

Toxoplasma Infection in Nakadori Island of Nagasaki Prefecture : A Community Survey

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Abstract: During December 1984, blood samples were collected from 260 healthy inhabitants aged forty years or more at five different localities in Nakadori island of Nagasaki prefecture. The sera were screened for toxoplasma IgG specific antibody by an enzyme-linked immunosorbent assay (ELISA). The positive rate of IgG antibody in the island ranged from 48.1 to 86 percent. There was significant difference, by χ^2 -test, in the antibody rates between localities. The level of antibody distribution as well as the rate of high antibody titer for one locality were significantly higher than those for other localities. The antibody rate at the 4th decade on the island was significantly lower than at the other decades. However, there was no significant difference in the antibody distribution with age. Our results lend support to the view that toxoplasma antibody rate is a very local matter and that data from one locality can not be applied to another localities however similar they may be. In addition the results showed that the positive rate tend to increase with age.

Key words: *Toxoplasma gondii*, Enzyme-linked immunosorbent assay, Seroepidemiology

INTRODUCTION

Toxoplasma gondii (*T. gondii*) are ubiquitous in nature and infect herbivorous, omnivorous and canivorous animals including all orders of mammals, some of birds and probably some of reptiles (Luft *et al.*, 1983). Although *Toxoplasma gondii* infections occur almost throughout the world, the prevalence varies from place to place for still undeter-

mined reasons (Feldman, 1968). Consequently, determination of the epidemiology of this condition should be a very local matter and reliable generalizations can not be made (Feldman *et al.*, 1956). Man is presumed to acquire toxoplasmosis through the oral route by ingestion of oocysts. As the oocysts are resistant to the acid and enzymes in the stomach, parasitemia and dissemination usually follow. Such acquired toxoplasmosis, when symptomatic, produces a variety of illnesses in which lymphadenopathy, myocaditis and encephalitis may predominate. However in most cases clinical manifestations are generally absent. Nonetheless it must be remembered that the parasite are widely distributed in the host tissues and remain viable in cysts for many years (Feldman, 1968). Because of the persistence of toxoplasma in the body, they may again disseminate in hosts whose immune mechanisms have been injured by other diseases or by immunotherapeutic agents (Ruskin *et al.*, 1976: Krick *et al.*, 1978). With increasing life expectancy in Japan in the last few decades, cancer patients and other such patients who may require immunosuppressive treatment have also increased (Kosei Tokei Kyokai, 1985). The present study is part of a larger program being conducted in Nagasaki prefecture aimed at: (a) determining the prevalence rate of toxoplasma IgG antibody in the community. (b) increasing awareness of practicing physicians of the presence and extent of toxoplasma infection albeit asymptomatic in the population. (c) attempting to identify local factors that may influence transmission of toxoplasmosis.

MATERIALS AND METHODS

Serum 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

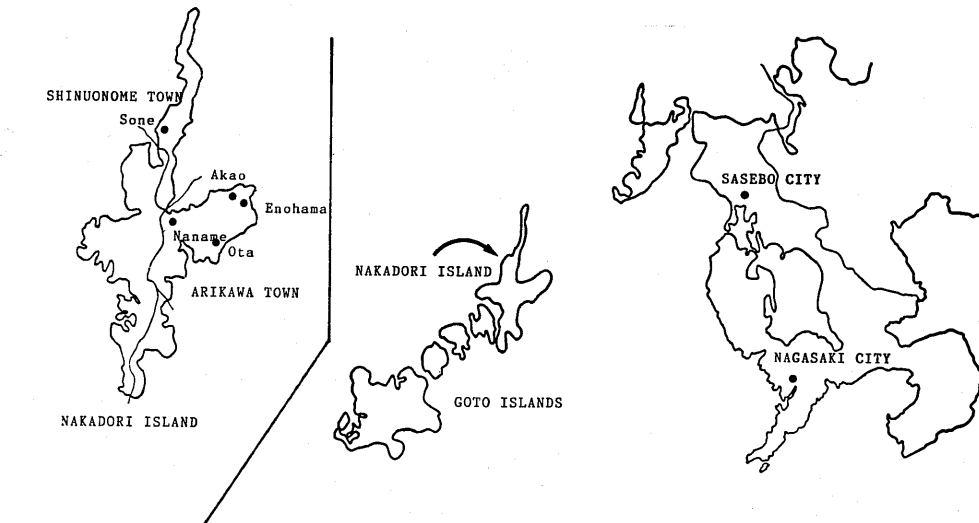


Fig. 1 Map of Nagasaki prefecture.

