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Human Case of Severe Fever with Thrombocytopenia Syndrome Virus Infection, Taiwan, 2019

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We report on a 70-year-old man with fever, leukopenia, thrombocytopenia, vomiting, malaise, dyspnea, and consciousness disturbance who was infected with severe fever with thrombocytopenia syndrome virus in northern Taiwan, 2019. This autochthonous case was confirmed by reverse transcription PCR, virus isolation, and genomic sequencing.

Severe fever with thrombocytopenia syndrome (SFTS) is a tickborne infection caused by the SFTS virus (SFTSV, also known as *Huaiyangshan banyangvirus*), which was identified in China in 2009 (*1*) and afterward in South Korea (*2*), Japan (*3*), and Vietnam (*4*). Since then, the number of SFTS cases in East Asia has risen rapidly. Therefore, laboratory-based surveillance of SFTS has been conducted in the routine molecular diagnosis of arboviral infections in the Taiwan Centers for Disease Control (Taiwan CDC) since 2013. We identified a patient in Taiwan with laboratory-confirmed SFTS who was originally suspected of having dengue or rickettsial infections.

In November 2019, a 70-year-old man who lived in northern Taiwan and had no travel history was admitted to the hospital with a 9-day history of fever (38.8°C–39.2°C), chills, nausea, vomiting, and malaise. The patient had underlying hypertension and type 2 diabetes mellitus that was controlled without medication. At hospital admission, we noted a generalized rash over the trunk and both feet. Laboratory examinations showed that the patient had leukopenia; thrombocytopenia; abnormal prothrombin time; elevated levels of aspartate transaminase, alanine transaminase, creatinine kinase, and C-reactive protein; and diagnostic disseminated intravascular coagulation (Table). Chest radiography and chest computed tomography showed patchy consolidations and ground-glass opacities of both lungs. A few hours after admission, the patient experienced a general tonic–clonic seizure, with worsening consciousness and dyspnea. He was transferred to the intensive care unit, where intubation and ventilator support began. He also received massive blood transfusions for severe thrombocytopenia, active mucosal (oral, nasal, and gastrointestinal tract) bleeding, and disseminated intravascular coagulation. Blood and sputum cultures revealed that the patient was infected with *Pseudomonas aeruginosa*; he received piperacillin/tazobactam, doxycycline, and clarithromycin as empirical therapy. Results of laboratory tests for hepatitis A and B viruses, cytomegalovirus, herpes simplex virus, adenovirus, and influenza were all negative. After the patient received a diagnosis of SFTSV infection, he received treatment with intravenous immunoglobulin for 5 days. However, his condition continued to deteriorate progressively. The patient died on day 40 after illness onset as a result of multiorgan failure. Delayed diagnosis and the presence of underlying conditions in this patient, including hypertension and diabetes mellitus, may be associated with his severe disease and death (*5*).

The patient often spent time on a vegetable farm in a mountainous area without wearing shoes, raising suspicions for arboviral and rickettsial infections. The hospital sent blood samples, collected from the patient before the blood transfusions on day 12 after illness onset, to the Taiwan CDC for

[1]These first authors contributed equally to this article.

Table. Laboratory findings of patient with severe fever with thrombocytopenia syndrome virus infection, Taiwan, 2019.

Laboratory finding	Patient value	Reference range
Leukocytes, cells/μL	1.550×10^3	$3.9–10.6 \times 10^3$
Erythrocytes, cells/μL	5.550×10^6	$3.9–5.4 \times 10^6$
Hemoglobin, g/dL	16.0	12–16
Platelets/μL	41×10^3	$150–400 \times 10^3$
% Neutrophils	68.4	42–74
% Lymphocytes	29.7	25–56
Aspartate transaminase, U/L	1,326	0–37
Alanine transaminase, U/L	569	0–40
Creatinine kinase, U/L	1,310	56–224
Creatinine, mg/dL	1.7	0.44–1.03
C-reactive protein, mg/L	7.8	<5
Total bilirubin, mg/dL	1.7	0.2–1.2
Glucose, mg/dL	250	70–100
Prothrombin time, s	14.4	6.6–11.6
Activated partial thromboplastin time, s	63.4	23.9–34.9

laboratory diagnosis of arboviral and rickettsial diseases. Arboviral infections were detected using primer sets (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/26/7/20-0104-App1.pdf) by SYBR-Green I-based real-time reverse transcription PCR (RT-PCR). In addition, we detected the SFTSV genome using SFTSV-specific primer sets targeting nonstructural protein and nucleocapsid protein genes. Results of RT-PCR and PCR for flavivirus and chikungunya virus infections, scrub typhus, murine typhus, spotted fever rickettsiae, and leptospirosis were all negative.

