Original Article

Presence of Antibodies against Severe Fever with Thrombocytopenia Syndrome Virus in Non-Endemic Areas of China

Xiaoxia Huang^{1†}, Zhongxin Zhang^{2†}, Guanghui Jin², Xiaofang Wang¹, Chusheng Tan³, Hongmei Yin⁴, and Shiwen Wang^{1*}

¹National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing;

²Yixian Center for Disease Control and Prevention, Anhui;

³Jiangxi Center for Disease Control and Prevention, Jiangxi; and

⁴Chinese Center for Disease Control and Prevention, Beijing, China

SUMMARY: To determine the seroprevalence of severe fever with thrombocytopenia syndrome (SFTS) virus (SFTSV) and identify potential risk factors in non-endemic areas, a cross-sectional study was conducted among a healthy population without previously reported SFTS in a mountainous area of Anhui Province. We recruited and collected blood samples from 270 participants in 4 age groups. An enzyme-linked immunosorbent assay kit was used to detect immunoglobulin G (IgG) antibodies against SFTSV. The overall seropositive rate of SFTSV-IgG among study participants was 6.3% (17/270). We found a significant difference in the SFTSV seropositivity rate between tea pluckers (9.4%, 14/149) and non-tea pluckers (2.5%, 3/121). An increasing trend in the seropositivity rate was found with age for all participants (1.5% to 14.3%) and tea pluckers (2.8% to 19.4%). The SFTSV seropositivity rate in men was higher than that in women for all participants and tea pluckers, but the differences were not significant. Critical risk factors for SFTSV infection are increasing age and being a tea plucker. Our findings suggest that health education should be enhanced to increase awareness among residents (especially, the older ones and tea pluckers) to protect them against SFTS.

INTRODUCTION

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by the SFTS virus (SFTSV), a newly identified member of *Phlebovirus* genus of *Bunyaviridae* family. SFTSV was thought to be mainly transmitted by ticks, based on evidence from tick exposure history, SFTSV detection, and sequence analysis (1,2). SFTS was first reported in 2009 in China (3); by 2013, more than 1,700 laboratory-confirmed cases had been reported in 14 provinces, with a fatality rate of 7–15% (4). In recent years, the number of provinces with SFTS cases has gradually increased to 23 provinces with 5,352 cases (5).

Since SFTSV is a leading public health issues, researches on its clinical characteristics, pathogenic mechanism, detection methods, and epidemiologic characteristics were conducted (6–13). With regards to SFTSV

epidemiology, seroprevalence of the healthy population in endemic areas has previously been investigated (14–16); however, in non-endemic areas, it is unclear, and more knowledge in this area is urgently needed.

As a vector borne disease, SFTSV mainly infects people who work outdoors (e.g., farmers). We chose to conduct our study in Yixian, a mountainous area in Anhui Province without any previously reported cases of SFTS. Furthermore, Yixian is an agricultural county in China, where tea picking is the main outdoor work for local residents. Our aim in this study is to explore evidence for SFTSV infection in a non-endemic area and identify relevant risk factors for SFTSV infection in this area.

MATERIALS AND METHODS

Ethical statement: The study was approved by the Ethics Committee of the National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention.

Study area: The study was conducted in Yixian (latitude 117°38'30"~118°6'E and longitude 29°47'~30°11'30"N), a county in southern Anhui Province, China (Fig. 1). This county includes 8 towns, with a total land area of 857 km² and a population of approximately 95,500. In Yixian, 85.3% of the terrain is mountainous and 84.4% is covered by forest. Yixian is a traditional agricultural

Received May 11, 2016. Accepted August 10, 2016.

J-STAGE Advance Publication August 31, 2016. DOI: 10.7883/yoken.JJID.2016.214

*Corresponding author: Mailing address: National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, 155#, Changbai Rd, Changping District, Beijing, China.

Tel: +86-10-58900675, E-mail: melindayin@163.com

[†]These authors contributed equally to this work.

Fig. 1. Location of Anhui Province (gray shading) in China (left panel) and Yixian (gray shading) in Anhui Province (right panel).

county, where tea is one of the main crops. During the year, tea is harvested from March to May. Outdoor activities provide numerous opportunities to contact ticks.

