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Research Paper

Prevalence of antibodies against severe fever with thrombocytopenia syndrome virus in shelter dogs in the Republic of Korea

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ABSTRACT

The purpose of this study was to investigate the seroprevalence of antibodies against severe fever with thrombocytopenia syndrome virus (SFTSV) in shelter dogs in the Republic of Korea (ROK) using an indirect immunofluorescence assay and virus neutralization test. Sera were collected from 426 dogs in 12 animal shelters throughout the ROK from March 2016 to November 2016. Overall, 59 of 426 (13.9%) samples were seropositive for antibodies against SFTSV. A significant difference was observed in accordance with the sampling region ($p < 0.001$), but not according to the sex ($p = 0.279$) or breed ($p = 0.729$) of the dogs. The seroprevalence of SFTSV showed an inversely proportional trend to the latitude of the sampling regions: the highest rate was observed in the southern region followed by the Jeju-do region. This is the first report on the nationwide prevalence of antibodies against SFTSV in companion dogs in animal shelters throughout the ROK.

1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne infectious disease caused by the SFTS virus (SFTSV), a novel phlebovirus, belonging to the family Bunyaviridae (Yu et al., 2011). The first patient with SFTS was reported in rural China in 2009 (Yu et al., 2011); the disease was subsequently identified in the Republic of Korea (ROK) and Japan (Kim et al., 2013; Takahashi et al., 2014). The disease can be fatal to humans, especially the elderly, and case fatality rates range from 6% to 30% depending on the study (Choi et al., 2016; Ding et al., 2013; Guo et al., 2016; Yoshikawa et al., 2015; Yu et al., 2011). Most human cases have been identified in China (Guo et al., 2016). In the ROK, 172 human cases were reported between 2013 and 2015, with 54 fatalities (Choi et al., 2016).

Transmission of SFTSV to humans usually occurs through tick bites (Luo et al., 2015), but human-to-human transmission within families and nosocomial transmissions have also been reported (Bao et al., 2011; Kim et al., 2015). Elderly people living in rural areas with domestic animals are at high risk for infection with SFTSV (Song et al., 2017). Recently, however, a pediatric case of SFTS in a child who had frequent contact with animals on her family farm in a rural area was also reported in the ROK (Song et al., 2017).

To date, no clinical case of SFTS in animals has been reported, and it is unknown whether the virus causes clinical symptoms in animals (Niu et al., 2013). Nevertheless, molecular or serological detection of SFTSV and its antibodies has been reported in various animals such as dogs, sheep, goats, cattle, pigs, chickens, and wild boar (*Sus scrofa*) in China and Japan (Ding et al., 2014; Hayasaka et al., 2016; Niu et al., 2013). In the ROK, SFTSV has been identified in shelter dogs and cats, feral cats, and wild animals such as the Korean water deer (*Hydropotes inermis*) and wild boar (*Sus scrofa*) (Hwang et al., 2017; Lee et al., 2017b; Oh et al., 2016). Further studies are required to investigate the animal-vector-human transmission cycle and the potential role of animals in maintaining the circulation and transmission of SFTSV.

As domestic animals, especially companion animals, have close contact with humans, it is important to study their SFTS infection status. However, to the best of our knowledge, although a few studies have been conducted on domesticated dogs in rural China (Ding et al., 2014; Gong et al., 2014; Niu et al., 2013), no studies have been conducted on SFTSV in companion animals in the ROK. Therefore, we investigated the prevalence of antibodies against SFTSV in companion dogs in animal shelters throughout the ROK.

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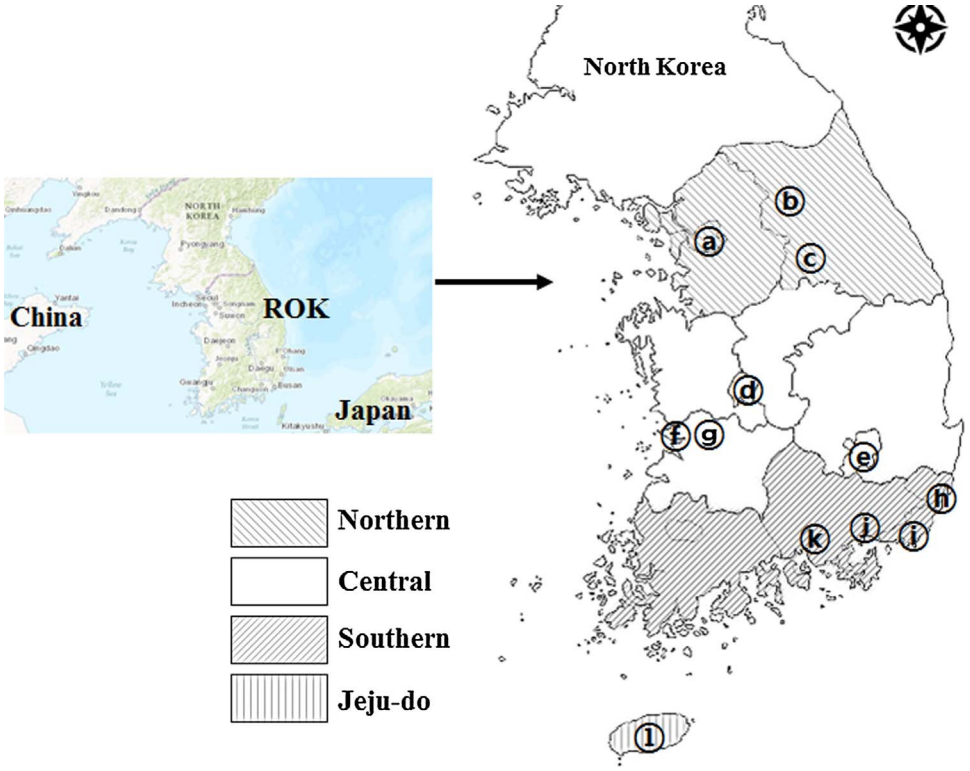

