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Epidemiological Report

High Prevalence of Antibody to *Toxoplasma gondii* among Humans in Surabaya, Indonesia

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SUMMARY: Infection with *Toxoplasma gondii* is of medical importance in relation to a recent increase in cases of acquired immunodeficiency syndrome (AIDS). In the present study, we surveyed antibody to *Toxoplasma* among 1,761 people in Surabaya, Indonesia. The overall prevalence was 58% with significant differences between males (63%) and females (52%; $P < 0.001$). Although antibody prevalences at 0-9 years in both genders were less than 10%, those at ages over 10 years were more than 50% in males or more than 40% in females, suggesting an extremely high transmission rate of the parasite to humans in this area. A bimodal pattern in the frequency distribution of *Toxoplasma* antibody levels suggested a persistent feature of *Toxoplasma* infection in humans.

INTRODUCTION

Toxoplasma gondii is an intracellular protozoan parasite that can infect a variety of hosts ranging from birds to mammals. Humans, once infected with *Toxoplasma*, persistently harbor this parasite throughout the life, since human defense mechanisms cannot eliminate the cyst form of *Toxoplasma*. Epidemiological studies have indicated that this parasite is ubiquitously distributed throughout the world (1). Although the majority of infections are found to be asymptomatic, this parasite can cause congenital infections in infants and acute infections in immunocompromised individuals. Therefore, the increase in patients with acquired immunodeficiency syndrome (AIDS) has contributed to the increase in *Toxoplasma* encephalitis (2) and pneumonia cases (3).

The region of South and Southeast Asia is reported to have an estimate of 6 million people infected with human immunodeficiency virus (HIV), the second largest such population in the world. Although the largest population with HIV/AIDS is distributed in sub-Saharan Africa, the recent rapid growth of HIV/AIDS populations in Southern and Eastern Asia is a serious concern (4). Therefore, the epidemiology of *Toxoplasma* infection in this region is important in relation to the possible acquisition of opportunistic *Toxoplasma* infection. We have previously surveyed *Toxoplasma* antibody among people in the Philippines and revealed an overall prevalence of 11% in an urban area (Metro Manila) and much higher prevalences in rural areas, such as Mindoro (61%), and Leyte (30%; Kawashima et al., in submission). These prevalences in rural areas were relatively high in comparison with our previous survey data (20%) from a rural area in Japan (5).

In this study, we surveyed the prevalence of *Toxoplasma* antibodies among people in Surabaya, Indonesia. Previous surveys carried out in this country have revealed a wide range from 2% to 63% (6-8) among a variety of human populations. Surabaya is the second largest city in Indonesia with a population of approximately 3 million. Two previous surveys of Surabaya populations revealed much different prevalences of 9% (9) and 63% (10). We revealed in the present survey of over 1,700 people that the overall prevalence was 58% with a significantly higher prevalence in males than females in some age groups.

MATERIALS AND METHODS

Study subjects: A total of 1,761 sera were collected from general patients at the Emergency Unit of Doctor Soetomo Hospital in Surabaya, Indonesia, from November 1999 through March 2000. The ages of the patients ranged from 0 to 100 years, and the patients were grouped at 10-year increments, except for those over 80 years which were grouped in one age group. Serum samples from babies less than 6 months old, which may have contained maternally transferred antibodies, were not used in this survey. Age and sex compositions of

Table. Age and sex compositions of the present survey population

Age (years)	Number of Individuals		
	Male	Female	Total
0-9	39	30	69
10-19	78	45	123
20-29	125	122	247
30-39	158	131	289
40-49	106	140	246
50-59	126	129	255
60-69	123	158	281
70-79	133	69	202
80-100	26	23	49
Total	914	847	1,761

