Transmission of Severe Fever with Thrombocytopenia Syndrome Virus by Haemaphysalis longicornis Ticks, China

Lu Zhuang, Yi Sun, Xiao-Ming Cui, Fang Tang, Jian-Gong Hu, Li-Yuan Wang, Ning Cui, Zhen-Dong Yang, Dou-Dou Huang, Xiao-Ai Zhang, Wei Liu, Wu-Chun Cao

We demonstrate maintenance and transmission of severe fever with thrombocytopenia syndrome virus by *Haemaphysalis longicornis* ticks in the larva, nymph, and adult stages with dissemination in salivary gland, midgut, and ovarian tissues. The *H. longicornis* tick is a competent vector to transmit this virus in both transovarial and transstadial modes.

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by SFTS virus (SFTSV), identified in China in 2009 (1) and subsequently in South Korea (2) and Japan (3). Symptoms of SFTS usually include fever, thrombocytopenia, and leukocytopenia; case-fatality rates are 10%–30% (1,4). SFTS is implicated as largely a tick-associated disease, supported by evidence that many patients had exposure to ticks before disease onset (1). The longhorned tick, *Haemaphysalis longicornis*, the most abundant human-biting tick species in most SFTS-endemic areas of China (5), was found to harbor SFTSV (1,6,7). These studies suggested that *H. longicornis* ticks might be competent vectors for SFTSV transmission. Our study was designed to determine the role of the *H. longicornis* tick as a vector in maintenance and transmission of SFTSV.

The Study

We randomly allocated 90 female *H. longicornis* ticks from an SFTSV-free colony into 2 equal groups, experimental and control. We injected the experimental group with SFTSV and the control group with phosphate-buffered saline (PBS). Seven days postinjection, we used 18 of the 35 live SFTSV-infected ticks for the detection of viral RNA by real-time reverse transcription PCR (rRT-PCR) (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/5/15-1435-Techapp1.pdf); all showed positive results, confirmed by subsequent rRT-PCR and sequencing analysis. Twelve days postinjection, we dissected 5 live ticks from the experimental group to detect SFTSV in salivary glands and ovaries by indirect fluorescence assay (IFA), which showed notable SFTSV-specific fluorescence (Figure 1, panel A). For the control group, none of the 19 ticks tested by rRT-PCR had SFTSV RNA, and none of the 5 ticks tested by IFA showed SFTSV-specific fluorescence (Figure 1, panel B).

We then let the remaining 12 live ticks from both groups feed on naive Balb/C mice (4 ticks/mouse) until the ticks detached from the mice. The engorged females were harvested and maintained to lay eggs. We determined transovarial transmission of SFTSV by further testing of SFTSV RNA from eggs, larvae, and nymphs using rRT-PCR. A total of 15 pools of eggs laid by 5 infected *H. longicornis* ticks (3 pools from each tick, each pool coming from a single female) were SFTSV RNA positive. In contrast, the egg pools from ticks of the control group were all negative. When hatched to larvae, 20 of 25 pools derived from the infected *H. longicornis* ticks (5 pools from each tick) tested positive for SFTSV RNA; all 25 larvae pools of the control group tested negative (Table 1).

We further performed transstadial transmission of SFTSV by rearing larvae to adults. All remaining larvae were reared to nymphs and adults by feeding on 20 naive Balb/C mice. We subjected 1 mouse to SFTSV RNA testing after it was bitten by each pool of larvae and the hatched nymphs and adults; we used the other mice for feeding multiple pools of larvae and the hatched nymphs and adults. We fed 3,195 larvae in the experimental group and 2,987 in the control group to engorgement and randomly selected and tested engorged larvae. We maintained the other engorged larvae for molting to nymphs. In all, 694 engorged larvae in the experimental group and 652 engorged larvae in the control group successfully molted to nymphs.

