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

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Abstract: A total of 9,281 larval chigger mites were collected from small mammals captured at Hwaseong-gun, Gyeonggi-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 mite, 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 mammals 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°02’53.3˝ 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 × 9 × 23 cm), i.e., aluminum folding traps (H.B. Sherman, Tallahassee, Florida, USA) baited with peanut butter and rolled oats (1:3 ratio by vol-
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\textsubscript{2}, 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
| Sites          | Month | No. traps | No. Collected (Trap Rate) | No. w/Chiggers (Infestation Rate) | No. Chiggers (Chigger Index) | No. Collected (Trap Rate) | No. w/Chiggers (Infestation Rate) | No. Chiggers (Chigger Index) | No. Collected (Trap Rate) | No. w/Chiggers (Infestation Rate) | No. Chiggers (Chigger Index) | Subtotal |
|---------------|-------|-----------|---------------------------|----------------------------------|----------------------------|----------------------------|----------------------------------|----------------------------|----------------------------|----------------------------------|----------------------------|----------|
| Hwaseong      | Apr.  | 30        | 5 (16.6)                  | 3 (60.0)                         | 162 (54.0)                 | 0                          | 0                                | 0                          | 0                          | 5 (16.6)                         | 3 (60.0)                 | 162 (54.0) |
|               | May   | 25        | 5 (20.0)                  | 5 (100)                          | 250 (50.6)                 | 0                          | 0                                | 0                          | 0                          | 5 (20.0)                         | 5 (100)                  | 253 (50.6) |
|               | Jun.  | n/d       | n/d                       | n/d                              | n/d                        | n/d                        | n/d                              | n/d                        | n/d                        | n/d                              | n/d                       | n/d       |
|               | Sep.  | 7         | 2 (30.0)                  | 0                                | 0                          | 0                          | 0                                | 0                          | 0                          | 2 (30.0)                         | 0                        | 0         |
|               | Oct.  | 35        | 7 (20.0)                  | 7 (100)                          | 579 (82.7)                 | 0                          | 0                                | 0                          | 0                          | 7 (20.0)                         | 7 (100)                  | 579 (82.7) |
|               | Nov.  | 32        | 11 (34.0)                 | 11 (100)                         | 1,760 (160.0)              | 0                          | 0                                | 0                          | 0                          | 11 (34.0)                        | 11 (100)                | 1,760 (160.0) |
|               | Subtotal | 129    | 30                       | 6 (54.5)                         | 136 (22.6)                 | 0                          | 0                                | 0                          | 0                          | 12 (64.0)                        | 6 (50.0)                 | 136 (22.6) |
| Asan          | Apr.  | 65        | 11 (17.0)                 | 6 (54.5)                         | 136 (22.6)                 | 0                          | 0                                | 1                          | 0                          | 12 (64.0)                        | 6 (50.0)                 | 136 (22.6) |
|               | May   | 30        | 6 (20.0)                  | 6 (100)                          | 23 (3.8)                   | 0                          | 0                                | 0                          | 0                          | 6 (20.0)                         | 6 (100)                  | 23 (3.8)   |
|               | Jun.  | 40        | 8 (20.0)                  | 1 (12.5)                         | 3 (3.0)                    | 0                          | 0                                | 0                          | 0                          | 8 (20.0)                         | 1 (12.5)                 | 3 (3.0)    |
|               | Sep.  | n/d       | n/d                       | n/d                              | n/d                        | n/d                        | n/d                              | n/d                        | n/d                        | n/d                              | n/d                       | n/d       |
|               | Oct.  | 65        | 10 (15.5)                 | 10 (100)                         | 1,739 (173.9)              | 0                          | 0                                | 4                          | 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                          | 8 (26.6)                         | 8 (100)                  | 1,457 (182.1) |
|               | Subtotal | 230    | 43                       | 31 (72.1)                        | 3,358 (108.3)              | 0                          | 0                                | 5 (10.4)                    | 0                          | 48 (31.64)                        | 3,358 (108.3)            |                     |
| Jangseong     | Apr.  | 90        | 6 (6.7)                   | 6 (100)                          | 102 (18.7)                 | 0                          | 0                                | 0                          | 0                          | 6 (6.7)                          | 6 (100)                  | 102 (18.7) |
|               | May   | 80        | 7 (8.9)                   | 5 (7.14)                         | 14 (3.6)                   | 1                          | 0                                | 0                          | 0                          | 8 (10.0)                         | 5 (62.5)                 | 14 (3.6)   |
|               | Jun.  | 90        | 19 (21.1)                 | 11 (57.9)                        | 34 (5.1)                   | 0                          | 0                                | 0                          | 0                          | 19 (21.1)                        | 11 (57.9)                | 34 (5.1)    |
|               | Sep.  | 90        | 7 (7.8)                   | 5 (7.14)                         | 446 (95.2)                 | 0                          | 0                                | 0                          | 0                          | 7 (7.8)                          | 5 (71.4)                 | 446 (95.2) |
|               | Oct.  | 60        | 17 (28.0)                 | 11 (64.7)                        | 2,423 (247.5)              | 1                          | 1 (100)                           | 73 (73.0)                  | 4 (7.0)                     | 4 (100)                           | 77 (77.0)                | 22 (37.0)  |
|               | Nov.  | n/d       | n/d                       | n/d                              | n/d                        | n/d                        | n/d                              | n/d                        | n/d                        | n/d                              | n/d                       | n/d       |
|               | Subtotal | 410    | 56                       | 38 (87.9)                        | 3,019 (89.1)               | 2                          | 1 (50.0)                          | 73 (73.0)                  | 4 (100)                    | 77 (77.0)                          | 62 (39.62)               | 3,169 (88.9) |
| Total         | Apr.  | 22        | 44.0)                    | 15 (68.2)                        | 400 (27.3)                 | 0                          | 0                                | 1                          | 0                          | 23 (46.0)                         | 15 (65.2)                | 400 (27.3) |
|               | May   | 18        | 60.0)                    | 16 (88.9)                        | 290 (18.4)                 | 1                          | 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                          | 27 (44.0)                         | 12 (44.4)                | 379 (4.9)  |
|               | Sep.  | 9         | 90.0)                    | 5 (55.6)                         | 446 (95.2)                 | 0                          | 0                                | 0                          | 0                          | 9 (90.0)                          | 5 (55.6)                 | 446 (95.2) |
|               | Oct.  | 34        | 57.0)                    | 28 (80.0)                        | 4,741 (180.0)              | 1                          | 1 (100)                           | 73 (73.0)                  | 8 (13.3)                   | 4 (100)                           | 77 (77.0)                | 43 (72.0)  |
|               | Nov.  | 19        | 63.0)                    | 19 (100)                         | 3,217 (169.3)              | 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)                        | 9                          | 4 (100)                    | 77 (77.0)                         | 140 (96.86)              | 9,281 (99.8) |

