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Study on vector mites of tsutsugamushi disease in Cheju Island, Korea

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Abstract: Because no reference on trombiculid mites (Acarina: Trombiculidae) in Cheju Island where tsutsugamushi disease is highly endemic had been available, studies on trombiculid mites in Cheju Island were implemented during the period of August 1991-April 1992, and the results obtained are summarized as follows: (1) The species and numbers of the field rodents collected were 143 *Apodemus agrarius chejuensis* (92.3%), 11 *Crocidura lasiura* (7.1%) and 1 *Micromys minutus* (0.6%). From total 12,075 chiggers harvested, 9 species of 4 genera in Trombiculidae were identified. (2) The predominant species through all seasons was *L. zetum* (43.3%), followed by *L. orientale* (27.4%) and *L. scutellare* (26.6%). However, in autumn when the most cases of tsutsugamushi disease occur, *L. scutellare* was prominently predominant, having 79.8% of the collected chiggers. (3) Among 1,142 *L. scutellare* examined for *Rickettsia tsutsugamushi* by means of IFA test, 6 individuals were found positive showing 0.5% of infection rate. This is the first finding that *L. scutellare* is the second vector species of tsutsugamushi disease in Korea. (4) Antibody positive rate of *A. agrarius chejuensis* sera were 31.2% (44/139), and 1 *M. minutus* serum was also found positive. The seropositive rates by season were not so significantly different.

Key words: Tsutsugamushi disease, epidemiology, vector species, Cheju Island, Korea.

INTRODUCTION

Tsutsugamushi disease which is a rickettsial disease of acute onset caused by *Rickettsia tsutsugamushi* is widely spread throughout the country. In Cheju Island, seropositive rate against *R. tsutsugamushi* among patients with acute febrile episodes were 52.9% (9 cases out of 17) in 1987 and 55.7% (34 cases out of 64) in 1988 (Chang *et al.*, 1989). Shin *et al.* (1989) carried out sero-epidemiological studies in Cheju Island and reported that 11 cases were found to be antibody positives against *R. tsutsugamushi* among 200 sera of healthy residents, showing 5.5% positive rate. Because no single reference on chigger mites in Cheju Island which are vector animals as well as reservoir hosts has been published, the authors implemented comprehensive epidemiological studies of tsutsugamushi disease in August 1991-April 1992, such as fauna of trombiculid mites, densities of chigger populations by season, determination of vector species and their infection rate against *Rickettsia tsutsugamushi*, antibody positive rate of the field rodents against rickettsial organisms and some others.

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MATERIALS AND METHODS

Study period and study areas: The studies were carried out seasonally, visiting Cheju Island in August and October 1991, and in January and April 1992. Field rodents and chigger mites were collected at three fixed stations: (1) Tonggui-ri, Aiwol-ub, Pukcheju-gun, (2) Kumdog-ri, Aiwol-ub, Pukcheju-gun and (3) Ara-1-dong, Cheju-shi, respectively. Collections were also carried out at a few other places.

Collection of field rodents: Field rodents were collected by Sherman live traps. Each time 30-40 traps baited with oats-peanut butter ball were set up at 4-5 p.m. and removed at 7-8 a.m. next morning. The rodents collected alive were transported to the laboratory of the Cheju-do Provincial Institute of Health and Environment. After identifying the species, their blood and spleen were taken for the detection of antibody and *R. tsutsugamushi* respectively.

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 and put in a chigger-rearing container, at 24 hour intervals for 2 days. A chigger-rearing plastic container is made of charcoal-plaster mixture and kept over 90% of relative humidity. The plastic containers with the harvested chiggers alive from each rodent were kept in a refrigerator(30°C), in which they were able to be kept alive at the larval stage for several months.

