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Annual Fluctuation in Chigger Mite Populations and *Orientia Tsutsugamushi* Infections in Scrub Typhus Endemic Regions of South Korea

Seong Yoon Kim [a], Byoungchul Gill [b], Bong Gu Song [a], Hyuk Chu [c], Won Il Park [a], Hee Il Lee [a], E-hyun Shin [a], Shin-Hyeong Cho [a], Jong Yul Roh [a,*]

[a] *Division of Vectors and Parasitic Diseases, Korea Centers for Disease Control and Prevention, Cheongju, Korea*
[b] *Division of Laboratory Diagnosis Management, Korea Centers for Disease Control and Prevention, Cheongju, Korea*
[c] *Division of Bacterial Disease Research, Korea Centers for Disease Control and Prevention, Cheongju, Korea*

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ABSTRACT

Objectives: Chigger mites are vectors for scrub typhus. This study evaluated the annual fluctuations in chigger mite populations and *Orientia tsutsugamushi* infections in South Korea.
Methods: During 2006 and 2007, chigger mites were collected monthly from wild rodents in 4 scrub typhus endemic regions of South Korea. The chigger mites were classified based on morphological characteristics, and analyzed using nested PCR for the detection of *Orientia tsutsugamushi*.
Results: During the surveillance period, the overall trapping rate for wild rodents was 10.8%. In total, 17,457 chigger mites (representing 5 genera and 15 species) were collected, and the average chigger index (representing the number of chigger mites per rodent), was 31.7. The monthly chigger index was consistently high (> 30) in Spring (March to April) and Autumn (October to November). The mite species included *Leptotrombidium pallidum* (43.5%), *L. orientale* (18.9%), *L. scutellare* (18.1%), *L. palpale* (10.6%), and *L. zetum* (3.6%). *L. scutellare* and *L. palpale* populations, were relatively higher in Autumn. Monthly *O. tsutsugamushi* infection rates in wild rodents (average: 4.8%) and chigger mites (average: 0.7%) peaked in Spring and Autumn.
Conclusion: The findings demonstrated a bimodal pattern of the incidence of *O. tsutsugamushi* infections. Higher infection rates were observed in both wild rodents and chigger mites, in Spring and Autumn. However, this did not reflect the unimodal incidence of scrub typhus in Autumn. Further studies are needed to identify factors, such as human behavior and harvesting in Autumn that may explain this discordance.

Introduction

Scrub typhus is a notorious, endemic, vector-borne disease that is prevalent in Asian countries, including Japan, Taiwan, China, Thailand, and South Korea [1]. In total, 10,365 cases were reported in South Korea in 2013 which translated to over 20 cases per 100,000 population in South Korea [2]. In contrast, less than 500 cases have been recorded in Japan and Taiwan since 2009 (unpublished data). Scrub typhus is transmitted by chigger mites (larval trombiculid mites) infected with the gram-negative intracellular rickettsial bacterium *Orientia tsutsugamushi* [3].

In South Korea, *O. tsutsugamushi* is transmitted by 2 major species of chigger mites, namely, *Leptotrombidium pallidum* and *L. scutellare. L. palpale*, *L. orientale*, *L. zetum*, *Neotrombicula japonica*, and *Euschoengastia koreaensis* also contribute to the

*Corresponding author: Jong Yul Roh
Division of Vectors and Parasitic Diseases, Korea Centers for Disease Control and Prevention, Cheongju, Korea
E-mail: rohling@korea.kr

transmission of *O. tsutsugamushi* [4,5]. Mites of the genus *Leptotrombidium* are known to be the major vectors for scrub typhus in many countries however, the dominant species varies. *L. pallidum* and *L. scutellare* are believed to be the major vectors in Japan [6]. *L. deliense* is a key vector in Taiwan, and *L. deliense*, *L. imphalum*, and *L. chiangraiensis* are primary vectors in Thailand [7]. In China, "Summer" and "Autumn/Winter" types of scrub typhus are transmitted by *L. deliense* and *L. scutellare*, respectively [8].

