

## Seroepidemiological Investigation on *Rickettsia* *Tsutsugamushi* in Nagasaki Prefecture

Hiroshi SUZUKI, Yoshie FUKUMOTO and Keizo MATSUMOTO,

*Department of Internal medicine, Institute for Tropical  
Medicine, Nagasaki University, Nagasaki 852*

Reizo MATSUO, Makoto KUWAZUKA and Youichi KASE

*Nagasaki Prefectural Institute of Public Health and  
Environmental Science, Nagasaki 852*

**Abstract:** Levels of serum IgG antibody to *Rickettsia tsutsugamushi* were determined by the immune peroxidase method on 314 healthy inhabitants in Nagasaki prefecture. Of the 314 inhabitants 120 were from Nagasaki city, 50 from Shimabara city, 50 from Fukue city, 50 from Oseto town and 44 from Wakamatsu town. Mean positive rate in Nagasaki prefecture was 5.1 per cent. The highest positive rate was 8.0 per cent from Oseto own and the lowest one was 2.0 per cent from Shimabara city. However these results were not significantly different according to the  $\chi^2$ -test. The level of antibody in positive cases ranged from 1:20 to 1:80 in serum dilution and the positive rate at each decade ranged from 4.0 per cent at the 4th decade to 15.0 per cent at the 3rd decade. These results were, however, not significant when checked by  $\chi^2$ -test. These results indicate that wild rodents had been already infected by *Rickettsia tsutsugamushi* in some areas before we found clinical cases of *Rickettsia tsutsugamushi* in Nagasaki prefecture.

*Key words:* *Rickettsia tsutsugamushi*, immune peroxidase method

### INTRODUCTION

Infections due to both the old and new strain of *Rickettsia tsutsugamushi* (R. *tsutsugamushi*) had decreased during the period 1965 to 1976 (Otsuru, 1984). However since 1977, infections due to the new strain of R. *tsutsugamushi* are reported to be on the increase in Japan. The occurrence of R. *tsutsugamushi* before 1976 was limited mainly to Akita, Niigata and a few other prefectures. The current infections however do not only occur in these prefectures but have recently spread to other prefectures (Otsuru, 1984). In Nagasaki prefecture there were no reports of R. *tsutsugamushi* infections from 1950 to 1982 (Annual report of health in Nagasaki prefecture, 1983). In 1982 and 1983 five and eight patients, respectively, who met both the clinical and the

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Received for Publication, November 17, 1984.

Contribution No. 1511 from the Institute for Tropical Medicine, Nagasaki University.

serological criteria for diagnosis of *R. tsutsugamushi* were reported in Nagasaki prefecture (Annual report of health in Nagasaki prefecture, 1983). These were sporadic and occurred at different areas of the Nagasaki prefecture. Infection due to *R. tsutsugamushi* are potentially fatal (Suzuki et al, 1981; Sasa et al, 1984). Prompt diagnosis and treatment reduce both the duration of illness and the mortality rate. Although chloramphenicol, tetracycline and in particular doxycycline remain effective, the antibiotics in current use in Japan are ineffective (Sheehy et al, 1973; Olson et al, 1980; Twarz et al, 1982). The foregoing reports and the potentially fatal outcome of the disease if untreated prompted the present seroepidemiological studies of *R. tsutsugamushi*. The results will hopefully help to map out endemic areas in Nagasaki prefecture.

### MATERIALS AND METHODS

**Subject.** Three hundred and fourteen healthy inhabitants of Nagasaki prefecture selected randomly were the basis of the present study. Of the 314 subjects, 120 resided at Nagasaki city, 50 at Shimabara city, 50 at Oseto town, 50 at Fukue city and 44 at Wakamatsu town. Their ages ranged from 20 to 80 years, with a mean of 49, at Nagasaki

