ISSN (Print) 0023-4001
ISSN (Online) 1738-0006

Korean J Parasitol Vol. 52, No. 2: 221-224, April 2014
http://dx.doi.org/10.3347/kjp.2014.52.2.221

▣ BRIEF COMMUNICATION

No Detection of Severe Fever with Thrombocytopenia Syndrome Virus from Ixodid Ticks Collected in Seoul

Heejin Ham*, Sukju Jo, Jungim Jang, Sungmin Choi

Seoul Metropolitan Government Institute of Public Health and Environment, Seoul 137-734, Korea

Abstract: Larvae, nymphs, and adult stages of 3 species of ixodid ticks were collected by tick drag methods in Seoul during June-October 2013, and their infection status with severe fever with thrombocytopenia syndrome (SFTS) virus was examined using RT-PCR. During the period, 732 *Haemaphysalis longicornis*, 62 *Haemaphysalis flava*, and 2 *Ixodes nipponensis* specimens were collected. Among the specimens of *H. longicornis*, the number of female adults, male adults, nymphs, and larvae were 53, 11, 240, and 446, respectively. Ticks were grouped into 63 pools according to the collection site, species, and developmental stage, and assayed for SFTS virus. None of the pools of ticks were found to be positive for SFTS virus gene.

Key words: *Haemaphysalis longicornis*, tick, SFTS virus, Seoul

Haemaphysalis longicornis is an important vector of zoonotic tick-borne pathogens that impact on medical and veterinary health worldwide [1]. Severe fever with thrombocytopenia syndrome (SFTS) virus is a tick-borne virus transmitted by *H. longicornis* and included in the family Bunyaviridae (genus Phlebovirus) [2-5].

Tick-borne disease surveillance is becoming increasingly important as zoonotic tick-borne pathogens are recognized to affect man and wild and domestic animals worldwide [6]. Dragging vegetation for questing ticks are often employed where ticks are present and provides information on habitats and seasonal life stage distributions. The purpose of the present study was to provide estimates of the distribution of life cycle stages during periods when ixodid ticks are active and their infection status with SFTS virus. The data would serve to provide information that is necessary for the development of tick-borne disease threat assessments.

Tick surveillance was conducted in Seoul during June-October 2013. Tick drags consisted of a 1.0 m long and 1.0 m wide flannel cloth attached to a stainless dowel (1.2 m long, 2.0 cm diameter). Collections were made by slowly walking and dragging the flannel cloth on the ground for approximately 5-10 m, turning the drag over and then removing the attached ticks using a fine forceps from both sides of the cloth. Nymphs and adults were placed in 2 ml cryovials, while larvae were placed separately in 2 ml cryovials. This was repeated twice until each collector surveyed an area 5-10 m length. Nymphs, adults, and larvae were identified to species and developmental stages under a dissecting microscope according to Yamaguti et al. [7].

A total of 796 ticks in 63 pools were assayed for SFTS virus. Ticks were collected at 51 sites from 7 parks (8 sites of Jamshil, 5 sites of Gwangnaru, 8 sites of Nanji, 5 sites of Gangseo, 5 sites of Worldcup, 5 sites of Seoul Forest, and 15 sites of Seoul Grand parks). Pools of larvae (n=40), nymphs (n=20), and adults (n=5) were pretreated by precellys 24 homogenizer (Bertin Technology, Orsay, France) at 6,000 rpm, for 25 sec, twice at -20˚C. Pools were then homogenized by MK28R (Bertin Technology) with 2.8 mm stainless-steel beads in 600 µl buffer solution containing autoclaved 10% (v/v) FBS and 5% (v/v) penicillin/streptomycin.

RNA was extracted from the tick suspensions using viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions [8]. Detection of the M gene was conducted using Diastar™ 2X onestep RT-PCR pre-mix (Solgent, Seoul, Korea) and conventional RT-PCR arrayed for SFTS virus.

The foward sequence primer was 5'-GATGAGATGGTCCATGCTGATTCTAA-3', and the reverse primer was 5'-CTCATG GG GTGGAATGTCCTCAC-3'. RT-PCR was conducted in a 2720

• Received 15 September 2013, revised 9 December 2013, accepted 25 December 2013.