The geographical distribution of chigger mites in South Korea are relatively evenly distributed throughout the Korean peninsula where there are populations of *L. pallidum*, *L. palpale*, and *L. orientale* [9]. *L. scutellare* is mainly distributed in the western and southern regions, where scrub typhus is prevalent [9].

Annual fluctuations in chigger mite populations suggest that populations of *L. scutellare* and *L. palpale* are predominant in the Shandong province in Northern China in Autumn and Winter [10]. In Jeollanam-do, in the southern part of South Korea, *L. pallidum* and *L. scutellare* populations are at their highest peaks in November and October (Autumn), respectively. *L. pallidum* has been reported to be perennial [except in Summer (June to August)], and *L. scutellare* has only been obtained between September and January [11].

In Japan, *L. pallidum* and *L. scutellare* are believed to transmit *O. tsutsugamushi* Gilliam and Karp genotypes, and Kawasaki and Kuroki genotypes, respectively [6]. In China, *L. deliense* and *L. scutellare* are believed to transmit Karp, Gilliam, and Kato, and Gilliam and Kawasaki genotypes, respectively [12].

To improve the current understanding of high incidences of scrub typhus in Autumn, 4 scrub typhus endemic areas of South Korea were surveyed. Annual fluctuations in wild rodent trapping (a measure of rodent activity), the prevalence of *O. tsutsugamushi* infections in wild rodents, the chigger index (CI), which represents the number of chigger mites per rodent, and infection rates in these mites were examined.

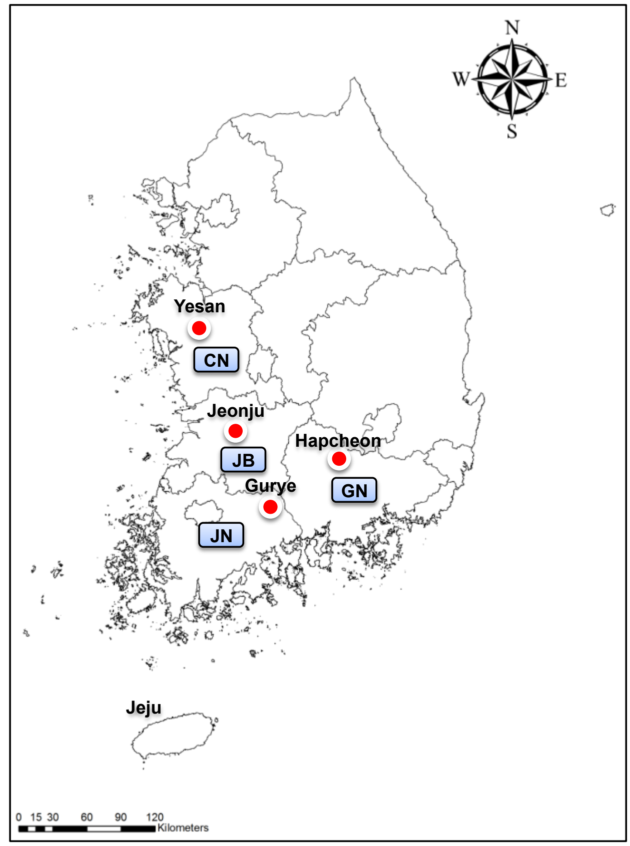

Figure 1. Collection sites for wild rodents in scrub typhus endemic regions of South Korea: Yesan in Chungcheongnam-do (CN), Jeonju in Jeollabuk-do (JB), Gurye in Jeollanam-do (JN), and Hapcheon in Gyeongsangnam-do (GN).

Materials and Methods

1. Localities and periods of surveillance

Chigger mites were collected from wild rodents captured from 4 scrub typhus endemic regions, namely, Yesan in Chungcheongnam-do, Jeonju in Jeollabuk-do, Gurye in Jeollanam-do, and Hapcheon in Gyeongsangnam-do, between 2006 and 2007 (Figure 1). To analyze the demographics of the vector species in the context of the seasonal prevalence of scrub typhus, it was essential to monitor monthly population fluctuations of chigger mite species. Therefore, rodents were collected monthly from each of the scrub typhus endemic regions, for 2 years. Data on the collection, including locality, collection year and month, number of traps installed, and number of rodents captured, have been summarized in Tables 1 and 2. The trapping locations included rice fields, crop fields, reservoirs, waterways, hillsides, grass fields, and riversides.

