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ORIGINAL ARTICLE

# Distribution of Tick Vectors of Severe Fever with Thrombocytopenia Syndrome Virus (SFTSV) Collected from Four Environments in Jeju

Kyoung A Chung†, Hyeon Je Song†, Hyeok Jae Lee, Chul Park, Min Yeung Seo

Department of Biomedical Laboratory Science, Gwangju Health University, Gwangju, Korea

# 제주지역 4개 환경에서 채집한 중증열성혈소판감소증후군 매개 참진드기 분포

정경아†, 송현제†, 이혁재, 박 철, 서민영

광주보건대학교 임상병리과

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**ABSTRACT**

The distribution of ticks and severe fever with thrombocytopenia syndrome virus (SFTSV) pathogens were investigated by collecting ticks from March to November 2018 in four environments (grass fields, copses, mountain roads, and tombs) in Jeju. Three thousand and ninety ticks were collected using a tick trap, and 1,569 ticks were collected using the flagging method. Of the 4,659 ticks collected, *Haemaphysalis longicornis* and *Haemaphysalis flava* accounted for 4,440 ticks (95.2%) and 219 ticks (4.7%), respectively. Nine hundred and fifty, 883, 847, and 410 ticks were collected from grass fields, copses, mountain roads, and tombs, respectively, using tick traps, whereas 704, 472, 197, and 196 ticks were collected from copses, mountain roads, tombs, and grass fields, respectively, using the flagging method. The largest fraction of ticks (2,978) was collected from April to August, and most were collected in May and June. Adult ticks comprised 94 percent of the total ticks from June to August. SFTSV was not detected in the 4,440 *H. longicornis* ticks or the 219 *H. flava* ticks collected in this study.



## INTRODUCTION

Zoonosis is an infectious disease that spreads from animals to humans and is classified as bacterial, viral, fungal, and parasitic depending on pathogen. Direct propagation involves transmission from animals to humans without an intermediate vector, whereas indirect propagation involves transmission through intermediate vectors such as mosquitoes and mites. Currently, about 300 types of zoonosis are known, and about 60 types of zoonosis require preventive management. Global warming caused by human activity has resulted in an increase in greenhouse gases such as $CO_2$. As the vectors are sensitive to climate change, recent climate change is mainly caused by global warming. In Korea, temperatures have risen 1.5˚C since 1900, with annual precipitation showing a large fluctuation, and more extreme weather (drought

Corresponding author: Min Yeung Seo
Department of Biomedical Laboratory Science, Gwangju Health University, Bungmun-daero, 419 beon-gil Gwangsan-gu, Gwangju 62287, Korea
E-mail: qraseo@ghu.ac.kr
ORCID: https://orcid.org/0000-0002-9129-4475
†The first two authors contributed equally to this work.

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and flood) than ever before [1]. Such climate change will change the environment and the ecosystems where vectors live. Because vectors are sensitive to climate change, the distribution of the vectors may be altered, which may also affect the distribution of various infectious diseases.

The class Arachnida includes spiders, scorpions, ticks, mites, harvestmen, and solifuges. Within the Arachnida is the order Acari, which includes ticks and mites, and is reported to have 50,000 species on Earth. Approximately 500 species of ticks and mites are distributed in Korea [2]. Among these, 5 genera and 27 species of ticks are known [3]. Our research included only members of the Acari known as hard ticks.

The tick form is largely divided into the mouthparts and the body. The mouthparts can be uniquely specialized so that ticks can live in various environments. The mouthparts allow ticks to penetrate the host skin and extract a blood meal from the tissue underneath. The upper side of the body consists of a protective plate, called the scutum. The lower side of the body consists of the legs and respiratory, digestive, and reproductive structures. In males, the scutum is large, whereas in females the scutum is much smaller [4].

The life includes a six-legged larva, followed by an eight-legged nymph, followed by an eight-legged adult. Ticks shed their exoskeleton between each of these stages. Hard ticks have a single larval and nymphal stage before becoming adults. The larva and adult have differences in the presence of reproductive cells and the number of bristles. Both sexes of ticks must feed on the blood of a vertebrate host in all of their life stages. Through the blood sucking process, pathogens inside the tick move to the host, causing illness. Ticks detect the host's movements due to temperature changes, odors, and vibrations of the ground, and then escape from the host to the ground and undergo metamorphosis.