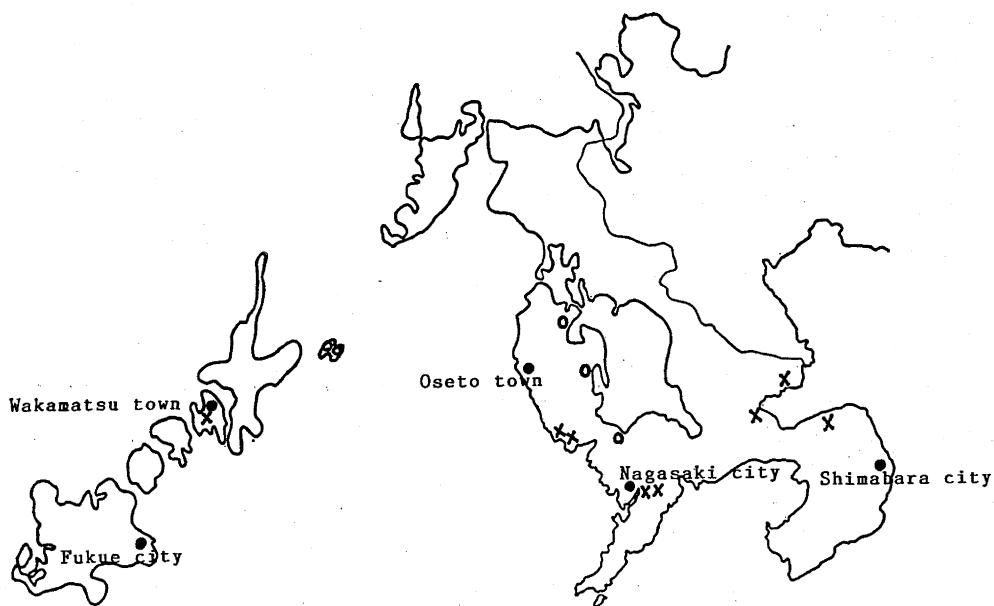


Fig. 1. Map of Nagasaki prefecture.

- — Shows areas from which serum samples were collected.
- — Localities where patients with *Rickettsia tsutsugamushi* were reported in 1982.
- × — Localities where patients with *Rickettsia tsutsugamushi* were reported in 1983.

city, from 41 to 69 years (mean 57) at Shimabara city, from 40 to 81 years (mean 61) at Oseto town, from 40 to 77 years (mean 61) at Fukue city and from 41 to 85 years (mean 61) at Wakamatsu town. These areas in Nagasaki prefecture are shown in Fig. 1.

Serum. Sera from healthy inhabitants at each area were taken from the beginning of December in 1983 to the end of January in 1984 and stored at  $-20^{\circ}\text{C}$  until use.

Immune peroxidase method. Antibody to *R. tsutsugamushi* was determined by following the method of Dr. Suto (Suto, 1983). Gilliam strain of *Rickettsia tsutsugamushi* was used as antigen. These were kindly supplied by Dr. Suto (Professor, Department of Bacteriology, Akita University School of Medicine) and Dr. Cho (Director, Institute of Virological Science, Denka Seiken Corporation). As peroxidase-immunoglobulin-conjugate, antihuman IgG immunoglobulin conjugate and antihuman IgM immunoglobulin conjugate, preparations from Tago Inc., Salingame, Ca., USA, were used.

Antibody level. Each serum was diluted in twofold serial dilution, starting at 1:20 by using phosphate buffered saline with 0.3 percent of bovine serum albumine. The level of antibody was positive for *R. tsutsugamushi*. A serum was considered to be positive for *R. tsutsugamushi* if it had a titer of 1:20 or more.

## RESULTS

### Positive rate

Positive rates for *R. tsutsugamushi* at each area in Nagasaki prefecture are shown in Table 1. The mean positive rate in Nagasaki prefecture was 5.1 percent. The highest positive rate was 8.0 percent at Oseto town, a place where 2 patients suffering from *R. tsutsugamushi* were reported in 1982. Furthermore Shimabara city had the lowest positive rate of 2 percent. From Shimabara city there have been no reports of patients suffering from *R. tsutsugamushi* (Fig. 1). The difference of positive rate among each area was not significant by  $\chi^2$ -test.