This study included data on the geographical distribution of *L. scutellare* and scrub typhus incidence in South Korea obtained from Yesan (April-October 2007), Jeonju (April-October 2007), Gurye (April 2006), and Hapcheon (November 2006 and March 2007) which was previously published [9].

2. Collection of wild rodents and chigger mites

Specific permission was not required for the collection of rodents because the sites were not located within national parks or protected areas. The selection of collection sites and the collection of wild rodents were supported by the local Research Institute of Health and Environment centers. The rodent species collected were not endangered or protected in Korea. The animal-handling protocol used in this study was reviewed and approved based on the guidelines for ethical procedures and scientific care of the Institutional Animal Care and Use Committee of the Korea Centers for Disease Control and Prevention (KCDC-046-13-2A).

The collection of wild rodents was performed monthly at all 4 collection sites. At each site, 10 to 15 Sherman folding live traps (3 × 3.5 × 9 inches, BioQuip, USA) baited with a peanut butter-spread biscuit, were set up at 5–7 points, at 3–5-meter intervals, and were collected the next morning. The wild rodents collected were euthanized with compressed carbon dioxide, and subsequently suspended for 24 hours over glass bowls filled with tap water to collect the shed chigger mites. The mites were then recovered from the surface of the water using a fine brush, and stored at 4°C until further examination. Half of the mites collected were used for morphological identification, and the remainder were used for the detection of *O. tsutsugamushi* infections.

3. Identification of chigger mites

Individual chigger mites were transferred to glass slides and mounted using polyvinyl alcohol mounting medium (BioQuip, Rancho Dominguez, CA, USA). The species of the specimens were identified by stereo-microscopic examination, using morphological keys [13].

4. Detection of *O. tsutsugamushi* in wild rodents and chigger mites

Deoxyribonucleic acid (DNA) was extracted from the blood of individual wild rodents, and pooled chigger mites (5–30 per rodent), using the G-spin total DNA extraction kit (iNtRON Biotechnology, Korea). The nucleotide sequence (475 bp) of the gene that encodes the 56 kDa antigen of *O. tsutsugamushi* was detected using a nested polymerase chain reaction (PCR) assay, as described in a previous report [14]. For the first PCR, 5 µL of the template DNA of chigger mites was added to the PCR premix (BIONEER, Korea). This was then incubated at 94°C for 5 minutes, followed by 30 cycles of 94°C for 30 seconds, 60°C for 2 minutes, 72°C for 2 minutes, and finally, 72°C for 10 minutes for amplification. Except for the use of a second pair of PCR primers, the procedure for amplification of 2 µL of the first PCR product, for the second PCR, was the same as described. The second PCR product was analyzed by electrophoresis on