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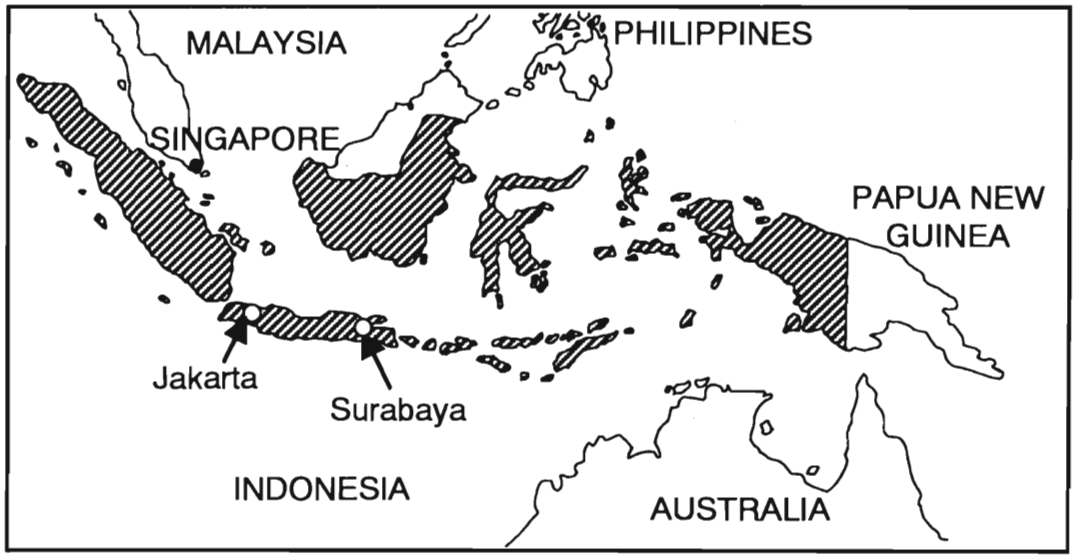

Fig. 1. Geographical map of Indonesia (hatched lines) and neighboring countries. Arrows show the locations of Surabaya and the capital, Jakarta.

the study subjects are shown in Table. Mean ages (± standard deviation [SD]) were 44.9 (±22.1) years in males, 45.2 (±19.5) years in females and 45.0 (±20.3) years in total with no significant differences between males and females ($t = 0.303$; $P > 0.05$). The location of Surabaya in Indonesia is shown in Fig. 1.

Enzyme-linked immunosorbent assay (ELISA) system: Crude soluble *Toxoplasma* antigen used for ELISA was prepared from tachyzoites of the RH strain of *T. gondii* as previously described (11). Briefly, tachyzoites harvested in the peritoneal cavity of mice were filtered through a polycarbonate membrane, homogenized and clarified. The supernatant was stored at −30°C until used for ELISA.

The ELISA for measuring antibodies to *Toxoplasma* was performed as previously described (12) with some modifications (13). In brief, microplate wells sensitized with *Toxoplasma* antigen were incubated serially with test sera, alkaline phosphatase-conjugated anti-human IgG, and *p*-nitrophenyl phosphate. Absorbance values obtained in duplicate at 410 nm were averaged and adjusted with the value for the positive control as 1.0 to minimize interplate variations. Sera showing adjusted values (ELISA values) of more than 0.357, which is the cutoff value established previously (12, 13), were determined to be positive for *Toxoplasma* antibody.

Statistical analysis: Significance of differences in prevalence rates was evaluated by the chi-square test with the Yates' correction factor. Significance of differences in mean ELISA values was evaluated by the Student's *t* test.

