Seroprevalence and Genetic Characterization of Severe Fever with Thrombocytopenia Syndrome Virus in Domestic Goats in South Korea

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Abstract

Severe fever with thrombocytopenia syndrome (SFTS) is a newly emerging tick-borne infectious disease caused by the SFTS virus (SFTSV). To investigate the prevalence of SFTSV in domestic goats in South Korea, we collected blood samples in commercial slaughterhouses in Chungbuk Province in 2017. Of the 207 samples tested, 4 (2%) were found to be positive for viral RNA by RT-PCR and 30 (14.4%) were positive for SFTSV antibody as detected by a nucleocapsid (NP) protein-based ELISA. Phylogenetic analysis of the non-structural protein (NS) sequences showed that all viruses belonged to the genotype B, although they were clustered into two different sublineages that showed the highest homology with the KR612076-JP01 and KY789441-CB3 human isolate from South Korea. Further, we confirmed the specificity of seropositive goat sera by FRNT50 and western blotting analysis and found differential cross-reactivity of the sera with genotype A and B SFTSV strains. Collectively, this study suggests that relatively high numbers of goats are infected by antigenically different SFTSV strains, which might have a potential for zoonotic infection.

Introduction

Since the first detection of severe fever with thrombocytopenia syndrome (SFTS) in China in 2009, cases have been identified in Japan and South Korea in 2012 with a case fatality rate of 6–30% in humans. The causative agent of this disease is the SFTS virus (SFTSV), a newly emerging tick-borne viral pathogen. Although human-to-human transmission through contact with infected blood or body secretions has been reported, SFTSV infection in humans is believed to be predominantly transmitted by tick bites. Further, the prevalence of SFTS in both humans and animals is closely correlated with the seasonal distribution of ticks. *Haemaphysalis longicornis* is a proven vector of SFTS virus. Other tick species, e.g. *Rhipicephalus microplus*, *Amblyomma testudinarium*, *Haemaphysalis flava* or *Ixodes nipponensis*, may be involved in the circulation of SFTSV, although this requires confirmation.

SFTSV infection is not limited to humans or ticks as it has also been found in domestic and wild animals (e.g., cattle, deer, goats, dogs, and cats). Therefore, continuous surveillance of the prevalence of SFTSV in animals is necessary to identify the potential for animal-to-human transmission. Goats are at high risk for SFTSV infection through uptake of infected ticks or by being fed on by infected ticks during grazing in meadows. Furthermore, as a domestic animal, goats have considerable contact with humans during which they could transmit the virus. However, the prevalence and genetic characterization of SFTSV in South Korean goats has not previously been investigated. Hence, in this study, we collected goat sera from two different commercial slaughterhouses in Chungbuk Province from March to October of 2017 and investigated the prevalence of SFTSV in goats by ELISA and RT-PCR.

Results

Phylogenetic Analysis

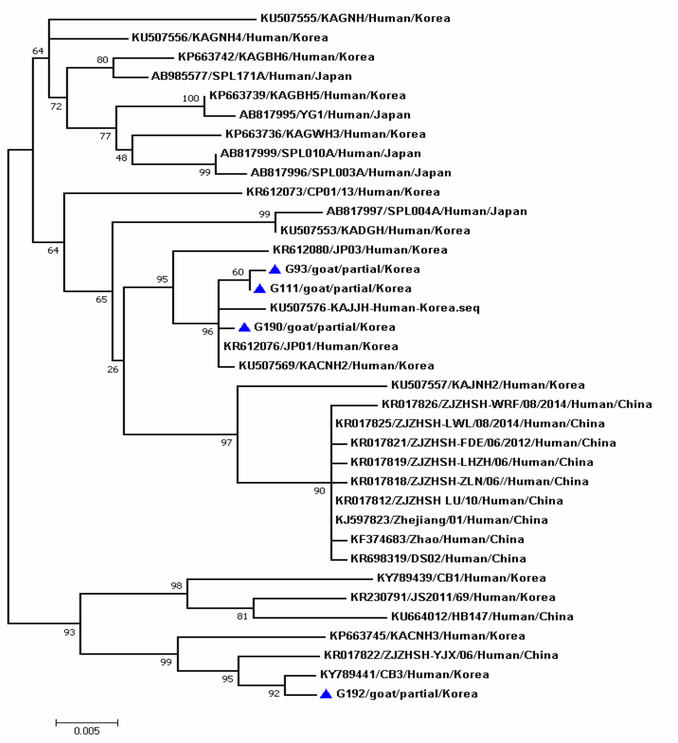

Figure 1. Phylogenetic trees comparing the nucleotide sequences of NS segments of SFTSV detected between March and October of 2017 in South Korean goats.

Sequences of selected SFTSV strains are available in GenBank. The tree was constructed using the maximum likelihood method with MEGA 7.0. The numbers on the branches indicate bootstrap percentages based on 1,000 replications, and the scale bar indicates the number of nucleotide substitutions per site. NS sequences of four SFTSVs detected in samples from goats are marked with blue triangles. For comparison, NS sequence data from viruses identified in China, South Korea, and Japan were obtained from GenBank.

