PREVALENCE OF *TOXOPLASMA* ANTIBODIES IN CHIANG MAI POPULATION

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INTRODUCTION

Toxoplasmosis is caused by a coccidian parasite, *Toxoplasma gondii*. Infection with *Toxoplasma* occurs among warm blooded animals and birds through out the world. Man acquires infection indirectly by ingestion of meat containing *Toxoplasma* cyst or by contamination of oocysts from cat feces, or directly by transplacental infection (Scott, 1978; Krick and Remington, 1978). Many Chiang Mai natives keep cats in their houses, and in addition, one of their popular dishes is composed of raw meat with spices so called "Lahb" and "Nahm". The possibility thus exists that the infection is prevalent, and since acquired infection is usually subclinical, it may easily pass unrecognized. This study is conducted, therefore, to determine the prevalence of *Toxoplasma* antibodies in certain groups of Chiang Mai population by means of serological methods.

MATERIALS AND METHODS

Human sera: Five hundred sera were randomly collected from Blood Bank Unit, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University. These were from apparently 513 healthy male blood donors with mean age of 24.2 years.

Blood donor sera were examined by both indirect hemagglutination antibody (IHA) and indirect fluorescent antibody (IFA) tests. In addition, 500 sera were randomly collected from 697 pregnant women with mean age of 25.3 who attended the antenatal care clinic of the Outpatient Department. Also 1,002 serum specimens of both inpatients and outpatients, from the Central Laboratory of this same hospital, were screened for *Toxoplasma* antibodies.

Parasite: *Toxoplasma gondii* RH strain was obtained from Medical Research Institute, Phra Mongkut Klao Hospital, Bangkok, Thailand. The parasite has been maintained in Swiss mice by serial passage of peritoneal fluid.

Antigen preparation: Mice were each injected intraperitoneally (ip) with 3×10^6 organisms. Three days later, 3-5 ml of phosphate buffer saline (PBS), 0.05M, pH 7.2 containing 10 units heparin/ml was injected, ip. The peritoneal fluid was collected and centrifuged at 600 × g for 7 min. The sediment containing the parasite was resuspended and washed with PBS for three times and the final cell suspension was examined under the microscope. Only the suspension containing white blood cells less than 5% was used for antigen preparation.

A soluble antigen for indirect hemagglutination test (IHA) was prepared based on Jacobs and Lunde (1957) as modified by Karim and Ludlam (1975). Briefly, parasite sediment was resuspended in cold distilled water so as to obtain 5×10^8 organisms/ml. The lysed *Toxoplasma* was kept in a refrigerator overnight, freezed and thawed once; and then an equal volume of 1.7% NaCl was

added. The suspension was centrifuged at 10,000 × g for 30 min and the supernatant fluid was collected and stored at –40°C.

Indirect hemagglutination test: Glutaraldehyde-treated human group O erythrocytes sensitized with *T.gondii* soluble antigen were utilized. Human blood group O was collected in Alsever's solution and stored at 4°C for at least 2 days. After extensive washing with 0.15M phosphate buffer saline (PBS), pH 7.2, a 10% (v/v) cell suspension was made. To 4 parts of the cell suspension, one part of 2.5% glutaraldehyde in PBS, pH 7.2, was added drop by drop with constant stirring. The reaction was allowed to take place at room temperature for 2 hours. Then, treated cells were washed 3 times with above buffer solution and finally resuspended to 2.5% (v/v) concentration.

Tanning and sensitization procedures were as described elsewhere (CDC, 1976) with the only exception that tanning procedure was done in an ice bath, sentitization with antigen was performed at 37°C, and the final cell suspension was 0.75% in PBS 7.2 containing 0.5% bovine serum albumin (BSA, fraction V, Sigma Chemical Co., St.Louis, Missouri) and 0.1% gelatin.

Test was carried out in U-bottom microtiter plates (Cooke, Dynatech Laboratories, Inc., Alexandra, Virginia). Heat-inactivated serum sample was diluted twofold with PBS 7.2 containing BSA and gelatin as described above. The final volume of diluted serum in each well was 50 µl. To each well, 25 µl of non-sensitized or sensitized cells was added. The plate was covered, then shaken for 5 min, and subsequently incubated at 30°C overnight. The highest serum dilution giving a 2 + hemagglutination was taken as the *Toxoplasma* antibody titer (CDC, 1976).

Indirect fluorescent antibody test. The IFA test was performed and interpreted according to the CDC (1976). Fluorescein-conjugated anti-human globulin (sheep) was purchased from Wellcome Reagents Limited, London; and used at 1:10 dilution in 0.0002% Evans blue. Serum samples were first screened at a 1:16 dilution. Positive serum was further diluted fourfold and examined for the end point titer. Antigen slides were examined under the Fluorescence microscope (Olympus, Japan) with a B1 exciter filter and a Y-52 barrier filter at a magnification of 400 ×.

