



Original article

Seroprevalence of severe fever with thrombocytopenia syndrome virus antibodies among inhabitants of Dachen Island, eastern China

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ARTICLE INFO

Keywords:

SFTSV
Seroprevalence
Antibody
Island

ABSTRACT

Severe fever with thrombocytopenia syndrome virus (SFTSV) has been emerging and has caused many human cases in China, Japan and Korea. Some studies speculated that SFTSV was transmitted with bird migration among these countries. Notably, SFTS cases have been identified in a Chinese island named Dachen which is situated southwest of Japan and Korea. In this study, we conducted a serum survey of SFTSV antibodies among inhabitants of the island. A total of 439 serum specimens were collected in June 2018. All serum samples were tested for total antibodies and IgM antibody with double-antigen sandwich ELISA method. The rates of seropositivity for SFTSV total antibodies and IgM antibody were 3.0% (95% CI 1.4–4.6) and 0.5% (2/439), respectively. The median age of all participants was 61 years and all seropositive samples were all from inhabitants aged > 50 years. The differences of seroprevalence between different gender groups and different age groups were not significant. However, seroprevalence varied significantly among different villages ($P = 0.033$). Our results showed that some inhabitants of Dachen Island had been infected with SFTSV, and some ticks and host animals of the island carry SFTSV. Comprehensive measures should be conducted to prevent the occurrence of SFTS cases in the island.

1. Introduction

Severe fever with thrombocytopenia syndrome virus (SFTSV) which was firstly reported in China in 2011 is the causative agent of severe fever with thrombocytopenia syndrome (SFTS; Yu et al., 2011). SFTSV is classified in Bunyavirales order, family *Phenuiviridae*, genus *Phlebovirus* and it consists of three segments of negative or ambisense polarity RNA, designated S, M and L segments. The major clinical characteristics and symptoms of SFTS include acute febrile, gastrointestinal symptoms, leukocytopenia, thrombocytopenia, and multi-organ dysfunction in severe cases (Yu et al., 2011). Of note, the case fatality rate of SFTS was reported up to 30% in China and 47.2% in Korea in hospitalized patients (Liu et al., 2014; Shin et al., 2015). Previous studies have determined that SFTSV could be transmitted through tick bites and direct contact with patient's blood or secretion (Luo et al., 2015; Bao et al., 2011; Gai et al., 2012; Gong et al., 2015).

In China, a total of 5360 laboratory-confirmed SFTS cases were reported from 2011 to 2016 (Sun et al., 2017). Moreover, SFTS cases

were also confirmed in southwestern areas of Japan and Korea (Takahashi et al., 2014; Shin et al., 2015). Some studies reported that SFTSV may be spread with bird migration among China, Japan and Korea (Keun et al., 2013; Yun et al., 2015). Interestingly, three cases were identified in a Chinese coastal island named Dachen from 2016 to 2018. It is a small island which affiliates to Jiaojiang District, Taizhou City, Zhejiang Province. The island is situated southwest of Japan and Korea. The identification of SFTS cases in the island indicated that some inhabitants might have been infected with SFTSV. In this study, we investigated the seroprevalence of SFTSV antibodies among inhabitants and explored tick species and density in the island.

2. Methods

2.1. Specimen collection

Dachen Island locates in the East China Sea, 52 km southeast of Jiaojiang District and about 3600 individuals live in the island. It

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Received 22 November 2018; Received in revised form 13 February 2019; Accepted 21 February 2019

Available online 22 February 2019

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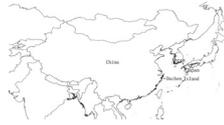


Fig. 1. Location of Dachen Island in Taizhou City, Zhejiang Province, China.

consists of “Shang Dachen Dao” (approximately 7 square kilometers) and “Xia Dachen Dao” (4.89 square kilometers). The highest point Fengwei Mountain is 228.6 m above sea level (28°26'N, 121°53'E). The island has a forest coverage rate of 50–60% and is a provincial-level marine forest park (Fig. 1). Residents are mainly engaged in fishing and farming.

Blood samples were collected from inhabitants of seven villages in Dachen Island in Jun 2018. Blood serum samples were separated in time and stored at -20°C prior to use. Additionally, the aims of our study were explained to all participants upon enrolment and demographic information including age, gender, place of residence was collected from each participant.

2.2. Tick surveillance

Flagging was used to collect host-seeking ticks in Dachen Island from March to October, 2018. Two sites were selected for tick surveillance and flagging lasted an hour in each site every month. All ticks were identified to the species level by standard guides (Teng et al., 1991).

