Transmission of Severe Fever with Thrombocytopenia Syndrome Virus to Human from Nonindigenous Tick Host, Japan

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We report a human case of severe fever with thrombocytopenia syndrome virus infection transmitted by a tick, confirmed by viral identification. *Haemaphysalis aborensis*, a tick species not native to Japan that has been observed to transmit the virus to humans, is now recognized as a potential vector of this virus in Japan.

Blood-feeding ticks can transmit viruses to vertebrates, including humans. A previously unknown flavivirus, Saruyama virus, was detected in Japan in 2018 (*1*); similar viral sequences have also been identified in wild deer and boars in Japan. Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tickborne disease caused by SFTS virus (SFTSV), which belongs to the family Phenuiviridae, genus *Bandavirus*. The first SFTS case was reported in China in 2010 (*2*), followed by cases in Japan and South Korea in 2013 (*3*,*4*); those 3 countries are the primary endemic areas for SFTSV.

Tick bites are the primary route of SFTSV transmission (*2*,*5*). *Haemaphysalis longicornis* ticks, native to east Asia, have been identified as a major SFTSV vector (*2*,*6*). SFTSV cases have been reported in several countries in Southeast Asia, including Vietnam and Thailand, and in South Asia, including Pakistan (*7*), suggesting that SFTSV might expand from endemic regions in tandem with its host animals or through tick migration. In Japan, several tick species, including *H. flava*, *H. megaspinosa*, *H. kitaokai*, *H. formosensis*, and *H. hystricis*, carry the SFTSV genome (*8*,*9*). The mortality rate for SFTS infection ranges from 5% to 28%; the elderly are at higher risk for fatal clinical outcomes (*10*), indicating its potential public health consequences. No antiviral drugs or vaccines are available for SFTSV infection.

We report a human case of SFTS transmitted by a novel tick host, *H. aborensis*, a tick not endemic to Japan that has been identified as a potential vector of SFTSV, indicating possible expansion of habitats of infectious ticks. That finding highlights the importance of comprehensive viral genome analysis as part of routine tick-borne viral surveillance. We obtained informed consent for publication from the patient and ethics approval from the Clinical Research Ethics Committee of Nagasaki University Hospital (record no. 23112012).

The Study

An 80-year-old female patient with a medical history of hypertension, bronchial asthma, and cerebral aneurysm (postoperative), but no history of smoking, alcohol consumption, or recent travel, experienced fever and dizziness. The case-patient resided in Nagasaki, Japan, close to the forest, and reported that she frequently encountered wild animals, such as wild boars and civets. She engaged in daily activities, regularly tended her garden, and had no companion animals. She sought medical consultation with a primary care physician on day 3 after onset of symptoms.

Blood tests revealed a drop in her leukocyte count to 3,180 cells/μL (reference range 3,300–8,600 cells/μL) and platelet count to 104,000/μL (reference range 158,000–348,000/μL). Subsequent blood tests showed further decreases in leukocytes to 1,690 cells/μL and platelets to 72,000/μL. On day 5, the case-patient was

