Seroepidemiology of *Toxoplasma* Infection in Two Islands of Nagasaki by ELISA

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Abstract: In Hirashima Island and Enoshima Island in Nagasaki prefecture, where human ecology and environmental conditions are the same, *Toxoplasma* infection of apparently healthy inhabitants aged from 6 to 79 years was investigated by determining speficic IgG antibody to *T. gondii* by ELISA. In 1985 serum specimens were collected from 283 and 270 inhabitants in Hirashima and Enoshima Islands, respectively. In 1986 serum samples were again collected from 196 and 205 inhabitants whose sera were collected in 1985 in the same islands. The overall positive rate of *Toxoplasma* infection was higher in Hirashima Island (44.2%) than in Enoshima Island (34.1%) and the difference was statistically significant. The positive rates at 7 areas in Hirashima Island were different from area to area, while at 3 areas in Enoshima Island the positive rates were similar. The calculated annual incidence rates were similar in Hirashima Island (0.9%) and in Enoshima Island (0.8%). The positive rate increased with a linear fashion with advancing age in Enoshima Island and in a stepwise fashion in Hirashima Island. Furthermore, the calculated annual new infection risk was high at the age of 15 to 35 years (2.8%) in Hirashima Island and at the age of 65 to 75 years (3.8%) in Enoshima Island. At an interval of 1 year, seroconversion (0.9%) was detected among inhabitants in Hirashima Island only. Rising rate of the antibody level was 5.8% in Hirashima Island and 2.6% in Enoshima Island. These results indicate that the prevalence pattern of *T. gondii* is different between the two Islands.

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

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INTRODUCTION

It is well known that the positive rates for *Toxoplasma* infection determined by serological tests differ from place to place and increase with age (Feldman and Miller, 1956; Tizard *et al.*, 1976; Van der Veen and Polak, 1980). In our previous study, similar results were obtained in urban and rural areas in Nagasaki prefecture, in which the level of the antibody production to *Toxoplasma gondii* (*T. gondii*) increased with age (Suzuki *et al.*, 1985; Suzuki *et al.*, 1987). It was also pointed out that the infection remains asymptomatic in many cases (Feldman, 1974), although congenital *toxoplasmosis* has been recognized as a serious condition (Koppe *et al.*, 1986) and *T. gondii* can be a lethal opportunistic pathogen in immunosuppressed host (Ruskin, et al., 1976; Wong, *et al.*, 1984). Due to the increase of patients with cancer and geriatric population, it is possible that toxoplasmic encephalitis might increase in these patients complicated with compromised condition. Recently, in our department we experienced toxoplasmic encephalitis in a patient with malignant thymona who had no clinical manifestations and diagnosis of *T. gondii* was only established after post-mortem examination (Uzuka *et al.*, 1983). Thus, a study was attempted to analyze background for such *Toxoplasma* infection among symtomless populations and the transmission routes of *T. gondii*. Not only the positive rates in the inhabitants but also changes in the antibody titers to *T. gondii* at an interval of 1 year were investigated by Enzyme-linked immunosorbent assay (ELISA) in 2 islands of Nagasaki.

MATERIALS AND METHODS

Location. The investigation was done at Hirashima and Enoshima Islands in Nagasaki prefecture with area of 9.9km^2, and 2.8km^2, respectively (Fig. 1). Inhabitants lived at 7 locations in Hirashima Island and at 3 locations in Enoshima Island. In both islands major occupations of inhabitants were fishery and agriculture.

Serum samples. Structure of the population and sample source in these islands are shown in Table 1. From July to August 1985, serum samples were collected from apparently healthy inhabitants of the 2 islands. The number of serum samples randomly selected were 283 in Hirashima island which corresponded to 55% of all inhabitants and 270 in Enoshima Island which corresponded to 61% of all inhabitants. The subjects examined aged from 6 to 79 years in both islands and sex ratio of male to female was 1:1.5 in Hirashima Island and 1:1.6 in Enoshima Island. Furthermore, during the same seasons in 1986 blood samples were again collected from 196 inhabitants in Hirashima Island and 205 inhabitants in Enoshima Island whose blood samples had been collected in 1985. The sera were stored at -20℃ until use.