RESULTS

Prevalence of *Toxoplasma* antibody: Figure 2 shows age-dependent prevalence curves obtained with 914 males, 847 females, and a total of 1,761 serum samples. The overall prevalence was 63.2% in males, 52.4% in females and 58.0% in the total population, with a significant difference between males and females ($\chi^2 = 20.7$; $P < 0.001$). The prevalence of antibody at 0-9 years was less than 10% in both males and females, which increased significantly to more than 50% in males ($\chi^2 = 19.4$; $P < 0.001$) and more than 40% in females ($\chi^2 = 9.6$; $P < 0.01$) at 10-19 years. These high antibody

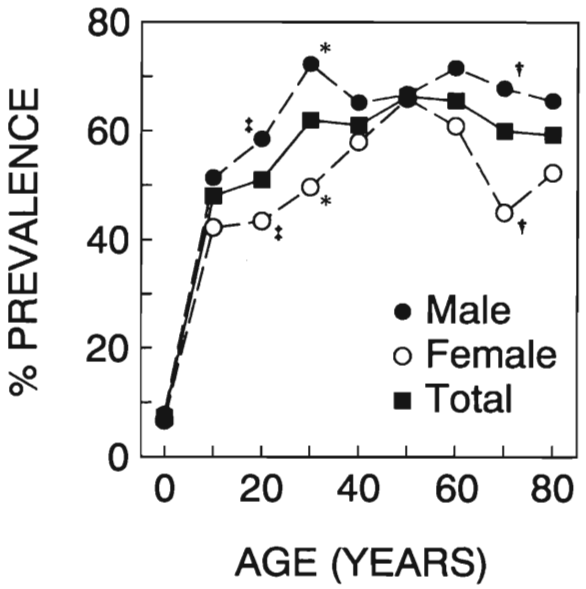

Fig. 2. Age-prevalence curve for *Toxoplasma* antibody in males (filled circle), females (open circle) and the total population (filled square). Significant differences between males and females are shown by *($P < 0.001$), † ($P < 0.01$) and ‡ ($P < 0.05$).

prevalences were also observed in age groups over 20 years for both males and females. Males showed significantly higher prevalences than females in age groups of 20-29 ($\chi^2 = 4.95$; $P < 0.05$), 30-39 ($\chi^2 = 14.5$; $P < 0.001$), and 70-79 ($\chi^2 = 8.86$; $P < 0.01$) years.

Distribution of *Toxoplasma* antibody levels: Figure 3 shows frequency distributions of ELISA values determined for male, female and the total populations. The maximum value was 1.750 in males and 1.636 in females. The distribution pattern was bimodal in both genders with the negative group at a peak of 0.0-0.1 and the positive group at a peak of 0.5-0.8. The mean ELISA values (±SD) of antibody-positive specimens were 0.758 (±0.261) in males and 0.763 (±0.261) in females without significant differences ($t = 0.346$; $P > 0.05$). Comparison of age groups in frequency distribution of ELISA

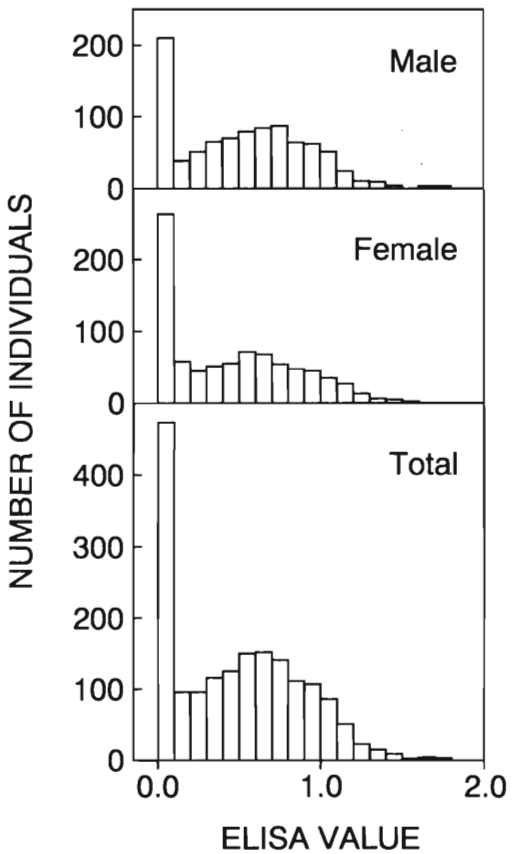

Fig. 3. Frequency distribution of ELISA values determined for 914 males, 847 females and a total of 1,761 serum samples.

values indicated a similar bimodal pattern (data not shown). The mean ELISA values of positive specimens were not significantly different among these age groups ($P > 0.05$).