Sample collection: The investigation of SFTSV seroprevalence was conducted during June 2012 in all 8 towns of Yixian country, where at that time, no SFTS cases had been reported. In our study, we divided potential participants into 4 age groups (i.e., < 20, 20–39, 40–59, ≥ 60 years); each group included at least 50 persons. A total of 270 volunteers were randomly recruited to participate in the study, and blood samples for analysis were collected. In addition, a questionnaire was used to collect demographic data from each participant, including age, sex, occupation (tea plucker or not), and address. Here, tea pluckers were defined as persons who engaged in tea picking during the harvest season, and it is the main economic source for their families.

Antibody detection: Sera were separated from collected blood and stored at -20°C . We used an enzyme-linked immunosorbent assay kit obtained from the National Institute for Viral Disease Control and Prevention and followed its instructions to test for SFTSV-specific immunoglobulin G (IgG) antibodies in sera samples (17,18). A sample of 100 μl (1 μl sera diluted in 100 μl total sample) was added to each well and incubated at 37°C for 40 min. After washing the well 6 times, 100 μl of horseradish peroxidase labeled reagent was added to each well, then incubated at 37°C for 40 min. Chromogenic agents A (mainly containing carbamide peroxide) and B (mainly containing 3, 3', 5, 5'-tetramethylbenzidine) were added to each well. After incubation, the optical density (OD) was read at 450 nm. For each test or plate, 2 negative and 2 positive controls were used. The OD value of a sample $\geq$ cut-off value was considered to be positive. The cut-off value = 0.10 + the average OD value of the negative controls (if the OD value was < 0.05, it was considered as 0.05).

Statistical analysis: SPSS software ver. 18.0 (Chicago, IL, USA) was used to conduct the statistical analysis; a P -value < 0.05 was considered statistically significant. We compared the seropositive rate by age groups, sex, and occupation (tea pluckers or not) with Pearson's chi-square test and Fisher's exact test. Risk factor analysis was conducted using binary logistic regression.

RESULTS

Overview of SFTSV seroprevalence: A total of 270 residents from all 8 towns participated in this study. The numbers of participants in each age group was as follows: i) < 20 years ($n = 66$); ii) 20–39 years ($n = 55$); iii) 40–59 years ($n = 93$); and iv) ≥ 60 years ($n = 56$). The median age of all participants was 42.5 years (range, 5–83 years), and 60.4% were women. Among all participants, 6.3% (17/270) were seropositive for SFTSV, varying from 1.5% to 14.3% in the different age groups ($\chi^2 = 8.090$, $P = 0.035$). Among participants, the seropositivity showed an increasing trend with age (Fig. 2). The youngest and oldest cases of SFTSV infection were a woman aged 18 years and a man aged 75 years, respectively. Seroprevalence was higher in men (8.4%, 9/107) than in women (4.9%, 8/163), but the difference was not statistically significant ($\chi^2 = 1.344$, $P = 0.246$).

SFTSV seroprevalence in tea pluckers: Among the participants 149 (55.2%) were tea pluckers. The median age of the tea pluckers was 46 years (range, 5–75 years), and 61.7% were women. SFTSV seropositivities in tea pluckers and non-tea pluckers were 9.4% (14/149) and 2.5% (3/121), respectively. The difference between these 2 groups was statistically significant ($\chi^2 = 5.414$, $P = 0.020$). The SFTSV seropositive rate among tea pluckers was higher than the total seropositive rate in each age group. We also found an increase in seropositivity with age in tea pluckers (Fig. 2). Seroprevalence was higher in men (10.5%, 6/57) than in women (8.7%, 8/92), but the difference was not statistically significant ($\chi^2 = 0.139$, $P = 0.710$).

Fig. 2. Age distribution of SFTSV-IgG antibody in subjects from Yuixian, China.

Risk factor analysis of SFTSV infection: Based on the results of our statistical analysis, we selected the variables of “tea pluckers or not” and “age” to analyze as SFTSV infection risk factors using binary logistic regression. Table 1 shows that tea pluckers and age were determined to be risk factors for SFTSV infection.

Table 1. Multi-factor analysis of SFTSV infection by logistic regression (Method = Enter)

Factor	B	SE	Wald	df	P	OR	95% OR CI	
							Low	Up
Tea pluckers	1.321	0.657	4.038	1	0.044	3.746	1.033	13.582
Age	0.043	0.016	7.025	1	0.008	1.043	1.011	1.077
Constant	-6.981	1.511	21.345	1	< 0.001	0.001		

B, beta; df, degrees of freedom; OR, odds ratio; CI, confidence interval.