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 were as follows: 50 for Naname, 50 for Ota, 56 for Akao, 54 for Enohama and 60 for Sone. Subsequently the sera were stored at -20°C until use.

Antigen. The antigen was prepared from the Rh strain of *T. gondii* using the method described by Bodner and coworkers (1972). It contained $40\mu\text{g}$ of protein per ml as determined by the method of Lowry and coworkers.

Control serum. A serum sample with Dye Test *T. gondii* antibody titer of 1:256 was used as positive control. Negative serum to *T. gondii* was prepared by the absorption method. The period of incubation was 1 hr at 37°C , followed by 48hr at 5°C . After that it was centrifuged at 6,000 rpm for 30min at 5°C . The supernatant was filtered through a membrane filter with $0.45\mu\text{m}$ pores and thereafter stored at -20°C until use.

ELISA procedure. For the determination of antibody to *T. gondii* micro-ELISA technique was used (Voller *et al.*, 1976; Palangyo *et al.*, 1985). The absorbance of each well was read in a "Microelisa Auto Reader" (Dynatech Instruments, Inc., Santa Monica, Ca., USA). Alkaline phosphatase labeled goat IgG fractions against human immunoglobulins were commercial preparations from Sigma Chemical Company USA.

Concentration of antigen. The appropriate concentration of antigen was determined by using positive and negative sera and by the method of checkerboard titration at different incubation times. It was denoted that the appropriate concentration of antigen was $0.4\mu\text{g}/100\mu\text{l}$ in a well at 60 min of incubation (Pallangyo *et al.*, 1985).

Antibody level. Two fold serial dilutions (beginning with a dilution of 1:20), were prepared using phosphate buffered saline. Optical density values greater than 3 times the optical density value of the negative control for the corresponding dilution were considered significant. The end point of titration was expressed by the reciprocal of highest dilution.

High titer of Antibody. Sera with antibody titers equal or more than 1:2560 were categorized as having high titer.

RESULTS

Positive rate at each locality.

As shown in Table 1, the positive rate ranged from 48.1 percent for Enohama to 86 percent for Ota. In comparison of the positive rate among localities the positive rate for Ota was significantly higher than that for Akao and Enohama, although difference of the positive rate between Ota and Naname or Sone was not significant. The positive rate for Enohama was significantly lower than that for Naname and Sone.

Antibody distribution at each locality.

Antibody distribution in the samples from the island was shown in Fig. 2. Comparison of these data by the Wilcoxon test showed that the level of antibody distribution

for Ota was significantly higher than that for Akao and Enohama. The level of antibody distribution for Enohama was significantly lower than that for Naname, but the level of antibody distribution for Naname and Ota was not significantly different.

Positive rate at age decade.

As shown in Table 2 the positive rate by age decade on the island ranged from 41.5 percent at the 4th decade to 79.2 percent at the 5th decade. The positive rate at the 4th decade was significantly lower than at the other decades. While, the difference in the positive rates at the 5th, 6th and 7th decades were not significant.

Antibody distribution at age decade.

As shown in Fig. 3 the level of antibody distribution by age decade on the Nakadori island did not show any significant difference.

Table 1 Rates of *Toxoplasma gondii* antibody in Nakadori island of Goto islands in Nagasaki prefecture.

Locality	No. of samples	No. of positive samples	No. of samples with high titer ($\geq 1:2560$)
Arikawa town			
Ota	50	43 (86.0)	26 (52.0)
Naname	50	40 (80.0)	15 (30.0)
Akao	54	35 (64.8)	16 (29.6)
Enohama	52	25 (48.1)	10 (19.2)
Shinuonome town			
Sone	54	38 (69.1)	11 (20.4)
Total	260	181 (69.6)	78 (30.0)

The numbers in parentheses indicate percentage. * : Significant at the level of 5 %. ** : Significant at the level of 1 %. Statistical evaluation was carried out by χ^2 -test.

