Seroprevalence and Risk Factors for Severe Fever with Thrombocytopenia Syndrome Virus Infection in Jiangsu Province, China, 2011

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Abstract. Severe fever with thrombocytopenia syndrome (SFTS), which is caused by a novel bunyavirus, is an emerging infectious disease in China. In 2011, this new virus was designated as severe fever with thrombocytopenia syndrome virus (SFTSV). The aim of the present study was to determine the seroprevalence and risk factors of SFTSV infection. The investigation was conducted among the general population in Jiangsu Province, China in 2011. A total of 2,510 serum samples were collected. Testing by enzyme-linked immunosorbent assay was conducted to determine the seroprevalence of SFTSV infection. Result showed that the overall seroprevalence of SFTSV infection was 0.44% (11 of 2,510) in seven counties in Jiangsu Province. Multiple variable logistic regression analysis showed that raising goats, farming, and grazing were risk factors for SFTSV infection. Raising goats, farming, and grazing might be important risk factors for virus exposure, and appropriate health education could be useful in preventing infections.

INTRODUCTION

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease in China. Since 2007, SFTS had been documented in six provinces of China, including Jiangsu Province. In 2009, infection with a novel type of bunyavirus was identified as causing the disease.¹ In 2011, the new virus was designated as severe fever with thrombocytopenia syndrome virus (SFTSV),² a novel phlebovirus in the family *Bunyaviridae* that was most closely related to Uukuniemi virus, as identified by metagenomic analysis, sequence-independent polymerase chain reaction, and high-throughput sequencing. Infection with SFTSV is characterized by acute onset of fever, low leukocyte cell and platelet counts, high levels of alanine and aspartate transaminases, and proteinuria, and has a mortality rate $\geq 10\%$ in hospitalized patients.³

Cases of infection with SFTSV have been found in six provinces in China (Henan, Hubei, Shandong, Jiangsu, Anhui, and Liaoning), and imported cases have been reported in Zhejiang and Beijing, China.^{4–8} Occurrence of SFTSV infection starts in March, peaks in May–July (96% of total cases), and lasts until November.⁹ A recent study demonstrated person-to-person transmission of SFTSV infection by direct personal contact.^{6,10} In addition, it has been shown that cases of infection with SFTSV are found predominantly in hilly rural areas.⁹ Severe fever with thrombocytopenia syndrome virus is believed to be transmitted by ticks because the virus has been detected in *Haemaphysalis longicornis* ticks.²

There have been few studies of the seroprevalence of SFTSV in humans and the risk factors involved in infection with this virus. The aim of the present study was to determine the seroprevalence and risk factors of SFTSV infection. Also, the study was performed to provide new knowledge to

guide future decisions on preventive measures of controlling SFTS in China.

MATERIALS AND METHODS

Sample and data collection. Jiangsu Province is located in eastern coastal China (Figure 1). The area consists of low-lying hills, with a continental monsoon humid climate and four distinct seasons. The annual average temperature is 13–19°C, and the average rainfall is 1,002.7 mm. A cross-sectional survey was conducted in seven counties in Jiangsu Province, China, in 2011 (Figure 1). The investigation targeted permanent urban and rural residents of the seven counties. The sampled population was divided into six age groups (10–19, 20–29, 30–39, 40–49, 50–59, and ≥ 60 years) in the seven counties where surveillance for SFTS was conducted.

In brief, 2,758 persons were visited and 2,510 (91%) agreed to participate. Each participant (≥ 10 years of age) was interviewed by using a structured questionnaire to obtain demographic information (e.g., name, age, sex, marital status); information on livestock (e.g., goats, dogs, and chickens); outdoor activities in recent months (e.g., farming, mowing grass, hunting, picking tea leaves, grazing, traveling); and history of bites by animals and insects in recent months (e.g., rats, ticks). Grazing was defined as movement by farmers of herds of animals to find food. A total of 2,510 serum samples were obtained during this study by collection of peripheral venous blood. The study was approved by the ethical review committee of Jiangsu Provincial Center for Disease Control and Prevention. Informed consent was obtained from all participants after they were provided with detailed descriptions of the potential benefits of the study.

