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Original article

Seroprevalence of severe fever with thrombocytopenia syndrome virus in medium-sized wild mammals in Miyazaki, Japan

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ABSTRACT

Severe fever with thrombocytopenia syndrome (SFTS) is a fatal emerging tick-borne zoonotic disease caused by the SFTS virus (SFTSV). SFTSV infection in humans and companion animals is a matter of concern in endemic areas. Various wild animals are involved in the transmission cycle of SFTSV with vector ticks. Because the home range of medium-sized wild mammals commonly overlaps with humans' living spheres, this study aimed to reveal the endemicity of SFTSV in such mammals. This study investigated the prevalence of antibodies against SFTSV and viral RNA in medium-sized wild mammals in Miyazaki Prefecture, Japan where human cases have been most frequently reported in Japan and performed a phylogenetic analysis to compare the detected SFTSV with those previously reported. Forty-three of 63 (68%) Japanese badgers (*Meles anakuma*) and 12 of 53 (23%) Japanese raccoon dogs (*Nyctereutes procyonoides viverrinus*) had antibodies against SFTSV. Japanese marten ($n = 1$), weasels ($n = 4$), and Japanese red fox ($n = 1$) were negative. Two of 63 (3%) badgers tested positive for SFTSV RNA, whereas the other species were negative. Phylogenetic analysis of the partial nucleotide sequence of SFTSV revealed that viral RNA detected from badgers exhibited 99.8% to 100% similarity to SFTSV, as previously reported in humans, cat, and ticks in the study area. This study demonstrated high seropositivity of antibodies in medium-sized wild mammals and suggested that SFTSV could be shared among these mammals, humans, and companion animals in endemic areas.

1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne zoonotic disease. The causative agent is *Dabie bandavirus* (SFTS virus [SFTSV]), belonging to the genus *Bandavirus*, family *Phenuiviridae*, order *Bunyavirales* (International Committee on Taxonomy of Viruses report in 2020; https://ictv.global/taxonomy/). The disease was first reported in China in 2011 (Yu et al., 2011), followed by South Korea (Kim et al., 2013), and Japan (Takahashi et al., 2014). Nowadays, the disease is reported in Vietnam (Tran et al., 2019) and Taiwan (Lin et al., 2020). Phylogenetically, eight genotypes of SFTSV have been reported in East Asian countries up to date (Fu et al., 2016; Yoshikawa et al., 2015; Yun et al., 2020). SFTS demonstrates a high case-fatality rate of 27% in human cases in Japan (Kobayashi et al., 2020). As of July 2021, 641 patients with SFTS were reported from 26 of the 47 prefectures in Japan, which are geographically concentrated in western parts of Japan (Center for Field Epidemic Intelligence, Research and Professional Development, Infectious Disease Surveillance Center, Department of Virology 1, 2021), but its distribution gradually expanded each year from western to central Japan (Kobayashi et al.,

Abbreviations: BSL, Biosafety level; CADIC, Center for Animal Disease Control; ELISA, Enzyme-linked immunosorbent assay; EMEM, Eagle's minimum essential medium; FBS, Fetal bovine serum; NP, Nucleocapsid protein; qRT-PCR, quantitative one-step reverse transcription-polymerase chain reaction; RT-PCR, Reverse transcription-polymerase chain reaction; SFTS, Severe fever with thrombocytopenia syndrome; SFTSV, SFTS virus.

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2020). SFTSV is transmitted to humans by tick bites (Yu et al., 2011; Zhang et al., 2012). However, another transmission mode, such as direct contact through body fluids from infected humans and animals, is also recognized (Gai et al., 2012; Kida et al., 2019; Yamanaka et al., 2020), highlighting that SFTS is a zoonotic disease transmitted without tick bites, raising another public health concern. Because of its etiology, workers in agriculture or forestry, hunters, veterinary doctors, and veterinary technicians are at high risk of SFTSV infection (Kimura et al., 2018; Yamanaka et al., 2020).

SFTSV infects various animals, including domestic and wild animals. The family Felidae, including domestic cats and cheetahs (*Acinonyx jubatus*), is more lethal than other mammals (Matsuno et al., 2018; Matsuu et al., 2021, 2019; Park et al., 2019). Antibody positivity or detection of viral RNA has been reported in various animals, including wild boars (*Sus scrofa*), Sika deer (*Cervus nippon*), raccoons (*Procyon lotor*), sheep, goats, cattle, dogs, pigs, and chickens (Hayasaka et al., 2016; Kang et al., 2019; Kimura et al., 2018; Lin et al., 2020; Maeda, 2016; Maeda et al., 2019; Matsuu et al., 2021; Niu et al., 2013; Yu et al., 2018). Based on a regional and temporal seroepidemiological study previously conducted among wild animals, it was suggested that SFTSV infection in wild animals spread from western to central Japan (Okada et al., 2021) following the above-mentioned spatial spread of human SFTS cases (Kobayashi et al., 2020). Therefore, wild animals play an important role in maintaining SFTSV in nature and can act as a sentinel in evaluating the endemicity and epidemicity of the virus in certain areas. The Miyazaki Prefecture is where SFTS infections in humans are most frequently reported in Japan (National Institute of Infectious Disease and Tuberculosis and Infectious Diseases Control Division, 2019). SFTS cases in companion animals are also regularly diagnosed in Miyazaki (Maeda et al., 2019; Yamanaka et al., 2020). Isolation of SFTSV from ticks collected near areas where SFTSV-infected human houses are located was also previously reported in Miyazaki (Sato et al., 2021). Furthermore, the seropositivity of antibodies against SFTSV among wild boars was high at 41.9% in Miyazaki, with 7.6% seroprevalence of viral RNA (Kirino et al., 2022).

While high antibody retention against SFTSV was recorded, particularly in large-sized wild mammals such as wild boars and Sika deer in Miyazaki and other prefectures of Japan (Kirino et al., 2022; Maeda et al., 2019), the situation in other wild mammals was less reported. This study focused on medium-sized wild mammals, particularly those belonging to Carnivora, whose head and body length is <1 m. The animals chosen for the study included Japanese badgers (*Meles anakuma*) and Japanese raccoon dogs (*Nyctereutes procyonoides viverrinus*) that widely inhabit mountainous and urbanized areas (Akihito et al., 2016; Kaneko et al., 2014; Mitsuhashi et al., 2018; Nakamura et al., 2021; Saeki et al., 2007; Sasaki and Kawabata, 1994; Shimada and Ochiai, 2016; Tanaka et al., 2002) and are likely to visit human and companion animal living spheres. This study aimed to elucidate the seropositivity of antibodies against SFTSV and detect viral RNA or infective virus in medium-sized wild mammals in the Miyazaki Prefecture to understand the endemicity of SFTSV in such medium-sized wild mammals.

2. Materials and methods

2.1. Study approval

This study used animal carcasses hunted by licensed hunters *via* vermin control conducted by the local governments under their policies and roadkills collected by the authors. Therefore, this study excluded animal experiments but laboratory experiments corresponding to biosafety level (BSL) 3. These experiments were conducted at the BSL 3 facility of the Center for Animal Disease Control (CADIC), University of Miyazaki (Miyazaki, Japan). The CADIC Infectious Disease Research Unit Management Committee approved the research plan on May 23, 2018, and was amended and approved on November 24, 2021.

