

SEROPREVALENCE OF SPECIFIC TOTAL IMMUNOGLOBULIN (Ig), IgG AND IgM ANTIBODIES TO *TOXOPLASMA GONDII* IN BLOOD DONORS FROM LOEI PROVINCE, NORTHEAST THAILAND

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Abstract. A total samples from 345 healthy blood donors from Loei Province, Northeast Thailand were examined for anti-*Toxoplasma gondii* antibodies by ELISA. The seroprevalence of the anti-*Toxoplasma gondii* total Ig, IgG and IgM antibodies was 4.9%, 4.1% and 4.3%, respectively. Overall seropositive rate was 33 out of 345 individuals (9.6%). Among the seropositive cases, 5 (15.2%), 2 (6.1%) and 13 (39.4%) of the samples were determined by using each type of anti-*T. gondii* total Ig, IgG and IgM antibodies, respectively. The seropositive sera was also determined by combining of anti-*T. gondii* antibodies (anti-*T. gondii* total Ig with IgG and anti-*T. gondii* total Ig with IgM antibodies). These results were 10 (30.3%) and 2 (6.1%) cases, respectively. Only one (3%) sample had all types of anti-*T. gondii* antibodies. In addition, the frequency distribution curves of ELISA optical densities of anti-*T. gondii* total Ig, IgG and IgM antibodies in blood donor presented "unimodal" curves. The negative results were found in the age group that less than 20 years old and more than 51. The highest seropositive results were found in two age groups (21-30 and 31-40 years old), and males were significantly higher than female ($p < 0.05$). These results demonstrated that when using anti-*T. gondii* total Ig, IgG and IgM antibodies for determining the seroprevalence, the sensitivity was twice that with the anti-*T. gondii*, total Ig antibody alone.

INTRODUCTION

Toxoplasmosis is caused by a coccidian parasite, *Toxoplasma gondii*. It has a worldwide distribution and shows a broad host range from warm-blooded animals to birds and reptiles. Man acquires the infection indirectly by ingesting oocysts from contaminated environments, by consuming *Toxoplasma* cysts from tissues of other intermediate hosts, or directly by transplacental infection (Krick and Remington, 1978; Scott, 1978). Human infection is generally asymptomatic, except in immunocompromised adults and congenitally infected children (Dubey and Beattie, 1988). The prevalence of toxoplasmosis antibodies in the world population has been estimated to range from none in Eskimos and Taiwanese Aborigines, to 72% in Brazilians (Feldman, 1982), and as high as 94% in Costa Rican and Guatemalan people (Gibson and Coleman, 1958). From different parts of Thailand, the prevalence is 2.8 to 8% in a healthy person (Nabnein, 1979; Morakote *et al*, 1984; Maleewong *et al*, 1989;

Nishigawa *et al*, 1989; Wongkamchai *et al*, 1995) and 2.1 to 15.2% among pregnant women (Morakote *et al*, 1984; Maleewong *et al*, 1989).

Most serological surveys rely on an anti-*T. gondii* total immunoglobulin (Ig) antibody in healthy people (Morakote *et al*, 1984; Maleewong *et al*, 1989). However, the seroprevalence data determined by using the overall total Ig, IgG and IgM antibodies to *T. gondii* in Thailand has not been reported. The present study investigated the seroprevalence of these antibodies from blood donor sera by the enzyme-linked immunosorbent assay (ELISA) in Loei Province, in the northeast part of Thailand, as a model. Many Loei natives keep cats in their houses, and in addition, one of their popular dishes is composed of raw meat with spices. Thus, it is possible that infection is prevalent.

MATERIALS AND METHODS

Study area and population

Loei Province is located approximately 600 km northeast of Bangkok, near the Mekong River on the Thai-Lao PDR border (Fig 1).

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Sample collection

During 1997, a total of 345 serum samples were randomly collected from apparent healthy blood donors of Loei Hospital. The sex distribution was 114 (33%) females and 231(67%) males, with the age range from 17 to 56 years and a mean age of 31.38 years. The blood samples were then centrifuged. The sera collected and stored at -70°C, until transported to Department of Parasitology, Faculty of Medicine, Khon Kaen University, Thailand, for analysis.

Pooled positive reference human sera were prepared by combining equal volumes of toxoplasmosis anti-sera and an antibody titer of more than 1:1,024 by an indirect agglutination test (Pastorex® Toxo, USA). Pooled negative reference human sera were prepared by combining equal volumes of apparent healthy volunteers and a *Toxoplasma* antibody titer of less than 1:64 by indirect agglutination test (Pastorex® Toxo, USA).

