

Geographical distribution of vectors and sero-strains of tsutsugamushi disease at mid-south inland of Korea

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Abstract: Studies on geographical distributions and relative population densities of the vector mites of tsutsugamushi disease were carried out in October 1996 at 12 locations of the mid-south inland of the Korean peninsula, where chigger mites have been never studied. Of 177 field rodents and insectivores collected, 154 (87.0%) were *Apodemus agrarius*. Total 25,707 chigger mites were collected and 14 species were identified, of which *Leptotrombidium pallidum* was predominant (79.8%) and *L. palpale* the next (8.9%). *L. pallidum*, the vector species, was widely distributed in all study areas, showing the highest density at Cho-o 2-dong, Sangju-si (chigger index 201.8), and the lowest at Tanwol-dong, Chungju-si (chigger index 40.7). The other vector species, *L. scutellare* was found only at the southern part of the study area such as Yobae and Mipyong, Kumrung-gun and Unsu, Kimchon-si. The northernmost areas of the *L. scutellare* distribution were coincided with the areas where annual mean air temperature is above 10.0°C. Among 157 *A. agrarius* sera tested, 48.3% was Karp, 1.7% Gilliam and 3.3% Kuroki. The rest of the sera were not able to determine the sero-type because of the cross antigen-antibody reactions among the tested sero-types.

Key words: tsutsugamushi disease, vector species, geographical distribution, sero-type, Korea

INTRODUCTION

Since the first Korean cases of tsutsugamushi disease were reported in 1985, cases have remarkably increased, and thousands of the serologically confirmed cases are reported every year throughout the country including Cheju Island (Chang, 1994). The disease is transmitted to man by the bite of infected chigger mites (Acari: Trombiculidae). It is

known that *Leptotrombidium deliense*, *L. fletcheri* and *L. arenicola* are main vector species in Southeast Asian countries (Kohls *et al.*, 1945; Campbell and Domrow, 1974; Shirai *et al.*, 1982), and *L. akamushi*, *L. pallidum* and *L. scutellare* in Japan (Kawamura, 1926; Sasa, 1954; Asanuma *et al.*, 1962). In Korea, total 39 species of chigger mites have been found so far, and *L. pallidum* and *L. scutellare* are confirmed as the vector species (Jackson *et al.*, 1957; Ree *et al.*, 1991a & 1992). Recently Ree *et al.* (1997) isolated *Orientia tsutsugamushi* from three species, *L. palpale*, *L. orientale* and *L. zetum* in addition to two previously confirmed vector species. Studies on geographical distributions of the vector mites have been sporadically carried out mainly at the middle of the Korean peninsula, the

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northern part of Kyonggi-do and Kangwon-do (Traub *et al.*, 1954; Lee *et al.*, 1983; Lee *et al.*, 1988; Shim *et al.*, 1989; Ree *et al.*, 1991b; Lee *et al.*, 1993), and at the southern part of the peninsula, Chollabuk-do, Chollanam-do and Kyongsangnam-do (Lee IY *et al.*, 1993; Ree *et al.*, 1995; Song *et al.*, 1996). In order to draw an overall figure of geographical distributions of the vector species, chigger mite studies were carried out at mid-south inland of Korea, where chigger mites have never been studied.

MATERIALS AND METHODS

Study area and period

The following localities were selected for the collection of wild rodents and chigger mites.

Kyongsangbuk-do:

- (1) Cho-o 1-dong, Sangju-si
 - (2) Cho-o 2-dong, Sangju-si
 - (3) Yobae-ri, Namseo-myon, Kumrung-gun
 - (4) Mipyong-ri, Kuseong-myon, Kumrung-gun
 - (5) Unsu-ri, Kimchon-si
 - (6) Jiksan-ri, Homyong-myon, Yechon-gun
 - (7) Jeokha-ri, Namseon-myon, Andong-si
 - (8) Imha-ri, Imha-myon, Andong-si
 - (9) Jinan-ri, Munkyeong-ub, Munkyeong-si
 - (10) Koyo-ri, Munkyeong-ub, Munkyeong-si
- Chungchongbuk-do:
- (11) Tanwol-dong, Chungju-si
 - (12) Sesong-dong, Salmi-myon, Chungju-si

The collections were carried out in October 1996, the period of which is the peak season of vector mite populations.

