Detection of severe fever with thrombocytopenia syndrome virus RNA and total antibodies in wild animals, Jiangsu, China, 2014–2019

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Background: Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by SFTS virus (SFTSV). Currently, SFTS is endemic to some areas in China. Moreover, wild animals are considered to play important roles in the circulation of SFTSV in the environment. Therefore, wild animals monitoring for SFTSV has been fulfilled in Jiangsu Province.

Methods: We studied the results of the detection to provide basic data for better diagnosis of wild animals. This research was conducted in Jiangsu Province from 2014 to 2019. Sera of wild animals (rodents, pheasants and hedgehogs) were collected to detect SFTSV both RNA and total antibodies by qRT-PCR and enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed by using SPSS 25.0.

Results: A total of 95.8% (1,298/1,355) of the specimens had the corresponding SFTSV RNA and total antibodies detection results. However, there was a significant difference between the detection rates of SFTSV RNA and total antibodies, and the detection consistency was very poor. The detection rate of SFTSV total antibodies was highest in hedgehogs (19.54%).

Conclusions: Hedgehog could be a competent reservoir for SFTSV. Also, SFTSV total antibodies testing was preferred method during wild animals surveillance.

Keywords: Severe fever with thrombocytopenia syndrome virus (SFTSV); wild animals; detection

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Introduction

Sporadic cases with clinical manifestations of acute onset of fever, low white blood cell and platelet counts, high levels of alanine and aspartate transaminases, and proteinuria, have been observed in China since 2005. A new bunyavirus, which was then named severe fever with thrombocytopenia syndrome virus (SFTSV) in 2010, was found to be related to this disorder. SFTSV is endemic in the central and eastern China. In addition, confirmed SFTS cases have been reported in Japan and South Korea in recent years (1-7). SFTSV is thought to be a tick-borne zoonotic virus (1,8,9), and has been detected in or isolated from several species of ticks including Haemaphysalis longicornis, Amblyomma testudinarium, and Ixodes nipponensis in China and Korea (10-13). Spotted doves, one of the most abundant bird species in China, could be a competent amplifying host for SFTSV and play an important role in its ecology (14). SFTS could be transmitted not only by means of tick bites, but also via human to human transmission caused by direct contact with blood (15). SFTS, as an emerging infectious disease with case fatality up to more than 10%, was listed as one

of the most severe infectious disease by WHO in 2007.

Virus RNA detection by real-time RT-PCR and antibody detection by enzyme-linked immunosorbent assay (ELISA) are common methods to identify virus infection. The former is usually used to confirm SFTSV infection. The latter is often used to know recent and past infection status. However, the detection results of SFTSV RNA or antibodies in routine SFTS monitoring were not very clear in wild animals. To fill this gap, we performed SFTSV RNA and antibody detection and analysis on the wild animals collected in Jiangsu Province in 2014–2019. The aim was to understand the surveillance of the virus distribution and the detection results of SFTSV RNA and antibodies, so as to explore appropriate conventional laboratory pathogenic detection strategies to provide a pathogenic and serological basis for better diagnosis of the wild animals. We present the following article in accordance with the MDAR reporting checklist (available at https://jphe.amegroups.com/article/view/10.21037/jphe-21-77/rc).

Methods

Data collection

This study was conducted in three cities (Lianyungang, Zhenjiang and Yancheng) and four counties (Yixing, Lishui, Dongtai and Xuyi), Jiangsu Province, eastern China, the main SFTS epidemic area where human SFTS cases had been reported before (*Figure 1*). We randomly selected 10 villages of the 3 cities and 4 counties as study site. The wild animals were selected in every study site, including small wild mammals (rodents and hedgehogs) and avian (pheasants). A total of 1,355 serum of wild animals were collected from 2014 to 2019. Blood samples from wild animals were collected directly in serum tubes. The samples were centrifuged at 2,560 ×g for 2 min and the serum was transferred to small vials, which were kept at –18 ℃ until the time of analysis. Rodents, pheasants and hedgehogs were collected with live traps in accordance with standard protocols, as previously described (16,17). Trapping grids were set up at sites adjacent to case households and in locations chosen to provide geographic diversity. We abided by established safety guidelines for rodent capture and processing. All trapped animals were anesthetized using ketamine, blood was drawn from the retro-orbital sinus.