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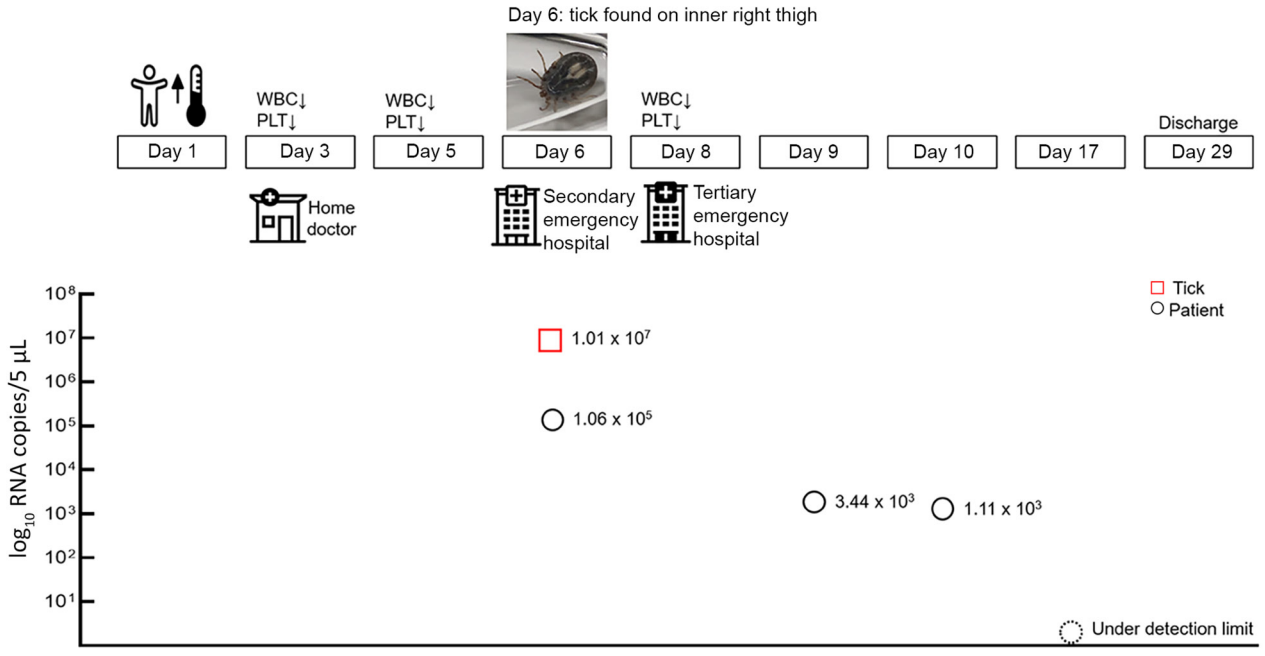

Figure 1. Timeline of SFTSV progression in a human patient in Japan and photograph of *Haemaphysalis aborensis* tick collected from the patient. WBC, white blood cells (leukocytes); PLT, platelets; SFTSV, severe fever with thrombocytopenia syndrome virus.

referred to a secondary emergency hospital for further evaluation. A blood-engorged tick was found on her inner right thigh (Figure 1). Presence of leukopenia, thrombocytopenia, and tick bites indicated SFTSV infection. A serum specimen from the patient was sent to the laboratory at Nagasaki City Health Center, which is responsible for administrative inspections for SFTS diagnosis. The results revealed SFTSV positivity on day 12 after symptom onset.

The patient was transferred from the secondary hospital to the Department of Internal Medicine of Infectious Diseases at Nagasaki University Hospital, a tertiary emergency hospital, on day 8 after onset. Physical examination at time of admission indicated a body temperature of 36.0°C, heartbeat of 66 beats/min, blood pressure of 121/72 mm Hg, SpO_2 of 94% (room air), and respiratory rate of 28 breaths/min. The patient's level of consciousness was unclear, but

