## Larval Chigger Mites Collected from Small Mammals in 3 Provinces, Korea

In-Yong Lee<sup>1,†</sup>, Hyeon-Je Song<sup>2,†</sup>, Yeon-Joo Choi<sup>3</sup>, Sun-Hye Shin<sup>3</sup>, Min-Kyung Choi<sup>3</sup>, So-Hyun Kwon<sup>3</sup>, E-Hyun Shin<sup>4</sup>, Chan Park<sup>4</sup>, Heung-Chul Kim<sup>5</sup>, Terry A. Klein<sup>6</sup>, Kyung-Hee Park<sup>3</sup>, Won-Jong Jang<sup>3,\*</sup>

<sup>1</sup>Department of Environmental Medical Biology, Yonsei University College of Medicine, Seoul 120-752, Korea; <sup>2</sup>Department of Clinical Pathology, Gwangju Health College, Gwangju 506-701, Korea; <sup>3</sup>Institute of Global Disease Control and Department of Microbiology, College of Medicine, Konkuk University, Seoul 143-701, Korea; <sup>4</sup>Division of Medical Entomology, National Institute of Health, Osong 363-951, Korea; <sup>5</sup>5th Medical Detachment, 168th Multifunctional Medical Battalion, 65th Medical Brigade, USA; <sup>6</sup>Public Health Command Region-Pacific, Camp Zama, Japan

**Abstract:** A total of 9,281 larval chigger mites were collected from small mammals captured at Hwaseong-gun, Gyeong-gi-do (Province) (2,754 mites from 30 small mammals), Asan city, Chungcheongnam-do (3,358 mites from 48 mammals), and Jangseong-gun, Jeollanam-do (3,169 for 62 mammals) from April-November 2009 in the Republic of Korea (= Korea) and were identified to species. *Leptotrombidium pallidum* was the predominant species in Hwaseong (95.8%) and Asan (61.2%), while *Leptotrombidium scutellare* was the predominant species collected from Jangseong (80.1%). Overall, larval chigger mite indices decreased from April (27.3) to June (4.9), then increased in September (95.2) and to a high level in November (169.3). These data suggest that *L. pallidum* and *L. scutellare* are the primary vectors of scrub typhus throughout their range in Korea. While other species of larval chigger mites were also collected with some implications in the transmission of *Orientia tsutsugamushi*, they only accounted for 11.2% of all larval chigger mites collected from small mammals.

Key words: Apodemus agrarius, Leptotrombidium pallidum, Leptotrombidium scutellare, chigger index

Orientia tsutsugamushi is gram-negative obligate intracellular bacteria and the causative agent of scrub typhus (tsutsugamushi disease), an acute febrile infectious disease [1,2]. O. tsutsugamushi is maintained in chigger mites (the family Trombiculidae) by transovarian transmission and transmitted by bite of zoonotic hosts and incidentally to humans [1,2]. Zoonotic hosts for larval chigger mites are small mammals and with mite species demonstrating preferential host-feeding patterns [3]. Apodemus agrarius, Micromys minutus, Mus musculus, Microtus fortis, Rattus norvegicus, and Myodes regulus are commonly collected from various habitats throughout the Republic of Korea (= Korea) and host to larval trombiculid mites (other stages are free-living); they were shown to be serologically positive for scrub typhus [3-5]. Leptotrombidium pallidum was the predominant larval chigger mite collected from small mam-

© 2014, Korean Society for Parasitology and Tropical Medicine
This is an Open Access article distributed under the terms of the Creative Commons
Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0)
which permits unrestricted non-commercial use, distribution, and reproduction in any
medium, provided the original work is properly cited.

mals that were captured from northern provinces (Gyeonggi-do and Gangwon-do) [3,4], while *Leptotrombidium scutellare* was the predominant chigger mite collected from small mammals captured from southern provinces of Korea [6].