SFTSV-RNA extraction and real-time RT-PCR

Total RNA prepared from the serum samples from wild animals were extracted using an RNeasy kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Real-time RT-PCR was performed using the QuantiTech RT-PCR kit (Qiagen). The primers were designed as previously described and used in a one-step real-time RT-PCR. The primers and MGB probe used in the real-time RT-PCR were targeted to the S segment of the viral genome (18). Conditions for the reaction were as follows: 50 ℃ for 30 min, 95 ℃ for 15 min, 40 cycles at 95 ℃ for 15 s, and 55 ℃ for 40 s. Amplification and detection were performed with an Applied Biosystems 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA). Data were analyzed using the software supplied by the manufacturer.

Ethics statement

Small wild mammals and avian were captured and handled in strict accordance with good animal practice according to the Animal Ethics Procedures and Guidelines of the People's Republic of China (Regulations for Administration of Affairs Concerning Experimental Animals, China, 1988). Handling and sampling blood collection were approved by the Animal Ethics Committee, Jiangsu Provincial Center for Disease Control and Prevention, with the certificates No. SCXK [su] 2012–0021 and No. JSCDCLL [2012] 039. The certificates also provided the permission on the protocol for sampling wild birds including Anser cygnoides.

ELISA for SFTSV antibody detection

Serum samples from wild animals were tested for SFTSV IgG and IgM antibodies with commercial ELISA kits from DAAN GENE (Zhongshan, China). For initial screening, a 1:40 diluted serum sample was used to determine whether the sample was positive for viral antibodies. Positive serum samples were further diluted in a 2-fold serial dilution starting at 1:80 for the assay to obtain endpoint titers determined by the cutoff values set by positive and negative controls as provided with the ELISA kit.

Statistical analysis

SPSS 25.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis and statistical significance level was set at 0.05. Pearson chi-square test was conducted to compare the detection rates of SFTSV RNA and total antibodies among samples. The McNemar test was applied to compare SFTSV RNA and total antibodies detection rates in the

