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Original Article

Seroprevalence of severe fever with thrombocytopenia syndrome (SFTS) virus antibodies in humans and animals in Ehime prefecture, Japan, an endemic region of SFTS☆

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ABSTRACT

Severe fever with thrombocytopenia syndrome (SFTS) was first identified as an emerging tick-borne infectious disease caused by the SFTS virus (SFTSV) in China and has also been found to be endemic to Japan and South Korea, indicating that SFTS is of great concern in East Asia. The aim of the present study was to determine the seroprevalence of SFTSV antibodies in humans and animals in SFTS-endemic regions of Japan. One of 694 (0.14%) healthy persons over 50 years of age and 20 of 107 (18.7%) wild and domestic animals in Ehime prefecture of western Japan were determined to be seropositive for SFTSV antibodies by virus neutralization test and ELISA, respectively. The seropositive person, a healthy 74-year-old woman, was a resident of the southwest part of Ehime prefecture engaged in citriculture and field work. This woman's sample exhibited neutralizing activity against SFTSV although she had neither a clear experience with tick bites nor SFTS-like clinical illness. These findings indicate that most people living in the endemic regions are not infected with SFTSV and suggest that most of the SFTS patients reported so far do not reflect the tip of an iceberg of people infected with SFTSV, but at the same time, that SFTSV infection does not always induce severe SFTS-associated symptoms. These findings also suggested that SFTSV has been maintained in nature within animal species and ticks.

1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is a newly identified tick-borne viral infection caused by a novel bunyavirus, SFTS virus (SFTSV), with a high case fatality rate in China [1–3]. SFTS is also endemic to Japan and South Korea [4,5] and is of great concern in East Asia. Japanese and Chinese SFTSV isolates formed distinct clusters in phylogenetic trees [5], suggesting that they have evolved independently. The clinical characteristics are the same between Chinese and Japanese cases [1,5,6], but the case fatality rate in Japan (approximately 23%) appears to be higher than that in China (approximately 12%) [1,3,5,6]. Japanese SFTS cases have been reported chiefly in western Japan so far. The seroprevalence of

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SFTSV antibodies (Ab) in the populations living in the SFTS-endemic regions in Japan has not yet been reported, and the SFTSV seroprevalence in animals, especially in wild animals, is not well understood. The aim of the present study was to determine the seroprevalence and risk factors of SFTSV infection to provide new insights into guiding future decisions on preventive measures for controlling SFTS in Japan.

2. Materials and methods

2.1. Collection of SFTS patients' data in Japan and in Ehime prefecture

The data regarding SFTS notifications in Japan were obtained from the National Epidemiological Surveillance of Infectious Diseases (NESID) database (https://nesid3g.wish.mhlw.hq.admix.go.jp), and those in Ehime prefecture were obtained from the Ehime Prefectural Epidemiological Surveillance of Infectious Diseases (EESID) database (http://www.pref.ehime.jp/h25115/kanjyo/index.html).

2.2. Sample collection from humans and animals

All of the protocols and procedures in this study were approved by the Research Ethics Committee of both the National Institute of Infectious Diseases (approval no. 594) and the Ehime Prefectural Institute of Public Health and Environmental Science (approval no. 236-2). Because most of the SFTS patients in Japan have been over 50 years of age, such people might be particularly susceptible to SFTSV. Therefore, in this first surveillance in Ehime prefecture, we focused on people living in the endemic areas who were over 50 years of age. Thus, blood samples were collected from 694 healthy participants over the age of 50 years living in the Chuyo, Yawatahama, and Uwajima public health center jurisdictions (PHCJ) from July to August 2015. The participants were informed of the study purposes upon enrollment, and written consent was obtained from each participant. All of the participants enrolled provided information regarding their sex, age, place of residence, outdoor activities, presence of pets, experience with tick bites, and chronic diseases under treatment including diabetes mellitus, hyperlipidemia, hypertension, kidney disease, heart disease, and stroke.

In addition to human sera, sera of 107 animals (40 wild boars, 20 wild deer, 33 outdoor dogs, and 14 impounded dogs) chiefly inhabiting the three jurisdictions were collected from November 2013 to February 2014.

