The Korean Journal of Parasitology
Vol. 33, No. 1, 27-36, March 1995

Comparative epidemiological studies on vector/reservoir animals of tsutsugamushi disease between high and low endemic areas in Korea

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Abstract: Comparative epidemiological studies on vector reservoir animals of tsutsugamushi disease were carried out in between south coast (the highest endemic) areas and east coast (low endemic) areas in October 1993. Fauna of field rodents and their population densities were not different between two areas. Antibody positive rate of *Apodemus agrarius* sera was higher in east coast (43.2% in south coast and 63.6% in east coast). High correlation (r = 0.87) was shown between antibody positive rate of *A. agrarius* sera and population density of the vector mites (*Leptotrombidium scutellare* and *L. pallidum*). *L. scutellare* was predominant in south coast, showing 110.6 chigger index (74.9% of the total chiggers), whereas *L. pallidum* was predominant in east coast, showing 126.3 chigger index (60.4% of the total). As higher population density of *L. scutellare* was found in south coast where the prevalence rate of tsutsugamushi disease is the highest, it is believed that *L. scutellare* is more important vector species than *L. pallidum*, which may result from more frequent vector-human contact.

Key words: Tsutsugamushi disease, vector species, field rodent, epidemiology, Korea

INTRODUCTION

Tsutsugamushi disease cases occur throughout the country every year in Korea, and the number of the reported cases has been increased recently, being 2,166 serologically confirmed cases reported in 1991 and 2,473 cases in 1992 (Chang, 1994). Jackson *et al.* (1957) isolated the pathogen (*Rickettsia tsutsugamushi*) from *Leptotrombidium pallidum* chiggers and from field rodents (*Apodemus agrarius*, *Microtus fortis* and *Micromys minutus*). Recently Ree *et al.* (1991c), confirmed that *L. pallidum* is the main vector species in the middle of the Korean peninsula, and Ree *et al.* (1992) found for the first time that *L. scutellare* is also the vector species in Korea. Studies on *L. scutellare* such as geographical distribution, behaviour and vector efficiency have not yet been done in Korea.

Geographical distribution of the total 8,073 cases reported in 1986-1992 showed the highest occurrence rate in Kyongsangnam-do (21.7%) and Chollanam-do (14.0%), both of which are located southern most of the Korean peninsula, and except large cities, the lowest occurrence rate in Kangwon-do (3.4%), which is located at east of the peninsula (Chang, 1994). As epidemiological studies of tsutsugamushi disease in relation to vector

• Received Dec. 1 1994, accepted after revision Jan. 4 1995.
• This paper was supported by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1993.
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mites and wild rodents have not been done in both high and low endemic areas, epidemiological features on vector mites and host animals were compared at south coast (high endemic areas) and east coast (low endemic areas) during the transmission season (October) of 1993.

MATERIALS AND METHODS

Collection sites

The following localities were selected for collection of wild rodents and chigger mites from 20 October to 30 October 1993 (Fig. 1).

South west coast:

Kunso-myon, Yongkwang-gun, Chollanam-do.

South coast:

Changhung-ub, Changhung-gun, Chollanam-do.

Yulchon-myon, Yochon-gun, Chollanam-do.

Hapo-gu, Masan-shi, Kyongsangnam-do.

Tosan-myon, Tongyong-gun, Kyongsangnam-do.

East coast:

Namjong-myon, Yongdok-gun, Kyongsang-buk-do.

Kundok-myon, Samchok-gun, Kangwon-do.

Chongok-dong, Tonghae-shi, Kangwon-do.