DISCUSSION

SFTS cases are mainly distributed among farmers living in mountainous or hilly rural areas (19). So far, more than two-thirds of the provinces in mainland China have reported cases of SFTS. However, some areas in China, with a similar environment as in endemic areas, SFTS cases have not been found. Furthermore, since 2010, when SFTSV was found to be the pathogen responsible for SFTS (3), serological surveys were conducted to understand SFTSV seroprevalence, which was found to be inconsistent (ranging from 0% to 9.2%) and rare, especially in non-endemic areas (14–16,20). Therefore, it is urgent to conduct investigations of SFTSV seroprevalence to know the infection status in such areas. In this study, we chose Yixian as our study site, which is an agricultural county of Anhui Province in China, where tea is the main economic source of income. Although cases identified as SFTSV infection had previously been reported in nearby areas, no cases of SFTS have been reported there since the first case of SFTS was reported in China. Our study showed that the seropositivity rate of SFTSV-IgG antibodies among participants in Yixian country was 6.3%, which was higher than the reported rate of 4.66% in endemic areas of Anhui Province (21). This discrepancy may be related to the number involved, age, sex, geographic location, and types of antibodies tested (total antibodies or IgG antibodies against SFTSV) (22).

In our study, participants were divided into 4 age groups based on 20-year intervals to analyze the SFTSV seropositivity rate. We found that participants in all age groups had SFTSV-IgG antibodies in their sera. We inferred that the population was exposed to SFTSV and was susceptible to SFTSV (1). Furthermore, the seropositivity rate increased with age for all participants and tea pluckers. The seropositivity rate was higher in men than in women in our study. This phenomenon could be caused by several factors (23–25). First, Yixian is an agriculture area, and young people often work outside Yixian, whereas middle-aged and older residents remain in the country to perform agricultural works (e.g., tea picking). Therefore, older participants had a greater chance of being exposed to SFTSV-amplifying vectors. The increasing trend with age in participants was mainly attributable to infection in the tea pluckers. Second, different chances of exposure (e.g., men wear less clothing during labor) may contribute to different rates of sero-

positivity by sex.

Our study is the first to show a detailed description of the SFTSV infection rate among tea pluckers (9.4%), which was much higher than some of the previously reported rates in healthy populations (14–16) and non-tea pluckers. We inferred from these findings that outdoor workers, like tea pluckers, have a greater risk of infection with SFTSV. Therefore, local health institutions should be attentive to this population regarding future effort to prevent SFTSV infection. Furthermore, we found that the rate of SFTSV-IgG seropositivity was consistent among male and female tea pluckers, but was much higher among men than women for all participants. Ding et al. showed that age is a critical risk factor for SFTS morbidity and mortality (17). In our study, we found that age is also a risk factor for SFTSV infection.

This study is important in understanding the risk factors for SFTSV infection. Our study was limited the number and type of participants due to the dispersed population in mountainous areas and non-ideal timing of blood collection, which coincided with the harvesting season for farmers. For further study of SFTSV infection, we will continue to collect more specimens to carry out deep and rigorous research, thus minimizing statistical bias.

In conclusion, SFTSV infection exists in non-endemic areas with a higher seroprevalence rate. Older persons who are engaged in outdoor work (e.g., tea pluckers) are at higher risk for infection. Health education should be provided to these residents, especially the older ones, to encourage individuals to protect themselves while performing agricultural work, especially outdoor work such as tea picking) to decrease the risk SFTSV infection.

Since the seroprevalence of SFTSV in our study was higher than expected rate, further surveillance should be done to investigate whether or not cases of SFTS exist (including subclinical ones) in non-endemic areas such as Yixian.

Acknowledgments This work was supported by the China Mega-Project for Infectious Diseases (2012ZX10004215) and National Science and Technology Support Program (2014BAI13B05).

Conflict of interest None to declare.

