Severe Fever with Thrombocytopenia Syndrome Virus in Humans, Domesticated Animals, Ticks, and Mosquitoes, Shaanxi Province, China

Huaiyu Tian,^{1†} Pengbo Yu,^{2†} Gerardo Chowell,^{3,4†} Shen Li,² Jing Wei,² Hui Tian,⁵ Wen Lv,² Zongqi Han,⁶ Jing Yang,¹ Shanqian Huang,¹ Sen Zhou,^{7,8} John S. Brownstein,⁸ Jingjun Wang,^{2*} and Bing Xu^{1,8*}

¹State Key Laboratory of Remote Sensing Science, College of Global Change and Earth System Science, Beijing Normal University, Beijing, China;

²Shaanxi Provincial Centre for Disease Control and Prevention, Xi'an, China; ³School of Public Health, Georgia State University, Atlanta, Georgia;

⁴Division of International Epidemiology and Population Studies, Fogarty International Center, National Institutes of Health, Bethesda, Maryland;

⁵Baoji Centre for Disease Control and Prevention, Baoji, China; ⁶Long County Centre for Disease Control and Prevention of Shaanxi Province,

Baoji, China; ⁷Department of Pediatrics, Harvard Medical School, Boston, Massachusetts; ⁸Ministry of Education Key Laboratory for Earth System Modelling, Department of Earth System Science, Tsinghua University, Beijing, China

Abstract. The first human infection with severe fever with thrombocytopenia syndrome virus (SFTSV) was detected in Shaanxi Province, China, in 2013, although the virus had been reported in 13 other provinces of China since 2010. We collected and analyzed a total of 4,011 samples, including 936 human serum samples, 155 animal serum samples, 895 ticks, 1,950 mosquitoes, 30 midges, and 20 sandflies. SFTSV antibodies were found in 44 human samples (4.7%) with no significant differences between males and females or across counties. The incidence rate of severe fever with thrombocytopenia syndrome was significantly higher among individuals 20–60 years of age. Moreover, SFTSV-specific antibodies were detected in goats (66.7%), cattle (13.2%), and dogs (15.0%), but not in pigs (0%). We detected the virus in *Haemaphysalis concinna* ticks with a prevalence rate of 21.3% (17/80 pools). All mosquito, midge, and sandfly samples were negative for SFTSV. These results support wide circulation of the virus in western China. *Haemaphysalis concinna* ticks may serve as a novel SFTSV vector, and the role of these ticks requires further investigation.

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging hemorrhagic fever that was first reported in China in 2009.¹ The causative agent, SFTS virus (SFTSV), is a newly identified pathogenic member of the *Phlebovirus* genus in the family *Bunyaviridae*.² Clinical symptoms of SFTS include abrupt onset of high fever, gastrointestinal symptoms, thrombocytopenia, leukocytopenia, multiorgan dysfunction, and hemorrhage. SFTSV is thought to circulate in an enzootic tick–vertebrate–tick cycle.³ In particular, *Haemaphysalis longicornis* ticks have been implicated as vectors of SFTSV,² where domestic animals could play the role of amplifying hosts. Person-to-person transmission through contact with an infected patient's blood or mucous has also been documented.⁴

About 2,500 cases of SFTS have been reported in at least 13 provinces of China since 2010.⁵ Most reported SFTS cases are hospitalized patients with an average case-fatality rate of 7.3%,⁶ and distributed in central and eastern China.⁷ The characteristic clinical symptoms of SFTS are nonspecific and could be confused with various other infectious diseases including hemorrhagic fever with renal syndrome, which is caused by hantavirus, as well as human anaplasmosis which is caused by *Anaplasma phagocytophilum*.⁸ SFTSV has also been detected in other countries including Korea and Japan.^{9,10}

We have reported the first human infection with SFTSV in Shaanxi Province, China, 2013.¹¹ On the basis of data from a primary investigation, enhanced epidemiological surveillance was conducted to investigate the infection sources of SFTS cases and assess the prevalence of SFTSV among humans and local domestic animals and arthropods between July and

September 2013. Cross-sectional studies were conducted in both patient and nonpatient counties that cover rural areas within the Shaanxi Province.

This study was conducted in the following locations: 1) one patient village and one nonpatient village in Long County and 2) one nonpatient village in Qianyang County. All the study sites are located in the province of Shaanxi in China (Figure 1). A total of 936 people of different age groups were selected randomly in study sites. A total of 363 individuals were selected in the patient village of Long County, 323 in the nonpatient village of Long County, and 250 in the nonpatient village of Qianyang County (Table 1, Figure 1). The sampled population was divided into five age groups (0–6, 7–19, 20–39, 40–59, and ≥ 60 years).

The most common domesticated animal species in the patient village include goats, cattle, dogs, and pigs, and approximately 50 animals of each species were sampled. We collected engorged ticks from domestic animals in 25 collection sites in the patient village for three consecutive nights. Ticks were placed in plastic tubes and transported to our laboratory. Mosquitoes were collected by light traps in 6-hour trapping periods after sunset for three consecutive nights in the patient village. A total of 25 light traps were applied in 25 collection sites in residential areas.