Detection of the vector species: The detection of *R. tsutsugamushi* organisms from individual chiggers principally followed the methods of Dohany *et al.* (1978). Each chigger mite was placed in a tiny drop of phosphate buffered saline, pH 7.2 (PBS) solution on a slide. Under a dissecting microscope, the exoskeleton of the mite was punctured in the dorsal posterior portion, and internal contents were squeezed out by using two minute pins. The exoskeleton of the chigger which remained mostly intact was moved to another slide and mounted in a drop of Hoyer's mounting solution. The mounted chiggers were identified following the key prepared by Ree(1990). The internal contents of the chigger in PBS were thoroughly broken into fragments by using a minute pen nib. A tiny amount of the internal content suspension was put on a six well-prepared slide by also using the pen nib. Each time after dissecting one chigger, the dissecting needles and the pen nib were cleaned by ethanol. The suspensions of four mites were dotted on each well so that total 48 mite suspensions were prepared on a slide. Two sheets of slides were prepared, one for Karp strain and the other for Gilliam strain. The slides were dried at room temperature and fixed with acetone for 10 minutes. The fixed slides were again dried at room temperature and kept in a deep freezer until indirect FA test was carried out. When testing, the slides were warmed at room temperature. The polyclonal antibodies of *R. tsutsugamushi* strains which were diluted to 1 : 50 with PBS were dropped on each well of the slide, Karp strain antibody on a slide and Gilliam strain anibody on the other slide. The slides were then incubated at 35°C in a moisture chamber for 30 minutes, washed twice with PBS for 10 minutes and air-dried. Then FITC-conjugated anti-mouse IgG which was diluted to 1 : 50 with PBS and added 0.2% Evans blue for counter stain was applied on each well. The slides were incubated again at 35°C in a wet container for 30 minutes, washed with PBS for 10 minutes and air-dried. With a drop of buffered glycerine pH 7.3, a cover slip was placed on each slide. The slides were examined with a fluorescence microscope.

Preparation of the antiserum of *R. tsutsugamushi*: Reference strains of Karp (ATCC VR-150) and Gilliam (ATCC VR-312) were inoculated in the monolayer cells of L-929. About 14 days after inoculation, rickettsia cells were harvested and used as immunizing antigen. Young normal BALB/c mice were inoculated intraperitoneally with two doses of 0.5 ml amo-

unt of cell count 4×10^6 cells/ml. The two injections were given at 10–15 days interval. Three days after the last inoculation, small amount of blood was obtained for testing, if the homologous titer of the serum is at least 1 : 1280, the mice were exsanguinated. The serum was harvested and stored at deep freezer until use.

Detection of antibodies of the field rodent sera: The blood in cryotube (1 cc) was left along at room temperature for 2 hours, and centrifuged at 15,000 rpm for 5 minutes for obtaining the sera. Two-fold serial dilutions of the serum, from 1 : 10 to 1 : 160, were prepared in PBS 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 35°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 of PBS 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 fluoresence 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 antigen of each strain of *R. tsutsugamushi* for positive control.

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 a room temperature, sealed in moisture-proof vinyl containers and stored at −70°C deep freezer until use.

RESULTS

Collection of field rodents: The result of field rodent collections is shown in Table 1. Total 794 Sherman live traps (alminium made) were set baited with a oat-peanut butter ball and 155 rodents were collected, showing 19.5% trapping rate in average. The trapping rate by season was 12.2% in August, 10.7% in October, 25.0% in January and 32.2% in April. Among a total of 155 field rodents collected during the study period, *Apodemus agrarius chejuensis* was

Table 1. Field rodent collections in Cheju Island

Month	Locality**	No. of traps set	No. of rodents collected				Trap rate (%)
			*A**	*C**	*M**	Total	
Aug. 1991	I	60	6	0	0	6	
	II	60	9	0	0	9	
	III	45	6	0	0	6	
	IV	15	1	0	0	1	
	Subtotal	180	22	0	0	22	12.2
Oct. 1991	I	70	11	2	0	13	
	II	70	3	0	0	3	
	III	70	4	2	0	6	
	V	24	3	0	0	3	
	Subtotal	234	21	4	0	25	10.7
Jan. 1992	I	70	16	1	0	17	
	II	70	18	0	0	18	
	III	60	14	1	0	15	
	Subtotal	200	48	2	0	50	25.0
Apr. 1992	I	46	11	1	0	12	
	II	44	16	2	1	19	
	III	60	13	2	0	15	
	VI	14	1	0	0	1	
	VII	16	11	0	0	11	
	Subtotal	180	52	5	1	58	32.2
Grand total		794	143	11	1	155	19.5
(%)		—	(92.3)	(7.1)	(0.6)	(100)	—

* *A: Apodemus agrarius chejuensis, C: Crocidura lasiura, M: Micromys minutus.*

** I : Tonggui-ri, Aiwol-ub, Pukcheju-gun; II : Kumdogri, Aiwol-ub; III : Ara-l-dong, Cheju-shi; IV : Odong-dong, Cheju-shi; V : Eorimog, Mt. Halla; VI : Iho-l-dong, Cheju-shi; VII : Kosong-ri, Aiwol-ub.

