formation is arguably needed before an animal can be designated a reservoir (*8*). Despite intense Zika outbreaks in humans, no active Zika virus infection and a low seroprevalence (2.9%) with low antibody titers was found in various NHP species in Brazil, suggesting that New World NHPs are unlikely to sustain sylvatic transmission cycles (*9*). Antibody responses after flavivirus infection are broadly cross-reactive and cross-neutralizing in the first few months after infection (*10*), but the effects against heterologous flaviviruses are poorly understood in wild macaques. Also, the circulation of Zika virus in macaques could be affected by the sylvatic cycles of other endemic flaviviruses. In conclusion, the low seroprevalence of Zika virus antibodies in long-tailed macaques reinforces the need to study other NHPs and mammals as reservoirs in Malaysia to elucidate Zika virus transmission and emergence.

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Severe Fever with Thrombocytopenia Syndrome Virus in Dogs, South Korea

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Of 103 serum samples collected from dogs in South Korea, 3 (2.9%) were positive for severe fever with thrombocytopenia syndrome virus (SFTSV) and 22 (21.4%) were positive for antibodies against SFTSV. A dog-derived isolate of SFTSV clustered with many South Korea SFTSV strains in the Japanese clade.

Severe fever with thrombocytopenia syndrome virus (SFTSV), a new tickborne phlebovirus of the *Phenuiviridae* family (previously *Bunyaviridae*), causes severe fever with thrombocytopenia syndrome (SFTS) in China, Japan, and the Republic of Korea (South Korea) (*1*). After identification of the first human case of SFTS in South Korea in 2013 (*1*), 335 cases (73 deaths; case-fatality rate 21.8%) were reported during 2013–2016 (*2*).

SFTSV is primarily transmitted through a tick bite. The *Haemaphysalis longicornis* tick is the main vector for SFTSV, promoting its circulation and transmission (*3*). Investigations have been conducted to determine the frequency of exposure of companion animals, wild animals, and livestock to SFTSV (*4*–*7*). Of particular importance, dogs are companion animals that have frequent contact with humans. Therefore, their infection status has major implications for public health. We isolated SFTSV from dog serum and determined the prevalence of SFTSV in dogs in South Korea.

We collected 103 serum samples during June–October 2016 from the following dog breeds: 42 Belgian Malinois, 58 German shepherds, and 3 Labrador retrievers. All dogs were military dogs in a training camp in Gangwon Province, South Korea, at the time of serum collection. The animals had no significant clinical signs associated with febrile disease. Information about body temperature, evidence of tick bites, blood chemistry, and complete blood count was unavailable.

Of the 103 samples, 3 (2.9%), obtained from dog 16, a German shepherd; dog 22, a Belgian Malinois; and dog 56, a German shepherd were positive for the small (S [346 bp]), medium (M [859 bp]), and L (large [1,165 bp]) segments of SFTSV by reverse transcription PCR (the L segment of dog 16 was not amplified). The sequences of the SFTSV S, M, and L segments differed from each other. The results of phylogenetic analysis of partial S, M, and L segments showed that sequences of SFTSV obtained from dogs were more related to strains from Japan than to strains from China (Appendix, https://wwwnc.cdc.gov/EID/article/25/2/18-0859-App1.pdf). Moreover, 22 (21.4%) samples were positive for SFTSV antibodies by immunofluorescence assay. SFTSV

