Trop. Med., 27 (3), 147–154, September, 1985

Seroepidemiology of Rickettsia Tsutsugamushi in Nakadori Island in Nagasaki Prefecture

Hiroshi SUZUKI[1], Kisali JUSTO PALLANGYO[2], Yoshie FUKUMOTO[1], Keizo MATSUMOTO[1], Takuro ASO[3] and Toshitaka HAMASAKI[3]

[1]*Department of Internal Medicine, Institute for Tropical Medicine, Nagasaki University, Nagasaki 852, Japan*

[2]*Department of Internal Medicine, Faculty of Medicine, Muhimbili Medical Center, P.O. Box 65066, Dar-es-Salaam, Tanzania*

[3]*Arikawa Medical Association, Arikawa-cho, Nagasaki 857-41, Japan*

Abstract: During December 1984, blood samples were collected from 270, healthy inhabitants of five different areas in Nakadori Island of Nagasaki Prefecture. Rickettsia tsutsugamushi specific IgG and IgM antibody were determined by immune peroxidase method using the Gilliam strain as antigen. The positive rate of IgG antibody at four areas ranged from 51.8 to 76.0 percent and were similar to those reported for another endemic areas for Rickettsia tsutsugamushi in Japan. However these rates were not significantly different. Another area had a positive rate of 9.3 percent. This was significantly lower than the positive rates at the other four areas. The IgG antibody distribution in the samples from the five areas were significantly different by the Wilcoxon test. Our results show that four of the five localities investigated are endemic for Rickettsia tsutsugamushi. In addition, the results suggest that the endemic zone has been spreading with time and further spread to presently non-endemic areas is anticipated.

Key words: Rickettsia tsutsugamushi, Immune peroxidase method, Seroepidemiology

INTRODUCTION

The Nakadori Island, one of the Goto group of Islands, of the Nagasaki Prefecture is thought to be endemic for Rickettsia tsutsugamushi (R. tsutsugamushi). But for a period of 35 years there were no reports of disease due to R. tsutsugamushi from these islands or any other part of the prefecture (Health Department of Nagasaki Prefectural Government, 1983). Since 1982, however, documented cases of disease due to R. tsutsuga-

Received for Publication, July 6, 1985
Contribntion No. 1651 from the Institute for Tropical Medioine, Nagasaki University

mushi have appeared sporadically not only in the Nakadori Island but also from several parts of the Nagasaki Prefecture, where the disease had never been reported before (Health Department of Nagasaki Prefectural Government, 1983).

In the light of these recent developments we conducted a seroepidemiological survey to evaluate the prevalence rate, the distribution of antibody to R. tsutsugamushi and the pathogenic organism for the inhabitants of the Nakadori Island.

MATERIALS AND METHODS

Samples. In December 1984, during routine annual medical examination done for all inhabitants aged 40 years and above at Naname, Ota, Akao, Enohama in Arikawa town and Sone in Shinuonome Town (Fig. 1) blood samples were collected. A random selection of the samples obtained during the exercise was done. The number of samples thus obtained and the corresponding population ratio studied from each locality were as follows: 50 (9.1 percent) for Naname, 50 (5.4 percent) for Ota, 56 (13.4 percent) for Akao, 54 (17 percent) for Enohama and 60 (10 percent) for Sone. Sample source and structure of the population studied are shown in Table 1.

Occupation and residential environment. The inhabitants of the five localities from which the blood samples were collected are mainly involved in agriculture. At times however, some of them may do fishing or participate in road construction. The residential areas of the study population are separated from each other by small hills around which are stretches of waste lands.

Immune peroxidase method. Antibody to R. tsutsugamushi were examined for by the method described by Suto (1984). The Gilliam strain of R. tsutsugamushi was used

