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Determination of the vector species of tsutsugamushi disease in Korea

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Abstract: In order to determine the vector species of tsutsugamushi disease in Korea, chiggers were individually dissected, and internal contents were tested for *Rickettsia tsutsugamushi* organisms by means of indirect FA test, and each exoskeleton was mounted on slide for identification. Among 4,142 chiggers collected from 48 *Apodemus agrarius* at nine different localities during the period of July-November, 1989, 990 chiggers of 10 species of Trombiculidae were dissected and tested. Rickettsiae were confirmed in two *Leptotrombidium pallidum* larvae out of 447 tested, giving 0.4% of the infection rate. The chiggers of the other species tested were found negative.

Key words: *Leptotrombidium pallidum*, *Rickettsia tsutsugamushi*, tsutsugamushi disease, vector species, epidemiology

INTRODUCTION

Tsutsugamushi disease (scrub typhus) is a rickettsial disease of acute onset caused by *Rickettsia tsutsugamushi*. The triangular endemic region extends from northern Japan and the Primorye territory of far eastern U.S.S.R. in the north, to northern Australia in the south, and to Afghanistan and Pakistan in the west, that includes the entire Orient, parts of the Palearctic and the Australasian zoogeographic regions. The disease is transmitted to man by the bite of an infected chigger mite of the family Trombiculidae, suborder Prostigmata, order Acarina. Many species have been established and/or suspected as vectors of the desease. *Leptotrombidium deliense*, *L. fletcheri* and *L. arenicola* were found to be important vector species in Southeast Asian countries (Kohls *et al.*, 1945; Philip *et al.*, 1946; Campbell and Domrow, 1974; Shirai *et al.*, 1982). *L. akamushi*, *L. pallidum* and *L. scutellare* were confirmed as vectors in Japan (Kawamura, 1926; Sasa, 1954; Asanuma *et al.*, 1959 & 1962). Those found to be infected with *R. tsutsugamushi* by direct FA test were *L. umbricola*, *L. bodense*, *L. keukenschrijveri*, *L. arvinum*, *Eutrombicula wichmanni* and *Microtrombicula chamlongi* in various countries, and other species such as *L. tosa*, *L. pavlovskyi*, *L. orientale*, *Ascoshoengastia indica*, *A. audyi* and *Neotrombicula japonica* are suspected of being vectors on the basis of laboratory and epidemiological studies (Oaks *et al.*, 1983).

Since the first Korean cases of tsutsugamushi disease were reported in 1985, cases have been remarkably increased, showing 460 cases in 1986, 784 cases in 1987, and 827 cases in 1988. The cases are widely distributed throughout the country including Cheju Island. Nevertheless, tsutsugamushi disease is relatively unknown in Korea, and very recently being studied in only

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a few laboratories mainly for the diagnosis. Therefore, very little information on epidemiological features of the disease in Korea is available until present. The determination of vector species in Korea was studied only by Jackson *et al.* (1957), who isolated the causative agent from the chigger mites of *L. pallidum* collected at Chipo-ri, Korea.

MATERIALS AND METHODS

Wild rodents were collected at nine different localities from the period of July–November, 1989, by setting modified-Sherman traps, baited with a oats-peanut butter mixed ball. The trapped rodents were transported to the laboratory and identified. The blood and spleen were taken for detection of *R. tsutsugamushi* antibody and antigen respectively, by means of indirect FA test. 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 ecto-parasites including the chiggers (larvae of Trombiculidae). 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 3 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 (3°C) until dissection. They were able to be kept alive at the larval stage for several months.

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 (prepared by the authors). 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 mite 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 procedure is schematically shown in Fig. 1. The slides were dried at room temperature and fixed with acetone for 10 min. 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 to 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 the slide A and Gilliam strain antibody on the slide B. The slides were then incubated at 36°C in a moist chamber for 30 min, washed twice with PBS for 10 min 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 36°C in a wet container for 30 min, washed with PBS for 10 min 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.

The antiserum of *R. tsutsugamushi* was prepared as follows. 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 inoculated intraperitoneally with two doses of 0.5 ml amount of cell count 4×10^6 cells/ml. The two