* Corresponding author (hhj3814@seoul.go.kr)

Table 1. Morphologic identification of ticks collected from natural/city parks from June to October 2013 and assayed for tick-borne severe fever with thrombocytopenia syndrome (SFTS) virus, by species and developmental stage

Collection sites[a]	Species	Stage	No. pools (No. ticks)	Detection on SFTS virus by RT PCR[c]
Jamsil park	*Haemaphysalis longicornis*	Nymph	1 (1)	
		Subtotal	1 (1)	Not detected
Gwangnaru park	*Haemaphysalis longicornis*	Nymph	4 (70)	
		Female	3 (15)	
		Subtotal	7 (85)[b]	Not detected
Nanji park	*Haemaphysalis longicornis*	Female	1 (3)	
		Subtotal	1 (3)	Not detected
Gangseo park	*Haemaphysalis longicornis*	Nymph	1 (5)	
		Female	1 (1)	
		Subtotal	2 (6)	Not detected
	Ixodes nipponensis	Nymph	1 (1)	
		Subtotal	1 (1)	Not detected
Worldcup park	*Haemaphysalis longicornis*	Nymph	2 (21)	
		Male	1 (3)	
		Female	2 (7)	
		Subtotal	5 (31)	Not detected
Seoul forest park	*Haemaphysalis longicornis*	Larvae	1 (4)	
		Nymph	5 (82)	
		Male	2 (8)	
		Female	5 (22)	
		Subtotal	13 (116)	Not detected
	Haemaphysalis flava	Nymph	1 (5)	
		Female	1 (1)	
		Subtotal	2 (6)	Not detected
Seoul grand park	*Haemaphysalis longicornis*	Larvae	22 (424)	
		Nymph	4 (61)	
		Female	1 (5)	
		Subtotal	27 (490)	Not detected
	Haemaphysalis flava	Larvae	1 (18)	
		Nymph	2 (38)	
		Subtotal	3 (57)	Not detected
	Ixodes nipponensis	Nymph	1 (1)	
		Subtotal	1 (1)	Not detected
Total	*Haemaphysalis longicornis*	Larvae	24 (446)	
		Nymph	17 (240)	
		Male	3 (11)	
		Female	13 (53)	
		Total	56 (732)	Not detected
	Haemaphysalis flava	Larvae	1 (18)	
		Nymph	3 (43)	
		Female	1 (1)	
		Total	5 (62)	Not detected
	Ixodes nipponensis	Nymph	2 (2)	
		Total	2 (2)	Not detected

[a]The areas which had hard ticks in 51 sites of 7 parks: Jamshil (8 sites), Gwangnaru (5 sites), Nanji (8 sites), Gangseo (5 sites), Worldcup (5 sites), Seoul Forest (5 sites), and Seoul Grand (15 sites) parks.
And, the areas which had no hard ticks in 59 sites of 15 parks: Yeouido (3 sites), Ttukseom (9 sites), Ichon (4 sites), Mangwon (4 sites), Yeouido (3 sites), Independence (2 sites), Seoseoul Lake (2 sites), North Seoul Iris (3 sites), Boramae (3 sites), Namsan (8 sites), Yongsan Family (2 sites), Gildong Ecological (2 sites), Jungnang Camping Forest (3 sites), Yangjae Citizen (3 sites), and Children's Grand (8 sites) parks.
[b]Including 5 pools (83 ticks) from Goduk Ecological park.
[c]Conventional RT-PCR.
★ Eight parks along Han river: Yeouido, Ttukseom, Ichon, Mangwon, Jamshil, Gwangnaru (included with Goduk Ecological), Nanji, and Gangseo parks.
★ Fourteen large scale Seoul citizen parks: Yeouido, Independence, Seoseoul Lake, Worldcup, North Seoul Iris, Boramae, Namsan, Yongsan Family, Seoul Forest, Gildong Ecological, Jungnang Camping Forest, Yangjae Citizen, Seoul Grand, and Children's Grand parks.

thermal cycler (Applied Biosystems, Foster City, California, USA). The PCR products were identified by electrophoresis in 2.0% agarose gel, and the M gene was confirmed at 560 bp.