*Trap rate = No. of small mammals captured/No. of traps set.

Infestation rate = No. of small mammals with larval chigger mites/No. of small mammals captured.

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

| Month | Hwaseong | Asan | Jangseong | Total | Hwaseong | Asan | Jangseong | Total |
|-------|----------|------|----------|-------|----------|------|----------|-------|
|       | *L. pallidum* |       |          |       | *L. scutellare* |       |          |       |
| May   | 158 (6.0)  | 86 (4.2) | 39 (9.9) | 283 |          |      |          |       |
| June  | 336 (13.7) | 131 (6.5) | 127 (3.1) | 594 |          |      |          |       |
| July  | n/d       | n/d   | 22 (5.6) | 22 |          |      |          |       |
| September | n/d | n/d | 36 (1.5) | 36 |          |      |          |       |

*A Percent of total for each species by location.

*b* n/d = not done.

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

| Genus/Species | Central Provinces | Southern Province |
|---------------|-------------------|-------------------|
|               | Hwaseong-si, Gyeonggi Province | Asan-si, Chungcheongnam Province | Jangseong-gun, Jeollanam Province | Total (%) |
| *Leptotrombidium pallidum* | 2,637 (51.8) | 2,056 (40.4) | 395 (7.8) | 5,088 (54.8) |
| *Leptotrombidium scutellare* | 110 (4.0) | 774 (23.0) | 2,538 (80.1) | 3,422 (36.9) |
| *L. palpale* | 4 (0.1) | 152 (4.5) | 18 (0.6) | 174 (1.9) |
| *L. orientale* | 3 (0.1) | 110 (3.3) | 115 (3.6) | 228 (2.5) |
| *L. zetum* | - | 83 (2.5) | 70 (2.2) | 153 (1.6) |
| *L. gemmaticulum* | - | 41 (1.2) | - | 41 (0.4) |
| *N. japonica* | - | 63 (1.9) | - | 63 (0.7) |
| *N. gardelai* | - | 6 (0.2) | - | 6 (0.1) |
| *Cheladonta ikaoensis* | - | 16 (0.5) | - | 16 (0.2) |
| *Euschoengastica koreaeensis* | - | 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) |

*Central Provinces include Hwaseong-Si (City Area), Gyeonggi-do and Asan-Si, Chungcheongnam-do.

*Southern 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.

*No. of mites collected from all collection sites, by species/Total no. of mites collected from all collection sites (provinces).

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.

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CONFLICT OF INTEREST

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

REFERENCES

1. 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-reensis, 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 IV, 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.
4. Lee IV, 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.
5. Ree HI, Cho MK, Lee IV, 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.
6. Ree HI, Lee IV, 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.
8. Lee IV, Ree HI, Hong HK. Seasonal prevalence and geographical distribution of trombiculid mites (Acarina: Trombiculidae) in Korea. Korean J Zool 1993; 36: 408-415.
9. Lee SH, Lee YS, Lee IV, 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.
