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Original Article

Seroprevalence for severe fever with thrombocytopenia syndrome virus among the residents of Miyazaki, Japan: An epidemiological study

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ABSTRACT

Introduction: Severe fever with thrombocytopenia syndrome (SFTS) is a tick-borne infectious disease caused by the SFTS virus (SFTSV). The Miyazaki Prefecture has the highest number of SFTS cases in Japan and requires countermeasures for prevention. In this study, we aimed to conduct an epidemiological survey in Miyazaki Prefecture to determine the exposure conditions of SFTSV by measuring the seroprevalence among residents of Miyazaki and to evaluate the factors that influence the endemicity of SFTS.
Methods: The survey was conducted between June 2014 and April 2019 in all 26 municipalities in Miyazaki Prefecture. SFTSV antibodies were detected using an enzyme-linked immunosorbent assay in the blood samples of 6013 residents (3184 men and 2829 women). A questionnaire-based survey of the living environment was also conducted.
Results: Multiple logistic regression analysis revealed that age and occupation were significant factors related to the proportion of participants with an optical density (OD) value > 0.2 and a seroprevalence of 0.9 % (54/6013). Seven seropositive individuals (0.1 %) with an OD value of >0.4 were identified (three men and four women, aged 54–69 years), and all were asymptomatic. One participant had a higher OD than the positive control.
Conclusion: Although SFTS is endemic in Miyazaki Prefecture, Japan, its seroprevalence is relatively low. Since some risk areas in Miyazaki prefecture have been identified, it is important to enhance awareness of SFTS in residences and reduce contact with ticks, especially in high-risk areas.

1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne infectious disease caused by SFTS virus (SFTSV). SFTSV is a member of the genus *Phlebovirus* within the family *Bunyaviridae* [1,2]; it was newly renamed "*Dabie bandavirus*" of the genus *Bandavirus* within the family *Phenuiviridae* by the International Committee on Taxonomy of Viruses in 2018 [3]. The clinical manifestations of this disease include fever, leukopenia, thrombocytopenia, liver dysfunction, and gastrointestinal problems. These symptoms are often followed by hemophagocytic syndrome and hemorrhage, leading to multiple organ dysfunction [2]. The case fatality rate (CFR) of this disease ranges from 2.5 % to 30 % [4].

Tick bites are the most common routes of virus transmission [5,6]. A previous study reported the detection of SFTSV in *Haemaphysalis longicornis, Amblyomma testudinarium*, and *Ixodes nipponensis* in China, South Korea, and Japan [7]. Furthermore, as non-tick-borne infections from infected animals to their owners or veterinary personnel have been reported in Japan, close contact with infected animals is also an important route of infection [8–10]. In 2009, the first patient with SFTS was identified in Hubei and Henan provinces in China [1,2]; since then, the number of SFTS cases has been increasing. Overall, 7419 laboratory-confirmed SFTS cases were reported in 23 provinces of China between 2010 and 2016 [11]. SFTS cases have also been confirmed in South Korea, Japan, Vietnam, and Taiwan [12–16]. The number of cases in South Korea and Japan has been increasing annually, reaching 866 in South Korea in 2018 and 792 in Japan in 2022 [12,17]. In Japan, the annual number of patients with SFTS has gradually increased since

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2013, and the highest number of cases is 116 in 2022. The CFRs of the SFTS were 11.6 % in 2022 and 35 % in 2013 [12]. Among the 47 prefectures in Japan, SFTS cases were confirmed in 29 prefectures in the western part of the country. In particular, Miyazaki, Yamaguchi, and Kagoshima prefectures in western Japan had a higher number of SFTS cases [12,18].

