Molecular detection of severe fever with thrombocytopenia syndrome and tick-borne encephalitis viruses in ixodid ticks collected from vegetation, Republic of Korea, 2014

Seok-Min Yun a, Ye-Ji Lee a, WooYoung Choi a, Heung-Chul Kim b, Sung-Tae Chong b, Kyu-Sik Chang c, Jordan M. Coburn d, Terry A. Klein e, Won-Ja Lee a,∗

a Division of Arboviruses, National Institute of Health, Korea Centers for Disease Control and Prevention, Cheongju-si, Chungcheongbuk-do (Province), 28159, Republic of Korea
b 5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, Unit 15247, APO AP, 96205-5247, USA
c Division of Medical Entomology, National Institute of Health, Korea Centers for Disease Control and Prevention, Cheongju-si, Chungcheongbuk-do (Province), 28159, Republic of Korea
d Carl R. Darnall Army Medical Center, Department of Preventive Medicine, 76022 Crockett Street, Ft Hood, TX 76544, USA
e Public Health Command District-Korea (Provisional), 65th Medical Brigade, Unit 15281, APO AP, 96205-5281, USA

ABSTRACT

Ticks play an important role in transmission of arboviruses responsible for emerging infectious diseases, and have a significant impact on human, veterinary, and wildlife health. In the Republic of Korea (ROK), little is known about information regarding the presence of tick-borne viruses and their vectors. A total of 21,158 ticks belonging to 3 genera and 6 species collected at 6 provinces and 4 metropolitan areas in the ROK from March to October 2014 were assayed for selected tick-borne pathogens. Haemaphysalis longicornis (n = 17,570) was the most numerous collected, followed by Haemaphysalis flava (n = 3,317), Ixodes nipponensis (n = 249), Ambyomma testudinarium (n = 11), Haemaphysalis phasiana (n = 8), and Ixodes turdus (n = 3). Ticks were pooled (adults 1–5, nymphs 1–30, and larvae 1–50) and tested by one-step reverse transcription polymerase chain reaction (RT-PCR) or nested RT-PCR for the detection of severe fever with thrombocytopenia virus (SFTSV), tick-borne encephalitis virus (TBEV), Powassan virus (POWV), Omsk hemorrhagic fever virus (OHFV), and Langat virus (LGTv). The overall maximum likelihood estimation (MLE) [estimated numbers of viral RNA positive ticks/1000 ticks] for SFTSV and TBEV was 0.95 and 0.43, respectively, while, all pools were negative for POWV, OHFV, and LGTV.

The purpose of this study was to determine the prevalence of SFTSV, TBEV, POWV, OHFV, and LGTV in ixodid ticks collected from vegetation in the ROK to aid our understanding of the epidemiology of tick-borne viral diseases. Results from this study emphasize the need for continuous tick-based arbovirus surveillance to monitor the emergence of tick-borne diseases in the ROK.

© 2016 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license. (http://creativecommons.org/licenses/by-nc-nd/4.0/)

1. Introduction

Ticks (Acari: Ixodidae) are important vectors of tick-borne pathogens, including protozoa, bacteria, and viruses that impact on human, veterinary, and wildlife health worldwide (de la Fuente et al., 2008; Mansfield et al., 2009; Dantas-Torres et al., 2012). A wide variety of pathogens of medical and veterinary importance [e.g., *Ehrlichia* and *Anaplasma* spp. (Chae et al., 2003; Kang et al., 2013, 2016), *Bartonella* spp. (Kim et al., 2005; Kang et al., 2013, 2016), *Borreli*a spp. (Park et al., 1992), *Rickettsia* spp. (Lee et al., 2013), tick-borne encephalitis virus (TBEV) (Kim et al., 2009; Ko et al., 2010; Yun et al., 2012), and severe fever with thrombocytopenia syndrome virus (SFTSV) (Kim et al., 2013; Park et al., 2014a,b; Yun et al., 2014)] have been reported from the Republic of Korea (ROK).

TBEV, the etiologic agent of tick-borne encephalitis (TBE), Family *Flaviviridae*, Genus *Flavivirus*, affects the human central nervous system (Dumuis et al., 1999). TBEV is endemic throughout much of Eurasia (Süss, 2011) and has been subdivided into 3 subtypes: European (=Western), Far-Eastern, and Siberian (Ecker et al., 1999). The primary vector of the European subtype is *Ixodes ricini*.

