Low Seroprevalence of Severe Fever with Thrombocytopenia Syndrome Virus Antibodies in Individuals Living in an Endemic Area in Japan

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SUMMARY: Severe fever with thrombocytopenia syndrome (SFTS), a tick-borne infection, was first reported in China in 2011. The disease is characterized by non-specific symptoms, including fever, gastrointestinal tract symptoms, and general fatigue, and by leukopenia and thrombocytopenia. The case fatality rate (CFR) is 12% (1). This infection is caused by SFTSV virus (SFTSV) and is endemic in some areas in western Japan, including the Kagoshima prefecture. In the present study, healthy individuals living in this prefecture were examined to assess for anti-SFTSV seroprevalence. An initial study was performed using the serum samples collected from a total of 646 individuals living in Kagoshima. At the same time, a questionnaire was used to collect information (such as occupation and a history of tick bite). Enzyme-linked immunosorbent assay and indirect immunofluorescence assay were used for the screening. Finally, the seroprevalence of anti-SFTSV antibodies was confirmed using a neutralization assay. Only 2 (0.3%) out of 646 study participants were positive for anti-SFTSV antibodies. No significant difference was observed between individuals who are at a high or low risk of tick bite in terms of seropositivity. Next, a total of 1,000 serum samples collected from general blood donors by the Japanese Red Cross Kyushu Block Blood Center were tested. None of these samples tested positive for anti-SFTSV antibodies. These results suggest a low seroprevalence of anti-SFTSV antibodies in healthy individuals living in an endemic area in Japan.

INTRODUCTION

Severe fever with thrombocytopenia syndrome (SFTS), a tick-borne infection, was first reported in China in 2011. The disease is characterized by non-specific symptoms, including fever, gastrointestinal tract symptoms, and general fatigue, and by leukopenia and thrombocytopenia. The case fatality rate (CFR) is 12% (1). This infection is caused by SFTSV virus (SFTSV), a novel phlebovirus that belongs to the family Phenuiviridae (1,2). The SFTSV genome is detected in some tick species (including Haemaphysalis longicornis and Rhipicephalus microplus) living in endemic areas. These ticks are most likely the vectors for the transmission of SFTSV to humans (1,3). SFTS cases have also been reported in South Korea in 2012 and in Japan in 2013 (4,5). SFTS is a notifiable disease (NIID) as of July 2017, mostly from the western part of Japan (Chugoku, Shikoku, and Kyushu regions) (6). In Japan, the CFR of SFTS is approximately 20% (6). Because SFTSV is carried by mammals and ticks, people living in the SFTS endemic area are at a risk of infection. Therefore, the risk of infection should be properly evaluated, and more attention should be provided in controlling SFTS in endemic areas.

The laboratory diagnosis of SFTS is based on the detection of its genome, the isolation of SFTSV from a patient’s blood sample and/or body fluids (e.g., throat swabs and urine), or a significant increase in the anti-SFTSV antibody titers of patients during the acute and convalescent phases (1,7). Serological surveys based on the detection of anti-SFTSV IgG antibodies in the general population provide important information about the overall picture of the infection among humans. Such data might help in evaluating the ratio of symptomatic and asymptomatic infections among individuals infected with SFTSV and in understanding the demographics and risk factors of the infection in endemic areas. Despite the simplicity and high sensitivity of the antibody detection assays, such as enzyme-linked immunosorbent assays (ELISAs) and immunofluorescent antibody assays (IFAs), false-positive results may still be obtained. Furthermore, anti-SFTSV antibodies may cross-react with other phleboviruses present in the serum of patients with SFTS during the convalescent phase (8). Therefore, the focus reduction neutralization assay (FRNA), which uses a susceptible cell line and infectious SFTSV, is used to detect SFTSV-specific neutralizing antibodies (9). In China, a double-antigen ELISA or an indirect ELISA has been used to identify anti-SFTSV IgG antibodies in the general population. Li et al. (10) recently conducted a systematic review of 21 studies on the seroprevalence of SFTSV in China.