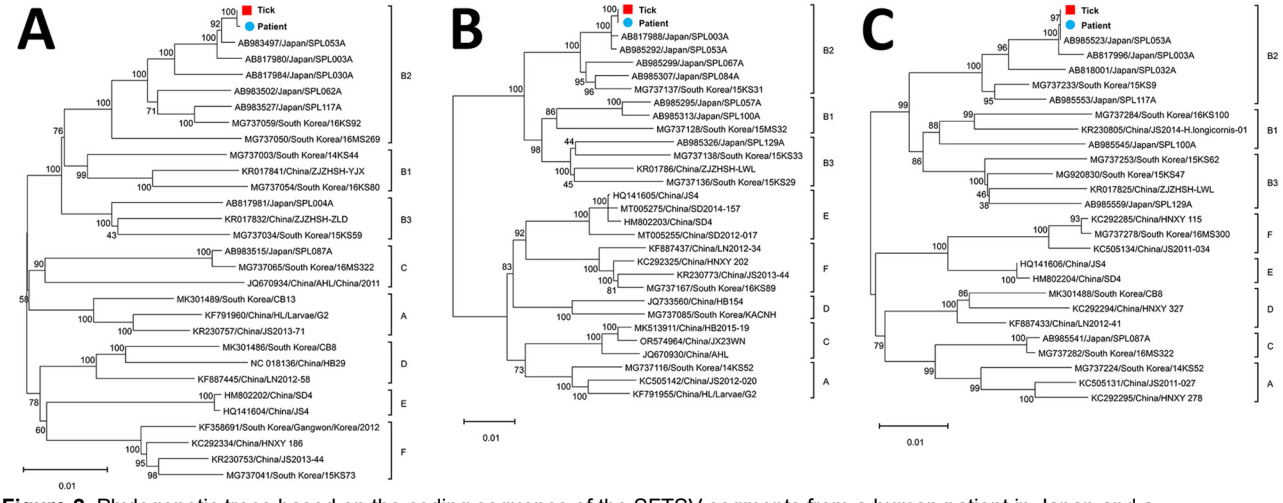

Figure 2. Phylogenetic trees based on the coding sequence of the SFTSV segments from a human patient in Japan and a *Haemaphysalis aborensis* tick collected from the patient. A) Large segment; B) medium segment; C) small segment. Blue circles indicate patient-derived SFTSV strains and red squares tick-derived strains from this study. Labels at right of each tree represent SFTSV genotypes A–F. We derived the phylogenetic trees using the maximum-likelihood method and general time-reversible model and ran 1,000 bootstrap replicates using MEGA 11.0.13 (https://www.megasoftware.net). Scale bars indicate the number of base differences per site. SFTSV, severe fever with thrombocytopenia syndrome virus.