Control serum. A serum with an antibody titer of 1:256 to *T.gondii* by the dye test modified by Kobayashi *et al.* (1968) was used as positive control. Negative control serum to *T. gondii* was prepared by the absorption method as described previously (Suzuki *et al.*, 1987).

ELISA procedure. For the determination of IgG antibody to *T.gondii*, a microELISA technique (Suzuki *et al.*, 1987) was used. Briefly, antigen diluted in coating buffer (0.05M carbonate buffer at pH 9.6) was applied to flat-bottomed microELISA plate (Immunolon of Dynatech MicroELISA System) in 100μl/well. The plates were kept at 5℃ overnight. The volume of serum samples, conjugate and substrate added were 100μl per well. In all the steps wells were washed 3 times with phosphate buffered saline (PBS)-Tween 20. The absorbance was read by MicroELISA Auto Reader (Dynatech Instrument Inc., Santa Monica, Ca., USA).

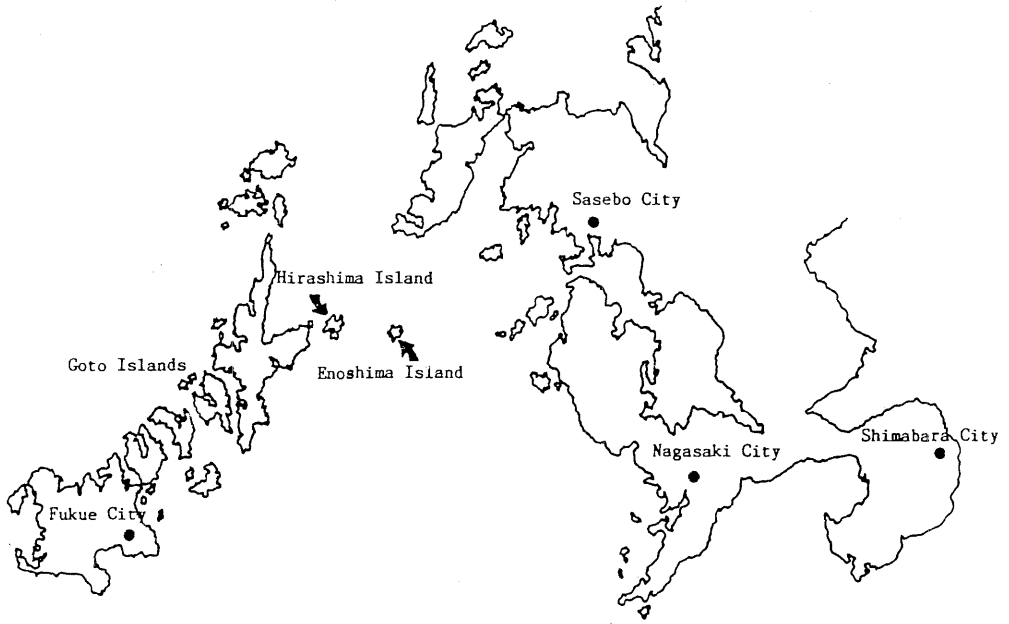

Fig. 1. Map of Nagasaki Prefecture

Table 1. Sample source and structure of the population in Hirashima and Enoshima Islands of Nagasaki Prefecture

Area	Hirashima Island		Area	Enoshima Island	
	No. of population	No. of specimens		No. of population	No. of specimens
Oda	59	30 (51)	Higashi	132	69 (52)
Tomari	87	47 (54)	Hama	156	98 (63)
Kurosaki	57	44 (77)	Nishi	154	103 (67)
Ura	108	44 (41)			
Haedomari	79	50 (63)			
Miyazaki	72	33 (46)			
Yatsubo	50	35 (70)			
Total	512	283 (55)		442	270 (61)

Numbers in parenthesis indicate percentage.

