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Epidemiological studies on host animals of tsutsugamushi disease in Korea

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Abstract: Epidemiological studies on host rodents of tsutsugamushi disease were carried out during the period of July~September 1990 at nine localities of central Korea. Among total 111 wild rodents trapped by the modified Sherman live traps, 103 were *Apodemus agrarius* (92.8%), seven were *Crocidura lasiura* (6.3%) and one was *Microtus fortis* (0.9%), showing 24.0% of trapping rate in winter, 11.7% in spring, 11.2% in summer and 12.0% in autumn. Out of 103 *A. agrarius* 84 were parasitized by chiggers, showing 81.6% of the infestation rate and 43.0 of the chigger index. The antibody positive rate of *A. agrarius* sera to *Rickettsia tsutsugamushi* was significantly variable by locality, being in the range of 0~78.6%. The seasonal change of the antibody positive rate at Dorai 5-ri, Goyang-gun was 75.8% in average during November~March, decreased to 30.3% in April and further decreased to 13.3% in average during May~August. Among 33 antibody positives, 31 were Karp strain and two were Gilliam. Seven *Crocidura lasiura* sera showed all negative. *R. tsutsugamushi* organisms were isolated from three *A. agrarius* out of 94 mice tested, showing 3.2% of the infection rate.

Key words: *Rickettsia tsutsugamushi*, tsutsugamushi disease, epidemiology, field mouse, Korea

INTRODUCTION

Since eight cases of tsutsugamushi disease among U.N. Army soldiers for the first time in Korea in 1951~1953 (Munro-Faure, 1951; MGL, 1953), the case had not been reported for more than 30 years until 1985, when 64 Korean cases of the disease were reported (Lee *et al.*, 1986; Yi *et al.*, 1986; Kim *et al.*, 1987; Chang *et al.*, 1987). Thereafter, the reported cases were increased to 460 cases in 1986, 784 cases in 1987, and 827 cases in 1988 (Kim *et al.*, 1987; Chang *et al.*, 1987 & 1989; Chang, 1988; Kim, M.H. *et al.*, 1988; Kim, Y.W. *et al.*, 1988; Choi *et al.*, 1989).

The trombiculid mites are known as both the vector and the reservoir host of tsutsugamushi disease, because *Rickettsia tsutsugamushi* organisms are transovarially transmitted to off-springs of the vector species. Besides the vector mites, many animals have been implicated as natural reservoirs of this disease because they serve as hosts for chiggers of the vector species. Quite many isolations of *R. tsutsugamushi* from naturally infected wild mammals such as *Microtus montebelli*, *Melomys cervinipes*, *Rattus rattus*, *R. flavipectus*, *R. norvegicus*, *Apodemus argenteus*, *A. speciosus*, *Bandicota bengalensis*, *Clethrionomys rufocanus*, *Micromys minutus*, *Mus musculus* and some others throughout many endemic countries (Oaks *et al.*, 1983).

In Korea, *R. tsutsugamushi* organisms were isolated from *Apodemus agrarius*, *Microtus fortis* and *Micromys minutus* by Jackson *et al.* (1957), and recently from *A. agrarius* by Shin *et al.* (1989). The authors carried out an epidemiological study of tsutsugamushi disease in Korea in relation to the host rodents, such as their geographical distribution, seroepidemiology, and infection rate with the causative agent.

MATERIALS AND METHODS

Collections of field rodents: Wild rodents were collected by modified Sherman live traps at nine different localities during the period of July 1989～August 1990. Each time 30～50 traps baited with oats-peanut butter ball were set up at 3～5 p.m. and removed at 6～7 a.m. next morning. The rodents collected alive were transported to the laboratory and identified. Their blood and spleen were taken for the detection of antibody and *R. tsutsugamushi* respectively. Each body of the killed rodents was hung over a beaker in which tap water was put to harvest the chiggers.

Detection of antibodies: The blood in a cryotube (1 cc) was left alone 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 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 of PBS and the slide was allowed to dry in the air at room temperature. Fluorescein isothiocyanate (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 7.2) for fluorescence microscope 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. The Karp, Gilliam and Kato strains of the *R. tsutsugamushi* antigen were propagated in L-929 cell cultures as described by Tamura *et al.* (1982 & 1984). The infected cells were spotted onto microscope slides at room temperature, sealed in moisture-proof vinyl containers and stored at −70°C until use.

