Ticks and Tick-borne Diseases 8 (2017) 626–630

Contents lists available at ScienceDirect

Ticks and Tick-borne Diseases

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

Short communication

Molecular detection and phylogenetic analysis of severe fever with thrombocytopenia syndrome virus in shelter dogs and cats in the Republic of Korea

Seung-Hun Lee[a], Hyun-Joo Kim[a], Jae-Won Byun[a], Min-Jung Lee[a], Neung-Hee Kim[b], Doo-Hwan Kim[b], Hae-Eun Kang[a], Hyang-Mi Nam[a,*]

[a] Foreign Animal Disease Division, Animal and Plant Quarantine Agency, Gimcheon, Republic of Korea
[b] Seoul Metropolitan Government Research Institute of Public Health & Environment, Gwacheon, Gyunggi, Republic of Korea

ARTICLE INFO

Keywords:
Severe fever with thrombocytopenia syndrome
Shelter dog
Shelter cat
Zoonosis

ABSTRACT

Severe fever with thrombocytopenia syndrome is a tick-borne infectious disease. The present study investigated the prevalence of severe fever with thrombocytopenia syndrome virus (SFTSV) in shelter dogs and cats in the Republic of Korea (ROK). Blood samples were collected from 426 dogs and 215 cats in animal shelters throughout the ROK in 2016. Of the tested samples, one (0.2%) dog and one (0.5%) cat were positive for SFTSV. Phylogenetic analysis of the sequences obtained in the present study showed that the viruses belonged to the J3 clade, which is considered the dominant clade in the ROK. This study reports the first molecular detection of SFTSV in shelter dogs and cats in the ROK.

1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is a fatal tick-borne disease caused by a novel phlebovirus, severe fever with thrombocytopenia syndrome virus (SFTSV) belonging to the family Bunyaviridae (Luo et al., 2015; Yu et al., 2011). The disease was first identified in a Chinese patient in 2009 (Yu et al., 2011) and was subsequently identified in humans in other countries, including the Republic of Korea (ROK) and Japan (Kim et al., 2013; Takahashi et al., 2014).

The SFTS can be fatal to elderly and/or immunocompromised individuals and the case fatality rate can be as high as 30% (Ding et al., 2013; Yu et al., 2011). In humans, SFTS is characterized by fever and thrombocytopenia (Gai et al., 2012; Liu et al., 2014a), whereas there is no evidence of the virus causing clinical symptoms in animals (Liu et al., 2014a; Niu et al., 2013). However, different studies have identified antigens and antibodies against SFTSV in various animals such as sheep, goats, cattle, dogs, chicken, pigs (Ding et al., 2014; Niu et al., 2013), and rodents (Liu et al., 2014b).

Since the first report of SFTS in humans in the ROK in 2013 (Kim et al., 2013), patients with SFTS have been reported in the ROK annually and 172 human cases, including 54 fatal cases, were reported from 2013 to 2015 (KCDC, 2016). In addition, previous studies detected SFTSV in feral cats and wild animals in the ROK (Hwang et al., 2017; Oh et al., 2016). The virus has also been identified in various ticks including *Haemaphysalis* spp., *Amblyomma testudinarium*, *Ixodes nipponensis*, and *Rhipicephalus microplus* (Lam et al., 2013; Oh et al., 2016; Yun et al., 2014; Zhang et al., 2012).

The recent nationwide incidence of patients with SFTS in the ROK and molecular identification of SFTSV in various animals and ticks has increased the attention given to this disease in the ROK (Choi et al., 2016; Hwang et al., 2017; Oh et al., 2016). Investigating the prevalence of SFTSV in dogs and cats and their role as possible reservoir hosts of SFTSV is important, especially because as companion animals, they frequently live in close contact with humans. Therefore, the purpose of the present study was to investigate the prevalence of SFTSV in shelter dogs and cats and to assess their phylogenetic characteristics.

2. Materials and methods

2.1. Sera collection

The survey and use of experimental animals were approved by the Animal and Plant Quarantine Agency Institutional Animal Care and Use Committee (Approval #2015-309).

From April to October 2016, whole-blood samples were collected from 426 dogs and 215 cats in animal shelters throughout the ROK (Fig. 1). The sampling numbers were determined by a simple random

* Corresponding author at: Foreign Animal Disease Division, Animal and Plant Quarantine Agency, 177 Hyeoksin 8-ro, Gimcheon 39660, Republic of Korea.
E-mail address: namhm@korea.kr (H.-M. Nam).

http://dx.doi.org/10.1016/j.ttbdis.2017.04.008
Received 20 February 2017; Received in revised form 8 April 2017; Accepted 11 April 2017
Available online 14 April 2017
1877-959X/

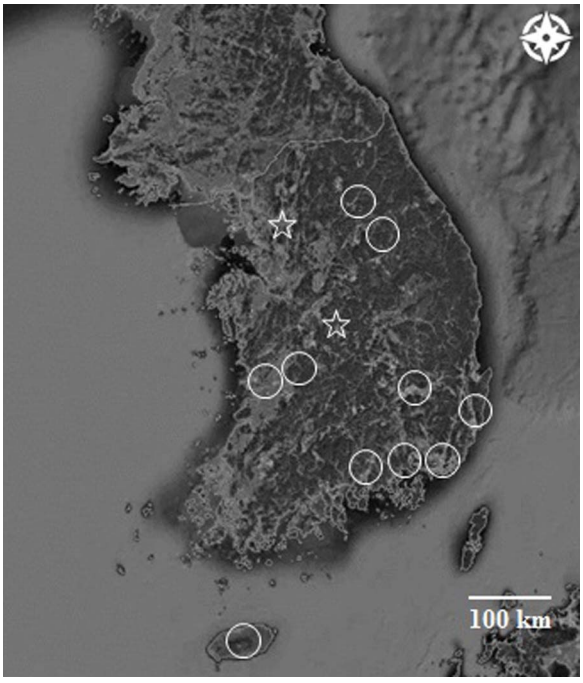

Fig. 1. Map of the Republic of Korea and regions of animal shelters where blood samples were collected. Regions corresponding to the positive samples are indicated by an asterisk. The map was generated using Google Earth.

sampling method with an expected prevalence of 10% and desired absolute precision of 5% (Thrusfield, 2005). According to the formula, at least 139 blood samples from each species were required.