Samples obtained from healthy volunteers and domesticated animals, ticks, and mosquitoes were sent to the laboratory of the Shaanxi Provincial Center for Disease Control and Prevention (CDC). Serum samples were tested for antibodies (IgG and IgM) against SFTSV by using a double-antigen sandwich enzyme-linked immunosorbent assay kit provided by the National Institute for Viral Disease Control and Prevention as described previously.⁵ Ticks and mosquitoes were divided into pools to detect the M segment gene of SFTSV by quantitative reverse transcription polymerase chain reaction (RT-PCR). We used the RNeasy Mini Kit (QIAGEN, Hilden, Germany) to extract RNA from the supernatant of the arthropod homogenates. RT-PCR was performed by using the QuantiTect Probe RT-PCR Kit

*Address correspondence to Bing Xu, Tsinghua University, No. 30 Shuangqing Road, Beijing 100084, China, E-mail: bingxu@tsinghua.edu.cn or Jingjun Wang, Shaanxi Provincial Centre for Disease Control and Prevention, No. 3 Jiandong Road, Xi'an, Shaanxi, China, E-mail: jingjunwang@china.com.

†These authors contributed equally to this work.

FIGURE 1. Sampling locations in study area, Shaanxi Province, China, in 2013. The gray shading represents the occurrence of human severe fever with thrombocytopenia syndrome virus (SFTSV) infection in China. Points represent sampling locations, red asterisk represents the occurrence of first human SFTSV infection in Shaanxi, green area is crop land, yellow represents grassland and shrubland, dark green is forest, blue is water body, and pink represents artificial surfaces and associated areas.

(QIAGEN) with designed primers, MF3 (5'-AAGAAGTGGCTGTTCATCATTATTG-3'), MR3 (5'-GCCTTAAGGACATTGGTGAGTA-3'), and M-Probe-3 (FAM-TCATCCTCCTTGGATATGCAGGCCTCA-BHQ-2) as described.¹² The RT-PCR product sequence is provided in the Supplemental Information.

All statistical analyses were performed using SPSS 17.0 (SPSS, Chicago, IL). Statistical significance level was set at 0.05. χ^2 test or Fisher's exact test was used to analyze the SFTSV antibody-positive proportions in the human population by regions, gender, and age groups.

A total of 936 serum samples were collected from human residents in Shaanxi Province. Seroprevalence was not statistically significantly different across study areas ($\chi^2 = 2.84$, $P = 0.24$) (Table 1). Overall, 4.7% (44/936) of serum samples were seropositive for SFTSV, whereas the difference between males (4.8%) and females (4.4%) was not statistically significant ($\chi^2 = 0.13$, $P = 0.72$) (Table 1).

SFTSV seroprevalence was significantly different among people at different age groups ($\chi^2 = 12.28$, $P = 0.02$, Table 1). The highest seroprevalence was among individuals 40–59 years of age (6.9%) and the lowest seroprevalence was among individuals ≤ 6 years of age (0.6%).

TABLE 2
Ticks collected in Shaanxi Province, 2013

Tick species	No. ticks	No. pools	No. positive pools	MIR*
<i>Haemaphysalis concinna</i>	860	80	17	2.0
<i>Haemaphysalis japonica</i>	18	3	0	0
<i>Haemaphysalis longicornis</i>	11	2	0	0
<i>Haemaphysalis yeni</i>	6	1	0	0

*MIR = minimum infection rate per 100 ticks (no. positive pools/total no. ticks assayed).

Of the 895 identified ticks, the most abundant tick was *Haemaphysalis concinna* ($N = 860$), followed by *Haemaphysalis japonica* ($N = 18$), *H. longicornis* ($N = 11$), and *Haemaphysalis yeni* ($N = 6$). We divided the ticks into 83 pools according to their species. SFTSV was detected only in *H. concinna*, with a minimum infection rate of 2.0%, based on 17 positive pools out of 80, containing a total of 860 ticks (Table 2). A total of 1,950 mosquitoes, 30 midges, and 20 sandflies were sampled in the patient village. However, viral RNA was not detected in any of the mosquitoes, midges, and sandflies that were tested.

Our results showed that seroprevalence levels in animals ranged from 0% among pigs to 66.7% among goats; 66.7% in goats, 15.0% in dogs, 13.2% in cattle, whereas no antibodies were detected in pigs.

In our study area, the SFTSV seroprevalence was 5.5% in the patient village, whereas SFTSV antibodies were also detected in the nonpatient village of Qianyang County where no cases were confirmed. These results suggest that subclinical SFTSV infections or a relatively mild form of SFTS illness affects humans in this region. Seroprevalence levels for 20–39 and 40–59 years age groups were significantly higher compared with ≤ 6 years age groups in our study. This age-specific difference in seroprevalence could be explained by age-specific differences in exposure rates through outdoor activities or an accumulation of antibodies among older populations where specific antibodies to SFTSV are detectable from about 7 days after disease onset.