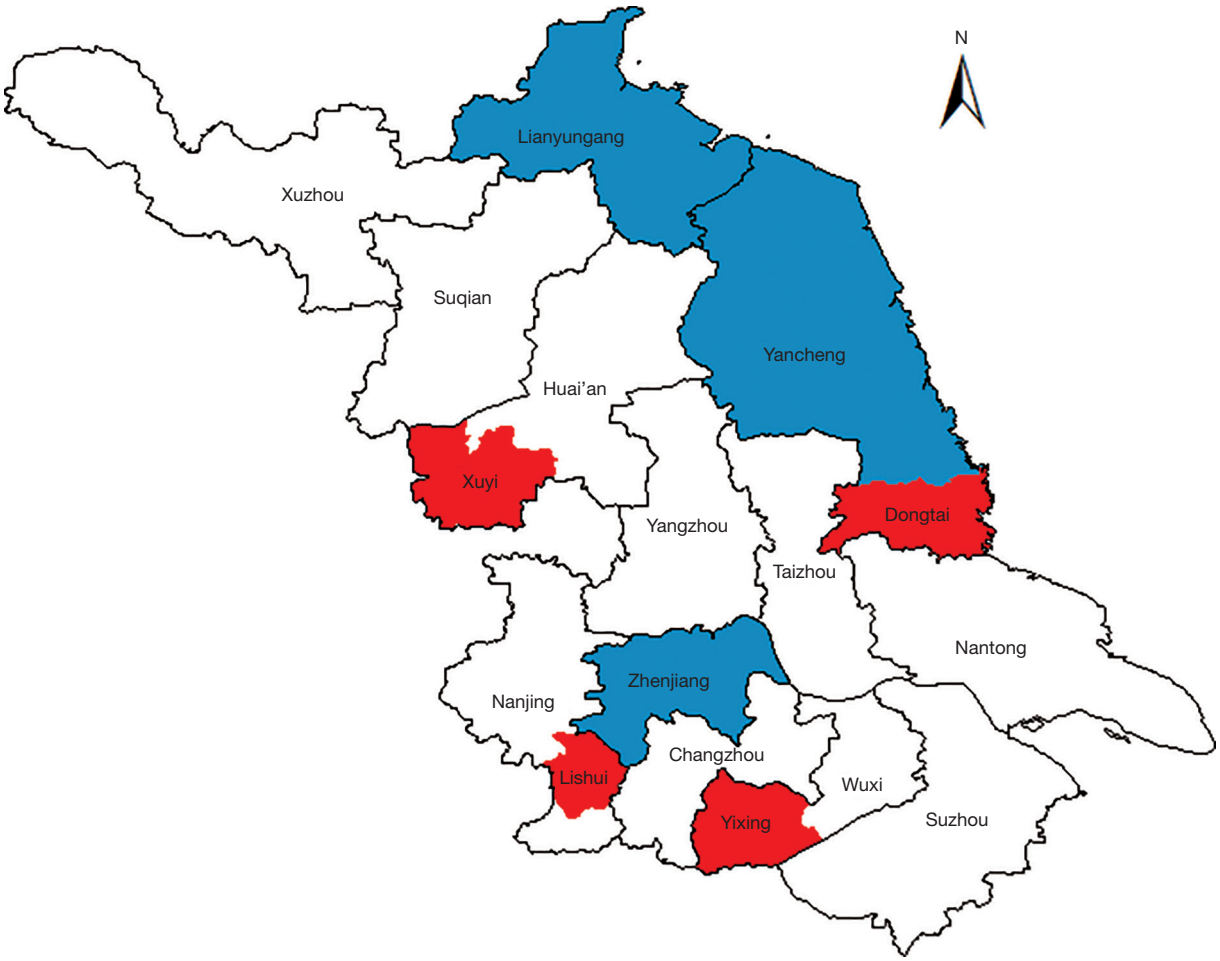

Figure 1 Location of the three cities (blue) and four counties (red) in Jiangsu Province.

paired specimens, and the kappa value was calculated to compare the detection consistency of the two methods.

Results

SFTSV RNA detection

Among the 1,355 samples, 16 (1.18%) were positive for SFTSV RNA detection. There was a significant difference in the detection rate of RNA among hedgehogs, rodents and pheasants. The highest positive rate of SFTSV RNA in hedgehogs was 5.75% (5/87). The lowest positive rate of SFTSV RNA in pheasants was 0.71% (*Table 1*).

SFTSV total antibody detection

Of the 1,355 samples, all the sera samples were chosen to conduct SFTSV total antibody (IgM and IgG) detection. The detection rate of SFTSV total antibodies was 4.06% (55/1,355) in all samples. There was a significant difference in the detection rate of total antibodies among three species of wild animals. The highest total antibodies detection rate was found in hedgehogs 19.54% (17/87) (*Table 1*).

Comparison of SFTSV RNA and total antibodies detection

The detection rates of SFTSV total antibodies were 43.7% (7/16) and 3.6% (48/1,339) in SFTSV RNA-positive and SFTSV RNA-negative samples, respectively (*Table 2*). A total of 95.8% (1,298/1,355) of the samples had the same SFTSV RNA and total antibodies detection results; 7 samples were found concordantly positive and 1,291 samples were found consistently negative by both methods. However, there was a significant difference

Table 1 SFTSV RNA, total antibodies detection results

Wild animals	Total detection No.	SFTSV RNA-positive samples No./total detection No. (%)	SFTSV total antibodies-positive samples No./total detection No. (%)
Hedgehogs	87	5/87 (5.75)	17/87 (19.54)
Rodents	1,128	10/1,128 (0.89)	29/1,128 (2.57)
Pheasants	140	1/140 (0.71)	9/140 (6.43)
Total	1,355	16/1,355 (1.18)	55/1,355 (4.06)

SFTSV, severe fever with thrombocytopenia syndrome virus.