Antibody level. Two-fold serial dilutions of serum samples (beginning from 1:20) were prepared using PBS-Tween 20 containing 0.6% bovine serum albumin. Optical density (OD) values higher than 3 times that of the negative control for the corresponding dilution were considered positive. The endpoint titer of the test showing positive reaction was expressed by the reciprocal of the highest dilution.

Annual new infection risk (K) by age. The risk of seronegative persons to acquire *Toxoplasma* infection was calculated by the formula of Van der Veen and Polak (1980).

New infection during 1 year. Seroconverted inhabitants during 1 year were presumed to have acquired new infection with *T. gondii.*

Fluctuation of antibody level. For demonstration of a significant difference in *T. gondii* antibody levels, sera in 1985 and 1986 of the same inhabitants were stored and tested in parallel. More than 4-fold titer difference between the initial and the sample taken 1 year later from same individual was considered as significant.

Statistical calculation. In order to examine statistical difference, chi-square test, Student's t-test and Wilcoxon test were used. Statistical analysis for antibody level was performed, after logarithmic conversion of the data.

RESULTS

Positive rate.

As shown in Table 2, overall positive rate of *T.gondii* in ELISA of all the inhabitants tested was higher in Hirashima Island (44.2%) than in Enoshima Island (33.7%), the difference was statistically different ($p<0.01$), although the age distribution of subjects in Enoshima Island (average age 54 ± 19 years) was higher than that in Hirashima Island (ibid 46 ± 20)(Table 3). In Hirashima Island, the positive rate at different age groups ranged from 0 to 61.5% and the positive rate at 30 years and older was signigicantly higher than that at 19 years and younger. On the other hand, in Enoshima Island the positive rate at different age groups ranged from 0 to 52.5% and the positive rate was significantly higher at 40 years and older than at 10-19 years. In fact the positive rate in both islands increased with advancing age. When positive rate was compared between the same age group in 2 islands, the positive rate at 40-59 years was significantly higher in Hirashima Island than in Enoshima Island. Linear regression which was calculated from the means at age group with more than 10 samples was shown in Fig. 2. In Enoshima Island the positive rate increased according to age to 52.5% of the inhabitants aged 70 to 79 years giving an annual incidence of 0.8%. While, the positive rate in Hirashima Island was high in children and reached 50% at the age of 30 years. Between 30 to 59 years the positive rate appeared to stabilize at 50%. But, after 60 years of age the positive rate increased again to 61.5%. The annual incidence of infection in Hirashima Island was 1.8% between 6 and 30 years and 0.5% between 50 and 79 years, however, the annual incidence in overall inhabitants was 0.9%. The positive rate at different areas in both islands is shown in Table 3. Among 7 areas in Hirashima Island, 5 areas with the same

Table 2. Result of ELISA assay detecting specific IgG antibody to *Toxoplasma gondii* in healthy inhabitants living in Hirashima Island and Enoshima Island in Nagasaki in 1985

Age in years	Island	No. of samples examined	No. of positive samples (%) #	Antibody level in positive samples (mean±S. D.)##
6− 9	Hirashima	14	0 (0.0)	
	Enoshima	7	1 (16.7)	
10−19	Hirashima	42	5 (11.9)	2.98±1.27
	Enoshima	23	1 (4.3)	
20−29	Hirashima	5	2 (40.0)	
	Enoshima	7	0 (0.0)	
30−39	Hirashima	16	8 (50.0)	2.65±0.99
	Enoshima	10	2 (20.0)	
40−49	Hirashima	40	20 (50.0)	2.41±0.74
	Enoshima	33	8 (24.2)	2.87±0.45
50−59	Hirashima	87	44 (50.6)	2.42±0.62
	Enoshima	62	21 (33.9)	2.60±0.68
60−69	Hirashima	53	30 (56.6)	2.64±0.63
	Enoshima	69	27 (39.1)	2.74±0.59
70−79	Hirashima	26	16 (61.5)	2.50±0.46
	Enoshima	59	31 (52.5)	3.00±0.68
Total	Hirashima	283	125 (44.2)	2.52±0.69
	Enoshima	270	91 (33.7)	2.83±0.65

Numbers in parenthesis indicate percentage. #: Statistical analysis was done by chi-square test. ##: After logarithmic conversion of data, statistical analysis was performed by t-test. Comparison of antibody level at age groups with less than 3 positive samples was not done. *: $p<0.05$, **: $p<0.01$.