The IHA and IFA tests were standardized with human anti-*Toxoplasma* serum, TOX-60 (1,000 international units/ml, Statens Seruminstitut, Copenhagen, Denmark). This serum gave both IHA titer and IFA titer of 1:4,096. Negative control serum for the IFA test was prepared by absorption of pooled normal human serum with *Toxoplasma* trophozoites according to Karim and Ludlam (1975).

RESULTS

In the 500 blood donors IHA titers ranged from negative to 1:256 (Table 1). If the titer of 1:64 or more was considered positive, 23 out of 500 (4.6%) were positive and 95.4%

Table 1

Distribution of IHA titers to *Toxoplasma* in blood donors.

Titer	No. of Sera	% of Total
Negative	38	7.6
Undiluted	60	12.0
1:2	56	11.2
1:4	103	20.6
1:8	113	22.6
1:16	65	12.6
1:32	42	8.4
1:64	18	3.6
1:128	2	0.4
1:256	3	0.6
Total	500	100.0

was negative. It was also found that 38 (7.6%) had no detectable antibodies. When the same 500 plus 3 sera were examined by IFA technique only 1.2% was positive at titer of 1:64 or more (Table 2).

The results of IHA test of 500 pregnant women are shown in Table 3, Only 14 (2.8%) were found to be positive, and 76 (15.2%) were found to have no detectable antibodies. This percentage of negativity was apparently higher than in blood donors.

Frequency distributions of IHA titers to *Toxoplasma* antigens in both blood donor and pregnancy groups are summarized in Fig.1. As Both curves show "unimodal" in distribution. However, the peak of the curve shifted to the right in blood donor group demonstrating that blood donors generally have higher antibody titer than that of pregnancy group.

Table 2

Distribution of IFA titers to *Toxoplasma* in blood donors.

Titer	No. of Sera	% of Total
< 1:16	376	74.7
1:16	121	24.1
1:64	6	1.2
Total	503	100.0

Table 3

Distribution of IHA titers to *Toxoplasma* in pregnant women.

Titer	No. of Sera	% of Total
Negative	76	15.2
Undiluted	112	22.4
1:2	138	27.6
1:4	61	12.2
1:8	46	9.2
1:16	32	6.4
1:32	21	4.2
1:64	12	2.4
1:128	2	0.4
1:256	0	0
Total	500	100.0

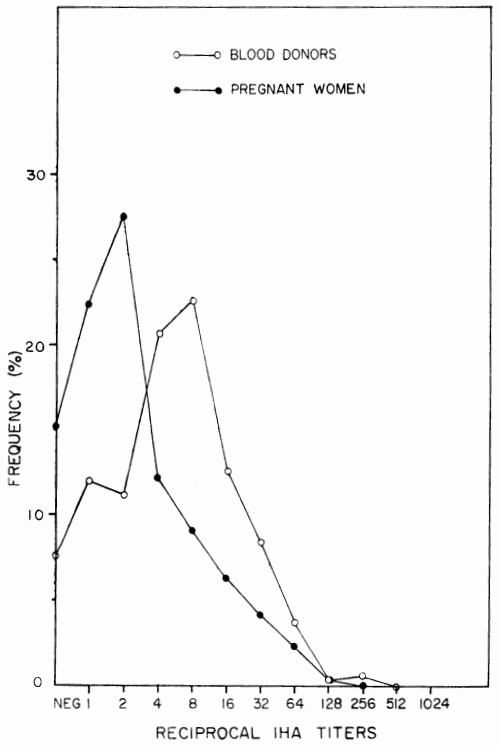

Fig. 1—Frequency distribution curves of IHA titers in blood donors and pregnant women.

In an attempt to find cases with possible recent exposure to *Toxoplasma* 1,002 sera of both inpatients and outpatients were screened for *Toxoplasma* antibodies by IHA method. Only one had a high titer of 1:2,048 and another had a titer of 1:1,024. A few showed titer of 1:512. Thus prevalence of toxoplasmosis seemed to be low in this group of patients as well as in blood donors and pregnant women.

DISCUSSION

Toxoplasma gondii has a worldwide distribution, and an estimated 25 to 50% of the world population has been exposed to this parasite (Lobel and Kagan, 1978). Detection of antibodies to *Toxoplasma* in population for epidemiologic study or for diagnostic purpose usually relies on serological methods. These include the Sabin-Feldman dye test (Sabin and Feldman, 1948; Feldman and Lamb, 1966), the indirect hemagglutination test (Jacobs and Lunde, 1957), the indirect fluorescent antibody test (Kelen *et al.*, 1962), and the enzyme-linked immunosorbent assay (Voller *et al.*, 1976). With the serological methods, prevalence of *Toxoplasma* antibodies, in world population has been estimated to range from none in Eskimos to 68% in Tahitians (Feldman, 1982).