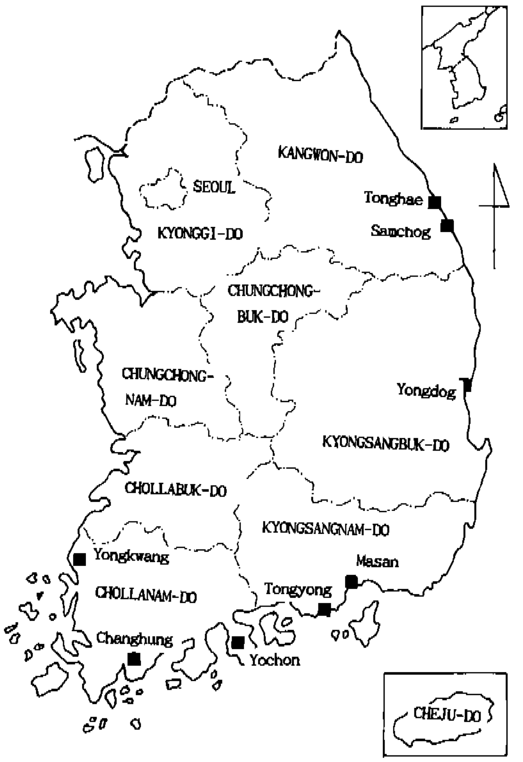

Fig. 1. Study sites along south and east coast of the Korean Peninsula.

Collection of field rodents

Field rodents were collected by Sherman live traps. Ninety eight traps, baited with oats-peanut butter ball, were set up at 4-5 p.m. and removed at 6-7 a.m. next morning. The rodents collected alive were transported to the field station. The field stations were temporarily set up at the laboratory of either the Provincial Institute of Health and Environment or the Quarantine Office. After identifying the species, their blood was taken for the detection of antibody of *Rickettsia tsutsugamushi*.

Collection of chigger mites

Each body of the killed rodents was hung over a beaker in which tap water was put 1 cm deep in order to harvest the chiggers. The chiggers which were 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 done every morning for two days. The chiggers were mounted on slides with Hoyer's solution and were heated by an alcohol lamp for clearing the specimens and stretching legs. The identification was done under a high power microscope by using the keys prepared by Ree (1991).

Preparation of antigen

The Karp and Gilliam strains of the *R. tsutsugamushi* antigen were propagated in L-929 cell cultures as described by Tamura *et al.* (1982). The infected cells were spotted onto microscope slides at room temperature, sealed in moisture-proof vinyl containers and stored at −70°C deep freezer until use.

Detection of antibodies of the field rodent sera

The blood in cryotube (1 cc) was left along for 2 hours at room temperature, and centrifuged at 15,000 rpm for 5 minutes for obtaining the

Table 2. Chigger index of each species of the field rodents for assessing host preferences of chigger mites

Rodent species	No. collected	*L. pallidum*	*L. scutellare*	*L. orientale*	*L. palpale*	*L. zetum*	Others	Total
A. agrarius	148	59 (35.1)	69 (41.1)	10 (6.0)	9 (5.4)	4 (2.4)	17 (10.1)	168 (100)[a]
M. minutus	2	0	0	0.5	0	0	3	3.5
E. rufocanus	4	17 (4.6)	7 (1.9)	272 (73.1)	20 (5.4)	37 (9.9)	19 (5.1)	372 (100)
C. laisura	30	0.1	0.2	0	0.0	0	1.0	1.3
R. norvegicus	1	24 (11.9)	0	21 (10.4)	136 (67.3)	1 (0.5)	20 (9.9)	202 (100)
M. musculus	1	18 (33.3)	0	0	25 (46.3)	0	11 (20.4)	54 (100)

[a]Per cent in parenthesis.

Table 3. Species and total numbers of the chigger mites collected in study areas

Species	No. collected	%
Cheladonta ikaoensis	27	0.1
Eushoengastia koreaensis	958	3.6
Leptotrombidium gemiticulum	6	0.02
Leptotrombidium orientale	2,561	9.6
Leptotrombidium pallidum	8,456	31.9
Leptotrombidium palpale	1,660	6.3
Leptotrombidium scutellare	10,493	39.5
Leptotrombidium zetum	608	2.3
Neotrombicula gardellai	103	0.4
Neotrombicula japonica	543	2.0
Neotrombicula mitamurai	11	0.04
Neotrombicula nagayoi	19	0.1
Neotrombicula talmiensis	21	0.1
Neotrombicula tamiyai	955	3.6
Neotrombicula sp. A	120	0.5
Total	26,544	100

