

# Monthly Occurrence of Vectors and Reservoir Rodents of Scrub Typhus in an Endemic Area of Jeollanam-do, Korea

Seung Hyun Lee<sup>1,7</sup>, Young-Sun Lee<sup>2</sup>, In Yong Lee<sup>3</sup>, Jae Won Lim<sup>4</sup>, Hee-Kwan Shin<sup>5</sup>, Jae-Ran Yu<sup>6</sup> and Seobo Sim<sup>6,7,\*</sup>

<sup>1</sup>Department of Microbiology, Konkuk University School of Medicine, Chungju 380-701, Korea; <sup>2</sup>Suncheon Health Service Center, Suncheon 540-260, Korea; <sup>3</sup>Department of Environmental Medical Biology and Institute of Tropical Medicine, Yonsei University College of Medicine, Seoul 120-752, Korea; <sup>4</sup>National Institute of Biological Resources, Incheon 404-708, Korea; <sup>5</sup>Seoul Science High School, Seoul 110-530, Korea; <sup>6</sup>Department of Environmental and Tropical Medicine, Konkuk University School of Medicine, Chungju 380-701, Korea; <sup>7</sup>IBST and Institute of Functional Genomics, Konkuk University, Chungju 380-701, Korea

**Abstract:** Monthly surveys were conducted to investigate the occurrence of chigger mites and seroprevalence of scrub typhus among small mammals in Jeollanam-do, the southwestern part of Korea, from November 2006 through October 2007. Fifty-eight small mammals, including 57 *Apodemus agrarius* (98.3%) and 1 *Crocidura lasiura* (1.7%), were captured, and a total of 4,675 chigger mites representing 4 genera and 8 species were collected from them. The chigger infestation rate among small mammals was 69.0%. The most predominant species in *A. agrarius* was *Leptotrombidium scutellare* (54.0%), followed by *Leptotrombidium pallidum* (39.4%), *Leptotrombidium orientale* (4.4%), *Leptotrombidium palpale* (1.1%), *Neotrombicula tamiyai* (0.6%), *Eushoengastia koreaensis* (0.3%), *Neotrombicula gardellai* (0.3%), and *Cheladonta ikaoensis* (<0.1%). The chigger index of *A. agrarius* was the highest in October (740.0), followed by November (242.0), September (134.6), March (98.3), February (38.2), January (35.3), December (34.5), April (30.8), and May (1.7). The average antibody positive rate of scrub typhus in wild rodents was 50.0%. The seropositive rates were high in October (100.0%) and November (83.3%), whereas those in other months were relatively low (28.6-57.1%). The chigger index of *L. scutellare* rapidly increased in September to form an acuminate peak in October, followed by a gradual decline. These results suggest that the outbreak of scrub typhus in the southwestern part of Korean peninsula is mostly due to *L. scutellare*.

**Key words:** *Leptotrombidium scutellare*, *Leptotrombidium pallidum*, *Apodemus agrarius*, scrub typhus, monthly occurrence

## INTRODUCTION

Scrub typhus (tsutsugamushi disease) caused by the obligate intracellular bacterium *Orientia tsutsugamushi* is an acute febrile infectious disease transmitted through larval bites of trombiculid mites, also known as chigger mites [1,2]. *O. tsutsugamushi* is naturally maintained with the cycles of chigger mites and wild rodents. It can be transovarially transmitted in chigger mites [3,4]. Scrub typhus is endemic in geographic regions, including Korea, China, Japan, Thailand, and other Asian countries [1,2]. Since the first Korean case of scrub typhus was reported in 1985 [5], the annual number of cases has increased to more than 5,000 in 2005-2008 [6]. The southwestern and

middle-western parts of Korea, such as Jeollanam-do, Jeollabuk-do, and Chungcheongnam-do, showed higher prevalence than other areas [7]. Although several epidemiological data showing relative abundance of vector mites, their hosts, and geographic distribution were published, there are no reports demonstrating monthly occurrence of chigger mites and wild rodents during a whole year in the Republic of Korea. The present study was conducted to identify monthly population dynamics of chigger mites and rodents, and seroprevalence rates of scrub typhus in wild rodents in an endemic area of Jeollanam-do which is located in the southwestern part of the Korean peninsula.