Table 2 Comparison of SFTSV RNA and total antibodies detection

Animals	SFTSV RNA	SFTSV total antibodies		P value (McNemar test)	Kappa value
		Positive, n	Negative, n		
Hedgehogs	Positive	4	1	0.002	0.302
	Negative	13	69		
Rodents	Positive	3	7	0.001	0.143
	Negative	26	1,092		
Pheasants	Positive	0	1	0.021	–0.013
	Negative	9	130		
Total	Positive	7	9	0.000	0.182
	Negative	48	1,291		

SFTSV, severe fever with thrombocytopenia syndrome virus.

between the detection rates of SFTSV RNA and total antibodies, and the detection consistency was verypoor.

Discussion

Pathogen detection mostly aims at RNA and antibodies. Real-time RT-PCR is an ordinary method for virus RNA detection, which directly targets the RNA of pathogens. However, ELISA is an indirect method to determine whether there is virus infection by detecting virus antibody (IgG and IgM). Real-time RT-PCR requires higher detection sensitivity in the acute phase. Whereas ELISA needs a simpler operating environment and is much simpler to operate by professionals in county level. These two methods are both applied in the early stage after disease onset. Thus, we used these two methods to detect pathogen in this study.

Jiangsu Province is a highly endemic area for SFTS. We compared the detection rates of SFTSV RNA and SFTSV-specific antibodies among wild animals in seven study sites of our province. We found that approximately 4.06% of surveillance wild animals had SFTSV total antibodies and 1.18% had SFTSV RNA. This result showed that the detection rates between SFTSV RNA and total antibodies were different, and the detection consistence were all very poor in hedgehog, rodents and pheasants. In addition, 3.6% of SFTSV RNA negative specimens were positive for SFTSV total antibodies. Therefore, SFTSV RNA negative specimens could not be completely ruled out as SFTS when only relying on RNA detection. It was suggested that the monitoring of wild animals was different from that of human beings (19). SFTSV antibodies detection was superior to RNA detection for wild animals. Besides, we found that the detection rate of SFTSV total antibodies was highest in hedgehogs (19.54%). However, Liu *et al.* and Li *et al.* (20,21) had investigated seropositive rate of SFTSV in rodents and hedgehogs by ELISA, and showed SFTSV antibody positive rate was low in these wild animals. In this study, the SFTSV antibody positive rate was similar with previous study in rodents, but

was high in hedgehogs. The results are in accordance with the previous study (22,23). It implied that hedgehogs might be the amplified hosts of SFTSV in maintaining the life cycle of SFTSV in nature.

Conclusions

Our study suggested that SFTSV antibody detection conducted with ELISA was preferred method for wild animals surveillance. For highly suspected SFTSV infection in wild animals, SFTSV RNA is suggested to make a comprehensive judgement. Also, we found that hedgehog could be a competent reservoir for SFTSV and play an important role in its ecology.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://jphe.amegroups.com/article/view/10.21037/jphe-21-77/rc

Data Sharing Statement: Available at https://jphe.amegroups.com/article/view/10.21037/jphe-21-77/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jphe.amegroups.com/article/view/10.21037/jphe-21-77/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Small wild mammals and avian were captured and handled in strict accordance with good animal practice according to the Animal Ethics Procedures and Guidelines of the People's Republic of China (Regulations for Administration of Affairs Concerning Experimental Animals, China, 1988). Handling and sampling blood collection including blood collection were approved by the Animal Ethics Committee, Jiangsu Provincial Center for Disease Control and Prevention, with the certificates No. SCXK [su] 2012–0021 and No. JSCDCLL [2012] 039. The certificates also provided the permission on the protocol for sampling wild birds including Anser cygnoides.

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