Laboratory and Epidemiology Communications

Investigation of Severe Fever with Thrombocytopenia Syndrome Virus Antibody among Domestic Bovines Transported to Slaughterhouse in Shimane Prefecture, Japan

Kenji Tabara†,*,**, Hiromi Fujita‡, Atau Hirata†, and Daisuke Hayasaka†,§

†Shimane Prefectural Laboratory of Meat Hygiene Inspection Station, Shimane 699-2212; ‡Mahara Institute of Medical Acarology, Tokushima 779-1510; §Institute of Tropical Medicine; and §Leading graduate school program, Nagasaki University, Nagasaki 852-8523, Japan

Communicated by Masayuki Saijo

Severe fever with thrombocytopenia syndrome (SFTS) is a highly lethal tick-borne viral disease that has been reported in China, South Korea, and Japan (1–7). In Japan, 163 patients with SFTS were identified between the autumn of 2012 and October 2015, with most cases occurring in the western part of Japan, including Shimane Prefecture, located in Chugoku region of Honshu island (7–9).

The causative agent, SFTS virus (SFTSV), belongs to the genus Phlebovirus in the family Bunyaviridae (1). Humans are infected by this virus through the bite of an infected tick, such as Haemaphysalis longicornis (1,9). Seroepidemiological surveys identified anti-SFTSV antibodies in domestic animals in endemic areas, indicating that SFTSV circulates between ticks and animals in nature (1,9–13). Antibody prevalence rates are particularly high among livestock in regions with a large number of SFTS patients in China; 74.8±6.0% in goats, 69.5% in sheep, 57.1%–60.4% in cattle, and 47.4%–52.1% in chickens (11–13). Therefore, seroepidemiological studies on animals may provide useful information on the prevalence of SFTS in endemic areas. However, seroepidemiological data on SFTS are limited in Japan, and the prevalence of SFTSV infections in livestock has not yet been examined.

In the present study, we investigated the prevalence of anti-SFTSV antibodies among livestock, particularly cattle, in Shimane Prefecture, Japan (Fig. 1).

Serum samples were collected from 510 slaughtered cattle, including 408 fattening cattle (reared for flesh), 43 nulliparous milk cows and stags, aged 27.7±3.7 months), 63 milk cows (reared for milking, aged 3.7 months), and 39 breeding cattle (reared for producing calves, aged 125.6±41.2 months), in Shimane Prefecture between June 2014 and March 2015 (Table 1). We obtained the cattle backgrounds, regions of Japan they were born and reared in, from the National Livestock Breeding Center website (https://www.id.nlbc.go.jp/top.html). Consequently, 392 cattle were born and reared in Shimane Prefecture (Group A), 105 cattle were reared in and outside Shimane Prefecture (Group B), and 13 cattle were born and reared outside Shimane Prefecture (Group C) (Table 1).

Serum samples were tested for the prevalence of anti-SFTSV antibodies using the indirect immunoperoxidase technique. Vero E6 cells were infected with the SFTSV YG1 strain (7) for 6 days and inactivated with 4% paraformaldehyde at 4°C overnight. Antigen slides were prepared by adding SFTSV- and mock-infected cells to glass slides. Serially diluted serum (1:40 to 1:320) was applied to these slides and then treated with peroxidase-labeled Protein G. Vero E6 cells infected with SFTSV that stained dark blue with 40-fold or higher serum dilution was determined as positive. In addition, we confirmed less than 40-fold dilutions as negative using the same indirect immunoperoxidase technique on other anti-Bunyavirus antibody-positive cattle sera, which had neutralizing antibodies against Akabane virus and Aino virus.