Collection of field rodents

About 60 Sherman live traps, baited with oats-peanut butter ball, were set up with 2-3 m intervals at 5-6 p.m. and removed at 5-6 a.m. next morning. The rodents collected alive were transported to the field station. The field stations were temporarily set up at the laboratories of Sangju-si Health Center, Department of Biology, Andong University and Department of Parasitology, Konkuk University College of Medicine. After identifying the species, their blood was taken for the detection of antibody of *Orientia tsutsugamushi* sero-

types.

Collection of chigger mites

Each body of the killed rodents was hung over a beaker in which tap water was put 1 cm deep for the harvest of chiggers and other ectoparasites. The chiggers fallen into the water of the beaker were picked up with a fine brush and put in 75% ethanol for preservation. The chigger harvest was repeated every morning for two days. Later, the chiggers were mounted on slides with Hoyer's solution and were heated by an alcohol lamp for clearing the specimens as well as stretching legs. The identification was done under a high power microscope by using the keys prepared by Ree (1990).

Preparation of antigen

The Karp, Gilliam, Kuroki and Kawasaki strains of the *O. tsutsugamushi* antigen were propagated in L-929 cell cultures as described by Tamura *et al.* (1982). The infected cells were spotted onto 8-well slides at room temperature, sealed in moisture-proof vinyl containers and stored at -70°C deep freezer until use for the detection of sero-types of the antibody.

Detection of antibodies of the field rodent sera

The blood in a microtube (1.5 ml) was left at room temperature for 2 hours, centrifuged at 15,000 rpm for 5 minutes for obtaining the sera at the field station, and kept in deep freezer until tested at the central laboratory in Seoul. The sera were tested for the presence of antibody against *O. tsutsugamushi* sero-types by IF test as described previously by Ree *et al.* (1991b).

RESULTS

The result of field rodent and insectivore collections is given in Table 1. A total of 801 Sherman traps was set at 12 locations and 177 mice and shrews were collected, showing 22.1% of the trap rate in average, the highest at Cho-o 1-dong, Sangju-si (42.5%) and the lowest at Yobae-ri, Kumrung-gun (8.5%). Out of 177 mice and shrews collected in total, 154

Table 1. Field rodent and insectivore collections at 11 locations of the inland of Korea in October 1996

Locality	No. traps	Trap rate (%)	<i>A. a.</i> ^{a)}	<i>M. m.</i>	<i>C. l.</i>	<i>E. r. r.</i>	Total
Kyongsangbuk-do:							
Cho-o 1, Sangju-si	40	42.5	16	0	1	0	17
Cho-o 2, Sangju-si	87	26.4	20	2	1	0	23
Yobae, Kumrung-gun	82	8.5	7	0	0	0	7
Mipyong, Kumrung-gun	35	34.3	12	0	0	0	12
Unsu, Kimchon-si	100	13.0	13	0	0	0	13
Jiksan, Yechon-gun	90	28.9	23	0	3	0	26
Jeokha, Andong-si	60	26.7	12	3	1	0	16
Imha, Andong-si	67	26.9	16	0	2	0	18
Jinan, Munkyong-si	60	16.7	8	0	1	1	10
Koyo, Munkyong-si	60	31.7	14	4	1	0	19
Chungchongbuk-do:							
Tanwol, Chungju-si	60	16.7	9	1	0	0	10
Sesong, Chungju-si	60	10.0	4	0	2	0	6
Total	801	22.1	154	10	12	1	177
%			87.0	5.6	6.8	0.6	100

^{a)}*A. a.*: *Apodemus agrarius*, *M. m.*: *Mus musculus*, *C. l.*: *Crocidura laisura*, *E. r. r.*: *Eothenomys rufocanus regulus*

Table 2. Number and species of the chigger mites collected in the inland of Korea in October 1996