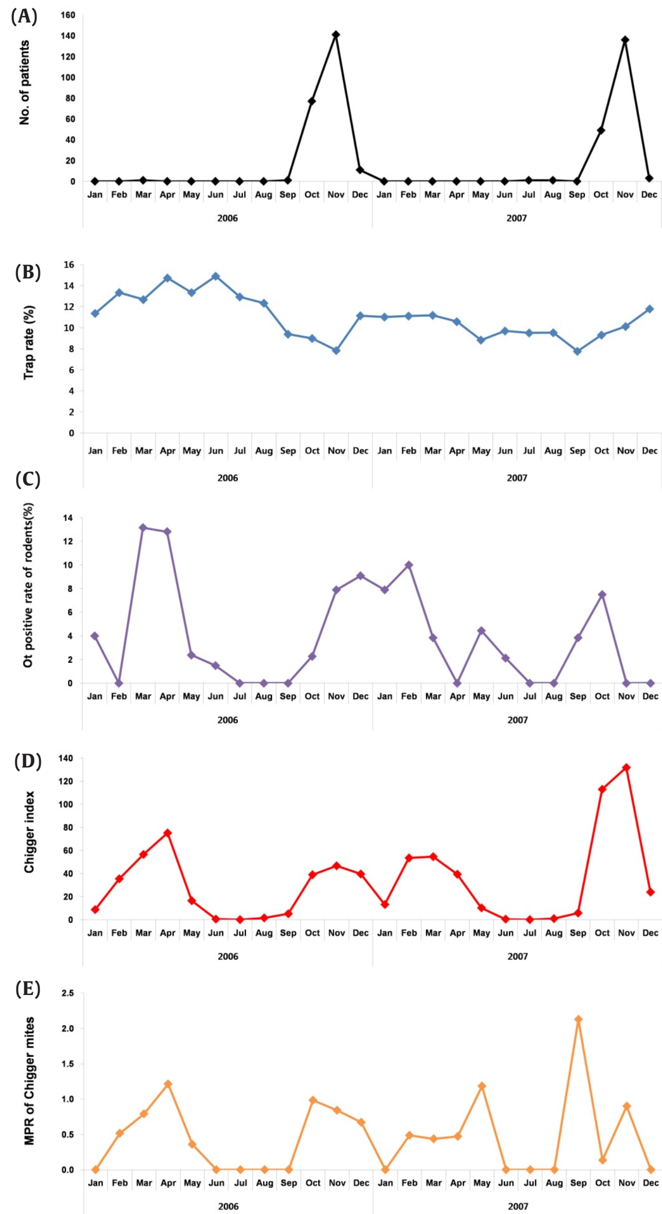

Figure 2. Annual fluctuation in the population of scrub typhus vectors and *O. tsutsugamushi* infections in these vectors. (A) Scrub typhus incidence in the collection regions in 2006–2007, (B) trapping rate of rodents, (C) *O. tsutsugamushi* infection rate in rodents, (D) chigger index, and (E) minimum *O. tsutsugamushi* positivity rate of chigger mites.

Table 1. Coordinates of the rodent collection sites.

Area	Detailed location	N	E
Yesan	Nojeon-ri, Gwangsi-myeon, Yesan-gun, Chungcheongnam-do	36˚ 32′ 2.25″	125˚ 45′ 40.7″
Jeonju	Samcheon-dong, Jeonju-si, Jeollabuk-do	35˚ 48′ 17.46″	127˚ 08′ 15.81
Gurye	Samsan-ri, Ganjeon-myeon, Gurye-gun, Jeollanam-do	35˚ 9′ 28.79″	127˚ 32′ 49.40″
Hapcheon	Jung-ri, Chogye-myeon, Hapcheon-gun, Gyeongsangnam-do	35˚ 33′ 40.75″	128˚ 15′ 15.37″

a 1.5% agarose gel. The nucleotide sequence of the nested PCR products was analyzed using the BLAST program from the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov) to confirm that the gene originated from the 56 kDa protein of *O. tsutsugamushi*. The minimum infection rate was calculated as a percent (%) ratio of the number of positive pools/numbers of tested chigger mites.

Results

1. Collection of chigger mites and *O. tsutsugamushi* infection rates in rodents

Overall, 9,486 traps were installed, and 1,028 wild rodents were captured from 4 regions of the scrub typhus endemic provinces (Figure 1; Table 1). The overall trapping rate of the

Table 2. Summary of collection data and *O. tsutsugamushi* infections in wild rodents and chigger mites.

