PREVALENCE OF ANTIBODIES TO *TOXOPLASMA GONDII* AMONG URBAN AND RURAL RESIDENTS IN THE PHILIPPINES

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Abstract. A survey of antibodies to *Toxoplasma gondii* was carried out among residents in urban (Metro Manila) and rural (Mindoro and Leyte) areas in the Philippines. A total of 1,173 serum samples were examined for *Toxoplasma* antibody by an ELISA method. The overall seropositivity was 11.1% (n=904, 12.4% in males, 10.0% in females) in Metro Manila, 61.2% (n=152, 63.3% in males, 53.1% in females) in Mindoro, and 30.1% (n=113, 34.3% in males, 22.5% in females) in Leyte, indicating significantly higher ($p<0.001$) seropositivities in rural than urban settings. No significant differences in seropositivities were observed between males and females. In each group, seropositivity tended to increase with age of the subjects.

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite of medical importance. This parasite is ubiquitously distributed throughout the world, and antibodies specific for *Toxoplasma* are observed in humans at various rates in almost all nations (Remington and Desmonts, 1990). Especially, old surveys in some human populations reported extremely high prevalence rates of approximately 90%. Once infected, humans cannot eliminate the parasite, and specific antibody persists throughout life. Therefore, antibody prevalence rates indicate the percentage of humans who harbor *Toxoplasma* parasites (Bearman *et al*, 1995).

Toxoplasma gondii causes severe congenital infections in infants and acute infections in immunosuppressed patients, although almost all cases of *Toxoplasma* infections are asymptomatic. When a woman acquires the first infection during pregnancy, the parasite may cause intra-uterine infection and eventually lead to developmental abnormality including hydrocephalus and mental retardation in her offspring. On the other hand, the recent increase in populations of acquired immunodeficiency syndrome (AIDS) patients have defined a significant opportunistic feature of *Toxoplasma* infection, since cerebral infection caused by pre-existing *Toxoplasma* parasites has been a major cause of death in AIDS patients in Europe and the USA (Porter and Sande, 1992). The increasing number of AIDS patients in a population with a high *Toxoplasma* infection rate may raise serious public health concern. This is especially true in the Asian region, which has one of the fastest growing populations of HIV/AIDS patients in the world (WHO, 1996). However, there have been few reports on how many people harbor *Toxoplasma* parasites in Asian countries. In this study, we surveyed the prevalence of *Toxoplasma* antibodies among rural and urban residents in the Philippines, where no report has been published in these past 22 years (Cross *et al*, 1977).

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MATERIALS AND METHODS

Study population

A total of 1,173 serum samples were used in the study (Table 1). These samples were collected from 908 urban residents in Metro Manila (Barzaga, 1996), and 152 and113 local residents in Mindoro and Leyte, respectively. Mean ages (±standard

deviations) of these residents were 27.0 (±15.1) years in Metro Manila, 28.3 (±17.6) years in Mindoro, and 28.6 (±16.5) years in Leyte: there were no significant differences in age composition among three populations ($p>0.05$).Subjects of Metro Manila belonged to upper middle income families in which 40% are students and 20% were employees. These populations in Metro Manila were composed of residents from northeastern (39%), southwestern (39%), and central parts (22%). The locations of Metro Manila, Mindoro and Leyte are shown in Fig 1. These serum samples were stored at -20ºC until tested.

ELISA

Toxoplasma antibody levels in human sera were measured by ELISA as previously described (Konishi and Takahashi, 1983) with some modifications (Khin-Sane-Win *et al*, 1997). Briefly, 96-well, flat-bottom non-ELISA microplates were sensitized with *Toxoplasma* crude antigen prepared from tachyzoites of the RH strain. These plates were incubated with a 1:100 dilution of test sera, and then with alkaline phosphatase-labeled anti-human IgG. The enzyme activity in each well was measured with *p*-nitrophenyl bisodiumphosphate. Each absorbance value was adjusted with the value obtained with the constant positive control serum to minimize interplate variations. Sera showing the adjusted values (ELISA values) of more than 0.357 were determined to be positive for *Toxoplasma* antibody (Konishi and Takahashi, 1983).

Statistical analysis

Significance of differences was evaluated by the chi-square test for seropositivities and the Student's *t*-test for mean ages. Probability levels (p) of less than 0.05 were considered significant.

RESULTS

Comparison among Metro Manila, Mindoro and Leyte

The seropositivity in the total population of each location was 11.0% in Metro Manila, 61.2% in Mindoro and 30.1% in Leyte (Table 2). There were significant differences between each of two rural areas (Mindoro and Leyte) and the urban area (Metro Manila) in age-matched populations ($p<0.001$).

