Original Article

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

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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 (5).

Since SFTSV is a leading public health issue, 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
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 county, 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 100μl (1μl sera diluted in 100μl total sample) was added to each well and incubated at 37°C for 40 min. After washing the well 6 times, 100μl 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 used 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 ≥ 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 (χ² = 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 (χ² = 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 (χ² = 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 (χ² = 0.139, P = 0.710).

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 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.
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, whereas middle-aged and older residents remain in the country to perform agricultural works (e.g., tea picking). Therefore, older participants had a greater risk of 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. 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.

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