Miyazaki Prefecture, located on the eastern coast of Kyushu Island, has the highest number of SFTS cases in Japan, with 107 confirmed cases reported in July 2023 [12]. Miyazaki Prefecture has a warm, humid climate. The Ministry of Agriculture, Forestry and Fisheries reported that three-fourths of Miyazaki prefecture or approximately 600,000 ha (1,480,000 acres) is covered by forest [19], which is a suitable environment for ticks and wild animals infected with SFTS [20]. Therefore, these conditions may have contributed to the increase in the number of SFTS cases in Miyazaki Prefecture.

Some SFTS patients remain asymptomatic; however, the actual situation remains unclear. Several studies have identified several asymptomatic patients in China, while the seroprevalence rates of SFTSV in healthy individuals were 0.4%–4.7 % [4,21]. However, the seroprevalence rates in Japan were not apparent. Therefore, this study aimed to investigate the seroprevalence of SFTSV on a large proportion of individuals in the Miyazaki area, which has the highest number of SFTS cases in Japan. It also aimed to evaluate the epidemiological factors that influenced the SFTS endemic situation.

2. Materials and methods

2.1. Study participants

This study was conducted between June 2014 and April 2019. The Miyazaki Prefecture has 26 municipalities. A total of 6013 adults aged 40–75 years were recruited during a health checkup conducted by the municipalities' health agency in Miyazaki. All participants provided informed consent after receiving an explanation sheet that contained information regarding the study. Each participant was interviewed using a questionnaire that included questions on sex, age, occupation, place of residence, livestock breeding, presence of pets, agricultural work, history of tick bites, and history of unidentified fever. Peripheral blood samples were collected from each participant. This study was approved by the Ethics Committee of the Faculty of Medicine at the University of Miyazaki (approval number: 2017-155).

2.2. Serological test

Serum samples were obtained from the participants and stored in a freezer at −20 °C until the serological checkup. SFTSV antibodies were detected using an enzyme-linked immunosorbent assay (ELISA) [22]. Viral antigens were obtained from Huh-7 cells infected with SFTSV. The plate was coated with infected and mock cell lysates, diluted at 1:800 with phosphate-buffered saline (PBS), and incubated overnight at 4 °C. After washing with PBS with 0.05 % Tween 20 (PBST), it was blocked with modified PBS with Tween 20 (MPBST) (5 % skimmed milk and 0.05 % MPBST) for 2 h at room temperature (RT). After washing with PBST, the samples were diluted with MPBST (1:100–1:400) and incubated for 2 h at RT. Following a PBST wash, goat anti-human immunoglobulin G (H + L) HRP conjugate (Zymax Grade, Zymed Laboratories Inc., CA, USA) was diluted with MPBST and incubated for 1 h at RT. A final wash with PBST was performed, and the cells were incubated with ABTS for 30 min at RT. The optical density (OD) was measured at 405 nm using a reference wavelength of 490 nm. The serum of patients with SFTS was used as a positive control, while the serum of healthy participants without a history of SFTS was used as a negative control.

2.3. Geographical distribution

QGIS version 3.12.0 (QGIS Development Team, 2020) was used to plot the addresses of patients with SFTS on the map. The geographic locations of patients with SFTS in Miyazaki Prefecture until June 2017 were derived from the Annual Report of the Miyazaki Prefectural Institute for Public Health and Environment [23,24]. A geospatial distribution of municipal seroprevalence was also constructed, indicating each OD value (>0.2, >0.3, and >0.4) using the post office number of the resident location. The land data were collected from the National Land Numerical Information Database [25].

2.4. Statistical analyses

Wilcoxon rank-sum and Kruskal–Wallis tests were used to compare continuous variables, while Fisher's exact test was used to compare categorical variables. Univariate analysis was conducted to identify the risk factors for SFTSV infection according to the OD value. Multivariate analysis was conducted to avoid the effects of confounding factors after adjusting for sex, age, occupation, livestock, pets, agricultural work, history of tick bites, and history of unidentified fever. P values of <0.05 and <0.01 were considered significant. All statistical analyses were performed using R ver. 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria) and JMP version 16 (SAS Institute, Cary, NC, USA).

