

## Letter

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

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Dear Editor,

Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne *Bandavirus* that can cause severe symptoms in patients include fever, gastrointestinal symptoms, thrombocytopenia, leukopenia and even death (Yu et al., 2011). SFTSV was first identified in China in 2009, and has subsequently been found in South Korea (Yun et al., 2014), Japan (Takahashi et al., 2014), Vietnam (Tran et al., 2019), Myanmar (Win et al., 2020) and Pakistan (Zohaib et al., 2020) in Asia. Initial outbreaks of SFTSV were mostly restricted to people from remote and mountainous country areas, with people from cities being relatively unaffected (Li et al., 2014). This was certainly true for Beijing residents, where no cases of severe fever with thrombocytopenia syndrome (SFTS) were reported before 2021.

The *Haemaphysalis longicornis* (Asian long-horned tick), is the primary vector for SFTSV and the dominant human-biting tick in the SFTSV-endemic areas (Zhuang et al., 2018). *H. longicornis* ticks have both bisexual and parthenogenetic populations, among which the parthenogenetic populations are widely distributed in China and strongly correlated with the distribution of SFTS cases (Zhang et al., 2022). Our previous studies suggest that the parthenogenetic *H. longicornis* ticks, are probably transported to naïve areas by migratory birds, and this mechanism plays a major role in the long-range spread of SFTSV (Zhang et al., 2022; Yun et al., 2015). Even though many domestic and companion animals show high SFTSV seroprevalence, our studies strongly suggest that only hedgehogs are the major amplifying host for SFTSV in China (Zhao et al., 2022). Hedgehogs are widely distributed throughout China, encompassing areas where SFTSV is endemic. Due to fast urbanization and strict ecological conservation, many cities, including Beijing, have recently experienced a rapid increase in hedgehog and *H. longicornis* populations (Zhao et al., 2022). This is a major concern since it implies that SFTSV can now circulate in urban as well as rural areas.

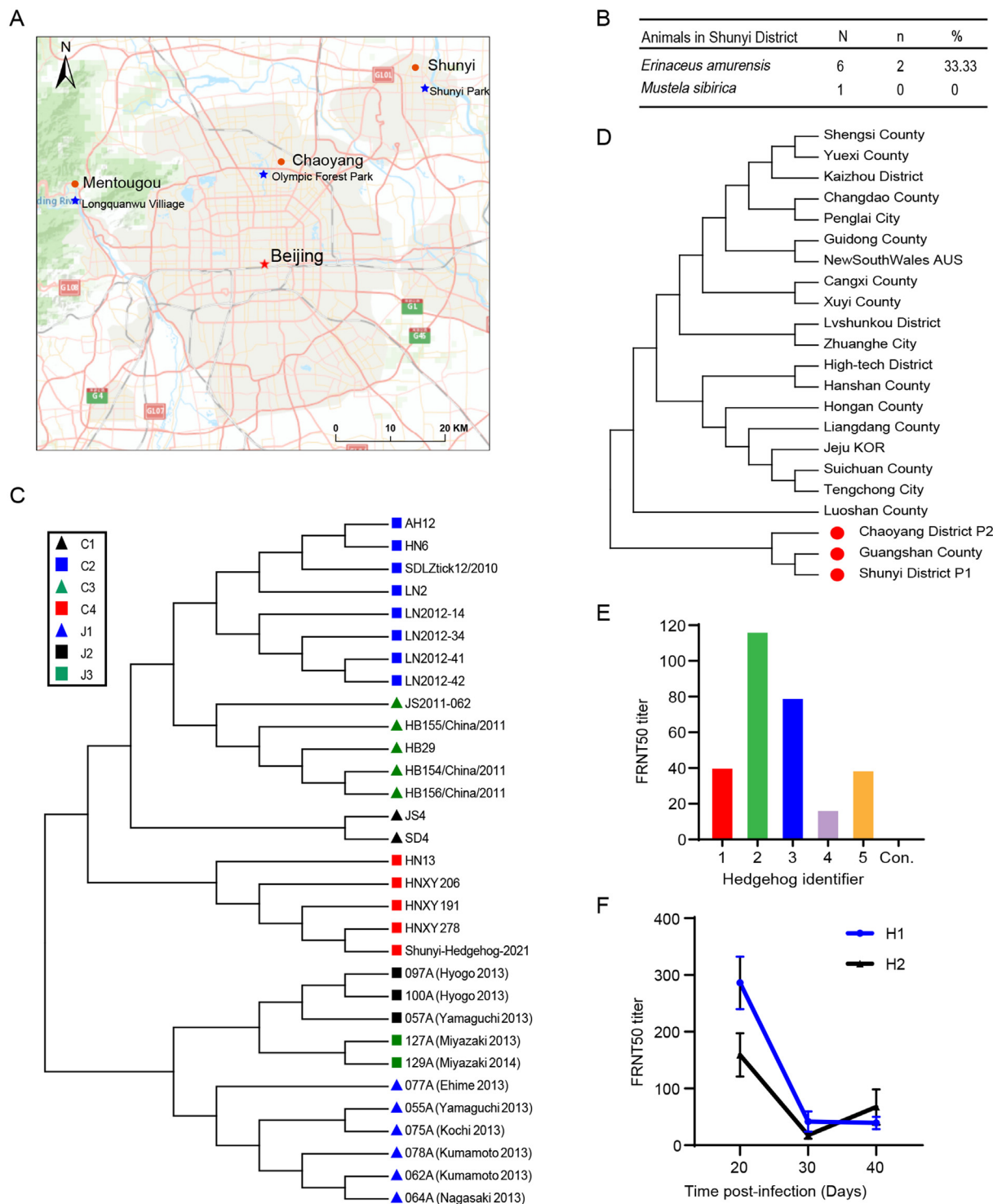
In October 2021, a suspected case of SFTS was reported at Beijing You'an Hospital, whose samples were tested positive for SFTSV RNA and antibody by the Beijing Center for Disease Control and Prevention (Sun et al., 2021). The 69-year-old male patient lives in Longquanwu Village, Mentougou District, Beijing, where is only 20 miles away from the center of Beijing City and is fully urbanized, with no poultry and livestock present (Fig. 1A).