## MATERIALS AND METHODS

### Collection localities

Monthly surveillance of field rodents was conducted from November 2006 through October 2007. The collection site was located in an endemic region of scrub typhus in Hoeryong-ri,

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\*Corresponding author (ssim@kku.ac.kr)

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Hwangjeon-myeon, Suncheon-si, Jeollanam-do, Korea (127° 30'12.8"E, 35°36'11.5"N) (Fig. 1). The monthly average temperature and precipitation in Jeollanam-do was based on the data of Korea Meteorological Administration.

#### Collection of field rodents and chigger mites

Field rodents were captured by Sherman live traps (7.6 × 8.9 × 22.9 cm; H.B. Sherman, Tallahassee, Florida, USA) baited with peanut butter placed between 2 saltine crackers. The traps were set up before sunset and collected early in the next morning. Live-captured rodents were transported to the laboratory, where they were anesthetized. After identifying the species, their blood was taken for detection of antibodies. The bodies of the rodents were hung individually over a 1,000 ml beaker filled to a depth of 1 cm with tap water for harvesting the larval mites. The mites which fell into the water were removed with a fine brush and placed in 75% ethanol until mounted on slides with polyvinyl alcohol media. The larval mites were identified under a light microscope using morphological keys prepared by Ree [8].

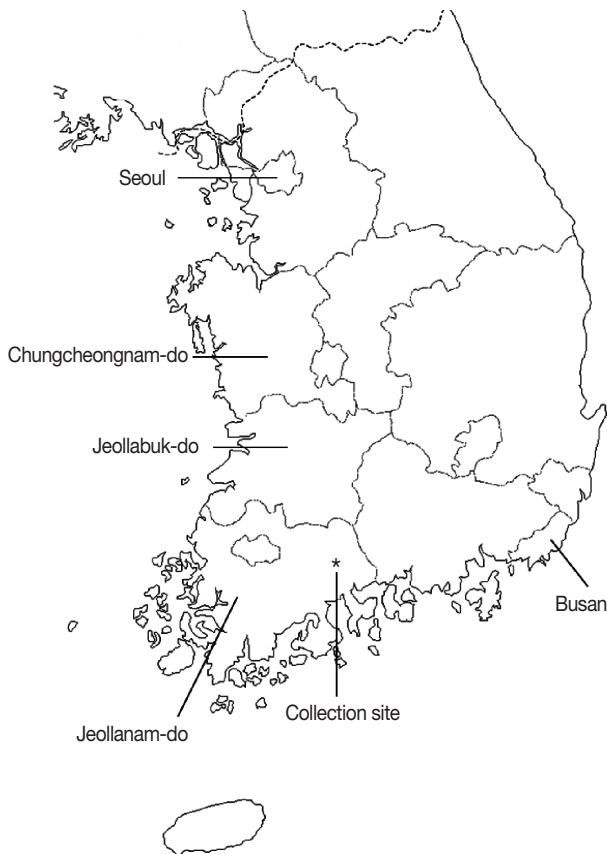


Fig. 1. Map of the collection site.

#### Detection of *O. tsutsugamushi*-specific antibodies

Blood samples were centrifuged at 1,000 g for 10 min, and sera were maintained at -70°C until assayed for the presence of antibodies against *O. tsutsugamushi*. The sera were diluted 1:32 in PBS and examined for IgG antibodies against *O. tsutsugamushi* Karp and Gilliam stains by the indirect immunofluorescence assay (IFA). The IFA antigen slide was placed in a moist chamber to maintain the humidity throughout the procedure. Diluted sera to be tested were deposited on a spot slide and incubated at 37°C for 30 min. After washing 3 times with PBS for 5 min, fluorescein isothiocyanate-conjugated goat anti-mouse antibody (MP Biomedicals, Aurora, Ohio, USA) was pipetted onto each spot, and the slide was incubated in a humidified chamber at 37°C for 30 min. The slide was washed 3 times each for 5 min with PBS and then air-dried. The slide spots were mounted with glycine-buffered glycerol under cover slips and examined for characteristic cytoplasmic fluorescent patterns with a fluorescence microscope (50W, Zeiss Co, Mainz, Germany).