	No. collected	%
<i>Cheladonta ikaoensis</i>	19	0.1
<i>Eushoengastia koreaensis</i>	362	1.4
<i>Leptotrombidium orientale</i>	305	1.2
<i>L. palpale</i>	2,294	8.9
<i>L. pallidum</i>	20,527	79.8
<i>L. scutellare</i>	648	2.5
<i>L. zetum</i>	10	0.0
<i>Leptotrombidium sp.</i>	1	0.0
<i>Neotrombicula gardellai</i>	34	0.1
<i>N. japonica</i>	734	2.9
<i>N. kwangnungensis</i>	446	1.7
<i>N. talmiensis</i>	1	0.0
<i>N. tamiyai</i>	324	1.3
<i>Neotrombicula sp.</i>	2	0.0
Total	25,707	100.0

was *Apodemus agrarius* (87.0%), 10 *Mus musculus* (5.6%), 12 *Crocidura laisura* (6.8%), and 1 *Eothenomys rufocanus regulus* (0.6%).

Total 25,707 chigger mites collected from 165 mice and shrews were slide-mounted and

12 species of 4 genera were identified as shown in Table 2. The predominant species was *L. pallidum* (79.8%), followed by *L. palpale* (8.9%), *Neotrombicula japonica* (2.9%), *L. scutellare* (2.5%), *N. kwangnungensis* (1.7%), *Eushoengastia koreaensis* (1.4%), *N. tamiyai* (1.3%) and *L. orientale* (1.2%). *Cheladonta ikaoensis* (0.1%), *L. zetum* (0.04%), *N. gardellai* (0.1%) and *N. talmiensis* (0.004%) were the rare species in the study areas. *Leptotrombidium sp.* and *Neotrombicula sp.* seemed to be new species. However, the number of the specimens were too few (one and two, respectively) to describe and designate as new species.

The chigger-infestation rate and the chigger indices of each host animal are given in Table 3. All *A. agrarius* collected were infested by chigger mites (100% of infestation rate), and the chigger index was 173.7, being predominantly infested by *L. pallidum*, the chigger index of which was 133.1 (76.6% of the total chiggers infested). Considerably large numbers of *L. pallidum*, *N. japonica* and *L. palpale* were infested on *E. rufocanus regulus*, showing 48, 31 and 28 chigger indices, respectively. In the case of *M. musculus*, the infestation rate was 90% and the chigger index was 14.8. The infestation rate of *C. laisura* was 41.7% and

Table 3. Chigger-infestation rates and chigger indices of field rodents and insectivores collected at the 12 study locations in October 1996

Host species	Total No. collected	No. of mite-infested hosts	Infestation rate (%)	Chigger index				
				<i>L. pll.</i> ^{a)}	<i>L. plp.</i>	<i>N. ja.</i>	Others	Total
<i>A. agrarius</i>	146	146	100	133.1	16.2	5.0	19.4	173.7
<i>E. r. regulus</i>	1	1	100	48	28	31	7	114
<i>M. musculus</i>	10	9	90	9.3	4.9	0.4	0.2	14.8
<i>C. laisura</i>	12	5	41.7	0	0.3	5.8	0.4	6.5

^{a)}*L. pll.*: *L. pallidum*, *L. plp.*: *L. palpale*, *N. ja.*: *N. japonica*

Table 4. Geographical distribution and population density of trombiculid mites infested on *Apodemus agrarius* in the inland of Korea in October 1996