DISCUSSION

Since the *Toxoplasma* parasite can evade human immune responses and establish a persistent infection, the presence of antibodies in an individual indicates that the individual was infected with the parasite at any time point before the survey. Therefore, the antibody prevalence indicates a cumulative rate of individuals with past exposure to *Toxoplasma*. The antibody prevalence is considered to reflect the rate of individuals who have a potential to develop toxoplasmosis upon acquisition of HIV infection. The relatively high prevalence (58%) obtained in the present survey confirms previous reports indicating that *Toxoplasma* is a serious public health concern in Indonesia (6-8). Varying antibody prevalences from 2% to 39% have been reported in other countries in Southeast Asia, such as Bangladesh (14,15), Laos (16), Malaysia (17, 18), Singapore (19, 20), Thailand (21-25), and Vietnam (26). This suggests that a wide area of Southeast Asia is contaminated with *Toxoplasma* by which people are frequently infected.

Three main mechanisms for *Toxoplasma* transmission to humans involve (i) congenital transmission, (ii) ingestion of the cyst contained in infected raw meat, and (iii) accidental ingestion of the oocyst defecated by cats (1). Since the rate of congenital transmission is considered to be less than the antibody prevalence in 0-9 years, this transmission mechanism does not greatly contribute to the main factor concerning high prevalence (58%) among the Surabaya population. A recent study of pregnant women across Europe pointed out that the ingestion of raw meat is the major transmission route of *Toxoplasma* infection (27). Concerns about food-borne parasitic diseases have been raised in some Southeast Asian countries (20, 28-30), including Indonesia (31). However, since Indonesian people do not have a habit of eating raw meat, the main mechanism of *Toxoplasma* transmission in the Surabaya population is considered to be the accidental ingestion of the oocyst. Positive *Toxoplasma* antibody prevalences among animal populations in several areas of Indonesia support that *Toxoplasma* oocysts are broadly distributed in this country (6, 8, 32).

Males had a higher *Toxoplasma* antibody prevalence than females in the Surabaya population. In the literature, the sex difference in prevalence has varied from population to population (1). Some behavioral factors, e.g., frequent contact with soil, are considered relevant to the difference in *Toxoplasma* prevalence between males and females. Further studies are needed to analyze the factors involved in transmission of *Toxoplasma* to the Surabaya population.

Based on the persistence of *Toxoplasma* infection in humans, its prevalence usually increases with age. For instance, our previous survey among a Japanese population revealed 1-4% in 0-39 years, 14% in 40-49 years, 18% in 50-59 years, and 30% in 60-69 years (13). Therefore, the age prevalence curve of the Surabaya population is very different from those of Japanese populations. One particularly distinct point is the difference between prevalences at 0-9 years and 10-19 years. Since the survey was a horizontal but not longitudinal one, this is not direct evidence that transmission is currently active. However, a dramatic increase in prevalence observed in the Surabaya population suggests that this population has a much higher transmission rate than Japanese populations. It is also important to note that the prevalence did not reach near 100% in spite of the possible high transmission rate of *Toxoplasma* infection.

A bimodal pattern in frequency distribution of *Toxoplasma* antibody levels was found in the Surabaya population. The bimodal patterns were also observed in our previous (5, 13) and other (33) studies using Japanese populations. Again, based on the persistence of *Toxoplasma* infection in humans, it is possible that an equilibrium could exist between host immunity and parasite activity (34). Equivalent mean antibody levels in seropositive specimens between males and females suggest that the equilibrium may be maintained in both genders at a similar level. Also, consistent mean antibody levels in seropositive specimens among age groups ranging from 0 to 100 years suggest that the equilibrium may be maintained throughout the life of infected individuals.