***Toxoplasma* antigen**

T. gondii RH strain has been maintained in Swiss mice by serial passage of peritoneal fluid. Details of antigen preparation was similar, as previously described by Morakote *et al* (1984) and Ee *et al* (1989).

ELISA for an anti-*T. gondii* total Ig, IgG and IgM antibodies

The solid phase of ELISA was performed in a microtiter plate as a modification of that previously described by Ee *et al* (1989). Optimal conditions were determined by a checker board titration against pooled positive and negative reference sera. The optimum antigen concentrations were 0.5 µg per well to determine all types of anti-*T. gondii* antibodies. The enzyme conjugate used was peroxidase-conjugated goat antihuman total Ig, IgG and IgM (Cappel Laboratory, USA).

The sera were tested at 1:800, 1 : 200 and 1:100 dilution for anti-*T. gondii* total Ig, IgG and IgM antibodies, respectively. The positive and negative reference serum was included in each microtiter plate to correct day to day variations. The tested sera that gave the optical density (OD) at 490 nm, which was greater than the mean plus 2 standard deviation were considered as positive.

Statistical analysis

The results were analysed by the SPSS program (SPSS Inc, Chicago, USA). The differences in proportions were tested with chi-square test and

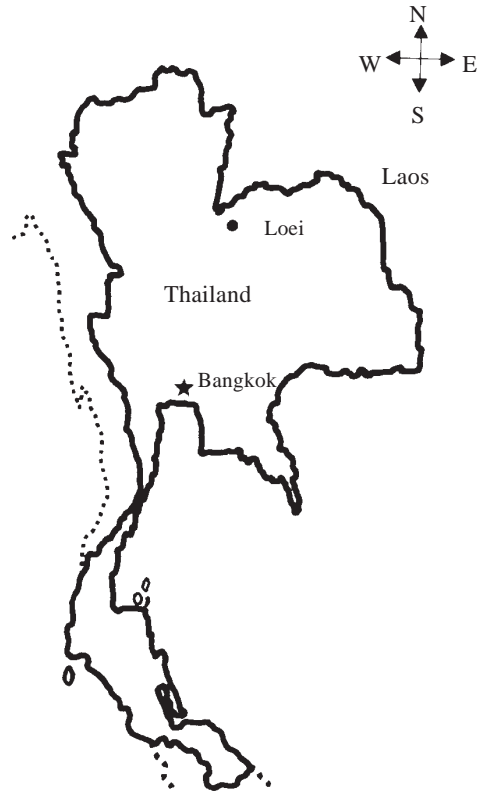


Fig 1—Map of Thailand showing Loei, the study area.

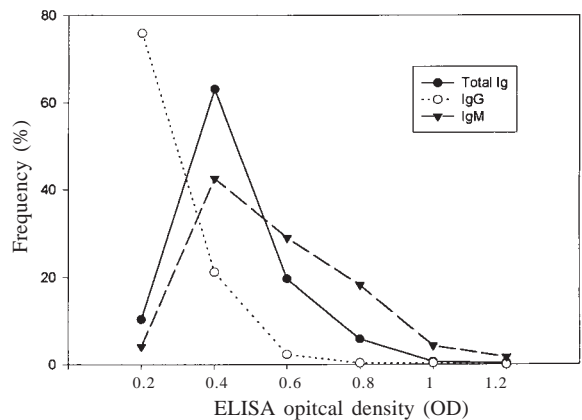


Fig 2—Frequency distribution curves of anti- *T. gondii* total Ig, IgG and IgM antibodies in blood donor sera.

differences in means by the student's *t*-test. A *p*-value of less than 0.05 was taken as significant.

RESULTS

The ELISA values were summarized in Table 1. The seroprevalence of anti-*Toxoplasma gondii*

total Ig, IgG and IgM antibodies was 4.9 %, 4.1 % and 4.3 %, respectively. The overall seropositive sera were 33 out of 345 individuals (9.6%). Among the seropositive cases, 5 (15.2%), 2(6.1%) and 13 (39.4%) samples were seropositive for each types of anti-*T. gondii* total Ig, IgG and IgM antibodies, respectively. The seropositive sera determined by combining the antibody responses represented by anti-*T. gondii* total Ig with IgG antibodies and anti-*T. gondii* total Ig with IgM antibodies were 10 (30.3%) and 2 (6.1%) cases, respectively. Only one (3%) sample had all types of anti-*T. gondii* antibodies. In addition, the frequency distribution of OD for anti- *T. gondii* total Ig, IgG and IgM antibodies in blood donors showed “unimodal” curves (Fig 2). The number of seropositive for anti-*T. gondii* total Ig, IgG and IgM antibodies in each age group were summarized in Table 2. Statistical comparison of the results demonstrated the seropositive from male was significantly higher than female ($p < 0.05$).