Table 2 Rates of *Toxoplasma gondii* antibody at age decade in Nakadori island.

Age decade	No. of samples	No. of positive samples	No. of samples with high titer ($\geq 1:2560$)
4th	41	17 (41.5)	10 (24.4)
5th	77	61 (79.2)	26 (31.7)
6th	60	47 (78.3)	22 (36.7)
≥ 7 th	77	56 (72.7)	20 (26.6)

The numbers in parentheses indicate percentage. ** : Significant at the level of 1%. Statistical calculation was carried out by χ^2 -test.

High titer of antibody.

High titer antibody (≥ 2560) rate ranged from 19.2 percent at Enohama to 52.0

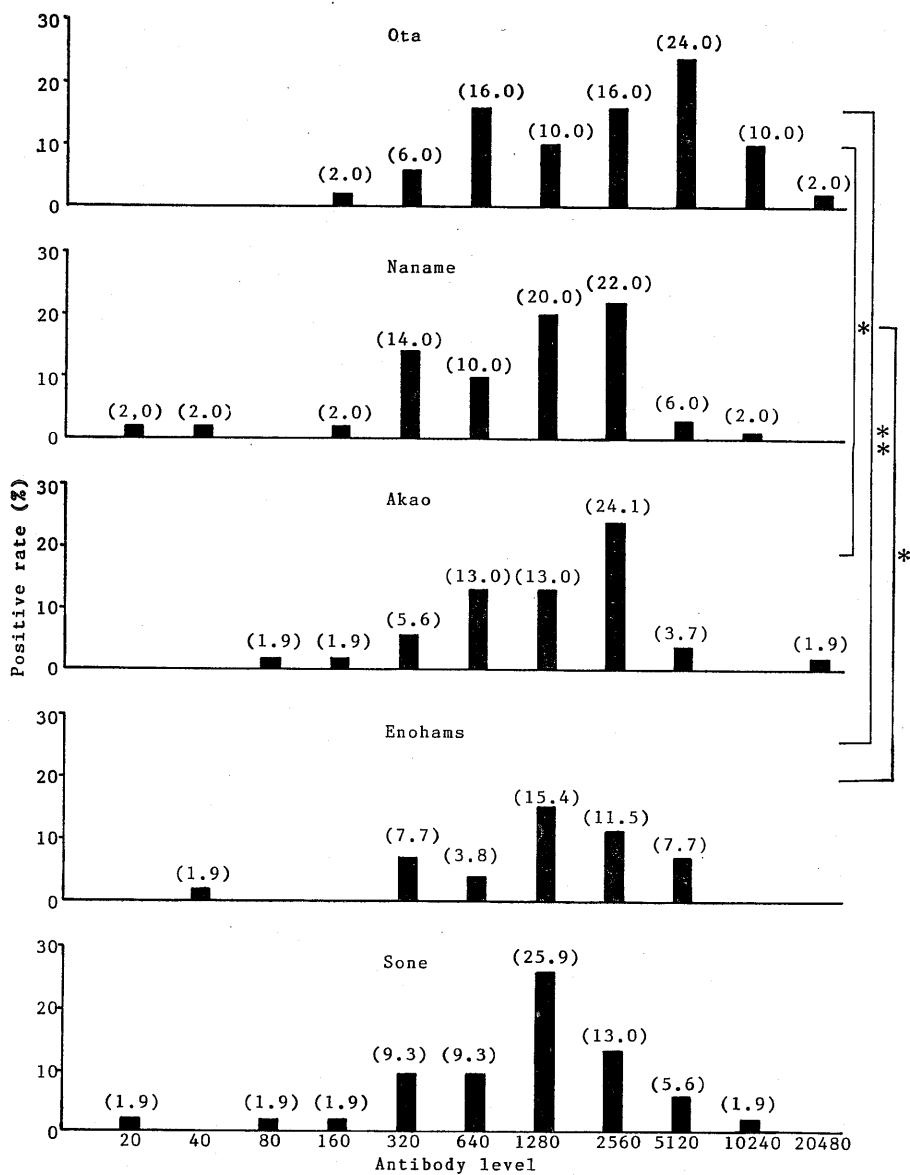


Fig. 2 Antibody distribution of *Toxoplasma gondii* at different localities in Nakadori island. The numbers in parentheses express percentage. The level of antibody was expressed by the reciprocal in serum dilution. Statistical calculation was done by the method of Wilcoxon test. **: Significant at the level of 1%. *: Significant at the level of 5%.