*Corresponding author at: 187 Osongsaengmyeong 2-ro, Osong-eup, Cheongju-si, Chungcheongbuk-do 28159, Republic of Korea.
E-mail address: leejonja@gmail.com (W.-J. Lee).

http://dx.doi.org/10.1016/j.ttbdis.2016.05.003
1877-959X/© 2016 The Authors. Published by Elsevier GmbH. This is an open access article under the CC BY-NC-ND license. (http://creativecommons.org/licenses/by-nc-nd/4.0/)
nus, while *Ixodes persulcatus* is reported to be the primary vector of the Far-Eastern and Siberian subtypes (Gritsun et al., 2003). Although transmission of TBEV has been reported by drinking unpasteurized milk or milk-products of virus-infected livestock (Lindquist and Vapalahti, 2008; Hudopisk et al., 2013), the primary route of transmission to humans is by tick bite. Previous studies implicated *Haemaphysalis longicornis*, *Haemaphysalis flava*, *Haemaphysalis japonica*, and *Ixodes nipponensis* for the first time as potential vectors TBEV in ticks collected from vegetation and from wild and domestic animals in the ROK (Kim et al., 2009; Yun et al., 2012). Similarly, TBEV was detected from lung tissues of the striped field mouse, *Apodemus agrarius* (Kim et al., 2008), and that *A. agrarius* collected at US and ROK operated military training sites near the demilitarized zone (DMZ) were infested with *I. nipponensis* (98.9%), *I. pomerantzevi* (1.1%), and *H. flava* (0.1%), but not *H. longicornis* or other ticks, even though the mice were collected among grasses and other vegetation where *H. longicornis* is commonly collected (Kim et al., 2010; Coburn et al., 2016).

SFTSV is a tick-borne virus, Family Bunyaviridae, Genus phlebovirus, is the causative agent of severe fever with thrombocytopenia syndrome (SFTS), an emerging infectious disease characterized by high fever, gastrointestinal symptoms, leukopenia, thrombocytopenia, and high mortality rate (Yu et al., 2011). Human cases of SFTS were first observed in China in 2009 (Yu et al., 2011) and subsequently reported in Japan in 2011 (Takahashi et al., 2014), and the ROK in 2013 (Kim et al., 2013; Park et al., 2014a). While ticks are the primary source of SFTSV transmission, there have been reports of nosocomial transmission through close patient contact and blood and mucus from infected patients (Liu et al., 2012; Gai et al., 2012; Kim et al., 2015). SFTSV has previously been reported in ticks belonging to 3 genera and 4 species (*H. longicornis*, *H. flava*, *Amblyomma testudinaria*, and *I. nipponensis*) and has been isolated from *H. longicornis* ticks (Park et al., 2014b; Yun et al., 2014, 2016).

Other tick-borne viruses, e.g., Powassan virus (POWV), Omsk hemorrhagic fever virus (OHFV), and Langat virus (LTV) have not yet been reported in the ROK. However, it is possible that these viruses are present in the ROK since the distribution of the vectors (*H. longicornis*, *I. persulcatus*, *I. granulatus*, *Dermacentor marginatus*, and *D. reticulatus*) for these viruses are present in the ROK (Smith, 1956; Lee, 1999; Dantas-Torres et al., 2012).
Table 1
Primer sets and RT-PCR or nested RT-PCR conditions used for detection of the envelope gene for tick-borne encephalitis virus (TBEV), Powassan virus (POWV), Omsk hemorrhagic fever virus (OHFV), and Langat virus (LGTV) and the medium (M) segment for severe fever with thrombocytopenia virus (SFTSV).