However, no studies have examined the seroprevalence of SFTSV in healthy individuals in Japan. Therefore, risk factors associated with the infection caused by this virus...
in endemic areas remain unclear. In Kagoshima prefecture (the southernmost prefecture on the island of Kyushu), more than 26 patients with SFTS were identified as of July 2017 (6). Therefore, this study aimed to examine the seroprevalence of SFTSV among healthy individuals in Kagoshima. The serum samples were screened using a combination of ELISA and IFA. FRNA was used to confirm whether the selected samples tested positive or negative for SFTSV antibodies.

**MATERIALS AND METHODS**

**Study design and setting:** A total of 646 participants were included in the initial study. From July 2015 to August 2015, blood samples were collected from the members of the hunting association in areas A, B, and C (Fig. 1). The samples were also collected from the general population (not involved in hunting-related activities) from July 2015 to January 2016 in area C (Fig. 1). In addition, a questionnaire was used to collect information from each participant, including age, occupation (hunting related or not), and a history of tick bite. Participants were divided into 2 groups: hunting group \((n = 125)\) and non-hunting group \((n = 521)\) (Table 1).

Furthermore, a total of 1,000 serum samples collected from the general blood donors (age range: 19–69 years) in 2016 by the Japanese Red Cross Kyushu Block Blood Center in Kagoshima prefecture were used in this study. The serum samples were provided by the Japanese Red Cross Society (Table 2). Information about the participants (e.g., occupation and a history of tick bite) was not obtained.

**Serological testing:** IgG ELISA was based on a viral antigen obtained from Huh7 cells infected with SFTSV (HB29 strain), as described previously (11). The cut-off optical density (OD) value (0.562) at a 100-fold serum dilution was determined using negative control human sera (11). Serum samples with OD values above the cut-off were subjected to IFA (11). The IFA titer was calculated as the reciprocal of the highest serum dilution at which the sample generated a specific fluorescent signal against the SFTSV antigen in the cells examined under a fluorescent microscope. FRNA was utilized, as described previously (9), to confirm SFTSV-neutralizing antibodies using the SFTSV strains YG1 and HB29. The neutralization titer was calculated as the reciprocal of the highest serum dilution at which the number of foci was less than 50% of that of the control. Serum samples that showed a positive reaction in the FRNA were considered positive for anti-SFTSV antibodies.

**Statistical analysis:** Differences in the seroprevalence of anti-SFTSV antibodies between the hunting and non-hunting groups were compared using Fisher’s exact test. A statistical analysis was performed using GraphPad Prism (San Diego, CA, USA).

**Ethical statement:** All protocols and procedures were carried out with the approval of the Research Ethics Committee of Kagoshima University, Graduate School of Medical and Dental Sciences (No. 549), the Research Ethics Committee of the Kagoshima Prefectural Institute for Environmental Research and Public Health (No. 28-1), and the Research Ethics Committee of the NIID (No. 626 and No. 727).

**RESULTS**

Patients with SFTS have been identified in several areas, including the study areas in Kagoshima prefecture. An initial study was conducted to compare anti-SFTSV antibody seropositivity between healthy individuals who are at a high and low risk of tick bite (hunting and non-hunting groups, respectively) using a total of 646 serum samples collected from the 3 areas in Kagoshima prefecture (Fig. 1). The hunting group included the members of the hunting association involved in hunting-related activities in the forest (Table 1). We used ELISA and IFA to screen the samples, and FRNA was used to confirm whether the samples were positive or negative for SFTSV antibodies. Among the 125 participants in the hunting group, 5 tested positive in the IgG ELISA, of which 2 also tested positive in the IFA (Table 2). Among the 521 participants in the non-hunting group, 7 tested positive in the IgG ELISA, of which 3 were also positive in the IFA (Table 2). Because most ELISA-positive sera showed OD values slightly higher than the cut-off value at a 100-fold dilution, FRNA was used to confirm anti-SFTSV antibodies in these sera using 2 SFTSV strains (YG1 [isolated in Japan] and HB29 [isolated in China]) to rule out the possibility that the serum neutralization activity was limited to a particular virus strain. One participant in the hunting group and one in the non-hunting group (Nos. 7 and 20, respectively) tested positive in the FRNA using both SFTSV strains, whereas all the remaining serum samples tested negative in this assay (Table 2). No significant difference was observed in the rate of SFTSV antibody
90.1%) reported a tick bite (p < 0.001, Table 1). No statistically significant difference was noted between the 2 groups in terms of the onset of fever after a tick bite (p = 0.86, Table 1).