	No. of scrub typhus patients	No. of installed traps	No. of collected rodents	Rate of rodent trapping (%)	No. of rodents infested by chigger mites	Proportion of rodents infested by chigger mites (%)	No. of collected chigger mites	Chigger Index	No. of *Ot* positive rodents	Proportion of *Ot* positive rodents (%)	No. of *Ot* positive chigger mite pools	No. of tested chigger mites	Minimum infection rate (%) of chigger mites
2006													
Jan	-	220	25	11.4	12	48.0	218	8.7	1	4.0	-	50	-
Feb	-	270	36	13.3	26	72.2	1,275	35.4	-	-	3	582	0.5
Mar	1	300	38	12.7	33	86.8	2,148	56.5	5	13.2	8	1,011	0.8
Apr	-	265	39	14.7	27	69.2	2,929	75.1	5	12.8	18	1,484	1.2
May	-	315	42	13.3	26	61.9	691	16.5	1	2.4	1	278	0.4
Jun	-	450	67	14.9	11	16.4	34	0.5	1	1.5	-	-	-
Jul	-	441	57	12.9	1	1.8	1	0.0	-	-	-	-	-
Aug	-	430	53	12.3	10	18.9	81	1.5	-	-	-	18	-
Sep	1	245	23	9.4	6	26.1	118	5.1	-	-	-	29	-
Oct	77	490	44	9.0	25	56.8	1,713	38.9	1	2.3	8	813	1.0
Nov	141	485	38	7.8	27	71.1	1,773	46.7	3	7.9	7	832	0.8
Dec	11	395	44	11.1	41	93.2	1,741	39.6	4	9.1	5	745	0.7
Subtotal	231	4,306	506	11.8	245	48.4	12,722	25.1	21	4.2	50	5,842	0.9
2007													
Jan	-	345	38	11.0	28	73.7	499	13.1	3	7.9	-	166	-
Feb	-	360	40	11.1	28	70.0	2,139	53.5	4	10.0	5	1,029	0.5
Mar	-	465	52	11.2	43	82.7	2,838	54.6	2	3.8	6	1,373	0.4
Apr	-	435	46	10.6	26	56.5	1,810	39.3	-	-	4	848	0.5
May	-	510	45	8.8	18	40.0	460	10.2	2	4.4	2	169	1.2
Jun	1	485	47	9.7	4	8.5	22	0.5	1	2.1	-	9	-
Jul	1	505	48	9.5	1	2.1	1	0.0	-	-	-	-	-
Aug	-	315	30	9.5	9	30.0	28	0.9	-	-	-	-	-
Sep	49	335	26	7.8	10	38.5	150	5.8	1	3.8	1	47	2.1
Oct	136	430	40	9.3	38	95.0	4,517	112.9	3	7.5	3	2,220	0.1
Nov	3	435	44	10.1	38	86.4	5,798	131.8	-	-	25	2,779	0.9
Dec	190	560	66	11.8	54	81.8	1,577	23.9	-	-	-	622	-
Subtotal	421	5,180	522	10.1	297	56.9	19,839	38.0	16	3.1	46	9,262	0.5
Total		9,486	1,028	10.8	542	52.7	32,561	31.7	37	3.6	96	15,104	0.6

Ot = Orientia tsutsugamushi.

study period was 10.8%; the highest (14.9%) and lowest (7.8%) trapping rates were recorded in June 2006, November 2006 and September 2007, respectively. Among the trapped rodents, *Apodemus agrarius* was the dominant species in all regions, accounting for 97.9% of the collection, followed by *Crocidura lasiura* and *Craseomys regulus* at 2.0% and 0.1%, respectively (data not shown). The detection rate of *O. tsutsugamushi* in wild rodents was 3.6% and infection rates peaked in March (13.2%), April (12.8%), December (9.1%), and November (7.9%) of 2006, and in February (10.0%), January (7.9%), and October (7.5%) of 2007 (Table 2).

2. Chigger indices and *O. tsutsugamushi* infections in mites

The CI represents the number of chigger mites per rodent. In total, 32,561 mites representing 5 genera and 15 species were collected, with an average CI of 31.7 (Figure 2; Table 2). The CIs in Spring (February, March, and April) and Autumn (October and November) were higher than the average value (Table 2). The predominant chigger mite species identified in this study was *Leptotrombidium pallidum* (43.5%), followed by *L. orientale* (18.9%), *L. scutellare* (18.1%), and *L. palpale* [10.6% (Table 3; Figure 3)].

The minimum infection rate in these mites was 0.6%. High infection rate peaks were observed in March (0.8%), April (1.2%), October (1.0%), November (0.8%), and December (0.7%) 2006, and in May (1.2%), September (2.1%), and November (0.9%) 2007. The Boryong genotype of *O. tsutsugamushi* was predominantly obtained from both positive rodents (59.5%) and chigger mites [72.9% (data not shown)].