Table 3. Positive rate of *Toxoplasma gondii* at different areas in Hirashima Island and Enoshima Islands in 1985

Area	No. of samples	Years in age (mean±s. d.)#	No. of positive samples (%)	Antibody level in positive samples (mean±s. d.)##
Hirashima Island				
Oda	30	41±22	11 (36.7)	2.77±0.84
Kurosaki	44	46±23	16 (36.4)	2.74±0.71
Ura	44	47±23	17 (38.6)	2.22±0.64
Haedomari	50	47±23	23 (46.0)	2.59±0.58
Tomari	47	46±20	28 (59.6)	2.51±0.63
Yatsubo	35	50±14	11 (31.4)	2.34±0.80
Miyazaki	33	51±17	19 (57.6)	2.47±0.71
Total	283	46±20	125 (44.2)	2.52±0.69
Enoshima Island				
Nishi	103	56±18	27 (30.3)	2.75±0.51
Hama	98	53±21	37 (46.8)	2.98±0.76
Higashi	69	69±18	28 (43.7)	2.85±0.65
Total	270	54±19	91 (33.7)	2.83±0.65

#: Statistical calculation was done by t-test. ##: After logarithmic conversion of data, statistical analysis was performed by t-test. *: $p<0.05$, **:$p<0.01$.

age distribution, the positive rate at Tomari (59.6%) was significantly higher than that at Oda (36.7%), Kurosaki (36.4%) and Ura (38.6%). Between the remaining 2 areas with the same age distribution, the positive rates at Yatsubo (31.4%) and Miyazaki (57.6%) were significantly different. While, among 3 areas in Enoshima Island where the age distribution was the same, no significant difference of the positive rates was detected.

Antibody level.

Antibody level at different age groups and areas in both islands is shown in Table 2 and Table 3, respectively. The antibody level of inhabitants in Enoshima Island (2.83 ± 0.65) was higher than that in Hirashima Island (2.52 ± 0.69) and the difference was statistically significant ($p < 0.05$). When antiody level between the same age group in 2 islands was compared, the antibody level at 70-79 years was higher in Enoshima Island (3.00 ± 0.68) than in Hirashima Island (2.50 ± 0.46) with statistical significance ($p < 0.05$). On the other hand, in comparison of the antibody level at different areas in both islands, significant difference of the antibody level was only detected between Kurosaki (2.74 ± 0.71) amd Ura (2.22 ± 0.64) in Hirashima Island.

Annual new infection risk by age.

Annual new infection risk was calculated in age groups with more than 9 samples as shown in Table 4. In Hirashima Island, the highest annual infection risk of 2.8% was found in the age group of 15-35 years. While, the highest annual infection risk, 2.5%, in Enoshima Island was detected in the 65-75 year group.

New infection during 1 year.

As shown in Fig. 3, in Hirashima Island, of 111 inhabitants with negative antibody in 1985 only one subject (0.9%) showed seroconversion in 1986. This case did not have