chiggers were infested on a head of *A. agrarius* in average, and 372 chiggers on *Eothenomys rufocanus*. Main mite species infested on *A. agrarius* were *Leptotrombidium pallidum* (35.1%) and *L. scutellare* (41.1%), both of which are the vector species of tsutsugamushi disease in Korea, whereas *L. orientale* (73.1%) was predominant species infested on *E. rufocanus*, and *L. palpale* (67.3%) on *R. norvegicus*. Very few chigger mites were infested on *Micromys minutus* and *C. laisura*, showing 3.5 and 1.3 chigger indices respectively.

Fauna of Trombiculidae and their geographical distribution

Total 26,544 chigger mites were collected at all study areas, and 15 species in 4 genera were identified as shown in Table 3. The most predominant species was *L. scutellare* (39.5%), and *L. pallidum* (31.9%) the next. The rare species are *L. gemiticulum* (0.02%), *Neotrombicula gardellai* (0.4%), *N. mitamurai* (0.04%), *N. nagayoi* (0.1%), *N. talmiensis* (0.1%) and *Neotrombicula* sp.A (0.5%). Table 4 compared the fauna of chigger mites and their densities between south coast and east coast. In south coast, *L. scutellare* was predominant species showing 110.6 chigger index (74.9% of the total) in average and *L. pallidum* was only 4.9% (7.2 chigger index). In contrast, in east coast *L. pallidum* was predominant species showing 126.3 chigger index (60.4%), and *L. scutellare* was collected only in Yongdok-gun, located at southern part of the east coast, where *L. scutellare* and *L. pallidum* coexisted, giving sera. Two-fold serial dilutions of the serum, from 1:10 to 1:160, were prepared in phosphate buffered saline (PBS, pH 7.2) diluent. A 0.01 ml aliquot of the diluted serum was layered on smears of each of the Karp and Gilliam antigens. The slides, kept horizontally, were placed in a plastic box kept humid with wet sponge, and incubated at 37°C for 30 minutes. The sera were removed by rinsing and immerging the slide in PBS for a total of 10 minutes in two changes and the slide was allowed to dry in the air at room temperature. FITC-labelled anti-mouse IgG (Cappel, Organon Teknika Corporation, West Chester, PA, U.S.A.), diluted 1:50 with PBS, added with 0.2% Evans blue for counter staining was layered on each smear. The slides were incubated in a moist chamber as before for 30 minutes. The conjugate was washed off twice by immerging the slides in PBS for 10 minutes. The smears were air-dried and mounted in buffered glycerin, pH 7.3 for fluorescence microscopy examination. A titer of 1:20 or greater was treated as antibody positive. The serum of a healthy mouse was tested at the same time for negative control and the positive serum of each strain of *R. tsutsugamushi* for positive control.

RESULTS

Collection of field rodents

The result of field rodent collections is shown in Table 1. Total 187 rodents were collected at all study areas. Among six identified species, *Apodemus agrarius* (the striped field mouse) was the predominant (79.7% of the total) and *Crocidura laisura* the next (16.0% of the total). One *Rattus norvegicus* (the Norway rat) and one *Mus musculus* (the house mouse) which are originally domestic rodents was also collected in the field. There was no marked difference in the fauna and population density between in south coast and east coast, as demonstrated that *A. agrarius* was predominant at both areas and the trap rate of *A. agrarius* was 20.4% and 19.4% respectively, which may represent the population density. Table 2 shows the host preference of the chigger mites on field rodents. Total 168

Table 1. Field rodent collections by using 98 Sherman traps at each study area in October 1993