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Primer designation</th>
<th>Polarity</th>
<th>Sequences (5′-3′)</th>
<th>RT-PCR or nested RT-PCR conditions</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBEV</td>
<td>TBE913F</td>
<td>sense</td>
<td>TGCACACAYYTGGAAAAACAGGGA</td>
<td>45°C, 30 min; 94°C, 5 min; 25 cycles (94°C 30 s, 52°C 30 s, 72°C 1 min); 72°C, 5 min</td>
<td>854</td>
<td>Ternovoi et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>TBE1738R</td>
<td>antisense</td>
<td>TGGCCACTTTTCAGGTGGTACTTGGTTCC</td>
<td>94°C, 2 min; 30 cycles (94°C 20 s, 62°C 10 s, 68°C 20 s); 70°C, 5 min</td>
<td>506</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TBE1192F</td>
<td>sense</td>
<td>CAGAGTGATCGAGGCTGGGGYAA</td>
<td>94°C, 30 s; 52°C 30 s; 72°C 1 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TBE1669R</td>
<td>antisense</td>
<td>AAGACTCCAGTCTGGTCTCCOR0AGGTTGTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFTSV</td>
<td>MF3</td>
<td>sense</td>
<td>GATGAGATGTCATCCGCTGATTC</td>
<td>50°C, 30 min; 95°C, 15 min; 35 cycles (95°C 20 s, 58°C 40 s, 72°C 30 s); 72°C, 5 min</td>
<td>560</td>
<td>Yun et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>MR2</td>
<td>antisense</td>
<td>CTCATGGGGTGTGAATGCTCACC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>POWV</td>
<td>POWV-E2-F</td>
<td>sense</td>
<td>ACCGGCCATGTACTGTTGA</td>
<td>45°C, 30 min; 94°C, 5 min; 30 cycles (94°C 30 s, 54°C 30 s, 72°C 40 s); 72°C, 5 min</td>
<td>463</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>POWV-E4-R</td>
<td>antisense</td>
<td>GGAATTGGACAGCGCGAGGAACAA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OHFV</td>
<td>OHFV-E2-F</td>
<td>sense</td>
<td>AGCTTTGCAATACCCGCCGTC</td>
<td>45°C, 30 min; 94°C, 5 min; 30 cycles (94°C 30 s, 54°C 30 s, 72°C 40 s); 72°C, 5 min</td>
<td>447</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>OHFV-E4-R</td>
<td>antisense</td>
<td>CTCCGCGTGTCAGGACGGAATTTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LGTV</td>
<td>LGTV-E2-F</td>
<td>sense</td>
<td>GTGCTCATGAGGTTGGCTTT</td>
<td>45°C, 30 min; 94°C, 5 min; 30 cycles (94°C 30 s, 54°C 30 s, 72°C 40 s); 72°C, 5 min</td>
<td>466</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>LGTV-E4-R</td>
<td>antisense</td>
<td>GGAAGCCCCATGGACAAGGTG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Y = C + T.
b R = A + G.
2. Materials and methods

2.1. Tick collection and identification

Ticks were collected by tick drag as described by Chong et al. (2013) from 139 sites located in 6 provinces (Gyeonggi, Gangwon, Chungcheongnam, Gyeongsangbuk, Gyeongsangnam, and Jeju) and 4 metropolitan areas (MA) (Seoul, Daegu, Ulsan, and Busan) in the ROK (Fig. 1) from March-October 2014. Ticks were identified to developmental stages and species using a stereo-microscope (×100) and keys developed by Yamaguti et al. (1971). After identification, ticks were placed in 2 ml cryovials in pools of 1–5 adults, 1–30 nymphs, and 1–50 larvae according to species and developmental stage and collection period, location, and habitat.

2.2. RNA extraction from ticks

A total of 21,158 ticks [H. longicornis (n = 17,570), followed by H. flava (n = 3317), I. nipponensis (n = 249), A. testudinarium (n = 11), H. phasiana (n = 8), and I. turdus (n = 3)] were assayed by one-step reverse transcription polymerase chain reaction (RT-PCR) or nested RT-PCR for the detection of SFTSV, TBEV, POWV, OHFV, and LGTV. Tick pools were homogenized in 600 µl of phosphate-buffered saline (PBS) using a Precellys® 24 homogenizer (Bertin Technologies, Bretonneux, France) and 2.8 mm stainless steel beads. After the tick homogenates were centrifuged at 8600 × g for 5 min, the supernatant was collected and RNA extracted using the Viral Gene-spin™ RNA extraction kit (INRON Biotechnology, Seongnam, ROK) according to the manufacturer’s instructions.