1These authors contributed equally to this article.
Transmission of SFTSV by *H. longicornis* Ticks

The remaining 569 nymphs in the SFTSV group and 527 nymphs in the control group were fed on 20 naive Balb/C mice; 453 nymphs in the SFTSV group and 437 in the control group were fully engorged. We divided the remaining engorged nymphs into 5 replicate cohorts; 166 engorged nymphs (39% ± 6% standard error [SE]) in the experimental group and 155 (38% ± 5% SE) in the control group matured to adults (online Technical Appendix Tables 1, 2). The overall hatching rate of eggs and molting rates of nymphs and adults in the 2 groups were comparable.

All 25 engorged larvae pools (5 pools from each mother) in the SFTSV-infected group and none from the control group were positive for SFTSV RNA (Table 1). After the larvae molted to nymphs, 23 of 25 nymph pools from the SFTSV-infected group and none from the control group tested positive for SFTSV RNA. Similarly, all 25 engorged nymph pools from the SFTSV-infected group and none from the control group were positive. When the second generation emerged, we tested 50 adults (25 females, 25 males) in each group for SFTSV RNA; in the SFTSV-infected group, 44% (11/25) of the females and 36% (9/25) of the males tested positive, whereas all 25 females and 25 males in the control group were negative. Positive samples were confirmed by identical sequences to that of the inoculated virus strain.

A total of 83 naive Balb/C mice were infested by ticks (online Technical Appendix Table 3). All 3 Balb/C mice fed by the SFTSV-infected females were positive for exposure to SFTSV 1 week after the ticks detached. Of the naive Balb/C mice that were bitten by larvae from the SFTSV-infected group, 4 of 5 were positive for SFTSV RNA, as were 4 of 5 mice bitten by nymphs, 4 of 5 mice bitten by adult female ticks, and 3 of 5 mice bitten by male ticks; mice bitten by ticks from the control group were negative (Table 2). We used IFA to test serum samples from the mice collected before and 3 weeks after detachment of ticks at different developing stages; all mice positive for SFTSV RNA demonstrated seroconversion against SFTSV (Table 2).

Three of 4 pools of saliva and hemolymph from the experimental group were SFTSV RNA positive. We selected 5 females at random from each group to detect SFTSV in tissues by IFA. The salivary glands, midguts, and ovaries of the SFTSV-injected group displayed SFTSV-specific fluorescence (Figure 2).

### Table 1. Detection of severe fever with thrombocytopenia syndrome virus RNA in experimental and control *Haemaphysalis longicornis* ticks

| Source                        | Experimental infection group | Control group |
|-------------------------------|-----------------------------|---------------|
| Mother tick carcasses         | 5                           | 5             |
| Egg pool*                     | 15                          | 15            |
| Larvae pool†                  | 25                          | 25            |
| Engorged larva pool‡          | 25                          | 25            |
| Nymph pool§                   | 25                          | 25            |
| Engorged nymph                | 25                          | 25            |
| Male adult                    | 25                          | 25            |
| Female adult                  | 25                          | 25            |
| Female hemolymph pool¶        | 3                           | 4             |
| Female saliva pool#           | 4                           | 4             |
| Male hemolymph pool¶          | 3                           | 4             |

*Eggs were tested in pools of 60.
†Larvae were tested in pools of 50.
‡Engorged larvae were tested in pools of 5.
§Nymphs were tested in pools of 5.
¶Hemolymph collected from 5 ticks was pooled as 1 sample.
#Saliva collected from 5 ticks was pooled as 1 sample.
DISPATCHES

We observed a significantly higher level of viral load in second-generation eggs than in second-generation adults (p< 0.001 by Mann-Whitney U-test). We also found a significantly higher level (p< 0.0001) of viral load in saliva of engorged second-generation adults than in saliva of unengorged adults, indicating that SFTSV had multiplied.