Locality	Chigger index by species ^{a)}									
	<i>L. pll.</i>	<i>L. sc.</i>	<i>L. plp.</i>	<i>L. or.</i>	<i>N. ja.</i>	<i>N. ta.</i>	<i>N. kw.</i>	<i>E. ko.</i>	Others	Total
Kyongsangbuk-do:										
Cho-o 1, Sangju-si	198.8	0	7.2	0.2	2	0.2	0	0	0	208.4
Cho-o 2, Sangju-si	201.8	0.1	0.7	0	0.1	0.4	0	0	0	203.1
Yobae, Kumrung-gun	158.9	1.4	8.3	6.9	6.4	7.8	34.7	20	1.1	245.5
Mipyong, Kumrung-gun	185.3	1.2	4.8	0.8	0.3	0.2	0	0.1	0	192.7
Unsu, Kimchon-si	128.1	61.6	17.1	8	0.2	0	0.2	0.9	4.0	220.1
Jiksan, Yechon-gun	157.4	0	13.7	1.3	0.2	0	0	5.2	0.4	178.2
Jeokha, Andong-si	55.2	0	81.8	7.6	7.1	0.3	0.1	0.5	0.5	153.1
Imha, Andong-si	85.9	0	4	0.1	0.1	0	0	0	0	90.1
Jinan, Munkyong-si	149.9	0	12.9	3.5	21.4	6.3	4.9	0.9	0.3	200.1
Koyo, Munkyong-si	127.4	0.1	21.4	0.3	20.1	14.1	11.4	1.1	0.1	196.0
Chungchongbuk-do:										
Tanwol, Chungju-si	40.7	0	0.1	0	0	0	0	0	0	40.8
Sesong, Chungju-si	107.8	0.3	22.5	4.3	2.0	0.5	0.3	17.8	0.6	156.1

^{a)}*L. pll.*: *L. pallidum*, *L. sc.*: *L. scutellare*, *L. plp.*: *L. palpale*, *L. or.*: *L. orientale*, *N. ja.*: *N. japonica*, *N. ta.*: *N. tamiyai*, *N. kw.*: *N. kwangnungensis*, *E. ko.*: *E. koreaensis*

the chigger index was only 6.5, and no *L. pallidum* was infested on *C. laisura*, whereas the predominant species was *N. japonica* (89.2% of the total chiggers infested).

Geographical distributions and population densities are given in Table 4. *L. pallidum* was the predominant species at all study locations, except one location, Jeokha-ri, Andong-si where *L. palpale* was predominant. The highest number of *L. pallidum* was collected at Cho-o 2-dong, Sangju-si (201.8 chigger index) and the lowest at Tanwol-dong, Chungju-si (40.7 chigger index). None or very few numbers (0.1-1.4 chigger indices) of *L. scutellare* were found at all study locations, except one, at Unsu-ri, Kimchon-si, where 61.6 chigger index

was shown though the predominant species was *L. pallidum* (128.1 chigger index). *L. palpale* was widely distributed with the largest chigger index (81.8) at Jeokha-ri, Andong-si and the smallest one (0.1) at Tanwol-dong, Chungju-si. *L. orientale* was also widely distributed with rather low density. High densities of *N. japonica* was found at Jinan-ri and Koyo-ri, Munkyong-si, showing 21.4 and 20.1 chigger indices, respectively. Geographical distribution of *N. tamiyai*, *N. kwangnungensis* and *Eushoengastia koreaensis* were uneven and population densities were significantly varied by locality, showing high chigger index (34.7) of *N. kwangnungensis* at Yobae-ri, Kumrung-gun.

Table 5. Antibody positive rate of *A. agrarius* sera against *O. tsutsugamushi* serotypes, using IFA test

Locality	No. tested	No. of positives ^{a)}									
		Kp	G	Kr	Kp /Kr	Kp /Kw	Kw /Kr	Kp /G	Kp/Kw /Kr	Total	%
Kyongsangbuk-do:											
Cho-o 1, Sangju-si	16	6	0	0	2	1	0	0	1	10	62.5
Cho-o 2, Sangju-si	20	5	0	0	7	0	0	0	4	16	80.0
Yobae, Kumrung-gun	7	2	0	2	2	0	0	0	0	6	85.7
Mipyong, Kumrung-gun	12	2	0	0	0	0	1	0	1	4	33.3
Unsu, Kimchon-si	13	0	0	0	0	0	0	0	0	0	0
Jiksan, Yechon-gun	23	3	0	0	2	0	0	0	1	6	26.1
Jeokha, Andong-si	13	6	0	0	0	1	0	0	0	7	53.8
Imha, Andong-si	16	3	1	0	0	1	0	0	0	5	31.3
Jinan, Munkyeong-si	9	0	0	0	0	0	0	0	2	2	22.2
Koyo, Munkyeong-si	14	0	0	0	0	0	0	0	1	1	7.1
Chungchongbuk-do:											
Tanwol, Chungju-si	10	1	0	0	0	0	0	0	0	1	10
Sesong, Chungju-si	4	1	0	0	0	0	0	1	0	2	50
Total	157	29	1	2	13	3	1	1	10	60	38.2
%	—	48.3	1.7	3.3	21.7	5.0	1.7	1.7	16.7	100	

^{a)}Kp: Karp, G: Gilliam, Kr: Kuroki, Kw: Kawasaki; Kp/Kr ... Kp/Kw/Kr: the antibody titers against both and/or three antigens identical.