The purpose of larval chigger mite surveillance was to analyze and identify small mammal hosts, larval chigger mite host preferences, and seasonal abundance of larval chigger mites associated with the seasonal prevalence of scrub typhus (prevalence of scrub typhus is reported separately), in addition to determine the northern limits of L. scutellare. Areas surveyed in this study were geographically separated and ecologically variable. Small mammals, including rodents and soricomorphs were live captured from Hwaseong-gun (1 site), Gyeonggi-do (Province) (37°02′25.3″ N, 126°52′11.0″ E) and Asan city (1 site), Chungcheongnam-do (36°45′43.2″ N, 126°52′04.2″ E) in the west central region, and Jangseong-gun (9 sites), Jeollanam-do (36°45′43.2″ N, 126°52′04.2″ E) in the southwestern region of Korea from April-November 2009 (Fig. 1).

Sherman® live capture traps  $(7.7 \times 9 \times 23 \text{ cm})$ , i.e., aluminum folding traps (H.B. Sherman, Tallahassee, Florida, USA) baited with peanut butter and rolled oats (1:3 ratio by vol-

<sup>•</sup> Received 14 October 2013, revised 24 February 2014, accepted 25 February 2014.

<sup>†</sup> In-Yong Lee and Hyeon-Je Song contributed equally to this work.

<sup>\*</sup>Corresponding author (wjjang@kku.ac.kr)

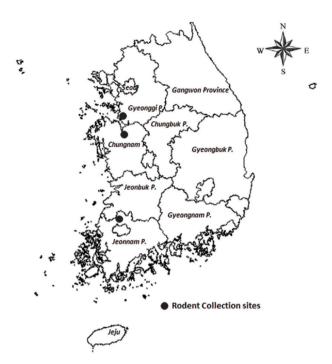


Fig. 1. Small mammal survey sites. (1) Jangan-ri, Jangan-myeon, Hwaseong, Gyeonggi-do (Province). (2) Gundeok-ri, Seonjang-myeon, Asan, Chungcheongnam-do, in the central region. (3) Bukil-myeon, Jangseong-gun, Jeollanam-do in southern region.

ume) were set in 6 trap lines, each consisting of 10 traps set at 1-3 m intervals. Traps were set at 15:00-17:00 hr and collected the following morning between 06:00-07:00 hr. Traps positive for small mammals were sequentially numbered according to the site and returned to the central laboratory, Konkuk University, Seoul, Korea. Each small mammal was given a unique identification code, anesthetized using CO<sub>2</sub>, identified to species, sexed, weighed, and then euthanized by cardiac puncture in accordance with Konkuk University Animal Use Guidelines. Following cardiac puncture, the spleen, kidneys, and liver tissues were removed, and the animal carcasses hung by the heels over a container with water to collect chigger mites as described by Ree et al. [6]. The population densities, by species of larval chigger mite, were calculated as the mean number of larval chigger mites per infested small mammal by species.

Overall, a total of 140 small mammals consisting of 3 species (A. agrarius, M. regulus, and Crocidura lasiura) were collected (Table 1). The trap rates were highest at Hwaseong (23.3%) and lowest at Jangseong (13.7%). Only A. agrarius (n = 30) was collected from Hwaseong, while A. agrarius and M. regulus were collected from Asan, and A. agrarius, M. regulus, and C. lasiura were collected at Jangseong. A. agrarius is the most commonly collected rodent throughout most areas of Korea [5],

and during this study it accounted for 92.1%, while C. lasiura and M. regulus accounted for 6.4% and 1.4% of the small mammals collected, respectively. Small mammals were trapped during the spring/early summer (April-June) and again during the fall (September-November) (Table 1). Overall, larval chigger mite infestation rates for A. agrarius were 72.1%, 67.9%, and 86.7% for Asan, Jangseong, and Hwaseong, respectively (Table 1). The mean larval chigger mite infestation rate for A. agrarius was 73.1%, ranging from a low level of 44.4% (June) to a high level of 100% (November) (Table 1). Seasonal larval chigger mite indices decreased from 27.3 in April to 4.9 in June, then increased from 95.2 in September to high levels of 180.0 and 169.3 in October and November, respectively. Only 1/2 M. regulus was infested with larval chigger mites during October when larval chigger indices were high for A. agrarius. Chigger indices for M. regulus and C. lasiura captured during October were similar, 73.0% and 77.0% respectively.