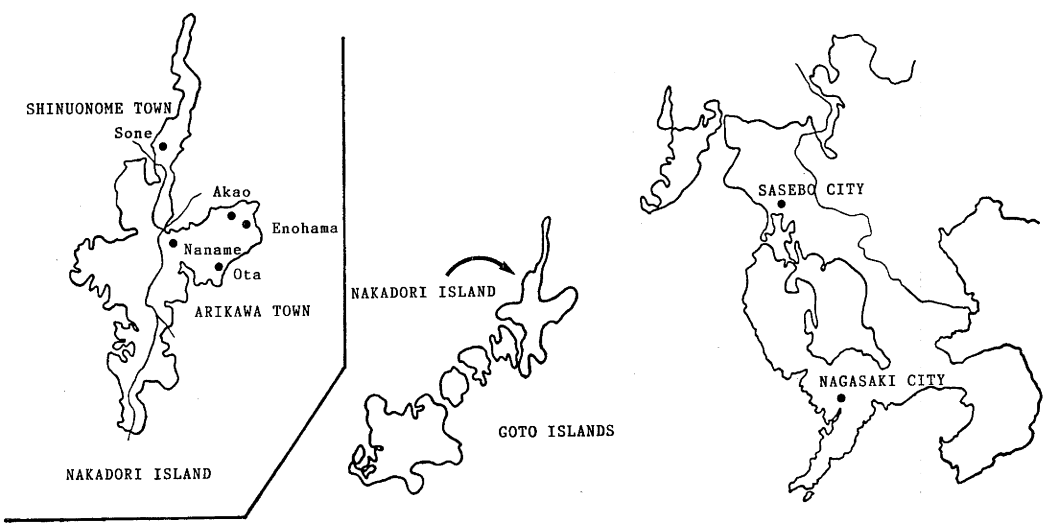

Fig. 1. Map of Nagasaki Prefecture.

Table 1. Sample source and structure of the population studied

Locality	Percent of populaton studied	No.of samples	Age in years		Sex
			mean	range	M : F
Arikawa town					
Naname	9.1	50	57	40−78	0.7 : 4.3
Ota	5.4	50	57	40−78	1.5 : 4.5
Akao	13.4	56	61	42−81	1.0 : 4.6
Enohama	17.0	54	62	40−85	1.7 : 3.7
Shinuonome town					
Sone	10.0	60	68	41−85	2.3 : 3.7
Total		270	61	40−88	

as the antigen. The antigen was kindly supplied by Dr. Cho (Director, Institute of Virological Science, Denka Seiken Corporation). Peroxidase-labeled goat IgG and IgM fractions against human immuno globulins were commercial preparations from Tago Inc. Salingame Ca. USA.

Antibody positive level. Two fold serial dilutions (begining with a dilution of 1 : 20) were prepared using phosphate buffered saline with 0.3 percent bovine serum albumin. Sera with antibody titers of 1 :20 or more were considered positive.

RESULTS

Positive rate.

The IgG antibody rates were as shown in Table 2. The positive rate ranged from 9.3 percent at Enohama to 76 percent at Ota. The rate at Enohama was found to be significantly lower (by χ^2-test) than the rates at the other four areas. On the contrary the

Table 2. Rates of Rickettsia tsutsugamushi antibody in Nakadori Island of Goto Islands in Nagasaki Prefecture

Locality	No. of cases	Positive antibody	
		IgG	IgM
Arikawa town			
Enohama	54	5 (9.3) *	0
Akao	56	29 (51.8)	0
Nanama	50	31 (62.0)	0
Ota	50	38 (76.0)	8 (16.0)
Shinuonome town			
Sone	60	36 (60.0)	10 (16.7)
Total	270	139 (51.5)	18 (6.7)