Detection of *R. tsutsugamushi* organisms: Immediately after extraction, the spleen of each field rodent was broken into fragments in 0.4～0.6 ml SPG solution (KH_2PO_4 0.0038 M, K_2HPO_4 0.0072 M, L-glutamic acid 0.0049M, sucrose 0.21 M; pH 7.0) and the suspension was intraperitoneally injected into a BALB/c mouse. The inoculated mice were sacrificed 14 days after inoculation whether or not symptoms were shown. The spleen was extracted and kept in a −70°C deep freezer for the second passage. Impression smears of the peritoneal tissue surface were made on three sheets of microscopic slides and air dried. One of three slides was fixed with methanol for 30 seconds and stained by Giemsa solution in order to observe rickettsia-like structures in the cytoplasm of cells under high power microscope (1,000×). The other two sheets of the slides were fixed with acetone for 10 minutes for indirect FA test, one for the Karp strain and the other for the Gilliam strain. The polyclonal antibodies against *R. tsutsugamushi* Karp and Gilliam strains which were diluted to 1:50 with PBS were dropped on the smear. The slides were then incubated at 37°C in a moist chamber for 30 minutes, washed twice with PBS for 10 minutes and air-dried. Then, FITC-conjugated anti-mouse IgG, diluted to 1:50 with PBS and added by 0.2% Evans blue for counter stain, was applied on the smear. The slides were

incubated at 37°C in a moist chamber for 30 minutes, washed twice with PBS for 10 minutes and air-dried. With a drop of buffered glycerine, pH 7.3, a cover slip was placed on the smear, which was examined with a fluorescent microscope. The antiserum of *R. tsutsugamushi* was prepared as follows. Reference strains of Karp and Gilliam were inoculated to 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 amounts 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 1:1,280, the mice were exsanguinated. The serum was harvested and stored at deep freezer until used.

RESULTS

Collection of field rodents: Among a total of 111 field rodents collected at 8 different localities, *Apodemus agrarius* was the predominent species at all collection sites, being 103 (92.8%) captured. The other species collected were 7 *Crocidura lasiura* (6.3%) and 1 *Microtus fortis* (0.9%) as shown in Table 1. Trapping rate of the field rodents by using modified Sherman live traps baited with a oats-peanut butter ball was given in Table 2. The trapping rate was significantly higher in winter (24.0%) compared to other seasons: spring (11.7%), summer (11.2%) and autumn (12.8%). The chigger positive rate of the rodents was 81.6% in *A. agrarius* (84 mice out of 103) and 28.6% in

Table 1. Field rodent collections during the period of July 1989~July 1990

Locality	Date	No. of traps set	No. of rodents collected			
			*A. ag.**	*C. la**	*M. fo.**	Total
Dorai, Goyang-gun	1989. 11. 1	105	16	0	0	16
	12. 12	50	20	2	0	22
	1990. 1. 30	45	6	1	0	7
	3. 14	30	4	0	0	4
	4. 18	30	3	0	0	3
	5. 16	15	1	1	0	2
	6. 13	45	4	0	0	4
	7. 13	45	8	0	1	9
Haingsin, Goyang-gun	1990. 3. 14	30	2	1	0	3
	5. 16	15	1	1	0	2
Goyang, Goyang-gun	1989. 11. 16	60	5	0	0	5
Wondang, Goyang-gun	1989. 11. 24	27	7	0	0	7
Gwangtan, Paju-gun	1989. 7. 5	75	5	0	0	5
	9. 5	36	3	0	0	3
	10. 5	38	2	0	0	2
	1990. 2. 14	30	1	0	0	1
Bucheon-si	1989. 10. 19	39	1	0	0	1
Dongduchon-si	1989. 8. 23	32	4	0	0	4
Deogsan, Chung-Nam	1989. 11. 11	29	2	0	0	2
Hyondo, Chung-Buk	1989. 11. 29	27	8	1	0	9
Total (%)		794	103 (92.8)	7 (6.3)	1 (0.9)	111 (100.0)

* *A. ag.*=*Apodemus agrarius*; *C. la.*=*Crocidura lasiura*; *M. fo.*=*Microtus fortis*.