A total of 5,088 L. pallidum and 3,422 L. scutellare were collected from A. agrarius (Tables 2, 3). The proportions of L. pallidum and L. scutellare collected from each site were highest during the late fall (October and November). Other larval chigger mites only accounted for 0.3% of the total number collected at Hwaseong, 16.6% at Asan, and 7.6% at Jangseong. The greatest diversity of larval chigger mites was observed at Asan, with 10 larval chigger mite species, while there were only 6 and 4 species observed at Jangseong and Hwaseong, respectively. Both Hwaseong and Asan had the highest proportion of L. pallidum, accounting for 95.8% and 61.2% of all chigger mites collected, respectively (Table 3). For A. agrarius, the lowest L. scutellare mite indices were observed at Hwaseong (3.2), the most northern collection site. The larval chigger mite indices increased for L. scutellare collected from A. agrarius at Asan (22.6), south of Hwaseong, and were highest at Jangseong (74.2), the most southern collection site.

In this study, as well as others, larval chigger indices were highest prior to and during periods when peak numbers of scrub typhus cases were observed [2]. These studies provide evidence that *L. scutellare* is the principal vector in the southern region, while *L. pallidum* is the principal vector in the northern region of Korea, and with transmission of *O. tsutsugamushi* correlated with high larval chigger mite indices for both species in the fall [7,8]. It has been shown that the northern limit line of *L. scutellare* is reported for areas where the annual mean temperature is greater than 10°C [9]. During this study, low numbers of *L. scutellare* were collected where the mean temperature

Table 1. Number of traps set, number of small mammals collected (trap rate®), number small mammals infested (infestation rate®) with larval chigger mites, number (chigger mite index®) of larval chigger mites collected from small mammals at Hwaseong (Gyeonggi-do), Asan (Chungcheongnam-do) and Jangseong (Jeollanam-do) from April-November 2009, Korea