Locality	Trap rate (%)	*Aodemus agrarius*	*Micromys minutus*	*Crocidura laisura*	*Mus musculus*	*Rattus norvegicus*	*Eothenomys rufocanus*	Total
South:								
Changhung	22.4	14	0	8	0	0	0	22
Yochon	23.5	22	0	1	0	0	0	23
Masan	31.6	30	0	0	0	0	1	31
Tongyong	17.3	14	1	1	0	0	1	17
Subtotal	23.7	80[a]	1	10	0	0	2	93
%		86.0	1.1	10.8	0	0	2.2	100
East:								
Yongdok	31.6	22	0	9	0	0	0	31
Samchok	24.5	19	0	4	0	0	1	24
Tonghae	26.5	16	1	6	1	1	1	26
Subtotal	27.6	57[a]	1	19	1	1	2	81
%		70.4	1.2	23.5	1.2	1.2	2.5	100
South west:								
Yongkwang	13.3	12	0	1	0	0	0	13
Total	23.9	149	2	30	1	1	4	187
%		79.7	1.1	16.0	0.5	0.5	2.1	100

[a]Trap rate of *A. agrarius*: 20.4% at South coast and 19.4% at East coast.

Table 4. Comparative density of chigger mites (chigger index) on *Apodemus agrarius*

Locality	No. of mice	*L. scutellare*	*L. pallidum*	*L. orientale*	*L. palpale*	*L. zetum*
South:						
Changhung	14	146.9	19.6	3.1	0.9	0
Yochon	21	212.3	8.2	4.1	3.1	0.1
Masan	30	51.1	0.9	16.5	2.3	2.8
Tongyong	14	31.9	0	32.7	3.5	11.8
Average	—	110.6	7.2	14.1	2.5	3.7
%	—	74.9	4.9	9.5	1.7	2.5
East:						
Yongdok	22	71.6	56.7	5.0	32.2	0.4
Samchok	19	0	237.4	6.4	7.9	5.5
Tonghae	16	0	84.9	7.5	20.4	2.3
Average	—	23.9	126.3	6.3	20.2	2.7
%	—	11.4	60.4	3.0	9.7	1.3
South west:						
Yongkwang	12	32.7	62.8	1.5	3.0	5.1
%	—	28.5	54.7	1.3	2.6	4.4

Locality	*N. tamiyai*	*N. japonica*	*N.* sp.	*E. koreaensis*	Others	Total
South:						
Changhung	0	0	0.6	0.7	3.4	175.2
Yochon	0	0.1	0.1	0.4	0.4	228.8
Masan	0	0.1	0.4	19.2	1.0	94.3
Tongyong	0	0	0	12.1	0.3	92.3
Average	0	0.1	0.3	8.1	1.3	147.7
%	0	0.1	0.2	5.5	0.9	100
East:						
Yongdok	9.6	7.0	0.2	2.3	1.4	186.4
Samchok	4.1	15.5	2.9	2.1	0.5	282.3
Tonghae	36.1	4.6	1.8	0.1	1.2	158.9
Average	16.6	9.0	1.6	1.5	1.0	209.1
%	7.9	4.3	0.8	0.7	0.5	100
South west:						
Yongkwang	0	0.3	0	8.3	1.2	114.9
%	0	0.3	0	7.2	1.0	100

71.6 and 56.7 chigger indices respectively. There was no *L. scutellare* at northern part of the east coast (Samchok-gun and Tonghae-shi). In Yongkwang-gun, where is located at southwest coast, both vector species were co-exist, showing 32.7 chigger index of *L. scutellare* and 62.8 chigger index of *L. pallidum*. Besides the vector species, *L. orientale* (9.5%) and *Eushoengastia koreaensis* (5.5%) showed higher densities in south coast (3.0% and 0.7% at east coast, respectively), whereas *L. palpale* (9.7%) and *N. japonica* (4.3%) showed higher densities in east coast (1.7% and 0.1% in south coast, respectively). *N. tamiyai* was found in the east coast only, not in southwest coast nor south coast.