2.3. Amplification of tick-borne viruses

The presence of SFTSV, TBEV, POWV, OHFV, and LGTV RNA in ixodid ticks was molecularly screened by one-step RT-PCR or nested RT-PCR using specific primer sets for each virus according to previously described methods or designed methods in this study (Ternovoi et al., 2003; Kim et al., 2008; Yun et al., 2014) (Table 1). The primer sets of the first and nested PCR were designed by using conserved DNA regions encoding envelope (E) gene of TBEV, strain 205 belonging to the Far-Eastern subtype (Ternovoi et al., 2003).

For the detection of POWV, OHFV, and LGTV, one-step RT-PCR was performed in a 20 µL reaction volume containing 5 µL extracted RNA and 10 pmol of each primer using a Maxime™ RT-PCR Pre-Mix kit (INRON), under the following conditions: an initial step of 30 min at 45 °C for reverse transcription and 5 min at 94 °C for denaturation, followed by 30 cycles of 30 s at 94 °C, 30 s at 54 °C,
and 30 s at 72 °C, and a final extension step of 5 min at 72 °C. In each one-step RT-PCR or nested RT-PCR reaction, positive controls containing genomic RNA of the target viruses and distilled water as negative control were included. The results were visualized by 1.5% agarose gel electrophoresis stained with 0.1 μL/mL of SYBR® Safe DNA Gel Stain (Invitrogen, Carlsbad, USA).

2.4. Sequencing and phylogenetic analysis

All positive RT-PCR products were purified with a QIAquick® Gel Extraction Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions and sequenced after cloning into a pCR™4-TOPO® plasmid (Invitrogen) for confirmation. Sequencing was performed with T7 promoter (S′-TAATACGACTCACTATAGGG-3’) and M13R-pUC (S′-CAGGAAACAGCTATGAC-3’) primers using an ABI Prism BigDye™ v3.1 Terminator Cycle Sequencing Kit and an ABI 3730xl Sequencer (Applied Biosystems, Foster City, USA) at Macrogen Inc. (Daejeon, ROK). Sequencing results were aligned using SeqMan PRO, Lasergene version 5.0.6 (DNASTAR Inc., USA) and compared for similarity to sequences deposited in GenBank using BLAST.

For phylogenetic analysis, sequence alignment and construction of the phylogenetic tree by the Maximum Likelihood (ML) method were performed using MEGA Version 6.0 software (Tamura et al., 2013).

3. Results

3.1. Tick identification

A total of 21,158 ticks belonging to 3 genera and 6 species, comprising 846 adults (310 males, 536 females), 12,491 nymphs, and 7821 larvae, collected by tick drag from vegetation in the ROK were assayed for SFTSV, TBEV, POWV, OHFV, and LGTV (Table 2). H. longicornis (83.04%, 17,570/21,158) accounted for the majority of the ticks assayed, followed by H. flava (15.68%, 3317), I. nipponensis (1.18%, 249), A. testudinarium (0.05%, 11), H. phasiana (0.04%, 8),
and *I. turdus* (0.01%, 3). All ticks collected from vegetation were unfed.

### 3.2. Detection of tick-borne viral pathogens

The minimum field infection rate (MFIR) estimates the lower bound of the infection rate while MLE estimates the infection rate itself (*Gu et al.*, 2003, 2004). The maximum likelihood estimation (MLE) takes into account the number of pools, number of positive pools, and variation in pool size thereby relaxing the assumption of MFIR that only one infected specimen exists in a positive pool, and therefore is a more accurate measure of infection rate (*Gu et al.*, 2003, 2004). MLE was calculated using PooledInRRate (Biggerstaff, CDC, [www.cdc.gov/ncidod/dvbid/westnile/software.htm](http://www.cdc.gov/ncidod/dvbid/westnile/software.htm)). The MLE (estimated number of viral RNA positive ticks per 1000 ticks) for SFTSV, by tick species and developmental stage, ranged from 0.45 to 14.70 for selected localities and habitats surveyed. Overall, the highest MLE for SFTSV was observed for *I. nipponensis* (4.0; 1 pool/249 individual ticks), followed by *H. flava* (2.42; 8 pools/3317 ticks), and *H. longicornis* (0.63; 11 pools/17,570 ticks) (*Table 2*). None of the pools of *A. testudinarium, I. turdus*, or *H. phasiana* were positive for SFTSV.