## RESULTS

#### Monthly occurrence of field rodents and chigger mites

A total of 58 small mammals representing 2 genera and 2 species were collected. The common striped field mouse, *Apodemus agrarius* (98.3%) was predominantly collected, while only 1 insectivore *Crocodyrus lasiura* (1.7%) was collected. Thirty-nine of 57 (68.4%) *A. agrarius* and 1 *C. lasiura* were infested with larval mites. The monthly trapping rates ranged from 4.4% to 16.0% (Table 1). In total, 4,675 chiggers, representing 4 genera and 8 species, were collected from small mammals. Of 4,658 chiggers (99.6%) collected from *A. agrarius*, *Leptotrombidium scutellare* was the most predominant species (54.0%), followed by *Leptotrombidium pallidum* (39.4%), *Leptotrombidium orientale* (4.4%), *Leptotrombidium palpale* (1.1%), *Neotrombicula tamiyai* (0.6%), *Eushoengastia koreaensis* (0.3%), *Neotrombicula gardellai* (0.3%), and *Cheladonta ikaensis* (<0.1%) (Table 2). The monthly occurrence and chigger indices of each chigger mite from *A. agrarius* are shown in Table 3 and Fig. 2. The chigger index was the highest in October (740.0), followed by November (242.0), September (134.6), March (98.3), February (38.2), January (35.3), December (34.5), April (30.8), and May (1.7). There was no chigger during the summer season (June-August). The chigger index of *L. scutellare* increased rapidly in September to form an acuminate peak in October, fol-

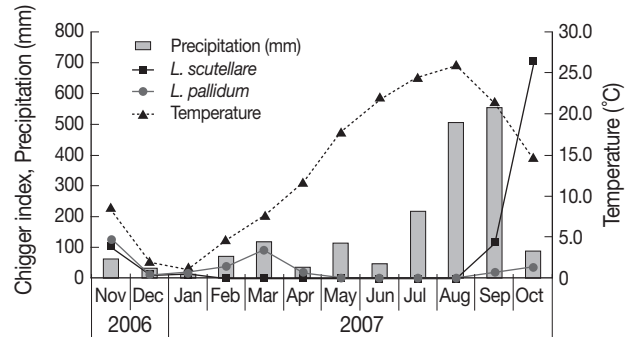
lowed by a gradual decline, whereas that of *L. pallidum* reached a peak in November.

**Table 1.** Monthly trapping rates and chigger infestation status of field small mammals in Jeollanam-do from November 2006 through October 2007

Month	Trapping rate (No. of collection /No. of traps)	<i>Apodemus agrarius</i>		<i>Crocidura lasiura</i>	
		No. of collection	No. with chiggers	No. of collection	No. with chiggers
Nov 5, 2006	14.0% (7/50)	6	6	1	1
Dec 5, 2006	16.0% (8/50)	8	7	0	0
Jan 5, 2007	6.6% (3/45)	3	3	0	0
Feb 12, 2007	8.8% (4/45)	4	4	0	0
Mar 12, 2007	12.0% (6/50)	6	6	0	0
Apr 12, 2007	12.0% (6/50)	6	6	0	0
May 22, 2007	14.0% (7/50)	7	2	0	0
Jun 18, 2007	4.4% (2/45)	2	0	0	0
Jul 21, 2007	6.6% (3/45)	3	0	0	0
Aug 21, 2007	15.5% (7/45)	7	0	0	0
Sep 21, 2007	6.0% (3/50)	3	3	0	0
Oct 21, 2007	4.4% (2/45)	2	2	0	0
Total	10.2% (58/570)	57	39	1	1

*O. tsutsugamushi*-specific antibodies in field rodents

Of 40 *A. agrarius*, 20 (50.0%) were positive for antibodies to *O. tsutsugamushi*. Seropositive rates of scrub typhus were higher in October (100%) and November (83.3%), whereas those in other months were relatively lower (28.6-57.1%) (Table 4).