Both in males and females, seropositivity in Mindoro was the highest (63.3% in males and

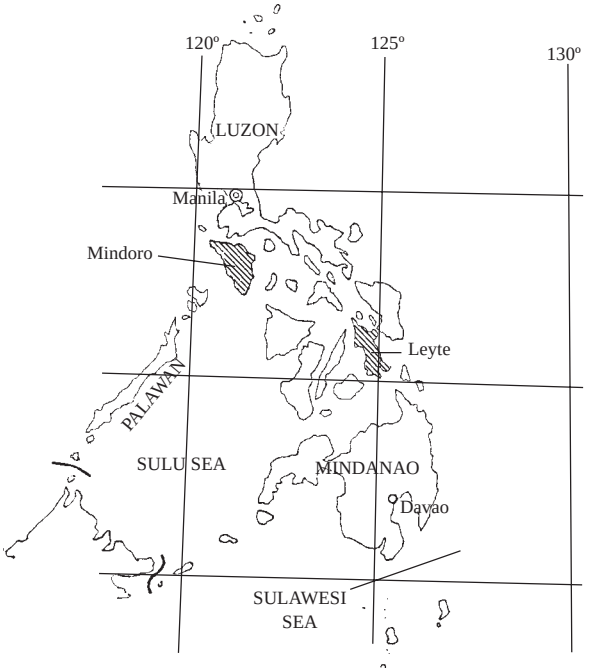

Fig 1–Map of the Philippine Islands, indicating locations of the survey areas, Manila, Mindoro, and Leyte.

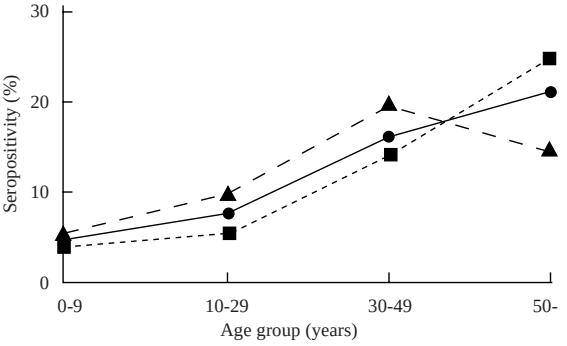

Fig 2–Age-dependent *Toxoplasma* antibody prevalence in total (●), male (▲), and female (■) populations in Metro Manila.

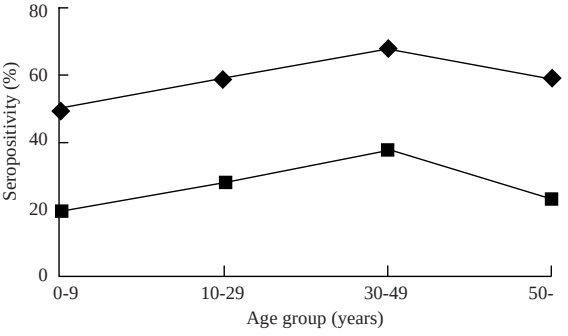

Fig 3–Age-dependent *Toxoplasma* antibody prevalence in Mindoro (◆) and Leyte (■).

Table 1
Number of subjects used in this survey.

Age (years)	Metro Manila		Mindoro		Leyte	
	Male	Female	Male	Female	Male	Female
1-9	55	72	4	4	6	4
10-29	197	203	61	20	38	15
30-49	122	193	35	6	23	14
50-	21	41	20	2	6	7
Total	395	509	120	32	73	40

Table 2
Prevalence of *Toxoplasma* antibody among residents in Metro Manila, Mindoro and Leyte.

Location	Seropositivity (No. positive/ no. tested)		
	Male	Female	Total
Metro Manila	12.4% (49/398)[a,b]	10.0% (51/509)[d,e]	11.1% (100/904)[g,h]
Mindoro	63.3% (76/120)[a,c]	53.1% (17/32)[d,f]	61.2% (93/152)[g,i]
Leyte	34.2% (25/73)[b,c]	22.5% (9/40)[e,f]	30.1% (34/113)[h,i]

[a,b,c,d,g,h,i]$p<0.001$, [e]$p<0.05$, [f]$p<0.01$

Table 3
Prevalence of *Toxoplasma* antibody among residents in subareas of Metro Manila.

