

Clinical and Epidemiological Study on Severe Fever with Thrombocytopenia Syndrome in Yiyuan County, Shandong Province, China

Feng Cui, Hai-Xia Cao, Ling Wang, Shou-Feng Zhang, Shu-Jun Ding, Xue-Jie Yu,* and Hao Yu

Zibo Municipal Center for Disease Control and Prevention, Zibo City, Shandong Province, China;

Yiyuan County Center for Disease Control and Prevention, Yiyuan, Shandong Province, China;

Shandong Province Center for Disease Control and Prevention, Jinan, Shandong Province, China; School of Public Health, Shandong University, Jinan, People's Republic of China; Department of Pathology, University of Texas Medical Branch, Galveston, Texas

Abstract. Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease in China. Polymerase chain reaction and enzyme-linked immunosorbent assay were used to detect SFTS virus (SFTSV) and *Anaplasma phagocytophilum* in previous clinically diagnosed human anaplasmosis patients and SFTS patients. A serosurvey for SFTSV infection was also conducted on healthy persons and animals in Yiyuan County in Shandong Province of China. Among 21 patients SFTSV was detected in 17 (81%) however *A. phagocytophilum* was not detected in any of the patients. The seroprevalence rate of IgG antibody to SFTSV antigens was 1.3% (1 of 78) in healthy persons, 95% (19 of 20) in goats, 50% (1 of 2) in dogs, 0% in cattle (0 of 21), and rats (*Rattus norvegicus*) (0 of 35). The conclusion of this study was that co-infection of SFTSV and *A. phagocytophilum* are rare in SFTS patients and goats might play an important role in transmission of SFTSV.

INTRODUCTION

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by SFTS virus (SFTSV), a *Phlebovirus* in the family *Bunyaviridae*.¹ The SFTS is a severe disease with a case fatality rate of ~12%; human cases have been reported in seven provinces in northeast and central China.^{1,2} The clinical symptoms and laboratory findings in SFTSV patients include fever, weakness, thrombocytopenia, leukopenia, abnormal coagulation times, elevated serum hepatic enzymes, and occasionally hemorrhagic manifestation in some fatal patients. The SFTSV is believed to be tick transmitted, because the virus has been detected in *Haemaphysalis longicornis* ticks in proximity to the patients' home.¹ The disease can also be transmitted from person to person through close contact with an infected patient's blood or mucous.^{3,4} In retrospect, SFTS-like cases have been reported in Yiyuan County, Shandong Province, China since 2004. During the period from 2007 to 2010, patients with fever, thrombocytopenia, and leukopenia were clinically diagnosed and treated as human anaplasmosis according to the Guideline of the Ministry of Health of China without etiological evidence.⁵ In 2010, SFTSV was isolated and identified as the true etiology of the so-called "human anaplasmosis" in China and the disease was renamed as severe fever with thrombocytopenia syndrome SFTS.¹ However, for some physicians the etiological agent of SFTS was changed from *Anaplasma phagocytophilum* to *A. phagocytophilum* and SFTSV co-infection. We retrospectively analyzed SFTS cases clinically diagnosed as "human anaplasmosis" during 2008 and cases clinically diagnosed as SFTS during 2010 in Yiyuan County to determine whether *A. phagocytophilum* was involved in SFTS. We also conducted a serosurvey on humans and animals living in endemic areas.

MATERIALS AND METHODS

The research protocol was approved by the Human Bioethics Committee of the Zibo Municipal Center for Disease

Control and Prevention (Zibo CDC) and all participants or a member of the patient's family provided written informed consent. The animal research protocol was also approved by the Zibo CDC Institutional Animal Care and Use Committee.

Case definition. Suspected SFTS cases were defined as sudden onset of febrile illness with body temperature $\geq 38^{\circ}\text{C}$, leukopenia and/or thrombocytopenia without any known blood system disease or other known infectious or chronic diseases. Confirmed SFTS patients were polymerase chain reaction (PCR) positive and/or had a 4-fold increase in antibody titer to SFTSV between acute and convalescent samples. We included patients who were clinically diagnosed as "human anaplasmosis" to determine whether these patients were infected with SFTSV and/or *A. phagocytophilum*.

Patient sample collection. All patients' blood samples and clinical information were submitted to Zibo CDC by two local hospitals in Yiyuan County for diagnostic study of human anaplasmosis in 2008 and SFTS in 2010. Acute sera were obtained within 1 week of onset of illness and convalescent sera were obtained 3–4 weeks after hospitalization.