SFTSV was isolated from patient serum with the Vero cell line and confirmed by RT-PCR and immunofluorescence assays. SFTSV RNA remained undetected in the urine sample. The viral loads in serum continuously decreased from day 12 after disease onset and became undetectable on day 29 after disease onset (Appendix Figure 1). SuperScript III 1-step RT-PCR (http://www.thermofisher.com) identified partial small (S), medium (M), and large (L) segments of SFTSV in the serum collected on day 12 after disease onset using a different set of primers (Appendix Table 2). SFTSV has been classified into 6 different genotypes according to its genome sequence (*6*). Phylogenetic analyses of the partial S (1,704 bp; GenBank accession no. MN830173), M (3,340 bp; GenBank accession no. MN830174), and L (6,332 bp; GenBank accession no. MN910270) segment sequences using MEGA7 (*7*) using the maximum-likelihood method (Appendix Figures 2, 3) showed that the partial S, M, and L segments of SFTSV from this patient belong to genotype B and are closely related to Japanese strains. SFTSV identified in *Rhipicephalus microplus* ticks in central Taiwan belongs to genotype A/C (*8*).

All of the patient's close contacts, including 8 family members, a friend, and 60 medical personnel, were healthy and without symptoms during the monitoring period. Six mites from 2 brown rats (*Rattus norvegicus*) and 2 Asian house shrews (*Suncus murinus*) were captured in the area surrounding the residence of the patient. All the RNA samples from animals and mites showed SFTSV negative results by RT-PCR.

Although the main SFTSV tick vector, *Haemaphysalis longicornis*, has not been documented in Taiwan, other tick vectors, such as *R. microplus* and *Amblyomma testudinarium*, have been found in wild and domestic animals (*8*–*10*). Further studies on the identification of natural vectors and routes of transmission are needed. The presence of an emerging SFTS case highlights the need for further studies of the prevalence, geographic distribution, and surveillance of SFTSV in Taiwan.

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Lesions of *Mycobacterium avium* spp. *hominissuis* Infection Resembling *M. bovis* Lesions in a Wild Mule Deer, Canada[1]

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[1]Preliminary results from this study were presented at the American College of Veterinary Pathologists Annual Meeting, November 9-13, 2019, San Antonio, Texas, USA

We used molecular analyses to confirm *Mycobacterium avium* spp. *hominissuis* infection in lung granulomas and pyogranulomas in the tracheobronchial lymph node in a wild mule deer in Banff, Canada. These lesions are similar to those found in *M. bovis*–infected animals, emphasizing the critical need for disease surveillance in wildlife populations.

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In November 2018, a wild yearling male mule deer (*Odocoileus hemionus*) was found dead in Banff National Park, Alberta, Canada. The carcass was submitted to the Canadian Wildlife Health Cooperative Alberta Region at the University of Calgary (Calgary, Alberta, Canada) for diagnostic investigation. The University of Calgary Veterinary Sciences Animal Care Committee approved this research (AC17-0010).

Necropsy revealed that the deer had died of blunt-force trauma, presumably having been struck by a vehicle. During the necropsy, we found mineralized granulomas in the right caudal lung lobe and multifocal pyogranulomatous lymphadenitis in the tracheobronchial lymph node (Figure). We fixed lesion samples in 10% neutral buffered formalin for 48 h, then processed the samples by routine methods; we stained 4-μm-thick sections of paraffin-embedded tissues with hematoxylin and eosin before examination with light microscopy by an anatomic veterinary pathologist (J.L.R.), certified by the American College of Veterinary Pathologists.