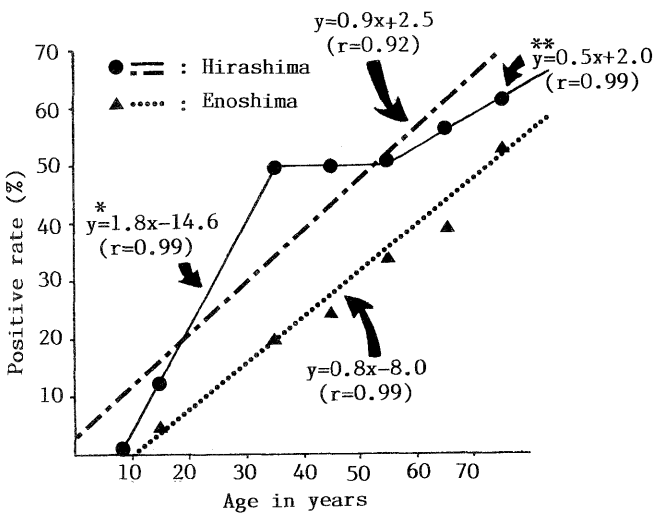

Fig. 2. Positive rate in ELISA detecting IgG Antibodies against *Toxoplasma gondii* by age groups in Hirashima and Enoshima Islands of Nagasaki.
*and **: Linear regression from 7.5 to 35 years and from 55 to 75 years in Hirashima Island, respectively.

Table 4. Frequency of negative sera in each age group and annual infection risk between median ages of successive age groups in Hirashima Island and Enoshima Island in 1985

Age group (years)	Island	No. of samples	No. of negative samples (%)	Median age group (years)	Annual infection risk (%) in# Hirashima	Enoshima
6−9	Hirashima	14	14 (100.0)			
				7−15	1.6	
10−19	Hirashima	42	37 (88.1)			
	Enoshima	23	22 (95.7)			
				15−35	2.8	0.9
30−39	Hirashima	16	8 (50.0)			
	Enoshima	10	8 (80.0)			
				35−45	0.0	0.5
40−49	Hirashima	40	20 (50.0)			
	Enoshima	33	25 (75.8)			
				45−55	0.1	1.4
50−59	Hirashima	87	43 (49.4)			
	Enoshima	62	41 (66.1)			
				55−65	1.3	0.8
60−69	Hirashima	53	23 (43.4)			
	Enoshima	69	42 (60.9)			
				65−75	1.2	2.5
70−79	Hirashima	26	10 (38.5)			
	Enoshima	59	28 (47.5)			

Numbers of parenthesis indicate percentage. #: Analysis of the annual new infection risk at each age group with more than 9 samples was done by the formula of Van der Veen *et al.* (1980).

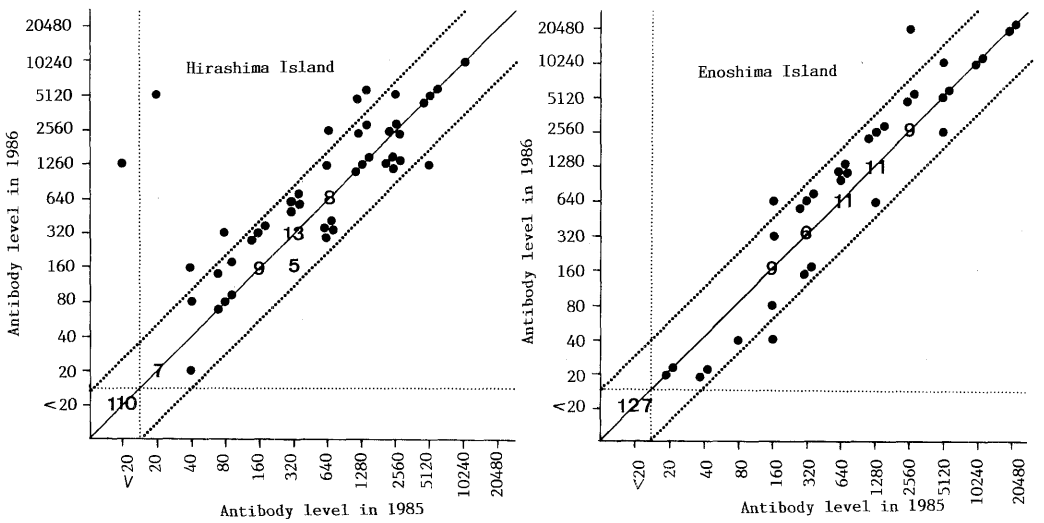

Fig. 3. Transition of antibody to *Toxoplasma gondii* of 196 and 205 inhabitants in Hirashima and Enoshima Islands, respectively, from 1985 to 1986.
●: One black circule indicate one inhabitant and numbers indicate inhabitant's number.

