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Short Communication

Severe fever with thrombocytopenia syndrome virus RNA in ticks from wild mongooses in Okinawa Prefecture, Japan

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Running head: SFTSV RNA in ticks from wild mongooses

Abstract

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging zoonotic tick-borne disease caused by the SFTS virus (SFTSV). SFTSV has a wide spectrum of animal hosts and is thought to circulate in an enzootic tick-vertebrate-tick cycle. A previous seroepidemiological study demonstrated the presence of anti-SFTSV antibodies in wild mongooses (*Herpestes auropunctatus*) and indicated that outdoor activity is associated with an increased risk of tick bites among Okinawa residents. However, the association of SFTSV between wild mongooses and ticks remains unknown. Thus, to understand the association between ticks and mongooses regarding SFTSV circulation, we investigated the presence of SFTSV RNA in ticks collected from wild mongooses on the main island of Okinawa. A total of 638 ticks, belonging to two genera and three species (*Haemaphysalis hystricis, Haemaphysalis formosensis,* and *Ixodes granulatus*), were collected from 22 wild mongooses from 2016 to 2020. SFTSV RNA was detected in two pools of *H. hystricis* larvae collected from a wild mongoose in the central area of the main island of Okinawa in 2017. Although the prevalence of SFTSV in ticks from wild mongooses is low, endemic circulation of the virus in Okinawa should be carefully monitored in the area to prevent future infections.

Severe fever with thrombocytopenia syndrome (SFTS) is a viral hemorrhagic fever caused by SFTS virus (SFTSV), which is thought to be a tick-borne zoonotic virus (1, 2). SFTSV is thought to have a tick-vertebrate-tick cycle and is maintained through vertical transmission in ticks (2, 3).

In Japan, the first case of SFTS was reported in 2013 (4). Subsequently, 0.03–0.06

cases/100,000 person-years of SFTS were reported, mostly from the western part of Japan (5).

Okinawa Prefecture consists of hundreds of remote islands. The main island is divided into three areas: northern, central, and southern. Most of the population resides in the central and southern areas. A large part of the northern area is covered with natural forests and mountains, which helps maintain biodiversity. In Okinawa Prefecture, the first laboratory-confirmed case of SFTS was reported in August 2016, and it remains the only case of SFTS reported in Okinawa to date. The SFTS patient was a 67-year-old male who lived in the central area of the main island of Okinawa and had no domestic and/or foreign travel history during the incubation period. A previous seroepidemiological study revealed the presence of anti-SFTSV antibodies in wild mongooses (*Herpestes auropunctatus*) (6). Although the seroprevalence survey results indicated a low frequency of SFTSV exposure among Okinawa residents, the survey results suggested that outdoor activity was associated with an increased risk of tick bites in Okinawa (6). Therefore, there are concerns about the risk of SFTSV infection transmitted by tick bites. However, the relationship between wild mongooses and ticks regarding SFTSV circulation remains unknown. Thus, to understand the relationship between ticks and mongooses regarding SFTSV circulation, we investigated the presence of SFTSV RNA in ticks that infested wild mongooses in Okinawa from 2016 to 2021.

Wild mongooses captured for pest control were obtained from the U.S. Naval Hospital in Okinawa from 2016 to 2021. The tick species that infested wild mongooses were identified by morphology using a stereomicroscope (Leica, Wetzlar, Germany) and taxonomic criteria (7-9). Each adult was put into a separate tube. One to five nymphs or one to twenty larvae from the same species, stage, and host were combined to create pooled samples and were placed

into separate tubes. The pooled ticks were homogenized, and RNA was extracted from the tick homogenates according to the manufacturer's instructions using ISOGEN II (Nippongene, Tokyo, Japan). The extracted RNA was used as a template for quantitative reverse transcription polymerase chain reaction (RT-qPCR) targeting the S segment of SFTSV, according to a previous study (10). The RT-qPCR product was sequenced using a 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

We obtained 46 wild mongooses from 2016 to 2021, of which 22 (48%) were infested with ticks (Table 1, Fig. 1.). A total of 638 ticks belonging to two genera and three species were collected from 22 wild mongooses (Table 2). The most dominant tick species were *H. hystricis* (n=537), followed by *H. formosensis* (n=91), and *Ixodes granulatus* (n=10). Majority of the ticks collected were in the larval development stage: 497 larvae and 40 nymphs of *H. hystricis*; 78 larvae and 13 nymphs of *H. formosensis*; and 1 larva, 1 nymph, and 8 females of *I. granulatus*. The ticks were divided into 83 pools. SFTSV RNA was detected in two pools of *H. hystricis* larvae. The larvae were obtained from the same wild mongoose in the central area in November 2017 (Table 2). SFTSV RNA and anti-SFTSV antibodies were not detected from the wild mongoose with SFTSV RNA positive-ticks. Of the two pools with SFTSV RNA positive in RT-qPCR, one was identified as a partial sequence of the nucleocapsid protein coding region of SFTSV (116 bp) by sequencing analysis (DDBJ/EMBL/GenBank accession no. LC653497). Phylogenetic analysis showed that the partial sequence of SFTSV from ticks (LC653497) was clustered into genotype J1 and had 100% similarity with the partial viral sequence of the SFTS patient in Okinawa in 2016 (LC670723). However, because this study could only identify the partial sequence of SFTSV, further studies are required to understand