2.2. Sample collection

One hundred twenty-two carcasses of medium-sized wild mammals were collected and used for this study: 63 Japanese badgers, 53 Japanese raccoon dogs, one Japanese marten (*Martes melampus melampus*), four weasels (without distinguishing *Mustela itatsi*, a native species, and *M. sibirica*, an introduced species), and one Japanese red fox (*Vulpes vulpes japonica*). All samples were collected within the same areas around Miyazaki City in Miyazaki Prefecture, Japan, between January 2019 and September 2021. These animals died of roadkill or vermin control by the local governments. The carcasses were stored at 4 °C and autopsied within 48 h after collection (101 carcasses). When autopsy within 48 h was impossible, carcasses were stored at 4 °C for up to 96 h, at the longest, and autopsied (nine carcasses). Twelve carcasses were temporarily stored at −20 °C for up to four months until autopsy. Sample information is summarized in Appendix 1. A blood sample was collected from each carcass' heart or great vessels and poured into a sterilized tube. When blood samples were unavailable, body fluids were collected. The collected blood (or body fluid) samples were settled at 4 °C overnight to exude serum. Serum (or body fluid without tissue debris) was collected *via* centrifugation at 1000 × *g* for 20 min at 4 °C. During autopsies, tissue samples were collected from the lungs, spleen, and kidney. Colonic contents were also sampled. Collected tissue samples were stored at −80 °C until use.

2.3. Antibody detection by ELISA

Aliquots of sera or body fluids were first heat-treated at 56 °C for 30 min. Then, a double-antigen enzyme-linked immunosorbent assay (ELISA) developed for detecting IgG and IgM was employed to detect anti-SFTSV nucleocapsid protein (NP) antibodies (Umeki et al., 2020). A specific antibody concentration was calculated for each sample based on the optical density value by referring to the standards whose antibody concentration was known. The details of the preparation of standard anti-SFTSV IgG were described by Umeki et al. (2020). GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA) was used to establish a four-parameter logistic regression model. Because known negative controls from naive animals against SFTSV were unavailable in this study, cutoff thresholds were determined by referring to change points detected by the change point analysis using the R package "changepoint" (Killick and Eckley, 2014; Killick et al., 2016; Lardeux et al., 2016) using the Pruned Exact Linear Time algorithm (Killick et al., 2012) with a normal assumption (Hinkley, 1970) in R version 4.1.2 (R Core Team, 2021). The analysis in this study was performed using values of log-transformed antibody concentration determined using ELISA. The detected values of log-transformed antibody concentration as change points were rounded up to the first decimal place and set as cutoff thresholds. Except for the change point corresponding to 0 ng/mL, this study detected two change points, which were 7.0 and 8.2, corresponding to 1096.6 and 3641.0 ng/mL, respectively. Therefore, a specific antibody concentration of ≥3641.0 ng/mL was regarded as antibody positive, a concentration between ≥1096.6 and <3641.0 ng/mL was regarded as slightly positive, and <1096.6 ng/mL was regarded as negative (Appendix 2).

2.4. RNA extraction from tissue samples

Total RNA was extracted from sera or body fluids using the NucleoSpin Virus (Macherey-Nagel, Düren, Germany) manually or the MagDEA Dx SV reagent (Precision System Science, Matsudo, Japan) *via* magLEAD 12gC (Precision System Science), an automated nucleic acid extraction system, according to the manufacturers' instructions. When the samples contained numerous cellular components, and separating them from supernatants was difficult, a combination of TRIzol LS Reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and Direct-zol RNA Miniprep Kits (Zymo Research, Irvine, CA, USA) was

applied for RNA extraction according to the manufacturers' instructions. Unless the collected sample volume was inadequate, 200 μL samples was used for RNA extraction *via* the methods mentioned above. When the volume of serum or body fluid was <200 μL, 50 or 100 μL samples were processed for RNA extraction according to the available amount of the samples. These RNA specimens extracted from sera or body fluids were used to screen SFTSV genes using quantitative one-step reverse transcription-polymerase chain reaction (qRT-PCR). RNA extraction from tissue and colonic contents was performed by combining TRIzol Reagent (Invitrogen, Thermo Fisher Scientific) and Direct-zol RNA Miniprep Kits, according to the manufacturers' instructions. Each 100 mg tissue or colonic content was used for RNA extraction. These specimens of total RNA from tissue and colonic contents were used to determine the viral copy number per tissue mass (100 mg) *via* qRT-PCR and genotyping through conventional reverse transcription-polymerase chain reaction (RT-PCR) when the serum or body fluid sample was detected positive for SFTSV.

2.5. Detection of SFTSV genomic RNA and phylogenetic analysis

qRT-PCR was performed for screening: One-step PrimeScrip RT-PCR Kit (Perfect Real Time; Takara Bio, Kusatsu, Japan) or One-step PrimeScript III RT-qPCR Mix (Takara Bio) was used for qRT-PCR using LightCycler 96 (Roche Diagnostics, Basel, Switzerland), according to the manufacturers' instructions. The primers and labeled probe used for qRT-PCR were referred to by Takahashi et al. (2014) and slightly modified as follows: forward primer 5'-GGGTGAGRGAYA-CAAAGTTYA-3', reverse primer 5'-CCCYGATGCCTTGACGATYT-3', and probe 5'-CATTGTCTTTGCCYTGACYCGAGGYA-3'.

Specimens detected positive by qRT-PCR were further subjected to conventional RT-PCR to determine the genotype of the SFTSV NP gene. Conventional RT-PCR was conducted using PrimeScript One-step RT-PCR Kit Ver.2 (Dye Plus; Takara Bio); 10.0 μL reaction mixture containing 5.0 μL of 2 × 1-step Buffer (Dye Plus), 0.4 μL PrimeScript 1-step Enzyme Mix, 10 μM of the primer set (final concentration: 0.3 μM each), and 2.0 μL template RNA. The primer set was SFTS NP-1F primer 5'-ATCGTCAAGGCATCAGGGAA-3' and SFTS NP-1Rd primer 5'-TTCAGC-CACTTCACCCGRA-3'. The primers used were referred to by Takahashi et al. (2014) with slight modifications. The reaction conditions were as follows: one cycle at 50 °C for 30 min, followed by 94 °C for 2 min; 35 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s. The RT-PCR products were observed in 1.5% agarose gel (Nacalai Tesque, Kyoto, Japan) stained with GelRed (Biotium, Fremont, CA, USA). Because nonspecific amplification that demonstrated a similar size to the target amplification (458 bp) was observed in the preliminary experiment, TA cloning was performed to select the target sequence as follows: deoxyriboadenosine was added at the 3' end of the amplicons using TaKaRa Ex Taq (Takara Bio) and visualized through 1.0% agarose gel (Nacalai Tesque). The target bands were purified using NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel). Subsequently, ligation was conducted using pGEM-T Easy Vector Systems (Promega, Madison, WI, USA), and transfection was performed using *Escherichia coli* DH5α as competent cells (Takara Bio). Plasmid purification was performed using NucleoSpin Plasmid EasyPure (Macherey-Nagel).