any clinical symptoms during the past 1 year, although the antibody level was high (1: 1,280). In Enoshima Island no case showed seroconversion during the past 1 year in 127 inhabitants with negative antibody to *T. gondii*.

Change in antibody level.

As shown in Fig. 3, in Hirashima Island 6 subjects among 85 inhabitants with positive antibody level in 1985 showed more than 4-fold antibody titer during 1 year. Of these 6 inhabitants, 5 (5.8%) showed significant rise and 1 (1.2%) showed a decrease in their antibody levels. Of 5 inhabitants with an increase of the antibody level 1 subject showed a rise of 256 fold from the initial level (from 1:20 to 1:5,120), while the remaining 4 showed a 4-fold rise. In Enoshima Island significant change of the antibody level was detected in 3 subjects among 78 positives in 1985. Of the 3 inhabitants 2 (2.6%) cases showed a rise and the remaining 1 (1.3%) showed a decrease. The rise or decrease of antibody level was variable and did not depend on certain specific age group.

Discussion

In this survey the positive rate in Hirashima Island (44.2%) was significantly higher than that in Enoshima Island (33.7%), in spite of the same human ecology and environmental conditions. As reported by other researchers (Tizard *et al.*, 1976; Van der Veen and Polak, 1980), the positive rate in both islands increased with age. Direct comparison of the rate with those in different places in Japan such as Nakadori Island 57.7% and 46.3% in Nagasaki city (Suzuki *et al.*, 1987), 12.6% in Nagasaki city (Murakami, 1964), 29.7% in Tokyo and 49.7% in Kagoshima (Kobayashi, 1977) and 15.7% in Hyogo (Takahashi *et al.*, 1985) was not performed. Because the age distribution of the sampled subjects and methods used for detection of the antibody to *T.gondii* varied by the reported papers. The estimated annual incidence was 0.9% in Hirashima Island and 0.8% in Enoshima. The incidence in Hirashima Island corresponded to the observed incidence (0.9%) during 1 year. While actual incidence was not detected in Enoshima Island during the same year. The estimated annual incidence in both islands was similar to that in Nagasaki city (0.9%) (Suzuki *et al.*, 1987) and in Paris (0.9%) (Feldman, 1974), lower than in Nakadori Island (1.3%) in Nagasaki, Where environmental conditions are similar as in Hirashima and Enoshima Islands (Suzuki *et al.*, 1987), in Ontario (1.7%) (Tizard *et al.*, 1976) and in El Salvador (5.5%) (Remington *et al.*, 1970), but was higher than in New York city (0.1%) and in Ohio (0.2%) (Feldman, 1974). Furthermore, the actual incidence during 1 year in Hirashima (0.9%) was similar to that of 6 month pregnant women in Tokyo (0.2%) (Kobayashi *et al.*, 1974) and in Melbourne (0.5%) (Sfameni *et al.*, 1986). The positive rate increased with age in different fashions, in linear mode in Enoshima Island and in stepwise fashion in Hirashima Island. It implies that the mode of transmission might be different in these islands, even though the inhabitants in 2 islands have similar socioeconomical conditions, relationship to animals and habits in meals.