REFERENCES

1. Li DX. Severe fever with thrombocytopenia syndrome: a newly discovered emerging infectious disease. *Clin Microbi-*

- ol Infect. 2015;21:614-20.
2. Zhao L, Zhai SY, Wen HL, et al. Severe fever with thrombocytopenia syndrome virus, Shandong Province, China. *Emerg Infect Dis*. 2012;18:963-5.
3. Yu XJ, Liang MF, Zhang SY, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. *N Engl J Med*. 2011;364:1523-32.
4. Liu K, Zhou H, Sun RX, et al. A national assessment of the epidemiology of severe fever with thrombocytopenia syndrome, China. *Sci Rep*. 2015;5:9679.
5. Li Y, Zhou H, Mu D, et al. Epidemiological analysis on severe fever with thrombocytopenia syndrome under the national surveillance data from 2011 to 2014, China. *Zhonghua Liu Xing Bing Xue Za Zhi*. 2015;36:598-602. Chinese.
6. Guo CT, Lu QB, Ding SJ, et al. Epidemiological and clinical characteristics of severe fever with thrombocytopenia syndrome (SFTS) in China: an integrated data analysis. *Epidemiol Infect*. 2016;144:1345-54.
7. Sun Y, Liang M, Qu J, et al. Early diagnosis of novel SFTS bunyavirus infection by quantitative real-time RT-PCR assay. *J Clin Virol*. 2012;53:48-53.
8. Jin C, Jiang H, Liang M, et al. SFTS virus infection in non-human primates. *J Infect Dis*. 2015;211:915-25.
9. Sun Y, Jin C, Zhan F, et al. Host cytokine storm is associated with disease severity of severe fever with thrombocytopenia syndrome. *J Infect Dis*. 2012;206:1085-94.
10. Jin C, Liang M, Ning J, et al. Pathogenesis of emerging severe fever with thrombocytopenia syndrome virus in C57/BL6 mouse model. *Proc Natl Acad Sci USA*. 2012;109:10053-8.
11. Ding F, Guan XH, Kang K, et al. Risk factors for bunyavirus-associated severe fever with thrombocytopenia syndrome, China. *PLoS Negl Trop Dis*. 2014;8:e3267.
12. Wang S, Li J, Niu G, et al. SFTS virus in ticks in an endemic area of China. *Am J Trop Med Hyg*. 2015;92:684-9.
13. Li D. A highly pathogenic new bunyavirus emerged in China. *Emerg Microbes Infect*. 2013;2:e1.
14. Zhang L, Sun J, Yan J, et al. Antibodies against severe fever with thrombocytopenia syndrome virus in healthy persons, China, 2013. *Emerg Infect Dis*. 2014;20:1355-7.
15. Wang YP, Wang QK, Ge HM, et al. Serological surveillance on severe fever with thrombocytopenia syndrome. *Jiangsu J Prev Med*. 2014;25:26-8. Chinese.
16. Zhang WS, Zeng XY, Zhou MH, et al. Seroepidemiology of severe fever with thrombocytopenia syndrome bunyavirus in Jiangsu province. *Dis Surveil*. 2011;26:676-8.
17. Ding SJ, Niu GY, Xu XH, et al. Age is a critical risk factor for severe fever with thrombocytopenia syndrome. *PLoS One*. 2014;9:e111736.
18. Chen K, He J, Xiu BS, et al. Establishment and evaluation of kit for detecting novel bunyavirus antibody. *Lett Biotech*. 2014;25:100-1. Chinese.
19. Liu K, Cui N, Fang LQ, et al. Epidemiologic features and environmental risk factors of severe fever with thrombocytopenia syndrome, Xinyang, China. *PLoS Negl Trop Dis*. 2014;8:e2820.
20. Zhang L, Ye L, Ojcius DM, et al. Characterization of severe fever with thrombocytopenia syndrome in rural regions of Zhejiang, China. *PLoS One*. 2014;9:e111127.
21. Lyu Y, Ding F, Sun J, et al. Seroprevalence and risk factors of severe fever with thrombocytopenia syndrome virus infection in endemic areas. *Infect Dis (Lond)*. 2016;48:544-9.
22. Liu S, Chai C, Wang C, et al. Systematic review of severe fever with thrombocytopenia syndrome: virology, epidemiology, and clinical characteristics. *Rev Med Virol*. 2014;24:90-102.
23. Li SB, Xue C, Ding XJ, et al. Analysis on clinical characteristics and genome sequence of sporadic severe fever with thrombocytopenia syndrome patients in Zhejiang province. *Chin J Infect Dis*. 2012;30:268-72. Chinese.
24. Peng DL, Huang LM, Liu CW, et al. The changes of the scale and structure of agricultural laborer and their impacts on food production: empirical study on Yinshan Po Village, Suizhou City. *J Anhui Agri Sci*. 2010;38:19192-6. Chinese.
25. Sun HY. The impact on agricultural production by the population structure change of family left in rural areas. *J Hunan Soc Sci*. 2008;2:123-6. Chinese.