Of the 139 sites where ticks were collected, SFTSV and TBEV were only detected in ticks collected from three provinces (Gyeongsangbuk, Gyeongsangbuk, and Chungcheongnam) and two metropolitan areas (Seoul and Busan). For positive sites and stage of development, the MLE for SFTSV ranged from 0.93–500.00 (*Table 3*). *H. longicornis, H. flava*, and *I. nipponensis* collected from mixed, deciduous forests and grass habitats near Andong, Gyeongsangbuk province were positive for SFTSV. *H. longicornis* and *H. flava* collected from grass and pine forest habitats near Danjin, Chungcheongnam province, Gijang, Busan MA, and Pohang, Gyeongsangbuk province were positive for SFTSV. *H. longicornis* was collected from grass habitat near Seongdong-gu, Seoul MA, and Yeoncheon, Gyeonggi province for positives for SFTSV. *H. flava* collected from pine forest habitats were positive near Yeoncheon, Gyeongsangbuk province for positives SFTSV (*Table 3*).

The MLE for TBEV, by tick species and developmental stage, ranged from 2.79–333.33. Both *I. nipponensis* and *H. flava* collected from grass and pine forest habitats near Andong, Gyeongsangbuk province were positive for TBEV, while *H. longicornis* and *H. flava* collected from grass and pine forest habitats near Uiseong, Gyeongsangbuk province were positive for TBEV. Only *H. longicornis* collected from grass habitat near Yangsan, Gyeongsangnam province was positive for TBEV, while only *H. flava* collected from grass habitat near Dong-gu, Daegu MA was positive for TBEV (*Table 3*).

The SFTSV M segment demonstrated 91.8–100% similarity to previously reported SFTSV strains from China, Japan, and the ROK and phylogenetic analysis indicated that the 18 Korean strains were closely related to those from China and Japan. A total of 11 of the Korean strains (KOR14-96, KOR14-97, KOR14-127-10, KOR14-127-20, KOR14-167, KOR14-190, KOR14-193, KOR14-205, KOR14-1168, KOR14-1173, and KOR14-1189) clustered with the SFTSV strains from humans and ticks collected from humans and vegetation in Japan and the ROK, while 7 of the strains (KOR14-11, KOR14-29, KOR14-44, KOR14-46, KOR14-59, KOR14-127-14, and KOR14-459) grouped with SFTSV strains from China obtained from humans, ticks, and a goat, a Japanese strain from human, and Korean strains from humans and ticks (*Table 3, Fig. 2A*).