The antibody level was significantly higher in Enoshima Island with lower positive

rate than in Hirashima Island with higher positive rate. In previous seroepidemiological survey of *T.gondii* infection in Nagasaki, the antibody level in Nakadori Island with high positive rate was higher than that in Nagasaki city with low positive rate (Suzuki *et al.*, 1987). From these results it was postulated that there is not always a consistency between the antibody positive rate and the antibody level, even though human ecology and environmental conditions are the same. Furthermore, difference of the antibody level among age groups was detected in Enoshima Island only, with high antibody level among subjects aged 70-79 years. The fact that high antibody level is detected at early stage of the infection (Remington and Mcleod, 1986) or on the recognition of *T. gondii* infection (Koppe *et al.*, 1986), the annual infection risk and the fluctuation of antibody level were investigated.

The highest annual new infection risk which was a parameter for the incidence of primary infection among age groups in Hirashima Island (2.8%) and Enoshima Island (2.5%) was detected in groups, 15 to 35 years and 65 to 75 years, respectively. The age group with the highest infection risk in Enoshima Island was older than that in Nakadori Island (25 to 35) and in Nagasaki city (35 to 45) (Suzuki *et al.*, 1987). Moreover, in Enoshima Island the high antibody level was found in the age group, 65 to 75 years. Thus, it was assumed that primary infection or reexpousure to either living *Toxoplasma* or its antigen in Enoshima Island occurs in old people than in any other place in Nagasaki. In Hirashima Island antibody level at the age groups with high infection risk was not well clarified because of a few number of samples. While, in comparison of ELISA values among age groups from 1 to 93 years in Hyogo prefecture (Takahashi *et al.*, 1985) did not detect the age group with high level of ELISA values. Their result was different from ours.

During 1 year, significant rise in specific antibody level in inhabitants with positive antibody was detected in 5.8% and 2.6% in Hirashima and Enoshima Islands, respectively. Although it has been pointed out that titers of *T. gondii* fluctuate (Krick and Remington, 1978; Koppe *et al.*, 1986), no correlation between age and rise of antibody level was detected in our study. Since these inhabitants are healthy, it was suspected that they might be recognized extrinsic *T. gondii* rather than intrinsic *T. gondii*. Other researchers have pointed out about reinfection in epidemiological studies (Frenkel and Ruiz., 1980; Coutinho, *et al.*, 1982; Koppe, *et al.*, 1986). Furthermore, these rates in both islands, 2.6% and 5.8%, were similar to the rate of 3% observed in a group of 6 month pregnant women with positive antibody to *T.gondii* in Tokyo (Kobayashi *et al.*, 1974).

In the present study it is clearly clarified that in two islands in Nagasaki where human ecology and environmental conditions are the same there was a marked differences in the positive rate and the antibody level in each inhabitant and the estimated infection risk at each age group. On the contrary, routes of transmission in these islands are still far from clear, although the postnatal infections may be elicited by two major routes of transmission: consumption of raw meat and close association with infectious feces of cats (Remington and Mcleod, 1986). To analyze the actual transmission routes in these islands further investigations for detection of antibodies against unique stage-specific

oocyst/sporozoite antigen (Kasper and Ware, 1985) or IgA antibody response relating to the route of transcutaneous infection (Partanen, *et al.*, 1984) may be necessary.

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長崎県の2島におけるトキソプラスマ感染症に関する血清疫学的研究

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長崎県において，生活環境ならびに生活様式が近似している平島 (283名) と江ノ島 (270名) 在住の6－79才の健康な住民を対象として，特異性 IgG 抗体を酵素抗体法にて測定することによりトキソプラスマ感染症に関する血清疫学的研究を行った．抗体陽性率は平島 (44.2%) が江ノ島 (34.1%) より有意に高値であった．地区別陽性率の差異は平島（7地区）では認められたが，江ノ島（3地区）では認められなかった．年代別の抗体陽転率は平島では15－35才 (2.8%) で高いのに比して，江の島では65才以上の年代で高値であったが，加令に伴う抗体陽性率は平島では段階的，江ノ島では直線的上昇傾向を示した．1年間隔での抗体の4倍以上の有意の変動検出率は平島 (5.8%) と江ノ島 (2.6%) で異なっていた．これらの成績はトキソプラスマの感染様態が2つの島の住民で異なっていることを示唆した．

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