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REFERENCES

1. Remington, J. S., Mcleod, R. and Desmonts, G. (1995): Toxoplasmosis. p.140-267. *In* J. S. Remington and J. O. Klein (eds.), Infectious Diseases of the Fetus and Newborn Infant. 4th ed., W. B. Saunders, Philadelphia.
2. Luft, B. J. and Remington, J. S. (1992): Toxoplasmic encephalitis in AIDS. Clin. Infect. Dis., 15, 211-222.

3. Pomeroy, C. and Filice, G. A. (1992): Pulmonary toxoplasmosis. Clin. Infect. Dis., 14, 863-870.
4. World Health Organization (2000): Report from the Joint United Nations Programme on HIV/AIDS.
5. Konishi, E. and Takahashi, J. (1987): Some epidemiological aspects of *Toxoplasma* infections in a population of farmers in Japan. Int. J. Epidemiol.,16, 277-281.
6. Gandahusada, S. (1991): Study on the prevalence of toxoplasmosis in Indonesia. Southeast Asian J. Trop. Med. Public Health, 22 Suppl., 93-98.
7. Chomel, B. B., Kasten, R., Adams, C., Lambillotte, D., Theis, J., Goldsmith, R., Koss, J., Chioino, C., Widjana, D. P. and Sutisna, P. (1993): Serosurvey of some major zoonotic infections in children and teenagers in Bali, Indonesia. Southeast Asian J. Trop. Med. Public Health, 24, 321-326.
8. Uga, S., Ono, K., Kataoka, N. and Hasan, H. (1996): Seroepidemiology of five major zoonotic parasite infections in inhabitants of Sidoarjo, East Java, Indonesia. Southeast Asian J. Trop. Med. Public Health, 27, 556-561.
9. Yamamoto, M., Tokuchi, M., Hotta, S. and Noerjasin, B. (1970): A survey of anti-*Toxoplasma* hemagglutinating antibodies in sera from residents and certain species of animals in Surabaya, Indonesia. Kobe J. Med. Sci., 16, 273-280.
10. van der Veen, J., Padmodiwirjo, S. and Basuki, L. (1974): Serologic study of toxoplasmosis in Indonesia. MKI, 5-6, 340-345. listed in reference 5.
11. Yamaoka, M. and Konishi, E. (1993): Prevalence of antibody to *Toxoplasma gondii* among inhabitants under different geographical and climatic conditions in Hyogo Prefecture, Japan. Jpn. J. Med. Sci. Biol., 46, 121-129.
12. Konishi, E. and Takahashi, J. (1983): Reproducible enzyme-linked immunosorbent assay with a magnetic processing system for diagnosis of toxoplasmosis. J. Clin. Microbiol., 17, 225-231.
13. Khin-Sane-Win, Matsumura, T., Kumagai, S., Uga, S. and Konishi, E. (1997): Prevalence of antibody to *Toxoplasma gondii* in Hyogo Prefecture, Japan: comparison at a 10-year interval. Kobe J. Med. Sci., 43, 159-168.
14. Samad, M. A., Dey, B. C., Chowdhury, N. S., Akhtar, S. and Khan, M. R. (1997): Sero-epidemiological studies on *Toxoplasma gondii* infection in man and animals in Bangladesh. Southeast Asian J. Trop. Med. Public Health, 28, 339-343.
15. Ashrafunnessa, Khatun, S., Islam, M. N. and Huq, T. (1998): Seroprevalence of *Toxoplasma* antibodies among the antenatal population in Bangladesh. J. Obstet. Gynaecol. Res., 24, 115-119.
16. Catar, G., Giboda, M., Gutvirth, J. and Hongvanthong, B. (1992): Seroepidemiological study of toxoplasmosis in Laos. Southeast Asian J. Trop. Med. Public Health, 23, 491-492.
