Original Article

A Cross-Sectional Survey of Severe Fever with Thrombocytopenia Syndrome Virus Infection of Domestic Animals in Laizhou City, Shandong Province, China

Shujun Ding^{1,2†}, Haiying Yin^{3†}, Xuehua Xu³, Guosheng Liu³,
Shanxiang Jiang³, Weiqing Wang³, Xinqiang Han³, Jingyu Liu⁴,
Guoyu Niu⁵, Xiaomei Zhang², Xue-jie Yu^{1,6*}, and Xianjun Wang²

¹*School of Public Health, Shandong University, Jinan;*

²*Shandong Center for Disease Control and Prevention, Shandong Provincial
Key Laboratory of Communicable Disease Control and Prevention, Jinan;*

³*Laizhou City Center for Disease Control and Prevention, Laizhou;*

⁴*Yantai City Center for Disease Control and Prevention, Yantai;*

⁵*China Center for Disease Control and Prevention, Beijing, China; and*

⁶*Department of Pathology, University of Texas Medical Branch, Galveston, Texas, USA*

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SUMMARY: A serosurvey of severe fever with thrombocytopenia syndrome virus (SFTSV) infection in domestic animals was conducted in the rural areas of Laizhou City, Shandong Province, China to determine strategies for control and prevention of SFTS. Serum samples were collected from cattle, goats, dogs, pigs, and chickens and antibodies against SFTSV were detected by double-antigen sandwich enzyme-linked immunosorbent assay (ELISA). Of 641 serum samples, the SFTSV seropositive rate was 41.8% (268/641): 74.8%, 57.1%, 52.1%, 35.9%, and 0%, for goats, cattle, dogs, chickens, and pigs, respectively. We also found that the SFTSV seropositive rates were high among the aged cattle, goats, dogs, and chickens. SFTSV infections existed among cattle, goats, dogs, and chickens in Laizhou City, and goats had the highest seroprevalence. SFTSV seroprevalence increased with an increase in age among animals. To control of animal infestation with ticks may prevent human SFTSV infections.

INTRODUCTION

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease in China, Japan, and South Korea, which is characterized by hemorrhagic fever. The causative agent of SFTS is SFTS virus (SFTSV), a novel *Phlebovirus* in the family *Bunyaviridae* (1–3), which is most likely transmitted by ticks. SFTS has been identified in 16 provinces in eastern China, and the incidence of the disease was very high in the Shandong Province, particularly among residents living in the hilly and mountainous areas in Yantai City, Tai'an City, and Zibo City (4–6). To determine potential animal hosts and the infection status of the host animals, we investigated SFTSV seropositive rates in domestic animals from Laizhou City of Yantai City, Shandong Province in 2011. In this study, we report the results of the serosurveillance.

MATERIALS AND METHODS

Geographical features of study site: Laizhou City is located in the northwest of Jiaodong Peninsula between

119°33' and 120°18'E longitude and 36°59' and 37°28'N latitude, with a total area of 1,878 km² and a population of 859,000. Laizhou City has a sub-humid, northern, temperate, monsoon-prone, continental climate with an annual rainfall of approximately 610 mm and an annual average temperature of approximately 12°C. The city has a high elevation in the southeast, marked by hills and mountains, and a low elevation in the northwest, marked by plains. The area comprises 10.3% low mountains, 48.1% hills, and 41.6% plains with several rivers. In 2010, the city had 3,612 households with medium- to large-scale farms that produced 50,149 cattle, 111,000 goats, and more than 1 million pigs for slaughter. Farmers grew peanuts, corn, and other food crops.

Selection of study site: The towns of Guojiadian and Zuocun were selected as study sites for this survey because the number of SFTS cases reported in these areas were slightly more than those reported in others areas.

Animals: We surveyed domestic animals, including cattle, dogs, pigs, and chickens. Five to 10 blood samples were collected from animals, except pigs, belonging to the patients and their neighbors in each village that reported at least 2 SFTS cases. The blood samples of pigs were collected at slaughter houses located in the towns.

Animal blood samples: Animal blood samples were collected 3 times a year: spring (April), summer (August), and autumn (November–December). Before the surveillance, investigators were trained to use ques-

*Corresponding author: Mailing address: School of Public Health, Shandong University, Jinan, Shandong 250012, China. Tel: +86-15069192031, E-mail: yuxuejie@sdu.edu.cn.

†These authors contributed equally to this work.

tionnaire forms to record the required information gained from farmers participating in the survey. Five milliliters of blood were obtained from each cattle, goat, and pig and 2 ml from each chicken. All blood samples were centrifuged, and the separated sera were stored at -70°C .