Serologic test for SFTS by enzyme-linked immunosorbent assay. Total antibodies (including IgG and IgM) in serum samples were detected by using a double-antigen sandwich enzyme-linked immunosorbent assay (ELISA)¹¹ For the ELISA, 96-well plates were coated overnight with

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FIGURE 1. Jiangsu Province in China (gray), location of the seven counties (red stars) in Jiangsu Province, and number of participants seropositive for severe fever with thrombocytopenia syndrome in each county.

recombinant nucleoprotein of SFTSV and 50 μ L of serum samples was added. Samples were incubated at 37°C for 30 minutes, washed three times with phosphate-buffered saline, 0.05% Tween 20, and horseradish peroxidase-conjugated nucleoprotein was added. After samples were incubated and washed, plates were developed by using a substrate solution (0.2% *o*-phenylenediamine, 0.05% hydrogen peroxide in citric acid-phosphate buffer, pH 5.0). The chromogenic reaction was stopped by using 50 μ L of 2 M sulfuric acid, and absorbance was read at 450 nm (including negative and blank controls).

Serum samples from goats known to be positive for SFTSV were used as a positive control. These samples were obtained from 20 goats infected with the Jiangsu-014 strain of SFTSV. Serum samples collected from 20 that were exposed to the virus were tested and found to be negative for SFTSV by using a serum neutralization test. These serum samples were used as a negative control. Estimates of sensitivity, specificity, and other combined measures of diagnostic accuracy were determined at cutoff values of the mean value of negative control serum plus 3 SD.

Statistical analysis. All statistical analyses were performed by statistical software SPSS version 17.0 (SPSS Inc., Chicago, IL). Comparisons of frequencies across groups was tested for statistical significance by using Fisher's exact probability method, and the risk factors for SFTSV infection were identified by using single-variable and multiple-variable logistic regression models. Statistical significance at a 0.05 risk was used in tests and for confidence intervals (CIs). Multivariate models were constructed (logistic regression) and included univariate analysis of risk factors with significance level of ≤ 0.05 . The backwards stepwise elimination procedure was applied. Odds ratios and exact 95% CIs were used to determine the association between risk factors and SFTSV seropositivity.

RESULTS

Seroprevalence of SFTSV infection by region of residence, sex, and age. A total of 2,510 participants (median age = 42 years, age range = 10–94 years) in seven counties of Jiangsu Province were enrolled in this study. Of the 2,510 participants, 1,021 were (40.7%) male and 1,489 (59.3%) were female.

TABLE 1

Sociodemographic characteristics of the study population and prevalence of total antibodies (IgG/IgM) against SFTSV, Jiangsu Province, China*

Characteristic	No. participants (n = 2,510)	No. (%) seropositive participants (n = 11)	95% CI	P†
County				0.002
Donghai	308	0 (0.00)	(0.00–1.19)	
Lishui	362	1 (0.28)	(0.06–1.50)	
Liyang	183	3 (1.64)	(0.33–4.70)	
Peixian	373	0 (0.00)	(0.00–0.98)	
Xuyi	426	6 (1.41)	(0.52–3.04)	
Yixing	495	1 (0.20)	(0.05–1.12)	
Jiangning	363	0 (0.00)	(0.00–1.01)	
Sex				> 0.99
M	1,021	4 (0.39)	(0.11–1.00)	
F	1,489	7 (0.47)	(0.19–0.97)	
Age, years				0.556
10–19	398	3 (0.75)	(0.16–2.19)	
20–29	399	2 (0.25)	(0.06–1.80)	
30–39	383	0 (0.00)	(0.00–0.96)	
40–49	411	3 (0.73)	(0.15–2.12)	
50–59	417	1 (0.24)	(0.01–1.33)	
≥ 60	502	2 (0.40)	(0.05–1.43)	
Total	2,510	11 (0.44)	(0.18–0.70)	

*SFTSV = severe fever with thrombocytopenia syndrome virus; CI = confidence interval.

†By Fisher's exact test.