In 2021, two field surveys were conducted to investigate the small mammals and parasitic ticks at two locations in Beijing City. The first location was a small park in Shunyi District, where is surrounded by up-market gated communities, and the second location was the Olympic Forest Park in Chaoyang District, where parthenogenetic *H. longicornis* ticks had previously been recorded in 2019 (Fig. 1A) (Zhang et al., 2022). The Shunyi Park is quite small, about 0.5 km<sup>2</sup>, and has a relatively simple ecosystem. The small mammal survey showed that only *Erinaceus amurensis* hedgehogs and *Mustela sibirica* yellow weasel in this park (Fig. 1B). On October 19th, 2021, six *E. amurensis* hedgehogs were caught in the Shunyi Park, two of which (33%) were seropositive for SFTSV (Fig. 1B). In addition, *H. longicornis* ticks collected from these hedgehogs were positive for SFTSV RNA and an L segment was sequenced (GenBank accession number: OL518989). Sequencing showed that the Shunyi SFTSV (Shunyi-Hedgehog-2021) was clustered into lineage C4, which is similar to the strain of SFTSV collected in Xinyang City, Henan Province, one of the original SFTS hot spots (Fig. 1C). In contrast, no SFTSV RNA or antibody was detected in hedgehog serum samples or parasitic ticks collected at the Chaoyang sample site.

Phylogenetic analysis of the whole mitochondrial sequences from the parthenogenetic *H. longicornis* ticks collected at Shunyi (Shunyi District P1) and Chaoyang (Chaoyang District P2), further established that these ticks were closely related to those from Guangshan County, Xinyang City in Henan Province (Fig. 1D). The results of the viral sequencing and phylogenetic analysis, when taken together, strongly

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**Fig. 1.** Natural circulation of SFTSV in Beijing City. **A** Sample collection sites. Shunyi Park in Shunyi District (Shunyi): ticks and hedgehog sera were collected in Oct. 2021. The Olympic Forest Park in Chaoyang District (Chaoyang): ticks and hedgehog sera were collected in Oct. 2021. Longquanwu Villiage in Mentougou District (Mentougou): ticks and hedgehog sera were collected in April 2023. **B** Seroprevalence of animals caught in Shunyi District. N, number of sampled animals; n, number of sampled animals positive for SFTSV antibody; %, percentage of sampled animals positive for SFTSV antibody. **C** Maximum likelihood tree was established based on partial L segments of SFTSV isolate in Shunyi (Shunyi-Hedgehog-2021) and isolates from SFTSV endemic areas (Zhang et al., 2022). Full names of the isolates were provided in [Supplementary Table S2](#). SFTSV lineages were illustrated by colors and shapes. **D** Phylogenetic analysis of the parthenogenetic population of *H. longicornis* ticks. Maximum likelihood tree was established based on the full-length mitochondrial genomes of *H. longicornis* ticks collected in Chaoyang District, Shunyi District, and SFTSV endemic areas (Zhang et al., 2022). Full names of the isolates were provided in [Supplementary Table S3](#). **E** Neutralizing antibody titer (FRNT50) against SFTSV Wuhan strain (GenBank accession numbers: S, KU361341.1; M, KU361342.1; L, KU361343.1) in *E. amurensis* hedgehogs caught near Longquanwu Village in Mentougou District was determined. Control (Con.) is naïve *E. amurensis* hedgehogs maintained in our lab. The detection limit is 10. **F** Two *E. amurensis* Hedgehogs (H1 and H2) were intraperitoneally challenged with  $4 \times 10^6$  FFU of SFTSV Wuhan strain. The SFTSV neutralizing antibody titer (FRNT50) were monitored at 20, 30, 40 days post infection.

suggest that both the tick and SFTSV collected in Shunyi District originated from Xinyang City, where the first SFTS case in China was reported. Although no local SFTS cases had been reported in Beijing at that time, our results suggested that a population of hedgehogs and *H. longicornis* ticks existed in the Shunyi District that could maintain SFTSV in an urban ecosystem.