3. Results

Overall, 6013 healthy serum samples were collected for this study, and none of the participants had a confirmed medical history of SFTS. Table 1 presents the characteristics of the study population. Among the 6013 participants, 53.0 % were women. The participants were aged 40–75 years; of the total participants, 50.7 % were aged 60–69 years, while 80 % were aged >60 years. Most were farmers (28.6 %). Approximately 4.9 % of the participants fed on livestock, while 36.1 % fed on pets. Additionally, 78.3 % of the participants were engaged in

Table 1
Characteristics of the participants of the study.

Variable	Participants (n = 6013) n (%)
Gender	
Female	3184 (53.0)
Male	2829 (47.0)
Age group	
40–49	473 (7.9)
50–59	775 (12.9)
60–69	3049 (50.7)
70–75	1716 (28.5)
Occupation	
Farmer	1718 (28.6)
Dairy	77 (1.3)
Company	278 (4.6)
Other	3940 (65.5)
Livestock	
No	5604 (93.2)
Yes	294 (4.9)
NA	115 (1.9)
Pet	
No	3730 (62.0)
Yes	2168 (36.1)
NA	115 (1.9)
Agricultural work	
No	1070 (17.8)
Yes	4707 (78.3)
NA	236 (3.9)
Tick bite history	
No	5012 (83.4)
Yes	886 (14.7)
NA	115 (1.9)
Unidentified fever history	
No	4792 (79.7)
Yes	30 (0.5)
NA	1191 (19.8)

NA: Not Available (unknown).

agricultural work, and 15 % had a history of tick bites.

Table 2 shows the seroprevalence rates of SFTSV based on the OD value (>0.2, >0.3, and 0.4) by municipality in the Miyazaki prefecture. The variation in the distribution of seroprevalence by municipality was not uniform. The relationship between the OD values and participant characteristics was evaluated. All negative controls were under OD of 0.2, and the mean OD of all samples was 0.013 (standard deviation [SD]: 0.047). Therefore, an OD of 0.2 is bigger than the "mean + 3 SD" and near the "mean + 4 SD". Therefore, using an OD of 0.2 for seroprevalence may be appropriate and valid. Seven seropositive individuals (OD values > 0.4) were confirmed in seven municipalities; among them, three were men aged between 54 and 69 years. Of these participants, one showed an OD value of 0.6, while the other had a higher OD value than the positive control. As confirmed cases were detected between spring and autumn in Miyazaki Prefecture, the seasonal variations in the distribution of seroprevalence were assessed. The distributions of seroprevalence were divided into "April to October" and "November to March" and compared. However, no significant differences were observed between the groups.

Fig. 1 shows the geographic distribution of seroprevalence based on an OD value of 0.2 in Miyazaki Prefecture. The dots in the figure indicate patients who were diagnosed and hospitalized until June 2017 [23,24]. These patients lived in the area between the farmland and forest, and areas with an OD of 0.2 were near the seaside and large rivers.

Table 3 shows the participants' characteristics by OD value. In the group with an OD value of >0.2, the proportion of participants aged 40–59 years was higher than that of participants aged 60–75 years (crude OR: 0.48, 95 % confidence interval [CI]: 0.27–0.89, $P = 0.02$), and the majority of participants were farmers (crude OR: 2.34, 95 % CI: 1.31–4.16, $P = 0.002$).

Table 4 shows the relationships between the OD values and characteristics using multiple logistic regression analysis. A higher proportion of the participants (OD values > 0.2) were aged 40–59 years and engaged in farm work (60–75 years: adjusted OR: 0.45, 95 % CI: 0.22–0.95, $P = 0.03$; farmers: adjusted OR: 2.35, 95 % CI: 1.03–5.46, $P = 0.04$).

Table 2
Seroprevalence of SFTSV by OD value by the municipality of Miyazaki prefecture.

