



Epidemiological Characteristics of Rodents and Chiggers with *Orientia Tsutsugamushi* in the Republic of Korea

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Abstract: A survey of rodents and chiggers associated with *Orientia tsutsugamushi* was conducted in a rural region of the Republic of Korea (Korea) between 2014 and 2018. Overall *Apodemus agrarius* 15.2% had the highest seropositive for *O. tsutsugamushi*, followed by *Myodes regulus* 11.4%. Monthly risk factors using logistic regression analysis were not associated with *O. tsutsugamushi* infections in rodents. The overall prevalence rate of *O. tsutsugamushi* among chiggers was 0.3%. The chigger (*Leptotrombidium scutellare*) and monthly (October) risk factors were associated with *O. tsutsugamushi* human infections ($P < 0.05$). *Orientia tsutsugamushi* infections are endemic in rodents in Korea and people, for example, soldiers who are active outdoors, must employ preventive measures, especially during October ($P < 0.05$). When there are many reports of *O. tsutsugamushi* infections in Korea. The Boryong strain 85.7% (2/14) was the most common strain detected in chiggers, followed by the Shimokoshi 7.1% (1/14) and Karp 7.1% strains.

Key words: *Orientia tsutsugamushi*, wild rodent, chigger, phylogenetic analysis

INTRODUCTION

Orientia tsutsugamushi, the causative agent of scrub typhus, is transmitted by chiggers (larval trombiculid mites), and can result in death if untreated. Additionally, chigger bites can cause severe itching in people spending time outdoors, especially during fall [1]. Scrub typhus is distributed throughout much of East and Western Central Asia to northern Australia; cases have also been reported in Africa and Europe [2]. Seasonal outbreaks of scrub typhus in Asia are more frequently reported in the fall and early winter in Korea [3]. Studies on scrub typhus prevalence during fall, the primary period of transmission, have primarily focused on chiggers as vectors of *O. tsutsugamushi* and their distribution [4-7].

The geographic distribution of scrub typhus depends on the population density and vector competence of local chigger vectors [6]. Statistical studies aimed at predicting and preventing the occurrence of scrub typhus infections have been con-

ducted both inside and outside Korea with respect to human outdoor activities and to the time spent on activities such as preventive measures as well as the duration of exposure in chigger habitats such as grasses [8,9]. A statistical model was recently presented for scrub typhus occurrence and included climatic factors such as precipitation and humidity, and the occurrence of scrub typhus cases [9]. It is thus necessary to understand the epidemiological parameters for the seasonal prevalence of *O. tsutsugamushi* infections, based on vector distributions and population densities to predict the occurrence of exposure-associated infections [17]; however, there have been few such studies in Korea.

Therefore, this study examined the seasonal trends of scrub typhus and established the appropriate preventive measures. It also includes a cross-sectional study to determine the risk factors associated with the relationship between the occurrence of *O. tsutsugamushi* infections in rodents and the associated chigger vectors from 2014 to 2018 using a statistical program (SPSS 20, IBM, Armonk, New York, USA).

MATERIALS AND METHODS

Ethics statement

All procedures were conducted under an animal use proto-

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col approved by the Chosun University Animal Ethics Committee (CIACUC 2016-S0003).

Rodent and chigger collections

Rodents were collected monthly from a suburban area (35°13'N126°54'E) with uncut grasses using Sherman live traps (3×3.5×9 inches) (BioQuip, Rancho Dominguez, California, USA). The study area was located in a densely populated area in the southwestern part of Korea, where scrub typhus patients are reported frequently. The information for the human infection rate was obtained from Korea Centers for Disease Control and Prevention (K-CDC) Disease Web Statistics System [3]. In total, 100 traps baited using crackers covered with peanut butter were set monthly from 10:00 to 14:00 hr the following day. Traps positive for rodents were numbered sequentially, placed in a secure container, and then transported to a central laboratory [4]. The rodents were euthanized using chloroform (Merck, New Jersey, USA) soaked in cotton (1×1 cm), and blood was collected by cardiac puncture using a 10-cc syringe. The collected blood was centrifuged at 3,000 rpm for 20 min, serum was separated, and then stored at 4°C. An indirect immunofluorescence assay was performed for antibody detection at 24 hr after the rodents were euthanized. After autopsy, the rodents were hung upside down over a dish containing distilled water for 24 hr. Chiggers were then collected from the water surface using a fine brush.

Indirect immunofluorescence assay for *Orientia tsutsugamushi* in wild rodents

In total, 10 µl of serum from each rodent was used. Antigen spot slides for *O. tsutsugamushi* were provided by the Korea Centers for Disease Control and Prevention (KCDC), and indirect immunofluorescence assays were conducted as described previously [14].

