Mixed Infection with Severe Fever with Thrombocytopenia Syndrome Virus and Two Genotypes of Scrub Typhus in a Patient, South Korea, 2017

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Abstract. Severe fever with thrombocytopenia syndrome (SFTS) is a tick-borne viral disease with a high mortality rate. Infection can also occur through close contact with an infected patient. Scrub typhus is an acute febrile illness caused by *Orientia tsutsugamushi*, a bacterium transmitted to humans through chigger mite bites. South Korea is an endemic region of SFTS and scrub typhus. In this study, we confirmed that a patient was coinfecting with SFTS virus and two (Boryong and Taguchi) genotypes of *O. tsutsugamushi*.

INTRODUCTION

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne viral hemorrhagic fever disease caused by the genus *Phlebovirus* in the family *Phenuiviridae*.^{1,2} *Phleboviruses* have a broad geographic range and have been found in sand flies, mosquitoes, and ticks in the Americas, Asia, Africa, and Mediterranean regions for many years; another tick-borne *Phlebovirus*, the Heartland virus (HRTV), was identified in northwestern Missouri in the United States in 2009.³ Maloor virus, a novel bat *Phlebovirus* closely related to SFTS virus (SFTSV) and HRTV, was isolated from the *Rousettus leschenaultii* species of bats in western India; there was also isolation and characterization of a phlebovirus from ticks in Australia that was similar to SFTSV and HRTV.^{4,5}

Most SFTSV infections occur via the *Haemaphysalis longicornis* tick, although SFTSV transmission can also occur through close contact with an infected patient.^{2,6–8} SFTS was first confirmed in middle China in 2009 and was also reported and confirmed in South Korea and the western regions of Japan in 2013; a SFTS Bunyavirus patient was also reported in the United Arab Emirates, an area not an endemic country for SFTS.^{2,6,9}

The disease is characterized by fever, gastrointestinal signs and symptoms, leukopenia, thrombocytopenia, and a high mortality rate.^{2,6–8,10}

Scrub typhus is a life-threatening bacterial disease caused by *Orientia tsutsugamushi* and is transmitted primarily by the bite of the *Leptotrombidium* spp. mite (Acari: Trombiculidae) infected with *Orientia* species and endemic in the “tsutsugamushi triangle” within the Asia-Pacific region.^{10,11} However, patients were documented in the Middle East and South America in 2016.¹¹ SFTS is endemic to South Korea and is a major public health concern between May and October; scrub typhus, also a major public health problem, is mostly present between October and November.¹⁰

The clinical presentations of SFTS and scrub typhus are similar: signs and symptoms typically develop within 1–2 weeks of infection and include fever, headache, malaise, and gastrointestinal symptoms, but SFTS exhibits a higher mortality rate than does scrub typhus.^{2,6–8,10,11}

In this study, we report the first confirmed case of mixed infection with SFTSV and two genotypes of *O. tsutsugamushi* in a patient.

MATERIALS AND METHODS

Patient. A healthy 74-year-old woman (JP13-Korea-2017) presented with fever, myalgia, headache, and abdominal pain in the periumbilical area of 3 days duration. The patient’s medical history was unremarkable. She resided in an urban area and went to the mountains 4 days prior, in the autumn season. She was unaware of a local insect bite. At presentation, a single eschar was found on the right flank area. Blood tests showed leukopenia and thrombocytopenia (white blood cell [WBC] count 1,300/μL [reference 4,000–10,000]), platelet count 100 × 10³/μL (reference 150–450), aspartate aminotransferase 134 IU/L (reference 8–38), alanine aminotransferase 77 IU/L (reference 4–44), C-reactive protein 0.39 mg/dL (reference 0.0–0.3), and ferritin 2,000 ng/mL (reference 13–150). She was administered ceftriaxone and azithromycin. A contrast-enhanced computed tomography scan revealed a wall enhancement in the distal esophagus. The patient became progressively more ill with altered mental status, profound leukopenia (WBC count 700/μL [reference 4,000–10,000]), and thrombocytopenia (platelet count 51 × 10³/μL [reference 150–450]). On day 2, SFTSV was diagnosed by reverse transcriptase–polymerase chain reaction (RT-PCR) was achieved. On day 3, the indirect immunofluorescence immunoglobulin G assay for *O. tsutsugamushi* was positive with a titer of 1/160 at the Jeju Institute of Health and Environment and Laboratory. We performed a nested PCR assay for the 56-kD type specific antigen from her blood specimen because of the differential diagnoses of coinfection of acute *O. tsutsugamushi* infection.¹² On day 4, her mental status, temperature, and laboratory values improved. Her overall illness improved after 3 days of azithromycin, followed by 4 days of doxycycline treatment and supportive treatment. The patient fully recovered without other complication at the time of this draft. This study was approved by the institutional review board (IRB) at the Jeju National University Hospital (IRB file no. 2015-11-001-003).