Ticks infect humans and animals with organisms such as viruses, bacteria, and protozoans. Tick-vectored diseases include severe fever with thrombocytopenia syndrome (SFTS), tickborne encephalitis, anaplasmosis, Ehrlichiosis, Lyme disease, and others [5-7].

SFTSV is a *Phlebovirus* belonging to the family Bunyaviridae, which includes group V negative single-stranded RNA viruses. In China, ticks such as *Haemaphysalis longicornis*, *H. flava*, *Amblyomma testudinarium*, and *Ixodes nipponensis* were reported to transmit SFTSV in humans and animals [5]. To date, SFTSV has no vaccine or therapeutic treatment and has a fatality rate of as much as 30%. The number of cases is increasing every year in Korea [6-8].

Because the number of infectious diseases caused by vectors is increasing with an increase in global warming and in outdoor leisure activities, it is important to study the influence of climate and environmental changes on the influx and spread of these diseases through long-term monitoring.

Jeju Island is located at the southernmost tip of the Korean Peninsula and corresponds to 33°10′33°34′N and 126°10′127°E east longitude. Recently, with the introduction of warm and humid air in the tropical and subtropical western Pacific, the average temperature has risen 1.31°C over the past decade, especially in Seogwipo City [9].

In this study, we collected ticks and conducted a survey on classification and population density and we determined whether any of the collected ticks were infected with the SFTS virus.

## MATERIALS AND METHODS

### 1. Period and place of tick collection

We collected ticks in the Jeju area from April to November 2018. Tombs, mountain roads, copses, and grass fields were selected as tick collection areas. In Jeju, ticks were collected once a month using tick traps, and in Seogwipo, ticks were collected in May and June using the flagging method (Table 1).

**Table 1.** Occurrence monitoring of ticks for environment

| Survey area | Survey environment | Location | Investigation method | Time of investigation | Frequency of investigation | Remarks |
|---|---|---|---|---|---|---|
| Jeju-city | Glass field | 33°27'20.1"N 126°34'15.3"E | Trap & flagging | April-November (Trap) May-Jun (Flagging) | Once a month | Classification identification |
| | Copse | 33°27'21.2"N 126°34'10.5"E | | | | |
| | Mountain road | 33°26'53.2"N 126°34'35.4"E | | | | |
| | Tomb | 33°26'55.2"N 126°34'42.9"E | | | | |

## 2. Classification of ticks

The collected ticks were fixed in 70% alcohol and classified by examination under the microscope (Olympus SZ61, Tokyo, Japan) according to methods of Yamaguti et al [8]. Each tick was identified as larva, nymph, or adult and adults were distinguished as male or female.

## 3. Detections of SFTSV in ticks

The collected ticks were treated as follows for the extraction of the SFTS RNA. Adults (N=5), nymphs (N=30), and larvae (N=50) were put into a 2 mL tube, and the tissues were homogenized twice for 20 sec at 5,000 rpm with an automatic homogenizer. The tick tissues were cooled for 5 min on ice and centrifuged for 1 min at 4°C and 13,000 rpm (13,250 RCF). After taking 140 µL of the upper layer solution and transferring it to a new 1.5 mL tube, RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Cat# 52906, Hilden, Germany). The solutions were stored in a freezer at −70°C. For the detection of the M-section gene, RT-PCR was performed using a primer set (5'-GATAGGATGTCCATTAA-3' and 5'-CTCATGGGGGGGGGGGAATGTCAC-3') [10] according to the protocols of the DiaStarTM 2X One-step RT-PCR Pre-Mix (SOLGENT, Daejeon, Korea). PCR products were separated using gel electrophoresis with a 2.0% agarose gel and 0.5 mg/mL ethidium bromide (Table 2). The SFTSV gene amplification product (560 bp) was identified using the standard molecular marker, positive control (480 bp) and SFTSV received from the Centers for Disease Control and Prevention.