Conclusions

We report the experimental maintenance and transmission of SFTSV in H. longicornis ticks. After microinjection of SFTSV, the virus disseminated in ovaries and salivary glands. Infected H. longicornis ticks could transmit SFTSV successfully in both transovarial and transstadial modes. The appearance of SFTSV in saliva and hemolymph suggests that the virus circulates in the tick hemocoel and is expressed in saliva. In addition, naive Balb/C mice infested with experimentally infected adults, larvae, and nymphs all became infected, evidenced by both detection of SFTSV-specific RNA and seroconversion.

These findings, together with data on natural infection in the field (1,6), implicate H. longicornis ticks as competent vectors for SFTSV. However, the evidence derived from IFA and rRT-PCR tests could not indicate that the virus is infectious. More efforts should be taken to demonstrate the infectivity of SFTSV in the transmission cycle.

H. longicornis ticks are widely distributed in the Asia-Pacific region (8–12). Predominant hosts of H. longicornis ticks include humans, poultry, livestock, wild rodents, and birds (12–14). As displayed in mice in the current research, SFTSV is likely to be maintained through vertical and horizontal transmission in ticks that infest these wild and domestic mammals. This maintenance has been evidenced by an extraordinarily high prevalence of SFTSV in sheep, cattle, dogs, pigs, and other animals (7,14). In areas where H. longicornis ticks are endemic, infested animals could be considered as key reservoirs in maintaining and transmitting SFTSV (15). The close contact between animals and their owners could pose another way of acquiring infection, in addition to tick bites.

Table 2. Detection of severe fever with thrombocytopenia syndrome virus in Haemaphysalis longicornis tick–infested mice*

| Stage (sex) | No. mice | No. ticks/mouse | No. positive by rRT-PCR | Titer ± SE |
|-------------|----------|-----------------|-------------------------|-----------|
| Adults (female) | 3 | 4 | 3 | 3.01 ± 0.30 |
| Larvae | 5 | 50 | 4 | 2.78 ± 0.15 |
| Nymphs | 5 | 10 | 4 | 3.16 ± 0.17 |
| Adults (female) | 5 | 5 | 4 | 3.09 ± 0.15 |
| Adults (male) | 5 | 5 | 3 | 2.81 ± 0.35 |

*IFA, indirect fluorescence assay; rRT-PCR, real-time reverse transcription PCR.

Figure 2. Specific detection of severe fever with thrombocytopenia syndrome virus (SFTSV) in tissues of adult Haemaphysalis longicornis ticks by indirect fluorescence assay. The green fluorescence represents the SFTS virus. A) Salivary gland of SFTSV-injected tick (original magnification x40). B) Midgut of SFTSV-injected H. longicornis tick (original magnification x10). C) Ovary of SFTSV-injected tick (original magnification x40). D) Salivary gland of phosphate-buffered saline (PBS)–injected tick (original magnification x40). E) Midgut of PBS-injected H. longicornis tick (original magnification x10). F) Ovary of PBS-injected tick (original magnification x40).
Transmission of SFTSV by *H. longicornis* Ticks

This study was supported by the Natural Science Foundation of China (grant nos. 81621005 and 81473023) and the China Mega-Project for Infectious Diseases (grant no. 2018ZX10713002). The funding agencies had no role in the design and conduct of the study, collection, management, analysis, interpretation of the data, preparation, review, or approval of the manuscript.

About the Author
Dr. Zhuang is an assistant professor in the Laboratory of Etiology of Affiliated Bayi Children’s Hospital, PLA Army General Hospital, Beijing, China. Her research interests include emerging infections, surveillance, and hospital infection.