)	)									,				
			Apo	Apodemus agrarius	ius	Z	Myodes regulus	S	Ŏ	Crosidura lasiura	ä		Total	
Sites	Month	No. traps	No. Collected (Trap Rate) <sup>a</sup>	No. w/Chiggers (Infestation Rate) <sup>b</sup>	No. Chiggers (Chigger Index)°	No. Collected (Trap Rate) <sup>a</sup>	No. w/Chiggers (Infestation Rate) <sup>b</sup>	No. Chiggers (Chigger Index)°	No. Collected (Trap Rate) <sup>a</sup>	No. w/Chiggers (Infestation Rate) <sup>b</sup>	No. Chiggers (Chigger Index)°	No. Collected (Trap Rate) <sup>a</sup>	No. w/Chiggers (Infestation Rate) <sup>b</sup>	No. Chiggers (Chigger Index)°
Hwaseong	Apr.	30	5 (16.6)	3 (60.0)	162 (54.0)	0	0	0	0	0 (	0	5 (16.6)	3 (60.0)	162 (54.0)
	May	52	5 (20.0)	5 (100)	253 (50.6)	0	0	0	0	0 ;	0	5 (20.0)	5 (100)	253 (50.6)
	Jun.	n/d	n/d	p/u	n/d û	n/d	n/d	n/d °	n/d	p/u	n/d °	p/u	p/u (	n/d
	Sep.	/ <sup>2</sup> C	2 (30.0)	0 7 (400)	0 620 2)	0 0	0 0	0 0	0 0	0 0	0 0	2 (30.0)	0 (7	(7 00) 023
	Nov.	32	11 (34.0)	(100)	1,760 (160.0)	0	0	0	0	0	0 0	11 (34.0)	11 (100)	1,760 (160.0)
	Subtotal	129	30	26 (86.7)	2,754 (105.9)	0	0	0	0	0	0	30	26 (86.7)	2,754 (105.9)
Asan	Apr.	65	11 (17.0)	6 (54.5)	136 (22.6)	0	0	0	-	0	0	12 (64.0)	(20.0)	136 (22.6)
	May	30	6 (20.0)	(100)	23 (3.8)	0	0	0	0	0	0	6 (20.0)	(100)	23 (3.8)
	Jun.	40	8 (20.0)	1 (12.5)	3 (3.0)	0	0	0	0	0	0	8 (20.0)	1 (12.5)	3 (3.0)
	Sep.	p/u	p/u	p/u	p/u	p/u	p/u	p/u	p/u	p/u	p/u	p/u	p/u	p/u
	Oct.	65	10 (15.5)	10 (100)	1,739 (173.9)	0	0	0	4	0	0	14 (15.5)	10 (71.4)	1,739 (173.9)
	Nov.	30	8 (26.6)	8 (100)	1,457 (182.1)	0	0	0	0	0	0	8 (26.6)	8 (100)	1,457 (182.1)
	Subtotal	230	43	31 (72.1)	3,358 (108.3)	0	0	0	5 (10.4)	0	0	48	31 (64.6)	3,358 (108.3)
Jangseong	Apr.	06	6 (6.7)	(100)	102 (18.7)	0	0	0	0	0	0	(6.7)	6 (100)	102 (18.7)
	May	80	7 (8.9)	5 (71.4)	14 (3.6)	-	0	0	0	0	0	8 (10)	5 (62.5)	14 (3.6)
	Jun.	06	19 (21.1)	11 (57.9)	34 (5.1)	0	0	0	0	0	0	19 (21.1)	11 (57.9)	34 (5.1)
	Sep.	06	7 (7.8)	5 (71.4)	446 (95.2)	0	0	0	0	0	0	7 (7.8)	5 (71.4)	446 (95.2)
	Oct.	09	17 (28.0)	11 (64.7)	2,423 (247.5)	1 (1.7)	1 (100)	73 (73.0)	4 (7.0)	4 (100)	(0.77) 77	22 (37.0)	12 (54.5)	2,573 (233.7)
	Nov.	p/u	p/u	p/u	p/u	p/u	n/d	p/u	p/u	p/u	p/u	p/u	p/u	n/d
	Subtotal	410	99	38 (67.9)	3,019 (89.1)	2	1 (50.0)	73 (73.0)	4	4 (100)	(0.77) 77	62	39 (62.9)	3,169 (88.9)
Total	Apr.		22 (44.0)	15 (68.2)	400 (27.3)	0	0	0	-	0	0	23 (46.0)	15 (65.2)	400 (27.3)
	May		18 (60.0)	16 (88.9)	290 (18.4)	-	0	0	0	0	0	19 (63.0)	16 (84.2)	290 (18.4)
	Jun.		27 (34.0)	12 (44.4)	37 (4.9)	0	0	0	0	0	0	27 (34.0)	12 (44.4)	379 (4.9)
	Sep.		(0.06) 6	5 (55.6)	446 (95.2)	0	0	0	0	0	0	(0.06) 6	5 (55.6)	446 (95.2)
	Oct.		34 (57.0)	28 (80.0)	4,741 (180.0)	-	1 (100)	73 (73.0)	8 (13.3)	4 (100)	(0.77) 77	43 (72.0)	29 (67.4)	4,891 (176.6)
	Nov.		19 (63.0)	19 (100)	3,217 (169.3)	0	0	0	0	0	0	19 (63.0)	19 (100)	3,217 (169.3)
	Total		129	95 (73.1)	9,131(100.0)	2	1 (50.0)	73 (73.0)	0	4 (100)	(77 (77.0)	140	96 (68.6)	9,281 (99.8)

<sup>a</sup>Trap rate=No. of small mammals captured/No. of traps set.