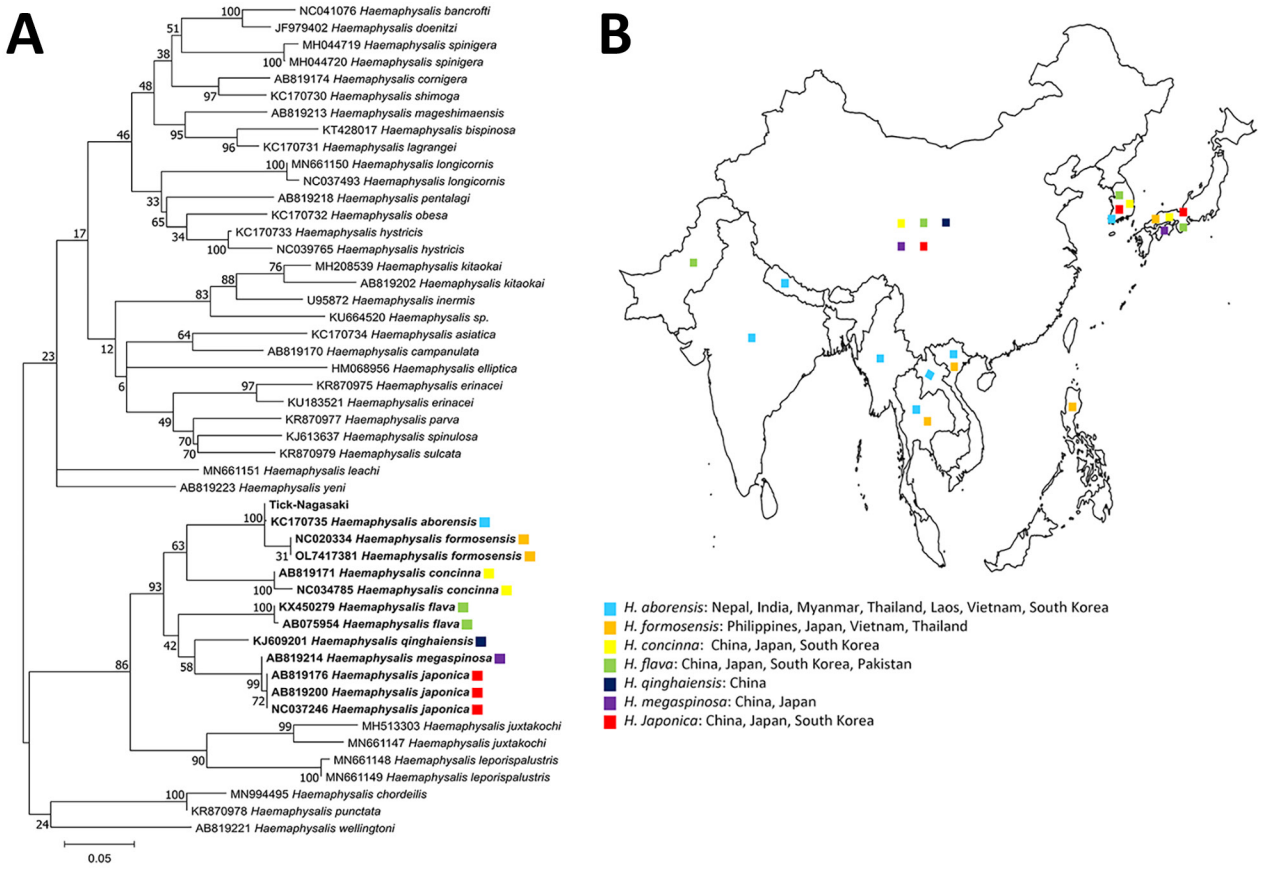

Figure 3. Phylogenetic tree (A) and geographic distribution (B) of 36 tick species from the genus *Haemaphysalis*. Bold indicates tick sequences analyzed in this study; Tick-Nagasaki indicates tick collected from a human patient in Japan who had severe fever with thrombocytopenia syndrome virus. Colors indicate locations where ticks have been found. We used 49 16S rRNA sequences to construct the maximum-likelihood tree based on 1,000 replicates in MEGA 11.0.13 (https://www.megasoftware.net). Bootstrap values are indicated next to the branches. Scale bar indicates nucleotide substitutions per site.

she responded when called, which is indicative of a II-10 rating on the Japan Coma Scale. Blood chemical examination demonstrated results with reference ranges for hemoglobin (12.3 g/dL), sodium (137 mEq/L), potassium (3.8 mEq/L), chloride (108 mEq/L), blood urea nitrogen (6 mg/dL), creatinine (0.7 mg/dL), and C-reactive protein (0.04 mg/dL). Compared with earlier test results, we noted further decreases in leukocyte count, to 2,300 cells/μL (neutrophils 920 cells/μL, lymphocytes 1,080 cells/μL), and platelet count, to 53,000/μL; we also saw increases in aspartate transferase (133 U/L, reference range 13–30 U/L), alanine transaminase (64 U/L, reference range 7–23 U/L), lactate dehydrogenase (770U/L, reference range 124–222 U/L), and creatine kinase (471 U/L, reference range 41–153 U/L). Urine examination revealed high protein 2+ and occult blood 2+ results. Results of blood cultures on days 6 and 10 and urine cultures on day 10 after onset were negative for bacterial infections. The patient gradually recovered and was discharged on day 29 after symptom onset without any specified lasting effects.

We sent the tick from the patient and serum specimens collected on days 6, 9, 10, and 17 after onset to the Department of Virology, Institute of Tropical Medicine, at Nagasaki University for examination. We extracted total RNA from the homogenized tick sample (Appendix, https://wwwnc.cdc.gov/EID/article/30/11/24-0912-App1.pdf) and subjected serum specimens to quantitative reverse-transcription PCR (qRT-PCR) (Appendix). The specimen from day 6 demonstrated the highest number of SFTSV RNA copies (1.06×10^5/5 μL). The SFTSV RNA copies in the serum specimens decreased and were undetectable on day 17 after onset (Figure 1). Homogenates from the tick demonstrated a substantially higher number of SFTSV RNA copies (1.01×10^7/5 μL) than the patient samples. We isolated viruses only from the tick, not from patient specimens.