References
1. Yu XJ, Liang MF, Zhang SY, Liu Y, Li JD, Sun YL, et al. Fever with thrombocytopenia syndrome in China. N Engl J Med. 2011;364:1523–32. http://dx.doi.org/10.1056/NEJMoa1010095
2. Kim KH, Yi J, Kim G, Choi SJ, Jun KI, Kim NH, et al. Severe fever with thrombocytopenia syndrome, South Korea, 2012. Emerg Infect Dis. 2013;19:1892–4. http://dx.doi.org/10.3201/eid1911.130792
3. Takahashi T, Maeda K, Suzuki T, Ishido A, Shigeoka T, Tominaga T, et al. The first identification and retrospective study of severe fever with thrombocytopenia syndrome in Japan. J Infect Dis. 2014;209:816–27. http://dx.doi.org/10.1093/infdis/jit603
4. Zhang YZ, He YW, Dai YA, Xiong Y, Zheng H, Zhou DJ, et al. Hemorrhagic fever caused by a novel bunyavirus in China: pathogenesis and correlates of fatal outcome. Clin Infect Dis. 2012;54:527–33. http://dx.doi.org/10.1093/cid/cir804
5. Chen Z, Yang XJ, Yang XH, Liu JZ. Geographical distribution and fauna of Chinese ticks. Sichuan J Zool. 2008;27:820–3. http://dx.doi.org/10.3969/j.issn.1000-7083.2008.05.034
6. Zhang YZ, Zhou D, Qin XC, Tian JH, Xiong Y, Wang JB, et al. The ecology, genetic diversity, and phylogeny of Huaiyangshan virus in China. J Virol. 2012;86:2864–8. http://dx.doi.org/10.1128/JVI.06192-11
7. Niu G, Li J, Liang M, Jiang X, Jiang M, Yin H, et al. Severe fever with thrombocytopenia syndrome virus among domesticated animals, China. Emerg Infect Dis. 2013;19:756–63. http://dx.doi.org/10.3201/eid1905.120245
8. Park SW, Song BG, Shin EH, Yun SM, Han MG, Park MY, et al. Prevalence of severe fever with thrombocytopenia syndrome virus in *Haemaphysalis longicornis* ticks in South Korea. Ticks Tick Borne Dis. 2014;5:975–7. http://dx.doi.org/10.1016/j.ttbdis.2014.07.020
9. Tateno M, Sanahara A, Nakanishi N, Izawa M, Matsu A, Setoguchi A, et al. Molecular survey of arthropod-borne pathogens in ticks obtained from Japanese wildcats. Ticks Tick Borne Dis. 2015;6:281–9. http://dx.doi.org/10.1016/j.ttbdis.2015.01.009
10. Hammer JF, Emery D, Bogema DR, Jenkins C. Detection of *Theileria orientalis* genotypes in *Haemaphysalis longicornis* ticks from southern Australia. Parasit Vectors. 2015;8:229. http://dx.doi.org/10.1186/s13071-015-0839-9
11. Mediannikov O, Davoust B, Cabre O, Rolain JM, Raoult D. Bartonellae in animals and vectors in New Caledonia. Comp Immunol Microbiol Infect Dis. 2011;34:497–501. http://dx.doi.org/10.1016/j.cimid.2011.09.002
12. Heath A. Biology, ecology and distribution of the tick, *Haemaphysalis longicornis* Neumann (Acari: Ixodidae) in New Zealand. N Z Vet J. 2016;64:10–20. http://dx.doi.org/10.1080/00480169.2015.1035769
13. Zhang YZ, Li J, Li JC, Hu XB, Liang DF, An DS. Preliminary investigation on ticks (Ixodidae: Ixodidae) in Yigong, Tibet. Med J Natl Def Forc Southeast Chin. 2007;4:519–20. http://dx.doi.org/10.3969/j.issn.1004-0188.2007.04.071
14. Teng KF, Jiang ZJ. Economic insect fauna of China. Beijing: Science Press; 1991.
15. Jiao Y, Qi X, Liu D, Zeng X, Han Y, Guo X, et al. Experimental and natural infections of goats with severe fever with thrombocytopenia syndrome virus: evidence for ticks as viral vector. PLoS Negl Trop Dis. 2015;9:e0004092. http://dx.doi.org/10.1371/journal.pntd.0004092