Result showed that the overall seroprevalence of SFTSV infection was 0.44% (95% CI = 0.18–0.70%) in this survey. With respect to specific regions, seroprevalence was significantly ($P < 0.05$) different among the seven counties. However, no significant difference by sex in the seroprevalence of SFTSV infection was detected. Statistical analyses showed that the seroprevalence of antibodies against the virus did not increase with age (Table 1).

Risk factor analysis by using a logistic regression model.

To control potential confounding effects from various risk factors, as previously selected by using univariate analysis, a multiple logistic regression model was constructed by

including significant risk factors associated with SFTSV infection. After including the factors of raising goats, farming and grazing, it was found that these three factors were still significantly associated with SFTSV infection in the study population (Table 2).

DISCUSSION

This study was an epidemiologic study conducted in Jiangsu Province, China, to investigate SFTSV infection. Results indicated an overall SFTSV seroprevalence of 0.44%, which is lower than that reported in Shandong Province (0.8%) (5). The differences between the two studies might be attributed to the different populations that participated in these studies (2,510 persons in Jiangsu Province and 237 persons in Shandong Province). Most importantly, this study indicated that raising goats, farming, and grazing were the risk factors of SFTS in humans. Educating persons to prevent this disease, especially for high-risk populations, is needed.

The study demonstrated that significant regional differences were observed in the seroprevalence of SFTSV among the counties, which might be attributed to variation in location. Infection with SFTSV infection was a serious problem in Lishui, Liyang, Yuyi, and Yixing Counties, which are characterized by hilly landscapes. Because tick reservoirs might be more frequently found in hilly landscapes, infection with SFTSV would be more likely in these areas. In a previous study, 111 (83%) of 134 goats in Yiyuan County, Shandong Province, China, were seropositive for this virus.⁵ On the basis of current evidence, we could speculate that goats are the potential reservoir for SFTSV. However, additional studies are needed to provide conclusive evidence.

Conversely, there was no significant sex difference observed, indicating that men and women are equally susceptible to SFTSV infections. In addition, there was no statistical difference among studied age groups for SFTSV infection.

TABLE 2

Single-variable and multiple-variable logistic regression analyses for identifying risk factors for SFTSV infection in Jiangsu Province, China*

Variable		Single-variable logistic regression analysis		Multiple-variable logistic regression analysis	
		OR (95% CI)	P	OR (95% CI)	P
Age		0.962 (0.922–1.004)	0.073	–	–
Raising goats	Yes	7.688 (1.050–56.284)	0.045	7.270 (1.292–40.893)	0.024
	No				
Raising dogs	Yes	0.962 (0.922–1.004)	0.798	–	–
	No				
Raising chickens	Yes	1.391 (0.323–5.983)	0.658	–	–
	No				
Rat bite	Yes	0.000	0.999	–	–
	No				
Farming	Yes	7.490 (1.549–36.215)	0.012	4.919 (1.269–19.058)	0.021
	No				
Grass mowing	Yes	0.000	0.662	–	–
	No				
Hunting	Yes	0.000	1.000	–	–
	No				
Picking tea leaves	Yes	0.000	0.997	–	–
	No				
Grazing	Yes	71.899 (6.301–820.432)	0.001	40.154 (6.385–252.530)	< 0.001
	No				
Traveling	Yes	0.000	0.999	–	–
	No				
Tick bite	Yes	0.736 (0.023–23.064)	0.861	–	–
	No				

*SFTSV = severe fever with thrombocytopenia syndrome virus; OR = odds ratio; CI = confidence interval.

Our results indicated that one age group was not at greater risk of being infected than another. Single-variable logistic regression analysis showed a significantly positive correlation of raising goats, farming, and grazing with SFTSV infection, and multiple-variable logistic regression analysis showed that these three variables were risk factors for the infection. Therefore, persons who engage in raising goats, farming, and grazing were the high-risk populations for SFTSV infection. Exposure to goats, farming, and grazing might increase the probability of tick bites and infection with SFTSV. Consequently, an understanding of the prevalence and risk factors of infection has been facilitated, which would provide evidence for SFTS prevention.

In conclusion, this serologic survey facilitated understanding of the current prevalence of SFTSV infection in seven counties of Jiangsu Province. Further studies are needed to isolate SFTSV from animals and ticks, identify the tick species involved in human transmission, and identify the potential role of wild animals as reservoirs. Health education on prevention and control of the disease should be provided to high-risk populations to increase their awareness of this infection, and a surveillance response system should be established to increase the ability for early detection and early diagnosis and to reduce incidence of SFTSV infections.