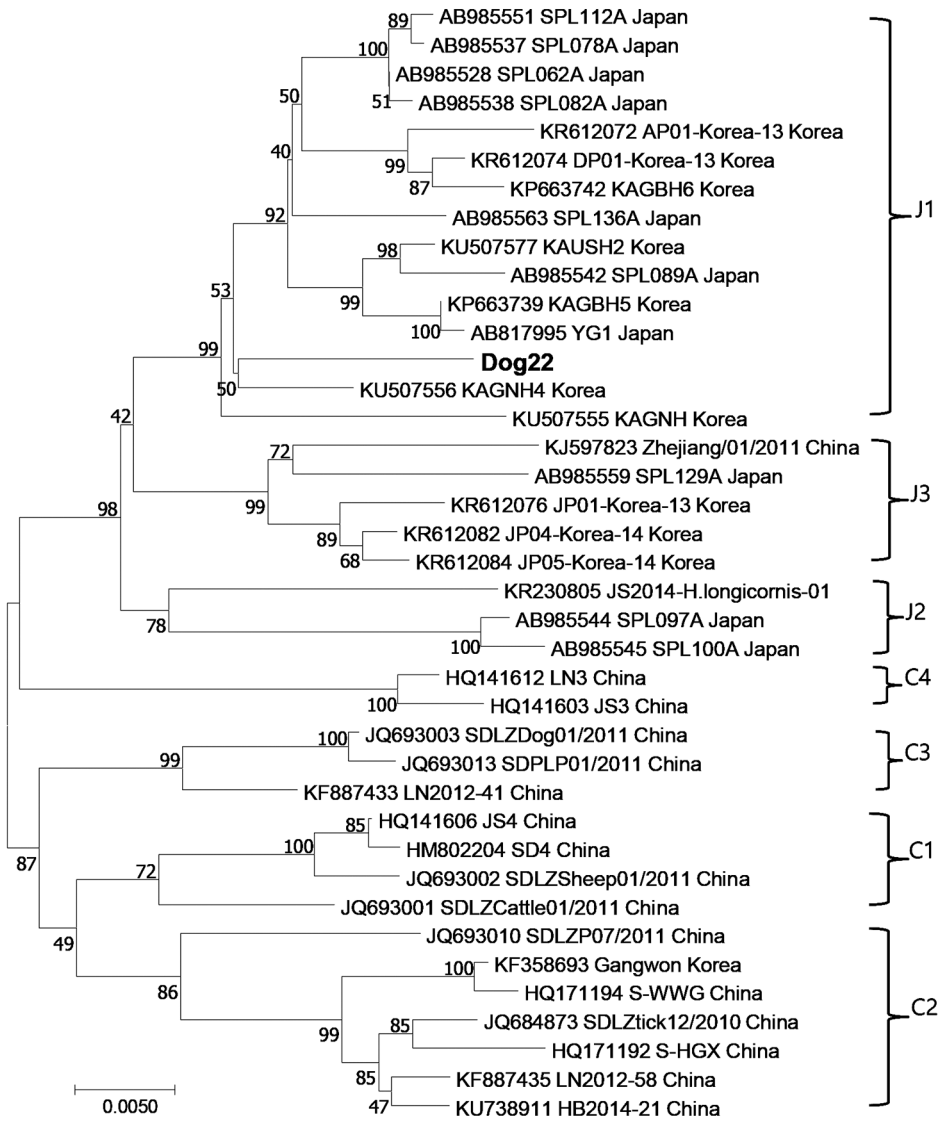

Figure. Phylogenetic analysis of severe fever with thrombocytopenia syndrome virus isolated from a military dog in South Korea (dog 22, bold) compared with reference viruses, based on the complete small segment. Evolutionary history was inferred using the maximum-likelihood method, based on the Kimura 2-parameter model (1,000 bootstrap replicates). The percentage of trees in which associated taxa clustered is shown next to the branches. The clades are designated by Japanese group. Scale bar indicates nucleotide substitutions per position.

seroprevalence was 25.9% (15/58) for Belgian Malinois, 16.7% (7/42) for German shepherds, and 0% (0/3) for Labrador retrievers. Among seropositive dogs, 22.2% (12/54) were male and 20.4% (10/49) were female.

We used Vero cells to isolate the virus from positive serum. We observed cytopathic effect in only 1 of 3 positive samples. The results of phylogenetic analysis of the complete S segment indicated that the SFTSV strain isolated from dog 22 had not previously been isolated; this strain clustered with many SFTSV strains from South Korea and Japan (Figure).

