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

Shujun Ding1,2†, Haiying Yin3†, Xuehua Xu3, Guosheng Liu3, Shanxiang Jiang3, Weiqing Wang1, Xinqiang Han3, Jingyu Liu4, Guoyu Niu5, Xiaomei Zhang2, Xue-jie Yu1,6*, and Xianjun Wang2

1School of Public Health, Shandong University, Jinan; 2Shandong Center for Disease Control and Prevention, Shandong Provincial Key Laboratory of Communicable Disease Control and Prevention, Jinan; 3Laizhou City Center for Disease Control and Prevention, Laizhou; 4Yantai City Center for Disease Control and Prevention, Yantai; 5China Center for Disease Control and Prevention, Beijing, China; and 6Department of Pathology, University of Texas Medical Branch, Galveston, Texas, USA

(Received May 5, 2013. Accepted July 16, 2013)

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, goats, 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-
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 μl aliquot of each sample (10 μl of serum diluted in 40 μl of PBS-Tween) was added to each well, except for the control wells, followed by addition of 50 μl 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 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 mining and processing. SFTSV seropositive rate in animals in different towns in Laizhou City

<table>
<thead>
<tr>
<th>Animal</th>
<th>Guojiadian Town</th>
<th>Zuocun Town</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goats</td>
<td>No. tested 56</td>
<td>No. positive 51</td>
</tr>
<tr>
<td>Cattle</td>
<td>No. tested 89</td>
<td>No. positive 45</td>
</tr>
<tr>
<td>Dogs</td>
<td>No. tested 27</td>
<td>No. positive 6</td>
</tr>
<tr>
<td>Chickens</td>
<td>No. tested 62</td>
<td>No. positive 24</td>
</tr>
<tr>
<td>Pigs</td>
<td>No. tested 89</td>
<td>No. positive 0</td>
</tr>
</tbody>
</table>

**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...
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.

Acknowledgments This study was supported by National Natural Science Foundation of China (Grant No. 81102171), Shandong Medical Science and Technology Development Program (Grant No. 2011HZ055) and Shandong Province Science and Technology Development Plan (Grant No. 2012GHZ30031).

Conflict of interest None to declare.

REFERENCES