Seropositive rates of *A. agrarius* which is predominant in Korea were investigated by means of the IFA test and the result is shown in Table 5. Out of 157 tested mouse sera, 60 positives were found, giving 38.2% of the antibody positive rate against *O. tsutsugamushi* antigen. The positive rate by locality were highly variable, showing the highest at Yobae-ri, Kumrung-gun (85.7%), and the next at Cho-o 2-dong, Sangju-si (80.0%), whereas the lowest rate was shown at Koyo-ri, Munkyeong-si (7.1%), and no positive serum was found at Unsu-ri, Kimcheon-si. Karp strain which was predominant (48.3%) was found at all study localities except Unsu-ri, Kimchon-si and Munkyeong-si. The positive rate of Gilliam and Kuroki strains was 1.7% and 3.3%, respectively. Distribution of these two strains were very limited, showing that Gilliam at Imha-ri, Andong-si and Kuroki at Yobae-ri, Kumrung-gun. The rest of the positive sera (46.8%) showed the same level of the titers against the antigens of two to three different strains, so that the serotypes were not able to determine.

DISCUSSION

In epidemiological point of view, studies on geographical distributions and population densities of the vector species of *tsutsugamushi* disease are of fundamental importance. Two vector species, *L. pallidum* and *L. scutellare* were confirmed in Korea. Geographical distributions and relative population densities, represented by the chigger index infested on *A. agrarius* mice during the period of October-November, are given in Fig. 1 for *L. pallidum* and Fig. 2 for *L. scutellare*, which were made from all the comparable sources of the previous workers (Lee *et al.*, 1983; Ree *et al.*, 1991b, 1995 & 1997; Lee *et al.*, 1993; Song *et al.*, 1996) and the present study. It is clear that *L. pallidum* is widely distributed with high densities throughout the Korean peninsula, except south coastal areas of Chollanam-do and Kyongsangnam-do where chigger indices of *L. pallidum* were significantly low. On the other hand, *L. scutellare* is distributed in southern part of Korea including Cheju Island, with higher densities along the south coast, and the

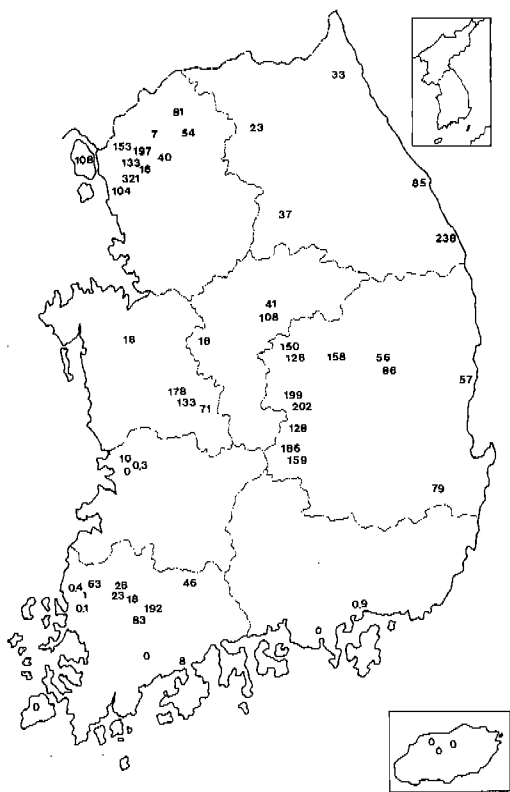


Fig. 1. Geographical distribution and population densities of *L. pallidum* in Korea. The figures are chigger indices of *L. pallidum*.