17. Yahaya, N. (1991): Review of Toxoplasmosis in Malaysia. Southeast Asian J. Trop. Med. Public Health, 22 Suppl., 102-106.
18. Hakim, S. L., Radzan, T. and Nazma, M. (1994): Distribution of anti-*Toxoplasma gondii* antibodies among Orang Asli (aborigines) in Peninsular Malaysia. Southeast Asian J. Trop. Med. Public Health, 25, 485-489.
19. Lim, K. C., Pillai, R. and Singh, M. (1982): A study on the prevalence of antibodies to *Toxoplasma gondii* in Singapore. Southeast Asian J. Trop. Med. Public Health, 13, 547-550.
20. Singh, M., Hian, Y. E. and Lay-Hoon, C. (1991): Current status of food-borne parasitic zoonoses in Singapore. Southeast Asian J. Trop. Med. Public Health, 22 Suppl., 27-30.
21. Morakote, N., Thamasonthi, W., Charuchinda, K. and Khamboonruang, C. (1984): Prevalence of *Toxoplasma* antibodies in Chiang Mai population. Southeast Asian J. Trop. Med. Public Health, 15, 80-85.
22. Chintana, T. (1991): Pattern of antibodies in toxoplasmosis of pregnant women and their children in Thailand. Southeast Asian J. Trop. Med. Public Health, 22 Suppl., 107-110.
23. Taechowisan, T., Sutthent, R., Louisirirotchanakul, S., Puthavathana, P. and Wasi, C. (1997): Immune status in congenital infections by TORCH agents in pregnant Thais. Asian Pac. J. Allergy Immunol., 15, 93-97.
24. Chintana, T., Sukthana, Y., Bunyakai, B. and Lekkla, A. (1998): *Toxoplasma gondii* antibody in pregnant women with and without HIV infection. Southeast Asian J. Trop. Med. Public Health, 29, 383-386.
25. Sukthana, Y. (1999): Difference of *Toxoplasma gondii* antibodies between Thai and Austrian pregnant women. Southeast Asian J. Trop. Med. Public Health, 30, 38-41.
26. Sery, V., Zastera, M., Prokopec, J., Radkovsky, J., The, P. H. and Canh, D. T. (1988): To the problem of toxoplasmosis in Vietnam. Bull. Inst. Marit. Trop. Med. Gdynia., 39, 181-185.
27. Cook, A. J., Gilbert, R. E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P. A., Foulon, W., Semprini, A. E. and Dunn, D. T. (2000): Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. Br. Med. J., 321, 142-147.
28. Eduardo, S. L. (1991): Food-borne parasitic zoonoses in the Philippines. Southeast Asian J. Trop. Med. Public Health, 22 Suppl., 16-22.
29. Singh, K. I. (1991): Current public health status of some food-borne parasitic zoonoses in Malaysia. Southeast Asian J. Trop. Med. Public Health, 22 Suppl., 11-15.
30. Shekhar, K. C. (1991): Epidemiological assessment of parasitic zoonoses in Malaysia. Southeast Asian J. Trop. Med. Public Health, 22 Suppl., 337-339.
31. Kusharyono, C. and Sukartinah, S. (1991): The current status of food-borne parasitic zoonoses in Indonesia. Southeast Asian J. Trop. Med. Public Health, 22 Suppl., 8-10.
32. Matsuo, K. and Husin, D. (1996): A survey of *Toxoplasma gondii* antibodies in goats and cattle in Lampung province, Indonesia. Southeast Asian J. Trop. Med. Public Health, 27, 554-555.
33. Kobayashi, A. (1977): Studies on toxoplasmosis. Tokyo Jikeikai Med. J., 92, 614-633 (in Japanese).
34. Konishi, E. (1989): Annual change in immunoglobulin G and M antibody levels to *Toxoplasma gondii* in human sera. Microbiol. Immunol., 33, 403-411.