Municipality	Participants (n = 6013)	Titer		
		>0.2 (n = 54)	>0.3 (n = 17)	>0.4 (n = 7)
		n (%)	n (%)	n (%)
A	615	8 (1.3)	4 (0.7)	1 (0.2)
B	102	0	0	0
C	312	1 (0.3)	1 (0.3)	1 (0.3)
D	87	0	0	0
E	388	0	0	0
F	393	2 (0.5)	1 (0.3)	1 (0.3)
G	215	1 (0.5)	1 (0.5)	0
H	276	0	0	0
I	239	1 (0.4)	1 (0.4)	1 (0.4)
J	146	2 (1.4)	1 (0.7)	0
K	202	0	0	0
L	151	1 (0.7)	1 (0.7)	1 (0.7)
M	357	0	0	0
N	307	6 (2.0)	1 (0.3)	0
O	141	16 (11.3)	4 (2.8)	0
P	134	7 (5.2)	1 (0.7)	1 (0.7)
Q	160	0	0	0
R	92	2 (2.2)	1 (1.1)	1 (1.1)
S	173	1 (0.6)	0	0
T	162	2 (1.2)	0	0
U	144	0	0	0
V	177	0	0	0
W	228	0	0	0
X	403	0	0	0
Y	183	4 (2.2)	0	0
Z	226	0	0	0

4. Discussion

Several studies have reported that farmlands in rural areas, especially those near the mountains or hills, are highly endemic areas for SFTSV infections [26,27]. By contrast, Yasuo et al. indicated that lowland areas near the rivers might also be high-risk areas for infection [24]. A seroprevalence surveillance was conducted among healthy individuals in Miyazaki Prefecture to detect high-risk areas for SFTSV exposure [21,27]. This study was the first large-scale serological survey performed in all municipalities of Miyazaki Prefecture.

The distribution of seropositive participants based on the OD values was evaluated (>0.2, >0.3, and >0.4). The prevalence of SFTS was higher in municipalities located in the eastern areas of Miyazaki, which directly faces the Pacific region and has a low altitude (Fig. 1). Yasuo et al. reported that the prevalence of SFTS is high in low-altitude areas near the rivers [24]. Therefore, areas with low altitudes near the rivers or coasts are at high risk of tick exposure. The area with high proportion of seropositive cases (over OD = 0.2, 0.3, 0.4) were not always matched the area where SFTS patients lived. First reason of this discrepancy could be that since subclinical SFTS infection was indicated some reports, people exposed to SFTS virus could not necessarily get ill. And the rate of subclinical infection was reported from 5 % to 1.1 % [28–32]. Second reason might be that since many of the patients are over 60 years old in Miyazaki prefecture, old people could be easy to illness. The iceberg phenomenon in SFTSV infections is considered a serious problem [33]. The seroprevalence rates were 0.1 % (7/6013) with an OD value of >0.4, 0.3 % (17/6013) with an OD value of >0.3, and 0.9 % (54/6013) with an OD value of >0.2. A previous study conducted in healthy individuals in the Shandong and Jiangsu provinces of China showed that the seroprevalence rate was lower than that reported in our study [21,27]. However, other studies have indicated higher seroprevalence. The Jeju Island in South Korea has a seroprevalence rate of 2.4 % [28]. Studies in China have reported a rate of >5 % [29,30]. In Japan, the seroprevalence rates in Kagoshima and Ehime prefectures were 1.1 % and 1.2 %, respectively [31,32]. Japan had a lower seroprevalence than China. However, the seroprevalence among healthy individuals is contradictory. The seroprevalence rates also differ depending on age, occupation, and environmental conditions [34,35]. However, the underlying mechanism remains unclear.

Several studies have identified the persistence of antibody titers among individuals who were infected by the SFTSV [36,37]. Unlike other infectious diseases with lifetime immunity, such as measles, the antibody titer of SFTSV decreased in 3–4 years, indicating that lifetime immunity may not be obtained [36]. However, owing to the insufficient number of studies investigating whether lifetime immunity can develop after infection with SFTSV, additional evaluation is needed. With regard to the seasonal changes in the seroprevalence of SFTSV, a 3-year study conducted in Xinyang, China, reported a higher seroprevalence in October than in other months [29]. This could be due to the high number of SFTS cases detected in August. However, the seroprevalence rates between "April to October" and "November to March" were not evaluated.