percent at Ota. These data are shown in Table 1. The rate at Ota was significantly higher than at the other localities. Furthermore, as shown in Table 2 there was no significant difference in the high antibody titer with age decade.

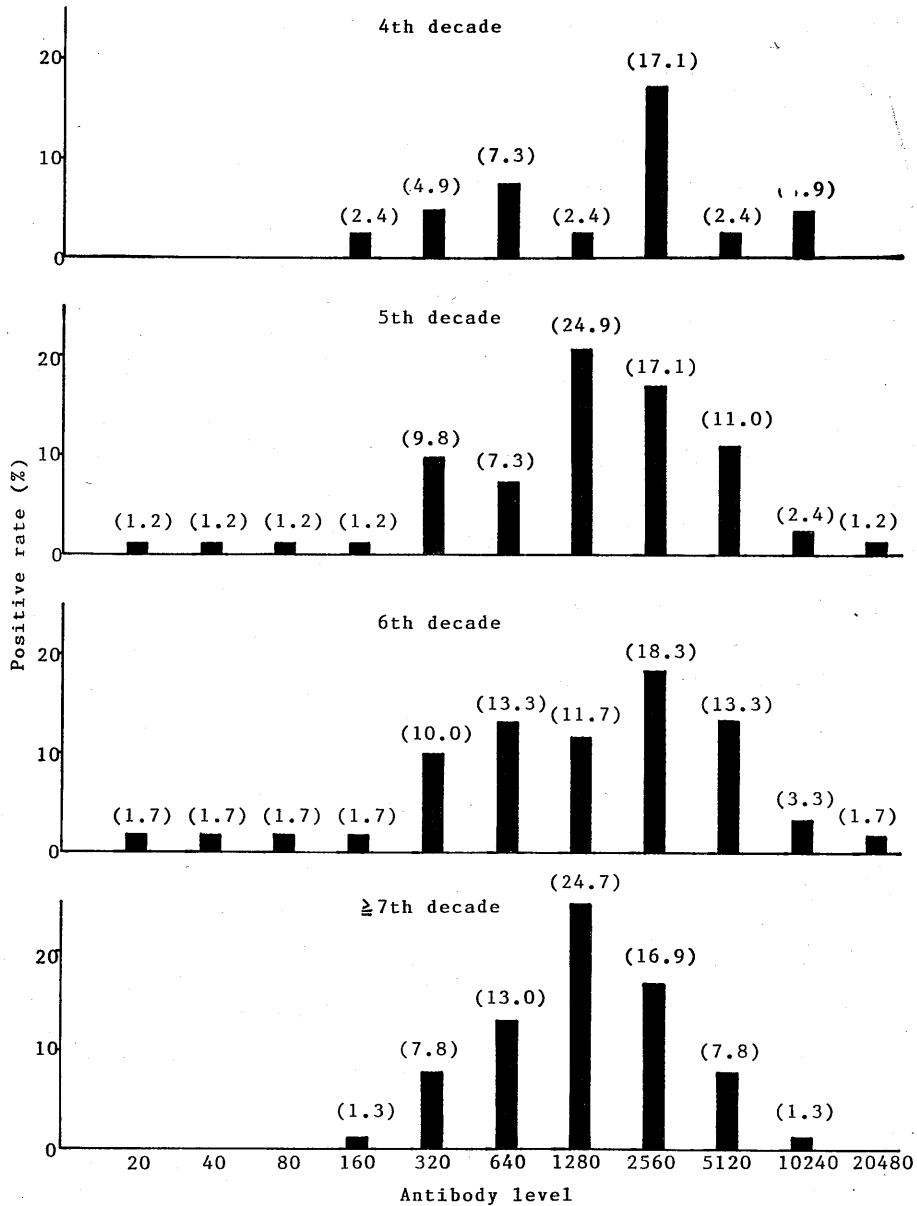


Fig. 3 Antibody distribution of *Toxoplasma gondii* at each age decade in Nakadori island of Nagasaki prefecture.

