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NOTE

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Risk assessment of infection with severe fever with thrombocytopenia syndrome virus based on a 10-year serosurveillance in Yamaguchi Prefecture

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ABSTRACT. In Japan, the first patient with severe fever with thrombocytopenia syndrome was reported in Yamaguchi in 2012. To understand the severe fever with thrombocytopenia syndrome virus (SFTSV) infection in this region, a retrospective surveillance in sika deer and wild boars in Yamaguchi was conducted using a virus-neutralizing (VN) test. The result revealed that 510 of the 789 sika deer and 199 of the 517 wild boars were positive for anti-SFTSV antibodies. Interestingly, seroprevalence in sika deer increased significantly from 2010–2013 to 2015–2020. The SFTSV gene was detected in one of the 229 serum samples collected from sika deer, but not from wild boars. In conclusion, SFTSV had spread among wild animals before 2012 and expanded gradually around 2013–2015 in Yamaguchi.

KEYWORDS: severe fever with thrombocytopenia syndrome, sika deer, wild boar

Severe fever with thrombocytopenia syndrome virus (SFTSV) is a triple-segmented and negative-stranded RNA virus belonging to the family *Phenuiviridae,* genus *Bandavirus*. The virus is transmitted through tick bites and direct contact with discharge from SFTS patients and animals [2, 5, 6, 10]. SFTSV infection causes hemorrhagic symptoms and is associated with high mortality rates in humans, cats and cheetahs [2, 5, 7, 9]. In Japan, the first patient with SFTS was reported in Yamaguchi Prefecture in autumn 2012 [14]. Since then, patients with SFTS have been reported in the western part of Japan; at present, over 60 cases are reported per year with a case fatality of 27% [5].

SFTSV has a wide range of hosts, including humans and livestock, zoo animals, companion animals, and wild animals. Epidemiological surveys conducted in China, Korea, and Japan have demonstrated anti-SFTSV antibody positivity in domesticated animals, including goats, sheep, cattle, dogs, chickens, and pigs, and wild animals, including deer, wild boars, and rodents in endemic areas [1, 3, 4, 8]. The anti-SFTSV antibody positivity rates in animals tend to be higher in endemic areas where human SFTS patients have been reported [1, 3, 4, 8], and this seroprevalence in wild animals may be a superior indicator of the risk of SFTSV infection in humans. In this study, surveillance of SFTSV infection in sika deer and wild boars in Yamaguchi Prefecture was conducted using serum samples collected for 10 years from 2010, before the first human patient in Japan was identified.

To detect anti-SFTSV antibodies in numerous animal species, we performed an enzyme-linked immunosorbent assay (ELISA) using protein A/G as a secondary antibody [15]. In raccoons, both, the specificity and sensitivity of ELISA were 100% [15]. However, the sensitivity and specificity of ELISA for sika deer and wild boars were low (unpublished data). Therefore, we performed a virus-neutralizing (VN) test for the surveillance of SFTSV infection in sika deer and wild boars.

Serum samples were collected from 789 sika deer (*Cervus nippon*) and 517 wild boars (*Sus scrofa*) in Yamaguchi Prefecture, Japan, from January 2010 to February 2020. The animals were captured by hunters as a countermeasure in the official population control program. All the serum samples collected were stored at −20°C until use. To detect anti-SFTSV antibodies, a VN test was performed by a 50% of focus-reduction neutralization test ($FRNT_{50}$) using the SFTSV HB29 strain [17]. Before the VN test, the complement in the sera was inactivated at 56°C for 30 min. $FRNT_{50}$ using Vero cells (Japanese Collection of Research Bioresources: JCRB9013)

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was performed with a final 10-fold dilution of sera according to our previous reports [13, 15].

The results of $FRNT_{50}$ showed that 510 of 789 sika deer (64.6%) and 199 of 517 wild boars (38.5%) possessed VN antibodies against SFTSV (Table 1). Sika deer and wild boars showed antibody prevalence rates of 46.7% and 11.4%, respectively, in 2010, which was two years before the first SFTS patient identified in Yamaguchi Prefecture. The antibody positivity rate in sika deer gradually increased. From 2010 to 2013, the positivity rate in sika deer was 42.3% (92/217), which further increased to 81.0% (324/400) from 2015 to 2020. Similarly, the anti-SFTSV antibody positivity rate in wild boars increased after 2015 (Fig. 1). These results indicate that the virus has been circulating more in the field since 2015, which may increase the risk of SFTSV transmission to humans.