H. longicornis ticks are the main vector of SFTSV and the dominant tick species collected from vegetation and animals in South Korea (*3*,*7*,*8*). However, because of the low SFTSV prevalence in ticks, mammalian hosts might be necessary for the circulation and maintenance of SFTSV in nature. Therefore, studies measuring the prevalence of SFTSV infection across various animal species have been undertaken (*4*–*7*). Only a few studies on SFTSV in dogs have been reported; these studies found that 1) the positive rates for SFTSV RNA were 5.3% (19/359) for domesticated dogs in China (*5*) and 0.2% (1/426) for shelter dogs in South Korea (*6*) and that 2) 28.9%–37.9% of domesticated dogs in China (*4*,*5*,*9*) and 13.9% of shelter dogs in South Korea (*10*) were seropositive for antibodies against SFTSV.

The detection rates of SFTSV RNA and antibodies in our study were 2.9% and 21.4%, respectively, which were higher than those observed in shelter dogs in South Korea (*6*). These results have 2 possible explanations. First, we collected samples during the summer, when dogs most easily and frequently have contact with ticks infected with SFTSV. In contrast, in the shelter dog study, the timing of sample collection was random and occurred throughout multiple seasons. Second, we drew serum from military dogs, which typically spend most of their time outside of the home; conversely, the shelter dog study examined small dogs that resided indoors before their relocation to a shelter.

Although we isolated only a few SFTSV strains from animals and our results could not represent all characteristics of SFTSV, our findings could indicate that SFTSV might not be host-specific and that various SFTSV clades circulate and are distributed in South Korea. Further studies continuously surveilling animals for SFTSV, along with whole-genome analysis of dog-derived Korean isolates of SFTSV, would help clarify the mechanisms of transmission and molecular evolution of SFTSV.

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Appendix

Methods

A total of 200 μL of serum was used for RNA extraction using the Gene-spin Viral DNA/RNA Extraction Kit (iNtRON Biotechnology, https://intronbio.com). Reverse transcription (RT), first PCR, and nested PCR were conducted to amplify S, M, and L segments of the SFTS viral RNA gene using a 1-step RT-PCR premix kit (Solgent, http://www.solgent.com/) with SFTSV genome–specific primer sets for PCR (Appendix Table). To identify the sequences for the SFTSV-positive samples, TA cloning with pGEM-T Easy Vectors (Promega, https://www.promega.com/) and sequencing using an automatic sequencer (3730xl capillary DNA Analyzer; Applied Biosystems, Foster City, CA, USA) were performed. The S segments (KY968712–KY9689714), M segments (KY968715–KY9689717), and L segments (KY968718 and KY9689719) of SFTSV were successfully sequenced and submitted to GenBank.

Vero cells were seeded in flat-bottomed 12-well plates at a concentration of 10^5 cells in 2 mL of Dulbecco's modified eagle medium (GE healthcare Life Sciences, https://www.gelifesciences.com/) containing 2% fetal bovine serum (Invitrogen, https://www.thermofisher.com/). After Vero cell monolayers were formed, 100 μL of positive serum was added into the 12-well plates. The plates were then incubated at 37°C with 5% CO_2 for 5–7 days. For identification of the isolated virus, viral RNA was extracted from the supernatants of passaged (passage 2–4) and infected cells. Sequencing of the complete S segment from isolated SFTSV was performed using the additional set of primers provided by Professor Lee (Jeju National University School of Medicine, Jeju, South Korea). Using the maximum-likelihood method in the MEGA 7 program (*2*) and based on the Kimura 2-parameter model, phylogenetic trees were constructed using the complete S segment sequences obtained from this study and GenBank.

An indirect immunofluorescence assay (IFA) was performed to detect antibodies against SFTSV. IFA slides were prepared using SFTSV-infected Vero cells. Vero cells were resuspended at 10^5 cell/well in media and were added to each well of 24 well slide glasses and incubated in 5% CO_2 for 16 h. Then the slides were fixed with 100% acetone for 10 min at –20°C. Then the diluted serum was added into IFA antigen slides and incubated in 5% CO_2 for 1 h. After washing with PBS, FITC-conjugated anti IgG (Sigma, https://www.sigmaaldrich.com/) was added to each well of the antigen slide and incubated in 5% CO_2 for 1 h. The visualization of the IFA slides was performed using EVOS® FL auto cell imaging system (Thermo Fisher Scientific, Inc., https://www.thermofisher.com/). The cutoff IFA value was determined based on the serial 2-fold dilution of positive and negative sample serum from 1:100 to 1:800 (data not shown). The goat serum that was positive against SFTSV (received from Korean Animal and Plant Quarantine Agency) was used as positive control.