* :percentage

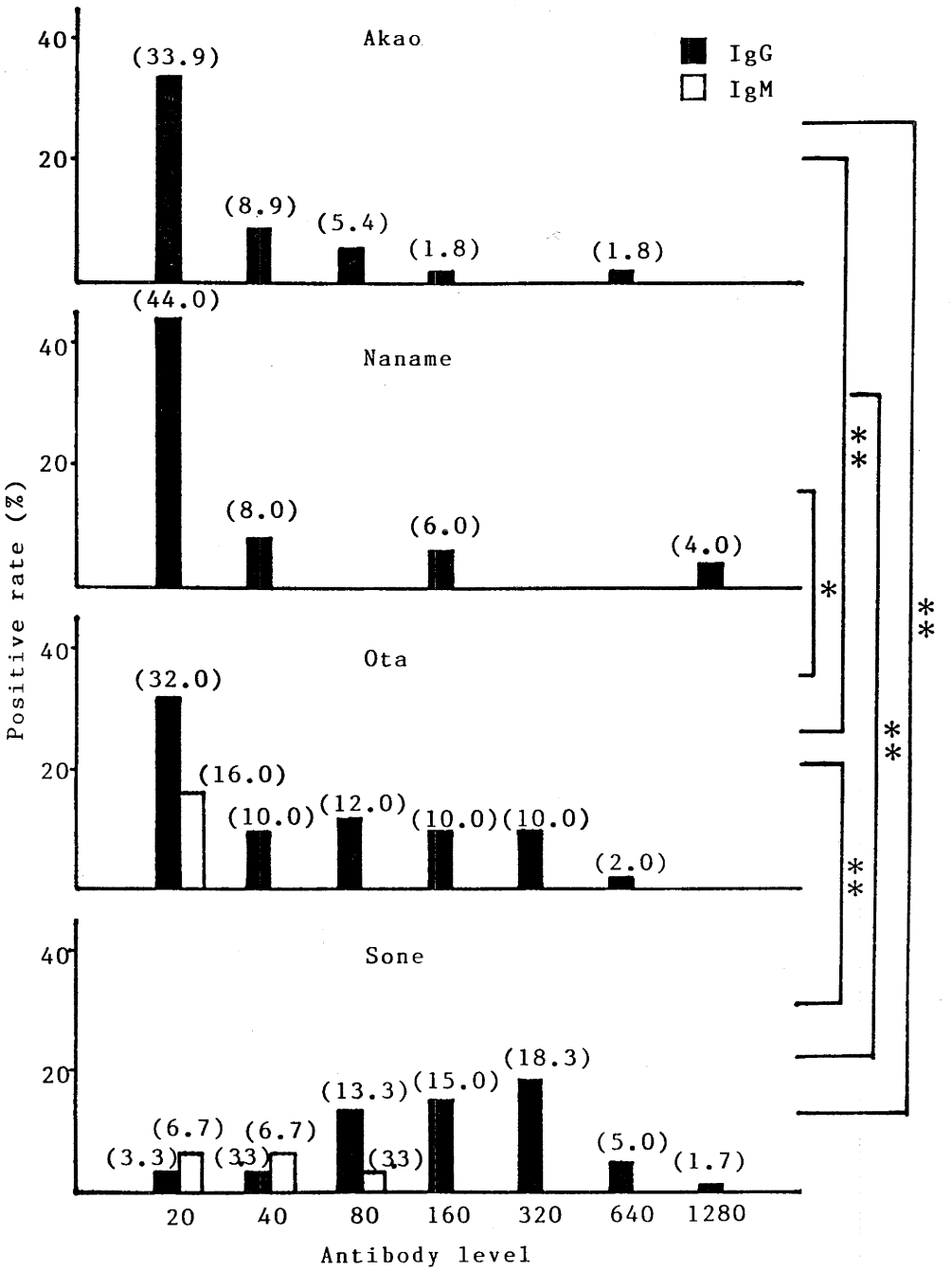

Fig. 2. Antibody distribution of Rickettsia tsutsugamushi at different areas in Nakadori Island. The number of parenthesis expresses percent. The level of antibody was expressed by the reciprocal in serum dilution.
*: significant at the level of 5%. **: significant at the level of 1%. Statistical calculation was done by the method of Wilcoxon test.

rates for Naname, Akao, Ota and Sone were not significantly different from each other.

IgG antibody distribution.

Antibody level distribution in the samples from the four areas with high positive rates are shown in Fig. 2 . Comparison of these data by the Wilcoxon test showed that the IgG antibody distribution was highest at Sone followed by Ota. The distribution at Akao and Naname were not significantly different from each other. Furthermore, antibody level distribution at Enohama was significantly lower than that at other four areas (data not shown).

IgM antibody distribution.

As shown in Table 2 R. tsutsugamushi specific IgM antibody were detected at Sone (16.7 percent) and Ota (16 percent) only. Whereas the reciprocal IgM antibody level for Sone ranged between 20 and 80, only titers of 20 could be detected at Ota, as shown in Fig. 2 .

IgG antibody distribution and age.

Analysis of IgG antibody rate (χ^2-test) and level (Wilcoxon test) by age decade at high prevalence areas (Akao, Naname, Ota and Sone) did not show any significant difference. As shown in Table 3 , however, there was general trend for the antibody positive rate to increase with increasing age.