Eleven samples from the 510 cattle tested were positive for anti-SFTSV antibodies (Table 1). Indirect immunoperoxidase antibody titers were 1:80 in 6 cattle, 1:160 in 3 cattle, and 1:320 in 2 cattle. The average age of the 11 anti-SFTSV antibody-positive cattle was 127.7±50.9 months, whereas that of the 499 negative cattle was 38.5±28.6 months. Furthermore, SFTSV seropositive rates in fattening cattle, milk cows, and breeding cattle were 0.5% (2 out of 408), 4.8% (3 out of 63), and 15.4% (6 out of 39), respectively, indicating that seropositivity was significantly higher in breeding cattle and milk cows than in fattening cattle (Fisher’s exact test on seropositivity age of cattle and milk cows vs. fattening cattle: P<0.01 and P<0.05 respectively; Table 1). As a result, the positive rate in older cattle (aged >120 months) was 28.6%, which was significantly higher than that in younger cattle (0.5% and 2.6% among ages <60 months and 60–120 months, respectively; Fisher’s exact test on seropositivity age of cattle and milk cows vs. fattening cattle: P<0.01 and P<0.05 respectively).
Table 1. Detection of anti-SFTSV antibodies among domestic bovine (Fattening cattle, Milk cows, and Breeding cattle) in groups A, B, C, and All

| Ages of month | Fattening cattle | Milk cow | Breeding cattle | Total |
|---------------|------------------|----------|----------------|-------|
| Group A<sup>4)</sup> |                  |          |                |       |
| <60           | 2/349            | 0/7      | 0/2            | 2/358 |
| 60–120        | —                | 1/11     | 0/8            | 1/19  |
| >120          | —                | 0/1      | 3/14           | 3/15  |
| total         | 2/349            | 1/19     | 3/24           | 6/392 (1.5) |
| Group B<sup>5)</sup> |                  |          |                |       |
| <60           | 0/58             | 0/27     | —              | 0/85  |
| 60–120        | —                | 0/12     | —              | 0/12  |
| >120          | —                | 2/5      | 1/3            | 3/8   |
| total         | 0/58             | 2/44     | 1/3            | 3/105 (2.9) |
| Group C<sup>6)</sup> |                  |          |                |       |
| <60           | 0/1              | —        | —              | 0/1   |
| 60–120        | —                | —        | 0/7            | 0/7   |
| >120          | —                | —        | 2/5            | 2/5   |
| total         | 0/1              | 2/12     | 2/12           | 2/13 (15.4) |
| All           | 2/408 (0.5)      | 3/63 (4.8) | 6/39 (15.4) | 11/510 (2.2) |

Positive numbers/tested numbers (%).

* Fisher’s exact test; P value < 0.05.
** Fisher’s exact test; P value < 0.01.
1): Fattening cattle; reared for flesh including nulliparous milk cows and stags.
2): Milk cows; reared for milking (parous cows).
3): Breeding cattle; reared for producing calves (parous cows and seed bulls).
4): Group A; born and reared in Shimane Prefecture.
5): Group B; reared both in and outside Shimane Prefecture.
6): Group C; born and reared outside Shimane Prefecture.

Fig. 1. Location of Shimane Prefecture in Japan, and indication of 4 areas in Shimane Prefecture.

Table 2. Born or reared areas of farms in which anti-SFTSV antibodies were detected in 6 individual cattle (a–f) in group A

| Area      | Fattening cattle | Milk cow | Breeding cattle | Total (number of farms) |
|-----------|------------------|----------|----------------|------------------------|
| Eastern   | a                | c        | d, e            | 4                      |
| Central   |                  | c        |                | 1                      |
| Western   | b                | d, f, f  |                | 4                      |
| Oki islands | a              |          |                | 1                      |

>120 months to ages < 60 months and 60–120 months; P < 0.01 and < 0.05, respectively: Table 1). Thus, these results suggest that SFTSV seropositivity in cattle is closely associated with ages.

In group A, anti-SFTSV antibodies were detected in 6 cattle (2 fattening cattle, 1 milk cow, and 3 breeding cattle; antibody prevalence rate: 1.5%: Table 1). These 6 cattle were born or reared in ten farms within Shimane prefecture, four farms each in the eastern and western areas, one farm in the central area, and one in the Oki islands (Fig. 1, Table 2). In group B, anti-SFTSV anti-
bodies were detected in 3 cattle (2 milk cows and 1 breeding cattle; antibody prevalence rate: 2.9%; Table 1). In group C, anti-SFTSV antibodies were detected in 2 breeding cattle (antibody prevalence rate: 15.4%; Table 1). Thus, we confirmed that cattle were infected with SFTSV both inside and outside Shimane Prefecture. SFTSV genes have been detected in many kinds of ticks, and anti-SFTSV antibodies have been confirmed in wild deer and wild boars in some parts of Japan, including Shimane Prefecture (10). We consider cattle reared in pastures or stock farms inhabited by ticks harboring SFTSV to be an epidemiological background. Cattle may be one of the sentry animals of SFTSV in Japan, similar to wild animals.