Haemaphysalis longicornis ticks had been considered the primary tick vector of SFTSV. Nevertheless, we detected the virus in *H. concinna* ticks, the most abundant tick in the study area, in a high proportion of the tested samples. Our results contrast with findings from other areas of China and Korea.^{2,13} However, this finding could be directly linked to

TABLE 1
Sociodemographic characteristics of the study population and prevalence of antibodies against SFTSV, Shaanxi Province, China

Factors	No. serum samples	No. (%) antibody positive	χ^2	P value
Sites			2.84	0.24
Patient village, Long County	363	20 (5.5)		
Nonpatient village, Long County	323	10 (3.1)		
Nonpatient village, Qianyang County	250	14 (5.6)		
Gender			0.13	0.72
Male	422	21 (5.0)		
Female	514	23 (4.5)		
Age			12.21	0.01
≤ 6	150	1 (0.7)		
7–19	159	5 (3.1)		
20–39	225	14 (6.2)		
40–59	304	21 (6.9)		
≥ 60	98	3 (3.1)		
Total	936	44 (4.7)		

SFTSV = severe fever with thrombocytopenia syndrome virus.

the tick's species distribution, which is associated with climate types and complex geography. Besides, since SFTSV was not detected in *H. longicornis* ticks in our study, we speculated this may result from small sample size of this species. Our results are consistent with prior analyses using data from Shandong Province,¹⁴ where *H. concinna* ticks were found to be potential vectors for SFTSV transmission. It is important to note that the presence of viral RNA in engorged ticks does not confirm a transmission link from ticks to domestic animals or humans. Further experimental studies are needed to demonstrate transmission links.

Most phleboviruses are considered to be associated with sandflies, with evidence of transovarial transmission.¹⁵ Besides, Rift Valley fever (family *Bunyaviridae*, genus *Phlebovirus*) is transmitted mainly by *Aedes* species mosquitoes. However, viral RNA was not detected in any of 1,950 mosquitoes, 30 midges, and 20 sandflies tested. Our result is in line with previous studies set in China.

Our results indicate that domestic animals should be evaluated as amplifying hosts of SFTS, and are consistent with findings from other studies.⁵ In previous studies, the seroprevalence level in goats was estimated at 75–95% in the Shandong Province,^{16,17} 57% in Jiangsu Province,¹⁸ and 67% in Hubei Province.¹⁹ In contrast, the seroprevalence level was very low in pigs.¹⁸ Our results suggest that animals with a higher likelihood of exposure due either to outdoor activities or farming practices that augmented risk of tick bite, were at higher risk of acquiring SFTSV infection, and play an important role in feeding the ticks that spread SFTSV.

Limitations of this study should also be acknowledged. First, the impact of socioeconomic factors, outdoor activities (e.g., farming, mowing grass, hunting, picking tea leaves, grazing, and traveling), and whether the patient had a history of bites by animals or arthropods in recent months (e.g., rats and ticks) were not explicitly considered in our study. Second, SFTSV seroprevalence of 4.7% in the population of the Shaanxi Province is similar to that reported for the Hubei Province (3.8–6.4%), Zhejiang Province (5.5%), and Shandong Province (0.8–3.3%), but much higher than that reported for the Jiangsu Province (0.4–0.9%, $\chi^2 = 94.33$, $P < 0.01$).^{18–20} Future studies should aim to elucidate epidemiological and ecological factors associated with SFTSV.

Our study confirmed that SFTSV antibodies are widespread across our study area even though disease cases were not known from all study sites. Our data also informed that some domestic animals are exposed at high rates. Our results showed that *H. concinna* ticks are potential vectors of SFTSV. More studies are needed to elucidate the SFTSV transmission model in nature and risk factors for human infection.

ETHICS

The study was approved by the ethical review committee of Shaanxi Provincial CDC. Informed consent was obtained from all participants after they were provided with detailed descriptions of the potential benefits of the study.

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Authors' addresses: Huaiyu Tian, Jing Yang, Shanjian Huang, and Bing Xu, State Key Laboratory of Remote Sensing Science, College of Global Change and Earth System Science, Beijing Normal University, Beijing, China, E-mails: tianhuaiyu@gmail.com, judyssister@163.com, huangshanjian@gmail.com, and bingxu@tsinghua.edu.cn. Pengbo Yu, Shen Li, Jing Wei, Wen Lv, and Jingjun Wang, Shaanxi Provincial Centre for Disease Control and Prevention, Xi'an, China, E-mails: xxdcy@21cn.com, lishen12345@163.com, weijingnwu@gmail.com, lvwen2008@gmail.com, and jingjunwang@china.com. Gerardo Chowell, School of Public Health, Georgia State University, Atlanta, GA, and Division of International Epidemiology and Population Studies, Fogarty International Center, National Institutes of Health, Bethesda, MD, E-mail: gchowell@gsu.edu. Hui Tian, Baoji Centre for Disease Control and Prevention, Baoji, China, E-mail: bjcdcth@sina.com. Zongqi Han, Long County Centre for Disease Control and Prevention of Shaanxi Province, Baoji, China, E-mail: lxcdchzq882@163.com. Sen Zhou and John S. Brownstein, Department of Pediatrics, Harvard Medical School, Boston, MA, E-mails: zhousenapril@gmail.com and john.brownstein@childrens.harvard.edu.

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