The present study was carried out in Chiang Mai, Thailand, where the climate is considered as tropical. Cat is one of the most common domestic pets. Two human groups were selected, namely blood donors and pregnant women, for the reason that sex difference could be obvious. Difference in mean age between these two group was slight (about 1 year). Using the titer of 1:64 or more as the positive titer for both IFA and IHA, 4.6% and 1.2% of blood donors were positive by IHA and IFA respectively (Table 1 and 3). The seropositivity in pregnant women was 2.8% by IHA (Table 3). The prevalence of *Toxoplasma* antibodies in both groups was quite low indicating low degree of transmission despite the fact that many cats were present in Chiang Mai and raw meat consumption was common. The reason for the low degree of transmission is not known at the present time. We have examined sera from some 60 local cats and found that 11 of them were positive (unpublished observation). Nabnien (1979) found 2.4% of stray cats in central Thailand shed *Toxoplasma* oocysts. In her study seroprevalence of antibodies in swine, a common food animal in Thailand, was 20.8%. This demonstrated the presence of infected intermediate and definitive hosts to complete cat-food animals-man transmission chain in Thailand.

A lower seropositive blood donors using IFA test was evident (Table 1). This demonstrated the neccessity to select only one test in serological survey of *Toxoplasma* antibodies in several groups of man or animals so that the results could be compared with one and the other. The IFA test was not suitable for a survey because it required the use of a fluorescence microscope, the human positive control sera was difficult to obtain, and the technique was laborious. It has the advantage, however, for the diagnosis of toxoplasmosis since the results can be obtained within a day.

A considerably high proportion of seronegativity in pregnant women (15%) (Table 3) indicated a risk of infection *in utero* if these susceptible women acquired infection during pregnancy. The percentage of seronegative pregnant women was higher than that of blood male donors (Table 1 and 3). When frequency distribution curves of IHA titers in both groups were superimposed, the curve of the pregnant group shifted to the left demonstrating generally a lower antibody titer than that of blood donors. The reason may be that men frequently consume dishes containing raw meat while the women seldom do so; also pregnant women and blood donors in this study came from different areas where there was a difference in a degree of *Toxoplasma* transmission; and pregnant women are possibly immunosuppressed (Loke, 1982).

It is noteworthy that IHA titer frequency distribution curves in this study was unimodal, unlike the bimodal curves obtained from blood donor groups in Tokyo, Japan (Kobayashi *et al.*, 1977). This may be explained by low endemicity of toxoplasmosis in Chiang

Mai, or low degree of transmission (Lobel and Kagan, 1978). This assumption was supported by the finding that only 2 out of 1,002 sera obtained from the Central Laboratory had high antibody titer.

This is the first serological study done in Chiang Mai, Thailand, a place that many conditions are suitable for *Toxoplasma* transmission, i.e., the presence of many cats, both stray and pet cats, the common habit of consumption of raw meat among Chiang Mai natives, and the presence of food animals which can serve as intermediate hosts in *Toxoplasma* life cycle. The results obtained from our study, however, demonstrated unexpectedly low seroprevalence in Chiang Mai population.

Studies of toxoplasmosis had been done in Bangkok, Sungkasuwan (1967) found 4 out of 265 slaughter workers seropositive. Bunyaratvej *et al.*, (1978) reported three fatal cases of human toxoplasmosis at Ramathibodi Hospital, Bangkok. Nabnien (1979) employed the IHA tests and found a seroprevalence of 7.4% in healthy subjects, 2.3% in pregnant women, and 12.6% in cases of fever with lymphadenopathy. More recently, seroprevalence among blood donors and pregnant women was estimated by the IHA test to be 2.8% and 2.1% respectively, similar to our results (Jiraporn Yuwawitayapanich, Khon Kaen University, pers. comm).

SUMMARY

Five hundred sera from blood donors were examined for antibodies to *Toxoplasma* by the indirect hemagglutination antibody (IHA) and the indirect fluorescent antibody (IFA) techniques. In addition, the IHA test was used to detect *Toxoplasma* antibodies in 500 pregnant women's sera.

It was found that 4.6% of the blood donors were positive by the IHA and only 1.2% by the IFA methods. The seroprevalence, however, was less in pregnant women as only 2.8% were positive. Interestingly, 7.5% of the blood donors and 15.2% of pregnant women showed no detectable antibodies. The frequency distribution curves of IHA titers were unimodal in both groups studied.

From the basis of these findings, it was concluded that there was a low degree of *Toxoplasma* transmission in Chiang Mai.

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