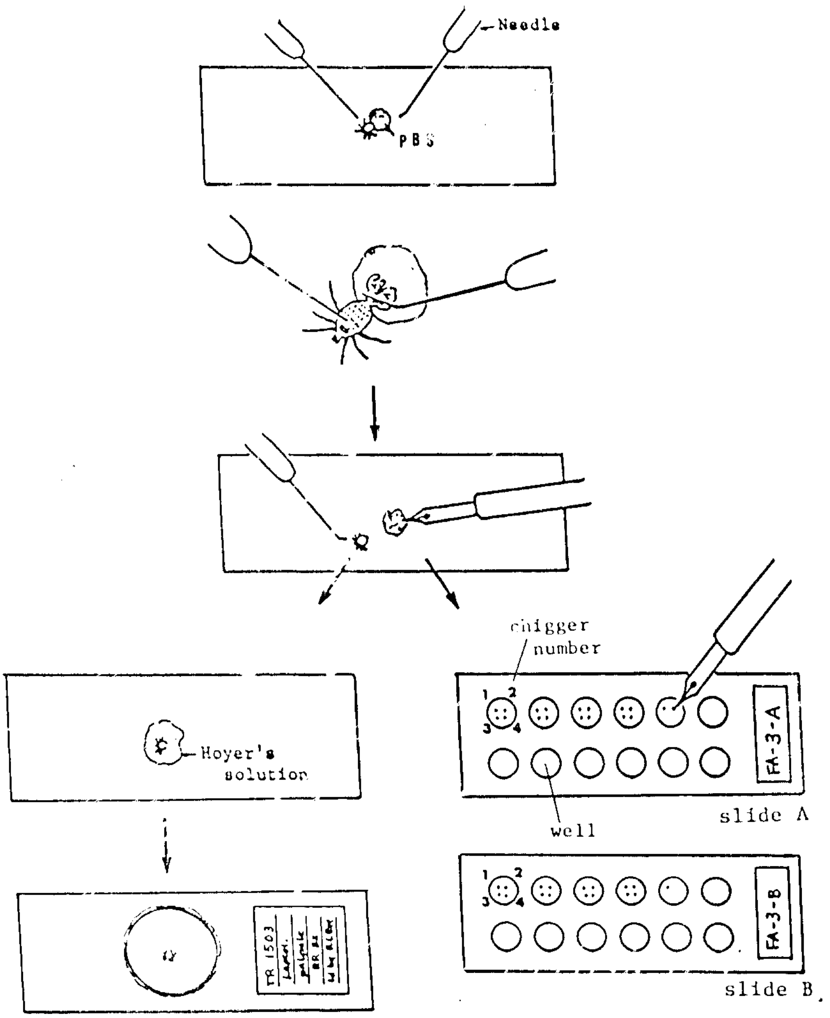

Fig. 1. Dissection of a chigger mite for preparing FA test slides and the chigger specimen.

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 use.

RESULTS

Total 4,142 chiggers were collected from 48 *Apodemus agrarius* and 7 *Crocidura laisura* trapped at nine different localities during the period of July-November, 1989. Out of 4,142 chiggers collected, 990 were dissected and studied their internal contents for *R. tsutsugamushi* antigen by means of indirect FA test and the result is shown in Table 1. The number of chiggers studied by species were 447 *Leptotrombidium pallidum*, 97 *L. palpale*, 12 *L. orientale*, two *L. zetum*, one *L. subintermedium*, one *L. scutellare*, 30 *Neotrombicula gardellai*, 131 *N. tamiyai*, 14 *Cheladonta ikaoensis* and 255 *Eushoengastia koreaensis*.

By the indirect FA test of the internal contents of 990 chiggers, *R. tsutsugamushi* was confirmed in two *L. pallidum* larvae among 447 larvae tested, showing 0.4% of infection rate.

Table 1. Detection of *R. tsutsugamushi* antigen from internal contents of the chigger mites, by means of IFA test in 1989

Locality**	Date of collection	*L. pallidum*	*L. palpale*	*L. orientale*	*N. gardellai*	*N. tamiyai*	*Ch. ikaoensis*	*Eu. koreanensis*	Others	Total No. examined
Ⅰ	23 Aug.	7	0	0	0	0	3	14	0	24
Ⅱ	5 Sep.	0	1	0	22	0	2	0	0	25
	5 Oct.	16	0	1	8	0	8	193	3	229
Ⅲ	19 Oct.	105	0	0	0	0	1	48	0	154
Ⅳ	1 Nov.	220(2)*	4	1	0	0	0	0	0	225
Ⅴ	11 Nov.	0	3	10	0	0	0	0	1	14
Ⅵ	16 Nov.	29	55	0	0	20	0	0	0	104
Ⅶ	24 Nov.	42	28	0	0	49	0	0	0	119
Ⅷ	29 Nov.	28	6	0	0	62	0	0	0	96
Total		447(2)*	97	12	30	131	14	255	4	990

(   )* : Number positive
Locality** :
Ⅰ : Jeonggam-dong, Dongducheon-si, Gyonggi-do.
Ⅱ : Gwangtan 3-ri, Jori-myon, Paju-gun, Gyonggi-do.
Ⅲ : Bugog-dong, Bucheon-si, Gyonggi-do.
Ⅳ : Dorai 5-ri, Wondang-up, Goyang-gun, Gyonggi-do.
Ⅴ : Deogsan-ri Deogsan-myon, Yesan-gun, Chungcheongnam-do.
Ⅵ : Goyang-ri, Byogje-myon, Goyang-gun, Gyonggi-do.
Ⅶ : Wonhung 2-ri, Wondang-up, Goyang-gun, Gyonggi-do.
Ⅷ : Simog-ri, Hyondo-myon, Cheongwon-gun, Chungcheongbuk-do.