A total of 9 pools of ticks were positive for TBEV by nested RT-PCR and confirmed by sequencing (overall MLE 0.43). *I. nipponensis* demonstrated the highest MLE (8.02), followed by *H. flava* (0.90), and *H. longicornis* (0.23%) (*Table 2*). None of the pools of *A. testudinarium, I. turdus*, or *H. phasiana* were positive for TBEV. Compared with other reported TBEV strains, the Korean strains...
### Table 3
Severe fever with thrombocytopenia syndrome virus and tick-borne encephalitis virus detected by RT-PCR (SFTSV) or nested RT-PCR (TBEV) in ticks collected from grass and forest habitats, by province/metropolitan area (MA), stage of development, number positive pools (number tested pools), strain, the minimum field infection rate, and the maximum likelihood estimation for each species by collection site and stage of development for ticks collected during 2014, ROK.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Collection site</th>
<th>TICK species</th>
<th>Developmental stage</th>
<th>Habitats</th>
<th>No. positive pools (No. tested pools)</th>
<th>Strain</th>
<th>MFR* (%)</th>
<th>MLE**</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFTSV</td>
<td>Seoul MA</td>
<td>H. longicornis</td>
<td>nymph</td>
<td>grass</td>
<td>1 (6)</td>
<td>KOR14-29</td>
<td>0.67</td>
<td>6.66</td>
</tr>
<tr>
<td></td>
<td>Gyeonggi</td>
<td>H. longicornis</td>
<td>nymph</td>
<td>grass</td>
<td>1 (7)</td>
<td>KOR14-11</td>
<td>0.70</td>
<td>7.19</td>
</tr>
<tr>
<td></td>
<td>Chungcheongnam</td>
<td>H. longicornis</td>
<td>nymph</td>
<td>grass</td>
<td>1 (6)</td>
<td>KOR14-127-20</td>
<td>0.34</td>
<td>3.44</td>
</tr>
<tr>
<td></td>
<td>Busan MA</td>
<td>H. longicornis</td>
<td>nymph</td>
<td>grass</td>
<td>1 (2)</td>
<td>KOR14-127-10</td>
<td>5.26</td>
<td>33.64</td>
</tr>
<tr>
<td></td>
<td>Gyeongsangbuk</td>
<td>H. longicornis</td>
<td>nymph</td>
<td>mixed forest</td>
<td>2 (22)</td>
<td>KOR14-1185</td>
<td>0.23</td>
<td>2.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H. flavia</td>
<td>nymph</td>
<td>deciduous forest</td>
<td>1 (20)</td>
<td>KOR14-97</td>
<td>0.37</td>
<td>3.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H. flavia</td>
<td>nymph</td>
<td>deciduous forest</td>
<td>2 (22)</td>
<td>KOR14-1189</td>
<td>1.29</td>
<td>14.17</td>
</tr>
<tr>
<td></td>
<td>Pohang</td>
<td>H. longicornis</td>
<td>adult male</td>
<td>grass</td>
<td>1 (7)</td>
<td>KOR14-96</td>
<td>12.5</td>
<td>123.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I. nipponensis</td>
<td>adult male</td>
<td>pine forest</td>
<td>3 (157)</td>
<td>KOR14-46</td>
<td>0.09</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H. flavia</td>
<td>nymph</td>
<td>pine forest</td>
<td>2 (49)</td>
<td>KOR14-459</td>
<td>0.58</td>
<td>5.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H. flavia</td>
<td>nymph</td>
<td>pine forest</td>
<td>1 (20)</td>
<td>KOR14-44</td>
<td>3.45</td>
<td>34.07</td>
</tr>
<tr>
<td></td>
<td>Yeongcheon</td>
<td>H. flavia</td>
<td>adult female</td>
<td>pine forest</td>
<td>1 (2)</td>
<td>KOR14-47</td>
<td>16.67</td>
<td>178.42</td>
</tr>
<tr>
<td>TBEV</td>
<td>Daegu MA</td>
<td>H. flavia</td>
<td>nymph</td>
<td>grass</td>
<td>1 (5)</td>
<td>KOR14-677</td>
<td>2.08</td>
<td>20.93</td>
</tr>
<tr>
<td></td>
<td>Gyeongsangnam</td>
<td>H. longicornis</td>
<td>nymph</td>
<td>grass</td>
<td>1 (17)</td>
<td>KOR14-1357</td>
<td>0.29</td>
<td>2.79</td>
</tr>
<tr>
<td></td>
<td>Gyeongsangbuk</td>
<td>I. nipponensis</td>
<td>adult male</td>
<td>pine forest</td>
<td>1 (6)</td>
<td>KOR14-117</td>
<td>14.29</td>
<td>151.81</td>
</tr>
<tr>
<td></td>
<td>Uiseong</td>
<td>H. longicornis</td>
<td>adult male</td>
<td>pine forest</td>
<td>1 (12)</td>
<td>KOR14-136</td>
<td>6.67</td>
<td>65.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H. flavia</td>
<td>nymph</td>
<td>pine forest</td>
<td>3 (36)</td>
<td>KOR14-131</td>
<td>0.39</td>
<td>4.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H. flavia</td>
<td>nymph</td>
<td>grass</td>
<td>1 (16)</td>
<td>KOR14-135</td>
<td>1.75</td>
<td>16.61</td>
</tr>
</tbody>
</table>

nd, not determined.

* MFR, minimum field infection rate per 100 ticks (No. of positive pools/No. of examined ticks in pools × 100), by species and stage of development.
** MLE, Maximum likelihood estimation = estimated numbers of viral RNA positive ticks per 1000 ticks.