Table 5. Antibody positive rate of *Apodemus agrarius* sera against *R. tsutsugamushi* in October 1993

Locality	Number tested	Number positive	Positive rate (%)	Chigger index of the vectors
South:				
Changhung	10	7	70.0	166.5
Yochon	21	13	61.9	220.5
Masan	30	9	30.0	52.0
Tongyong	13	3	23.1	31.9
Subtotal	74	32	43.2	117.7
East:				
Yongdok	22	13	59.1	128.3
Samchok	17	15	88.2	237.4
Tonghae	16	7	43.8	84.9
Subtotal	55	32	63.6	150.2
South west:				
Yongkwang	12	9	75.0	95.5
Total	141	76	53.9	127.1

Antibody positive rate of the field rodents

Sero-positive rates of *A. agrarius* sera against *R. tsutsugamushi* antigen by means of indirect FA test were shown in Table 5. Out of 141 *A. agrarius* sera tested, 76 sera was positive, giving 53.9% of the antibody positive rate. In south coast, 32 mice out of 74 were positive (43.2%), and in east coast, 32 mice out of 55 were positive (63.6%). In Yongkwang-gun (southwest coast), the sera-positive rate was 75.0% (9 out of 12). High correlation was shown between the population densities (represented by chigger index) of both vector species (*L. scutellare* and *L. pallidum*) and the infection rates (represented by antibody positive rate) of *A. agrarius* (Table 5 and Fig. 2). The regression line was Y = 19.01 + 0.268X and r value was 0.87.

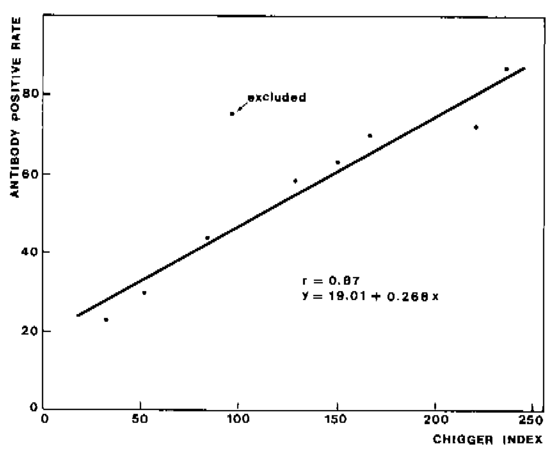

Fig. 2. Correlation between chigger indices of the vectors and antibody positive rate of *Apodemus agrarius* sera in October 1993.

DISCUSSION

Case distribution of tsutsugamushi disease in Korea was annually reported by Chang *et al.* (1989, 1990, 1991, 1992) and well documented by a monograph by Chang (1994), all of which showed that occurrence rate in Kangwon-do was always lower than that in Kyonggi-do. However the prevalence rate (Number of the cases/total population of the area x 1000) was higher in Kangwon-do than in Kyonggi-do, showing 0.0509 in 1991 and 0.0484 in 1992 in Kangwon-do, whereas 0.0301 in 1991 and 0.0411 in 1992 in Kyonggi-do (Table 6). The previous works in Kyonggi-do (Ree *et al.*, 1991 a, b; Lee *et al.*, 1993; Park and Cho, 1991; Lee *et al.*, 1990) and in Kangwon-do (Lee *et al.*, 1993; Shin, 1992) as well as the present study result in Kangwon-do showed that the epidemiological features in relation to human cases, vector mites and field rodents, such as

Table 6. Prevalence rate of tsutsugamushi disease by province in Korea in 1991 and 1992