Sequencing was conducted using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) based on the Sanger method using a capillary DNA sequencer (SeqStudio Genetic Analyzer; Applied Biosystems), according to the manufacturers' instructions. Phylogenetic analysis of the partial sequence of the SFTSV NP gene in the S segment and several sequences of the SFTSV NP gene available in GenBank was performed. ClustalW protocol (Thompson et al., 1994) was used to align the sequences. A phylogenetic tree was constructed using the maximum likelihood method based on the Kimura 2-parameter model+G (Kimura, 1980) with 1000 bootstrap replications in MEGA 11 software (Tamura et al., 2021). The partial nucleotide sequences for the SFTSV NP gene detected in this study were deposited in the DDBJ/EMBL/GenBank databases under accession numbers LC705280 and LC705281 (for specimen nos. 105 and 108, respectively).

2.6. Virus isolation

Virus isolation was conducted using tissue samples that tested positive for the SFTSV NP gene *via* qRT-PCR. Each viral RNA-positive tissue sample was crushed with beads in a sterilized 2.0 mL tube containing Eagle's minimum essential medium (EMEM; Sigma-Aldrich, St. Louis, MO, USA) to create 10% suspensions. The samples were centrifuged at 10,000 × *g* for 5 min at 4 °C, and the supernatants were used for virus isolation. Vero cells maintained with EMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS; Biowest, Nuaillé, France), 100-units/mL penicillin, and 100 μg/mL streptomycin (FUJIFILM Wako Pure Chemical, Osaka, Japan) were prepared for virus isolation. Six-well plates were seeded with Vero cells adjusted to 1×10^5 cells/mL and incubated for 12 h. The 500 μL supernatant collected from the 10% homogenate of each tissue sample was inoculated onto Vero cells. After adsorption for 60 min, the inoculum was removed, and the cells were washed with phosphate-buffered saline. EMEM supplemented with 2% heat-inactivated FBS was added to the wells. For each sample, a blind passage incubation was completed every fifth to seventh day at 37 °C and 5% CO_2 and continued until the third passage. This process was conducted in a BSL 3 facility. Confirmation of virus propagation in the supernatants from the third-passaged cell culture was performed *via* qRT-PCR, as described above, with RNA extracted using Prep Buffer A (Precision System Science), MagDEA Dx SV reagent, and magLEAD 12gC.

2.7. Statistical analysis

The association between the seropositivity of antibodies and animal species (Japanese badgers and Japanese raccoon dogs), the association between the seropositivity of antibodies and animal species in each season, and the association between the antibody retention and seasons in each animal species were tested using Fisher's exact test with the R package "fmsb" (Nakazawa, 2021) in R version 4.1.2. The association between the seropositivity of antibodies and sampling source (vermin control and roadkill) and animal species was also tested using the same method. The difference in median antibody concentration in seropositive animals by animal species and seasons was tested using the Brunner–Munzel test (Brunner and Munzel, 2000) with the R package "brunnermunzel" (Ara, 2020) in R version 4.1.2. A p-value < 0.05 was considered statistically significant. The p-values in the multiple tests were adjusted using the Benjamini–Hochberg method (Benjamini and Hochberg, 1995).

3. Results

3.1. Seropositivity of antibodies against SFTSV in wild mammals

Forty-three of 63 (68%) Japanese badgers presented with antibodies; 24 of 43 were positive, while 19 of 43 were slightly positive for antibodies against SFTSV NP. Twelve of 53 (23%) Japanese raccoon dogs were seropositive; five of 12 were positive, while seven of 12 were slightly positive. The collected weasels ($n = 4$), Japanese red fox ($n = 1$), and Japanese marten ($n = 1$) were all seronegative against SFTSV NP. Antibody positivity was higher in Japanese badgers than in Japanese raccoon dogs ($p < 0.01$; Fisher's exact test). The association between antibody positivity and sampling seasons in each animal species was not observed in both Japanese badgers and Japanese raccoon dogs ($p = 1.0$ for each combination of seasons in badgers, $p \geq 0.98$ for each combination of seasons in raccoon dogs; Fisher's exact test). Regarding the association between antibody positivity and animal species in the same season, antibody positivity was higher in Japanese badgers than in Japanese raccoon dogs in the same sampling season of spring ($p < 0.01$;

$p \geq 0.11$ between badgers and raccoon dogs in the other seasons; Fisher's exact test). No association was observed between the seropositivity of antibodies and sampling sources (vermin control and roadkill) in each animal species ($p = 1.0$ between vermin control and roadkill in badgers and $p = 0.11$ between vermin control and roadkill in raccoon dogs; Fisher's exact test), whereas Japanese badgers exhibited higher seropositivity of antibodies than Japanese raccoon dogs in vermin control and roadkill groups ($p < 0.01$ between vermin control badgers and vermin control raccoon dogs and $p = 0.03$ between roadkill badgers and roadkill raccoon dogs; Fisher's exact test). The results are summarized in Table 1, and the detailed information is shown in Appendix 1.

The median antibody concentrations in all Japanese badgers ($n = 63$) and all Japanese raccoon dogs ($n = 53$) were 2,258.5 and 499.3 ng/mL, respectively, and the medians differed significantly ($p < 0.01$; Brunner-Munzel test; Appendix 3A and B). Besides, among samples with an antibody concentration of >0 ng/mL, the median antibody concentrations in Japanese badgers ($n = 55$) and Japanese raccoon dogs ($n = 32$) were 2799.0 and 858.8 ng/mL, respectively, and the medians were differed significantly ($p < 0.01$; Brunner–Munzel test; Appendix 3C and D). In contrast, the median antibody concentrations in seropositive Japanese badgers ($n = 43$) and Japanese raccoon dogs ($n = 12$) were 4783.5 and 2990.0 ng/mL, respectively (Appendix 3E and F), and had no significant difference between them ($p = 0.09$; Brunner–Munzel test). A seasonal difference in median antibody concentrations was not confirmed in badger and raccoon dog samples with an antibody concentration of >0 ng/mL ($p = 1.0$ among all combinations of seasons; Brunner–Munzel test) or in seropositive samples ($p = 0.92$ among all combinations of seasons; Brunner–Munzel test; Appendix 4). The seasonal difference in the median antibody concentrations in all samples is shown in Appendix 5.

3.2. Detection of SFTSV RNA in tissue samples and virus isolation

qRT-PCR using serum or body fluid samples detected SFTSV RNA in two of 63 (3%) Japanese badgers (Table 1). These badgers were collected in spring (April and May) and were slightly positive for antibodies. Japanese raccoon dogs, weasels, Japanese red fox, and Japanese marten were negative for viral gene detection using qRT-PCR. In the above-mentioned positive Japanese badgers (specimen nos. 105 and 108), SFTSV RNA was detected in the spleen and kidney, whereas SFTSV RNA was undetected in the lungs and colonic contents using qRT-PCR. Viral loads were 1.48×10^4 copies/mL in body fluid, 9.97×10^5 copies/g in the spleen, and 3.92×10^5 copies/g in the kidney in specimen no. 105. Viral loads were 8.81×10^3 copies/mL in body fluid, 2.23×10^6 copies/g in the spleen, and 1.02×10^5 copies/g in the kidney in specimen no. 108 (Fig. 1). Regarding the virus isolation, the supernatants collected from the third blind passage were negative for SFTSV RNA by qRT-PCR.