Histopathology of the lung confirmed granulomatous pneumonia. Affected multifocal areas were characterized by central necrotic debris that was variably mineralized, surrounded by macrophage aggregates, multinucleated giant cells, lymphocytes, plasma cells, and variably thick fibrous capsules. Histopathology of the lymph node revealed similar multifocal areas of necrosis, variable mineralization, and infiltration of macrophages in the edges of some affected areas. Because of autolysis and freeze-thaw artifact, further characterization of the inflammatory cell population was not possible.

We designated the lymph node lesions as pyogranulomatous lymphadenitis because of the suppurative gross appearance of the lymph node combined with the microscopic presence of macrophages and mineralization revealed during histologic examination. We identified acid-fast organisms in multifocal macrophages at the margin of necrotic areas in both the lung and lymph node lesions. We submitted frozen samples to the Prairie Diagnostic Services laboratory

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Appendix

RNA Extraction and Reverse Transcription PCR (RT-PCR)

We extracted RNA from 140 μL acute-phase sera specimens using the QIAamp Viral RNA Mini Kit (QIAGEN, https://www.qiagen.com) according to the manufacturer's instructions. We performed real-time RT-PCR amplification using Quantitect SYBR green RT PCR kit (QIAGEN) with the following parameters: 50°C for 30 min; 95°C for 15 min and 45 cycles of 95°C for 15 sec, 55°C for 30 sec, 72°C for 20 sec, and 77°C for 20 sec; and 95°C for 1 min, melting curve program from 68°C to 95°C. We analyzed the results using the software program of the LightCycler 96 or 480 Real-Time PCR system (Roche Diagnostics, https://www.roche.com). The plasmid containing S genomic segment of severe fever with thrombocytopenia syndrome virus (SFTSV) HB29 strain (GenBank accession no. NC 018137) served as positive control for the RT-PCR assay.

RT-PCR and Nucleotide Sequencing

We extracted RNA from the patient's serum sample and further subjected it to RT-PCR for sequencing. The primers were designed based on the S (AB985557), M (AB985653 and AB985320), and L (AB983531) segment sequences. We performed RT-PCR using Superscript III One-Step RT-PCR system with Platinum Taq High Fidelity (Invitrogen, https://www.thermofisher.com) under the following conditions: 55°C for 30 min; 94°C for 2 min and 40 cycles of 94°C for 15 sec, 50°C or 54°C for 30 sec, and 68°C for 1 min; and a prolonged extension at 68°C for 5 min. We sequenced the RT-PCR products directly by using the BigDye Terminator Cycle Sequencing Kit and the ABI 3730xl DNA analyzer (Applied Biosystems,

https://www.thermofisher.com) according to the manufacturer's protocol. We used each forward and reverse primer for sequencing and the overlapping sequences were combined.

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Appendix Table 1. Primers used for SYBR green I-based real-time RT-PCR assay for severe fever with thrombocytopenia syndrome virus infection, Taiwan, 2019.*