Serological testing: Animal sera were tested for SFTSV total antibody using a double antigen sandwich enzyme-linked immunosorbent assay (ELISA) kit described previously (7), which was provided by the Chinese Center for Disease Control and Prevention (CDC) (8). The experiment included negative, positive, and blank controls. In brief, $50\ \mu\text{l}$ aliquot of each sample ($10\ \mu\text{l}$ of serum diluted in $40\ \mu\text{l}$ of PBS-Tween) was added to each well, except for the control wells, followed by addition of $50\ \mu\text{l}$ of HPR reagents, and then incubated at 37°C for 1 h. After washing each well 5 times, chromogenic agents (solution A and B) were added to each to develop color, and the optical density (OD) was read at 450 nm. The sample was considered as positive if the OD value was greater than the threshold (cut-off) value. The threshold value was calculated as $0.10 + \text{the average OD value of the negative control}$ (if the OD value of a negative control was <0.04 , it was considered as 0.04).

Statistical analysis: Statistical analysis was performed using SPSS 18.0 software and $P < 0.05$ was considered as statistically significant.

RESULTS

The towns of Guojiadian and Zuocun are located in the southeast mountainous area of Laizhou City. In the survey area, 20% of households had cattle, 5% had goats, and 60%–70% had chickens, dogs, and/or pigs. Ticks were commonly found on goats, dogs, and grasslands. Villagers planted peanuts, corns, fruit trees, or seedlings and were also involved in marble mining and processing.

Serologic testing: Of the 641 animal blood samples collected from Laizhou City, 268 (41.8%) were positive for SFTSV total antibody by ELISA. The SFTSV antibody-positive rates were 74.8%, 57.1%, 52.1%, 35.9%, and 0% for goats, cattle, chickens, dogs, and pigs, respectively, and these rates were significantly different (chi-square $[\chi^2] = 199.54$; $P < 0.01$; Table 1).

SFTSV seroprevalence among different towns: The SFTSV antibody-positive rates among all animal species were significantly lower in Guojiadian Town (39.0%, 126/323) than in Zuocun Town (52.4%, 142/271; Table 2). There was no significant difference in the antibody levels among the tested animals between the towns, except for goats, which was significantly higher in Guojiadian Town.

Cross-sectional survey of SFTSV infection in animals: The SFTSV antibody-positive rates were 42.0% (86/205), 30.7% (42/137), and 46.8% (140/299) for animals collected in April, August, and November, respectively, which were significantly different ($\chi^2 = 10.09$, $P < 0.01$). Bovine serum antibody level to SFTSV in November was significantly higher than that in August ($\chi^2 = 4.16$, $0.01 < P < 0.05$) and April, but the differences were not statistically significant ($\chi^2 = 1.34$, $P > 0.05$). In April, goats had the highest positive rate, followed by cattle. In August, goat sera were not tested because of insufficient samples. Cattle and chicken antibody-positive rates were similar. In November, goats had the highest positive rate.

SFTSV infection in animals of different ages: With aging, the serum SFTSV antibody levels among the different animals gradually increased. Further analysis revealed that the OD values by ELISA of the animal sera also elevated with an increase in age (Fig. 1), which suggested that the animals were frequently infested with SFTSV-carrying ticks during their growing years. The antibody-positive rate was highest ($>60\%$) at the age of 36 months for all animals, except for chickens, as sera samples were not collected at 36 months. The SFTSV

Table 1. Seroprevalence of SFTSV in animals in Laizhou City

Month	Cattle	Goat	Chicken	Pig	Dog
April	54.9 (28/51)	76.5 (39/51)	N/A	0 (0/50)	35.9 (19/53)
August	47.2 (25/53)	N/A	46.0 (17/37)	0 (0/47)	N/A
November	65.0 (52/80)	73.3 (44/60)	55.0 (44/80)	0 (0/79)	N/A
Total	57.1 (105/184)	74.8 (83/111)	52.1 (61/117)	0 (0/176)	35.9 (19/53)

Data are SFTSV antibody-positive rates % (no. of SFTSV-positive animals/total no. of animals tested).

Table 2. SFTSV seropositive rate in animals in different towns in Laizhou City

Animal	Guojiadian Town			Zuocun Town			χ^2	P
	No. tested	No. positive	Positive rate, %	No. tested	No. positive	Positive rate, %		
Goats	56	51	91.1	55	32	58.2	10.6	<0.01
Cattle	89	45	50.6	95	60	63.2	0.82	>0.05
Dogs	27	6	22.2	26	13	50.0	2.11	>0.05
Chickens	62	24	38.7	55	37	67.3	2.99	>0.05
Pigs	89	0	0	40	0	0		
Total	323	126	39.0	271	142	52.4	10.67	<0.01

SFTSV Serosurvey

Fig. 1. OD value variation in different ages of goats.