3.3. Phylogenetic analysis

The partial sequence (458 bp) of the SFTSV NP gene was detected in the above-mentioned Japanese badgers (specimen nos. 105 and 108) using spleen samples *via* conventional RT-PCR. The partial nucleotide sequence of the NP gene detected in this study exhibited 99.8% (457 of 458) and 100% (458 of 458) homology with the SFTSV NP gene

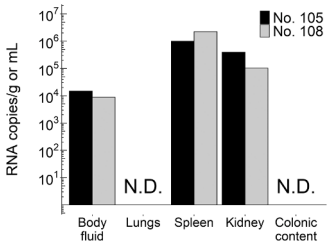

Fig. 1. *Viral loads in each tissue sample among viral RNA-positive animals (copies/g or mL).* Viral loads were 1.48×10^4 copies/mL in body fluid, 9.97×10^5 copies/g in the spleen, and 3.92×10^5 copies/g in the kidney in specimen no. 105. Viral loads were 8.81×10^3 copies/mL in body fluid, 2.23×10^6 copies/g in the spleen, and 1.02×10^5 copies/g in the kidney in specimen no. 108. Lungs and colonic contents were negative for specimen nos. 105 and 108. N.D., not detected.

Table 1
Antibodies and viral RNA positivity for severe fever with thrombocytopenia syndrome virus in medium-sized wild mammals in Miyazaki Prefecture, Japan.

Species	Category		Total no. carcasses	Antibodies positive			Viral RNA-positive		
				Number	%[†]		Number	%[†]	
Japanese badgers	Total		63	43	68	(55–79)	2	3	(0–11)
	Sex	Male	37	25	68	(50–82)	1	3	(0–14)
		Female	26	18	69	(48–86)	1	4	(0–20)
	Body weight (kg)[‡]	<5	22	12	55	(32–76)	2	9	(1–29)
		≥5–<8	31	23	74	(55–88)	0	0	(0–11)
		≥8	10	8	80	(44–97)	0	0	(0–31)
	Seasons	Spring (Mar–May)	15	12	80	(52–96)	2	13	(2–40)
		Summer (Jun–Aug)	33	21	64	(45–80)	0	0	(0–11)
		Autumn (Sep–Nov)	12	8	67	(35–90)	0	0	(0–26)
		Winter (Dec–Feb)	3	2	67	(9–99)	0	0	(0–71)
	Source	Vermin control	39	27	69	(52–83)	1	3	(0–13)
		Roadkill	24	16	67	(45–84)	1	4	(0–21)
Japanese raccoon dogs	Total		53	12	23	(12–36)	0	0	(0–7)
	Sex	Male	24	2	8	(1–27)	0	0	(0–14)
		Female	29	10	34	(18–54)	0	0	(0–12)
	Body weight (kg)[‡]	<3	16	1	6	(0–30)	0	0	(0–21)
		≥3–<4	29	9	31	(15–51)	0	0	(0–12)
		≥4	8	2	25	(3–65)	0	0	(0–37)
	Seasons	Spring (Mar–May)	16	2	13	(2–38)	0	0	(0–21)
		Summer (Jun–Aug)	8	2	25	(3–65)	0	0	(0–37)
		Autumn (Sep–Nov)	13	5	38	(14–68)	0	0	(0–25)
		Winter (Dec–Feb)	16	3	19	(4–46)	0	0	(0–21)
	Source	Vermin control	25	3	12	(3–31)	0	0	(0–14)
		Roadkill	28	9	32	(16–52)	0	0	(0–12)

[†] Numbers in parentheses denote a 95% confidence interval.
[‡] Subdivision of body weight in each species was grouped considering the distribution of body weight values according to a previous study (Kaneko, 2001) and authors' observation.

sequences previously identified in humans (accession nos. LC462230 and LC462231) and cat (accession no. LC462229) in Miyazaki in specimen nos. 105 and 108, respectively (Fig. 2). Furthermore, phylogenetic analysis revealed that partial sequences of the NP gene detected from Japanese badgers in this study were clustered into the same group as the previously reported SFTSV identified in the humans, cat, and ticks in Miyazaki (Fig. 2).

4. Discussion

This study investigated the seroprevalence of SFTSV in medium-sized wild mammals in Miyazaki Prefecture, where SFTS infection in humans and companion animals is endemic and frequently reported. This study demonstrated the detection of viral RNA and high seropositivity of antibodies against SFTSV in these wild mammals that inhabit close environments to human and companion animal living spheres.

This study demonstrated 68% and 23% seropositivity of antibodies against SFTSV in Japanese badgers and raccoon dogs, respectively. Previous studies showed seropositivity in various domestic and wild animals (Huang et al., 2019; Niu et al., 2013). In Japan, comparatively high seropositivity of antibodies was reported in wild boars and Sika deer, which was 8.6% to 53.9% and 5% to 43.2%, respectively, mainly from the western parts of Japan (Hayasaka et al., 2016; Kimura et al., 2018; Kirino et al., 2022; Lundu et al., 2018; Maeda, 2016; Matsuu et al., 2021). Besides, a yearly increase in seropositivity (reaching ~50% and 30% in 2015) was reported in raccoons and raccoon dogs, respectively, in the SFTSV-endemic area (Maeda, 2016). Total seropositivity, including all samples, regardless of sampling year, was reported as 10.9% and 7.3% in raccoons and raccoon dogs, respectively (Maeda, 2016). Regarding Japanese badgers, the seropositivity of antibodies was 8% in total regardless of sampling years in the same area (Maeda, 2016). In contrast, seropositivity of antibodies in Japanese badgers and raccoon