Table 2. Infestations of chigger mites on field rodents by season in 1991~1992

Month	*A.a. chejuensis*			*C. lasiura*			*M. minutus*	
	No. collected	No. infested	Chigger index	No. collected	No. infested	Chigger index	No. collected	Chigger index
Aug. 1991	22	9	3.0	0	0	0	0	0
Oct. 1991	20	20	122.0	4	1	0.3	0	0
Jan. 1992	48	48	122.0	2	2	4.0	0	0
Apr. 1992	50	49	74.3	5	2	0.4	1	15
Tot./Ave.	139	136	86.9	11	5	1.0	1	15

the predominent species at all collection sites, being 143 (92.3%) captured. The other species and numbers collected were 11 *Crocidura lasiura* (7.1%) and 1 *Micromys minutus* (0.6%) as shown in Table 1.

The most of *Apodemus agrarius chejuensis* collected were infested with chiggers through all the seasons showing a 97.8% infestation rate (136/139 mice), whereas a 45.5% infestation rate (5/11 mice) in *Crocidura lasiura* as shown in Table 2. One *Micromy minutus* collected was infested by 15 chiggers. The chigger index (number of chiggers per mouse) on *A. a. chejuensis* was 3.0, 122.0, 122.0 and 74.3 in summer(August), autumn(October), winter (January) and spring(April) respectively, showing 86.9 in average. In case of *C. lasiura*, the chigger index was extremely low, showing 1.0 in average.

Fauna of Trombiculidae and their seasonal prevalences: Total 12,075 chiggers were collected from 794 field rodents collected during the whole study period, and 9 species in 4 genera were identified. The predominant species through all the seasons in Cheju Island was *Leptotrombidium zetum* (43.3%), followed by *L. orientale* (27.4%), *L. scutellare* (26.6%), and *Eushoengastia koreaensis* (2.4%). The other five rare species were *L. palpale* (0.1%), *Leptotrombidium* sp. A (0.0%), *Neotrombicula gardellai* (0.1%), *N. kwangneungensis* (0.0%) and *Cheladonta ikaoensis* (0.1%), as shown in Table 3.

Seasonal prevalences of chigger mite populations infested on *Apodemus agrarius chejuensis*

Table 3. Species and total numbers of chiggers collected during the whole study period

Species	Total No. collected	Chigger index	%
Leptotrombidium orientale	4,075	22.0	27.4
Leptotrombidium palpale	18	0.1	0.1
Leptotrombidium scutellare	1,819	21.4	26.6
Leptotrombidium zetum	5,929	34.8	43.3
Leptotrombidium sp. A	1	0.0	0.0
Neotrombidium gardellai	3	0.1	0.1
Neotrombidium kwangneungensis	1	0.0	0.0
Cheladonta ikaoensis	15	0.1	0.1
Eushoengastia koreaensis	214	1.9	2.4
Total	12,075	80.4	100.0

Table 4. Seasonal prevalences of the chigger mites infested on *A. a. chejuensis* at three fixed study areas (Tongguiri, Kumdog-ri and Ara-1-dong) in Cheju Island

Season	No. of mice	*L. orientale*	*L. scutellare*	*L. zetum*	*E. koreaensis*	Others
Summer	21	2.9 (3.1)*	0	0.0	0.0	0.1
Autumn	18	2.1 (2.3)	91.0(96.0)	14.9(11.0)	5.8(70.7)	0.3
Winter	48	53.1(57.7)	3.7 (3.9)	62.7(46.2)	2.1(25.6)	0.4
Spring	38	34.0(36.9)	0.1 (0.1)	58.2(42.9)	0.3 (3.7)	0.2
Total	125	92.1 (100)	94.8 (100)	135.8 (100)	8.2 (100)	1.0

* The figures indicate chigger index and percent in parenthesis.

are given in Table 4. The density of *L. orientale* was kept very low in summer (3.1%) and autumn (2.3%), the peak in winter (57.7%) and decreased in spring (36.9%). In case of *L. scutellare*, no single chigger was found in summer, absolute majority of the population was appeared in autumn (96.0%), thereafter sharply decreased in winter (3.9%) and spring (0.1%). *L. zetum* was not collected in summer, started to increase from autumn (11.0%), showed the peak in winter (46.2%), and kept high (though slightly decreased) in spring (42.9%). In autumn when the most cases of tsutsugamushi disease are reported, *L. scutellare* was aparently predominant giving 79.8% of the total chiggers collected, and the other species were found very low. In epidemiological point of view, this finding gives strong evidence that *L. scutellare* is the main vector species of the disease in Cheju Island.