Address for correspondence: Wu-Chun Cao or Wei Liu, State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, 20 Dong-Da St, Fengtai District, Beijing 100071, China; email: caowc@bmi.ac.cn or liuwei@bmi.ac.cn
Transmission of Severe Fever with Thrombocytopenia Syndrome Virus by *Haemaphysalis longicornis* Ticks

**Materials and Methods**

**SFTSV Strain and Culturing**

The severe fever with thrombocytopenia syndrome virus (SFTSV) (Phlebovirus WCH/97/HN/China/2011, GenBank accession nos. JQ341190, JQ341189, and JQ341188, for L, M, and S segments) used in this study was isolated from a patient in Henan Province of China in 2011 (1), and maintained in the Vero E6 cell line with complete Dulbecco’s modified Eagle’s medium (DMEM), 10% fetal serum, and 10 U/mL penicillin and streptomycin. After we determined viral loads by quantitative real-time PCR, we harvested the virus for artificial infection of ticks by microinjection.

**Tick Colony and Rearing**

*H. longicornis* ticks were collected by flagging on vegetation in Shangcheng County, Henan Province, China in 2011. We established SFTSV-free tick colonies in our laboratory from engorged females. Briefly, the *H. longicornis* ticks were allowed to feed on Balb/C mice. All mice in this study were 2-week-old males, specific-pathogen free, supplied by the Center of Experimental Animals, Academy of Military Medical Sciences, China. The fully engorged females were kept individually until they laid eggs. We randomly sampled 10 batches (30 eggs in each batch) of eggs to screen for SFTSV, along with the corresponding adult mother tick, by isolation and RT-PCR assays, described later. The eggs from the groups in which both the mother tick and the filial eggs were negative for SFTSV were incubated to larvae. The larvae and
the following nymphs were fed on Balb/C mice, and the molted adults were subjected to the trial. The transmission cycle of SFTSV in *H. longicornis* ticks was simulated following the procedures shown in Technical Appendix Figure 1, with each step described as follows.

**Artificial Infection of Ticks with SFTSV by Microinjection**

Adult female *H. longicornis* ticks from the aforementioned SFTSV-free colony were infected with SFTSV by the microinjection protocol developed by Kocan et al. (2) with modification. We injected 1 µL of virus culture (5.9×10⁵ copies/mL) into each tick through its anal pore with a microsyringe (1 inch, 33 gauge needle) under a dissecting stereomicroscope (Technical Appendix Figure 2). We injected the same volume of phosphate-buffered saline (PBS) into ticks that were used as the control group. The ticks that were crawling and active after injection were maintained in an Intelligent Climate Cabinet (Saife Company, Ningbo City, China) with a relative humidity of 95 ± 5% at 22°C.

**Transmission Cycle of SFTSV in Ticks**

Two weeks after injection, the female ticks were fed on Balb/C mice so we could investigate transovarial transmission. The engorged female ticks were maintained until they laid eggs, which were allowed to hatch to larvae under the same conditions as described earlier. We screened subsequent larvae for SFTSV infection to assess the efficiency of transovarial transmission. Larvae and subsequent nymphs were allowed to feed on Balb/C mice until fully engorged and molt to nymphs and adults. At each developmental stage, ticks were starved for 3 weeks between molting and the next feeding. We tested the derived nymphs and adults for SFTSV to evaluate the efficiency of transstadial transmission.