Nested polymerase chain reaction (nPCR) for amplifying the 56-kDa TSA gene of *O. tsutsugamushi* in chiggers

Nested polymerase chain reaction (nPCR) was performed as described previously for *O. tsutsugamushi* detection in chigger mites [11]. A total of 30 chiggers/rodent were examined and placed individually on glass slides with 20 µl of PBS. The abdomen of each chigger was gently pressed using 2 fine needles until the internal contents were expelled and then observed after suspension in 30 µl of PBS under a stereomicroscope (Carl Zeiss, Oberkochen, Germany). The chiggers were mount-

ed with polyvinyl alcohol medium (BioQuip) and identified using a morphological key [12]. DNA was extracted from 20 µl of chigger material using a G-spin Total DNA Extraction Kit (iNtRON Biotechnology, Daejeon, Korea), and then analyzed by PCR for the *O. tsutsugamushi* 56-kDa TSA gene as described previously [13,14].

Risk factors for *O. tsutsugamushi* in rodent hosts

This study was conducted to identify the relationship between the risk factors (month, species) and prevalence of *O. tsutsugamushi* infection in rodents and chiggers. The month associated with the mean distribution of disease was evaluated as a risk factor in rodents by logistic regression analysis using SPSS version 20 software (IBM). The 95% confidence interval (CI), and the chi-square analysis was performed for the rodent species and proportion of infection by month as the risk factors in chiggers ($P < 0.05$).

Sequence and phylogenetic analysis

Amplified PCR products were submitted to Cosmogenetech (Daejeon, Korea) for sequencing using an ABI 3730XL DNA analyzer (Applied Biosystems, Foster City, California, USA). For phylogenetic tree construction, the twelve sequences (KX363949-KX363951, KX363952-KX363953, KY266824-KY266830) examined by Park in previous studies [13,14] and 2 sequences (MK451703 and MK451704) that were newly identified in 2017 were compared with *O. Tsutsugamushi* sequences downloaded from the National Center for Biotechnology Information database, and phylogenetic analyses were conducted using MEGA X. A neighbor-joining tree with 1,000 bootstrap replicates was constructed using the Kimura 2-parameter model.

RESULTS

In total, 99/652 (15.2%) serum samples of *Apodemus agrarius* were seropositive for *O. tsutsugamushi*, and s were positive for *O. tsutsugamushi* antibodies throughout the year, with the highest seropositive rate observed during October (Table 1). The summer months of July (odds ratio, 1.156; 95% CI, 0.379-3.528) ($P = 0.800$) and August (odds ratio, 2.022; 95% CI, 0.472-8.657) were not associated with seropositivity.

In total, 4,646/10,732 (42.3%) of the chiggers collected from the 723 rodents collected were individually tested for *O. tsutsugamushi*; of these, 14/4,646 (0.3%) chiggers were positive

for *O. tsutsugamushi* (Table 2). In total, 4,170/9,597 (43.4%) of the chiggers collected from 652 *Apodemus agrarius*, of which 13 were positive for *O. tsutsugamushi*; only 1 (*Leptotrombidium orientale*) of 476/1,135 (41.9%) of the chiggers collected from 35 *Myodes regulus* was positive for *O. tsutsugamushi*. No chiggers were collected from 38 *Crocidura lasiura*. The 56-kDa TSA gene of *O. tsutsugamushi* was detected in 12 *Leptotrombidium scutellare* individuals and one individual each of *L. orientale* and *L. palpale*. The mean number of chiggers collected monthly (except July) from *Apodemus agrarius* increased significantly beginning in September, and sharply declined in December (Fig. 1).

The results of a chi-squared analysis, by month and species

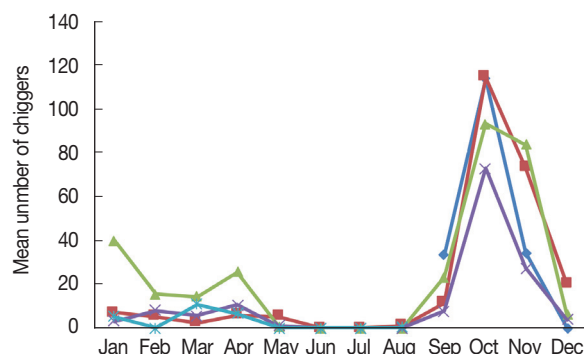


Fig. 1. Monthly mean number of chiggers collected from *Apodemus agrarius* in Korea from September 2014 to August 2018.