Table 1. Rates of *Rickettsia tsutsugamushi* antibody among Nagasaki prefecture.

Area	Antibody		Total
	Positive	Negative	
Nagasaki city	7* (5.8%)	113 (94.2%)	120
Shimabara city	1 (2.0%)	49 (98.0%)	50
Oseto town	4 (8.0%)	46 (92.0%)	50
Fukue city	2 (4.0%)	48 (96.0%)	50
Wakamatsu town	2 (4.5%)	42 (95.5%)	44
Total	16 (5.1%)	298 (94.9%)	314

\*Number of cases. The difference of positive rate among each area was not significant.

Antibody distribution

Fig. 2 shows the antibody distribution in Nagasaki prefecture. Serum samples with positive antibody titers of more than 1:20 were less than 2.9 percent. None of samples had a positive antibody titer above a dilution factor of 1:80. The antibody distribution at each area were as shown in Fig. 3. Although Oseto town had a higher rate than that at other areas, the difference was not significant by  $\chi^2$ -test.

Age distribution

Fig. 4. shows the age distribution of positive cases in Nagasaki prefecture. The distribution of antibody at the fifth and the sixth decades were higher than at the other

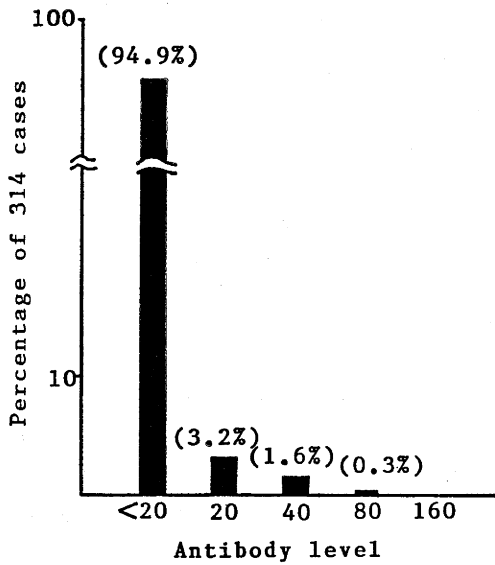


Fig. 2. Distribution of Rickettsia tsutsugamushi antibody levels among the positive cases. Antibody level is expressed by the reciprocal number.

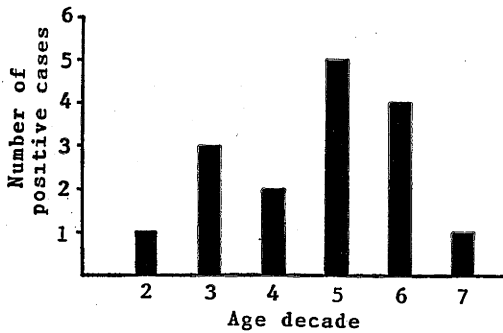


Fig. 4. Age distribution of positive cases in Nagasaki prefecture.

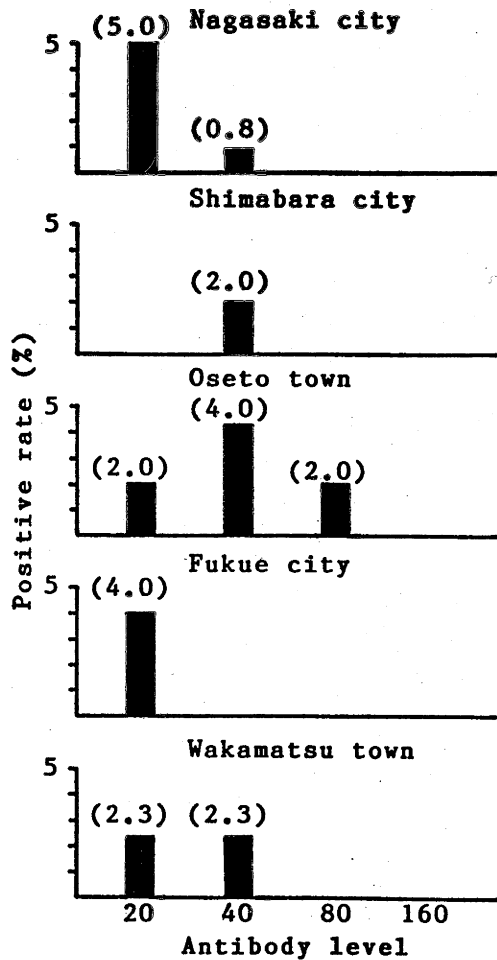


Fig. 3. Rate and titer of antibody levels for the different areas in Nagasaki prefecture. Antibody level is expressed by the reciprocal number.