Table 3. Positive rate and antibody distribution of Rickettsia tsutsugamushi at each decade in high prevalence areas

Age decade	cases	No. of positive cases (%)	Antibody distribution of posiive cases						
			20	40	80	160	320	640	1280
4	40	21 (52.5)	13 (32.5)	2 (5.0)	3 (7.5)	2 (5.0)	1 (2.5)		
5	66	38 (57.6)	17 (25.8)	3 (4.5)	3 (4.5)	7 (10.6)	5 (7.6)	1 (1.5)	2 (3.0)
6	50	49 (66.0)	13 (26.0)	5 (10.0)	6 (12.0)	4 (8.0)	3 (6.0)	1 (2.0)	1 (2.0)
≧ 7	60	44 (73.3)	18 (30.0)	6 (10.0)	5 (8.3)	5 (8.3)	7 (11.7)	3 (5.0)	

High prevalence areas of Rickettsia tsutsugamushi in Nakadori Island were Akao, Naname, Ota and Sone.

DISCUSSION

The antibody positive rate of 9.3 at Enohama was significantly lower than the rate at the other four areas. On the other hand the antibody rate for Enohama does not differ significantly with the rates of 2 to 8 percent reported from non-endemic areas in Nagasaki Prefecture (Suzuki *et al.* 1984). The present data, therefore, indicate that Enohama is a non-endemic area for R. tsutsugamushi. However, the possibility of low endemicity of R. tsutsugamushi at Enohama can not be ruled out by our data.

Akao, Naname, Ota and Sone had antibody positive rates of 51.8, 62.0, 76.0 and 60.0 percent respectively. These rates are higher than those reported for Yamanashi Prefecture (36.5 percent), Toshima Islands (23.5 percent) and Miyake Island (26.1 percent) areas known to be endemic for R. tsutsugamushi (Otsuru 1984). However, the rates at the four areas are similar to those reported for Hachijo Island of Tokyo-To (56.3 percent) another endemic area for R. tsutsugamushi (Otsuru 1984). Akao, Naname, Ota and Sone are therefore endemic areas for R. tsutsugamushi. However, the higher antibody level distribution and the presence of IgM antibodies in sera from Sone and Ota indicate recent infections. On the other hand infections at Naname and Akao were old as suggested by the lower IgG antibody levels and absence of IgM antibodies. It was, furthermore, assumed that the infection at Enohama was the oldest as evidenced by lower positive rate and antibody level distribution.

The similar positive rates for the samples from the four locations herein reported indicate that the pathogenic strain of R. tsutsugamushi endemic in these areas might be the same.

Following confirmed scrub typhus infection, the IgG antibodies to R. tsutsugamushi in most patients disappear within one year (Saunders *et al.* 1980). However persistence of IgG antibodies for over five years have been reported (Shishido 1962; Bozeman & Elisberg *et al.* 1963; Suto 1983). Also it is noteworthy that atypical, mild and subclinical infection by R. tsutsugamushi have been reported (Shishido 1962; Brown *et al.* 1983). Furthermore, inapparent infections with avirulent strains of R. tsutsugamushi are known to occur (Kawamura *et al.* 1980). The low IgG antibody levels detected in some inhabitants of Enohama could therefore have been due to either old infections of scrub typhus, avirulent strains of R. tsutsugamushi or both. It is interesting to note that the antibody level range of 1:20 to 1:80 at Enohama is similar to those for Miyake and Toshima reported by Kawamura and coworkers (1980). These researchers ascribed the antibodies found in their study population to inapparent infections with avirulent strains of R. tsutsugamushi.

In a given population, the seropositivity for R. tsutsugamushi is said to be influenced by the habitat, occupation, consumption of antibiotics (tetracycline, doxycycline and chloramphenicol) and the use of agricultural chemicals (Robinson *et al.* 1976; Cadigan *et al.* 1972; Sheehy *et al.* 1973; Olson *et al.* 1980; Twartz *et al.* 1982; Otsuru 1984). The occupation for the residents of the five areas studied are similar. We have no good reason to think that the antibiotic consumption or use of agricultural chemicals in these communities is different either. The fields around the residential areas were managed well until about ten years ago. Since then however, these fields have become waste land and therefore good for rodent habitation.