Table 2. Trapping rate of wild rodents by season in 1989~1990

Season	No. of traps set	No. of mice collected	Trapping rate(%)
Spring(Mar.~May)	120	14	11.7
Summer(Jun.~Aug.)	197	22	11.2
Autumn(Sep.~Nov.)	352	45	12.8
Winter(Dec.~Feb.)	125	30	24.0
Total	794	111	14.0

C. laisura (2 mice out of 7). The chigger index, defined as the total number of the chiggers parasitized divided by total number of the mice collected, of *A. agrarius* and *C. laisura* was 43.0 and 0.9 respectively.

Seroepidemiology of field rodents: Seropositive rates of the field rodents by means of indirect FA test were shown in Table 3. Out of 79 *A. agrarius* tested, 33 positives were found, giving 41.8% of the antibody positive rate. The seropositive rates by locality during November 1989 were highly variable, showing 78.6% at Dorai 5-ri, Goyang-gun, Gyonggi-do, 20% at Goyang-ri, Goyang-gun, 28.6% at Wonhung 2-ri, Goyang-gun, 0% at Deogsan-ri, Yesan-gun, Chungcheong-namdo (the test number was only 2) and 40% at Simog-ri, Cheongwon-gun, Chungcheong-bugdo. Seven *C. lasiura* were also tested and found all negative. Seasonal change of the seropositive rate of *A. agrarius* captured at the same site (a bank of the stream at Dorai 5-ri, Goyang-gun) from November 1989 to August 1990 was given in Fig. 1. Significantly high seropositive rates (73.3~78.6%) were kept from autumn through winter until March, started declining in April (30.3%) and kept low from May through summer showing 13.3% in average.

The antibody titers of the Karp, Gilliam and Koto strains of 33 positive sera of *A. agrarius* were shown in Table 4. The highest titers were demonstrated from the Karp strain in 18 sera, and from the Gilliam strain in 2 sera. The same value of the titers both for the Karp and Kato strains were seen in 13 sera, which would probably be the Karp strain because the Kato has not been found in Korea (Chang, 1988; Park and Chun, 1988; Chang *et al.*, 1990). Much stronger cross-reaction between the Karp and the Kato strains indicates a closer antigenic

Table 3. Antibody positive rates of *Apodemus agrarius* sera against *Rickettsia tsutsugamushi**

Locality	Date of collected	No. of *A. agra.* tested	No. of positive	%
Dorai 5-ri, Goyang-gun	1 Nov.	14	11	78.6
	12 Dec.	15	11	73.3
	14 Mar.	4	3	75
	18 Apr.	3	1	30.3
	16 May	1	0	0
	13 Jun.	4	1	25
	13 Jul.	9	1	11.1
	23 Aug.	1	0	0
	Subtotal	51	28	54.9
Goyang-ri Goyang-gun	16 Nov.	5	1	20
Wonhung 2-ri, Goyang-gun	24 Nov.	7	2	28.6
Haingsin 1-ri, Goyang-gun	14 Mar., 1 May	3	0	0
Kwangtan 3-ri, Paju-gun	5 Sept., 5 Oct.	5	0	0
Bucheon-si, Gyonggi-do	19 Oct.	1	0	0
Deogsan-ri, Yesan-gun	11 Nov.	2	0	0
Simog-ri, Cheongwon-gun	29 Nov.	5	2	40
Total		79	33	41.8

* Seven *Crocidura lasiura* were also tested, and found all negative.

Table 4. Antibody titer of *Apodemus agrarius* positive sera against *R. tsutsugamushi* strains by meas of IFA test.

Locality	Coll. date	Antibody titre of		
		Karp	Gilliam	Kato
Dorai 5-ri, Goyang-gun	1 Nov.	40	20	40
		160	20	80
		20	10	10
		80	20	40
		160	80	80
		80	10	20
		80	20	20
		160	40	80
		20	10	10
		160	80	160
		160	80	160
	12 Dec.	160	20	40
		40	20	40
		20	20	10
		20	10	20
		40	20	20
		40	10	20
		80	40	40
		80	20	40
		40	20	20
		40	10	20
		20	10	20
	14 Mar.	40	20	40
		20	10	20
		160	40	160
	18 Apr.	160	40	40
	13 Jun.	80	20	80
	13 Jul.	160	80	160
Goyang-ri, Goyang-gun	16 Nov.	160	20	80
Wonhung-ri, Goyang-gun	24 Nov.	160	80	160
		160	40	160
Simog-ri, Cheongwon-gun	29 Nov.	40	160	80
		40	80	40

relationship between them than between the Karp and the Gilliam.