In this study, an OD value of 0.2 was set as the cutoff value. The relationship between the participants' OD values (>0.2, >0.3, and >0.4) and their background characteristics was also evaluated (Tables 3 and 4). Age is an important risk factor for SFTSV infection. A previous study indicated that patients with SFTS were aged 1–93 years, but the majority were aged 40–79 years [27]. The present study found that people in the 40–59-year age group were more likely to be exposed to SFTSV, although the incidence of SFTS infection in Miyazaki Prefecture was higher in those aged >60 years [12,23,38]. Another study also revealed that older age was a risk factor for SFTS [33,39,40], although the risk of exposure to SFTSV among patients aged 40–59 years was higher than that among patients aged >60 years [30]. People of all ages may be

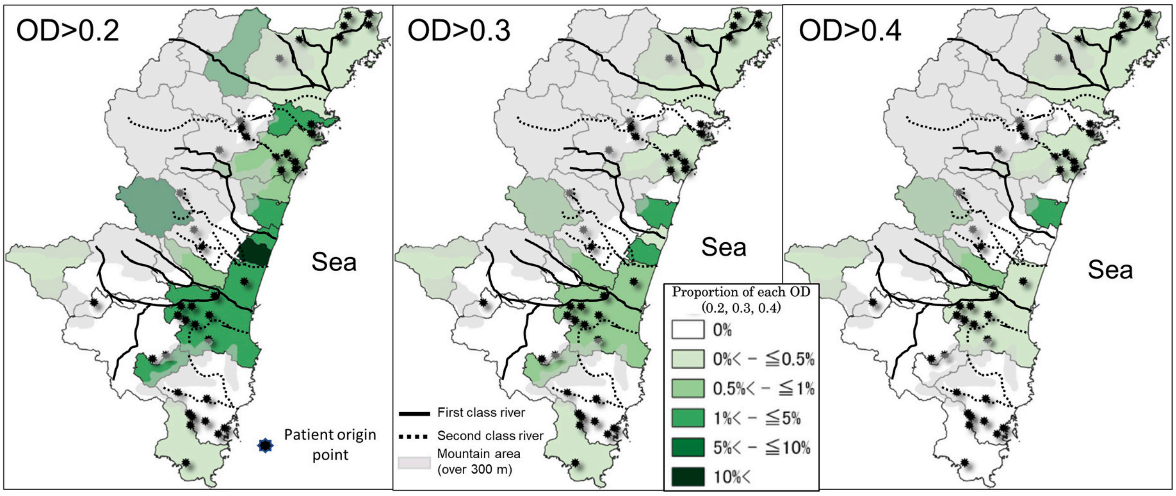

Fig. 1. Geographic distribution of SFTS seroprevalence and the resided point of SFTS patients in the Miyazaki prefecture.
Seroprevalence is designated by the percentage of participants with OD > 0.2 value in each municipality. The dot in the figure indicates the resided area of confirmed patients of SFTS by June 2017 [23].

Table 3
The characteristic of participants indicated over OD 0.2 value (>0.2, >0.3, >0.4).