Table 5. Detection of *R. tsutsugamushi* antigen from internal contents of the chigger mites by means of IFA test

Locality	Month	*L. scut.*	*L. orie.*	*L. jetu.*	*E. kor.*	Other spp.	Total
Tonggui-ri	Oct. 1991	972(4)*	1	33	41	3	1,050
Kumdog-ri	Oct. 1991	131(2)*	15	16	5	0	167
Ara-1-dong	Oct. 1991	1	6	128	28	0	163
Tonggui-ri	Jan. 1992	38	12	46	0	0	96
Total		1,142(6)*	34	223	74	3	1,476

( )* Number of positives.

Table 6. Antibody positive rate of *Apodemus agrarius chejuensis* sera against *R. tsutsugamushi* in Cheju Island in August 1991–April 1992

Date	Locality	Number tested	Number positive	Positive rate (%)
Aug. 1991	Tonggui-ri	6	2	33.3
	Kumdog-ri	9	2	22.2
	Ara-1-dong	6	1	16.7
	Odong-dong	1	0	0
	Subtotal	22	5	22.7
Oct. 1991	Tonggui-ri	11	3	27.3
	Kumdog-ri	3	0	0
	Ara-1-dong	4	2	50
	Eorimog	3	1	33.3
	Subtotal	21	6	28.6
Jan. 1992	Tonggui-ri	16	5	31.3
	Kumdog-ri	17	4	23.5
	Ara-1-dong	14	4	28.6
	Subtotal	47	13	27.7
Apr. 1992	Tonggui-ri	11	6	54.5
	Kumdog-ri	15	5	33.3
	Ara-1-dong	13	6	46.2
	Kosong-ri	10	2	20
	Subtotal	49	19	38.8
Total		139	44	31.2

Determination of the vector species: Total 1,476 chiggers were dissected and examined their internal contents for *R. tsutsugamushi* organisms by means of indirect FA test and the result is shown in Table 5. Among 1,142 *Leptotrombidium scutellare* examined, *R. tsutsugamushi* was confirmed in 6 individuals, showing 0.5% infection rate. Four positive chiggers were collected at Tonggui-ri, Aiwol-ub, Pukcheju-gun, and two positives were collected at Kumdog-ri, Aiwol-ub, giving 0.4% and 1.5% of the infection rate respectively. The *R. tsutsugamushi* antigens from six all positive chiggers reacted to both Gilliam and Karp strain antisera, showing that five positives reacted more strongly to Gilliam strain and one to Karp strain. Thirty four *L. orientale*, 223 *L. zetum*, 74 *Eushoengastia koreaensis* and 3 others were also examined and found all negative.

Antibody positive rate of the field rodents: Sero-positive rates of the field rodents by means of indirect FA test were shown in Table 6. Out of 139 *Apodemus agrarius chejuensis* tested, 44 individuals were found positive, giving

31.2% of antibody positive rate against *R. tsutsugamushi* antigen. The seropositive rates by season in Cheju Island were not so significantly different, showing 22.7% in August, 28.6% in October, 27.7% in January and 38.8% in April. Also the seropositive rates by locality through all seasons were not much different, showing 36.4% (16 out of 44) at Tonggui-ri, Aiwol-ub, Pukcheju-gun, 25% (11 out of 44) at Kumdog-ri, Aiwol-ub, and 35.1% (13 out of 37) at Ara-l-dong, Cheju-shi. Nine *Crocidura lasiura* were tested and found all negative. One *Micromys minutus* was also tested and found positive. Surprisingly, a head of Quelpart Island mink (*Mustela sibirica quelpartis*) was captured in a Sherman rodent trap at Tonggui-ri in January 1992 and her serum was tested, giving negative result.

DISCUSSION

As only one specimen of *Leptotrombidium* sp. was collected, it was not convinced whether it is a new species or merely a variation of *L. zetum*. More specimens are required to confirm. It is interesting to point out that *L. zetum* and *L. scutellare* were predominant in Cheju Island, forming 43.3% and 26.6% of the total chiggers respectively, whereas they were relatively rare in the middle part of the Peninsula of Korea (Traub *et al.*, 1954; Lee *et al.*, 1988; Shim *et al.*, 1990; Ree *et al.*, 1991c). Ree *et al.* (1991c) reported that *L. zetum*, *L. orientale* and *L. scutellare* were collected only 0.2%, 1.4% and 0.02% of the total chiggers resepectively.

