Ticks and Tick-borne Diseases xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

journal homepage: www.elsevier.com/locate/ttbdis

Original article

# Prevalence of severe fever with thrombocytopenia syndrome virus in black goats (*Capra hircus coreanae*) in the Republic of Korea

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A R T I C L E I N F O

*Keywords:*
Severe fever with thrombocytopenia syndrome virus
Goat
Prevalence
RT-PCR
IFA
Republic of Korea

A B S T R A C T

Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne pathogen in China, Japan, and the Republic of Korea (ROK). The aim of this study was to investigate the prevalence of SFTSV antigens and anti-SFTSV antibodies in black goats (*Capra hircus coreanae*) throughout the ROK. Sera were collected from 737 black goats in nine provinces in the ROK. Eighteen of 737 (2.4%) goat sera were positive for SFTSV on one-step reverse transcription nested polymerase chain reaction. The amplified 346-bp S segments of SFTSV sequences were classified into three genotypes (BG1, BG2, and BG3), and were included in the Japanese clade rather than the Chinese clade, based on phylogenetic analysis. Forty-three of 624 (6.9%) serum samples were seropositive for anti-SFTSV antibodies on enzyme-linked immunosorbent assay analysis. This study is the first to examine the molecular prevalence of SFTSV in goats and the first to perform serological detection of anti-SFTSV antibodies in livestock in the ROK. Moreover, the results indicate that SFTSV is widely distributed in goats and that additional monitoring for SFTSV is needed in livestock in the ROK.

## 1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) virus is a novel tick-borne *Phlebovirus* in the family of *Bunyaviridae* and a causative agent of an emerging infectious disease, SFTS, in China, Japan, and the Republic of Korea (ROK); this disease is mainly characterized by fever, leukopenia and thrombocytopenia (Yu et al., 2011; Kim et al., 2013; Takahashi et al., 2014). The SFTS virus (SFTSV) exhibits three single-stranded negative-sense RNA segments, which consist of S, M, and L segments (Yu et al., 2011). The S segment encodes non-structural and nucleocapsid proteins, which are essential for viral RNA encapsidation and replication; the M segment encodes glycoproteins C and N, essential viral envelope components; and the L segment encodes an RNA-dependent RNA polymerase (Yu et al., 2011; Zhou et al., 2013).

The RNA of SFTSV has been detected in *Haemaphysalis longicornis*, *Haemaphysalis flava*, *Ixodes nipponensis*, and *Rhipicephalus microplus* (Yu et al., 2011; Liu et al., 2014; Park et al., 2014; Oh et al., 2016; Yun et al., 2016). Recently, SFTSV was isolated from unfed *H. longicornis* that was collected by the dragging and flagging, rather than from fed ticks (Yun et al., 2016). Moreover, *H. longicornis* could transstadially survival and transovarially transmit SFTSV to ticks in other developmental stages; it could also transmit SFTSV to goat and mouse (Luo et al., 2015; Jiao et al., 2015). In the ROK, *H. longicornis* is a dominant tick species in the environment and on several mammals (Kim et al., 2006 and 2011; Park et al., 2014; Kang et al., 2016; Oh et al., 2016; Yun et al., 2016); most ROK residents have been exposed to this tick species

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https://doi.org/10.1016/j.ttbdis.2018.04.018
Received 27 December 2017; Received in revised form 24 April 2018; Accepted 25 April 2018
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(Yun et al., 2014). These studies indicate that *H. longicornis* serves as a main vector for SFTSV transmission.

Many clinical cases of SFTS in humans have been documented in Japan, China, and the ROK; however, clinical cases of SFTS in animals have not yet been reported (Liu et al., 2015; Choi et al., 2016; Kato et al., 2016). However, molecular and serological surveys have been conducted to determine SFTSV exposure in various animals in China and Japan, including dogs, sheep, goats, chickens, wild boar, pigs, minks, and cattle (Niu et al., 2013; Ding et al., 2014; Li et al., 2014; Hayasaka et al., 2016; Wang et al., 2017). In addition, experimental studies have been conducted to investigate transmission and circulation of SFTSV in mice, dogs, and goats in China (Niu et al., 2013; Jiao et al., 2015; Ni et al., 2015). In the ROK, the RNA of SFTSV has been detected in dogs, cats, Korean water deer (*Hydropotes inermis*) and wild boar (*Sus scrofa*) (Oh et al., 2016; Hwang et al., 2017; Lee et al., 2017a). Moreover, the seroprevalence of anti-SFTSV antibodies in dogs, within the ROK, has been reported using indirect immunofluorescence assay (IFA) and virus neutralization (Lee et al., 2017b).

The highest seroprevalence of anti-SFTSV antibodies among domestic mammals has been reported in goats raised in China (Jiao et al., 2012; Zhao et al., 2012), suggesting that goats experience significant exposure to SFTSV in their natural environment. In addition, several studies have shown that *H. longicornis* is a dominant tick species on goats (Kang et al., 2016; Zhang et al., 2017). However, to the best of our knowledge, there have been no published studies of the prevalence of SFTSV infection in livestock, or in goats, in the ROK. Therefore, the aim of this study was to determine the prevalence of SFTSV antigens and anti-SFTSV antibodies in black goats throughout the ROK.

## 2. Materials and methods

### 2.1. Sample collection

All procedures with animals were performed in accordance with the guidelines of the National Research Council for Care of Laboratory Animals. Whole-blood samples were collected from black goats (*Capra hircus coreanae*) in 2014–2015, throughout the ROK. Sera were separated from whole-blood samples by centrifugation, then stored at −80 °C until use. A total of 200 μL serum was used for RNA extraction via the Gene-spin™ Viral DNA/RNA Extraction Kit (Intron Biotechnology, Korea), according to the manufacturer's protocol. Resultant RNA samples were stored at −80 °C deep freezer until assayed.