A whole-blood sample was collected from each animal and transported to our laboratory, storing in a cold box (0–4 °C) within 24 h of collection. Sera were separated by centrifugation at 15,000 rpm for 10 min and immediately used for RNA extraction. Data regarding the sex, age, breed, and sampling region were collected for epidemiological analysis. Since the tested animals were abandoned or were lost pets that ended up in shelters, it was not possible to collect the detailed travel history of each animal.

2.2. RNA extraction and molecular detection of SFTSV

RNA was extracted from the sera using the Maxwell® 16 viral total nucleic acid purification kit (Promega, Madison, WI, USA) according to the manufacturer's instructions and the extracted RNA was stored at −70 °C until analysis.

To detect SFTSV, the 346-bp S-segment of SFTSV was amplified using reverse transcriptase nested PCR as previously described (Hwang et al., 2017). Reverse transcriptase PCR was performed using the OneStep RT-PCR Kit (Qiagen, Hilden, Germany) and nested PCR was performed using the AccuPower® HotStart PCR premix kit (Bioneer, Daejeon, Korea). SFTSV, previously isolated in our laboratory from a goat (unpublished data), was included as a positive control in each experiment. All PCR-positive samples were sent to Solgent (Daejeon, Korea) for bidirectional sequencing.

2.3. Phylogenetic analysis and statistical analysis

A phylogenetic tree was constructed based on the S-segment of SFTSV using MEGA 6.0 (Tamura et al., 2013) to estimate the genetic relatedness among the sequences and the region or host specificity. The tree was constructed based on the neighbour-joining method with 1,000 replications. Clades were designated as described by Yoshikawa et al. (2015).

For epidemiological analysis, chi-square test or Fisher's exact test were performed to detect associations among the sex, age, breed, and sampling region. A *P*-value less than 0.05 was considered statistically significant.

3. Results

Of the 426 dog and 215 cat sera samples, one dog (male, six months old, poodle) from Daejeon and one cat (male, two years old, mixed) from Seoul tested positive (Fig. 1). Owing to the small number of positive cases, statistical analysis of the epidemiological data did not produce meaningful results.

A 346-bp S-segment of SFTSV was obtained by bidirectional sequencing. The two sequences obtained in this study showed 99.1% identity with each other. The basic local alignment search tool (NCBI, Bethesda, MD, USA) revealed 99.4–99.7% similarity between the SFTSV sequences identified in this study and the sequence identified in a human in the ROK (KU507553). Phylogenetic analysis showed that the obtained sequences belonged to the J3 clade (Fig. 2). The obtained sequences in the present study were submitted to GenBank (accession nos. KY560448 and KY560449).

4. Discussion

The SFTS is a tick-borne infectious disease that has been reported in Asian countries, including China, ROK, and Japan (Kim et al., 2013; Takahashi et al., 2014; Yu et al., 2011). In contrast to the human cases, a limited number of studies are available in animals. For example, SFTSV was molecularly identified in 1.0% (5/517) of rodents (Liu et al., 2014b), 3.8% (18/472) of sheep, 4.2% (35/842) of cattle, 5.3% (19/359) of dogs, 2.6% (22/839) of pigs, and 1.7% (9/527) of chicken in China (Niu et al., 2013). In addition, 4.8% (1/21) of Korean water deer (*Hydropotes inermis*) and 3.7% (2/54) of wild boar (*Sus scrofa*) in the ROK were found to be positive for SFTSV (Oh et al., 2016). In contrast to the limited molecular detection of SFTSV in animals, antibodies against SFTSV have been identified in various animals such as goats, sheep, cattle, dogs, chicken, and rodents, with relatively high seroprevalence (> 35%) (Ding et al., 2014; Niu et al., 2013).

In the present study, 0.2% (1/426) of dogs and 0.5% (1/215) of cats tested positive for SFTSV by reverse transcriptase PCR. To the best of author's knowledge, only two studies have identified SFTSV in domesticated dogs (5.3%, 19/359) in China and in feral cats (17.5%, 22/126) in the ROK using molecular technique (Hwang et al., 2017; Niu et al., 2013). The prevalence of SFTSV in dogs and cats in this study was lower when compared to the values reported in those two studies.

The lower prevalence observed in this study compared to those reported in previous studies may be explained by two factors. First, the target animals tested in this study were pet animals mostly reared inside houses before being lost or abandoned and living in shelters. In contrast, the domesticated dogs in China (Niu et al., 2013) or feral cats in the ROK (Hwang et al., 2017) spend most of their time outside houses in fields or in the streets, and thus had a greater chance of contacting the ticks that transmit SFTSV. Second, sampling times differed between animals in this study and those in previous studies. To collect blood samples, we visited shelters and randomly selected animals among the lost or abandoned pet animals that had been living in the shelter for varying periods. In contrast, blood samples from domesticated dogs in China and feral cats in Seoul, ROK were collected immediately after capture while the animals were roaming outside the houses or in the streets. Considering that SFTSV lasts 7–10 days in dogs and non-human primates (Jin et al., 2015; Niu et al., 2013), even if the