more northern part of the peninsula, the less density was observed. The northernmost border line of the *L. scutellare* distribution seems to be the line of Iksan and Iri, Chollabuk-do — Kongju and Taejon, Chungchongnam-do — Kimchon, Kyongsangbuk-do — Kyongju, Kyongsangbuk-do — Yongdok, Kyongsangbuk-do, as shown in Fig. 2. Here, less than 1 chigger index of *L. scutellare* was excluded, because extremely low density of the vector population is not significant in epidemiological point of view. Fig. 3 shows the annual mean air temperature in 30 years average at each meteorological station in Korea, which was obtained from the Climatic Table of Korea (1951-1980), Vol. 1 (Central Meteorological Office, 1982). The line of the locations where the annual mean air temperature is above 10.0°C is coincided with the northernmost border line of the *L. scutellare* distribution. This finding indicates

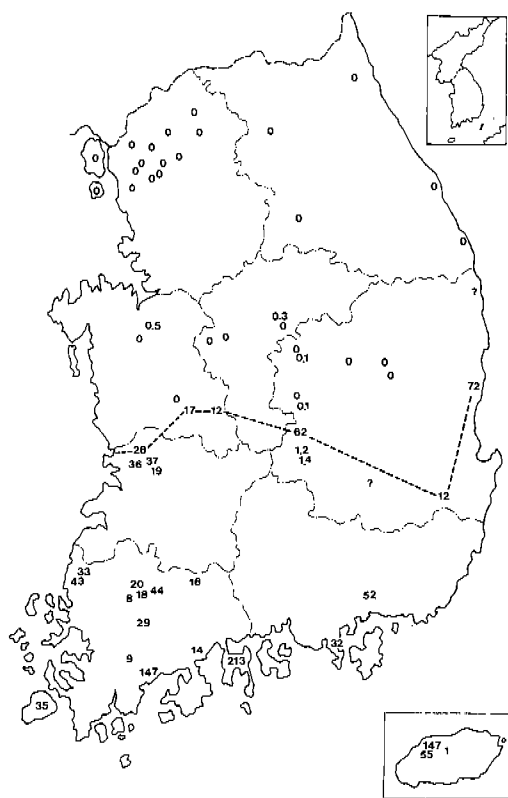


Fig. 2. Geographical distribution and population densities of *L. scutellare* in Korea. The figures are chigger indices of *L. scutellare*. The dot line is the northernmost distribution of *L. scutellare*.

that the most important limited factor for *L. scutellare* distribution is temperature of the habitat, and they require higher than 10.0°C in the annual mean temperature.

Studies on serotypes of *O. tsutsugamushi* including their geographical distribution have been little done so far in Korea. Chang *et al.* (1990) reported that a total of 137 positive serotypes of *O. tsutsugamushi* isolated from patients was analyzed for antigenic characteristics using strain-specific monoclonal antibodies and showed 7 Gilliams, 7 Karps and 3 Boryongs in Kyonggi-do, and 1 Gilliam in Kangwon-do, 4 Karps and 4 Boryongs in Chungchongbuk-do, 1 Gilliam, 2 Karps and 70 Boryongs in Chungchongnam-do, 13 Boryongs in Chollabuk-do, and 1 Gilliam and 21 Boryongs in Kyongsangnam-do. His report clearly indicates that Boryong strain is predominant at the South of the peninsula.