It was confirmed that *Leptotrombidium pallidum* which is the predominant species in middle part of Korean peninsula was the sole vector species of tsutsugamushi disease in Korea (Jackson *et al.*, 1957; Ree *et al.*, 1991a). The present study, however, revealed that *L. pallidum* was not inhabited in Cheju Island as shown in Table 1. Instead, it is confirmed that *L. scutellare* is the main vector species of tsutsugamushi disease in Cheju Island, which is the first recorded incidence of infected chigger species other than *L. pallidum* in Korea.

It was found that *L. scutellare* is one of the vector species of tsutsugamushi disease in Japan, Malaysia and Thailand. Asanuma *et al.* (1959) attempted to isolate rickettsiae from *L. scutellare* in 11 series, and obtained a positive pool consisting of 56 individuals collected from 2 *Apodemus speciosus speciosus* and 3 *Urotrichus talpoides hondonis* in February 1953 in Chiba Prefecture, Japan. Shirai *et al.* (1981a) studied infection rates of *Leptotrombidium* chiggers by applying the same methodology of the authors' present study in the Peninsula Malaysia. The infection rate was 2.6% (6 positives among 235 chiggers examined) in *L. scutellare*, 4.8% (116 positives among 2394 examined) in *L. deliense*, 3.5% (14 positives among 404 examined) in *L. fletcheri*, 1.9% (15 positives among 791 examined) in *L. umbricola*, and 2.3% (15 positives among 646 examined) in *L. keukenschrijveri*. Shirai *et al.* (1981b) reported 9 vector species infected with *R. tsutsugamushi* from Thailand, one of which was *L. scutellare* with a 3.5% infection rate (34 positives out of 985 tested).

Kim *et al.* (1990) carried out sero-epidemiological survey of rickettsial infections of rodents for the first time in Cheju Island in 1988 and reported 15.5% antibody positive rate of *Apodemus agrarius chejuensis* against *R. tsutsugamushi* in average, showing 0% (0/9) in March, 2.9% (1/34) in September and 35.7% (10/28) in October. Compared to their result, the infection rate obtained by the authors' study was much higher (31.2%) in average and not much different by season, giving the highest infection rate in spring (38.8%) and the lowest in summer (22.7%). However, a 31.2% antibody positive rate of *A. a. chejuensis* obtained by the authors in Cheju Island was relatively lower than those of the Peninsula of Korea. A antibody positive rate of *A. agrarius coreae* collected at three different areas of Kyonggi-do was 68% in average (Lee *et al.*, 1990). Ree *et al.* (1991b) carried out seroepidemiological study of *A. a. coreae* collected at 8 different localities of the

middle part of Korea and reported a 41.8% antibody positive rate (33/79) in average.

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=국문초록=

제주도의 쯔쯔가무시병 매개 털진드기에 관한 연구

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1991년 8월부터 1992년 4월에 걸쳐, 쯔쯔가무시병 환자 다발생 지역의 하나인 제주도에서 털진드기류에 관한 연구를 실시하여 다음과 같은 결과를 얻었다. (1) 채집된 들쥐 155마리 중 등줄쥐(*Apodemus agrarius chejuensis*)가 143바리(92.3%)로 우점종이었고, 땃쥐(*Crocidura laciura*) 11마리(7.1%)와 멧밭쥐(*Micromys minutus*) 1바리 (0.6%)였다. 이들 들쥐에 기생한 12,075 개체의 털진드기를 동정한 결과 4속 9종이 확인되었다. 그 중, 사계절을 통한 우점종은 *L. zetum*(43.3%)이었다. (2) 털진드기 개체군의 계절별 밀도를 비교하면 *L. zetum*은 겨울(46.2%)과 봄(42.9%)에 주로 출현하였고, *L. orientale*는 겨울(57.7%)에 피크를 보였다. *L. scutellare*는 가을에 96.0%로 집중적인 발생을 보였고, 가을에 출현하는 모든 털진드기의 79.8%를 차지하여 가을의 우점종으로 쯔쯔가무시병 환자 발생시기와 일치하였다. (3) 총 1,476 개체의 털진드기 유충을 해부하여 그 내용물을 간접면역형광현미경법으로 관찰한 결과 1,142 개체의 *L. scutellare* 중 6 마리가 항원 양성으로 확인되어(감염률 0.5%), 우리 나라에서는 처음으로 *L. scutellare*도 매개종임이 밝혀졌다. (4) 채집된 139바리의 등줄쥐 가운데 44바리가 *R. tsutsugamushi*에 대한 항체 양성으로 나타나 양성률은 31.2%이었다. 사계절을 통한 양성률의 큰 차이는 보이지 않았다.

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