decades. The positive rate at each decade for each area in Nagasaki prefecture were as shown in Table 2. The apparent differences in the positive antibody rates between the different age groups was not significant by  $\chi^2$ -test.

Table 2. Distribution of *Rickettsia tsutsugamushi* antibodies at each age decade in Nagasaki prefecture.

Area	Age decade					
	2	3	4	5	6	7
Nagasaki city	1/20* (5.0%)	3/20 (15.0%)	1/19 (5.3%)	1/20 (5.0%)	1/21 (4.8%)	0/20
Shimabara city			0/9	1/21 (4.8%)	0/20	
Oseto town			1/8 (12.5%)	1/10 (10.0%)	2/23 (8.7%)	0/9
Fukue city			0/7	1/15 (6.7%)	1/18 (5.6%)	0/10
Wakamatsu town			0/7	1/10 (10.0%)	0/16	1/11 (9.1%)
Total	1/20 (5.0%)	3/20 (15.0%)	2/50 (4.0%)	5/76 (6.6%)	4/98 (4.1%)	1/50 (2.0%)

\*Number of positive cases/number of specimens per decade. Number in parenthesis shows positive rate of *Rickettsia tsutsugamushi*. The difference of positive rate among each age decade was not significant by  $\chi^2$ -test.

## DISCUSSION

Seroepidemiological studies for *R. tsutsugamushi* were performed following reports of 11 documented patients of *R. tsutsugamushi* in Nagasaki prefecture during 1982 through 1983. To date, the available serological diagnostic procedures for *R. tsutsugamushi* are the complement fixation test (CF) (Iida et al, 1966; Shishido et al, 1969), the indirect immunofluorescence test (IIF) (Iida et al, 1966; Bozeman et al, 1963), the microimmunofluorescence test (Robinson and Brown et al, 1976), the indirect immune peroxidase test (IIP) (Suto, 1983; Yamamoto et al, 1982), the enzyme-linked sorbent assay (ELISA) (Dasch et al, 1979) and paper enzyme-linked immunosorbent technique (Crum et al, 1981). In comparison of these tests CF requires more time than the other tests. The sensitivity of IIF, IIP and ELISA are almost same but are considerably higher than that of CF. Furthermore only IIP can prepare permanent preparation for reexamination in addition to the fact that one can observe both the infected and uninfected cells (Suto, 1983; Yamamoto et al, 1982). However, objective determination of the antibody is possible by ELISA method only (Dasch et al, 1979). In the present study IIP was used so as to reconfirm the result that were obtained at different times. The Gilliam strain of *R. tsutsugamushi* was used as antigen, because consistently higher titers were obtained with the strain than with the Karp or Kato strains of rickettsiae for patients reported in 1982 and 1983 in Nagasaki prefecture.

The mean positive rate for *R. tsutsugamushi* in Nagasaki prefecture was 5.1 percent. Rate for other prefectures as determined by IIF were 2.2 for Shizuoka prefecture, 4.2 for Gunma prefecture, 4.5 for Niigata prefecture, 36.5 for Yamanashi prefecture, 23.5 for Toshima island, 26.1 for Miyake island and 56.3 percent for Hachijo islands in Izu Shichito Islands of Tokyo-to. (Otsuru, 1984; Kawamura et al, 1980). The rate for Nagasaki prefecture was similar with that for Niigata, Gunma and Shizuoka prefectures, but lower than that in Toyama prefecture, Yamanashi prefecture and the three islands in Tokyo-to. The positive rate in other prefectures ranged more widely than that in Nagasaki prefecture: It ranged from 18.9 to 49.5 percent in Toyama prefecture, from 23.5 to 56.8 percent at the three islands in Tokyo-to and from 8.1 to 50.2 percent in Yamanashi prefecture. Different researchers have pointed out that wide range of seropositivity for *R. tsutsugamushi* may be related to occupations and habitats (Robinson and Gan et al, 1976; Cadigan et al, 1972). However the explanation for the difference of positive rate and range in each prefecture cited above was not clear.