**Fig. 2.** Monthly average temperature, precipitation, and chigger indices of *A. agrarius* in Jeollanam-do from November 2006 through October 2007.

**Table 2.** Number of chigger mites by species collected from small mammals in Jeollanam-do from November 2006 through October 2007

Hosts	No.	<i>L. scu</i>	<i>L. pall</i>	<i>L. palp</i>	<i>L. ori</i>	<i>N. tam</i>	<i>N. gar</i>	<i>C. ika</i>	<i>E. kor</i>	Subtotal (%)
<i>Apodemus agrarius</i>	57	2,514	1,837	49	203	27	12	1	15	4,658 (99.6)
<i>Crocidura lasiura</i>	1	15	1	0	1	0	0	0	0	17 (0.4)
Total (%)	58	2,529 (54.1)	1,838 (39.3)	49 (1.0)	204 (4.4)	27 (0.6)	12 (0.3)	1 (<0.1)	15 (0.3)	4,675 (100.0)

*L. scu*: *Leptotrombidium scutellare*; *L. pall*: *L. pallidum*; *L. palp*: *L. palpalis*; *L. ori*: *L. orientalis*; *N. tam*: *Neotrombicula tamiyai*; *N. gar*: *N. gardellai*; *C. ika*: *Cheladonta ikaensis*; *E. kor*: *Eushoengastia koreaensis*.

**Table 3.** Monthly chigger indices by species collected from *A. agrarius* in Jeollanam-do from November 2006 through October 2007

Month	<i>L. scu</i>	<i>L. pall</i>	<i>L. palp</i>	<i>L. ori</i>	<i>N. tam</i>	<i>N. gar</i>	<i>C. ika</i>	<i>E. kor</i>	Total
Nov 5, 2006	105.0	122.3	5.2	6.0	0.3	1.3	0	1.9	242.0
Dec 5, 2006	11.6	11.8	1.3	6.6	2.2	0.5	0	0.5	34.5
Jan 5, 2007	11.6	19.7	0	4.0	0	0	0	0	35.3
Feb 12, 2007	0	37.8	0	0.2	0.2	0	0	0	38.2
Mar 12, 2007	0	91.8	1.3	4.2	1.0	0	0	0	98.3
Apr 12, 2007	0	19.7	0	11.1	0	0	0	0	30.8
May 22, 2007	0	0.4	0	1.3	0	0	0	0	1.7
Jun 18, 2007	0	0	0	0	0	0	0	0	0
Jul 21, 2007	0	0	0	0	0	0	0	0	0
Aug 21, 2007	0	0	0	0	0	0	0	0	0
Sep 21, 2007	116.0	18.6	0	0	0	0	0	0	134.6
Oct 21, 2007	704.0	35.5	0	0	0	0	0.5	0	740.0

*L. scu*: *Leptotrombidium scutellare*; *L. pall*: *L. pallidum*; *L. palp*: *L. palpalis*; *L. ori*: *L. orientalis*; *N. tam*: *Neotrombicula tamiyai*; *N. gar*: *N. gardellai*; *C. ika*: *Cheladonta ikaensis*; *E. kor*: *Eushoengastia koreaensis*.

## DISCUSSION

Scrub typhus is regarded as one of the most widespread diseases in Korea which occur mainly in autumn. In this study, we have shown that *A. agrarius* (98.3%) accounted for nearly all of the live-captured small mammals in the field. This agrees to previous results that *A. agrarius* was predominant in rural areas of the Korean peninsula [5,9-11]. *Leptotrombidium* species, including *L. pallidum*, *L. scutellare*, *L. palpale*, and *L. orientale*, were known as vectors of scrub typhus in Korea [5,12,13], of which *L. pallidum* was predominant in the middle and northern parts of Korea, whereas *L. scutellare* was predominant in the southern parts of Korea [12,13]. Our data showed that *L. scutellare* was the dominant species in Jeollanam-do, followed by *L. pallidum*.