Fig. 1. A map of the Republic of Korea indicating the location of the 12 animals shelters where whole blood samples were collected from 426 dogs. Sample collection regions were classified into four categories: northern [Seoul (a), Chuncheon (b), and Wonju (c)], central [Daejeon (d), Daegu (e), Gunsan (f), and Iksan (g)], southern [Ulsan (h), Busan (i), Changwon (j), and Jinju (k)], and Jeju-do [Jeju (l)], according to their administrative boundaries and geographical position. Landsat imagery courtesy of NASA Goddard Space Flight Center and U.S. Geological Survey (http://landsatlook.usgs.gov).

2. Materials and methods

2.1. Sample collection and data collection

Approval for the survey and use of experimental animals was obtained from the Animal and Plant Quarantine Agency Institutional Animal Care and Use Committee (Approval #2015-309).

The ROK is located between 34°20′ and 37°11′ northern latitude and 126°07′ and 129°19′ eastern longitude. Annual mean temperature and annual precipitation in 2015 were 13.4 °C and 948.6 mm, respectively (Jung et al., 2014; Korea Meteorological Administration, 2016).

The sample size was calculated with a simple random sampling method using an expected prevalence of 30% and an absolute precision of 5% (Thrusfield, 2005). The expected prevalence used in the calculations was based on previous studies from China (Ding et al., 2014; Gong et al., 2014; Niu et al., 2013). The calculations showed that a minimum of 323 samples would be required for the present study.

Whole blood was collected from 426 dogs in 12 animal shelters throughout the ROK from March 2016 to November 2016 (Fig. 1). Whole blood samples were placed on ice immediately and sent to the Foreign Animal Disease Division, Animal and Plant Quarantine Agency, Gimcheon, Republic of Korea within 24 h of collection. Sera were obtained by centrifugation at 15,000 rpm for 10 min.

For epidemiological analysis, data on the sampling regions (Seoul, Chuncheon, Wonju, Daegu, Daejeon, Gunsan, Iksan, Ulsan, Busan, Changwon, Jinju, and Jeju) and the sex (male or female) and species of the dogs were collected. The sampling regions were classified into four categories based on administrative boundaries and geographical position (northern, central, southern, and Jeju-do; Fig. 1). Unavailable data were marked as “unknown.”

2.2. Slide preparation for indirect immunofluorescence assay

The SFTSV used in this study was previously isolated from a naturally infected goat in our laboratory and was confirmed by PCR and electron microscopy (unpublished data). An indirect immunofluorescence assay (IFA) was performed to detect antibodies against SFTSV as previously described with slight modifications (Lee et al., 2017a). In brief, Vero E6 cells were inoculated with 500 μL of 4×10^5 50% tissue culture infectious dose per milliliter ($TCID_{50}$) SFTSV and incubated at 37 °C for 2 h 30 min. After removing the supernatant and exchanging the medium with Dulbecco’s modified eagle medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA), the infected cells were incubated at 37 °C for 7 days. The incubated cells were detached by trypsin (Thermo Fisher Scientific, USA) and spotted onto 12-well microscopic slides at a concentration of 2×10^3 cells per well. The slides were incubated overnight at 37 °C and then fixed with acetone at −20 °C for 10 min. The slides were stored at −70 °C until further testing was performed.

2.3. IFA

The IFA was performed as follows. Briefly, 20 μL of test sera diluted 1:20 with phosphate-buffered saline (PBS) was inoculated into each well and incubated at 37 °C for 1 h. After washing with PBS, 20 μL of fluorescein-labeled anti-dog IgG (KPL, Gaithersburg, MD, USA) diluted to 2.5 μg/mL was applied and the slide was incubated at 37 °C for 1 h in the dark. The slide was covered with mounting solution (Sigma-Aldrich, St. Louis, MO, USA) and fluorescence was observed using an IX71 fluorescence microscope (Olympus, Tokyo, Japan).

Sera that showed reactivity at dilutions of 1:20 with PBS were considered positive. The cut-off IFA value was determined based on the serial two-fold dilution of positive control serum and negative control serum from 1:10 to 1:80 (Fig. 2). As a positive control, a serum obtained from naturally SFTSV-infected dog was used and commercially available uninfected canine serum (ImprobioAH, Gyeongbuk, ROK) was used as a negative control. The seropositivity of each control was confirmed by a virus neutralization test. The results were reviewed and confirmed by two independent researchers.