A total of 796 ticks (larvae, nymphs, and adults) belonging to 2 genera and 3 species, *H. longicornis* (732), *H. flava* (62), and *I. nipponensis* (2), were collected from 7 parks (Jamshil, Gwangnaru, Nanji, Gangseo, Worldcup, Seoul Forest, and Seoul Grand parks) in Seoul, Korea, June-October 2013 (Table 1). Overall, *H. longicornis* (adults 64, nymphs 240, and larvae 446) accounted for 91.9% of the 3 species collected, followed by *H. flava* 7.8% (adults 1, nymphs 43, and larvae 18) and *I. nipponensis* 0.3% (nymphs 2). Significantly more *H. longicornis* adults, nymphs, and larvae were collected than *H. flava* and *I. nipponensis* (Table 1).

Similarly, *H. longicornis* was the most frequently collected ticks in northern Gyeonggi-do (Province) (75.8%), while *H. flava* and *I. nipponensis* accounted for 19.6% and 4.6% from April to October 2004 and 2005 [6]. In the southern province and Jeju-do (Island), *H. longicornis* was the most frequently collected ticks (73.4%), whereas *H. flava* and *I. nipponensis* accounted for 22.4% and 0.4% in 2007 [9]. However, in Hongdo, *H. longicornis* accounted only for 5.7% of all ticks collected, while *H. flava* and *I. nipponensis* accounted for 18.9% and 6.1%, respectively, from 2008 to 2009 [10]. The areas surveyed were grasses and not forested areas. Higher numbers of *H. flava* may have been collected if forested habitats were surveyed.

Also, Yamaguti [7] reported that *H. longicornis* and *H. flava* were the most commonly collected ticks in Korea and Japan. Lee [11] reported that *H. longicornis* were ectoparasites of mammals, whereas *H. flava* were ectoparasites of birds and mammals. None of these ticks were collected among grasses where people walk or sit. At the Seoul Forest and Seoul Grand parks, 116 and 490 ticks were collected, respectively. At the Seoul Forest Park, ticks were collected in grasses near the places where deers were kept, while at Seoul Grand Park, they were collected in grasses surrounding a wolf enclosure. No ticks were collected from 15 parks that were surveyed: Yeouido, Ttukseom, Ichon, Mangwon, Yeouido, Independence, Seoseoul Lake, North Seoul Iris, Boramae, Namsan, Yongsan Family, Gildong Ecological, Jungnang Camping Forest, Yangjae Citizen, and Children's Grand parks (Table 1; Fig. 1)

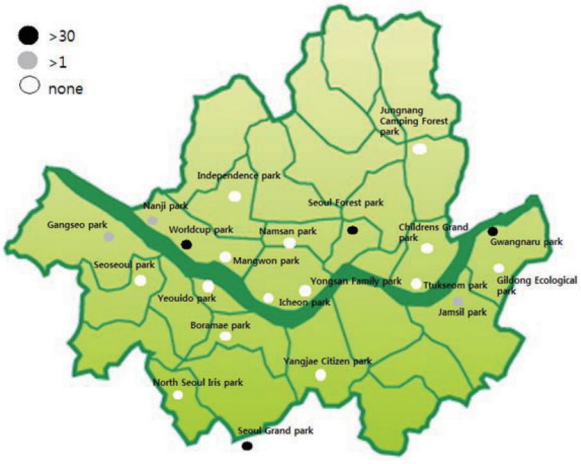

Fig. 1. The survey sites on ticks in citizen parks from April through October 2013 in Seoul, Korea by periodic surveillance.

All of the 63 pools of 796 ticks (larvae, nymphs, and adults) belonged to 3 species, and none were positive for SFTS virus by conventional RT-PCR. The reason that all the tick pools were negative, may be due, in part, to a small sample-size and lack of zoonotic hosts.