The aforementioned results indicate that only 2 (one in the hunting group and another in the non-hunting group) out of the 646 study participants (0.3%) were positive for anti-SFTSV antibodies. To examine the seroprevalence with a larger number of serum samples obtained from healthy individuals in Kagoshima, an additional 1,000 serum samples collected from the general blood donors by the Japanese Red Cross Kyushu Block Blood Center in Kagoshima prefecture were screened for the presence of anti-SFTSV antibodies (Table 2). In total, 6 out of 1,000 serum samples (0.6%) had OD values higher than the cutoff in the ELISA. However, none tested positive in both the IFA and FRNA (Table 2), indicating that all these serum samples were negative for anti-SFTSV antibodies. Based on the aggregate data obtained in this study, only 2 of 1,646 healthy participants (0.1%) living in Kagoshima had antibodies against SFTSV.

**DISCUSSION**

Herein, we examined the prevalence of anti-SFTSV antibodies in healthy individuals living in Kagoshima prefecture, an SFTS endemic region in Japan. Because the primary mode of transmission by which SFTSV infects humans is a bite by a SFTSV-infected tick, an occupational risk was noted for individuals working in farms as well as for those in contact with domestic and/or wild animals (12–14). SFTSV is known to circulate in Japan as well as for those in contact with domestic and/or wild animals (12–14). SFTSV is known to circulate in Japan because patients with SFTS are being notified (6) because the SFTS Seroprevalence in Kagoshima, Japan

| Table 1. Characteristics, SFTS seropositivity, and tick bite history of the study population |
|----------------------------------------|------------------|------------------|---------------|
| Gender and age                        | Hunting group (n = 125) | Non-hunting group (n = 521) | p            |
|                                       | Male | 120 | 250 |               |
|                                       | Female | 5 | 271 |               |
| Age range in years                    |      |      |      |               |
| < 30                                  | 1    | 32  |     |               |
| 30–49                                 | 8    | 134 |     |               |
| 50–69                                 | 81   | 270 |     |               |
| > 70                                  | 31   | 63  |     |               |
| NR                                    | 4    | 22  |     |               |
| SFTSV antibody-positive               |      |      |      |               |
| Experience of tick bite               |      |      |      |               |
| yes                                   | 109 (90.1%) | 264 (52.0%) |     |               |
| Frequency of tick bite/year           |      |      |      |               |
| multiple                              | 85 (83.3%) | 135 (51.3%) | <0.001 |               |
| once                                  | 7 (6.9%) | 78 (29.7%) | <0.001 |               |
| at least once in recent years         | 10 (9.8%) | 50 (19.0%) | 0.04  |               |
| Fever appeared after tick bite        |      |      |      |               |
| yes                                   | 13 (11.9%) | 29 (11.0%) | 0.86  |               |

NR, no response.

None of these samples tested positive for anti-SFTSV antibodies, and the rate of seropositivity was similar in the 2 groups (Tables 1 and 2). According to the questionnaire, neither of these 2 participants who were positive for the antibodies developed fever after the tick bite (data not shown). Therefore, mild or asymptomatic infection is not frequent in this SFTS endemic area in Japan.