**Detection of SFTSV in Ticks of Different Developmental Stages**

We subjected ticks of different developmental stages to real-time PCR and RT-PCR to determine their SFTSV infection status. We extracted RNA from egg pools (60/pool), larva pools (50/pool), nymph pools (5/pool) (Technical Appendix Table 2), and individual adult ticks
using the RNeasy Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer’s instructions. For real-time PCR assay, we used the one-step Primer Script RT-PCR Kit (TaKaRa, Dalian, China) according to the manufacturer’s instructions with 1 μL PCR primer mix (20 μM of sense and antisense each), 0.5 μL probe (10 μM) and 2 μL total RNA in LightCycler 2.0 (Roche Diagnostics, Mannheim, Germany). The real-time PCR primers (5’-ACCTCTTTGACCCCTGAGTTWGACA-3’ and 5’-CTGAAGGAGACAGGTGGAGATGA-3’) and probe (5’-Hex-TGCCCTTGACGATCTT-MGB-3) were targeted at the S-segment of the SFTSV (3). We performed RT-PCR and sequencing of the S-segment on positive samples (BNYS1-F: 5’-TCTTCTCCATCAAGAACGC-3’, BNYS1-R: 5’-TTCGACAAAAATTAGACCTCC-3’) to verify the real-time PCR results.

We prepared the positive control standard (nt. 1456–1557 of the SFTS virus segment S sequence, reference sequence GenBank accession no. KC505134) as described previously (4). We prepared serial dilutions from 10⁸ to 10³ copies/mL in diethylpyrocarbonate-treated water and stored them in RNase-free tubes at −80°C.

We performed quantification of SFTSV as described earlier in the 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). Absolute RNA quantification was included in every assay and was generated by using RNA transcripts produced earlier. Standard curves included 5 dilutions and 3 replicate wells for each dilution. All samples were quantified in 3 replicate wells. Levels of SFTSV RNA concentrations were expressed as copies/mL.

Detection of SFTSV in Saliva and Hemolymph of Adult Ticks

To prepare the molted adult ticks to salivate, we allowed them to engorge partially by feeding on Balb/C mice. Saliva was collected (Technical Appendix Figure 3, panel A) from the engorged ticks as described previously (5). After saliva collection, we obtained hemolymph samples from each tick by clipping a front leg and placing the tip of a glass micropipette to the wound (Technical Appendix Figure 3, panel B). The saliva from 5 ticks in each group was
pooled and the hemolymph from the same 5 ticks was pooled, mixed with 140 μL PBS, and subjected to the RNA extraction procedure using the QIAamp Viral RNA Mini Kit (QIAGEN). We then applied the RNA to RT-PCR assay, for which positive amplicons were sequenced as mentioned earlier.

**IFA Detection of SFTSV in Ticks**

Twelve days following injection with SFTSV, we embedded the whole bodies of the ticks in paraffin and cut them longitudinally at a cryostat (Leica CM 3050; Leica Microsystems, Wetzlar, Germany). We put the frozen slices on glass slides and subjected them to immunofluorescent assay (IFA) for SFTSV detection.

We then selected 10 females at random from the SFTSV and control groups and individually dissected their guts, salivary glands, and ovaries under sterile conditions using a dissecting microscope. After marginal cuts and scutum removal with lancets, we carefully removed the hemolymph around the tissues with filter papers and replaced it with sterilized PBS 3 times. We placed the tick tissues on glass slides and subjected them to IFA for SFTSV detection.

We soaked sheet glasses with prepared tissues in PBS with 5% skim milk to deparaffinize. We incubated the slices at 37°C for 1 hour with mAb that was previously prepared (6) in PBS with 0.05% Tween20. We used serum from Balb/C mice as a negative control. Following triple washing with PBS and 1 final washing with distilled water, we incubated the slices at 37°C for 30 minutes with fluorescence-conjugated goat antimouse antibodies (Zhongshanjinqiao, Beijing, China) at 1:100 dilution with Evans blue. We washed the slices in PBS 3 times and finally washed them with distilled water, visualizing with an Olympus BX51 Microscope until dried.
Detection of SFTSV in Mice

We collected serum samples from the mice 3 times (before tick feed, 1 week after tick engorgement, and 3 weeks after tick repletion) and extracted RNA using a QIAamp Viral RNA mini kit and detection by real-time PCR, as described earlier. By using the viral antigen of the SFTSV patient source from the Vero E6 cell line, we detected specific IgG against SFTSV by indirect IFA, as previously described. We measured antibody titers with serum dilution starting at 1:16 and then serially 2-fold to determine endpoint titers.