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REFERENCES

1. Feldmann H, 2011. Truly emerging: a new disease caused by a novel virus. *N Engl J Med* 364: 1561–1563.
2. Yu XJ, Liang MF, Zhang SY, Liu Y, Li JD, Sun YL, Zhang L, Zhang QL, Popov VL, Li C, Qu J, Li Q, Zhang YP, Hai R, Wu W, Wang Q, Zhan FX, Wang XJ, Wang Kan B, SW, Wan KL, Jing HQ, Lu JX, Yin WW, Zhou H, Guan XH, Liu JF, Bi ZQ, Liu GH, Ren J, Wang H, Zhao Z, Song SD, He JR, Wan T, Zhang JS, Fu XP, Sun LN, Dong XP, Feng ZJ, Yang WZ, Hong T, Zhang Y, Walker DH, Wang Y, Li DX, 2011. Fever with thrombocytopenia associated with a novel bunyavirus in China. *N Engl J Med* 364: 1523–1532.
3. Xu BL, Liu LC, Huang XY, Ma H, Zhang Y, Du Y, Wang P, Tang X, Wang H, Kang K, Zhang S, Zhao G, Wu W, Yang Y, Chen H, Mu F, Chen W, 2011. Metagenomic analysis of fever, thrombocytopenia and leukopenia syndrome (FTLS) in Henan Province, China: discovery of a new bunyavirus. *PLoS Pathog* 7: e1002369.
4. Chen H, Hu K, Zou J, Xiao J, 2013. A cluster of cases of human-to-human transmission caused by severe fever with thrombocytopenia syndrome bunyavirus. *Int J Infect Dis* 17: e206–e208.
5. Zhao L, Zhai S, Wen H, Cui F, Chi Y, Wang L, Xue F, Wang Q, Wang Z, Zhang S, Song Y, Du J, Yu XJ, 2012. Severe fever with thrombocytopenia syndrome virus, Shandong Province, China. *Emerg Infect Dis* 18: 963–965.
6. Bao CJ, Guo XL, Qi X, Hu JL, Zhou MH, Varma JK, Cui B, Yang HT, Jiao YJ, Klena JD, Li X, Tao WY, Li X, Chen Y, Zhu Z, Xu K, Shen AH, Wu T, Peng HY, Li ZF, Shan J, Shi ZY, Wang HJ, 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: 1208–1214.
7. Bao CJ, Qi X, Wang H, 2011. A novel bunyavirus in China. *N Engl J Med* 365: 862–863.
8. Zhang YZ, Zhou DJ, Xiong Y, Chen XP, He YW, Sun Q, Yu B, Li J, Dai YA, Tian JH, Qin XC, Jin D, Cui Z, Luo XL, Li W, Lu S, Wang W, Peng JS, Guo WP, Li MH, Zhang S, Chen C, Wang Y, de Jong MD, Xu J, 2011. Hemorrhagic fever caused by a novel tick-borne bunyavirus in Huaiyangshan, China. *Chin J Epidemiol* 32: 209–220.
9. Ding F, Zhang W, Wang L, Hu W, Soares Maqalhaes RJ, Sun H, Zhou H, Sha S, Li S, Liu Q, Li Q, Yang W, Huang L, Li C, Yin W, 2013. Epidemiologic features of severe fever with thrombocytopenia syndrome in China, 2011–2012. *Clin Infect Dis* 56: 1682–1693.
10. Tang XY, Wu WL, Wang HF, Du YH, Liu LC, Kang K, Huang X, Ma H, Mu F, Zhang S, Zho G, Cui N, Zhu BP, You A, Chen H, Liu G, Chen W, Xu B, 2013. Human-to-human transmission of severe fever with thrombocytopenia syndrome bunyavirus through contact with infectious blood. *J Infect Dis* 207: 736–739.
11. Jiao YJ, Zeng XY, Guo XL, Qi X, Zhang X, Shi ZY, 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: 372–377.