Neutralizing Antibody Titers

	Serum 1	Serum 2	Serum 3
CB1/2014 (genotype B)[a]	20[b]	20[b]	20[b]
CB2/2015 (genotype A)[a]	80[b]	40[b]	80[b]

Table 2. Serum neutralizing antibody titers in genotypes A and B of SFTSV strains

(a) Virus strains were infected in Vero-E6 for the FRNT50 assay. (b) FRNT50 values were determined as the reciprocal of the highest dilution at which the number of the foci was <50% of the number obtained without serum.

SFTSV Antibody Detection

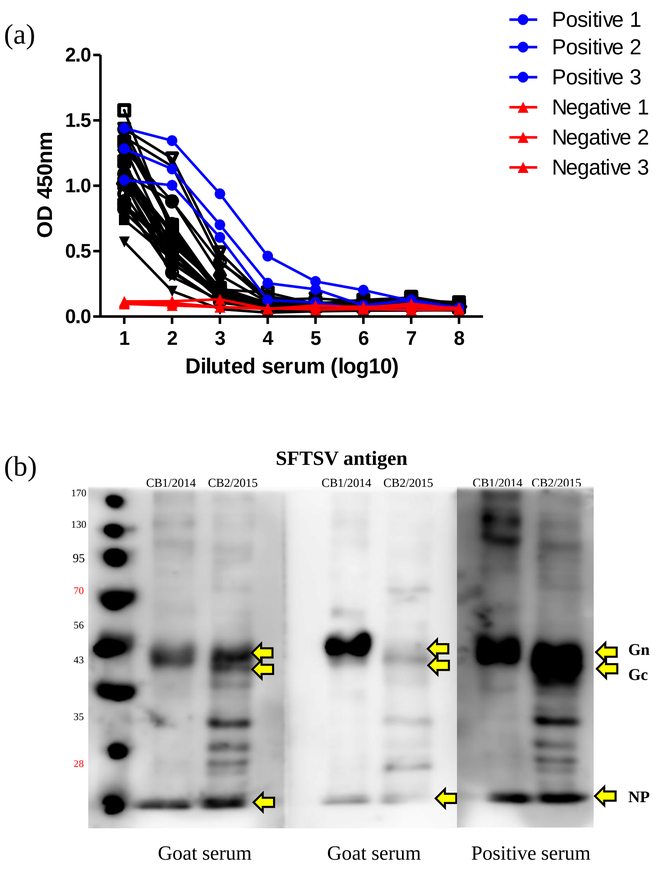

Figure 2. Detection of SFTSV protein by goat serum. An r-NP based ELISA was performed using the r-NP SFTSV protein of CB1/2014 (genotype B).

(a) Positive samples demonstrated an OD greater than 0.4 (Black). Mouse positive and negative control sera are colored blue and red, respectively. (b) Proteins purified from SFTSV CB1/2014 (genotype B) and CB2/2015 (genotype A) strains were electrophoresed on 8% SDS-PAGE gels and transferred onto polyvinylidene difluoride membranes. After blocking with Tris-buffered saline containing 0.1% Tween 20 and 5% skim milk, membranes were probed with serum from each goat, followed by further incubation with a secondary antibody conjugated with HRP- anti-goat IgG. Proteins were visualized using the ECL western blot detection reagents and viewed using a Fujifilm LAS- 3000 Imaging System. Positive SFTSV Gn or Gc glycoprotein and NP protein bands are indicated with yellow arrows (molecular masses of 56 kDa and 26 kDa, respectively).

Conclusions

1. **The detection of SFTSV RNA was showed that 2% (n=4) of samples were positive with viral RNA quantities ranging from 1.3 to 2.3 Log10 copies/ml.**
2. **Sequencing results (G93, G111, G190, and G192) revealed that all four viruses showed high homology with each other (95.9 to 99.8% nucleotide identity).**
3. **G93, G111, and G190 showed the highest homology with KR612076-JP01-Human-Korea (97.1 to 99.9% nucleotide identity) which was isolated from a human patient in Jeju, South Korea in 2013, while G192 was closely related with KY789441-CB3-Human-Korea (99.2% nucleotide identity) which was isolated from a human patient in Chungbuk, South Korea in 2016.**
4. **Phylogenetic analysis showed that according to the NS gene sequences, all sequences obtained belonged to the B genotype although they differently clustered into subclusters. G93, G111, and G190 were closely clustered with KR612076-JP01-Human and G192 clustered with the KY789441-CB3-Human-Korea strain.**
5. **Among the 207 sera samples, 14.4% (n=30) were positive for SFTSV-NP as assessed by ELISA.**
6. **Out of 30 ELISA-positive serum samples, 27 showed more than 40 $FRNT_{50}$ neutralization against the CB1/2014 (genotype B) strain with a geometrical mean titer (GMT) of 74.2 and the remaining three serum samples exhibited less than 40 $FRNT_{50}$ results, which may be caused by the relatively low sensitivity of the serum $FRNT_{50}$ neutralization assay.**
7. **Western blotting results showed the presence of antibodies against Gn/Gc of CB1/2014 (genotype B) and NP antigens of either CB1/2014 (genotype B) or CB2/2015 (genotype A) in all 30 SFTSV positive sera.**
8. **All three of the samples exhibited FRNT50 neutralization titers of more than 40 against the CB2/2015 strain (genotype A), with a GMT of 63.9, although the FRNT50 titer was less than 20 against the CB1/2014 (genotype B) strain, with a GMT of 20.**

Reference

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