**Table 2.** Conditions for RT-PCR

A. RT-PCR reaction component

| Component | Volume |
|---|---|
| 2X One-step RT-PCR Pre-Mix | 15 µL |
| Primer F (10 pmol/µL) | 12.5 µL |
| Primer R (10 pmol/µL) | 1.25 µL |
| Template RNA | 1.75 µL |
| RNase, DNase-Free water | 1.75 µL |
| Total reaction volume | 25 µL |

B. RT-PCR reaction conditions

| Temperature | Time | Number of cycle |
|---|---|---|
| 50° C | 30 min. | 1 cycle |
| 95° C | 15 min. | 1 cycle |
| 95° C | 20 sec. | |
| 58° C | 40 sec. | 35 cycles |
| 72° C | 30 sec. | |
| 72° C | 5 min | 1 cycles |

# RESULTS

## 1. Collection of ticks using a tick trap

In total, 3,090 ticks from the genus *Haemaphysalis* were collected using tick traps. We collected 950 ticks from grass fields (30.7%), 883 from copses (28.6%), 847 from mountain roads (27.4%), and 410 from tombs (13.3%) using tick traps (Table 3). The monthly collection results included 106 ticks in March, followed by 720 in May and 618 in June (Table 4). The tick population increased in May, then decreased for several months, and then increased again in October. Two species of ticks collected, 2,944 ticks were identified as *H. longicornis* and 146 were identified as *H. flava* (Figure 1). *H. longicornis* was the dominant

species, making up 95.3% of the total. We collected 709 *H. longicornis* ticks in May and 617 in June. We collected 59 *H. flava* ticks in November and 29 in September (Table 4). The distribution by stage of development of the 2,944 *H. longicornis* collected was 610 larvae, 1,556 nymphs, and 778 adult ticks (230 males and 548 females). For *H. flava*, we collected 54 nymphs and 94 adult ticks (44 males and 50 females), but no larvae were observed. In May, the distribution of *H. longicornis* according to the collection environment was 360 ticks from copses, 244 from mountain roads, 52 from grass fields, and 53 from tombs. In June, the distribution of *H. longicornis* according to the collection environment was 231 ticks from grass fields, 200 from mountain roads, 163 from copses, and 24 from tombs (Table 5).

## 2. Distribution of ticks collected using the flagging method

In total 1,569 ticks from the genus *Haemaphysalis* were collected using the flagging method. We collected 864 ticks in May and 705 ticks in June. *H. longicornis* was the dominant species with 1,496 individuals, making up 95.3% of the total, whereas for *H. flava*, we collected 73 individuals. In May, 800 *H. longicornis* ticks were collected, including 752 nymphs and 48 adults (10 males and 38 females). At the same time, 64 *H. flava* ticks were collected, including 2 larvae, 25 nymphs, 33 adults (13 males and 22 females). In June, 696 *H. longicornis* ticks were collected, including 2 larvae, 673 nymphs, and 21 adults (9 males and 12 females). Only nine *H. flava* were collected, all were female adults.

**Table 4.** Monthly distributional studies of tick species using tick trap

| Month | *H. longicornis* | *H. flava* | Total |
|---|---|---|---|
| | N (%) | | |
| Mar | 85 (2.7) | 21 (0.7) | 106 (3.4) |
| Apr | 246 (8.0) | 23 (0.7) | 269 (8.7) |
| May | 709 (22.9) | 11 (0.4) | 720 (23.3) |
| Jun | 617 (20.0) | 1 | 618 (20.0) |
| Jul | 404 (13.1) | 1 | 405 (13.1) |
| Aug | 401 (13.0) | 1 | 402 (13.0) |
| Sep | 35 (1.1) | 29 (0.9) | 64 (2.1) |
| Oct | 141 (4.6) | 0 | 141 (4.6) |
| Nov | 306 (9.9) | 59 (1.9) | 365 (11.8) |
| Total | 2,944 (95.3) | 146 (4.7) | 3,090 (100) |

Abbreviation: *H*, *Haemaphysalis*.