In this survey, 8 chigger mite species were analyzed based on their relative abundance, and 3 patterns of incidence were identified (Figure 3). The populations of *L. pallidum*, *L. orientale*, and *L. zetum* demonstrated similar high peaks in Spring and Autumn. Interestingly, *L. palpale* and *E. koreaensis* showed low and high peaks in Spring and Autumn, respectively. Additionally, *L. scutellare* and 2 *Neotrombicula* species showed no peak in Spring (1-digit collection recorded) and a high peak in Autumn.

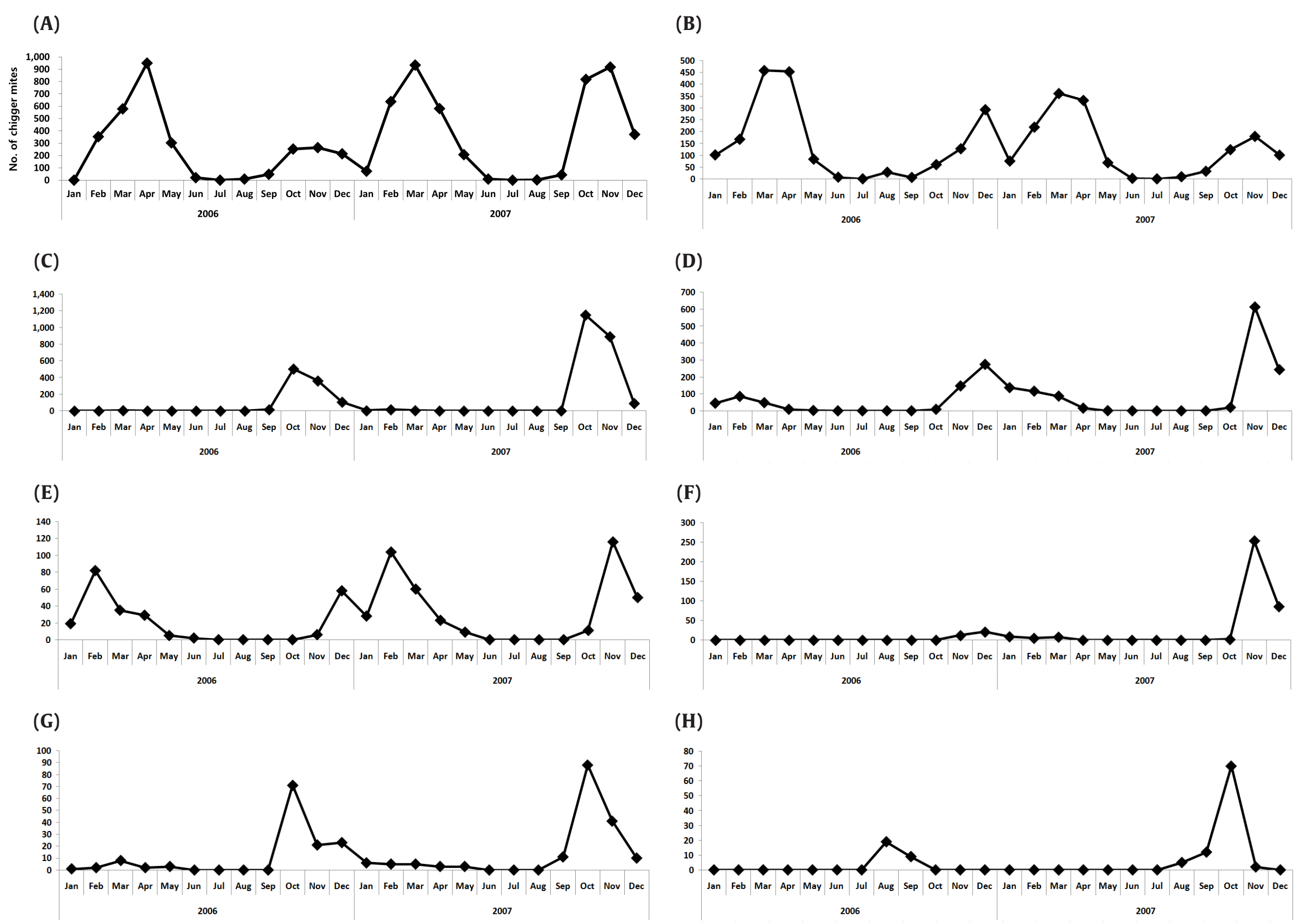

Figure 3. Annual fluctuations in the populations of the 8 dominant chigger mite species. (A) *L. pallidum*, (B) *L. orientale*, (C) *L. scutellare*, (D) *L. palpale*, (E) *L. zetum*, (F) *N. tamiyai*, (G) *E. koreaensis*, (H) *N. gardellai*.