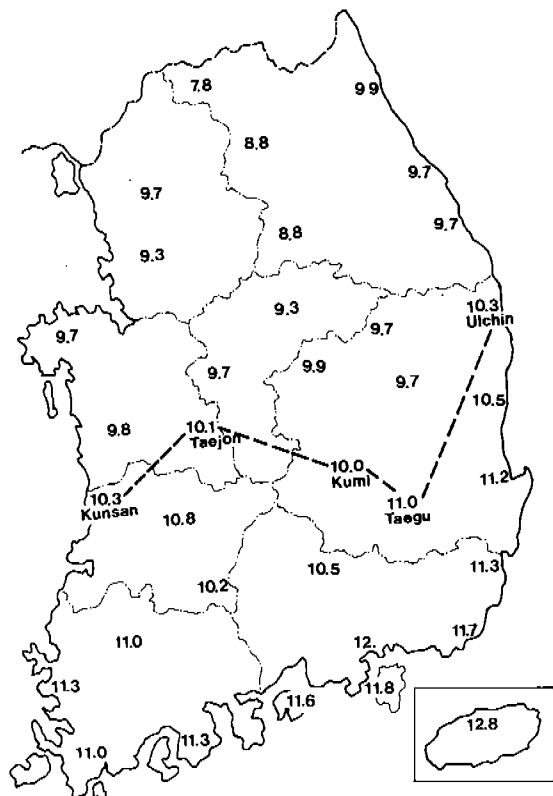


Fig. 3. Thirty years annual mean air temperature of each meteorological station in Korea. The dot line is the northernmost area where the mean air temperature is above 10.0°C.

The result of present study showed that Karp was predominant at most of the study areas, and 1 case of the Gilliam (1.7%) and 2 cases of Kuroki (3.3%) were found at Inha, Andong-si and Soksu, Kumrung-gun, respectively. Because the antigen of Boryong strain could not be tested, it is not clear whether it was real Kuroki or resulted from cross reaction between Boryong and Kuroki strains. Chang and Kang (1992) analyzed the antigenic relation between Boryong and other strains by IFA using the strain-specific monoclonal antibodies, and found that there was strong cross reactivity between Boryong and Kuroki strains, and Chang (1995) reported that serotype Boryong had almost 100% homology to the serotype Kuroki and 89% homology with Karp in comparison of nucleotide sequences. The present study simply indicates indefinable features on the local distribution of *O. tsutsugamushi* serotypes in the study areas,

because the specific serotypes of the mouse sera cannot be determined, by observing antibody titers of the IFA test which shows high cross reactions among them. Further studies are required for classification of the *O. tsutsugamushi* serotypes in Korea.

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=초록=

한반도 중남부 내륙지방에서의 쭈쭈가무시병 혈청형과 매개종의 지리적 분포상

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쭈쭈가무시병을 매개하는 털진드기에 대한 연구는 주로 경기도와 강원도 일대의 중부지방과 전북, 전남 및 경남 등 남부지방에서 부분적으로 이루어져 왔다. 저자들은 지금까지 전혀 연구된 바 없는 중남부 내륙지방 12개 지역에서 들쥐와 털진드기를 채집 조사하여 다음과 같은 결과를 얻었다. 채집된 총 177개체의 들쥐 중 등줄쥐(*Apodemus agrarius*)가 154(87.0%)였다. 들쥐에 기생하고 있던 털진드기 25,707개체를 채집하여 동정한 결과 14종이 확인되었는데 대일털진드기(*Leptotrombidium pallidum*)가 79.8%로 우점종이었고, 다음이 수염털진드기(*L. palpale*)로 8.9%였다. 쭈쭈가무시병의 매개종인 대일털진드기가 12개 조사지역 중 11개 지역에서 우점종이었고 밀도가 가장 높은 지역인 상주시 초소 2동의 chigger index는 201.8이었고 가장 낮은 지역인 충주시 단월동은 40.7이었다. 또 활순털진드기(*L. scutellare*)의 경우 김천시 운수리에서만 chigger index 61.6으로 높은 개체군 밀도를 보였고 금릉군 여배리와 미평리에서는 각각 1.4와 1.2였으며 기타 지역에서는 극소수 채집되었거나 전혀 채집되지 않았다. 활순털진드기 서식 북방 한계선은 전북 익산군 — 충남 공주군 — 경북 김천시 — 경북 경주시 — 경북 영덕군으로 연평균기온이 10.0°C 이상인 지역과 일치하였다. 등줄쥐 157개체의 혈청을 간접형광항체법으로 검사한 결과 60 개체가 양성으로 항체양성률 38.2%였고 가장 높은 지역은 금릉군 여배리로 85.7%였고 가장 낮은 지역은 문경시 고요리의 7.1%였으며 김천시 운수리에서는 양성률 0으로 나타났다. 혈청형은 Karp 48.3%, Gilliam 1.7% 및 Kuroki 3.3%였으며, 나머지 46.7%(28개체)는 2개 혈청형 이상이 같은 항체가를 보여 혈청형을 확인할 수 없었다.

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