### 4. Discussion

Tick-borne pathogens transmitted by ixodid ticks are the causative agents of important zoonotic disease affecting humans, domestic animals and birds, and wildlife worldwide (de la Fuente et al., 2008; Dantas-Torres et al., 2012; Lani et al., 2014). A number of tick-borne pathogens, including bacteria (Ehrlichia, Anaplasma, Bartonella, Borrelia, and Rickettsia), and viruses (TBEV and SFTSV) have been identified in the ROK (Park et al., 1992; Chae et al., 2003; Kim et al., 2005; Kim et al., 2009; Ko et al., 2010; Yun et al., 2012; Kang et al., 2013, 2016; Lee et al., 2013; Kim et al., 2013; Park et al., 2014a,b; Yun et al., 2014).

In addition to the tick-borne viruses (TBEV and SFTSV) known to be present in the ROK, ticks also were assayed for POWV, OHFV, and LGTV based on the potential of these viruses being present in the ROK. However, results from this investigation only detected SFTSV and TBEV in ixodid ticks collected from forest, forest + grass/herbaceous vegetation, and grass/herbaceous vegetation habitats.

SFTSV has taken on greater importance as the numbers of SFTS cases have increased annually from 36 (2013), 55 (2014), 79 (2015) (Korea Centers for Disease Control and Prevention, 2016) since it was first recognized in 2013. Geographical and ecological factors (e.g., forests and other herbaceous vegetation habitats) and human behavior (e.g., dairy and beef farming, tending to gravesites during Chusok, agriculture that include small gardens), and recreational and military activities are highlighted where ticks and their associated pathogens are important considerations when identifying risk factors associated with exposure for civilian and military populations in the ROK (Kim et al., 2013; Park et al., 2014a; Shin et al., 2015). In addition, the mean mortality rate was very high (2013, 47.2%; 2014, 32.1%; 2015, 26.6%), with nearly all mortality reported among persons aged 60 years of age or greater and are similar to those reported by Japan (Takahashi et al., 2014), but much higher than mortality rates of approximately 6.3–12.0% in China (Yu et al., 2011; Ding et al., 2013). Thus, younger populations may demonstrate milder symptoms that are not recognized as SFTS infections. Although SFTS is endemic in the ROK, there is limited information for the prevalence and distribution of SFTSV for various habitats and species of ticks that humans and domestic animals are exposed. Park et al. (2014b) and Yun et al. (2014) previously identified SFTSV in all developmental stages of H. longicornis (MFR 0.46%; 55 pools/11,856 ticks) and for three tick species (H. longicornis, A. testudinarium, and I. nipponensis) collected from humans (MFR 6.9%; 18 pools/261 ticks). A recent study of ticks collected from a SFTS outbreak area in the ROK found both H. longicornis and H. flavata collected...
from vegetation positive for SFTSV (MFIR 0.11%; 9 pools/8313 ticks) (Yun et al., 2016). For comparison, the overall MFIR for ticks collected from vegetation in this study was 0.10% was 2-fold lower than the overall SFTSV MFIR (0.20%) observed in ticks collected from vegetation from China (Luo et al., 2015). SFTSV is a zoonotic disease and a number of domestic and wild animals and birds have been reported positive for SFTSV antibodies. Few studies have been done to determine the natural reservoir or amplifying hosts for the transmission and maintenance of SFTSV (Jiao et al., 2012; Zhao et al., 2012; Niu et al., 2013; Cui et al., 2013; Liu et al., 2014). Transovarial and transstadial transmission are one potential mode for the maintenance of SFTSV since all stages of unfed ticks were found positive for SFTSV (Park et al., 2014b). The partial M segment phylogenetic analysis showed SFTSV Korean strains detected from ticks collected from vegetation are consistent with previous studies and were closely related to the SFTSV strains from Japan and China isolated from humans and domestic animals, ticks collected from vegetation, humans, and goats (Park et al., 2014a; Yun et al., 2014) (Fig. 2A).

TBE is one of the most important tick-borne viral diseases in Europe and Asia. Although there have not been any reported/confirmed human cases of TBE in the ROK, it was suggested that TBE cases may occur in the ROK based on the following evidence: (a) TBEV has been identified in ixodid ticks collected from vegetation and from ticks collected from wild and domestic animals (Kim et al., 2009; Ko et al., 2010; Yun et al., 2012), and (b) TBEV strains isolated from the striped field mouse, A. agrarius, a potential maintenance host for TBEV (Kim et al., 2008).