In April 2023, a field survey was conducted in Longquanwu Village, Mentougou District, around the 2021 patient's house (Sun et al., 2021). Five *E. amurensis* hedgehogs were caught, which showed 100% SFTSV seroprevalence with SFTSV neutralizing antibody titers (FRNT50) ranging from 20 to 110 (Fig. 1E). In addition, 190 *H. longicornis* ticks were collected from vegetation and the hedgehogs, all of which was negative for SFTSV RNA (Supplementary Table S1). Interestingly, the sequencing results of mitochondrial DNAs from 40 *H. longicornis* ticks showed that they were all bi-sexual.

The immune response of hedgehogs against SFTSV is unusual. In contrast to the stable humoral immune response of experimental dogs (Park et al., 2021), the neutralizing antibody titer of *E. amurensis* hedgehogs decreases quickly and almost eliminated by day 40 after intraperitoneal inoculation with SFTSV (Fig. 1F). Thus, it is plausible that active transmission existed between the hedgehogs and *H. longicornis* ticks around the patient's location, although no SFTSV RNA positive *H. longicornis* ticks were detected.

Our previous work raised two hypotheses, the first that parthenogenetic *H. longicornis* ticks are strongly associated with SFTSV disease outbreaks (Zhang et al., 2022) and the second that SFTSV can be maintained between *H. longicornis* ticks and hedgehogs in the urban ecosystem in China (Zhao et al., 2022). In our state-wide survey of *H. longicornis* ticks in 2019, parthenogenetic *H. longicornis* ticks were quite uncommon in Beijing, where no SFTS cases were reported at that time. Parthenogenetic *H. longicornis* ticks were only found in a small park in Shunyi District, and the Olympic Forest Park in Chaoyang District in Beijing (Zhang et al., 2022). Two years later, SFTSV was detected in the *H. longicornis* ticks collected in the Shunyi Park. In our opinion, these parthenogenetic *H. longicornis* ticks were probably imported to Beijing from Xinyang City, the hot SFTS endemic area, by migratory birds, since Beijing is located on the East Asian-Australasian Flyway. In 2021, the first SFTS patient diagnosed in Mentougou District, where is not far from the Shunyi location. Considering that the patient had not traveled more than 1 km from home and the *H. longicornis* tick density was high in his neighborhood, we speculate that he got infected by *H. longicornis* bites near his house.

Shunyi Park has a quite simple ecosystem where is surrounded by up-market gated communities. Only *E. amurensis* hedgehogs and yellow weasel (*Mustela sibirica*) were trapped there. *E. amurensis* hedgehogs were common in Shunyi District and 33% of the hedgehogs collected from the Shunyi Park were seropositive for SFTSV. Since there are almost no livestock, poultry, and stray dogs in this location, our discovery reinforces our hypothesis that SFTSV circulation can be maintained in an urban area by just hedgehogs and *H. longicornis* ticks. Experimentally infected beagle dogs only develop limited SFTSV viremia and SFTSV causes lethal viral haemorrhagic fever in cats, thus stray dogs and cats are unlikely to support efficient SFTSV circulation in nature (Park et al., 2019, 2021). However, the ticks caught by cats and dogs still have the chance to transfer to human, which need further attention.

Longquanwu Village is close to the mountains, but already urbanized, with no poultry and livestock, and is only 26 miles away from the Shunyi Park. Hedgehogs caught near the village showed 100% SFTSV seroprevalence. However, so far, no SFTSV RNA has been detected from both *H. longicornis* ticks collected from vegetation or hedgehogs. In experimentally infected hedgehogs, the neutralizing antibody titer decreased quickly after SFTSV infection and almost eliminated by 40 days post infection (Fig. 1F). Thus, we believe that all the hedgehogs were infected by *H. longicornis* bites in this spring. Maybe, the SFTSV-RNA positive rate is too low in this area and more ticks need to be tested in the future, which may also explain the low human case numbers in Beijing. Another

hand, birds may also play a role in natural circulation of SFTSV in the city ecosystem of China (Li et al., 2019). More intensive investigation of seroprevalence and SFTSV RNA in migratory birds need to be done. Overall, our results suggest that the natural circulation of SFTSV have been established in Beijing and need more surveillance of ticks and their animal hosts in the city ecosystem.

## Footnotes

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