	Seropositivity (No. positive/ no. tested)	Mean age±SD (years)
Northeastern[a]	9.3% (32/341)[d,f]	27.6±15.0[g,l]
Southwestern[b]	11.5% (42/350)[d,e]	24.5±15.4[g,h]
Central[c]	13.0% (26/213)[d,e]	29.8±14.0[h,i]

[a]Consisting of Quezon City, Markina, Caloocan, Valenzuela, Bulacan, Makati, Mandaluyong, Pasig, San Juan, Taguig and Rizal.
[b]Consisting of Las Pinas, Paranaque, Pasay City, Alabang, Languna, Cavite and Batangas.
[c]City of Minila
[g]$p<0.01$, [h]$p<0.0005$, [d,e,f,i]$p<0.05$

53.1% in females) followed by Leyte (34.2% in males and 22.5% in females) and Metro Manila (12.4% in males and 10.0% in females). No significant differences were observed between males and females in each area (Table 2) ($p>0.05$).

Comparison among three subareas in Metro Manila

Table 3 shows the prevalence of *Toxoplasma* antibodies among urban residents in three subareas of Metro Manila (northeastern, southwestern and central parts). No significant differences in seropositivity were observed among populations in these subareas. Although the southwestern population had a significantly lower mean age than other populations, age-adjusted seropositivities of the northwestern (8.3% at 24.5 years) and Central (11.3% at 24.5 years) populations were not significantly different from the seropositivity of the southwestern population (11.5% at 24.5 years; $p>0.05$), assuming a yearly increase of seropositivity in Manila population to be 0.32% based on the data shown in Fig 2 (see below: 5% at 5 years and 21% at 55 years).

Age-dependent prevalence

In Metro Manila, prevalence of *Toxoplasma* antibody increased with age (Fig 2). Statistically significant differences were observed among all age groups ($p<0.05$) except between groups of 1-9 years and 10-29 years, and between groups of 30-49 years and 50 years or more. In each age group, the differences between males and females were not significant.

In both Mindoro and Leyte, the pattern of increasing seropositivity (Fig 3) was similar to that observed in the population of Manila. However, the differences among age groups were not sig-

nificant due to the relatively high seropositivity of the age group of 1-9 years and lower seropositivity at 50 years or more than 30-49 years.

DISCUSSION

This study revealed a high prevalence of *Toxoplasma* antibody in Filipino populations, especially those in rural settings. *Toxoplasma* infection usually occurs asymptomatically in immunocompetent hosts, and the diagnosis depends mainly on serological tests. We have surveyed *Toxoplasma* antibody in Japanese populations by the ELISA method, and revealed overall prevalence of 10-20% in rural populations (Konishi and Takahashi, 1987). Since the same diagnostic method was used in this study, *Toxoplasma* antibody prevalence in the Philippines can be compared with that obtained in Japan.

Serological surveys of *Toxoplasma* antibody in Asian countries have been performed mainly by indirect hemagglutination assays (IHA). The previous survey carried out in the Philippines 22 years ago (Cross *et al*, 1977) was done in North Samar, which is located in the center of Philippine Archipelago, close to one of our study areas, Leyte. This survey revealed an overall seropositivity of 2.5% (32 positive individuals out of 1,274 samples). In Taiwan, where the location is close to the Philippines, seropositivity varied from 1.9% to 18% (Cross and Hsu, 1989). In other Asian countries, the prevalence of *Toxoplasma* antibody varied from 4.6% to 38.8% in Malaysia (Yahaya, 1991), from 2% to 63% in Indonesia, (Gandahusada, 1991), from 2.3% to 13.4% in Thailand. In Nepal, a survey done by latex agglutination and ELISA revealed seropositivities from 38% to 68% (Rai *et al*, 1996).

Toxoplasma is one of the pathogens which show the highest prevalence among humans in the temperate zone where most other parasites were eradicated. Recently, we compared age-dependent prevalence between two surveys at the same location with an interval of approximately 10 years in Japan (Khin-Sane-Win *et al*, 1997). The result indicated that the prevalence rate obtained in each age group at the first survey did not significantly increase at the second survey in the corresponding age group, suggesting a relatively low transmission rate during recent years. On the other hand, the present survey in rural areas in the Philippines revealed high seropositivities in young age groups of 1-9 years, suggesting high transmission rates. Surveys among humans in some other tropical and subtropical countries also indicated high seropositivities in infant groups (Remington *et al*, 1970). In tropical and subtropical regions, it appears that toxoplasmosis has not been a major concern because of a high incidence with other parasitic diseases. However, toxoplasmosis is now considered as one of the most important tropical and subtropical diseases.