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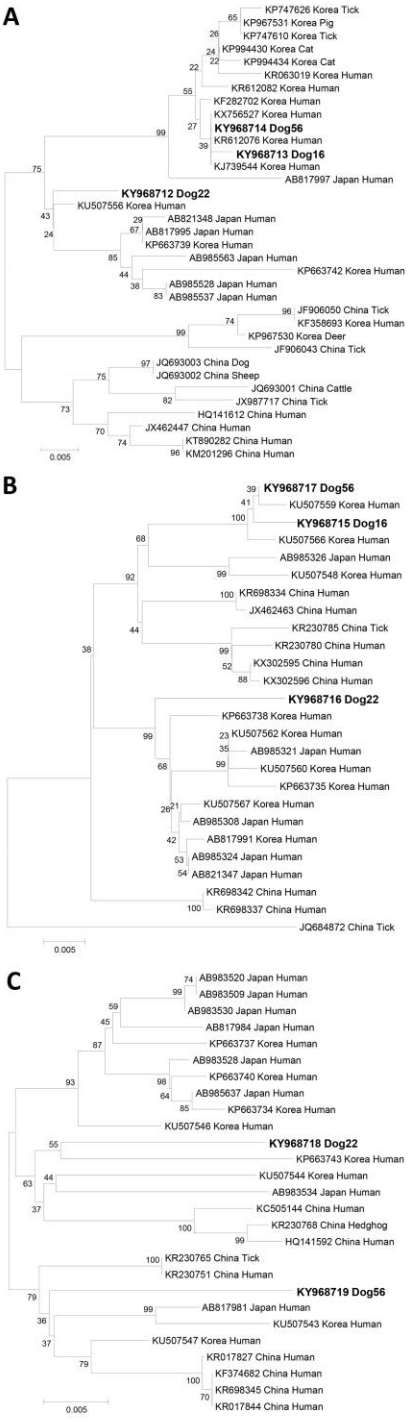
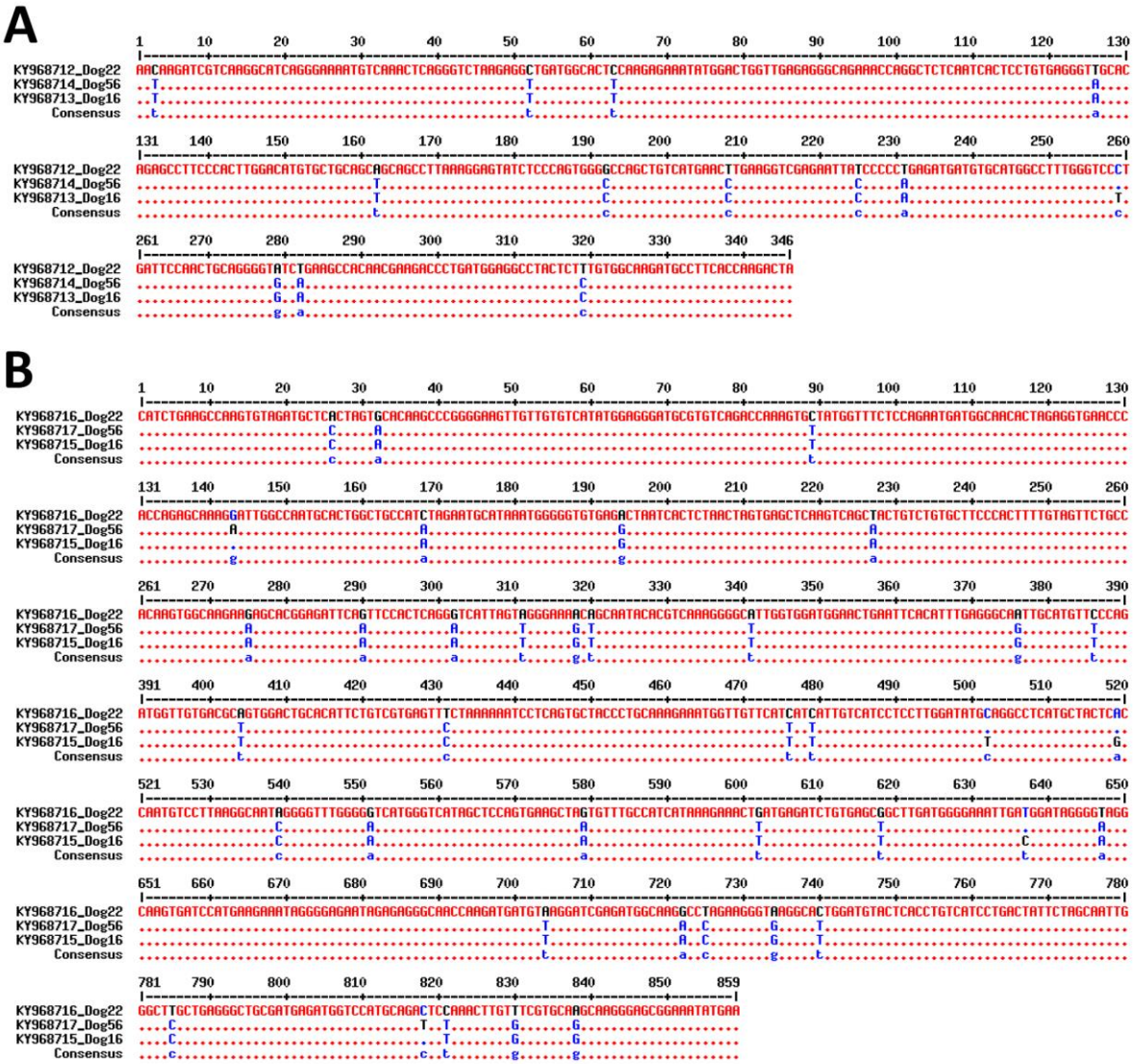
Appendix Table. Nucleotide sequences of PCR primers and conditions for amplification of severe fever with thrombocytopenia syndrome virus genes