In the present study, the prevalence of anti-SFTSV antibodies reflected the rate in Shimane Prefecture and not in all of Japan, because most cattle (76.9%, 392 out of 510) were born and reared in Shimane Prefecture. In the future, the prevalence rates of anti-SFTSV antibodies need to be examined in regions of Japan other than Shimane Prefecture, especially Kyushu and Shikoku regions, which have large number of patients with SFTS.

Acknowledgments We thank Ken Maeda (Joint Faculty of Veterinary Medicine, Yamaguchi University) for providing SFTSV YG-1, and Keigo Shitade (Domestic Livestock Disease Identification Office, Shimane Prefectural Government) for providing anti-Akabane virus and anti-Aino virus antibody-positive cattle sera. This work was supported by a Grant-in-Aid for Scientific Research (B) (25304045) from the Japan Society for the Promotion of Science and also by a Health Labour Sciences Research Grant on Emerging and Re-emerging Infectious Diseases (H25-Shinko-Shitei-009) from the Japanese Ministry of Health, Labour and Welfare, Health and Labour Sciences Research Grants, AMED.

Conflict of interest None to declare.

REFERENCES
1. Yu XJ, Liang MF, Zhang SY, et al. Fever with thrombocytopenia associated with a novel bunyavirus in China. N Engl J Med. 2011;364:1523-32.
2. Kim KH, Yi J, Kim G, et al. Severe fever with thrombocytopenia syndrome, South Korea, 2012. Emerg Infect Dis. 2013;19:1892-4.
3. Lam TT, Liu W, Bowden TA, et al. Evolutionary and molecular analysis of the emergent severe fever with thrombocytopenia syndrome virus. Epidemics. 2013;5:1-10.
4. Takahashi T, Maeda K, Suzuki T, et al. The first identification and retrospective study of severe fever with thrombocytopenia syndrome in Japan. J Infect Dis. 2014;209:816-27.
5. Wei J, Li S, Dong JH, et al. The first human severe fever with thrombocytopenia syndrome virus in Shaanxi Province, China. Int J Infect Dis. 2015;35:37-9.
6. Saito T, Fukushima K, Umeki K, et al. Severe fever with thrombocytopenia syndrome in Japan and public health communication. Emerg Infect Dis. 2015;21:487-9.
7. Yoshikawa T, Shimojima M, Fukushima S, et al. Phylogenetic and geographic relationships of severe fever with thrombocytopenia syndrome virus in China, South Korea and Japan. J Infect Dis. 2015;212:889-98.
8. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare. Surveillance data table. National Institute of Infectious Diseases in Japan (NIID). Available at <http://www.nih.go.jp/niid/en/surveillance-data-table-english.html>. Accessed November 1, 2015.
9. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare. Severe fever with thrombocytopenia syndrome (SFTS) in Japan. Japanese. Available at <http://www.nih.go.jp/niid/ja/sfts/3143-sfts.html>. Accessed November 1, 2015.
10. Morikawa S, Uda A, Kimura M, et al. Geographical distribution of severe fever with thrombocytopenia syndrome (SFTS) virus in Japan (2nd report). Infect Agents Surveillance Rep. 2014;35:75-6. Japanese.
11. Wu G, Li J, Liang M, et al. Severe fever with thrombocytopenia syndrome virus among domesticated animals, China. Emerg Infect Dis. 2013;19:756-63.
12. Zhao L, Zhai S, Wen H, et al. Severe fever with thrombocytopenia syndrome virus, Shandong Province, China. Emerg Infect Dis. 2012;18:963-5.
13. Ding S, Yin H, Xu X, et al. A cross-sectional survey of severe fever with thrombocytopenia syndrome virus infection of domestic animals in Laizhou City, Shandong Province, China. Jpn J Infect Dis. 2014;67:1-4.