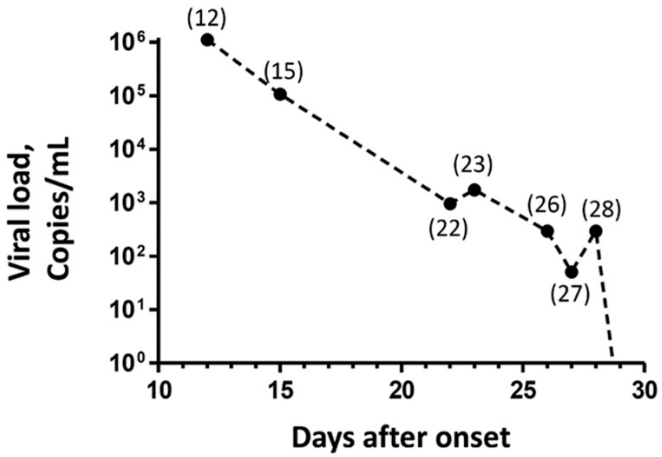
Name	Sequence (5' to 3')	Amplicon (bp)	Reference
SFTSV-SF	ACCTCTTTGACCCTGAGTTWGACA	125	(*1*)
SFTSV-SR	CTGAAGGAGACAGGTGGAGATGA		
SFTSV-NP-1F	ATCGTCAAGGCATCAGGGAA	458	(*2*)
SFTSV-NP-1R	TTCAGCCACTTCACCCGAA		
SFTSV-NP-2F	CATCATTGTCTTTGCCCTGA	461	(*2*)
SFTSV-NP-2R	AGAAGACAGAGTTCACAGCA		
SFTS-1F	GGAAACTGGRAGAGAGAACT	240	This study
SFTS-1R	GAAGTGAACAAGTGGTGGTT		
SFTSV-LF	AGTCTAGGTCATCTGATCCGTTYAG	92	(*3*)
SFTSV-LR	TGTAACTTCGCCCTTTGTCCAT		
DN-F	CAATATGCTGAAACGCGAGAGAAA	171	(*4*)
DN-R1	CCCCATCTAACCAATATTCCTGCT		
DN-R2	CCCCATCTGTTCAGTATCCCTGCT		
FL-F1	TGYGTBTACAACATGATGGG	272 or 200	This study
FL-F2	ATATGGTACATGTGGCTAGGAGC		
FL-R	GTGTCCCANCCHGCTGTGTCA		
F-CHIK	AAGCTYCGCGTCCTTTACCAAG	209	(*5*)
R-CHIK	CCAAATTGTCCYGGTCTTCTT		

*RT-PCR, reverse transcription PCR; SFTSV, severe fever with thrombocytopenia syndrome virus.

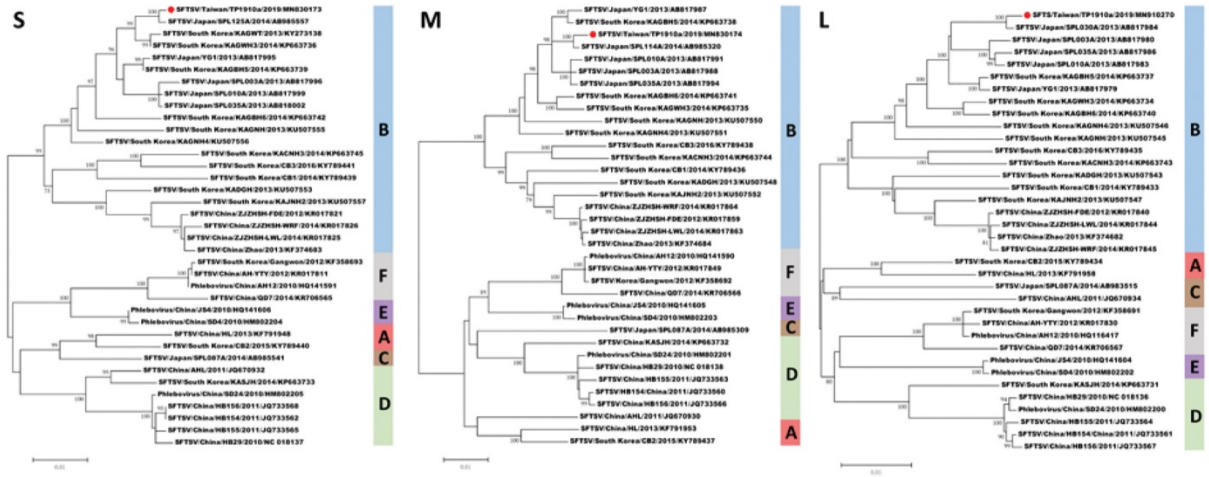
Appendix Table 2. Primers used for conventional RT-PCR assay and sequencing for severe fever with thrombocytopenia syndrome virus infection, Taiwan, 2019.*