Table 3. Species of chigger mites collected from wild rodents.

	C. ika	*E. kor*	*L. ful*	*L. gem*	*L. ori*	*L. pall*	*L. palp*	*L. scu*	*L. sub*	*L. zet*	*N. gar*	*N. jap*	*N. kwa*	*N. tam*	*W. fra*	*L. spp*	Total
2006																	
Jan	-	1	-	-	102	-	45	-	1	19	-	-	-	-	-	-	168
Feb	-	2	-	-	168	353	85	1	2	82	-	-	-	-	-	-	693
Mar	1	8	-	1	458	579	48	5	2	35	-	-	-	-	-	-	1,137
Apr	-	2	-	-	453	949	9	1	2	29	-	-	-	-	-	-	1,445
May	1	3	-	-	84	303	2	-	5	5	-	-	-	-	10	-	413
Jun	-	-	-	-	8	22	-	-	2	2	-	-	-	-	-	-	34
Jul	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
Aug	-	-	-	-	29	9	-	-	-	-	19	-	-	-	-	6	63
Sep	-	-	-	-	7	48	-	16	-	-	9	-	-	-	-	9	89
Oct	1	71	-	-	61	253	9	504	-	-	-	-	1	-	-	-	900
Nov	-	21	-	-	128	264	147	361	-	6	-	1	1	12	-	-	941
Dec	1	23	-	-	293	215	274	107	4	58	-	-	-	21	-	-	996
Subtotal	4	131	-	1	1,792	2,995	619	995	18	236	28	1	2	33	10	15	6,880
2007																	
Jan	-	6	-	-	76	74	137	3	-	28	-	-	-	9	-	-	333
Feb	-	5	-	-	219	638	116	16	7	104	-	-	-	5	-	-	1,110
Mar	1	5	-	-	361	934	87	5	4	60	-	-	-	8	-	-	1,465
Apr	-	3	-	-	332	582	16	1	5	23	-	-	-	-	-	-	962
May	1	3	-	-	69	208	1	-	-	9	-	-	-	-	-	-	291
Jun	-	-	-	-	3	10	-	-	-	-	-	-	-	-	-	-	13
Jul	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
Aug	3	-	7	-	10	2	-	-	1	-	5	-	-	-	-	-	28
Sep	-	11	1	-	33	43	-	2	1	-	12	-	-	-	-	-	103
Oct	2	88	8	-	124	817	20	1,151	1	11	70	1	-	2	2	-	2,297
Nov	-	41	-	-	180	918	613	892	2	116	2	2	-	253	-	-	3,019
Dec	-	10	-	-	101	372	243	91	2	50	-	-	-	86	-	-	955
Subtotal	7	172	16	-	1,509	4,598	1,233	2,161	23	401	89	3	-	363	2	-	10,577
Total	11	303	16	1	3,301	7,593	1,852	3,156	41	637	117	4	2	396	12	15	17,457
%	0.1	1.7	0.1	0.0	18.9	43.5	10.6	18.1	0.2	3.6	0.7	0.0	0.0	2.3	0.1	0.1	100.0

C. ika = Cheladonta ikaoensis; E. kor = Euschoengastia koreaensis; L. ful = Leptotrombidium fuller; L. gem = Leptotrombidium gemiticulum; L. ori = Leptotrombidium orientale; L. pall = Leptotrombidium pallidum; L. palp = Leptotrombidium palpale; L. scu = Leptotrombidium scutellare; L. sub = Leptotrombidium subintermedium; L. zet = Leptotrombidium zetum; N. gar = Neotrombicula gardellai; N. jap = Neotrombicula japonica; N. kwa = Neotrombicula kwangneungensis; N. tam = Neotrombicula tamiyai; W. fra = Walchia fragilis; L. spp. = Leptotrombidium species.