**Table 3.** Number of tick collected by environment using tick trap and flagging

| Environment | Tick trap | Flagging | Total (%) |
|---|---|---|---|
| Glass field | 950 | 196 | 1,146 (24.6) |
| Copse | 883 | 704 | 1,587 (34.1) |
| Mountain road | 847 | 472 | 1,319 (28.3) |
| Tomb | 410 | 197 | 607 (13.0) |
| Total | 3,090 | 1,569 | 4,659 (100) |


**Figure 1.** Photograpic featutes of *Ixodes spp.* collected at Jeju Island in 2018. (A) *H. longicornis* (male), (B) *H. longicornis* (female), (C) *H. flava* (male), (D) *H. flava* (female), (E) *H. longicornis* Nymph (left) and *H. flava* Nymph (right).

**Table 5.** Monthly distributional studies of *H. longicornis* and *H. flava* based on the developmental stages collected from the four environments in Jeju

| Month | Environment | Larva | | Nymph | | Male | | Female | | Tick number |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *H. longicornis* | *H. flava* | *H. longicornis* | *H. flava* | *H. longicornis* | *H. flava* | *H. longicornis* | *H. flava* | |
| Mar | Glass field | | | | | | | | | 106 |
| | Copse | | | 3 | 2 | | | 3 | 3 | |
| | Mountain road | | | 32 | 1 | 4 | 1 | 3 | 1 | |
| | Tomb | | | 38 | 8 | | 2 | 2 | 3 | |
| Apr | Glass field | | | 155 | 2 | 10 | | 13 | | 269 |
| | Copse | | | 4 | 3 | 1 | 1 | | 4 | |
| | Mountain road | | | 59 | 6 | | 4 | 1 | 2 | |
| | Tomb | | | 1 | 1 | | | 2 | | |
| May | Glass field | | | 50 | | | | 2 | | 720 |
| | Copse | | | 360 | 1 | | | | | |
| | Mountain road | | | 200 | 4 | 15 | 3 | 29 | 3 | |
| | Tomb | | | 34 | | 5 | | 14 | | |
| Jun | Glass field | | | 220 | | 3 | | 8 | | 618 |
| | Copse | | | 120 | | 10 | | 33 | | |
| | Mountain road | | | 160 | | 11 | 1 | 28 | | |
| | Tomb | | | 11 | | 6 | | 7 | | |
| Jul | Glass field | | | 13 | | 71 | | 6 | 1 | 405 |
| | Copse | 30 | | 14 | | 15 | | 58 | | |
| | Mountain road | | | 2 | | 11 | | 67 | | |
| | Tomb | | | 42 | | 22 | | 53 | | |
| Aug | Glass field | 11 | | 4 | | 5 | | 40 | | 402 |
| | Copse | 110 | | | | 3 | | 12 | 1 | |
| | Mountain road | | | 13 | | 27 | | 112 | | |
| | Tomb | 11 | | | | 6 | | 47 | | |
| Sep | Glass field | | | | | | | | | 64 |
| | Copse | | | 3 | 2 | | 7 | | 5 | |
| | Mountain road | 28 | | 2 | 4 | 1 | 4 | | 5 | |
| | Tomb | | | | | | 2 | 1 | | |
| Oct | Glass field | | | | | | | | | 141 |
| | Copse | 50 | | 1 | | | | | | |
| | Mountain road | | | | | | | | | |
| | Tomb | 90 | | | | | | | | |
| Nov | Glass field | 280 | | 13 | 18 | | 10 | 1 | 14 | 365 |
| | Copse | | | 2 | | 3 | 9 | 2 | 8 | |
| | Mountain road | | | | | | | 3 | | |
| | Tomb | | | | | 1 | | 1 | | |
| Total | | 610 | 0 | 1,556 | 52 | 230 | 44 | 548 | 50 | 3,090 |

In May, the distribution of *H. longicornis* according to the collection environment was 361 from copses, 265 from mountain roads, 131 from grass fields, and 43 from tombs. The distribution of *H. flava* according to the collection environment was 32 from copses, 14 from mountain roads, and 9 from tombs and grass fields. In June, the distribution of *H. longicornis* according to the collection environment was 306 ticks from copses, 193 from mountain roads, 145 from tombs, and 52 from grass fields. For *H. flava*, the distribution was 5 ticks from copses and 4 ticks from grass fields (Table 6).