Province	Population[b)]	1991			1992		
		No. of cases[a)]	%	P.R.	No. of cases[a)]	%	P.R.
Seoul	10,627,790	78	3.6	0.0074	77	3.1	0.0072
Inchon	1,818,293	41	1.9	0.0225	41	1.7	0.0225
Kyonggi-do	6,154,321	185	8.5	0.0301	253	10.2	0.0411
Kangwon-do	1,592,512	81	3.7	0.0509	77	3.1	0.0484
Chungchongbuk-do	1,414,295	52	2.4	0.0368	99	4.0	0.0700
Taejon	1,062,084	45	2.1	0.0424	32	1.3	0.0301
Chungchongnam-do	2,027,766	188	8.7	0.0297	243	9.8	0.1198
Taegu	2,228,834	86	4.0	0.0386	108	4.4	0.0485
Kyongsangbuk-do	2,865,676	203	9.4	0.0708	258	10.4	0.0900
Pusan	3,797,566	148	6.8	0.0390	164	6.6	0.0432
Kyongsangnam-do	3,679,396	374	17.3	0.1016	496	20.1	0.1348
Chollabuk-do	2,069,848	240	11.1	0.1160	270	10.9	0.1304
Kwangju	1,144,695	54	2.5	0.0472	47	1.9	0.0411
Chollanam-do	2,522,515	363	16.8	0.1439	287	11.6	0.1138
Cheju-do	514,608	15	0.7	0.0291	16	0.6	0.0311
Unidentified	—	12	0.6	—	5	0.2	—
Total	43,520,199	2,166	100	0.0498	2,473	100	0.0568

[a)]Data from Chang, 1994; [b)]Data from the Korea Census, 1991.

distribution and population density of the vector mites (*L. pallidum*), antibody positive rate of *A. agrarius* sera against *R. tsutsugamushi* and some others are not significantly different between Kangwon-do and Kyonggi-do. On the other hand, the prevalence rates of the cases in Kyongsangnam-do, Chollabuk-do and Chollanam-do (southern most provinces of the peninsula) were significantly higher than those in any other provinces, as demonstrated about three times higher than that of Kangwon-do or Kyonggi-do (Table 6). Particularly, the prevalence rate of the south coast areas of Kyongsangnam-do and Chollanam-do was the highest (0.2724 in 1991 and 0.3017 in 1992) as shown in Table 7, being 5.3 times higher than that of the east coast areas and 8.1 times higher than that of Kyonggi-do; it was even 2.3 times higher than those of the entire areas of Kyongsangnam-do and Chollanam-do.

The present comparative study result between south coast and east coast areas demonstrated that most of epidemiological features, such as population density of *A. agrarius*, the predominant species of the field rodent in both areas, antibody positive rate of the field rodents against *R. tsutsugamushi*, and total population densities of the vector mites (*L. pallidum* and *L. scutellare*) were not significantly different. However one very important finding was the difference of the vector species. The predominant species in south coast was *L. scutellare*, whereas it was *L. pallidum* in east coast. Population densities of both vector species were not much different between two compared areas, showing variance in density according to the collection sites.

The facts that the sera-positive rate of the field rodents was higher in east coast (low endemic area) than in south coast (the highest endemic area) and that there was a high correlation between the population densities of two vector species (regardless either *L. pallidum* or *L. scutellare*) and infection rates of the field rodents indicate that the higher density of the vector mites, the higher infection of field rodents at a given area regardless the vector species. It means that the vector efficacy of both species in transmission of the pathogen to field rodents is equally high and that the vector-field rodent relationship is quite different from vector-human relationship in

Table 7. Prevalence rate of tsutsugamushi disease along east and south coastal areas of Korea in 1991 and 1992

Province	Population[b]	1991		1992	
		No. of cases[a]	P.R.	No. of cases[a]	P.R.
East coast:					
Kangnung	151,921	8	0.0527	5	0.0329
Yangyang	32,299	2	0.0619	2	0.0619
Sokcho	74,798	2	0.0267	1	0.0134
Kosong	41,606	3	0.0721	1	0.0240
Tonghae	96,336	4	0.0415	6	0.0623
Samchok	58,553	15	0.256	6	0.1025
Uljin	71,072	0	0	1	0.0141
Yongdok	63,544	2	0.0315	6	0.0944
Total	590,129	36	0.0610	28	0.0474
South coast:					
Hainam	111,778	17	0.1521	24	0.2147
Changhung	63,978	49	0.7659	50	0.7815
Posong	75,574	10	0.1323	2	0.0265
Kohung	121,864	22	0.1805	38	0.3118
Yochon	71,560	15	0.2096	23	0.3214
Kosong	74,524	14	0.1879	13	0.1744
Kimhae	139,832	43	0.3075	57	0.4076
Sachon	56,836	25	0.4396	9	0.1584
Total	715,976	195	0.2724	216	0.3017

[a]Data from Chang, 1994; [b]Data from the Korea Census, 1991.

epidemiological point of view.