 $<sup>\</sup>label{eq:proposed_property} $^{\circ}$ In restance of small mammals with larval chigger mites/No. of small mammals captured. $^{\circ}$ Chigger mite index = No. of larval chigger mites collected/No. of small mammals infested with chiggers. $^{\circ}$ In the chigger mite index = No. of larval chigger mites collected/No. of small mammals infested with chiggers.$ 

**Table 2.** Seasonal prevalence of *L. pallidum* and *L. scutellare* and the percent (%) collected by month from small mammals captured at 3 primary localities in Korea

Marath		Leptotrombio	dium pallidum	Leptotrombidium scutellare				
Month	Hwaseong	Asan	Jangseong	Total	Hwaseong	Asan	Jangseong	Total
May	158 (6.0)	86 (4.2)	39 (9.9)	283	n/d <sup>b</sup>	n/d	10 (0.4)	10
June	252 (9.6)	21 (1.0)	14 (3.5)	287	n/d	n/d	n/d	n/d
July	n/d	n/d	22 (5.6)	22	n/d	n/d	5 (0.2)	5
September	n/d	n/d	56 (14.2)	56	n/d	n/d	360 (14.2)	360
October	487 (18.5)	928 (45.1)	264 (66.8)	1,679	92 (83.6)	600 (77.5)	2,163 (85.2)	2,855
November	1,740 (66.0)	1,021 (49.7)	n/d	2,761	18 (16.4)	174 (22.5)	n/d	192
Totala	2,637 (51.8)	2,056 (40.4)	395 (7.8)	5,088	110 (3.2)	774 (22.6)	2,538 (74.2)	3,422

<sup>&</sup>lt;sup>a</sup>Percent of total for each species by location.

Table 3. Number (% of total/collection site) of larval chigger mites, by species, collected from small mammals captured at Hwaseong (Gyeonggi-do), Asan (Chungcheongnam-do) and Jangseong (Jeollanam-do) from April-November 2009, Korea

	Centra	l Provinces <sup>a</sup>	Southern Province <sup>b</sup>		
Genus/Species	Hwaseong-si, Gyeonggi Province°	Asan-si, Chungcheongnam Province <sup>c</sup>	Jangseong-gun, Jeollanam Province <sup>c</sup>	Total (%)d	
Leptotrombidium pallidum	2,637 (95.8)	2,056 (61.2)	395 (12.5)	5,088 (54.8)	
Leptotrombidium scutellare	110 (4.0)	774 (23.0)	2,538 (80.1)	3,422 (36.9)	
Leptotrombidium palpale	4 (0.1)	152 (4.5)	18 (0.6)	174 (1.9)	
Leptotrombidium orientale	3 (0.1)	110 (3.3)	115 (3.6)	228 (2.5)	
Leptotrombidium zetum	-	83 (2.5)	70 (2.2)	153 (1.6)	
Leptotrombidium gemiticulum	-	41 (1.2)	-	41 (0.4)	
Neotrombidium japonica	-	63 (1.9)	-	63 (0.7)	
Neotrombidium gardellai	-	6 (0.2)	-	6 (0.1)	
Cheladonta ikaoensis	-	16 (0.5)	-	16 (0.2)	
Euschoengastica koreaensis	-	57 (1.7)	33 (1.0)	90 (1.0)	
Total (%)	2,754 (29.7)	3,358 (36.2)	3,169 (34.2)	9,281 (100.0)	

<sup>&</sup>lt;sup>a</sup>Central Provinces include Hwaseong-Si (City Area), Gyeonggi-do and Asan-Si, Chungcheongnam-do.

was <10°C. According to a recent report, *L. scutellare* was also collected at Songsan (chigger index, 0.4) and Jangan (3.9), Gyeonggi-do [1].