### 3. Monitoring of SFTSV in the collected ticks

We monitored 3,090 ticks (Trap) and 1,569 ticks (Flagging method) in pooled samples for the presence of SFTSV using RT-PCR. 235 Pools (Trap) and 129 Pools (Flagging method) consisted of 50 larvae, 30 nymphs, and 5 adult ticks per pool were set up at each collected site. All the ticks tested were confirmed to be negative

**Table 6.** Distributional studies of ticks based on the developmental stages collected from the four environments in Jeju

| Month | Environment | *H. longicornis* | | | | | *H. flava* | | | | | Tick number |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Larva | Nymph | Male | Female | Total | Larva | Nymph | Male | Female | Total | |
| | | N (%) | | | | | | | | | | |
| May | Mountain road | | 262 | 1 | 2 | 265 | | 6 | 3 | 5 | 14 | 279 (17.8) |
| | Tomb | | 41 | | 2 | 43 | | 6 | 2 | 1 | 9 | 52 (3.3) |
| | Copse | | 323 | 8 | 30 | 361 | | 11 | 8 | 13 | 32 | 393 (25.1) |
| | Glass field | | 126 | 1 | 4 | 131 | 2 | 4 | | 3 | 9 | 140 (8.9) |
| Jun | Mountain road | | 184 | 5 | 4 | 193 | | | | | | 193 (12.3) |
| | Tomb | 2 | 140 | 2 | 1 | 145 | | | | | | 145 (9.2) |
| | Copse | | 305 | 1 | | 306 | | | | 5 | 5 | 311 (19.8) |
| | Glass field | | 44 | 1 | 7 | 52 | | | | 4 | 4 | 56 (3.6) |
| Total | | 2 (0.1) | 1,425 (90.8) | 19 (1.2) | 50 (3.2) | 1,496 (95.3) | 2 (0.1) | 27 (1.7) | 13 (0.8) | 31 (2.0) | 73 (4.7) | 1,569 (100) |

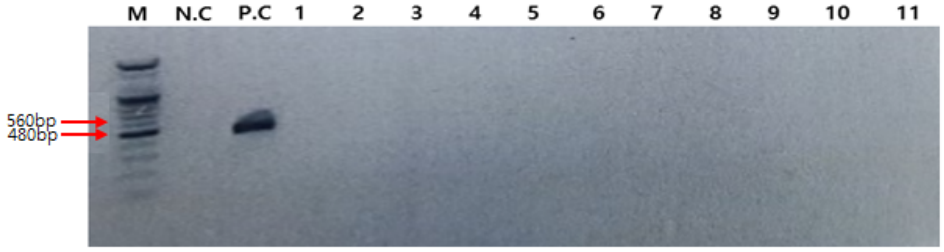


**Figure 2.** The results of amplification of SFTSV RNAs by RT-PCR from the pooling ticks collected from the four environment in Jeju city, 2018. Lane M show molecular weight marker, lane N.C, negative control; lane P.C, positive control (560 bp); lane 1~11, PCR products from the pooling ticks.

for SFTSV (Figure 2).

## DISCUSSION

Infectious-disease vectors, such as mosquitoes and ticks, are sensitive to climate change. Rising temperatures and increased precipitation may lead to increased lifespans, increased metabolic rates, and increased reproductive rates. These changes in ecological factors may result in higher incidence and greater spread of infectious diseases, emergence of new allergens, and genetic mutations [1].

Ticks are usually collected along the boundary of a forested area, a hillside with copses of trees, and burial sites. These environments provide opportunities for population growth and proliferation while using wild animals as hosts. In these situations, common infectious diseases vectored by external parasites can take hold.

When using tick traps, we collected the most ticks in grass fields, followed by copses, mountain roads, and tombs, in descending order. When using the flagging method, we collected the most ticks in copses, followed by mountain roads, tombs, and grass fields, again in descending order (Table 6). Therefore, continuous monitoring for SFTSV will be required in all collection environments. Ticks were collected monthly using tick traps. Ticks were collected in May and June using the flagging method.