That in 1982 two patients from the Nakadori Island met both the clinical and serological criteria for scrub typhus could be a result of several factors. It is of note that none had been reported for 35 years in Nagasaki Prefecture. On the first place it has been reported that in endemic areas disease due to R. tsutsugamushi occurs every 15 to

25 years (Kawamura *et al.* 1980). Secondly atypical, mild or subclinical infections which escaped clinical attention could have occured all along the years. Nonetheless the provision of good habitat for rodents during the last ten years favors persistence as well as increased risk of scrub typhus infections in these islands. Furthermore, results of the analysis of IgG and IgM antibody levels and distribution in the five areas suggest spreading R. tsutsugamushi infections from Akao and Naname to Ota and Sone. These results therefore, indicate that the change in the environment has been a major factor for the reappearance of scrub typhus disease in the Nakadori Island. That destruction of rodent habitat is mandatory for effective control and eradication of disease due to R. tsutsugamushi can not be overemphasized. Failure to do this simple but effective control measure will only encourage continued endemicity of R. tsutsugamushi and its consequences in the Nakadori Island.

REFERENCES

1) Bozeman, F. M. & Elisberg, B. L. (1963): Serological diagnosis of scrub typhus by indirect immunofluorescence. Proc. Soc. Exp. Med., 112, 568−573.

2) Brown, G. W., Shirai, A. & Groves, M. G. (1983): Development of antibody to Rickettsia tsutsugamushi in soldiers in Malaysia. Trans. R. Soc. trop. Med. Hyg., 77, 225−227.

3) Cardigan, F. C. Jr., Andre, R. G., 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, 582−587.

4) Health Department of Nagasaki Prefectural Government. (1984): Annual report of health, 211−212.

5) Kawamura, A. Jr., Murata, M., Osono, M., Nogami, S., Shirasaka, A., Tanaka, H., Sudo, K., Miyairi, T. & Kijima, H. (1980): Studies on inapparent infection of tsutsugamushi disease in Izu Shichito Islands: Seroepidemiology and demonstration of an avirulent rickettsia strain for mice. Japan. J. Exp. Med., 50, 91−105.

6) Olson, J. G., Bourgeois, A. L., Fang, R. C. Y., Coolbaugh, J. C. & Deniss, 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.

7) Otsuru, M. (1984): Rickettsial disease. Jap. J. clin. Med., 42, 225−239. (in Japanese)

8) 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.

9) Saunders, J. P., Brown, G. W., Shirai, A. & Huxsoll, D. L. (1980): The longevity of antibody to Rickettsia tsutsugamushi in patients with confirmed scrub typhus. Trans. R. trop. Med. Hyg., 74, 253−257.

10) 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.

11) Shishido, A. (1962): Inapparent infection of scrub typhus in Japan. Japan. J. Med. Sci. Biol., 15, 330−335.

12) Suto, T. (1983): Recent trend of Rickettsia tsutsugamushi in Japan and rapid diagnostic procedure by the immune peroxidase method. Rinsho and Virus, 11, 23−30. (in Japanese)
13) Suzuki, H., Fukumoto, Y., Matsumoto, K., Matsuo, R., Kuwazuka, M. & Kase, Y. (1984): Seroepidemiological investigation on Rickettsia tsutsugamushi in Nagasaki prefecture. Trop. Med., 26, 157−164.
14) Twartz, 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.

長崎県中通島におけるつつが虫病に関する血清疫学的研究

[1]鈴木　寛，[2]キサリ・ジャスト・パランギョ，[1]福本美枝，[1]松本慶蔵，[3]麻生卓郎，[3]浜崎利孝

([1]長崎大学熱帯医学研究所臨床部門，　[2]タンザニア　ムヒンビリ医学センター医学部内科，
[3]長崎県有川医師会)

長崎県五島列島に属する中通島の5地域住民270名を対象としてリケッチア・ツツガムシに関する血清疫学的研究を行った．抗体は immune peroxidase 法により，抗原として Gilliam 株を用いて測定した．IgG 抗体陽性率は江ノ浜（9％）のみが有意に他の4地域（赤尾52％，七目62％，太田76％，曽根60％）より低かった．しかし，高い陽性率を示した4地域間の陽性率には有意差を認め得なかった．さらに，各地における抗体分布レベルの比較では，有意に高い順位より曽根，太田，七目と赤尾，そして江ノ浜であった．尚，IgM 抗体は高レベルの抗体分布を示した曽根および太田の対象より検出された．これらの成績より限定された地域においても経時的感染順位があることが示唆され，さらに，高い陽性率を示した地域間の陽性率に有意差が認められなかったことより，これらの地域におけるリケッチア・ツツガムシの感染株は同一のものであろうと推測された．さらに，低陽性率の江ノ浜には今後リケッチアの浸淫するであろうことが予測される．

熱帯医学　第27巻　第3号，147−154頁，1985年9月