There was no significant difference in seroprevalence between female and male sika deer or wild boars (Table 1). In contrast, the prevalence of anti-SFTSV antibodies increased in a body weight-dependent manner in both, the sika deer and wild boar populations (Table 1). The rates of anti-SFTSV antibody positivity in sika deer weighing less than 30 kg, 30–59 kg, and 60 kg or more were 44.5%, 64.2%, and 73.3%, respectively. Similarly, the rates of anti-SFTSV antibody positivity in wild boars weighing less than 30 kg, 30–59 kg, and 60 kg or more were 28.2%, 29.1%, and 55.5%, respectively. These results indicate that heavier animals (i.e., older animals)

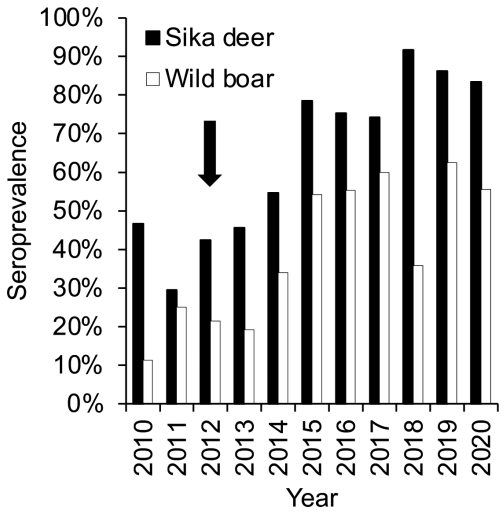

Fig. 1. Seroprevalence of anti-severe fever with thrombocytopenia syndrome virus (SFTSV) antibodies in sika deer (black bar) and wild boar (white bar) by year. The arrow shows the year of the first report on a human patient in Yamaguchi.

Table 1. Prevalence of anti-severe fever with thrombocytopenia syndrome virus (SFTSV) antibodies in sika deer and wild boars in Yamaguchi by year, sex and body weight

		Sika deer			Wild boar		
		Number of examined animals	Number of positive animals	Percentage of positive animals (%)	Number of examined animals	Number of positive animals	Percentage of positive animals (%)
Year	2010	45	21	46.7	44	5	11.4
	2011	34	10	29.4	24	6	25.0
	2012	59	25	42.4	42	9	21.4
	2013	79	36	45.6	47	9	19.1
	2014	172	94	54.7	100	34	34.0
	2015	79	62	78.5	59	32	54.2
	2016	77	58	75.3	85	47	55.3
	2017	89	66	74.2	30	18	60.0
	2018	84	77	91.7	53	19	35.8
	2019	65	56	86.2	24	15	62.5
	2020	6	5	83.3	9	5	55.6
Sex	Male	310	195	62.9	278	103	37.1
	Female	457	300	65.6	220	91	41.4
	No data	22	15	68.2	19	5	26.3
Body weight	−29 kg	119	53	44.5	142	40	28.2
	30–59 kg	419	269	64.2	172	50	29.1
	60 kg-	146	107	73.3	137	76	55.5
	No data	105	81	77.1	66	33	50.0
Total		789	510	64.6	517	199	38.5

have more chances of getting infected with the SFTSV. A similar result was reported for raccoons in Wakayama Prefecture [15].

Next, to detect SFTSV RNA in sika deer and wild boars, RT-PCR was performed using their serum samples. Sera were collected from 229 sika deer and 116 wild boars captured between January 2019 and January 2022 in Yamaguchi Prefecture. A portion of the sera used for the detection of SFTSV RNA was also analyzed using $FRNT_{50}$. RNA was extracted using a QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) and RT-PCR was performed using a One-Step RT-PCR Kit (QIAGEN) with two primers targeting the nucleocapsid protein gene of SFTSV (S2-200:5′-GACACAAAGTTCATCATTGTCTTTGCCCT-3′, S2-360:5′-TGCTGCAGCACATGTCCAAGTGG-3′) [12]. SFTSV RNA was detected in one of the 229 sika deer (0.4%) and in none of the 116 wild boars (0%). Captured in January 2019, the SFTSV-positive sika deer was a male, weighing 40 kg, and possessed anti-SFTSV antibodies. The sequence of the amplified fragment (201 bp) from this sika deer was determined and deposited in the DNA Data Bank of Japan (DDBJ: accession number: LC709266). The SFTSV sequence obtained from the deer was phylogenetically classified into the Japanese genotype J1, which was also detected in human patients in Yamaguchi Prefecture [14, 16].

Seroprevalence in Yamaguchi Prefecture was already high before 2012, and further increased after 2015. These results indicate that the risk of infection increased in humans and animals in this area from 2013 to 2015. The reason behind an increase in the positivity rate of SFTSV infection between 2013 and 2015 remains unknown. Further examination and analysis are needed to identify the factors responsible for the increase in SFTSV infection. In Yamaguchi Prefecture, several SFTS cases have been reported each year since 2013 [11], and some cases of cats with SFTS have been reported since 2017 (unpublished data). Sika deer may be a superior sentinel for assessing the regional spread of SFTSV and the risk of infection in humans in endemic areas.

SFTSV RNA was detected in only one serum sample from a sika deer. However, the kinetics of SFTSV in sika deer remains poorly characterized. Further studies are required to understand the transmission cycle of SFTSV in sika deer. SFTSV RNA was not detected in wild boars, and they possessed a lower positive ratio of anti-SFTSV antibodies than sika deer. In China, pigs were less infected with SFTSV than cattle and goats [1], suggesting that pigs and wild boars might be less sensitive to SFTSV infection than other animals belonging to the order *Artiodactyla*.

In conclusion, SFTSV infection had spread among wild animals in Yamaguchi Prefecture before 2010 and has recently expanded, resulting in annual reports of several cases of incidence in humans in this region. Surveillance of SFTSV infection among wild animals, especially the sika deer, is useful for assessing the risk of SFTSV infection in humans.

CONFLICTS OF INTEREST. The authors declare no conflicts of interest in association with the present study.

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