All the chiggers of the other species were found negative. The *R. tsutsugamushi* antigens of two positive cases reacted strongly to Karp strain antiserum and weakly responded to Gilliam strain antiserum.

DISCUSSION

The vector species of tsutsugamushi disease in Korea was reported by Jackson *et al.* (1957) for the first time in 1957. They collected *Apodemus agrarius* at Chipo-ri, Korea and the engorged chigger mites of *L. pallidum* parasitized on them, both of which were transported alive by air to Walter Reed Army Institute of Research, Washington, U.S.A., and *L. pallidum* larvae were maintained for colony. By means of laboratory mice inoculation, *R. tsutsugamushi* organisms were isolated from both *A. agrarius* and the off-springs of *L. pallidum.* Though *L. pallidum* were parasitized on the rickettsia-infected host, the study results indicated that *L. pallidum* was a vector species because *R. tsutsugamushi* organisms were transovarially transmitted to the off-springs. They did not mention how many chiggers of *L. pallidum* were used for colony, and other species of Trombiculidae were not tested.

The 447 chiggers of *L. pallidum* tested were collected at seven different localities as shown in Table 1, and two positive chiggers were found among 220 chiggers collected in November, 1989 at Dorai 5-ri, Wondang-up, Goyang-gun, Gyonggi-do, where 54.9% of the antibody positive rate of *A. agrarius* serum (78.6% in November only) and 4.7% of the infection rate of *A. agrarius* were shown. As the most chiggers were engorged and/or half-engorged when collected, it was possible that even the chiggers of non-vector species would possibly pick up rickettsiae from the host (if the host was infected stage), and rickettsiae would be kept alive, with or without propagation, during some period of the larval stage. In order to check such a possibility, the host rodents were tested whether they were infected with the causative agent and also observed their antibody titers. One positive chigger of *L. pallidum* (RTR-1483) was parasitized on the host *A. agrarius* (RR-17) that show 1:80 Karp strain antibody titer and was antigen negative, *i.e.* not infected with rickettsiae, and the other positive chigger (RTR-1525) was parasitized on the host *A. agrarius* (RR-30) that showed 1:40 Karp strain antibody titer and was also antigen negative. The authors' study result is the first finding that *R. tsutsugamushi* organisms were confirmed from individual chigger mites of natural populations of *L. pallidum* and that the infection rate was given (0.4%).

Infection rate of *Leptotrombidium* chiggers in Peninsula Malaysia were studied by Shirai *et al.* (1981), following the methods of Dohany *et al.* (1978), which were also followed by the authors. The infection rate was 4.8% (116 positive chiggers among 2,394 chiggers examined) in *L. deliense*, 3.5% (14/404) in *L. fletcheri*, 2.6% (6/235) in *L. scutellare*, 1.9% (15/791) in *L. umbricola*, 2.6% (2/77) in *L. bodense* and 2.3% (15/646) in *L. keukenschrijveri*, all of which showed much higher infection rate compared to 0.4% of *L. pallidum* in Korea. On the other hand, Minimum Infection Rate (MIR), defined as the number of pools positive divided by the number of chiggers examined multiplied by 100, of *L. pallidum* to *R. tsutsugamushi* was studied in two Prefectures of Japan; in Kanagawa Prefecture 1,414 chiggers of *L. pallidum*, divided by 17 pools were inoculated into laboratory mice and 3 pools were positive for rickettsiae showing 0.2% of MIR, and in Nigata Prefecture 722 chiggers, divided by 17 pools were inoculated into laboratory mice and 3 pools were positive for rickettsiae showing 0.2% of MIR, and in Nigata Prefecture 722 chiggers, divided into 8 pools were inoculated into mice and one pool had rickettsiae, giving 0.12% of MIR (Tamiya, 1962).

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=국문초록=

국내 쭈쭈가무시병(양충병)의 매개종 규명

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1989년 7월～11월에 걸쳐 9개 지역에서 생포한 48마리의 등줄쥐(*Apodemus agrarius*)로부터 4,142개체의 털진드기(Acarina: Trombiculidae) 유충을 채집하였다. 이들 중 10종 990개체를 해부하여 그 내용 물질로부터 쭈쭈가무시병의 병원체를 간접 면역형광항체법으로 조사하였다. *L. pallidum* 유충 447개체 가운데 2개체에서 병원체인 *Rickettsia tsutsugamushi*를 발견하여 본종이 쭈쭈가무시병의 매개종임을 확인하였고, 감염률은 0.4%이었다.

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