Molecular diagnosis and phylogenetic analysis of SFTSV.

RNA was extracted from stored patient serum using a QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany). Reverse transcriptase–polymerase chain reaction was performed to amplify the complete small (S) segment of the viral RNA from the stored serum and confirmed SFTSV infection.⁶

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FIGURE 1. Phylogenetic tree constructed based on complete S segments of SFTSV. The tree was constructed using the maximum likelihood method with MEGA 6.¹³ The complete S sequences from stored patient serum are shown in bold (JP13-Korea-2017). The S sequence data for the viruses identified from China, South Korea, and Japan were obtained from the National Center for Biotechnology Information/Basic Local Alignment Search Tool. SFTSV = severe fever with thrombocytopenia syndrome virus.

Reverse transcriptase–polymerase chain reaction products (1,746 bp) were sequenced using the BigDye Terminator Cycle Sequencing kit (Perkin Elmer Applied Biosystems, Warrington, United Kingdom). Phylogenetic analysis of S

segment sequences (1,746 bp) was performed with MEGA6, and phylogenetic trees were constructed using the maximum likelihood method, which confirmed SFTSV infection (Figure 1 and Supplemental Figure 1).¹³

Molecular diagnosis and phylogenetic analysis of genotype of *O. tsutsugamushi*. DNA was extracted from stored patient serum using a QIAamp DNA Mini kit (QIAGEN). Nested PCR was performed to amplify the 56-kDa antigen of *O. tsutsugamushi* from the stored serum, confirming *O. tsutsugamushi* infection.¹² Nested-PCR products (483 bp) were cloned using a TA cloning kit (Invitrogen, Carlsbad, CA). Five clones of each product were randomly picked and sequenced using the BigDye Terminator Cycle Sequencing kit (Perkin Elmer Applied Biosystems) because PCR product signals were weak when visualized in a gel and TA cloning and sequencing would lead to differentiation of a mixed infection with two genotypes (Supplemental Figure 1). Phylogenetic analysis of *O. tsutsugamushi* 56-kDa gene sequences (483 bp) was performed with MEGA6, phylogenetic trees were constructed using the maximum likelihood method, and the two sequences obtained from the patient clustered within the Boryong and Taguchi genotype, respectively, of *O. tsutsugamushi* (Figure 2 and Supplemental Figure 1).¹³

RESULTS AND DISCUSSION

Severe fever with thrombocytopenia syndrome is a tick-borne viral disease with a high mortality rate that infects humans primarily through tick bites.^{2,6-8,10} Transmission from person-to-person has been confirmed in close contact person such as family members and health-care workers, and asymptomatic infection via person-to-person close contact has also been reported.⁶⁻⁸

Scrub typhus is an acute febrile illness caused by *O. tsutsugamushi*, a bacterium transmitted to humans through chigger mite (Acari: Trombiculidae) bites.^{10,11}

The vectors of SFTSV and *O. tsutsugamushi* are different; *H. longicornis* is a major vector of SFTSV and *Leptotrombidium* spp. mites are vectors of *O. tsutsugamushi*.^{2,6,10,11} However, a previous report showed that SFTSV was detected by RT-PCR in *L. scutellare* mite bites in China, suggesting that the mites are a potential vector for SFTSV; Wi et al.¹⁰ suggested the possibility of coinfection with SFTSV and *O. tsutsugamushi* in South Korea.¹⁴ However, there has been no report of mixed infection

FIGURE 2. Phylogenetic tree constructed based on the *Orientia tsutsugamushi* 56-kDa gene sequences. The tree was constructed using the maximum likelihood method with MEGA 6.¹³ The *O. tsutsugamushi* 56-kDa gene sequences from stored patient serum are shown in bold as JP13-Korea-2017 (Boryong) and JP13-Korea-2017 (Taguchi). The *O. tsutsugamushi* 56-kDa gene sequence data identified from China, South Korea, Japan, and India were obtained from NCBI/BLAST.

with both SFTSV and different genotypes of *O. tsutsugamushi* in patients, although Zhang et al.¹⁵ showed two genotypes of *O. tsutsugamushi* coinfecting a patient in China. In this study, we first confirmed the case of mixed infection with both SFTSV and two (Boryong and Taguchi) genotypes of *O. tsutsugamushi* in a patient (Figures 1 and 2, and Supplemental Figure 1); we suggest that mixed infection with both SFTSV and single or different genotypes of *O. tsutsugamushi* could have occurred in the patient at the same time in endemic regions, such as China, South Korea, and Japan.¹⁰

The signs and symptoms of SFTS and scrub typhus are similar, but SFTS exhibits a higher mortality rate than does scrub typhus.² Therefore, it is important to consider SFTSV infection in patients having scrub typhus, who may be coinfecting with SFTSV.¹⁰ Thus, there is a need to better understand the ecological transmission dynamics and geographic distribution of SFTSV and *O. tsutsugamushi* in endemic countries, such as China, South Korea, and Japan.

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