Statistical Analysis

The antibody reciprocal titers were log-transformed. We used the Mann-Whitney test to determine the difference of SFTSV viral load in generation 2 eggs and adults, as well as the difference between the viral load of unengorged generation 2 adults and saliva collected from engorged generation 2 adults.

References

1. Lam TT, Liu W, Bowden TA, Cui N, Zhuang L, Liu K, et al. Evolutionary and molecular analysis of the emergent severe fever with thrombocytopenia syndrome virus. Epidemics. 2013;5:1–10. PubMed [http://dx.doi.org/10.1016/j.epidem.2012.09.002

2. Kocan KM, Blouin E, de la Fuente J. RNA interference in ticks. J Vis Exp. 2011; (47):2474. [http://dx.doi.org/10.3791/2474

3. Zhang YZ, He YW, Dai YA, Xiong Y, Zheng H, Zhou DJ, et al. Hemorrhagic fever caused by a novel bunyavirus in China: pathogenesis and correlates of fatal outcome. Clin Infect Dis. 2012;54:527–33. [http://dx.doi.org/10.1093/cid/cir804

Page 5 of 10
4. Sun Y, Liang M, Qu J, Jin C, Zhang Q, Li J, et al. Early diagnosis of novel SFTS bunyavirus infection by quantitative real-time RT-PCR assay. J Clin Virol. 2012;53:48–53. http://dx.doi.org/10.1016/j.jcv.2011.09.031

5. Spielman A, Ribeiro JMC, Mather TN, Piesman J. Dissemination and salivary delivery of Lyme disease spirochetes in vector ticks (Acari: Ixodidae). J Med Entomol. 1987;24:201–5. http://dx.doi.org/10.1093/jmedent/24.2.201

6. Hofmann H, Li X, Zhang X, Liu W, Kühl A, Kaup F, et al. Severe fever with thrombocytopenia virus glycoproteins are targeted by neutralizing antibodies and can use DC-SIGN as a receptor for pH-dependent entry into human and animal cell lines. J Virol. 2013;87:4384–94. http://dx.doi.org/10.1128/JVI.02628-12
Technical Appendix Table 1. Numbers (± standard error) of generation 2 ticks used in each stage of the transmission cycle

| Source                                      | SFTSV group     | Control group   |
|---------------------------------------------|-----------------|-----------------|
| Eggs                                        | 1305.6 ± 47.7   | 1436.6 ± 159.9  |
| Eggs for detection (eggs × pools)           | 60×3            | 60×3            |
| Eggs left                                   | 1125.6 ± 47.7   | 1256.6 ± 159.9  |
| Hatched larvae                              | 889 ± 66.2      | 847.4 ± 114.2   |
| Larvae for detection (larvae × pools)        | 50×5            | 50×5            |
| Larvae left                                 | 639 ± 66.2      | 597.4 ± 114.2   |
| Engorged larvae                             | 185.2 ± 24.5    | 179.4 ± 5.6     |
| Engorged larvae for detection (larvae × pools) | 5×5            | 5×5            |
| Engorged larvae left                        | 160.2 ± 24.5    | 154.4 ± 5.6     |
| Nymphs                                      | 138.8 ± 19.7    | 130.4 ± 10.6    |
| Nymphs for detection (nymphs × pools)       | 5×5             | 5×5             |
| Nymphs left                                 | 113.8 ± 19.7    | 105.4 ± 10.6    |
| Engorged nymphs                             | 90.6 ± 14.9     | 87.4 ± 10.7     |
| Engorged nymphs for detection (nymphs × pools) | 5×1            | 5×1            |
| Engorged nymphs left                        | 85.6 ± 14.9     | 82.4 ± 10.7     |
| Adults                                      | 33.2 ± 6.1      | 31 ± 4.6        |
| Female adults                               | 24.2 ± 8.9      | 23.2 ± 5.3      |
| Female adults for detection                 | 5               | 5               |
| Hemolymph (females × pools)                 | 5×1             | 5×1             |
| Saliva (females × pools)                    | 5×1             | 5×1             |
| Male adults                                 | 9 ± 4           | 7.8 ± 2.5       |
| Male adults for detection                   | 5×1             | 5×1             |
| Hemolymph (males × pools)                   | 5×1             | 5×1             |
**Technical Appendix Table 2.** Mean days in each period of development of *H. longicornis* ticks in the SFTSV study (± standard error)