The seroprevalence of anti-SFTSV antibodies in healthy individuals living in Kagoshima was further examined using an additional 1,000 serum samples collected from the general blood donors in Kagoshima prefecture (Table 2). None of these samples tested positive for anti-SFTSV antibodies. Therefore, a low seroprevalence of anti-SFTSV antibodies in healthy individuals living in Kagoshima prefecture was noted (0.1%, 2/1,646).

Similar to our findings, a previous study reported a low seroprevalence (2/237, 0.8%) of SFTSV antibodies in the general population of Yiyuan County, Shandong Province, China, in which more than 80% of the goats were positive for anti-SFTSV antibodies (16). In contrast, studies conducted in SFTS endemic regions in China have shown a higher seroprevalence (more than 5%) among healthy in-
dividuals (17–20). Furthermore, a recent study in South Korea reported that the seroprevalence of SFTS among patients visiting a tertiary hospital was 2.1%. The reasons for these discrepancies may be attributed, at least in part, to occupation, geographical location, and environmental conditions (10,21). However, it is more likely that the higher seroprevalence reported in China and South Korea might be because of the differences in the SFTSV antibody detection methods used. Previous studies have used a double-antigen ELISA or an indirect ELISA to detect anti-SFTSV IgG antibodies (10,21). However, we used ELISA and IFA to screen the samples, and FRNA was used to confirm if the samples tested positive or negative for SFTSV antibodies. ELISA makes a rapid, sensitive, and simple to perform, and easy to standardize antibody screening test. However, it may yield false-positive results (e.g., 1–2% of the test samples), resulting in an erroneous interpretation of seropidemiology. Therefore, a neutralization assay is required to confirm the presence of virus-specific antibodies in serum samples (22–24).

Among the 1,646 sera examined, 18 (1.1%) tested positive in the IgG ELISA. However, at a dilution of 1:100, most ELISA-positive sera had OD values slightly higher than the cut-off value (Table 2). One participant (No. 38) in the hunting group had a high antibody titer (> 640) in the IFA but tested negative in the SFTSV FRNA. The neutralization assay is a gold standard method for measuring serum antibody responses to viral infection. Therefore, we defined neutralization antibody-positive sera as SFTSV antibody true-positives and concluded that the serum of participant No. 38 was a false-positive in the ELISA and IFA.

Notably, sera obtained during the SFTS convalescent phase cross-react with Bhanja and Heartland viruses, which are tick-borne phleboviruses closely related to SFTSV (8). This cross-reactivity among tick-borne phleboviruses may be because of conserved amino acids in the nucleocapsid protein but not glycoproteins, which is the target for neutralizing antibodies (8). Further studies must be conducted to determine whether any viruses antigenically related to SFTSV are present in Japan, China, or South Korea.

Overall, the results suggest a low seroprevalence of SFTS antibodies in healthy individuals living in an endemic area. Furthermore, hunters (who were thought to be at a higher risk of tick bite) are not at a high risk of infection. This study enhances the understanding of the seroprevalence of anti-SFTSV antibodies in an endemic area in Japan. Further surveillance of healthy individuals living in endemic and non-endemic areas must be conducted to validate the risk factors associated with SFTS in humans.

Acknowledgments This work was supported in part by the Japan Agency for Medical Research and Development (AMED) and by the Ministry of Health, Labour, and Welfare (grant number H25-Shinko-Shitei-009). We thank Drs. Nobuyuki Minami, Hiroshi Fukuda, Junichiro Nishi, and Koumei Shirabe for help with blood sampling and testing. We also thank Drs. Masayuki Shimojima, Takeshi Kurosu, Shumpei Watanabe, Hideki Tani, Satoshi Taniguchi, and Aiko Fukuma, and Ms. Momoko Ogata (Department of Virology 1, NID), for technical support and helpful discussion.

Conflict of interest None to declare.

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