2.3. Antibodies and viral RNA testing

All serum samples were tested for total antibodies (including immunoglobulin G [IgG] and immunoglobulin M [IgM]) with double-antigen sandwich ELISA method (Zhejiang Saijien Stem Cell Genetic Engineering Co., Ltd.), which had 100% sensitivity, and 99.57% specificity and a high correlation ($R^2 = 0.986$) (Jiao et al., 2012). All samples positive for total antibodies were also tested for IgM antibody and SFTSV RNA. If the total antibodies and IgM antibody of one sample were all positive, it was considered to be recent infection, and if the total antibodies were positive and IgM antibody was negative, it was considered to be previous infection.

Viral RNA was extracted using the MagMAX™-96 Viral Isolation Kit (AMB1836, Thermo Fisher Scientific, America) according to the manufacturer's instructions. The 50 μ l RNA elution was stored at -80°C until further use. Real-time RT-PCR was performed with ABI 7500 (Applied Biosystems, USA) using AgPath-ID™ One step RT-PCR kits (AM1005, Ambion, America). The primers and probes used for specimen screening of SFTSV RNA targeted L, and S segments of SFTSV (Sun et al., 2012). Serum samples with Cycle threshold (Ct) values below 40 were considered to be positive.

2.4. Statistical analysis

All statistical analyses were performed using the Statistical Product and Service Solutions software (spss v20; SPSS, Chicago, IL, USA). Differences in categorical variable levels were compared using a two-sided Chi square or Fisher's exact probability method. P-value of < 0.05 was considered statistically significant.

The dependent variable in the logistic regression was assigned as the serological status and the independent variables were village, gender, age group, body mass index (BMI) and underlying condition. The method of logistic regression used was forward-conditional. The step-wise probability was set to 0.05 for entry and 0.10 for removal. The classification cutoff was 0.5 and the maximum number of iterations was 20.

Table 1

Seroprevalence of SFTSV in samples from Dachen Island, southeastern China.

	Total (n)	Positive (n)	Positive rate (%)	χ^2 or Fisher	P
Gender				2.323	0.161
Male	192	3	1.6		
Female	247	10	4.1		
Age group				4.708	0.268
20–50 year	90	0	0		
51–60 year	119	4	3.4		
61–70 year	126	6	4.8		
71–80 year	68	2	3.0		
> 80 year	36	1	2.8		
Village				13.697	0.033
Bei'ao	49	5	10.2		
Nan'ao	99	4	4.0		
Fengwei	67	2	3.0		
Weixing	50	1	2.0		
Yanjiang	37	1	2.7		
Shengli	96	0	0		
Dashatou	41	0	0		
Total	439	13	3.0 (1.4–4.6)		

3. Results

A total of 439 serum samples were collected and tested for SFTSV antibodies in this study. The median age of participants was 61 years (range 22–99 years), 349 (79.5%) participants aged > 50 years, and 247 (56.3%) participants were female. Samples were collected from individuals living in seven villages of Dachen Island (Table 1). Most participants were from Nan'ao Village (99/439), and followed by Shengli Village (96/439).

The overall rate of seropositivity for SFTSV total antibodies was 3.0% (13/439) and rate of seropositivity for IgM antibody was 0.5% (2/439). The rate of seropositivity for SFTSV antibodies were 1.6% in males and 4.1% in females, but the difference was not significant ($\chi^2 = 2.323$, $P = 0.161$). Seropositive samples were all from inhabitants aged > 50 years, and the 61–70 years group showed the highest rate of seropositivity (4.8%), followed by 51–60 years group (3.4%) and 71–80 years group (3.0%). However, the differences of seroprevalence between different age groups were not significant (Fisher = 4.708, $P = 0.268$). Rates of seropositivity among samples from different villages were significantly different ($\chi^2 = 13.697$, $P = 0.033$; Table 1), and samples from Bei'ao Village had the highest rate (10.2%).

According to results of logistic analysis, the Chi-square value in omnibus tests of model coefficients was determined to be 60.779 ($P < 0.05$). Furthermore, the overall correct percentage was found to be 97.0%. Variables in the equation were residence village and the wald was 4.720. The equation also indicated that residence village was significantly associated with seropositivity of SFTSV.

Two residents whose blood samples were positive for SFTSV IgM antibody were a 74 year male and a 64 year female, respectively. The results of SFTSV RNA detection for all seropositive samples were negative. After follow-up interviews, both residents did not have any SFTSV related-symptoms within one month, and they could not recall whether they had been bitten by animals or insects within one year.