Thirty-two species of ticks have been reported in Korea. In this survey, *H. longicornis* was the most common tick collected: 2,944 (95.3%) were collected with tick traps and 1,496 (95.3%) were collected with the flagging method. For *H. flava*, 146 (4.7%) were collected with tick traps and 73 (4.7%) were collected with the flagging method. In other studies, 161 (85%) *H. longicornis* were collected in Chungju in 2002 and 2003 [11], 158 (95.7%) were collected in Gangwon-do in 2015 [3], and 3,823 (98.8%) were collected in Jeonnam in 2016 [12], showing similar percentage patterns to this survey. It has been confirmed that *H. longicornis* is distributed widely not only in inland areas, but also in

all parts of Korea, including Jeju Island.

Ticks consist of the steps of egg-larva-nymph-adult. When hatching from an egg, a tick becomes a larva with three pairs of legs, adhering to the host during the larval and nymph infestation period, and then molting to develop into an adult. Differences in the developmental stages occur according to the season. In the case of the widely distributed *H. longicornis*, larvae were collected starting in July and 132 were collected in August, 28 in September, 140 in October, and 280 in November. Nymphs showed a higher collection rate over the entire survey period compared to other stages of development, with the most collected in May and June (Table 5, 6). Adults were collected regularly from March to November, but were concentrated between May and August, showing a distinct seasonal difference. One report [12] states that ticks were collected in October (325 ticks) from the Dulle-gil of Jirisan Mountain in Jeollanam-do. Nymphs hit a record high in May, showing a much higher collection rate than the other stages of development over the entire survey period. Larvae have been reported to appear in August [11, 13]. According to another report [12], nymphs were collected intensively from April to June, and 94% of adults were collected from June to August, showing a distinct seasonal difference. Compared with these previous reports, the results of this survey are not very different. Due to the climatic characteristics of the Jeju area, collection took place as early as March and as late as November. The number of ticks collected in September dropped sharply, which is believed to be due to the heavy precipitation caused by a seasonal typhoon.

As a way to diagnose SFTSV, Yun et al [14] examined the medium segment gene, a specific gene of SFTSV, using RT PCR for *H. longicornis* in 189 pools, and found 18 positive pools. In a study conducted in Jeollanam-do Province in 2016, SFTSV was not detected in gene analysis using the 2X OneStep RT-PCR method with 127 pooled ticks [12]. In the present study, 3,090 ticks (235 pools) collected using tick traps and 1,569 (129 pools) collected using the flagging method were monitored for SFTSV by RT-PCR. All pools tested were confirmed to be negative. Detection of the virus in the ticks varies depending on the existence of the virus in the ticks or the titer of the virus. In the future, collection areas and collection timing should be diversified and research on detection methods that can enhance sensitivity and specificity should be developed.

## 요 약

제주지역의 4개 환경(초지, 잡목림, 산길, 무덤)에서 2018년 3월부터 11월까지 진드기 채집을 통하여 진드기의 분포 특성 및 SFTSV 병원체 보균 여부를 조사하였다. Tick trap을 이용해 채집된 3,095마리와 flagging을 이용하여 채집된 1,569마리를 채집하였다. 총 4,664마리의 채집된 진드기 중에서 *Haemaphysalis longicornis*가 4,440마리(95.2%)로 대부분을 차지하였고, *H. flava*는 224마리(4.8%)가 채집되었다. 환경적으로는 Tick trap은 초지(953마리), 잡목림(883마리), 산길(847마리), 무덤(411마리)가 채집되었고, flagging은 잡목림(704마리), 산길(472마리), 무덤(197마리), 초지(196마리)가 채집되었다. 발육단계별로는 유충은 5월부터 채집되어 10월에 140마리, 11월에 280마리가 채집되었다. 약충은 4월부터 8월에 집중적으로 채집(2,978마리)되었고, 5월과 6월에 가장 많이 채집되었다. 성충은 6월부터 8월까지 전체 성충 중 94%가 채집되었다. 채집된 *H. longicornis* 4,440마리와 *H. flava* 224마리에서 SFTSV는 확인되지 않았다.

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**Conflict of interest:** None

**Author's information (Position):** Chung KA, Professor; Song HJ, Professor; Lee HJ, Professor; Park C, Professor; Seo MY, Professor.

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