The effect of global warming may have contributed to the increase of scrub typhus cases in Korea over the past several years by extending the geographical range of *L. scutellare*. Continued surveillance of small mammals and associated larval chigger mites should be conducted to determine the northern limit of *L. scutellare*, as well as the southern limit of *L. pallidum* and potential changes in geographical distributions due to global warming. In addition, detailed studies that identify specific habitats, hosts of larval chigger mites, and associated risks for transmission of *O. tsutsugamushi* are needed to develop disease risk assessments.

## **ACKNOWLEDGMENTS**

We thank Dr. Jong-Koo Lee, former Director General, Korea Centers for Diseases Control and Prevention (K-CDC) for his support during this study. We sincerely thank Ms. Suk-Hee Yi, Force Health Protection and Preventive Medicine, 65th Medical Brigade, for providing GIS maps of collection sites. Funding for this work was provided by the Korea National Institute of Health, Korea Centers for Disease Control and Prevention, Osong, Chungcheongbuk-do (Province), Korea (Title: Environmental cues on the aggregated distribution of *Leptotrombidium* mites, vector of scrub typhus, Project code no. 2009 E00549-00). Portions of this study were supported by the Armed Forces Health Surveillance Center, Global Emerging Infections Surveillance and Re-

bn/d = not done.

bSouthern province includes Jangseong-Gun (County) area.

No. of mites collected, by species, from each collection site (province)/Total no. of mites collected from that province.

<sup>&</sup>lt;sup>a</sup>No. of mites collected from all collection sites, by species/Total no. of mites collected from all collection sites (provinces).

sponse System, Silver Spring, Maryland, and the Public Health Command Region-Pacific, Camp Zama, Japan. The opinions and assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Department of the Army or the Department of Defense.

## **CONFLICT OF INTEREST**

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

## **REFERENCES**

- Lee HI, Shim SK, Song BG, Choi EN, Hwang KJ, Park MY, Park C, Shin EH. Detection of *Orientia tsutsugamushi*, the causative agent of scrub typhus, in a novel mite species, *Eushoengastia ko*reaensis, in Korea. Vector-borne Zoo Dis 2011; 11: 209-214.
- 2. Noh MS, Lee YJ, Chu CS, Gwack J, Youn SK, Huh S. Are there spatial and temporal correlations in the incidence distribution of scrub typhus in Korea? Osong Public Health Res Perspect 2013; 4: 39-44.
- 3. Kim HC, Lee IY, Chong ST, Richards AL, Gu SH, Song JW, Lee JS, Klein TA. Serosurveillance of scrub typhus in small mammals

- collected from military training sites near the DMZ, northern Gyeonggi Province, Korea, and analysis of the relative abundance of chiggers from mammals examined. Korean J Parasitol 2010; 48: 237-243.
- Lee IY, Kim HC, Lee YS, Seo JH, Lim JW, Yong TS, Klein TA, Lee WJ. Geographical distribution and relative abundance of vectors of scrub typhus in the Republic of Korea. Korean J Parasitol 2009; 47: 381-386.
- Ree HI, Cho MK, Lee IY, Jeon SH. Comparative epidemiological studies on vector/reservoir animals of tsutsugamushi disease between high and low endemic areas in Korea. Korean J Parasitol 1995; 33: 27-36.
- Ree HI, Lee IY, Jeon SH, Yoshida Y. Geographical distribution of vectors and sero-strains of tsutsugamushi disease at mid-south inland of Korea. Korean J Parasitol 1997; 35: 171-179.
- 7. Ree HI. Fauna and key to the chigger mites of Korea (Acarina: Trombiculidae and Leeuwenhoekiidae). Korean J Syst Zool 1990; 6: 57-70.
- Lee IY, Ree HI, Hong HK. Seasonal prevalence and geographical distribution of trombiculid mites (Acarina: Trombiculidae) in Korea. Korean J Zool 1993; 36: 408-415.
- Lee SH, Lee YS, Lee IY, Lim JW, Shin HK, Yu JR, Sim SB. Monthly occurrence of vectors and reservoir rodents of scrub typhus in an endemic area of Jeollanam Province, Korea. Korean J Parasitol 2012; 50: 327-331.