/54	54/31	82/169	139/237	83/194	50/262	54/263	178/34	55/168	
WBC count ($\times 10^9$) ^a	2.01/25.04	1.5/4.18	2.25/3.87	1.98/5.98	1.29/4.36	1.73/4.74	2.81/4.65	1.83/4.15	2.60/6.30	
ALT (U/L) ^a	322/892	36/468	61/44	88/26	27/36	121/23	62/17	28/269	35/107	
AST (U/L) ^a	608/2010	118/2607	65/38	95/16	33/38	283/19	101/17	33/289	37/45	
LDH (U/L) ^a	581/3608	465/3506	193/180	256/240	189/267	473/223	275/203	185/1140	206/205	
CK (U/L) ^a	172/2061	176/391	81/37	613/108	430/66	988/39	134/41	78/297	142/34	
Creatinine (U/L) ^a	53.3/40.7	69.0/199.0	70.2/54.9	75.0/60.2	70.4/53.6	84.3/63.6	55.2/48.0	59.0/47.7	66.6/52.3	
APTT (s) ^a	46.7/95.0	54.1/85.5	38.3/32.1	34.3/23.9	36.1/31.4	46.5/26.4	37.3/20.7	49.9/60.1	36.8/37.5	
Fibrinogen (g/L) ^a	1.72/0.59	2.19/1.56	2.43/2.06	1.56/2.31	2.38/2.95	1.83/2.73	1.72/2.15	2.07/2.26	2.60/2.74	
Proteinuria	+++	NT	+++	-	-	+++	+++	+++	++	
Haematuria	++	NT	+	-	-	+	+	-	-	
Virus isolation	Yes	Yes	No	No	Yes	Yes	Yes	No	Yes	
Viral RNA (copies/mL)	6.04×10^9	6.92×10^8	6.69×10^3	8.45×10^3	9.02×10^3	8.70×10^3	8.75×10^3	1.55×10^3	2.96×10^4	
IgM antibody	-	-	+	+	+	+	+	+	+	
IgG antibody	-	-	+	+	+	+	+	+	+	

-, negative; +, weak positive; +++, strong positive; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; NT, not tested; PLT, platelets; SFTS, severe fever with thrombocytopenia syndrome; WBC, white blood cells.

^aThe two numbers represent laboratory value of samples collected in the first and second week after disease onset, respectively.

TABLE 3. Risk factors assessment of person-to-person transmission of SFTSV

	Close contacts, n		Attack rate, %	Relative risk (95% CI) ^a	p ^b
	Overall	Developed secondary case			
Blood					<0.001
Exposed	41	8	19.5		
Unexposed	85	0	0		
Urine and faeces				1.377 (0.261–7.269)	1.000
Exposed	25	2	8.0		
Unexposed	101	6	5.9		
PPE					<0.001
Did not use	40	8	20.0		
Use	86	0	0		

PPE, personal protection equipment; SFTSV, severe fever with thrombocytopenia syndrome virus.
^aInfinite or not able to be calculated.
^bContinuity correction χ^2 tests.

with protective equipment. All eight secondary patients were unprotected when exposed to the index patient. Continuity correction χ^2 tests showed that blood contact was the most likely mode of transmission ($p < 0.001$) and PPE could effectively protect contacts from infection ($p < 0.001$, Table 3).

Discussion

Here, we presented a cluster of nine SFTS patients in China under the SFTS surveillance program. The index patient was diagnosed for SFTS 8 days after disease onset because fever and gastrointestinal symptoms are generally the first to appear, and can be easily confused with haemorrhagic fever with renal syndrome and human anaplasmosis, and may lead to misdiagnosis, and then affect the therapy and prognosis of the disease. Thus, the clinical differential diagnosis of SFTS is of great importance. We were more inclined to consider that the unique disease status of the index patient and a lack of precaution contributed to this sporadic person-to-person transmission. Seven to 13 days after the index patient's death, eight persons who had close exposure to the index patient's dead body presented typical SFTS symptoms, and clinical incubation time was mostly focused on 9–10 days.

It is notable that the clinical futures and laboratory tests showed that the two dead patients presented unusually high levels of ALT, AST, and LDH, and had significantly elongated APTT. The index patient also showed a largely reduced platelet count and a high CK value, accompanied with a quite low level of fibrinogen, which indicated a largely impaired coagulation function. The virus copies were also extremely high when they died. But in the surviving SFTS patients, the serum tissue enzymes reached maximal values at day 9–11 and declined

thereafter; the levels almost reverted to normal at the end of the second week. Therefore, the second week was critical in the course of the disease, and these disease-severity-related parameters might be used as indicators to determine the prognosis of SFTS.

After the index patient's death, the tracheal tube was removed and intravenous needles were withdrawn from the corpse; there was still bleeding from the dead body, which indicated a severe thrombocytopenia of the patient. Besides, the genus *Phlebovirus* was reported to remain infectious for a long time period of 40 days in wet conditions at room temperature. Therefore, we postulated that the index patient worked as a powerful source to spread virus to people having close contact with her. We also noticed that SFTSV nucleic acid testing in serum samples of three contacts were positive, but they had no clinical symptoms, suggesting the possibility of latent infection of SFTS virus [15].

A probable route for this person-to-person spread of SFTSV was direct contact transmission. The eight secondary patients who had unprotected close contact with the index patient's dead body surface or blood and other secretions successively became ill, and one secondary patient died. After epidemiological follow-up, no patients with SFTS were identified in those who had been exposed to the patients but with protection. Risk factors assessment of the person-to-person transmission revealed that the major exposure factors were close contact, in particular, contact with blood ($p < 0.001$). The possible mechanisms for this direct transmission between persons may be through open wounds, impaired skin, or through mucosa of the conjunctive, oral, or nasal cavity. Among these eight secondary patients, six had contact with the index patient only after death, indicating that the SFTSV-infected blood of the dead patient, in whom the level of virus copies was extremely high, may remain infectious even after patient death. In addition, regarding the healthcare workers, almost all that were exposed to the index patient or secondary patients were wearing PPE and none got infected. Risk factors assessment showed that PPE could effectively protect contacts from infection ($p < 0.001$).

The lessons of person-to-person transmission of SFTSV we learned from this study provided solid references of SFTS incubation time, clinical and laboratory parameters related to SFTS severity and outcome, and biosafety issues for preventing person-to-person transmission or nosocomial infection of SFTSV.

Transparency declaration

The authors declare that they have no conflicts of interest.

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