The antibody levels for *R. tsutsugamushi* in healthy inhabitants in Nagasaki prefecture ranged from 1:20 to 1:160 and were similar results for Toshima and Miyake islands (Kawamura et al, 1980). It was assumed that the positive cases of *R. tsutsugamushi* in these islands might have been due to inapparent infections with avirulent *R. tsutsugamushi* since these individuals had no previous history suggestive of *R. tsutsugamushi* infection. Inapparent infection by *R. tsutsugamushi* in an endemic area of *R. tsutsugamushi* has been reported by Shishido et al (Shishido, 1962). Furthermore Brown et al. found low levels of antibody to *R. tsutsugamushi* in individuals without clinical symptoms living in endemic areas in Malaysia. But they could not analyze the significance of their observations (Brown et al, 1983). Therefore our positive antibody cases in Nagasaki prefecture might have had inapparent infections. It has been reported that following infection by *R. tsutsugamushi*, the serum antibodies, as detected by IIA may persist for several years (Suto, 1983). Therefore, it was assumed that the low antibody titers of *R. tsutsugamushi* in individuals living in Nagasaki prefecture were due to infections acquired several years back. These results indicate that some areas had wild rodents, infected with *R. tsutsugamushi*, before clinically apparent cases of *R. tsutsugamushi* were experienced in Nagasaki prefecture.

#### REFERENCES

- 1) Annual report of health (1983). Health department of Nagasaki prefectural government.
- 2) Bozeman, F. M. & Elisberg, B. L. (1963) Serological diagnosis of scrub typhus by indirect immunofluorescence. *proc. Soc. Exp. Biol. Med.*, 112, 568-573.
- 3) Brown, G. W., Shirai, A. & Groves, M. G. (1983) Development of antibody to *Rickettsia tsutsugamushi* in soldiers in Malaysia. *Transaction of the Royal Society of Tropical Medicine and Hygiene.* 77, 225-227.