To explore the genomic similarity of SFTSV strains derived from tick and human samples, we determined the full-length protein-coding sequences of the large (L), medium (M), and small (S) segments of viruses from the tick (GenBank accession nos. PP813867–9) and patient (accession nos. PP839300–2) by using next-generation sequencing (Appendix). We conducted phylogenetic analysis using MEGA11 (https://www.megasoftware.net) to determine the genetic relationships between the sequences from our study and previously identified SFTSV sequences from countries in Asia, including Japan (*11*). The sequences of patient- and tick-derived SFTS L/M/S segments were identical. SFTSV identified in our study's belonged to B-2 clade (Figure 2, panels A–C), the genotype most prevalent in Japan and South Korea (*10*).

Morphologic characteristics (Figure 1) identified the tick collected from the patient as belonging to the genus *Haemaphysalis*. To confirm species identification, we sequenced the 16S ribosomal RNA (accession no. PP813416) (Appendix). Phylogenetic analysis identified it as most closely related to *H. aborensis*, a species not endemic to Japan (Figure 3, panel A). We found no previous reports of SFTSV isolation or gene detection in *H. aborensis* ticks.

H. aborensis ticks are primarily distributed in Nepal and India in South Asia and Laos, Vietnam, and Thailand in Southeast Asia (Figure 3, panel B) (*12*); porcupines, wild boars, and deer are the primary hosts (*12*). A previous study identified *H. aborensis* ticks collected from *Turdus pallidus* (pale thrush) on Hong Island, South Korea (*13*). The *T. pallidus* thrush is a migratory bird that breeds in areas from northeast China to far eastern Russia and overwinters in southern and central Japan, South Korea, and southern China (*14*). Because the B-2 clade has been isolated only in Japan and South Korea (*10*), SFTSV-infected ticks were likely carried by infected birds from South Korea. Although it is possible that ticks were carried by birds from South Korea, then acquired and transmitted SFTSV through infected animals in Japan, this scenario is unlikely because *H. aborensis* ticks had not been previously identified in Japan.

Conclusions

We report a case of tick-transmitted SFTSV infection in a human patient. Virus isolation and identification of the tick species confirmed that *H. aborensis* ticks can transmit SFTSV to humans. The phylogenetic analysis revealed no differences between sequences of SFTSV from the tick and the patient. Identifying an additional host tick highlights the importance of routine tick surveillance for monitoring SFTSV expansion.

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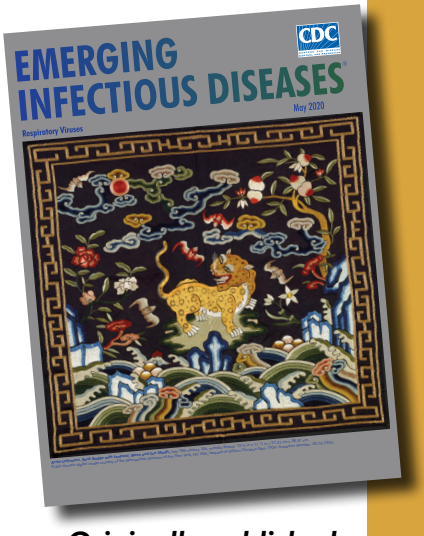

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Coronavirus

The first coronavirus, avian infectious bronchitis virus, was discovered in 1937 by Fred Beaudette and Charles Hudson. In 1967, June Almeida and David Tyrrell performed electron microscopy on specimens from cultures of viruses known to cause colds in humans and identified particles that resembled avian infectious bronchitis virus. Almeida coined the term "coronavirus," from the Latin *corona* ("crown"), because the glycoprotein spikes of these viruses created an image similar to a solar corona. Strains that infect humans generally cause mild symptoms. However, more recently, animal coronaviruses have caused outbreaks of severe respiratory disease in humans, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and 2019 novel coronavirus disease (COVID-19).