DISCUSSION

The inhabitants of the five localities forming the basis of the present study are mainly involved in agriculture. At times however, some may do fishing or participate in road-construction. The altitude and climatic conditions of these localities are the same. Furthermore, the social economic status as well as the cultural habits of these inhabitants are basically similar. It is therefore interesting to note that Ota had a significantly higher rate for toxoplasma antibody than Akao and Enohama. In addition the rates for Naname and Sone were significantly higher than Enohama. We can not account adequately for these differences, since the climatic, geographical, altitude, economic status and cultural habits for the study populations are similar. There is no evidence that people at Ota keep more cats or eat more raw meat than those at Enohama. It is of note, however, that the results of a seroepidemiological study for *Rickettsia tsutsugamushi* (Suzuki *et al.*, 1985) have a positive correlation with those of present study. The results indicate that toxoplasmosis and *Rickettsia tsutsugamushi* infections have parallel occurrence in the Nakadori island. Given that wild rodents may play a role, although different, in transmission of both toxoplasmosis and *Rickettsia tsutsugamushi*, it is tempting to incriminate these as an important factor in the transmission of the two conditions in the Nakadori island of Nagasaki prefecture in Japan. Similar differences in antibody rates in populations living in the same geographical locations and with similar cultural habits and economic status have been reported by other workers (Tizard *et al.*, 1977).

The toxoplasma IgG antibody rate was significantly lower at the 4th decade when compared with the other decades. This could be due to a number of factors either acting alone or in combination. First it may reflect improved hygienic conditions in the population due to improved socioeconomic status reported in Japan over the last few decades. Secondly it may reflect positive changes in the environmental sanitation, ecology, personal habits and the like with time. Younger population studies may provide useful information on this point.

Ota had significantly higher titer antibody rate than the other four localities. This could be due to relatively more recent infection at Ota compared to the other localities. Whereas, the rates at Sone, Akao and Naname were similar, Enohama had a much lower rate. It is noteworthy that in the seroepidemiological study for *Rickettsia tsutsugamushi*, similar observations were obtained (Suzuki *et al.*, 1985). The *Rickettsia tsutsugamushi* IgG antibody rate at Ota was only second to Sone. The results of the present study and those of *Rickettsia tsutsugamushi* seroepidemiological study cited above do not exclude wild rodents as an important factor in the transmission of both *Rickettsia tsutsugamushi* and *Toxoplasma gondii* in the Nakadori island of Nagasaki prefecture. We will therefore deliberate on this interesting relationship in our future studies.

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長崎県中通島におけるトキソプラズマ抗体陽性率

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近年, 免疫不全症例における終末期感染症の一つとしてトキソプラズマ感染症が注目されている。そこで, 健康人におけるトキソプラズマ抗体陽性率を地域別ならびに年齢別に検討した。抗体測定用の血清は長崎県五島列島に属する中通島の2町5郷(太田, 七日, 赤尾, 江の浜, 曾根)の住民270名から, 定期健康診断時に無作為に採取された。尚, 抗体価は抗原として *Toxoplasma gondii* 株を用いた免疫酵素抗体法により IgG 抗体が測定された。地域別の抗体陽性率は48.1%から86%に分布し, 地域間における陽性率に有意の差がみられた。尚, 高い陽性率を示した地域住民の抗体は高いレベルに分布していた。さらに, 年齢別抗体陽性率では, 50才代以上の年代の陽性率が40才代の陽性率より有意に高い値を示していた。以上の成績をみると, トキソプラズマの抗体陽性率は加齢と共に増加するが, 地域別の陽性率は地域により有意に異なっていた。そこで, 特定の地域における抗体陽性率を知るためには, その地域の抗体陽性率を調査する必要があることが示唆された。