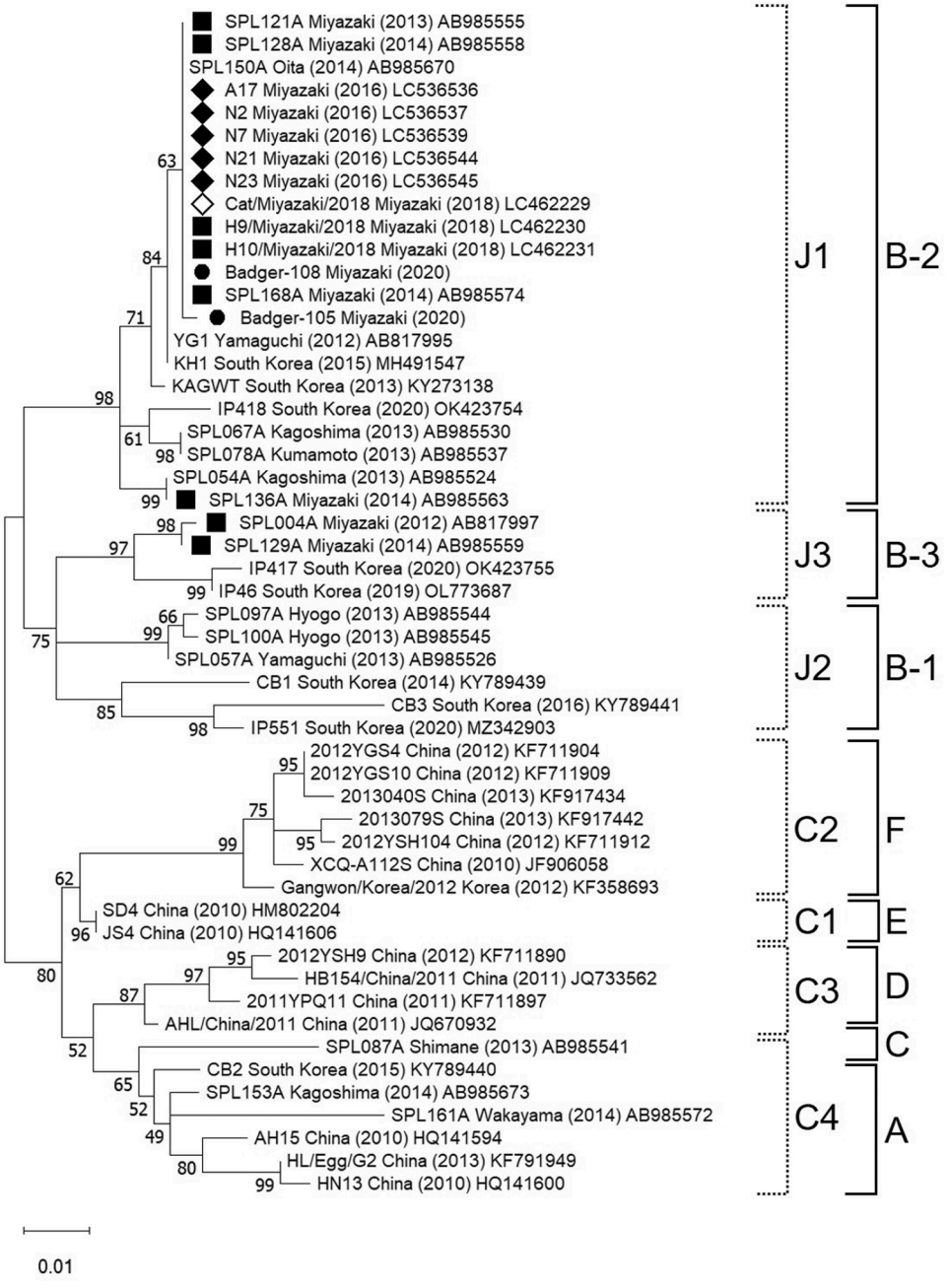

Fig. 2. *Phylogenetic analysis of severe fever with thrombocytopenia syndrome virus (SFTSV) identified in this study based on the partial cRNA nucleotide sequences of the nucleocapsid protein (NP) gene.* A phylogenetic tree was constructed using the partial 458 bp of cRNA nucleotide sequences of the NP gene detected in this study and other strains previously reported. The scale bar indicates nucleotide substitutions per site. All bootstrap values from 1000 replications are presented on the corresponding nodes. The black circle denotes the cRNA sequence detected in Japanese badgers in Miyazaki in this study. The black square represents SFTSV identified in humans in Miyazaki. The black rhombus represents SFTSV identified in ticks in Miyazaki. The white rhombus shows SFTSV identified in cat in Miyazaki. Genotype classification indicated with dotted line is according to Yoshikawa et al. (2015). Genotype classification indicated with solid line is based on Fu et al. (2016) and Yun et al. (2020).

dogs was retrospectively both 0% (zero of two and zero of 24, respectively) in areas where human SFTS cases have been unreported (Okada et al., 2021). This study highlighted the high seropositivity of antibodies against SFTSV among Japanese badgers and raccoon dogs in the Miyazaki Prefecture, signifying frequent virus transmission. Maeda previously suggested that increased seropositivity in wild animals indicates an increased risk of SFTS infection in humans (Maeda, 2016). Therefore, it would be natural that seropositivity against SFTSV in wild animals in Miyazaki is high because Miyazaki is a prefecture where human SFTSV infection cases have been most frequently recorded in Japan (National Institute of Infectious Disease and Tuberculosis and Infectious Diseases Control Division, 2019). At the same time, this study demonstrated that Japanese badgers and raccoon dogs function as sentinels for monitoring SFTSV endemicity.

Meanwhile, this study demonstrated the higher antibody positivity in Japanese badgers than in Japanese raccoon dogs collected from the same area of Miyazaki. Although the number of samples in this study was still small, and could not conclude any causal association, this study suggested that there are factors influencing the differences in SFTSV transmission or antibody productivity between Japanese badgers and raccoon dogs in the fields. These would include a difference in their behavior (such as different frequencies of contact with each other among the population), habitat density, vector tick species that frequently infest these two species, the virulence of the virus against different host species resulting in different mortality rates, the immune response to the virus, and so on. Regarding tick fauna, a previous study has investigated various wild mammals in Japan, such as Japanese raccoon dogs, Japanese badgers, wild boars, Reeves's muntjacs (*Muntiacus reeves*), Japanese hares (*Lepus brachyurus*), masked palm civets (*Paguma larvata*), Japanese weasels (*M. itatsi*), and others (Tsunoda, 2012). According to this study, Japanese raccoon dogs were most frequently infested by *Haemaphysalis flava*, whereas Japanese badgers were infested by various species of ticks, such as *H. flava, H. longicornis, H. megaspinosa, Ixodes ovatus*, and so on, compared to other mammals (Tsunoda, 2012). Although *H. flava, H. longicornis*, and *H. megaspinosa* are particularly known to harbor SFTSV (Sato et al., 2021; Yu et al., 2011), epidemiological factors related to the differences in antibody positivity need to be further investigated.

In contrast to the comparatively high seropositivity of antibodies in Japanese badgers and raccoon dogs, the prevalence of SFTSV RNA was low. This would imply that these species are frequently exposed to/infected with SFTSV, and viremia/virus excretion is not persistent. Maeda reported 2.4% (16 of 671) prevalence in raccoons (Maeda, 2016). Even in large-sized wild animals, the prevalence of SFTSV RNA was low: 0% (0 of 107) in deer and 1.0% (1 of 102) in wild boars in the Kagoshima Prefecture, neighboring Miyazaki (Matsuu et al., 2021). Meanwhile, the nucleotide sequence of viral RNA detected in Japanese badgers showed 99.8% to 100% homology with SFTSV, previously reported in humans, cat, and ticks in the Miyazaki Prefecture, indicating that humans, cats, and medium-sized wild mammals share the virus and are involved in the same transmission cycle. In this study, infective SFTSV was not isolated from tissue samples (body fluid, spleen, and kidney) collected from badgers, although these samples tested positive for SFTSV RNA. A previous study demonstrated that SFTSV RNA was detected in various organs in raccoons, such as the lungs, colons, and feces (Maeda, 2016). This differed in this study, as viral RNA was detected in the body fluid, spleen, and kidney except for lungs and colonic contents from badgers. This study could not clarify why viral RNA was not detected in animals' lungs or colonic contents and why the infective virus was not isolated. However, the possible reason is that SFTSV RNA-positive badgers exhibited a moderate level of antibodies, implying that they were at the latter stage of infection and in the process of clearing the virus from their bodies. Although the duration of virus excretion would be short according to the data of quite low viral RNA positivity in this study, and it is also unclear if these species exhibit obvious clinical signs, the above-mentioned findings indicated that such wild animals could excrete SFTSV into environments through urine, feces, or any types of body fluid, nevertheless.

Medium-sized wild mammals, particularly Japanese badgers and raccoon dogs, are common in Japan. They are widely distributed throughout mountainous, rural, and urbanized areas, utilizing similar environments (Akihito et al., 2016; Kaneko et al., 2014; Mitsuhashi et al., 2018; Nakamura et al., 2021; Saeki et al., 2007; Sasaki and Kawabata, 1994; Shimada and Ochiai, 2016; Tanaka et al., 2002). Because areas such as agricultural fields or human residential areas overlap with the common inhabitation of Japanese badgers and raccoon dogs, public awareness for general preventive measures against zoonoses is needed, particularly for agricultural workers.