The monthly prevalence of scrub typhus in an endemic region, Jeollanam-do, from November 2006 through October 2007 was shown in Table 5 [6]. In Jeollanam-do, the outbreaks increased drastically in October, peaked in November, and started to decrease in December, when the coldest season begins. Of 617 cases, 598 (96.9%) were reported during these 3 months, while several cases were reported in other months. In the present study, 50% of *A. agrarius* were positive for *O. tsutsugamushi*-specific antibodies, and the seropositive rate in *A. agrarius* was higher in autumn (November and October) than in other seasons (Table 4). *L. scutellare* and *L. pallidum* are active and take meals in cool dry autumn conditions. These findings indicate that *A. agrarius* is the main reservoir animal of scrub typhus. The chigger index was also the highest in October (740.0), fol-

lowed by November (242.0) (Table 3). The discrepancy of the peak period between the outbreaks of scrub typhus and the population density of chiggers might be due to the incubation period of the disease. Scrub typhus has an incubation period of 5-20 days [1].

The chigger index of *L. scutellare* in the autumn season (September and October) was higher than that of other species, while none were collected from February through August (Table 3). The chigger index of *L. palpale*, *L. orientale*, and *E. koreaensis*, which are also known as vectors of scrub typhus, was quite low in the autumn season. In October, the chigger index of *L. scutellare* in Jeollanam-do was 704.0, whereas those in Jeollabuk-do which is located in the middle-western part of the Korean peninsula ranged from 19.4 to 77.6 [14].

The population density of *L. scutellare* in Jeollanam-do was 10 times higher than that in Jeollabuk-do. The annual mean air temperature of the collection site was 13.5°C (Fig. 2). These results support the hypothesis that the most important limited factor for *L. scutellare* distribution is annual mean air temperature above 10°C [13]. Previous reports indicated that the infection rate of *O. tsutsugamushi* among chiggers in Korea was the highest for *L. palpale* (5.3%), followed by *Neotrombicula japonica* (4.3%), *L. scutellare* (3.7%), *L. orientale* (3.6%), *E. koreaensis* (1.9%), and *L. pallidum* (1.5%) [15]. Based on our results and also previous studies, we suggest that the outbreak of scrub typhus in autumn is mainly by *L. scutellare*, whereas a few cases in other seasons are possibly by other species, including *L. pallidum*, *L. palpale*, *L. orientale*, and *E. koreaensis*. The incidence of scrub typhus was positively correlated with temperature and humidity during summer [7]. The arthropod vectors of scrub typhus are ectothermic. Temperature and precipitation can strongly influence their development, reproduction, and population dynamics [16]. As shown in Fig. 2, the chigger index was increased after the hot and humid summer. Because of global warming, *L. scutellare* could expand its distribution to middle or northern parts of the Korean peninsula, and its abundance and population peak could be changed.

The epidemiological study on monthly distributions and population densities of the vectors and reservoir hosts of scrub typhus will be helpful to establish strategies for monitoring the disease transmission, management, and preventive plans.

**Table 4.** Seropositive rates of scrub typhus in *A. agrarius* in Jeollanam-do from November 2006 through October 2007

Month	No. of tested	No. of positive	Seropositive rate (%)
Nov 5, 2006	6	5	83.3
Jan 5, 2007	3	1	33.3
Mar 12, 2007	6	2	33.3
Apr 12, 2007	6	3	50.0
May 22, 2007	7	4	57.1
Jul 21, 2007	3	1	33.3
Aug 21, 2007	7	2	28.6
Oct 21, 2007	2	2	100.0
Total	40	20	50.0

**Table 5.** Monthly prevalence of scrub typhus in Jeollanam-do from November 2006 through October 2007

Month	Nov 2006	Dec 2006	Jan 2007	Feb 2007	Mar 2007	Apr 2007	May 2007	Jun 2007	Jul 2007	Aug 2007	Sep 2007	Oct 2007	Total
No. of cases	421	89	2	1	1	0	1	2	3	2	7	88	617

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