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Appendix

Additional Methods

Tick Homogenization and Extract Total RNA

The tick collected from the patient was homogenized following procedures from a previous study (*1*). Briefly, tick samples were homogenized in 500 μL of phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS) with P/S (Biowest, https://biowest.net), and 100 IU/mL penicillin and 100 μg/mL streptomycin (Sigma-Aldrich, https://www.sigmaaldrich.com), using the Bead Crusher μT-12 (TAITEC Corporation, https://e-taitec.com) and 2 mm stainless beads. RNA was extracted using ISOGEN-II (Nippon Gene, https://nippongene.com/index.html), following manufacturer instructions.

RT-qPCR Reaction

The quantitative reverse-transcription PCR (RT-qPCR) for SFTSV RNA detection was described previously (*2*). Briefly, total RNA was extracted from the patient's serum by ISOGEN-LS (Nippon Gene). The total RNA from the patient’s serum and tick homogenate was subjected to RT-qPCR using the One-Step PrimeScript RT-PCR Kit (Takara Bio,

https://www.takarabio.com) with a specific primer set and probe (Appendix Table), on the 7500 real-time RT-PCR System (Applied Biosystems, https://www.thermofisher.com).

Virus Isolation

Virus isolation procedures followed a previous study (*3*). Briefly, patient serum specimens collected on day 6 after onset and tick lysate were filtered through a Millipore 0.22 μm column (https://www.sigmaaldrich.com), and 50 μL of each was inoculated into T25 flasks containing Vero E6 African green monkey kidney cells. Next, 500 μL of Dulbecco modified Eagle medium (DMEM; Fujifilm Wako Pure Chemical, https://www.fujifilm.com) without FBS was added, and the cells were incubated at 37°C for 1 hour. Subsequently, 7 mL of DMEM with P/S and 2% FBS were added, and the cells were incubated at 37°C under 5% CO_2 for 7 days. On day 7 after inoculation, the cell supernatant was collected and 500 μL inoculated into fresh Vero E6 cells in T75 flasks for a secondary passage. Each passage was confirmed by observing the cytopathic effect and RT-qPCR.

Sequencing of SFTSV Full-Length Coding Region and Tick 16S rRNA

The RNA was extracted from tick lysate to achieve the complementary DNA synthesis by using ReverTra Ace-α-TM (Toyobo, https://www.toyobo-global.com) with random primers (Takara Bio). The 16S ribosomal RNA (rRNA) gene was amplified according to a previous study (*4*) (Appendix Table) and cloned into the Topo vector using the Zero Blunt TOPO PCR Cloning Kit (ThermoFisher Scientific, https://www.thermofisher.com). Subsequently, sequencing was performed with specific primers (Appendix Table) on an ABI 3500 Genetic Analyzer (Applied Biosystems).

RNA was extracted for next-generation sequencing from the patient's serum specimens from day 6 after onset and the tick lysate. First, a whole transcriptome library was constructed using the Ion Total RNA-Seq Kit v2 (ThermoFisher Scientific) and sequenced on the Ion Proton semiconductor sequencer (ThermoFisher Scientific). The raw sequencing data was processed

using FASTX-Toolkit (*5*) and de novo transcriptome assembly using Trinity RNA-Seq (*6*). The sequences were aligned with the ref-Seq from the National Center for Biotechnology Information (NCBI) to obtain the SFTSV sequencing results.

Appendix Table. The details of the primers used in this study include the following information for each primer: gene region, and nucleotide sequence of the primers and probes.

Primer	Region of gene	Sequence
RT-qPCR		
SFTSV-S-qPCR-F	S segment	5'- TGTCAGAGTGGTCCAGGATT-3'
SFTSV-S-qPCR-R		5'- ACCTGTCTCCTTCAGCTTCT-3'
SFTSV-S-qPCR probe		FAM-TGGAGTTTGGTGAGCAGCAGC-BHQ1
Tick identification		
16S-F	16S rRNA	5'-TTAAATTGCTGTRGTATT-3'
16S-R		5'-CCGGTCTGAACTCASAWC-3'
M13-F	Tick_16S rRNA-Topo	5'-GTAAAACGACGGCCAG-3'
M13-R	clone	5'-CAGGAAACAGCTATGAC-3'

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