5. Conclusions

This study highlighted the high prevalence of SFTSV among medium-sized wild mammals in an endemic area. The study also demonstrated that SFTSV could be shared among the medium-sized wild mammals, humans, and companion animals in endemic areas. Because the home range of medium-sized wild mammals commonly overlaps with human and companion animal living spheres, further public awareness for general preventive measures against zoonoses, coupled with preventing tick bites, should be enhanced. Further comprehensive studies are awaited to deeply understand SFTSV dynamics in nature.

Ethics statement

No ethical approval was required as this study did not include conducting animal experiment or handling clinical samples.

Declaration of Competing Interest

The authors declare no conflicts of interest associated with this manuscript.

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CRediT authorship contribution statement

Chiho Kaneko: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Hirohisa Mekata:** Investigation, Methodology, Resources, Validation, Writing – review & editing. **Kazumi Umeki:** Investigation, Methodology, Resources, Writing – review & editing. **Putu Eka Sudaryatma:** Investigation, Methodology, Writing – review & editing. **Takao Irie:** Investigation, Writing – review & editing. **Kentaro Yamada:** Investigation, Writing – review & editing. **Naoaki Misawa:** Investigation, Writing – review & editing. **Kunihiko Umekita:** Investigation, Methodology, Resources, Writing – review & editing. **Tamaki Okabayashi:** Investigation, Methodology, Funding acquisition, Resources, Writing – review & editing.

Data availability

All relevant data are shown within the manuscript and available in supplementary materials.

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Supplementary materials

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References

Akihito Sako, T., Teduka, M., Kawada, S.I., 2016. Long-term trends in food habits of the Raccoon Dog, nyctereutes viverrinus, in the Imperial Palace, Tokyo. Bull. Natl. Mus. Nat. Sci. Ser. A Zool. 42, 143–161.

Ara, T., 2020. brunnermunzel: (Permuted) Brunner–Munzel Test. R package version 1.4.1. Retrieved from https://CRAN.R-project.org/package=brunnermunzel.

Benjamini, Y., Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300.

Brunner, E., Munzel, U., 2000. The nonparametric Behrens–Fisher problem: asymptotic theory and a small-sample approximation. Biom. J. 42, 17–25. https://doi.org/10.1002/(SICI)1521-4036(200001)42:1<17::AID-BIMJ17>3.0.CO;2-U.

Center for Field Epidemic Intelligence, Research and Professional Development, 2021. Infectious Disease Surveillance Center, Department of Virology 1. National Institute of Infectious Diseases. Severe fever with thrombocytopenia syndrome (SFTS). https://www.niid.go.jp/niid/ja/sfts/3143-sfts.html (accessed October 24, 2021).

Fu, Y., Li, S., Zhang, Z., Man, S., Li, X., Zhang, W., Zhang, C., Cheng, X., 2016. Phylogeographic analysis of severe fever with thrombocytopenia syndrome virus from Zhoushan Islands, China: implication for transmission across the ocean. Sci. Rep. 6, 19563. https://doi.org/10.1038/srep19563.

Gai, Z., Liang, M., Zhang, Y., Zhang, S., Jin, C., Wang, S.W., Sun, L., Zhou, N., Zhang, Q., Sun, Y., Ding, S.J., Li, C., Gu, W., Zhang, F., Wang, Y., Bian, P., Li, X., Wang, Z., Song, X., Wang, X., Xu, A., Bi, Z., Chen, S., Li, D., 2012. Person-to-person transmission of severe fever with thrombocytopenia syndrome bunyavirus through blood contact. Clin. Infect. Dis. 54, 249–252. https://doi.org/10.1093/cid/cir776.

Hayasaka, D., Fuxun, Y., Yoshikawa, A., Posadas-Herrera, G., Shimada, S., Tun, M.M.N., Agoh, M., Morita, K., 2016. Seroepidemiological evidence of severe fever with thrombocytopenia syndrome virus infections in wild boars in Nagasaki. Jpn. Trop. Med. Health 44, 6. https://doi.org/10.1186/s41182-016-0009-6.

Hinkley, D.V., 1970. Inference about the change-point in a sequence of random variables. Biometrika 57, 1–17. https://doi.org/10.1093/biomet/57.1.1.

Huang, X.Y., Du, Y.H., Wang, H.F., You, A.G., Li, Y., Su, J., Nie, Y.F., Ma, H.X., Xu, B.L., 2019. Prevalence of severe fever with thrombocytopenia syndrome virus in animals in Henan Province. China. Infect. Dis. Poverty 8, 56. https://doi.org/10.1186/s40249-019-0569-x.

Kaneko, Y., 2001. Life cycle of the Japanese Badger (*Meles meles anakuma*) in Hinode Town, Tokyo (in Japanese with English abstract). Mamm. Sci. 41, 53–64. https://doi.org/10.11238/mammalianscience.41.53.

Kaneko, Y., Kanda, E., Tashima, S., Masuda, R., Newman, C., Macdonald, D.W., 2014. The socio-spatial dynamics of the Japanese badger (*Meles anakuma*). J. Mammal. 95, 290–300. https://doi.org/10.1644/12-MAMM-A-158.

Kang, J.G., Cho, Y.K., Jo, Y.S., Chae, J.B., Joo, Y.H., Park, K.W., Chae, J.S., 2019. Severe fever with thrombocytopenia syndrome virus in dogs. South Korea Emerg. Infect. Dis. 25, 376–378. https://doi.org/10.3201/eid2502.180859.

Kida, K., Matsuoka, Y., Shimoda, T., Matsuoka, H., Yamada, H., Saito, T., Imataki, O., Kadowaki, N., Noguchi, K., Maeda, K., Mochizuki, Y., Kishimoto, T., 2019. A case of cat-to-human transmission of severe fever with thrombocytopenia syndrome virus. Jpn. J. Infect. Dis. 72, 356–358. https://doi.org/10.7883/yoken.JJID.2018.526.

Killick, R., Eckley, I.A., 2014. changepoint: an R package for changepoint analysis. J. Stat. Softw. 58, 1–19. https://doi.org/10.18637/jss.v058.i03.

Killick, R., Fearnhead, P., Eckley, I.A., 2012. Optimal detection of changepoints with a linear computational cost. J. Am. Statist. Assoc. 107, 1590–1598.

Killick, R., Haynes, K., Eckley, I.A., 2016. changepoint: an R package for changepoint analysis. R package version 2.2.2. Retrieved from https://CRAN.R-project.org/package=changepoint.

Kim, K.H., Yi, J., Kim, G., Choi, S.J., Jun, K.I., Kim, N.H., Choe, P.G., Kim, N.J., Lee, J.K., Oh, M.D., 2013. Severe fever with thrombocytopenia syndrome, South Korea, 2012. Emerg. Infect. Dis. 19, 1892–1894. https://doi.org/10.3201/eid1911.130792.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120. https://doi.org/10.1007/BF01731581.

Kimura, T., Fukuma, A., Shimojima, M., Yamashita, Y., Mizota, F., Yamashita, M., Otsuka, Y., Kan, M., Fukushi, S., Tani, H., Taniguchi, S., Ogata, M., Kurosu, T., Morikawa, S., Saijo, M., Shinomiya, H., 2018. Seroprevalence of severe fever with thrombocytopenia syndrome (SFTS) virus antibodies in humans and animals in Ehime prefecture, Japan, an endemic region of SFTS. J. Infect. Chemother. 24, 802–806. https://doi.org/10.1016/j.jiac.2018.06.007.