The overall prevalence of TBEV (0.04%) in ticks was lower than for previous studies for ixodid ticks collected from vegetation in the ROK (Ko et al., 2010; Yun et al., 2012). The maintenance of TBEV in nature is poorly understood and there is no current evidence of transovarial transmission of TBEV in ixodid ticks in Korea. The phylogenetic analysis based on the partial E genes showed that the 9 TBEV strains from Korean ticks collected from vegetation were genetically close to the other TBEV strains belonging to the European (=Western) subtype. These results agree with previously published findings that the Korean strains were phylogenetically closely related to the European subtype (Kim et al., 2009; Ko et al., 2010; Yun et al., 2012) from both ticks and the striped field mouse (Fig. 2B).

For the validation of specificity and sensitivity of the nested RT-PCR assay used for the detection of TBEV, RNA extracted from 9 TBEV strains, including 6 strains belonging to the European subtype (Krl 93, Krl 213, Krl 215, Krl 216, Krl 219, and Neudoerfl strains) and 3 strains belonging to the Far-Eastern subtype (Sofjinho, Oshima 5–10, and KH98–5 strains) were subjected to nested RT-PCR analysis. Using nested RT-PCR, it was possible to detect viral RNA in samples that had a concentration corresponding to 30 plaque forming units (PFU) per nested RT-PCR reaction. In addition, we determined that this method could detect TBEV strains of the European and Far-Eastern subtype. Unfortunately, we could not confirm whether this method could detect the TBEV Siberian subtype, as this strain was unavailable in our lab. However, it may be possible that the Korean TBEV strains are of low pathogenicity or non-pathogenic for humans and therefore few cases are reported in Korea.

The detection of SFTSV and TBEV in H. longicornis, H. flava, and I. nipponensis implicates them as potential vectors of SFTSV and TBEV in the ROK. The detection of unfed larvae of H. longicornis and H. flava collected from vegetation positive for SFTSV is indicative of transovarial transmission among these two species. Laboratory studies to confirm transovarial transmission of SFTSV for various tick species in Korea should be done to identify how the virus is maintained in the environment. Attempts to isolate SFTSV and TBEV-positive tick homogenates using Vero E6 cells and ICR mice, respectively, failed (data not shown), but should be continued for further investigations.

POWV, OHFV, and LGTV were not detected in ixodid ticks collected from vegetation. However, this is the first investigation regarding the presence of POWV, OHFV, and LGTV in ticks collected from vegetation in the ROK, and more extensive tick-based surveys should be conducted to determine if they are present in the ROK.

In summary, SFTSV and TBEV were detected in ixodid ticks in the ROK and although there have been no reported cases of TBE in Korea, both pose serious health risks among Korean and US military personnel and civilian populations, as well as causing infection of domestic animals and wildlife (e.g., sheep, cattle, deer, and rodents).

5. Conclusions

SFTSV and TBEV were detected in three species of ixodid ticks collected from vegetation by tick drag and distributed throughout-out much of the ROK. These results aid our understanding of the epidemiology of SFTSV and TBEV and emphasize the need for continuous tick-based arbovirus surveillance to monitor the emergence of tick-borne viruses in the ROK. Additional studies, including larger scale surveys of ticks collected from vegetation and domestic/wild animals, rodent-borne disease surveys to identify potential zoonotic hosts, and transovarial and transmission studies should be conducted to determine the prevalence of tick-borne viruses and the risks they pose to human populations in the ROK.

Acknowledgements

The authors thank Eun-Ji Jeong and Jin-Yong Ahn for their laboratory assistance. Special thanks to Seung P. Seo, Nicolas W. Chang, and other personnel at the 65th Medical Brigade for the collection of ticks. This research was funded by an intramural grant of the Korea National Institute of Health (grant number: 2014-ND53001-00), the Public Health Command District-Korea, the 65th Medical Brigade, Seoul, Korea, and the Armed Forces Health Surveillance Branch–Global Emerging Infections Surveillance and Response System (AFHSB-GEIS).

The opinions expressed herein are those of the authors and are not to be construed as official or reflecting the views of the U.S. Department of the Army, Department of Defense, or the U.S. Government.

References


