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**Supplementary Data**

**Natural circulation of tick-borne severe fever with thrombocytopenia syndrome virus in the city ecosystem, China**

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**Materials and methods**

**Virus and cells**

SFTSV Wuhan strain (GenBank accession numbers: S, KU361341.1; M, KU361342.1; L, KU361343.1) and rabbit anti-SFTSV-NP polyclonal antibody were provided by Wuhan Institute of Virology, Chinese Academy of Sciences. Vero cells (African green monkey kidney epithelial cells) were obtained from American Type Culture Collection (ATCC) and maintained in Dulbecco’s modified Eagle’s medium (DMEM, Hyclone, USA) supplemented with 8% FBS and penicillin (100 U/mL), streptomycin (100 μg/mL; Gibco, USA) and L-glutamine in a 37 °C incubator supplemented with 5% CO2. SFTSV was propagated at 37 °C in Vero cells at a multiplicity of infection of 0.1. Cell culture supernatant was collected at 4 days post-infection and stored at − 80 °C as the working virus stock for animal studies.

**Animal trapping and sample collection**

The animals were captured using rodent capture cages (cage size: 14 × 14 × 26 cm) and baited with fried bread sticks (trap numbers varied between 30 and 50 traps/night depending on the availability of sites in the locations). The cages were setup into fields and collected the next morning (Fernando Torres-Pérez, 2004). Animals were anesthetized by inhalation using isoflurane with a dose of 1 mL per kilogram weight in a closed container. Blood samples were drawn from heart, and animals were released after blood collection. Blood samples were centrifuged at 3000 ×*g* for 10 minutes and the serum was transferred to small vials, which were kept at −80 ℃ until analysis.

**Tick collection and identification of tick species and phylogenetic analysis**

We collected ticks of all life stages by using flag-dragging from vegetation and removal directly from the skin surface from hedgehogs using tweezers. Ticks were identified based on morphological characteristics, and further molecular confirmation by sequencing the partial mitochondrial 16S ribosomal RNA (16S rRNA) gene. The primers were as follows: (16S-1) CTGCTCAATGATTTTTTAAATTGCTGTGG (Forward primer) and (16S-2) CGCTGTTATCCCTAGAGTATT (Reverse primer). Phylogenetic analysis was performed using the full-length mitochondrial genomes. Tick DNA was extracted using the MightyPrep reagent for DNA Kit (Takara, Japan) according to the manufacturer’s instructions. The mitochondrial DNA were sequenced by next generation sequencing (Tsingke Biotech, Beijing, China) and deposited in GenBank (Parthenogenetic *H.* *longicornis* in Shunyi District: OL335941; Chaoyang District: OL335942). Mitochondrial genomes of *H. longicornis* ticks from SFTS endemic areas were included in Supplementary Table S3. The phylogenetic tree was constructed by using the maximum likelihood method, MEGA-X with the bootstrap value set at 1000.

**SFTSV-RNA extraction and real-time RT-PCR**

Total RNA prepared from the homogenates of the ticks and the blood samples collected from hedgehogs’ heart were extracted using TRIzol reagent (Thermo Fisher Scientific, USA) or the RNeasy kit (Qiagen, Germany) according to the manufacturer’s instructions. Samples were analyzed using a One-Step SYBR PrimerScript reverse transcription (RT)-PCR kit (TaKaRa, Japan) on Applied Biosystems QuantStudio. Each sample was measured by triplicate. The primers were designed as previously described (Dong et al., 2019). Conditions for the reaction were as follows: 42 °C for 5 min, 95 °C for 10 sec, 40 cycles at 95 °C for 5 sec, and 60 °C for 20 sec.

**SFTSV sequencing and phylogenetic analysis**

The SFTSV sequences from Shunyi *H. longicornis* ticks were sequenced by Sanger sequencing (Tsingke Biotech, Beijing, China) and deposited in the GenBank (Shunyi-Hedgehog-2021, Accession number: OL518989). SFTSV sequences obtained in previous studies were downloaded from GenBank (Yoshikawa et al., 2015, Shi et al., 2017) (Supplementary Table S2). The phylogenetic trees were constructed by using the maximum likelihood method, MEGA program. The confidence of the tree was tested using 1000 bootstrap replications.

**SFTSV antibody detection by ELISA**

Serum samples from animals were tested for SFTSV antibodies including IgG and IgM with a commercial double antigen sandwich ELISA kit from Nanjing Immune-detect Bio-tech Co., Ltd. (Jiangsu, China).

**Virus titration and neutralization test**

Focus-forming assay was performed in Vero cells to titrate the viral titers. Cells were plated in triplicates 24 h before infection in 96-well plates at 1.5 × 104 cells per well in DMEM supplemented with 10% FBS. The virus samples were diluted 10-fold in DMEM with 2% FBS. After removal of medium, the cells were incubated with the 10-fold diluted viral solution at 37 °C. Three to four hours later, the cells were washed once and incubated with DMEM plus 2% FBS and 20mmol/L NH4Cl at 37 °C. Two days post infection, the cells were fixed with cold methanol and stained using a rabbit anti-SFTSV-NP polyclonal antibody at 1:700 dilution and Alexa 488-labeled goat anti-rabbit IgG at 1:700 dilution.

For the FRNT assay, 100 FFU of SFTSV Wuhan strain were incubated with heat-inactivated sera diluted from 1:10 to 1:1280 by two-fold serial dilution at 37 °C for 1 h and then layered onto the cells in 96-well plates. Neutralization titers were calculated as 50% inhibition of virus infection (FRNT50) using the Reed-Muench method.

**Experimental Infection**

All experimental infection study was conducted in a Bio-safety Level-3 Animal Laboratory in the Beijing Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences. Amur hedgehogs were purchased from Heze animal store in Shandong Province. Following acclimation, hedgehogs were challenged with 4 × 106 FFU (focus forming unit) of SFTSV Wuhan strain via intraperitoneally injection, with the 200 μL volume divided between two injection sites. Serum samples were collected on day 20, 30, and 40 post infection.

**Ethics statement**

All animal studies were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of the People’s Republic of China. The protocols for animal studies were approved by the Committee on the Ethics of Animal Experiments of the Institute of Zoology, Chinese Academy of Sciences (Approval number: IOZ20180058).

**References**

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Table S1. Tick collection in Longquanwu Village, Mengtougou District, Beijing City, China in 2023.

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| --- | --- | --- | --- | --- |
| Samples | Tick species | | | |
| *Haemaphysalis longicornis* | | *Dermacentor silvarum* | |
| Nymph | Adult | Nymph | Adult |
| Hedgehog 1 | 18 | 0 | 0 | 0 |
| Hedgehog 2 | 60 | 6 | 0 | 9 |
| Hedgehog 3 | 14 | 0 | 0 | 3 |
| Hedgehog 4 | 2 | 0 | 0 | 27 |
| Hedgehog 5 | 0 | 0 | 0 | 3 |
| Vegetation | 111 | 0 | 0 | 0 |
| Total | 205 | 6 | 0 | 42 |

Table S2. Detail information of the SFTSV isolates.

(Excel file in Supplementary Materials)

Table S3. Detail information of the mitochondrial genomes of *H. longicornis* ticks.

(Excel file in Supplementary Materials)