Why is the prevalence rate higher in Chollanam-do, Chollabuk-do and Kyongsang-nam-do than any other provinces and the highest in south coast areas? It would be answered from the geographical distribution and population density of *L. scutellare*. In Yongkwang-gun, located at northern part of Chollanam-do, population density of *L. scutellare* was much lower than those in south coast (32.7 chigger index in Yongkwang-gun and 110.6 in south coast). Lee *et al.* (1993) reported that chigger index of *L. scutellare* in October 1991 was 77.6 in Iksan-gun and 19.4 in Iri-shi of Chollabuk-do, which were also lower than those in south coast. Even though the investigated localities is too limited to get conclusion, there is a strong evidence that population density of *L. scutellare* is closely related to the prevalence of human cases. Therefore *L. scutellare* is more important vector species than *L. pallidum*. The higher vector efficacy of *L. scutellare* may result from more active human biting activity than *L. pallidum*, not from higher population density. Taking the prevalence rate into account, *L. scutellare* bites humans 3-5 times more preferably compared with *L. pallidum*. Kasuya and Yamashita (1994) and Fujimagari *et al.* (1994) also reported that *L. scutellare* might have more important role in transmission of tsutsugamushi disease than *L. pallidum* in their study areas (Gihu prefecture and Chiba prefecture, respectively) in Japan.

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=초록=

쯔쯔가무시병 다발생지역과 저발생지역간의 매개/숙주동물의 역학적 비교조사

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쯔쯔가무시병 환자의 유병률이 가장 높은 남해안 연안지역과 유병률이 낮은 동해안 연안 지역에서 매개 진드기 및 들쥐와 연관되는 제반 역학적 비교조사를 1993년 10월에 실시하였다. 채집된 6종의 들쥐(187개체) 중 등줄쥐(*Apodemus agrarius*)가 양지역에서 모두 절대 우점종이었고 개체군 밀도에 있어서도 차이가 없었다(트랩 채집률 각각 20.4%, 19.4%). 국내 매개종으로 확인된 *Leptotrombidium pallidum*과 *L. scutellare*는 모두 채집된 6종의 들쥐중 등줄쥐에 주로 기생하였는데, 두당 각각 59마리와 69마리가 기생하고 있었다. 등줄쥐의 *Rickettsia tsutsugamushi* 병원체에 대한 항체 양성률은 남해안 43.2%, 동해안 63.6%로 동해안 지역에서 약간 높게 나타나 환자 발생과는 다른 양상을 보였다. 지역에 관계없이 등줄쥐의 감염률(항체 양성률)과 매개종인 *L. scutellare*와 *L. pallidum*의 개체군 밀도간에 높은 상관관계를 보였다(r = 0.87). 이와같은 사실은 들쥐에 대한 양종의 매개 효율성(vector efficacy)이 똑같이 높다는 것을 의미한다. 양지역간의 가장 중요한 역학적 차이는 매개종으로, 남해안 지역에서는 *L. scutellare*가 절대 우점종(74.9%)인 반면에 동해안 지역에서는 *L. pallidum*이 절대 우점종(60.4%) 이었다. 두종의 개체군 밀도에는 큰 차이가 없었다. 조사지역이 불충분하여 확실하게 결론지을 수는 없지만, *L. scutellare*의 개체군 밀도가 높을수록 환자 유병률도 높게 나타나, *L. scutellare*가 인체에 대한 매개효율성(vector efficacy)이 *L. pallidum* 보다 월등하게 높은 중요한 매개종이라 생각된다.

〔기생충학잡지 33(1): 27-36, 1995년 3월〕