Name	Sequence (5' to 3')	Amplicon (bp)	Reference
SFTSVS1_F	ACACAAAGAACCCCCAAAAAAG	605	This study
SFTSVS1_R	CTCCGCGCATCTTCACATTG		
SFTSVS2_F	GTCAGAGTGGTCCGGAGTTG	560	
SFTSVS2_R	CTCCGCGCATCTTCACATTG		
SFTSVS3_F	GGTTGTGGAATGGGCACCTA	730	
SFTSVS3_R	ACTGTCAGGCTTGAGCCATC		
SFTSVS4_F	CTGATTCCAACTGCAGGGGT	747	
SFTSVS4_R	GCCATAGAGAAGCGAAGGCT		
SFTSVS5_F	AGCCTTCGCTTCTCTATGGC	523	
SFTSVS5_R	ACACAAAGAACCCCCTTCAT		
SFTSVM1_F	ACACAGAGACGGCCAACA	774	
SFTSVM1_R	GATGAGCTCTCTTGCACCCA		
SFTSVM2_F	ACCTCAGCCCCTTGATGTTG	690	
SFTSVM2_R	TTTGCAGGGTAGCACTGAGG		
SFTSVM3_F	GTGGCAAGAAAAGCACGGAG	660	
SFTSVM3_R	GCAGACATGCCTCTTGTCCT		
SFTSVM4_F	TCTGTGAGCTGCTTGATGGG	836	
SFTSVM4_R	TCACACAGGTCAGTCAAGCC		
SFTSVM5_F	GGCCCCTTCATGCATCTTCT	746	
SFTSVM5_R	ACGCAGTCTCATCCCCTTTG		
SFTSVM6_F	CCCTTGGACATCACAGCCAT	640	
SFTSVM6_R	CAACTCCCCTGAGAGCACTG		
SFTSVM7_F	CCCTTGGACATCACAGCCAT	723	
SFTSVM7_R	ACACAAAGACCGGCCAACAC		
SFTSVL1_F	ACACAGAGACGCCCAG	857	
SFTSVL1_R	GATCAGCATAGGCCTCCACC		
SFTSVL2_F	CCAGGCCTGTACGACCAAAT	834	
SFTSVL2_R	CTCTAGCCGCTCCTGAATGG		
SFTSVL3_F	CCATTCAGGAGCGGCTAGAG	901	
SFTSVL3_R	TCCCCAGCATCAATGGTGTC		
SFTSVL4_F	CATCTGGATTGCATGGTGCG	728	
SFTSVL4_R	GGGCTCAACTCACAGACACA		
SFTSVL5_F	CAGAACCTTGAGGAGCGTGT	886	
SFTSVL5_R	TGACATCCACCACCCCATTG		
SFTSVL6_F	CAATGGGGTGGTGGATGTCA	748	
SFTSVL6_R	CTGCTCCACCCAGTCTTCAG		
SFTSVL7_F	CCTCATGGACAACCCTGCAT	856	
SFTSVL7_R	AAAGGGGCCATCCCTCAATG		
SFTSVL8_F	AGGTGTGGTTTGGCCTGAAA	857	
SFTSVL8_R	ACAGCCGTCAGTCCTTGATG		
SFTSVL9_F	TGCATGGTTAGGCTGAGTGG	997	
SFTSVL9_R	ACACAAAGACCGCCCAGATC		
SFTSV_1324LF	GGCAGCAAACCAGAAGAAAG	1003	(*6*)
SFTSV_2326LR	CATTTCTCCGAGGGCATTTA		