Target gene	PCR primers and conditions	Primer sequences, 5′–3′				PCR product size, bp	Reference
		Denaturation, °C/sec	Annealing, °C/sec	Extension, °C/sec	Cycle		
S segment	NP-2F	CATCATTGTCTTTGCCCTGA				461	(*1*)
	NP-2R	AGAAGACAGAGTTCACAGCA					
	Conditions	94/20	52/40	72/30	40		
	N2-F	AAYAAGATCGTCAAGGCATCA				346	
	N2-R	TAGTCTTGGTGAAGGCATCTT					
	Conditions	94/20	54/20	72/30	25		
M segment	MF1	CATCTGAAGCCAARTGYAGA				859	This study
	MR1	TTCATATTTCCGCTCCCTTG					
	Conditions	94/30	56/40	72/60	38		
L segment	LF1	GGCAGCAAAYCAGAAGAAAG				1,165	This study
	LR2	TTGTCTTCCATGTCGCTGAG					
	Conditions	94/30	56/40	72/60	38		



Appendix Figure 1. Phylogenetic analysis of severe fever with thrombocytopenia syndrome virus based on the partial small (A), medium (B), and large (C) segments. The sequences identified in this study are indicated by bold letters. Evolutionary history was inferred using the maximum-likelihood method, based on the Kimura 2-parameter model (1,000 bootstrap replicates). The percentage of trees with associated taxa clustered together is shown next to the branches. Scale bars indicates the number of nucleotide substitutions per position. The clades are designated by Japanese group.



Appendix Figure 2. Genetic variation between 1 isolate (dog 22) and 2 amplified sequences (dog 16 and dog 56) on the partial small (A) and medium (B) segments.