Table 3. SFTSV antibody positive rate in different ages of animals

Age (mo)	Goat	Cattle	Dog	Chicken	Total
≤ 6	59.6 (28/47)	20 (1/5)	0 (0/5)	38.6 (17/44)	45.5 (46/101)
6–12	70.4 (19/27)	47.4 (9/19)	11.8 (2/17)	48 (24/50)	47.8 (54/113)
12–24	100 (9/9)	25 (8/32)	25 (1/4)	75 (3/4)	42.9 (21/49)
24–36	93.8 (15/16)	60 (15/25)	50 (7/14)	89.5 (17/19)	73.0 (54/74)
> 36	100 (12/12)	69.9 (72/103)	69.2 (9/13)	ND	72.7 (93/128)
Total	74.8 (83/111)	57.1 (105/184)	35.8 (19/53)	52.1 (61/117)	57.6 (268/465)

Data are SFTSV antibody-positive rates % (no. of SFTSV-positive animals/total no. of animals tested).

Table 4. SFTSV seroprevalence in different sexes of animals

Animal	Male			Female			χ^2	<i>P</i>
	No. tested	No. positive	Positive rate, %	No. tested	No. positive	Positive rate, %		
Goats	46	18	39.1	86	70	81.4	24.09	<0.01
Cattle	11	4	36.4	171	101	59.1	1.35	>0.05
Dogs	19	7	36.8	34	12	35.3	0.01	>0.05
Chickens	49	19	38.8	68	42	61.8	6.03	0.01 < <i>P</i> < 0.05
Total	125	48	37.6	359	225	62.7	22.22	<0.01

antibody-positive rates were significantly different at different ages in all animal species ($\chi^2 = 16.17, 23.98, 14.8$, and 14.75 , for goats, cattle, dogs, and pigs, respectively; $P < 0.01$; Table 3).

SFTSV seroprevalence in different genders of animals: In all animal species tested, except for dogs, females had higher SFTSV antibody-positive rates than males. Male dogs had slightly higher SFTSV-positive rates than females. Female goats and chickens had significantly higher SFTSV antibody-positive rates than males (Table 4).

DISCUSSION

SFTSV is a novel bunyavirus, which causes hemorrhagic fever in humans. Although the route of transmission and animal hosts of SFTSV remain unclear, ticks are the most likely vector (9). An epidemiological survey of SFTSV-infected patients revealed that the families of some of the patients raised cattle, goats, dogs, and other livestock that were infested with ticks. We collected

blood samples from cattle, dogs, and chickens during the survey period and tested the SFTSV seropositive rates of the animals by double-antigen sandwich ELISA. Our results demonstrated that the incidence of SFTSV infection was correlated to the life style of the animals. The animals including goats, cattle, dogs, and chickens were all positive for SFTSV antibody, while pigs were negative. This may be due to the free-range life style of goats, cattle, dogs and chickens that exposes them to a greater risk of tick bites than pigs that are usually held in pens. The present study and previous studies demonstrated that goats, cattle, dogs, and chickens were SFTSV seropositive in different provinces in China, and all studies showed that SFTSV seropositive rates were highest in goats among all animal species (10–12). These studies demonstrated that domestic animals may be intermediate animal hosts during SFTSV amplification. Niu et al. (13) recently reported SFTSV seroprevalence by RT-PCR among animals in Laizhou City and Penglai City in Shandong Province. Both the study by Niu et al. and the present study

demonstrated that animals in Laizhou City had high SFTSV seroprevalence rates. However, in our study, antibody analysis was studied in detail based on endemic distribution, seasonal prevalence, and sex distribution among different animal species. The number of SFTSV-positive samples from cattle, goats, dogs, and chickens from Guojiadian Town was significantly lower than those from the Zuocun Town. In 2011, there were 5 SFTSV cases reported in Zuocun Town (incidence rate, 12.15/100,000) and 2 in Guojiadian Town report 2 cases (incidence rate, 3.71/100,000). Thus, there is a positive correlation between the number of reported human SFTSV cases and the SFTSV seropositive rate of animals in the 2 towns. Villagers in Guojiadian Town are primarily engaged in planting fruit trees or seedlings, whereas those in Zuocun Town are primarily engaged in marble mining and processing. Thus, villagers engaged in different activities may have also affected the incidence of SFTSV infections. The survey found that the SFTSV seropositive rate in male animals, except for dogs, was lower than that in female animals. Male animals may be less frequently exposed to tick bites than female animals because farmers usually raise a few male animals for breeding, which are more often housed in pens compared with female animals. Our study further clarified SFTSV animal hosts. Based on the results of the present and previous studies, we concluded that the strategies for control and prevention of SFTSV should consider improving the environments in which animals are raised and controlling the tick population by using chemicals.

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Conflict of interest None to declare.

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