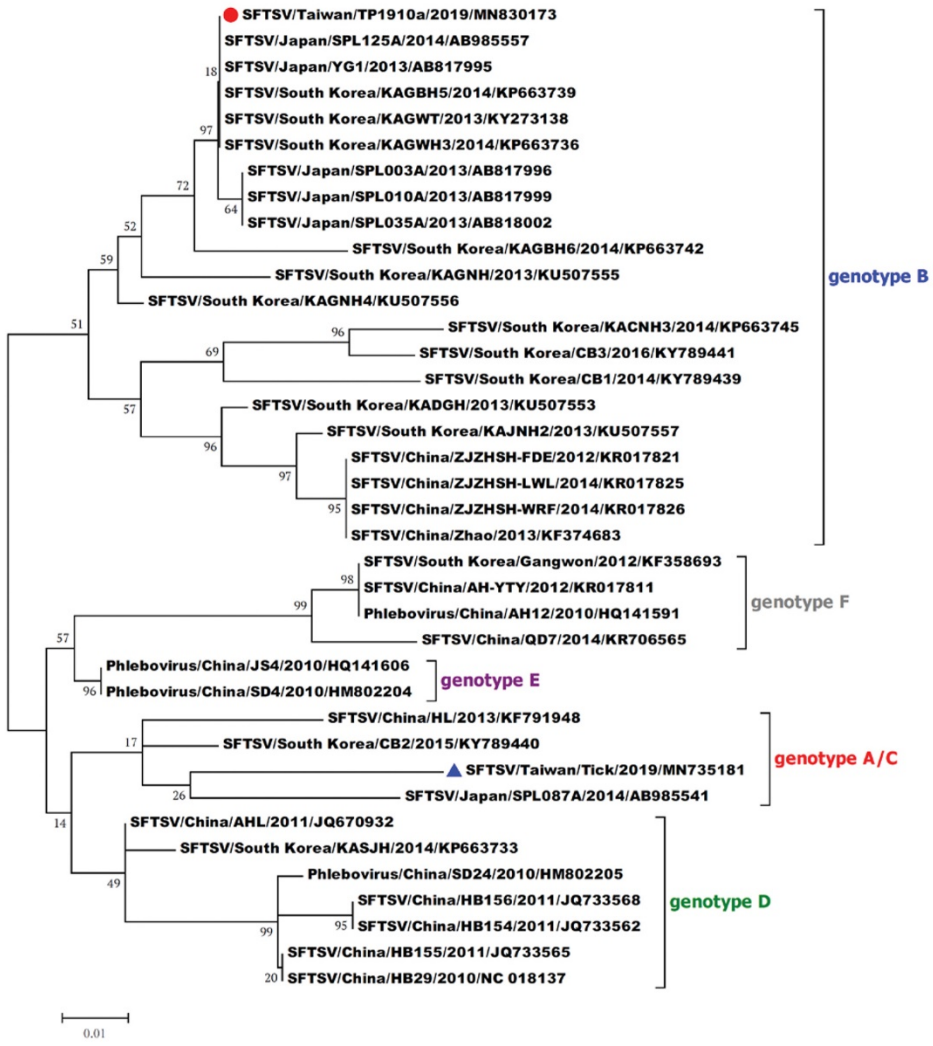
*RT-PCR, reverse transcription PCR; SFTSV, severe fever with thrombocytopenia syndrome virus.



Appendix Figure 1. Viral loads in serum samples of the severe fever with thrombocytopenia syndrome case in Taiwan. Viral copy numbers were calculated by SYBR-Green I-based real-time one-step RT-PCR with an SFTSV-specific primer set (SFTSV-SF and SFTSV-SR). The days after disease onset are marked.



Appendix Figure 2. Phylogenetic analysis of severe fever with thrombocytopenia syndrome case in Taiwan. Trees show the genetic relationships of six genotypes according to the partial S segment (1,704 bp) containing the complete nucleocapsid and nonstructural protein gene sequences of SFTSV strains (S), the partial M segment (3,340) containing the complete membrane glycoprotein gene sequence of SFTSV strains (M) and the partial L segment (6,332 bp) containing the complete RNA polymerase gene sequence of SFTSV strains (L). Viruses were identified by virus/country/strain/year of isolation/GenBank accession no. The analysis was performed by using MEGA7 software and the maximal-likelihood method (Kimura 2-parameter model). Bootstrap support values ≥ 75 are shown (1,000 replicates) along the branches. Scale bars indicate nucleotide substitutions per site.



Appendix Figure 3. Phylogenetic analysis of severe fever with thrombocytopenia syndrome virus identified in the human case (red solid circle) and *R. microplus* tick (blue solid triangle) in Taiwan. Trees show the genetic relationships of six genotypes according to the partial S segment nucleotide sequences (304 bp) of nucleocapsid protein gene of SFTSV strains. Viruses were identified by virus/country/strain/year of isolation/GenBank accession no. The analysis was performed by using MEGA7 software and the maximal-likelihood method (Kimura 2-parameter model). All bootstrap support values are shown (1,000 replicates) along the branches. Scale bars indicate nucleotide substitutions per site.