Variable	Participants (n = 6013)	>0.2 (n = 54)			>0.3 (n = 17)			>0.4 (n = 7)		
		n	Crude OR (95 % CI)	*P*	n	Crude OR (95 % CI)	*P*	n	Crude OR (95 % CI)	*P*
Gender										
Female	3184	31	Reference		10	Reference		4	Reference	
Male	2829	23	0.83 (0.46–1.48)	0.58	7	0.79 (0.25–2.29)	0.81	3	0.84 (0.12–4.99)	>0.99
Age group										
40–59	1248	19	Reference		4	Reference		2	Reference	
60–75	4765	35	0.48 (0.27–0.89)	0.02*	13	0.85 (0.26–3.59)	0.77	5	0.65 (0.11–6.88)	0.64
Occupation										
Non-farmer	4295	28	Reference		9	Reference		4	Reference	
Farmer	1718	26	2.34 (1.31–4.16)	0.002*	8	2.23 (0.75–6.52)	0.11	3	1.88 (0.27–11.10)	0.42
Livestock										
No	5604	40	Reference		13	Reference		6	Reference	
Yes	294	4	1.92 (0.50–5.36)	0.28	0	–	–	0	–	–
NA	115	10	–	–	4	–	–	1	–	–
Pet										
No	3730	28	Reference		11	Reference		6	Reference	
Yes	2168	16	0.98 (0.50–1.89)	>0.99	2	0.31 (0.03–1.43)	0.15	0	–	–
NA	115	10	–	–	4	–	–	1	–	–
Agricultural work										
No	1070	7	Reference		2	Reference		1	Reference	
Yes	4707	29	0.94 (0.40–2.55)	0.83	10	1.14 (0.24–10.69)	>0.99	5	1.14 (0.13–53.82)	>0.99
NA	236	18	–	–	5	–	–	1	–	–
Tick bite history										
No	5012	41	Reference		13	Reference		6	Reference	
Yes	886	3	0.41 (0.08–1.30)	0.14	0	–	–	0	–	–
NA	115	10	–	–	4	–	–	1	–	–
Unidentified fever										
No	4792	32	Reference		10	Reference		5	Reference	
Yes	30	0	–	–	0	–	–	0	–	–
NA	1191	22	–	–	7	–	–	2	–	–

Non-farmer included dairy, company and other occupations besides farmer.
NA: Not Available (unknown).
OR: odds ratio, 95%CI: 95 % confidence interval.
*: P < 0.05, -: No evaluation.

susceptible to SFTSV infection, but older individuals tend to experience more severe symptoms and are hospitalized or even die from the infection [39,41]. This finding indicates that relatively young individuals may be more prone to subclinical infections than older individuals.

Sex was not associated with SFTSV infection in the present study; therefore, men and women had similar probabilities of exposure. In Japan, sex does not influence the probability of SFTSV infection, and the sex distribution of patients is similar [12]. However, a previous study indicated that men had a higher risk of SFTSV infection than women because of their greater involvement in agriculture-related activities [42]. By contrast, women are more likely to experience SFTSV infection due to their higher involvement in tea picking than men [6,43]. The crucial factors for this infection were not related to sex, but to living conditions, role, and workplace [34].

Occupation also plays an important role in SFTSV infection. A meta-analysis concluded that farmers had a higher seroprevalence than those involved in other occupations [34]. This could indicate that farmers are

Table 4
The relation between over OD 0.2 value and characteristics.

Variable	Participants (n = 6013)	>0.2 (n = 54)		
		n	Adjusted OR (95 % CI)	*P*
Gender				
Female	3184	31	Reference	
Male	2829	23	0.95 (0.46–1.92)	0.88
Age group				
40–59	1248	19	Reference	
60–75	4765	35	0.45 (0.22–0.95)	0.03*
Occupation				
Non-farmer	4295	28	Reference	
Farmer	1718	26	2.35 (1.03–5.46)	0.04*
Livestock				
No	5604	40	Reference	
Yes	294	4	1.55 (0.36–4.75)	0.49
NA	115	10	–	–
Pet				
No	3730	28	Reference	
Yes	2168	16	0.68 (0.30–1.44)	0.33
NA	115	10	–	–
Agricultural work				
No	1070	7	Reference	
Yes	4707	29	0.61 (0.24–1.68)	0.31
NA	236	18	–	–
Tick bite history				
No	5012	41	Reference	
Yes	886	3	0.60 (0.14–1.72)	0.40
NA	115	10	–	–
Unidentified fever				
No	4792	32	Reference	
Yes	30	0	–	–
NA	1191	22	–	–