| Period                  | SFTSV group | Control group |
|-------------------------|-------------|---------------|
| Preoviposition period   | 8.20 ± 2.39 | 7.8 ± 1.80    |
| Oviposition period      | 7.73 ± 1.69 | 7.7 ± 1.72    |
| Egg hatching period     | 38.50 ± 1.24| 38.4 ± 1.46   |
| Larva feeding period    | 3.52 ± 0.64 | 3.4 ± 0.63    |
| Larva premolt period    | 14.50 ± 1.85| 14.2 ± 2.37   |
| Larva molting period    | 7.70 ± 1.67 | 7.9 ± 1.64    |
| Nymph feeding period    | 5.80 ± 1.28 | 5.55 ± 1.20   |
| Nymph premolt period    | 16.90 ± 2.79| 16.75 ± 2.52  |
| Nymph molt period       | 10.20 ± 1.35| 9.95 ± 1.42   |
| Adult feeding period    | 9.12 ± 1.42 | 9.04 ± 1.84   |

**Technical Appendix Table 3.** Numbers of mice for feeding in the life cycle of the *H. longicornis* ticks in the SFTSV study

| Source                  | SFTSV group | Control group |
|-------------------------|-------------|---------------|
|                         | Mice for feeding and detection (ticks/mouse) | Mice for feeding detection (ticks/mouse) | Mice for feeding |
| Generation 1 adults     | 3 (4)       | 3 (4)         | 0 |
| Eggs                    | 0           | 0             | 0 |
| Larvae                  | 5 (50)      | 5 (50)        | 5 |
| Nymphs                  | 5 (10)      | 5 (10)        | 5 |
| Generation 2 female adults | 5 (5)   | 5 (5)         | 5 |
| Generation 2 male adults | 5 (5)     | 4             | 3 |
Technical Appendix Figure 1. Experimental framework of *H. longicornis* ticks microinjected with SFTSV. We microinjected 45 adult *H. longicornis* ticks with SFTSV cell culture dilution (5.92×10^5 copies/mL) and microinjected 45 other adult ticks with PBS for negative control. Ticks were then fed on Balb/c mice. All control ticks were found to be SFTSV negative. To evaluate the SFTSV transmission from ticks to mice, we performed reverse transcription PCR and IFA of mice serum samples. To evaluate transovarial and transstadial transmission, we detected SFTSV RNA by PCR of 15 pools of eggs, 25 pools of larvae, 25 pools of nymphs, 25 males, and 25 females. We collected 4 pools of saliva and blood lymph from molted adults (generation 2) and used reverse transcription PCR assay for detection. We performed IFA of tissue smears of molted adults (generation 2) to show the localization of SFTSV. OT, oral transmission; TO, transovarial transmission; TT, transstadial transmission.
Technical Appendix Figure 2. Microinjection of adult *H. longicornis* ticks with SFTSV or PBS.

Technical Appendix Figure 3. Saliva and hemolymph collection from *H. longicornis* ticks. A) Collection of saliva from generation 2 adults. B) Collection of hemolymph from generation 2 adults.