- 4) Cadigan, F. C. Jr., Andre, R., G. Bolton, M., Gan, E. & Walker, J. S. (1972) The effect of habitat on the prevalence of human scrub typhus in Malaysia. *Trans. R. Soc. trop. Med. Hyg.*, 66, 588-587.
  - 5) Crum, J. W., Hanchajay, S. & Eamsila, C. (1980) New paper enzymelinked immunosorbent technique compared with microfluorescence for detection of human serum antibodies to *Rickettsia tsutsugamushi*. *J. Clin. Microbiol.*, 11, 584-588.
  - 6) Dasch, G. A., Halle, & Bourgeois, A. L. (1979) Sensitive microplate enzyme-linked immunosorbent assay for detection of antibodies against the scrub typhus rickettsia, *Rickettsia tsutsugamushi*. *J. Clin. Microbiol.*, 9, 38-48.
  - 7) Iida, T., Okubo, K. & Ishimaru, M. (1966) Immunofluorescence for sero-epidemiological study of tsutsugamushi disease rickettsia. *Japan. J. Exp. Med.*, 36, 435-447.
  - 8) Kawamura, A. Jr., Murata, M., Osono, M., Nogami, S., Shirasaka, A., Tanaka, H., Sudo, K., Suzuki, K., Miyairi, T. & Kijima, H. (1980) Studies on inapparent infection of tsutsugamushi disease in Izu Shichito Ilands: [Seroepidemiology and demonstration of an avirulent rickettsia strain for mice. *Japan. J. Exp. Med.*, 50, 91-105.
  - 9) Otsuru, M. (1984) Rickettsial disease. *Jap. J. clin. Med.*, 42, 225-239 (in Japanese)
  - 10) Olson, J. G., Bourgeois, A. L., Fang, R. C. Y. Coolbaugh, J. C. & Dennis, D. T. (1980) Prevention of scrub typhus. Prophylactic administration of doxycycline in a randomized double blind trial. *Am. J. Trop. Med. Hyg.*, 29, 989-997.
  - 11) Robinson, D. M., Brown, G., Gan, E. & Huxsoll, D. L. (1976) Adaptation of a microimmunofluorescence test to the study of human rickettsia tsutsugamushi antibody. *Am. J. Trop. Med. Hyg.*, 25, 900-905.
  - 12) Robinson, D. M., Gan, E. & Donaldson, J. R. (1976) The prevalence of scrub typhus antibodies in residents of west Malaysia. *Trop. geogr. Med.*, 28, 303-308.
  - 13) Sasa, H., Shibata, T., Oba, M., Okubo, M., Niwa, T. & Matsui, E. (1984) An autopsy case of the first occurrence of tsutsugamushi disease in Gifu prefecture. *J. Jap. Soc. Intern. Med.*, 73, 401-407.
  - 14) Sheehy, T. W., Hazlett, D. & Turk, R. E. (1973) Scrub typhus, A comparison of chloramphenicol and tetracycline in its treatment. *Arch. Intern. Med.*, 132, 77-80.
  - 15) Shishido, A. (1962) Inapparent infection of scrub typhus in Japan. *Japan. J. Med. Sci. Biol.*, 15, 330-335.
  - 16) Shishido, A., Hikita, M., T. & Kohno, S. (1968) Particulate and soluble antigens of *Rickettsia tsutsugamushi* in the complement fixation test. *J. immunol.*, 103, 480-490.
  - 17) Suto, T. (1983) Recent trend of *Rickettsia tsutsugamushi* in Japan and rapid diagnostic procedure by the immune peroxidase method. *Rinsho and Viruse.* 11, 23-30. (in Japanese)
  - 18) Suzuki, T. & Sekikawa, H. (1981) Four cases of tsutsugamushi disease (scrub typhus) complicated with disseminated intravascular coagulation. *J. J. A. Inf. D.*, 55, 642-648.
  - 19) Twarz, J. C., Shirai, A. Selvaraju, G., Saunders, J. P. Huxsoll, D. L. & Groves, M. G. (1982) Doxycycline prophylaxis for human scrub typhus. *J. Infect. Dis.*, 146, 811-818.
  - 20) Yamamoto, S. & Minamishima, Y. (1982) Serodiagnosis of tsutsugamushi fever (scrub typhus) by the indirect immunoperoxidase technique. *J. Clin. Microbiol.*, 15, 1128-1132.
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## 長崎県におけるつつが虫病の血清疫学的研究

鈴木 寛, 福本美枝, 松本慶蔵 (長崎大学熱帯医学研究所臨床部門)  
松尾礼三, 鍛塚 真, 嘉勢洋一 (長崎県衛生公害研究所ウィルス課)

長崎県においては, 昭和57年に5例のつつが虫病患者が始めて報告された. そこで, 和58年冬期に長崎県各地における住民に対するリケッチア・ツツガムシ抗体を Immune Peroxidase 法 (Gilliam strain) により測定した. 対象地区は, 長崎市 (120名), 島原市 (120名), 大瀬戸町 (50名), 福江市 (50名), 若松町 (44名) で, 対象数は総計314名であった. 長崎県全体としての陽性率は5.1%で, 地域別にみた陽性率の最高は大瀬戸町の8.0%, 最低は島原市の2.0%であった. 陽性者の抗体は, 1:20~1:80に分布していた. 尚, 陽性率の地域差および年代差はみられなかった. しかし, これらの成績から, 本県においてもリケッチア・ツツガムシに感染した野ネズミが既に存在していることが示唆された.

熱帯医学 第26巻 第4号, 157-164頁, 1984年12月