Detection of *R. tsutsugamushi* from field rodents: As shown in Table 5, a total of 94 *A. agrarius* captured at 8 different localities were tested for the isolation of *R. tsutsugamushi* by means of mouse passage and indirect FA test, and resulted that the rickettsial organisms were found in 3 *A. agrarius* among 64 which

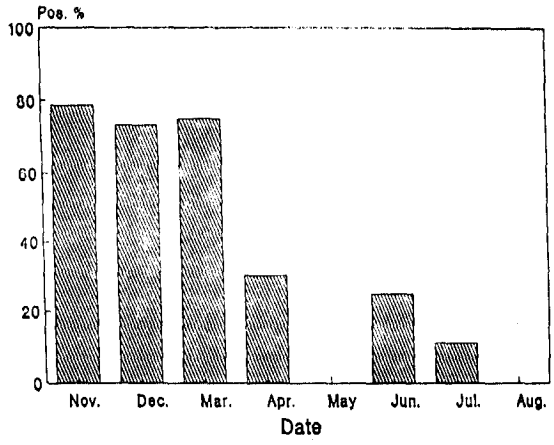

Fig. 1. Seasonal change of the antibody positive rates of *A. agrarius* to *R. tsutsugamushi* at Dorai 5-ri, Goyang-gun in 1989~1990.

were collected at Dori 5-ri, Goyang-gun, Gyonggi-do. The infection rate of *A. agrarius* to *R. tsutsugamushi* was 3.2% at all the study areas (3/94) and 4.7% at Dorai 5-ri, Goyang-gun only. Seven *C. lasiura* were also tested and found all negative. In the 3 positive cases, the spleen suspension inoculated BALB/c mice did not die but showed very mild symptoms until sacrificed 14 days after the inoculation. Their spleens were significantly enlarged, and *R. tsutsugamushi* organisms were detected from the peritoneum smears both by IFA test and by direct observation under a high power micro-

Table 5. Detection of *R. tsutsugamushi* antigen from wild rodents by means of IFA test

Locality	*A. agrar.*		*C. las.*	
	No.	Pos.	No.	Pos.
Gwangtan 3-ri, Paju-gun	4	0	0	0
Bugog-dong, Bucheon-si	1	0	0	0
Goyang-ri, Goyang-gun	5	0	0	0
Wonhung 2-ri, Goyang-gun	7	0	0	0
Haingsin 1-ri, Goyang-gun	3	0	2	0
Dorai 5-ri, Goyang-gun	64	3	4	0
Simog-ri, Cheongwon-gun	8	0	1	0
Deogsan-ri, Yesan-gun	2	0	0	0
Total	94	3(3.2)*	7	0

( )*: Positives rate

scope.

DISCUSSION

Seasonal observation of the seropositive rate of *A. agrarius* against *R. tsustsugamushi* at Dorai 5-ri, Goyang-gun showed that the peak season was November∼March (75.8% in average), decreased to 30.3% in April, and further decreased to 13.3% in average during the period of May∼August. It would be explained that the most of the *A. agrarius* population in the study site were infected by chiggers in Octobe∼November when 76% of the larval population of the vector species, *Leptotrombidium pallidum*, were appeared (Ree *et al.*, 1991), and young litters began earning their own living in April so that many of the old infected population of *A. agrarius* were replaced by youngs in May and seropositive rate was kept low until coming October.

Seropositive rate of *A. agrarius* of each locality was compared with the population density of *L. pallidum* of the same locality as reported by Ree *et al.* (1991). It was found that they were closely correlated to each other, *i.e.*, the higher the density of *L. pallidum*, the higher seropositive rate of *A. agrarius* in most of the study sites. The chigger index and the seropositive rate was 132.6 and 54.9% respectively at Dorai 5-ri, Goyang-gun, 39.1 and 28.6% at Wonhung 2-ri, Goyang-gun, 15.4 and 20% at Goyang-ri, Goyang-gun, and 17.5 and 40% at Simog-ri, Cheongwon-gun, whereas both the chigger index and the seropositive rate were zero at Haingsin-ri, Goyang-gun and at Kwangtan 3-ri, Paju-gun. Their positive correlation strongly supports that *L. pallidum* be the main, effective vector species of the desease.