A total of 607 adult ticks including *Haemaphysalis longicornis*, *Ixodes sinensis*, and *H. megaspinosa* were captured in Dachen Island in 2018. The dominant tick species was *H. longicornis* and the tick density peaked in May (Table 2).

4. Discussion

Herein, we firstly reported the seroprevalence of SFTSV among inhabitants of a coastal island named Dachen in southeast China where SFTSV case was emerging. Dachen Island is a coastal island which is far from the inland. The seroprevalence of SFTSV was found to be 3.0%

Table 2
Tick species and abundance in Dachen Island, 2018.

	<i>H. longicornis</i>	<i>I. sinensis</i>	<i>H. megaspinosa</i>	Total
March	90	43	1	134
April	34	58	0	92
May	238	0	0	238
June	87	0	0	87
July	8	0	0	8
August	16	0	0	16
September	30	0	0	30
October	2	0	0	2
Total	505	101	1	607

among Dachen Island residents, which was lower than that in other areas of Zhejiang Province (Xiangshan 10.57%, Liangdu 8.18%) and Hubei Province (6.37%), but it was much higher than that in Xianju County (1.5%), Shandong Province (0.84%), Jiangsu Province (0.94%), and Japan (0.30%; Gokuden et al., 2018; Zhan et al., 2013; Liang et al., 2014; Sun et al., 2015; Zhao et al., 2012). The discrepancy of seroprevalence might be attributed to different land cover, resident characteristics, ecology of tick and domesticated animals, and different testing methods (Gokuden et al., 2018; Rocha et al., 2013).

Two individuals presented no SFTS-related symptoms, whereas their blood samples were positive for SFTSV IgM. The result indicated that subclinical or mild SFTSV infection might be common among health populations, which was similar to the results of other studies (Han et al., 2018; Zeng et al., 2015). In addition, there was no significant difference observed in gender, suggesting that males and females are equally susceptible to SFTSV (Li et al., 2017). Furthermore, SFTSV seropositive samples were all from residents aged > 50 years, which may be due to the fact that inhabitants under 50 years old are mainly engaged in fishing and inhabitants aged > 50 years are mainly engaged in farming with higher exposure probability to risk factors for SFTS. Similarity, another study also reported that age was a risk factor of SFTSV infection (Ding et al., 2014). However, the seroprevalence of SFTSV antibodies varied significantly among seven villages and no positive results were observed in samples from two villages. The result informed that ticks in some villages carried SFTSV, but ticks in other villages might not carry SFTSV. The infection risk of residents from different villages was different. The fact that two SFTS cases were reported from Nan'ao Village and one case was reported from Bei'ao Village also contribute the hypothesis.

SFTSV is believed to be mainly transmitted by *H. longicornis* ticks (Luo et al., 2015). In this study, we found that the dominant tick species in Dachen Island was *H. longicornis*. Surprisingly, the dominant tick species in Japan and Korea was also *H. longicornis* (Iwakami et al., 2014; Yun et al., 2016). Moreover, a study on SFTSV phylogenetical analysis reported that SFTSV strains from China were classified in five lineages (A, B, C, D, E), strains from South Korea were classified into three lineages (A, D, and E), and all strains from Japan were classified into only lineage E. Four genotypes were found in the inland China and one genotype was found in the Zhoushan Islands of China and Jeju Island of Korea and Japanese Archipelagos (Liu et al., 2016). Dachen Island is very close to Zhoushan Island. The same dominant tick species and similar SFTSV genotypes suggest that SFTSV might be carried into the Dachen Island by migratory bird from Japan or Korea vice versa.

There are several limitations in our research. Firstly, there may be some bias in population selection and the seropositivity of SFTSV might not represent the overall rate in the Dachen Island. Secondly, we didn't investigate the distribution of SFTSV host animal species in the island. Finally, no SFTSV RNA was detectable in all samples in our study and phylogenetic analysis was not conducted accordingly.

In conclusion, this serologic survey results indicated that the SFTSV was endemic in an island of China which is situated southwest of Japan and Korea. These results informed that SFTSV might be transmitted by

bird migration between China, Japan and Korea. Comprehensive measures should be conducted to prevent the occurrence of SFTS cases in the island. More studies are needed to explore the prevalence of SFTSV among small mammals and ticks in this island and relationship of SFTSV stains from China, Japan and Korea.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical approval

This study was approved by the Ethics Committee of the TaiZhou CDC of Zhejiang Province, China (No. 201806). Human research was conducted in compliance with the Helsinki Declaration.

Acknowledgements

This study was supported by National Natural Science Funds of China (No.81872675) and Medical Research Program of Zhejiang Province (No.2017KY291 and 2018KY338).

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