The human and animal sera were first tested for SFTSV Ab detection with an IgG-enzyme-linked immunosorbent assay (ELISA). The human sera that showed a positive reaction in the ELISA were further tested by indirect fluorescence assay (IFA) and virus neutralization test (VNT), whereas the animal sera were not.

2.3. ELISA

IgG-ELISA was performed as described previously [7] with some modifications. Briefly, ELISA plates that had been pre-coated with SFTSV (HB29 strain)- or mock-infected Huh7 cell lysates were blocked and incubated with 100-fold diluted serum samples. The plates were further incubated with horseradish peroxidase (HRP)-conjugated goat anti-human IgG (Thermo Fisher Scientific, Inc.) for human samples or HRP-conjugated protein A/G for animal samples (Thermo Fisher Scientific, Inc.). Colorization was done using ABTS substrate [2,2azinobis (3-ethylbenzthiazolinesulfonic acid)], and the optical density (OD) at 405 nm was measured with a reference at 490 nm. Serum samples showing an adjusted OD value > 0.1 were regarded as positive.

2.4. IFA

IFA was performed as described previously [5,8] with some modifications. To prepare the antigens for the IFA, a mixture of SFTSV (HB29 strain)-infected and -uninfected Vero cells was spotted onto multi-well glass slides, air-dried, UV-treated, and fixed with acetone/methanol. The slides were incubated with diluted serum samples (1:10 to 1:640), followed by FITC-labeled goat anti-human IgG (Thermo Fisher Scientific, Inc.). The slides were then examined for the staining patterns of fluorescent signals under a fluorescence microscope (BZ-X710, KEYENCE). The reciprocals of the maximum dilution levels, at which the fluorescent signals were positive, were regarded as the SFTSV IFA-Ab titers. IFA Ab-titers of less than 10 were regarded as negative.

2.5. VNT

A VNT was performed as described previously [5,9] with some modifications. Approximately 100 focus-forming units of SFTSV (YG1 strain) were incubated with diluted serum samples for 1 h and then inoculated onto Vero cell monolayers. After 1 h incubation, the cells were washed and overlaid with 1% methylcellulose-containing culture medium. At 8 days of cell culture, the cells were fixed with 10% formalin and treated with UV. The cells were permeabilized with 0.1% Triton X-100 and stained with rabbit anti-N polyclonal Ab followed by HRP-conjugated goat anti-human IgG (Thermo Fisher Scientific, Inc.). The HRP activities were detected by 3,3′-diaminobenzidine (DAB) to visualize the foci induced by SFTSV replication. Experiments were performed in duplicate. The reciprocals of the maximum dilution levels, at which the number of the focuses decreased to less than 50% of the control, were regarded as the SFTSV-VNT Ab titers. VNT Ab-titers of less than 20 were regarded as negative.

3. Results

3.1. SFTSV cases in Ehime prefecture as of September 8, 2017

As of September 8, 2017, 297 patients with SFTS had been registered in the NESID database from 23 prefectures in western and central Japan (Fig. 1A). In total, 25 patients, all over 50 years of age, were reported from Ehime prefecture, and the number of notifications ranked in the top five among 23 prefectures at the time. Twenty-four of these patients, including 8 patients who died (case fatality rate, 33.3%), resided in Ehime, and 21 of them (87.5%) lived in the Chuyo, Yawatahama, and Uwajima PHCJ according to the EESID database, where farming and forestry are the main industries (Fig. 1B).

3.2. Seroprevalence of SFTSV Ab in the Chuyo, Yawatahama, and Uwajima PHCJ

The population composition of the enrolled participants is shown in Table 1. Among the 694 samples, 8 (1.15%) showed positive reactions in the IgG-ELISA (Table 2). These 8 samples were further assessed with IFA and VNT. Two of the 8 samples (0.29%) firmly reacted with SFTSV antigens (IFA-Ab titers: #302, 640; #584, 320) in IFA. The ELISA-positive samples were also tested further with a VNT, revealing that one (#302) of the 8 samples (0.14%) exhibited VNT-Ab positivity at a titer of 80. Sample #302 had the highest ELISA value, IFA titer, and VNT titer of all samples tested.

3.3. Interview with the VNT Ab-positive individual

The SFTSV Ab-positive participant (#302) was a healthy 74-year-old woman living in the Yawatahama PHCJ who was engaged in

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