Kirino, Y., Yamamoto, S., Nomachi, T., Mai, T.N., Sato, Y., Sudaryatma, P.E., Norimine, J., Fujii, Y., Ando, S., Okabayashi, T., 2022. Serological and molecular survey of tick-borne zoonotic pathogens including severe fever with thrombocytopenia syndrome virus in wild boars in Miyazaki Prefecture. Jpn. Vet. Med. Sci. 8, 877–885. https://doi.org/10.1002/vms3.696.

Kobayashi, Y., Kato, H., Yamagishi, T., Shimada, T., Matsui, T., Yoshikawa, T., Kurosu, T., Shimojima, M., Morikawa, S., Hasegawa, H., Saijo, M., Oishi, K., SFTS Epidemiological Research Group Japan, 2020. Severe fever with thrombocytopenia syndrome, Japan, 2013–2017. Emerg. Infect. Dis. 26, 692–699. https://doi.org/10.3201/eid2604.191011.

Lardeux, F., Torrico, G., Aliaga, C., 2016. Calculation of the ELISA's cut-off based on the change-point analysis method for detection of *Trypanosoma cruzi* infection in Bolivian dogs in the absence of controls. Mem. Inst. Oswaldo Cruz 111, 501–504. https://doi.org/10.1590/0074-02760160119.

Lin, T.L., Ou, S.C., Maeda, K., Shimoda, H., Chan, J.P.W., Tu, W.C., Hsu, W.L., Chou, C. C., 2020. The first discovery of severe fever with thrombocytopenia syndrome virus in Taiwan. Emerg. Microbes Infect. 9, 148–151. https://doi.org/10.1080/22221751.2019.1710436.

Lundu, T., Yoshii, K., Shintaro Morikawa, S., Tsubota, T., Misawa, N., Hayasaka, D., Kariwa, H., 2018. Serological survey of severe fever with thrombocytopenia syndrome virus infection in Sika deer and rodents in Japan. Jpn. J. Vet. Res. 66, 21–28. https://doi.org/10.14943/jjvr.66.1.21.

Maeda, K., 2016. Prevalence of SFTSV infection among wild and domestic animals, 2010–2015 (in Infectious Agents Surveillance Report, 37, 51–53; in Japanese with English abstract). National Institute of Infectious Disease and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare. Retrieved from https://www.niid.go.jp/niid/images/idsc/iasr/37/433j.pdf.

Maeda, K., Noguchi, K., Tatemoto, K., Sakai, Y., Shimoda, H., Takano, A., Morikawa, S., Matsuu, A., Kirino, Y., Okabayashi, T., Hayasaka, D., Mizutani, T., Matsuno, K., Shiranaga, N., Suzuki, K., 2019. SFTSV infection in animals (in Infectious Agents Surveillance Report, 40, 116–117; in Japanese with English abstract). National Institute of Infectious Disease and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare. Retrieved from https://www.niid.go.jp/niid/images/idsc/iasr/40/473.pdf.

Matsuno, K., Nonoue, N., Noda, A., Kasajima, N., Noguchi, K., Takano, A., Shimoda, H., Orba, Y., Muramatsu, M., Sakoda, Y., Takada, A., Minami, S., Une, Y., Morikawa, S., Maeda, K., 2018. Fatal tickborne phlebovirus infection in captive cheetahs. Jpn. Emerg. Infect. Dis. 24, 1726–1729. https://doi.org/10.3201/eid2409.171667.

Matsuu, A., Hamakubo, E., Yabuki, M., 2021. Seroprevalence of severe fever with thrombocytopenia syndrome virus in animals in Kagoshima Prefecture, Japan, and development of Gaussia luciferase immunoprecipitation system to detect specific IgG antibodies. Ticks Tick Borne Dis. 12, 101771 https://doi.org/10.1016/j.ttbdis.2021.101771.

Matsuu, A., Momoi, Y., Nishiguchi, A., Noguchi, K., Yabuki, M., Hamakubo, E., Take, M., Maeda, K., 2019. Natural severe fever with thrombocytopenia syndrome virus infection in domestic cats in Japan. Vet. Microbiol. 236, 108346 https://doi.org/10.1016/j.vetmic.2019.06.019.

Mitsuhashi, I., Sako, T., Teduka, M., Koizumi, R., Saito, M.U., Kaneko, Y., 2018. Home range of raccoon dogs in an urban green area of Tokyo. Jpn. J. Mammal. 99, 732–740. https://doi.org/10.1093/jmammal/gyy033.

Nakamura, N., Haginouchi, T., Asano, Y., Ikeda, M., Ryuno, M., Akai, K., Oshima, I., Nakanishi, Y., Takayama, K., 2021. Emergence of Japanese Badger (*Meles anakuma*) and its damage to crops at Kagoshima University Campus in Regional City. Bull. Fac. Agric. Kagoshima Univ. 71, 23–30.

Nakazawa, M., 2021. fmsb: functions for medical statistics book with some demographic data. R package version 0.7.2. Retrieved from https://CRAN.R-project.org/package=fmsb.

National Institute of Infectious Disease and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare, 2019. Severe fever with thrombocytopenia syndrome (SFTS) in Japan, as of June 2019 (in Infectious Agents Surveillance Report, 40, 111–112; in Japanese with English abstract). Retrieved from https://www.niid.go.jp/niid/en/iasr-vol40-e/865-iasr/9021-473te.html (accessed October 24, 2021), https://www.niid.go.jp/niid/images/idsc/iasr/40/473.pdf.

Niu, G., Li, J., Liang, M., Jiang, X., Jiang, M., Yin, H., Wang, Z., Li, C., Zhang, Q., Jin, C., Wang, X., Ding, S., Xing, Z., Wang, S., Bi, Z., Li, D., 2013. Severe fever with thrombocytopenia syndrome virus among domesticated animals. China. Emerg. Infect. Dis. 19, 756–763. https://doi.org/10.3201/eid1905.120245.

Okada, A., Hotta, A., Kimura, M., Park, E.-s., Morikawa, S., Inoshima, Y., 2021. A retrospective survey of the seroprevalence of severe fever with thrombocytopenia syndrome virus in wild animals in Japan. Vet. Med. Sci. 7, 600–605. https://doi.org/10.1002/vms3.400.

Park, E.S., Shimojima, M., Nagata, N., Ami, Y., Yoshikawa, T., Iwata-Yoshikawa, N., Fukushi, S., Watanabe, S., Kurosu, T., Kataoka, M., Okutani, A., Kimura, M., Imaoka, K., Hanaki, K., Suzuki, T., Hasegawa, H., Saijo, M., Maeda, K., Morikawa, S., 2019. Severe fever with thrombocytopenia syndrome phlebovirus causes lethal viral hemorrhagic fever in cats. Sci. Rep. 9, 11990. https://doi.org/10.1038/s41598-019-48317-8.

R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Saeki, M., Johnson, P.J., Macdonald, D.W., 2007. Movements and habitat selection of raccoon dogs (*Nyctereutes procyonoides*) in a Mosaic Landscape. J. Mammal. 88, 1098–1111. https://doi.org/10.1644/06-MAMM-A-208R1.1.

Sasaki, H., Kawabata, M., 1994. Food Habits of the Raccoon Dog *Nyctereutes procyonoides viverrinus* in a mountainous area of Japan. J. Mammal. Soc. Jpn. 19, 1–8. https://doi.org/10.11238/jmammsocjapan.19.1.