### 2.2. One-step reverse transcriptase-nested polymerase chain reaction (RT-nested PCR)

One-step RT-nested PCR was performed to amplify the S segment of the SFTS viral RNA genome, using SFTSV genome-specific primer sets for PCR. The first set of PCR primers used were forward primer (NP-2F: CATCATTGTCTTTGCCCTGA) and reverse primer (NP-2R: AGAAGACA GAGTTCACAGCA). The nested PCR primers used were forward primer (NP-2F: AAYAAGATCGTCAAGGCATCA) and reverse primer (NP-2R: TAGTCTTGGTGAAGGCATCTT) (Yoshikawa et al., 2014; Oh et al., 2016). The SFTSV used for positive control was kindly provided by Dr. Park (Gachon University of Medicine and Science, Inchon, Korea). PCR amplifications were performed in a reaction mixture (30 μL) containing 1.5 μL (10 pmol) of each forward and reverse primer, 4 μL of extracted RNA, 8 μL of TE buffer and 15 μL of one-step RT-PCR premix (Solgent, Korea). Reverse transcription (RT) reactions were incubated at 50 °C for 30 min, followed by 94 °C for 5 min. The amplification was performed according to the following conditions: 20 s at 94 °C, 40 s at 52 °C, and 30 s at 72 °C, for 40 cycles (first PCR) and 25 cycles (nested PCR), followed by a final extension step at 72 °C for 5 min. PCR products were visualized by gel electrophoresis using a 1.5% agarose gel, then purified using QIAquick Gel Extraction Kits (Qiagen, Germany).

### 2.3. Nucleotide sequencing and phylogenetic analysis

Purified PCR products were cloned with pGEM-T Easy Vectors (Promega, USA), followed by transformation into *Escherichia coli* JM109, then plated onto LB agar containing 100 ug/mL of ampicillin. Plasmid DNA was purified using the Wizard® Plus SV Minipreps DNA Purification system (Promega) and was sequenced using an automatic sequencer (3730xl capillary DNA Analyzer; Applied Biosystems, USA). Sequence data were analyzed using Chromas software (Ver 2.44). To compare sequences from this study with sequences that had previously been deposited in GenBank, sequences were aligned using Clustal X (Ver 2.1) and analyzed with MEGA 7 (Kumar et al., 2016). Phylogenetic trees were constructed using a Maximum Likelihood method based on the Kimura 2-parameter model; the data set was resampled 1000 times to generate bootstrap values.

### 2.4. Double-antigen sandwich enzyme linked immunosorbent assay (ELISA)

Double-antigen sandwich ELISA procedure was performed to detect total antibodies (including IgG and IgM) in serum samples, as described previously (Jiao et al., 2012). Recombinant nucleocapsid protein (rNP) of the first Korean SFTSV strain (GenBank accession no. KF358693) was adsorbed to the solid phase, and horseradish peroxidase-conjugated rNP was added after application of serum. Each serum sample was tested in duplicate, and the positive and negative controls were tested in quadruplicate. Optical density (OD) at 450 nm was measured after adding tetramethylbenzidine (Thermo Scientific, Waltham, MA, USA) and a stop solution. Each result was expressed as a percentage of the positive control serum, using the following formula: (OD of test serum/mean OD of positive-control serum) × 100. Cutoff value was determined as mean value plus three standard deviations (mean + (3 × SD), derived from a percentage of the positive control values in the negative-control serum.

## 3. Results

A total of 737 goat sera were collected from Gwangwon-do (n = 20), Gyeonggi-do (n = 31), Gyengsangbuk-do (n = 25), Gyengsangnam-do (n = 13), Chungcheongbuk-do (n = 115), Chungcheongnam-do (n = 24), Jeollabuk-do (n = 375), Jeollanam-do (n = 130), and Jeju-do (n = 4) provinces (Table 1, Fig. 1). Eighteen of 737 (2.4%) goat sera were positive for SFTSV on RT-PCR. The amplified 346-bp S segments of SFTSV sequences were classified into three genotypes including BG1 (KX672013), BG2 (KX672014), and BG3 (KX672015). The BG1 genotype was detected in nine different samples and was identical to the sequence identified in a cat in the ROK (KP994430). The BG2 genotype was detected in two goat sera and exhibited 99.4% identity to the KP994430 sequence (the BG1 genotype). In contrast, the BG3 genotype, detected in seven goat sera, was included in a different sub-clade from BG1 and BG2 (Fig. 1), and was

**Table 1**
Prevalence of severe fever with thrombocytopenia syndrome virus in RT-PCR and ELISA assay of goat sera from the Republic of Korea.

| Province | No. of samples | No. of PCR positive/ Tested samples (Positive rates, %) | No. of ELISA positive/Tested samples (Positive rates, %) |
|---|---|---|---|
| Gwangwon-do | 20 | 0/20 (0) | 3/20 (15.0) |
| Gyeonggi-do | 31 | 2/31 (6.5) | 2/31 (6.5) |
| Gyengsangbuk-do | 25 | 2/25 (8.0) | 1/25 (4.0) |
| Gyengsangnam-do | 13 | 0/13 (0) | 1/13 (7.7) |
| Chungcheongbuk-do | 115 | 2/115 (1.7) | 4/114 (3.5) |
| Chungcheongnam-do | 24 | 0/24 (0) | 6/24 (25.0) |
| Jeollabuk-do | 130 | 5/130 (3.9) | 0/30 (0) |
| Jeollanam-do | 375 | 7/375 (1.9) | 25/363 (6.9) |
| Jeju-do | 4 | 0/4 (0) | 1/4 (25.0) |
| Total | 737 | 18/737 (2.4) | 43/624 (6.9) |

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