Serosurvey of healthy populations. Study sites were five villages in Yiyuan County where SFTS cases were reported in the past and eight villages in which no SFTS were reported. We obtained blood samples from 78 healthy volunteers in the villages and the volunteers represented the age groups 10–29, 30–49, and ≥ 50 years of age, with at least 20 persons in each age group. All volunteers denied having SFTS symptoms previously. A standardized questionnaire was used to obtain information on age, sex, history of prior illness, tick exposure, and occupation of each participant. Five milliliters of blood sample was collected by venipuncture by basilic vein from each volunteer.

Serosurvey of vertebrate animals. The study sites were two villages in Yiyuan County where SFTS cases were diagnosed. Blood samples were collected from cattle, dogs, and goats of patients' families and/or their neighbors with their permission. Blood (10 mL) was obtained from each cow, goat, or dog by venipuncture by a jugular vein. Rats (*Rattus norvegicus*) were captured by snap traps inside and outside the patients' houses and rats were dissected in the field to collect tissue samples (spleen, kidney, liver, and lungs). A small amount of blood was also obtained from each rat using

*Address correspondence to Xue-Jie Yu, Department of Pathology, University of Texas Medical Branch, 301 University Blvd., Keiller Bldg. Galveston, TX 77555-0609. E-mail: xuyu@utmb.edu

a filter paper to absorb blood remaining in the heart. Serum was obtained by centrifugation and frozen until use.

Quantitative real-time PCR (qPCR). Acute sera of patients were tested for presence of SFTSV RNA genomes by quantitative PCR (qPCR) amplification of L-, M-, and S-segments as previously described⁶; if two or three segments were amplified from a patient's blood sample, the sample was considered as SFTSV positive. The RNA was extracted using QIAamp Viral RNA Mini Kit (Shanghai, China).

Enzyme-linked immunosorbent assay (ELISA). A double antigen sandwich ELISA kit was used to detect antibodies to SFTSV including immunoglobulin G (IgG) and IgM as described previously.⁷⁻⁹ Undiluted human sera (50 µL) was added to a well of the plate and was incubated for 30 minutes at 37°C to allow SFTSV antibodies to bind to the nucleoprotein of SFTSV antigen on the plate. After washing, horseradish peroxidase-labeled recombinant SFTSV nucleoprotein was added to the plate and detected by substrates for the horseradish peroxidase enzyme. The plate was read for absorbance at 450 nm wavelength. When the absorbance of the serum sample was three standard deviations above the mean optical density at 450 nm of the persons sampled, it was considered to be positive for SFTSV antibody. The same ELISA kit and same criterion were used for animal samples.

All SFTS patients' sera were also tested for *A. phagocytophilum* IgG and IgM antibody by indirect immunofluorescence assay and were tested for *A. phagocytophilum* genomic DNA by PCR as described previously.⁵

RESULTS

Patients. The blood samples of 10 patients who were clinically diagnosed as human anaplasmosis in 2008 and 11 patients who were clinically diagnosed as SFTS in 2010 were submitted to Zibo CDC by the Yiyuan County Hospital. The indirect fluorescent antibody detection of *A. phagocytophilum* IgG and IgM antibody and PCR amplification of the 16S RNA gene of *A. phagocytophilum* indicated that none of these patients were positive to *A. phagocytophilum*, the causative agent of human anaplasmosis. We also tested for SFTSV RNA by qPCR and SFTSV antibodies by ELISA on these 21 patients' blood samples. Fifteen of the 21 acute sera were confirmed to be infected with SFTSV by qPCR and 13 of 20 patients with acute and convalescent sera showed ≥ 4-fold increases for SFTSV IgG. Combining the PCR results and serological results together, 17 of the 21(81%) patients were confirmed to be infected with SFTSV Table 1.

Clinical laboratory data was available for 16 of the 17 laboratory-confirmed SFTS cases. Clinical manifestations obtained from these 16 laboratory-confirmed patients are summarized in Table 2. Epidemiological analyses indicated that 2 weeks before the onset of illness all patients had

TABLE 1
Laboratory detection of SFTSV in clinically diagnosed SFTS cases*

Year	Acute sera		Convalescent sera		Confirmed cases	Positive rate
	No. of patients	Positive qPCR	No. of patients	No. of patients with 4-fold increase		
2008	10	8	9	6	8	80.0%
2010	11	7	11	7	9	81.8%
Total	21	15	20	13	17	81.0%

* qPCR = quantitative polymerase chain reaction.

TABLE 2

Clinical symptoms of confirmed patients*

Symptoms	Number of persons (N = 16)	(%)
Fever	16	100
Fatigue	8	50
Lymphadenopathy	8	50
Diarrhea	8	50
Vomiting	7	43.8
Myalgia	7	43.8
Dizziness	5	31.3
Nausea	5	31.3
Abdominal pain	4	25
Joint pain	4	25
Chills	3	18.8
Headache	3	18.8
Abdominal distension	3	18.8
Loss of appetite	2	12.5
Laboratory results		
Elevated AST	15	93.75
Leukopenia (< 4,000)	13	81.5
Elevated ALT	11	68.75
Thrombocytopenia (< 150)	10	62.5

* AST = aspartate aminotransferase; ALT = alanine aminotransferase.

worked in fields harvesting grasses, herding goats, or lived in the areas that were infested with ticks, however, only one person recalled tick bite before the onset of illness.