Table 1. The number and percents of *Apodemus agrarius*, by month from September 2014 to August 2018 that were seropositive for *Orientia tsutsugamushi*

Month	No. of rodent	Percent	P-value (0.05)	Odds ratio	95% confidence interval
January	37	40.0	0.032	0.308	0.105-0.901
February	40	15.0	0.489	0.667	0.212-2.098
March	55	5.5	0.555	0.767	0.238-2.159
April	42	2.4	0.404	0.615	0.197-1.924
May	90	11.1	0.333	0.619	0.235-1.634
June	86	15.1	0.444	0.676	0.248-1.842
July	72	9.7	0.800	1.156	0.379-3.528
August	42	7.1	0.343	2.022	0.472-8.657
September*	39	15.4	-	-	-
October	34	47.0	0.017	0.283	0.101-0.796
November	53	15.1	0.160	0.490	0.181-1.325
December	62	20.1	0.057	0.393	0.151-1.027
Total	652	15.2			

*Reference category.

Table 2. The total number and percent of chiggers, by species, positive (%) for *Orientia tsutsugamushi* in the Republic of Korea from September 2014 to August 2018

Total	Species of chigger*									
	<i>L. scu</i>	<i>L. ori</i>	<i>L. pal</i>	<i>L. palp</i>	<i>L. zet</i>	<i>N. gar</i>	<i>N. jap</i>	<i>E. kor</i>	<i>W. fra</i>	
No. chiggers	4,646	2,065	1,053	734	301	71	51	9	290	72
No. positive	[14]	[12]	[1]	[0]	[1]	[0]	[0]	[0]	[0]	[0]
Percent	0.3	0.58	0.09	0	0.3	0	0	0	0	0
<i>A. agrarius</i> (652)	4,170	1,974	782	732	270	67	51	9	285	0
No. positive	[13]	[12]	[0]	[0]	[1]	[0]	[0]	[0]	[0]	[0]
Percent	0.31	0.61	0	0	0.4	0	0	0	0	0
<i>M. regulus</i> (35)	476	91	271	2	31	4	0	0	5	72
No. positive	[1]	[0]	[1]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
Percent	0.21	0	0.37	0	0	0	0	0	0	0
<i>C. lasiura</i> (38)	0	0	0	0	0	0	0	0	0	0
No. positive	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]	[0]
Percent	0	0	0	0	0	0	0	0	0	0

**L. scu*: *Leptotrombidium scutellare*, *L. ori*: *L. orientale*, *L. pal*: *L. palpale*, *L. zet*: *L. zetum*, *N. gar*: *Neotrombicula gardellai*, *N. jap*: *N. japonica*, *E. kor*: *Euchoengastia koreaensis*, *W. fra*: *Walchia fragilis*.

Table 3. The number and percents of rodents and soricomorph that were antibody positive for *Orientia tsutsugamushi* and relative host factors from September 2014 to August 2018 in the Republic of Korea

Host	No. of Tested	No. of Positive (%)	P-value (0.05)	Relative risk	95% confidence interval
<i>Apodemus agrarius</i>	652	98 (15.1)	0.204	1.826	0.711-4.689
<i>Myodes regulus</i>	35	4 (11.4)	-	-	-
<i>Crocidura lasiura</i>	21	1 (4.8)	-	-	-
Total	708	-	-	-	-

Table 4. The number and percents of chiggers, by species, collected from *A. agrarius* and *M. regulus* in the Republic of Korea from September 2014 to August 2018, which were positive for *Orientia tsutsugamushi*

Speices	No. of tested	No. of Positive (%)	P-value (0.05)	Relative risk	95% confidence interval
<i>L. scutellare</i>	2,065	12 (0.58)	0.002	7.537	1.685-33.715
<i>L. orientale</i>	1,053	1 (0.09)	-	1	-
<i>L. palpale</i>	301	1 (0.88)	-	1	-
<i>L. pallidum</i>	734	0	-	1	-
<i>L. zetum</i>	71	0	-	1	-
<i>N. gardellai</i>	51	0	-	1	-
<i>N. japonica</i>	9	0	-	1	-
<i>E. koreaensis</i>	290	0	-	1	-
<i>W. fragilis</i>	72	0	-	1	-
Total	4,646	-	-	-	-

as risk factors for *O. tsutsugamushi* infection are shown in Table 3. *Apodemus agrarius* was the predominant rodent collected and was not associated (relative risk, 1.826; 95% CI, 0.711-4.689) ($P=0.204$) with *O. tsutsugamushi* infections. The predominant chigger species, *L. scutellare*, was associated with scrub typhus infections (relative risk, 7.537; 95% CI, 1.685-33.715) ($P=0.002$), and October was very strongly associated with scrub typhus infections (relative risk, 14.421; 95% CI, 4.015-51.794) ($P=0.000$) (Table 4). The phylogenetic analysis results for the 56-kDa TSA gene of *O. tsutsugamushi* are shown in Fig. 2. Two nucleotide sequences were obtained from chiggers in 2017 and deposited to GenBank (*L. scutellare*: MK451703, *L. palpale*: MK451704). One sequence (MK451703) was related to the Karp cluster (percent similarity: 89.5-91.7%) (Fig. 2) (Table 5), and the other sequence (MK451704) was homologous to the Shimokoshi strain (88.7%) (Fig. 2) (Table 5).