the genetic characteristics of SFTSV circulating in Okinawa.

From January 1999 to March 2003, most of the tick species collected from wild mongooses in Okinawa were *I. granulatus*, *Amblyomma testudinarium*, *H. hystricis*, and *H. formosensis* (11). Therefore, the tick species collected in this study are similar to those found in the previous study. In a recent study conducted in Okinawa, *H. hystricis* and *A. testudinarium* were also frequently collected from other small mammals, such as stray cats and dogs, and rodents (*Diplothrix legata*) (12). These previous studies showed that *A. testudinarium* was mainly collected in the northern area while other tick species including *H. hystricis* were found in the entire area of the main island of Okinawa, and these tick infestations in small mammals were confirmed throughout the year.

In Japan, *H. longicornis* and *A. testudinarium* are considered the primary vectors of SFTSV. However, a recent study conducted in the SFTSV-endemic area of Japan has found SFTSV in other tick species, including *H. hystricis* (13). This indicates that *H. hystricis* is also a potential vector for SFTSV and may be the cause of sporadic transmission to humans (13). Although the prevalence of SFTSV in ticks from wild mongooses was low, the presence of anti-SFTSV antibodies in wild mongooses in a previous study (6), and the presence of SFTSV RNA in *H. hystricis* larvae that infested wild mongoose in this study, suggest that *H. hystricis* and wild mongooses may be associated with the maintenance of SFTSV in nature on the main island of Okinawa. However, due to small sample size and sampling sites number in this study, further continuous monitoring of SFTSV in ticks is necessary to understand SFTSV circulation in nature of Okinawa.

In conclusion, to our knowledge, this is the first study to report the presence of SFTSV RNA

in *H. hystricis* larvae collected from a wild mongoose. Although the prevalence of SFTSV in ticks and mongooses are low, these results, combined with the results of the previous seroprevalence survey which found anti-SFTSV antibodies in wild mongooses (6), suggest that *H. hystricis* and wild mongooses may be associated with the maintenance of SFTSV in nature on the main island of Okinawa. Continuous surveillance of SFTSV in ticks and animals in Okinawa is needed to prevent future SFTSV infections in the area.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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Figure legends

Fig. 1. Geographical location of sampling sites for ticks in Okinawa Prefecture, Japan. The main island of Okinawa is represented by three areas: the northern (black), central (gray), and southern (white) areas.

Table 1. The number of collected mongoose in the main island of Okinawa during 2016-2021 and infestation rate of tick.

Characteristics	Collected year						Total
	2016	2017	2018	2019	2020	2021	
No. of mongooses collected	2	16	2	11	11	4	46
Northern area	2	1	1	3	1	0	8
Central area	0	15	1	8	10	4	38
No. of mongooses infested with ticks	2	4	0	7	5	4	22
Northern area	2	1	0	3	1	0	7
Central area	0	3	0	4	4	4	15
Infestation rate of tick, %	100	25	0	63.6	45.5	100	47.8
Northern area	100	100	0	100	100	—	87.5
Central area	—	20	0	50	40	100	39.5

Table 2. The number of ticks from 22 wild mongooses and the detection result of SFTSV RNA by RT-qPCR in the pooled tick samples