Only three references on seroepidemiological investigations of field rodents to *R. tsutsugamushi* in Korea are available so far. Lee *et al.* (1990) reported that 68% (133/196) of seropositive rate in *A. agrarius*, 75% (6/8) in *Microtus fortis*, 0% (0/17) in *Crocidura lasiura* and 5% (7/139) in *Rattus norvegicus* were found in Yeocheon, Paju and Pocheon areas in 1986∼1987, and Kim *et al.* (1990) reported 22.4% in *A. agrarius* collected at 7 localities throughout the country in 1988. Shin *et al.* (1989) reported 28.8% (6/21) of the antibody positive rate in *A. agrarius* at Yoncheon and Yoju areas in 1989. In Japan, 52.9% (100/189) of seropositive rate of *Apodemus speciosus* were found at Miyake Island in 1987∼1988 (Kawamura and Tanaka, 1988), and 35.7% (50/140) at Saga city in 1983 (Nakao *et al.*, 1988).

Jackon *et al.* (1957) reported that the infection rates of wild Korean rodents with *R. tsutsugamushi* were 14.3% (11/77) in *A. agrarius* and 11.1% (1/9) in *M. fortis* collected in the Chulwon-Kumhwa-Yonchon area in 1952∼1953, and Shin *et al.* (1989) reported 19.0% (4/21) in *A. agrarius* collected at Yoju and Yoncheon areas in 1989. Their infection rates were extremely higher than the authors' result (3.2%). In Indonesia, infection rates were 8.3% (1/12) in *Rattus argentiventer*, 6.7% (1/15) in *R. rattus diardii*, and 2.8% (1/36) in *Bandicota indica* (Dennis *et al.*, 1981), and in Malaysia, it was 0.5% in *R. tiomianicus* and 6.7% in *Tupia glis* (Shirai *et al.*, 1978).

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=국문초록=

쭈쭈가무시병의 숙주동물에 관한 역학적 조사

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최근 전국적으로 높은 이환율을 보이고 있는 쭈쭈가무시병에 대한 방제 대책이 시급한 현실에 반해 본 질병에 대한 연구가 거의 이루어지 있지 않기 때문에 저자들은 1989년 7월부터 1990년 9월까지 9개 지역에서 숙주동물에 대한 역학적 조사를 실시하여 다음과 같은 결과를 얻었다.

1. 모두 794개의 Sherman식 live trap을 설치하여 111마리의 들쥐로 포획하여 trapping rate가 평균 14.0%이었다. 계절별로 보면 봄, 여름, 가을에 각각 11.7%, 11.2%, 12.8%이었고 겨울에는 24.0%로 가장 높았다. 포획한 111마리 중 103마리가 등줄쥐(*Apodemus agrarius*)로 92.8%를 차지하여 우점종이었고, 땃쥐(*Crocidura lasiura*)가 7마리로 6.3%이었으며. 갈밭쥐(*Microtus fortis*)는 1마리가 채집되어 0.9%이었다. 들쥐의 털진드기 기생률을 보면, 등줄쥐의 경우 103마리 중 84마리로 81.6%이었고, 땃쥐의 경우 7마리 중 2마리로 28.6%이었다 Chigger index는 등줄쥐의 경우 43.0개체였고 땃쥐는 0.9개체였다.

2. 등줄쥐 혈청의 *Rickettsia tsutsugamushi* 항체 양성률을 보면 평균 41.8%이었는데, 조사 지역에 따라 큰 차이가 있어 고양군 도래 5리 54.9%, 고양군 고양리 20%, 고양군 원흥 2리 28.6%, 청원군 시목리 40%이었고 고양군 행신 1리, 파주군 광탄 3리 와 예산군 덕산리에서는 모두 음성이었다. 땃쥐의 경우는 7마리 모두 음성이었다. 동일 장소에서의 등줄쥐 혈청의 *R. tsutsugamushi* 항체 양성률의 계절적 변동은 11월부터 3월까지는 평균 75.8%이었는데 4월에는 30.3%로 감소하였고 5월부터 8월에 걸쳐 평균 13.3%로 크게 감소하였다. 조사한 모든 양성 혈청의 항체가를 보면 Karp, Gilliam 및 Kato 주에 대해 서로 교차반응을 나타냈는데 Karp주와 Kato 주 간에 보다 강한 교차반응을 보였다.

3. 총 94마리의 등줄쥐로부터 마우스 복강접종법과 간접 면역형광법으로 *R. tsutsugamushi*의 분리를 시도한 바 3마리에서 분리되어 감염률은 3.2%이었다.

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