DISCUSSION

The present study was carried out with blood donors of Loei, Thailand, near the Thai-Lao PDR

border by using ELISA. When the cut off OD values, which more than mean plus 2 standard deviation is used as the positive criterion. The seroprevalence of the anti-*T. gondii* total Ig, IgG and IgM antibodies was 4.9%, 4.1% and 4.3%, respectively. This finding was also similar to the prevalence of 4.6%, as reported from Chaing Mai (Morakote *et al*, 1984) and this result might be due to the same geotopographic and climatic conditions (Rai *et al*, 1996). However, this observation was slightly lower than the prevalence of 7.4%, 8%, 6.3% and 6.4% reported from Bangkok, Songkhla, Nakorn Si Thamarat, Khon Kaen (Nabnein, 1979; Nishigawa *et al*, 1989; Maleewong *et al*, 1989), respectively. Interestingly, the seroprevalence determined from all types of anti-*T. gondii* antibody in this study (9.6%) was 2 times greater than that determined by anti-*T. gondii* total Ig antibody (4.9%). This prevalence is the highest reported in healthy people in Thailand.

The seroprevalence in this study was lower than previous reports in other Asian countries *ie*; 15.3% in Lao PDR (Catar *et al*, 1992), 15% in India (Bowerman, 1991), 0.33-11.97% in China (Yi-sheng *et al*, 1997), 10.6-17.5% in Malaysia (Hakim *et al*, 1994), 12.4% in Bangladesh (Samad *et al*, 1997),

Table 1
Optical density values and number of positive sample, mean, standard deviation, variance and range of ELISA optical density in blood donor sera.

Antibody types	Range	Mean ± SD	CV	No. positive/ total(%)
Total Ig	0.111 – 1.041	0.3429 ± 0.1402	40.89	17 (4.9)
IgG	0.060 – 0.994	0.1644 ± 0.1005	61.13	14 (4.1)
IgM	0.102 – 1.199	0.4623 ± 0.1986	42.87	15 (4.3)

Table 2
Frequency of anti – *T. gondii* total Ig, IgG and IgM antibodies in blood donor sera.

Age range (years)	No. of examined sera (no. of positive)	
	Males	Females
< 20	9 (0, 0, 0)*	14 (0, 0, 0)*
21-30	102 (4, 4, 3)*	43 (3, 2, 2)*
31-40	81 (6, 4, 6)*	48 (2, 2, 3)*
41-50	37 (2, 2, 1)*	9 (0, 0, 0)*
> 51	2 (0, 0, 0)*	0 (0, 0, 0)*
Total	231 (12, 10, 10)*	114 (5, 4, 5)*

(-, -, -)* : Numbers positive for anti-*Toxoplasma gondii* specific total Ig, IgG and IgM antibodies, respectively.

51-64% in Nepal (Rai *et al*, 1996) and 64% in Indonesia (Uga *et al*, 1996). These variations of the seroprevalence rate of *T. gondii* infection in humans might due to differences in food habits, ethnic groups, ages, sex, social classes, residences, cat contacts, environmental conditions and socio-economic status (Etheredge and Frenkel, 1995; Samad *et al*, 1997). Moreover, sensitivity and specificity of the test (Suthehall and Wreghitt, 1989), the type of antibodies for detection and the types of test kit are also variables (Hofgartner *et al*, 1997).

In addition, the seropositive rate was higher in males than in females, as similarly shown in a report from Chaing Mai Province (Morakote *et al*, 1984). The reason may be due to men frequently consuming dishes containing raw meat. The seropositivity was found in the age group of >21 years old, but not in the age group < 20 years old. This indicated that the seroprevalence might increase with age. This result has been also reported from another source (Rai *et al*, 1996; Sousa *et al*, 1988). The frequency distribution curves were unimodal, which indicated that toxoplasmosis was not endemic or a low degree of transmission in Loei.

This is the first serological study done in Loei, Thailand, a place that has many conditions suitable for *Toxoplasma* transmission, *ie*, the presence of many stray and pet cats, the consumption of raw meat among Loei natives and the presence of animals which can serve as intermediate hosts in the *Toxoplasma* life cycle. The results obtained from this study, however, demonstrated an unexplainably low seroprevalence in Loei. One reason may be a considerable time gap between the onset of the disease and laboratory investigation (Mohan *et al*, 1991). None the less, toxoplasmosis is widespread in Thailand as seropositivity is seen in blood donors, pregnant women and HIV positive patients (Wongkamchai *et al*, 1995).

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