In Shandong Province, China, from April to November 2011, SFTS virus-specific antibodies were detected in 69.5% of sheep, 60.5% of cattle, 37.9% of dogs, 3.1% of pigs, and 47.4% of chickens [2]. In addition, ELISA showed that 3.6% and 47.7% of human and animal serum samples were positive for SFTS virus antibodies, indicating that SFTS virus has circulated widely among domestic animals and birds in China [12]. By the end of 2011, SFTS had been reported in 11 provinces including Henan [3,4]. In China, the initial fatality rate for SFTS was 30%. However, with improved diagnosis and supportive care, the fatality rate decreased to 10-15% with an annual incidence of approximately 5 cases among 100,000 rural populations according to the 2011 surveillance data [5].

ACKNOWLEDGMENTS

We thank Prof. Hee-Jung Youn, Seoul National University for his support and Kyongshin Scientific Co., Ltd. for the use of precellys 24 homogenizer (Bertin Technology), and Hyunjung Seung, Eunyoung Cho, and Sanghyuk Ahn, for their assistance.

CONFLICT OF INTEREST

We declare that we have no conflict of interest related to this study.

REFERENCES

1. Ree HI. Third Edition Medical Entomology. Komoonsa 2003,

365-368.
2. Niu GY, Li JD, Liang MF, Jiang XL, Jiang M, Yin HY, Wang ZD, Li C, Zhang QF, Jin C, Wang XJ, Ding SJ, Xing Z, Wang SW, Bi ZQ, Li DX. Severe fever with thrombocytopenia syndrome virus among domesticated animals, China Emerging Infect Dis 2013; 19: 756-763.
3. Lam TT, Liu W, Bowden TA, Cui N, Zhuang L, Liu K, Zhang YY, Cao WC, Pybus OG. Evolutionary and molecular analysis of the emergent severe fever with thrombocytopenia syndrome virus. Epidemics 2013; 5: 1-10.
4. Zhang XS, Liu Y, Zhao L, Bing LI, Hao YU, Wen HL, Yu XJ. An emerging hemorrhagic fever in China caused by a novel bunyavirus SFTSV. Sci China Life Sci 2013; 56: 697-700.
5. Pan H, Hu J, Liu S, Shen H, Zhu Y, Wu J, Zhang X, Zhou X, Wang C, Qu J, Yuan Z. A reported death case of a novel bunyavirus in Shanghai, China. Virol J 2013; 7: 187-192.
6. Chong ST, Kim HC, Lee IY, Kollars TM, Sancho AR, Sames WJ, Chae JS, Klein TA. Seasonal distribution of ticks in four habitats near the demilitarized zone, Gyeonggi-do (province), Republic of Korea. Korean J Parasitol 2013; 51: 319-325.
7. Yamaguti N, Tipton VJ, Keegan HL, Toshioka H. Ticks of Japan, Korea, and the Ryukyu islands, Harvard University. Biological Series 1971; 16: 59-68, 94-100, 129-135.
8. Ham HJ, Oh SA, Kim CG, Jang JI, Jo SJ, Choi SM. Molecular characteristics of human noroviruses genogroup I and genogroup II detected from acute gastroenteritis patients in Seoul. J Environ Health Sci 2012: 38: 363-371.
9. Ko SJ, Kang JG, Kim SY, Kim HC, Klein TA, Chong ST, Sames WJ, Yun SM, Ju YR, Chae JS. Prevalence of tick-borne encephalitis virus in ticks from southern Korea. J Vet Sci 2010; 11: 197-203.
10. Kang JG, Kim HC, Choi CY, Nam HY, Chae HY, Chong ST, Klein TA, Ko S, Chae JS. Molecular detection of *Anaplasma, Bartonella* and *Borrelia* species in ticks collected from migratory birds from Hong-do Island, Republic of Korea. Vector Borne Zoonotic Dis 2013; 13: 215-225.
11. Lee WK, Lim JW, Lee SY, Lee IY. Redescription of *Haemaphysalis flava* and *Ixodes tanuki* collected from a raccon dog in Korea. Korean J Parasitol 1997; 35: 1-8.
12. Jiao Y, Zeng X, Guo X, Qi X, Zhang X, Shi Z, Zhou M, Bao C, Zhang W, Xu Y, Wang H. Preparation and evaluation of recombinant severe fever with thrombocytopenia syndrome virus nucleocapsid protein for detection of total antibodies in human and animal sera by double-antigen sandwich enzyme-linked immunosorbent assay. J Clin Microbiol 2012; 50: 372-377.