Sato, Y., Mekata, H., Sudaryatma, P.E., Kirino, Y., Yamamoto, S., Ando, S., Sugimoto, T., Okabayashi, T., 2021. Isolation of severe fever with thrombocytopenia syndrome virus from various tick species in area with human severe fever with thrombocytopenia syndrome cases. Vector Borne Zoonotic Dis 21, 378–384. https://doi.org/10.1089/vbz.2020.2720.

Shimada, M., Ochiai, K., 2016. Behavioral differences around setts and temporal niche differentiation between Japanese badgers (*Meles anakuma*) and raccoon dogs (*Nyctereutes procyonoides*) (in Japanese with English abstract). Mamm. Sci. 56, 159–165. https://doi.org/10.11238/mammalianscience.56.159.

Takahashi, T., Maeda, K., Suzuki, T., Ishido, A., Shigeoka, T., Tominaga, T., Kamei, T., Honda, M., Ninomiya, D., Sakai, T., Senba, T., Kaneyuki, S., Sakaguchi, S., Satoh, A., Hosokawa, T., Kawabe, Y., Kurihara, S., Izumikawa, K., Kohno, S., Azuma, T., Suemori, K., Yasukawa, M., Mizutani, T., Omatsu, T., Katayama, Y., Miyahara, M., Ijuin, M., Doi, K., Okuda, M., Umeki, K., Saito, T., Fukushima, K., Nakajima, K., Yoshikawa, T., Tani, H., Fukushi, S., Fukuma, A., Ogata, M., Shimojima, M., Nakajima, N., Nagata, N., Katano, H., Fukumoto, H., Sato, Y., Hasegawa, H., Yamagishi, T., Oishi, K., Kurane, I., Morikawa, S., Saijo, M., 2014. The first identification and retrospective study of severe fever with thrombocytopenia syndrome in Japan. J. Infect. Dis. 209, 816–827. https://doi.org/10.1093/infdis/jit603.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38, 3022–3027. https://doi.org/10.1093/molbev/msab120.

Tanaka, H., Yamanaka, A., Endo, K., 2002. Spatial distribution and sett use by the Japanese badger, *Meles meles anakuma*. Mammal Study 27, 15–22. https://doi.org/10.3106/mammalstudy.27.15.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680. https://doi.org/10.1093/nar/22.22.4673.

Tran, X.C., Yun, Y., Van An, L., Kim, S.H., Thao, N.T.P., Man, P.K.C., Yoo, J.R., Heo, S.T., Cho, N.H., Lee, K.H., 2019. Endemic severe fever with thrombocytopenia syndrome. Vietnam. Emerg. Infect. Dis. 25, 1029–1031. https://doi.org/10.3201/eid2505.181463.

Tsunoda, T., 2012. The tick fauna parasitizing middle- and large-sized mammals in Chiba Prefecture, Central Japan (in Japanese with english abstract). J. Nat. Hist. Mus. Inst., Chiba 12, 33–42.

Umeki, K., Yasuda, A., Umekita, K., Megumi, R., Nomura, H., Kawaguchi, T., Matsuda, M., Takajo, I., Shimojima, M., Okayama, A., 2020. Detection of anti-SFTSV nuclear protein antibody in the acute phase sera of patients using double-antigen ELISA and immunochromatography. J. Virol. Methods 285, 113942. https://doi.org/10.1016/j.jviromet.2020.113942.

Yamanaka, A., Kirino, Y., Fujimoto, S., Ueda, N., Himeji, D., Miura, M., Sudaryatma, P., Sato, Y., Tanaka, H., Mekata, H., Okabayashi, T., 2020. Direct transmission of severe fever with thrombocytopenia syndrome virus from domestic cat to veterinary personnel. Emerg. Infect. Dis. 26, 2994. https://doi.org/10.3201/eid2612.191513.

Yoshikawa, T., Shimojima, M., Fukushi, S., Tani, H., Fukuma, A., Taniguchi, S., Singh, H., Suda, Y., Shirabe, K., Toda, S., Shimazu, Y., Nomachi, T., Gokuden, M., Morimitsu, T., Ando, K., Yoshikawa, A., Kan, M., Uramoto, M., Osako, H., Kida, K., Takimoto, H., Kitamoto, H., Terasoma, F., Honda, A., Maeda, K., Takahashi, T., Yamagishi, T., Oishi, K., Morikawa, S., Saijo, M., 2015. Phylogenetic and geographic relationships of severe fever with thrombocytopenia syndrome virus in China, South Korea, and Japan. J. Infect. Dis. 212, 889–898. https://doi.org/10.1093/infdis/jiv144.

Yu, K.M., Yu, M.A., Park, S.J., Kim, Y.I., Robles, N.J., Kwon, H.I., Kim, E.H., Si, Y.J., Nguyen, H.D., Choi, Y.K., 2018. Seroprevalence and genetic characterization of severe fever with thrombocytopenia syndrome virus in domestic goats in South Korea. Ticks Tick Borne Dis. 9, 1202–1206. https://doi.org/10.1016/j.ttbdis.2018.05.001.

Yu, X.J., Liang, M.F., Zhang, S.Y., Liu, Y., Li, J.D., Sun, Y.L., Zhang, L., Zhang, Q.F., Popov, V.L., Li, C., Qu, J., Li, Q., Zhang, Y.P., Hai, R., Wu, W., Wang, Q., Zhan, F.X., Wang, X.J., Kan, B., Wang, S.W., Wan, K.L., Jing, H.-Q., Lu, J.X., Yin, W.W., Zhou, H., Guan, X.H., Liu, J.F., Bi, Z.Q., Liu, G.H., Ren, J., Wang, H., Zhao, Z., Song, J.D., He, J.R., Wan, T., Zhang, J.S., Fu, X.P., Sun, L.N., Dong, X.P., Feng, Z.J., Yang, W.Z., Hong, T., Zhang, Y., Walker, D.H., Wang, Y., Li, D.X., 2011. Fever with thrombocytopenia associated with a novel bunyavirus in China. N. Engl. J. Med. 364, 1523–1532. https://doi.org/10.1056/NEJMoa1010095.

Yun, S.M., Park, S.J., Kim, Y.I., Park, S.W., Yu, M.A., Kwon, H.I., Kim, E.H., Yu, K.M., Jeong, H.W., Ryou, J., Lee, W.J., Jee, Y., Lee, J.Y., Choi, Y.K., 2020. Genetic and pathogenic diversity of severe fever with thrombocytopenia syndrome virus (SFTSV) in South Korea. JCI Insight 5, e129531. https://doi.org/10.1172/jci.insight.129531.

Zhang, Y.Z., Zhou, D.J., Qin, X.C., Tian, J.H., Xiong, Y., Wang, J.B., Chen, X.P., Gao, D.Y., He, Y.W., Jin, D., Sun, Q., Guo, W.P., Wang, W., Yu, B., Li, J., Dai, Y.A., Li, W., Peng, J.S., Zhang, G.B., Zhang, S., Chen, X.M., Wang, Y., Li, M.H., Lu, X., Ye, C., de Jong, M.D., Xu, J., 2012. The ecology, genetic diversity, and phylogeny of Huaiyangshan virus in China. J. Virol. 86, 2864–2868. https://doi.org/10.1128/JVI.06192-11.