Mechanisms of transmission of *Toxoplasma* to humans other than congenital transmission are (1) accidental ingestion of the oocyst defecated by cats and (2) ingestion of the cyst contained in infected raw meat. Variations of seropositivities among previous surveys are based on several factors: geographic, climatic, hygienic and socioeconomic factors, as well as lifestyles of the human population. These factors are closely related to the above two transmission mechanisms. In the present study, seropositivities in the rural area (61.2% in Mindoro and 30.2% in Leyte) were higher than that in the urban area (11.0% in Manila). Since people in the Philippines do not have the habit of consuming raw meat, it is highly probable that accidental ingestion of oocysts is the primary route of transmission in Filipinos. Therefore, the difference in seropositivity between rural and urban areas may be due to the hygienic and socioeconomic status that relates to oocyst shedding by cats and peoples' contact with the soil. Specifically, the pavement widely seen in the urban area is considered to contribute to the reduced surviving period of oocysts shed by cats. The relation of hygienic conditions to the prevalence of infectious diseases is also observed in epidemiological studies on hepatitis A in the Philippines (Barzaga *et al*, 1996). The persistent exposure to the contaminated environment could be a risk factor in acquisition of such infectious diseases.

In conclusion, a survey of antibodies to *Toxoplasma gondii* in the Philippines has shown that higher seropositivities were observed in the rural population, suggesting hygienic conditions and exposure to a contaminated environment is associated to the prevalence of *Toxoplasma gondii*.

REFERENCES

Barbier D, Ancelle T, Martin Bouyer G. Seroepidemiological survey of toxoplasmosis in La Guadeloupe, French West Indies. *Am J Trop Med Hyg* 1983; 32: 935-42.

Barzaga NG, Florese RH, Roxas JR, Francisco ZT. Seroepidemiology of hepatitis A virus among Filipinos living in selected communities in and around Metro Manila. *Philip J Microbiol Infect Dis* 1996; July-Dec: 39-47.

Bearman MH, McCabe RE, Wong S-Y, Remington JS. *Toxoplasma gondii.* In: Mandell GL, Benett JE, Dolin R, eds. Principles and Practice of Infectious Disease, 4th ed. New York: Churchill Livingstone 1995; 2455-75.

Cross JH, Banzon T, Wheeling CH, Cometa H, *et al.* Biomedical survey in North Samar Province, Philippine Islands. *Southeast Asian J Trop Med Public Health* 1977; 8: 464-75.

Cross JH, Hsu HM. Seroepidemiology of toxoplasmosis on Taiwan and some of the offshore islands: *Kaoshiung J Med Sci* 1989; 5: 493-7.

Gandahusada S. Study of the prevalence of toxoplasmosis in Indonesia: A review. *Southeast Asian J Trop Med Public Health* 1991; 22 (suppl): 93-8.

Khin-Sane-Win, Matsumura T, Kumagai S, Uga S, Konishi E. Prevalence of antibody to *Toxoplasma gondii* in Hyogo Prefecture, Japan: Comparison at a 10-year Interval. *Kobe J Med Sci* 1997; 43: 159-68.

Konishi E, Takahashi J. Reproducible enzyme-linked immunosorbent assay with a magnetic processing system for diagnosis of toxoplasmosis. *J Clin Microbiol* 1983; 17: 225-31.

Konishi E, Takahashi J. Some epidemiological aspects of *Toxoplasma* infections in a population of farmers in Japan. *Int J Epidemiol* 1987; 16: 277-81.

Porter SB, Sande MA. Toxoplasmosis of the central nervous system in the acquired immunodefficiency syndrome. *N Engl J Med* 1992; 327: 1643-8.

Rai SK, Kubo T, Yano K, *et al.* Seroepidemiological study of *Toxoplasma* infection in Central and Western Regions in Nepal. *Southeast Asian J Trop Med Public Health* 1996; 27: 548-53.

Remington JS, Efron B, Cavanaugh E, Simon HJ, Trejos A. Studies on toxoplasmosis in El Salvador. *Trans R Soc Trop Med Hyg* 1970; 64: 252-67.

Remington JS, Desmonts G. Toxoplasmosis. In: Remington JS, Klein JO, eds. Infectious diseases of the fetus and newborn infants. Philadelphia: WB Saunders 1990; 89-195.

World Health Organization. Infectious diseases according to mode of transmission. In: The World Health Report 1996. World Health Organization, 1996.

Yahaya N. Review of toxoplasmosis in Malaysia. *Southeast Asian J Trop Med Public Health* 1991; 22 (suppl): 102-6.