Non-farmer included dairy, company and other occupations besides farmer.
NA: Not Available (unknown).
OR: odds ratio, 95 % CI: 95 % confidence interval.
*: $P < 0.05$, -: No evaluation.
Adjusted OR: gender, age, occupation, livestock, pet, agricultural work, tick bite history and unidentified fever history were adjusted.

more likely to be exposed to ticks, which are known vectors of SFTSV. Grass mowing and bush grazing are additional risk factors for tick contact [30]. This study also found that farmers had a higher probability of exposure to SFTSV than non-farmers. In this study, the proportion of farmers was higher in the 40–59-year age group than in the 60–75-year age group. This may explain the higher seroprevalence in individuals aged 40–59 years than in individuals aged >60 years.

Additionally, people with livestock and pets are more likely to be exposed to the SFTSV [21,40,44]. However, no association was found between SFTSV infections in livestock and pets in the present study. SFTSV infection in animals has been reported in 69.5 % of sheep, 60.5 % of cattle, 3.1 % of pigs, 37.9 % of dogs, and 47.4 % of chickens in China [45]; meanwhile, it was only reported in 2 % of cattle in Japan [46]. In Japan, the seropositivity rate of wild animals such as wild boar and deer is high in SFTSV endemic areas [47–49]. Symptomatic SFTS in animals also reported including captive cheetahs, dogs, and cats [50–52]. The clinical features of these animals are similar to those of human SFTS disease, but the fatality rate is higher in cats and dogs than in humans [51,52]. In addition, there have been reports of transmission of SFTSV from an infected domesticated cat to humans [53,54]. Therefore, close contact with infected animals is considered as another important route of transmission. Further epidemiological studies on SFTSV infection in animals are required to estimate the risk of SFTSV transmission from domestic animals and livestock to humans.

This study has some limitations. First, as our participants underwent specific health checkups by prefectural health agencies, their age range was limited to 40–75 years old. The overall median age of patients with SFTS in Japan is 75 years [12], which is higher than the median age of our sample (65 years). The association between younger age and SFTSV infection could not be evaluated. Therefore, the study sample may have been subject to selection bias. However, as more than 70 % of the participants were aged >60 years and nearly 30 % were aged >70 years, this study could be useful for evaluating seroprevalence in a healthy population in Japan. Second, the percentage of samples per population was not the same among the municipalities due to physical and financial constraints. Therefore, the detection power varies depending on the municipality in question. Third, the relationship between an OD value of 0.2 and participant characteristics was evaluated. ELISA is not suitable for evaluating the SFTSV antibody titers. Neutralization could be needed to confirm the presence of antibodies of SFTS. However, as some studies have used ELISA for testing the seroprevalence, ELISA was used for evaluation in the present study. Since an OD value of 0.2 was bigger than the "mean OD + 3 SD," an OD value of >0.2 could be an appropriate and suitable cut-off value. Kim, K.H. also set cut-off level using same method: mean + 3 SD [35]. Despite these limitations, this is the first large-scale study to report the seroprevalence in Miyazaki Prefecture.

5. Conclusions

The seroprevalence of SFTSV infection among the residents of Miyazaki Prefecture, an endemic area in Japan, was lower than that in China. The risky areas could be relatively concentrated near the seaside and large rivers in Miyazaki Prefecture. Awareness of the risk factors and areas of this infection is required to prevent its spread. These results may be useful for public health organizations in preventing SFTSV infection.

Authorship statement

Contributor Takuji Hinoura was responsible for the organization and coordination of the trial. Kazuhiro Hidaka was the chief investigator and responsible for the data analysis. Shuya Mitoma, Junzo Norimine, Masayuki Shimojima and Yoshiki Kuroda developed the trial design. All authors contributed to this study and the writing of the final manuscript.

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Declarations of competing interest

None of declare.

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