Table 5. The number and percents of chiggers, by month, collected from *A. agrarius* and *M. regulus* in the Republic of Korea from September 2014 to August 2018 that were positive for *Orientia tsutsugamushi*

Month	No. of tested	No. of Positive (%)	P-value (0.05)	Relative risk	95% confidence interval
October	950	11 (1.16)	0.000	14.421	4.051-51.794
Other months*	3,696	3 (0.08)	-	1	-
Total	4,646	-	-	-	-

*January, February, March, April, May, June, July, August, September, November, December.

DISCUSSION

O. tsutsugamushi is endemic in Korea throughout the year, and personal protection should be reported to prevent chigger bites, especially a month before the onset of increased numbers of human infections.

Although *O. Tsutsugamushi* was present in rodents throughout the year, analysis of *O. tsutsugamushi* and the monthly factors in rodents did not demonstrate any association (Table 1). However, this study showed a lower prevalence of infection in rodents than that observed in other studies [15-20]. Because previous studies have only been conducted seasonally (spring, fall), they do not reflect the monthly *O. tsutsugamushi* antibody prevalence in rodents.

The monthly distribution of chiggers revealed a particularly high prevalence during the fall season (Fig. 1), when the incidence of scrub typhus patients was the highest in the population of the country. However, *O. tsutsugamushi* positive chiggers were unexpectedly found to occur throughout the year, except during summer. Chiggers were first observed in September and a sharp increase in the mean number of chiggers was observed in October, after which the number of chiggers declined to low numbers in December and persisted until July. Therefore, people should be especially aware of the risks associated with outdoor activities in the spring and fall. There have been reports of scrub typhus onset in humans during spring when a large number of chiggers are present; the increased incidence of spring scrub typhus has also raised concerns related to global warming [17].

The prevalence of *O. tsutsugamushi* in chiggers was most commonly associated with *L. scutellare*, and is consistent with *L. scutellare* as the primary vector of *O. tsutsugamushi* in Korea [4,5,21]. Chigger species as a risk factor for rodents was not as-



Fig. 2. Phylogenetic tree of *Orientia tsutsugamushi* based on the 56-kDa gene fragment sequences detected in chiggers in the Republic of Korea from September 2014 to August 2018; open circles, (○) (KX363949-KX363951) [14], and closed circles, (●) (KX363952-KX363953, KY266824-KY266830) [13], indicate sequences obtained by Park in previous surveys from 2014-2016 [13,14]; open triangles, (△) (MK451703, MK451704), indicate sequences newly identified in 2017. The phylogenetic tree was constructed via the neighbor-joining method with Kimura's 2-parameter model (1,000 bootstrap replicates) using Mega X. The GenBank accession numbers for *O. tsutsugamushi* are indicated for each sequence.

sociated with *O. tsutsugamushi* infections, and *O. tsutsugamushi* infections were found to occur in rodent species other than the dominant species collected *A. agrarius*. *Leptotrombidium scutellare* showed a strong association with *O. tsutsugamushi* infection (relative risk, 7.537; $P=0.002$). Although *L. scutellare* is a known vector of *O. tsutsugamushi* in Korea [6], it showed a stronger association with *O. tsutsugamushi* infection during October (relative risk, 14.421; $P=0.002$). According to the KCDC, patient incidence reports were the highest in November [3]. Our results, supported by the incubation period (7-30 days) of scrub typhus in humans [8], which correspond to the time interval from infection to visiting the hospital to receiving treatment after symptom development.

In a study by Park in 2014-2016 [13,14] conducted in the same region, the *O. tsutsugamushi* genotype for the 56-kDaTSA gene detected in *L. scutellare* belonged to the Boryong strain, whereas other genotypes (Karp, Shimokoshi) were identified in 2017. Although there was only one unique type, the Shimokoshi strain was identified in *L. palpale*.

In conclusion, these results indicate that people must be aware of the risks of the exposure to chiggers that are vectors of

O. tsutsugamushi, especially during October when transmission is the highest. According to our study, soldiers, farmers, and other people that spend time outdoors belong to high-risk group and should be careful when performing outdoor activities, especially during October. To establish effective preventive measures for *O. tsutsugamushi* infections, long-term data on meteorological and ecological factors are needed. Studies conducted in additional areas might increase the reliability of disease prediction throughout Korea.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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