The patients ranged from 27 to 82 years of age with a median age of 67 and most patients (88%, 15 of 17) were farmers Table 3. All patients were healthy before onset of illness except for two patients. Before onset of illness one patient had a history of hypertension and coronary heart disease and one patient had a history of asthma.

Seroprevalence of SFTSV. Sera were collected from 78 volunteers who denied having previous SFTS symptoms. The ELISA showed that only one person was IgG positive to SFTSV among 78 persons. The positive person was a 29-year-old male country doctor. The ELISA showed that 95% (19) of goats and 50% (1) of dogs were positive to SFTSV IgG. None of the 21 cattle tested positive to SFTSV IgG and none of the 35 rats tested positive by indirect fluorescent antibody or by qPCR.

DISCUSSION

In China SFTS was named after isolation of SFTSV from patients in 2010¹; SFTS was reported as an unknown infectious

TABLE 3
SFTSV positive patients' epidemiological data

No.	Age	Sex	Occupation	Disease history
1	27	Male	Official	Healthy
2	40	Male	Farmer	Healthy
3	45	Male	Farmer	Healthy
4	50	Female	Farmer	Healthy
5	51	Male	Farmer	Healthy
6	54	Male	Farmer	Healthy
7	59	Female	Farmer	Healthy
8	62	Female	Farmer	Healthy
9	67	Male	Farmer	Healthy
10	69	Female	Farmer	Asthma
11	69	Male	Official	Healthy
12	70	Male	Farmer	Healthy
13	73	Female	Farmer	Healthy
14	75	Female	Farmer	Hypertension
15	78	Female	Farmer	Healthy
16	78	Male	Farmer	Healthy
17	82	Male	Farmer	Healthy

disease before 2007 and as human anaplasmosis from 2007 to 2010. In 2008 surveillance for human anaplasmosis was started in Yiyuan and 22 cases of "human anaplasmosis" were reported in 2008 and 21 cases in 2009. Subsequently, 42 SFTS cases were reported in 2010. We analyzed 10 cases of "human anaplasmosis" from 2008 and 11 SFTS cases from 2010 whose blood samples were available. We showed that 81% of the cases were positive to SFTSV. However, no patient was infected by *A. phagocytophilum*. Because *A. phagocytophilum* was considered to be the causative agent of SFTS in China before the discovery of SFTSV in 2010, even now tetracycline is routinely prescribed to SFTS patients by many physicians because of a concern of *A. phagocytophilum* co-infection. On the basis of our results, treatment of SFTS patients with tetracycline is not justified unless co-infection with *A. phagocytophilum* can be established.

We also confirmed subclinical SFTSV infection in healthy people. In addition, we showed there is a high SFTSV antibody rate in goats, but no antibody in cattle. Coincidentally, a 2011 serosurvey in Yiyuan County showed that a SFTSV seroprevalence rate of SFTS in healthy persons was 0.8% and the SFTSV antibody in goats (83%).⁸ A serosurvey in Jiangsu Province found an SFTSV antibody positive rate of 0.9% in healthy persons, 57% in goats, 32% in cattle, 6% in dogs, 5% in pigs, and 1% in chickens.⁹ The discrepancy in the seroprevalence in cattle between our study and the previous study might be caused by the fact that the cattle in Yiyuan were mainly kept in pens. Goats were probably exposed to tick bites more often than cattle, because goats are herded in the hills. The clinical manifestations of SFTS have been defined previously by Yu and others¹; we found that some SFTS patients had dizziness (31.3%), joint pain (25%), and chills (18.8%), which were not reported previously.¹

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Authors' addresses: Feng Cui, Hai-Xia Cao, and Ling Wang, Zibo Center for Disease Control and Prevention, Zibo, Shandong Province of China, Epidemiology, Zibo, Shandong, China, E-mails: cuifeng@126.com, haixiacao@yahoo.cn, and Lucywl120@sina.com. Shou-Feng Zhang, Yiyuan County CDC, Infectious Diseases, Yiyuan, China, E-mail: yxycdc@126.com. Shu-Jun Ding, Shandong Provincial Dis-

ease Prevention and Control Center, Department of Infectious Disease Control, Jinan, China, E-mail: dsj_jn@126.com. Xue-Jie Yu and Hao Yu, Shandong University, Jinan, China, and University of Texas Medical Branch, Pathology, Galveston, TX, E-mails: xuyu@utmb.edu and pnphappy@gamil.com.

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