Species	Collected area	Collected Date (year/month)	Total number of ticks (n=638)				The number of positive pools/the number of tested pools			
			Male	Female	Nymph	Larva	Male	Female	Nymph	Larva
Haemaphysalis hystricis (n=537)	Northern	2016/12	0	0	13	0	0/0	0/0	0/3	0/0
		2017/11	0	0	4	176	0/0	0/0	0/1	0/18
		2019/11	0	0	4	193	0/0	0/0	0/2	0/12
		2020/1	0	0	0	4	0/0	0/0	0/0	0/1
	Central	2017/11	0	0	2	46	0/0	0/0	0/1	2/9
		2019/11	0	0	0	47	0/0	0/0	0/0	0/3
		2020/1	0	0	0	2	0/0	0/0	0/0	0/1
		2020/2	0	0	7	0	0/0	0/0	0/1	0/0
		2020/3	0	0	4	1	0/0	0/0	0/0	0/0
		2021/7	0	0	6	28	0/0	0/0	0/3	0/3
Haemaphysalis formosensis (n=91)	Northern	2016/12	0	0	9	0	0/0	0/0	0/2	0/0
		2017/11	0	0	0	23	0/0	0/0	0/0	0/1
		2019/11	0	0	0	31	0/0	0/0	0/0	0/4
		2020/1	0	0	0	1	0/0	0/0	0/0	0/1
	Central	2019/11	0	0	1	18	0/0	0/0	0/1	0/2
		2020/1	0	0	2	3	0/0	0/0	0/1	0/1
		2020/4	0	0	1	0	0/0	0/0	0/1	0/0
		2021/7	0	0	0	2	0/0	0/0	0/0	0/1
Ixodes granulatus (n=10)	Northern	2019/11	0	0	0	1	0/0	0/0	0/0	0/1
	Central	2017/9	0	1	0	0	0/0	0/1	0/0	0/0
		2019/11	0	1	0	0	0/0	0/1	0/0	0/0
		2019/12	0	2	0	0	0/0	0/2	0/0	0/0
		2021/4	0	3	0	0	0/0	0/3	0/0	0/0
		2021/7	0	1	1	0	0/0	0/1	0/1	0/0

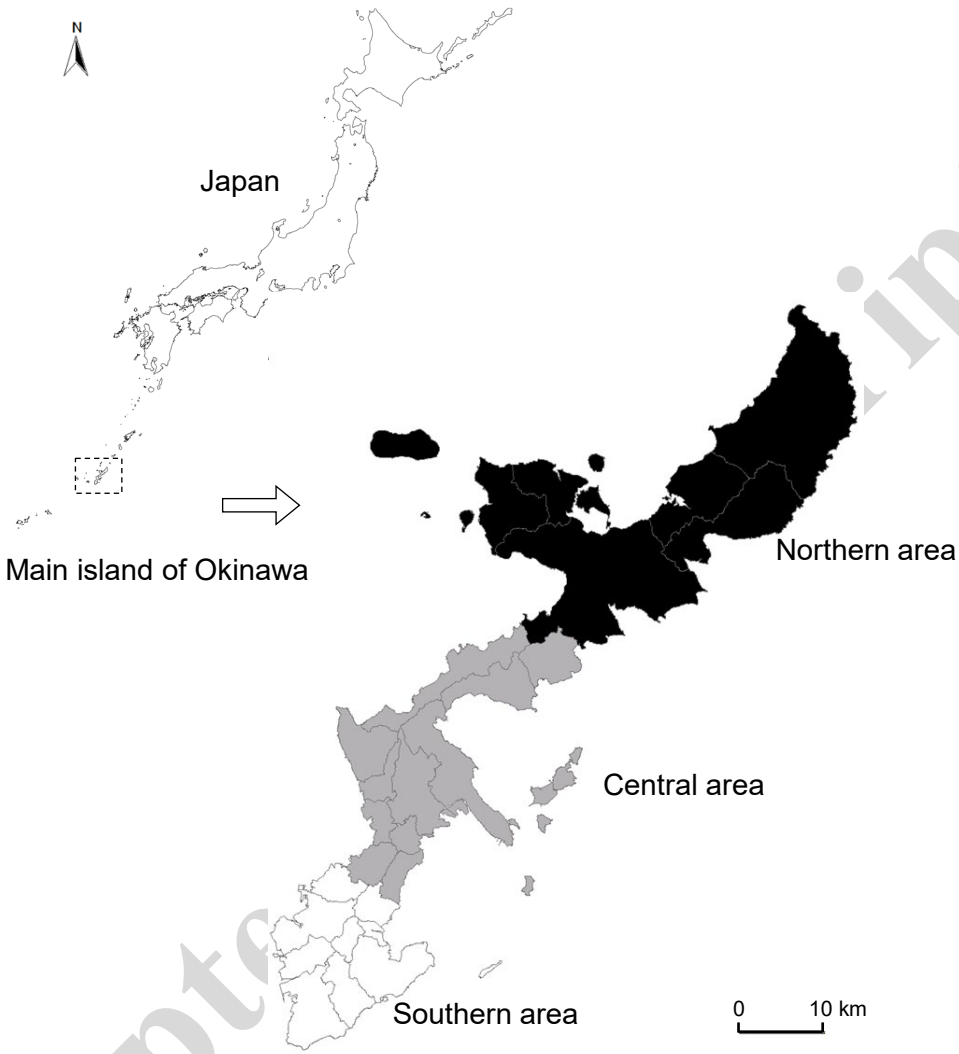

Fig. 1.