Discussion

The egg-laying season for adult trombiculid mites in South Korea is Summer [15]. The population density of chigger mite larvae is therefore very low in Summer, and increases from September. Therefore, in South Korea, the high density of chigger mites may affect the high incidence of scrub typhus in the Autumn (October to November). However, the high CI in Spring does not explain the low incidence of scrub typhus. In terms of agricultural activity, Spring and Autumn are the seeding and harvesting seasons, respectively. This implies that there are fewer grasses or weeds to shelter chigger mites in the

Spring. However, there is an abundance of crops and grasses in the Autumn, increasing the probability of contact between humans and vectors, including chigger mites.

In this survey, *L. pallidum* was determined to be the predominant species, followed by *L. orientale* and *L. scutellare*. This finding was similar to that of several previous reports conducted in Korea. In 1995, Ree et al [5] reported that *L. pallidum* and *L. scutellare* were predominant in Chungcheongnam-do and Jeollanam-do, respectively. Song et al [16] also reported that *L. pallidum* (76.3%) was the dominant species, followed by *L. scutellare* (12.9%). *L. scutellare* was mainly distributed in the southern parts of Korea, including Jeju Island. The northernmost areas of distribution of *L. scutellare* included regions where the mean annual temperature was above 10ºC [11]. In another survey, Lee et al [17] reported that *L. pallidum* was the predominant species collected between October and November 2006 in Chungcheongnam-do (100%), Jeollabuk-do (73.9%), and Jeollanam-do (77.0%). However, in Gyeongsangnam-do, *L. scutellare* was the predominant species (77.9%). In recent years, Lee et al [11] also surveyed chigger mite populations in Jeollanam-do between November 2006 and October 2007, and reported that *L. scutellare* (54.0%) was the predominant species, followed by *L. pallidum* (39.4%), *L. orientale* (4.4%), *L. palpale* (1.1%), and *Neotrombicula tamiyai* (0.6%).

The *O. tsutsugamushi* infection rates in both rodents and chigger mites were relatively high in Spring and Autumn, with similar patterns of fluctuation of the CI. The predominant genotype in this survey was Boryong. Ree et al [18] also reported that Boryong was the predominant genotype in mice (78.3%) and chigger mite pools (82.9%), and was distributed widely in the Korean peninsula; the Karp genotype was confined to central Korea. They also reported that the Karp genotype was found in areas of *L. pallidum* distribution, whereas Boryong was collected in areas where both, *L. pallidum* and *L. scutellare*, were prevalent. In this current study, pooled chigger mites were used therefore, the *O. tsutsugamushi* genotypes, based on individual chigger mite species could not be determined. This difference in *O. tsutsugamushi* genotypes in relation to individual chigger mite species warrants further investigation to improve the understanding of the epidemiological relationship between the vector and pathogen of scrub typhus.

Conclusion

In South Korea, scrub typhus has a unimodal incidence pattern during the epidemic season of Autumn. Conversely, in Taiwan and Japan, a bimodal incidence pattern (Spring and Autumn) has been reported [19]. The findings from this current 2-year survey demonstrated the seasonal relationship between scrub typhus and chigger mites in the endemic regions of South Korea, highlighting the epidemiology of this disease. In this survey, the annual fluctuation in the trapping rate, CI, and infection rates in wild rodents and chigger mites showed a bimodal pattern over the 2-year surveillance period; this differed from the incidence pattern of scrub typhus. However, the numbers of chigger mites collected and their infection rates reflected the incidence pattern of human scrub typhus in Autumn, but not in Spring. Further studies are needed to determine the factors responsible for this inconsistency. In South Korea, the discordance between the prevalence of wild rodents and chigger mites, and the incidence of scrub typhus in Spring may be attributed to human behavior, including agricultural activities (cultivation and harvesting).

Conflicts of Interest

The authors have no conflicts of interest to declare.

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