

# DISTRIBUTION OF ANTI-*TOXOPLASMA GONDII* ANTIBODIES AMONG ORANG ASLI (ABORIGINES) IN PENINSULAR MALAYSIA

S Lokman Hakim<sup>1</sup>, T Radzan<sup>1</sup>, and M Nazma<sup>2</sup>

<sup>1</sup>Department of Tropical Medicine, and Infectious Diseases, Institute for Medical Research, Kuala Lumpur;

<sup>2</sup>Medical and Health Division, Aboriginal Affairs Department, Gombak Hospital, Selangor, Malaysia

**Abstract.** The distribution of anti-toxoplasma antibodies among the aborigines in Malaysia and its association with other soil transmitted infections and eosinophilia were studied. A total of 415 serum samples were collected and tested by IFA test. Overall prevalence was 10.6%, lower than previously reported. The antibody titers showed a unimodal distribution peaking at 1 : 8 dilution. There was a higher proportion of high antibody titer (>1 : 128) in the adult compared to the children with no significant difference in prevalence rate by sex. The pattern of infection does not differ from other soil transmitted infections and there was no association between raised *Toxoplasma* antibodies with eosinophilia.

## INTRODUCTION

Toxoplasmosis is a disease cause by a protozoan parasite, *Toxoplasma gondii*. It has a world wide distribution and the parasite itself shows very little host specificity. Various animals in Malaysia and Singapore have been shown to be infected with the parasite (Singh *et al*, 1967; Chooi *et al*, 1988). It has been established that infected animals are one of the sources of infection in man.

Previous studies have shown that all the major ethnic groups including the aborigines (Orang Asli) in Malaysia were affected with the highest rate noted among the Malays (Sinniah *et al*, 1984). Close association of the Malays with cats was the reason given. Lokman Hakim *et al* (1989) showed a high prevalence of antibody against *Toxoplasma gondii* among the wild caught monkeys, which are still hunted by the aborigines for food. It could therefore serve as a source of infection to this community. The Orang Aslis are known to keep dogs rather than cats as pets and therefore the source of infection would theoretically be through infected meat rather than oocysts from cat feces. The prevalence of toxoplasmosis should then be distinct from other soil-transmitted infections such as helminthiasis and toxocariasis which are common infections in this group of people (Dissanaike *et al*, 1977; Lokman Hakim *et al*, 1992). Differences in route of infection may also be reflected in the distribution of antibody titer by age group (van der Veen and Polak, 1980) which was not considered in previous studies.

Eosinophilia is a common finding in parasitic diseases such as helminthiasis and toxocariasis. Generally, protozoan infection is not associated with eosinophilia. However, it has been reported that in congenital toxoplasmosis mild eosinophilia may occur (Rajantie *et al*, 1992). Some even suggest that eosinophilia may serve as an early diagnostic marker for disseminated toxoplasmosis (Anderson *et al*, 1983). In order to study the distribution of anti-toxoplasma antibodies in this community, its association with other soil transmitted diseases and eosinophilia, we examined specimens collected from Gombak Hospital, Selangor, Malaysia.

## MATERIALS AND METHODS

### Specimens

Three ml of venous blood were collected from patients who were admitted to the Gombak Hospital and also from their accompanying relatives. The hospital is a special hospital that only caters for the medical needs of the Aborigines who come from all over Peninsular Malaysia. The blood samples were then centrifuged, sera collected and stored at -70°C until used. Stool samples were collected for intestinal helminthic infections examined by direct smear methods. Blood was also collected for eosinophil count expressed as the percentage of the total white cell in the peripheral blood.

### Toxoplasma antigen slides

Balb/c mice were infected intraperitoneally with RH strain *Toxoplasma gondii* under aseptic technique. After 3 to 4 days post-infection, peritoneal larvae with PBS pH 7.2 was performed. The parasites were then washed three times with PBS pH 7.2 by centrifuging at 5,000 rpm for 10 minutes. The parasites were resuspended with PBS pH 7.2 to give a suspension of about 30 parasites per microscopic field (at 40 $\times$  objective). Antigen smears were then made in each wells of the PTFE coated slides. The smears were air dried and later fixed in cold acetone for 30 minutes. After fixing, the slides were stored at -70°C until used.

### Immunofluorescent antibody test (IFAT)

Antigen slides were allowed to thaw at room temperature. Two-fold serial sera dilutions were made and working from highest to lowest dilution, serum sample diluted in PBS pH 7.2 were added to each well of the antigen coated slide and incubated for 30 minutes at 37°C in a moist chamber. After incubation, the slides were rinsed thrice in PBS and finally with distilled water. Fluorescein-labeled IgG antihuman conjugate in Evan's blue were then added to each well and incubated for 30 minutes. After rinsing, a drop of buffered glycerol were added and cover slides were superimposed. The slides were then examined with the 43 $\times$  objective and 10 $\times$  eyepiece on a fluorescent microscope equipped with BG-12 exciter and OG-1 ocular filters. Each slide was for one serum sample with a positive and negative control sera. A titer of 1 : 64 or more is taken as positive.

### Enzyme-linked immunosorbent assay (ELISA) for toxocarasis

The details of the method used have been described elsewhere (Lokman Hakim *et al*, 1992). Basically, the excretory-secretory antigen of *Toxocara canis* L2 was coated onto a microtiter plate overnight. After washing, serum dilution was then added and incubated for 2 hours at room temperature followed by washing and incubation for 3 hours with antihuman IgG peroxidase conjugate. The plates were then washed before adding substrate solution and incubated for 30 minutes. The reaction was stopped using sulphuric acid and the optical density (OD) read at 492 nm using an ELISA reader. An OD reading of more than 0.643 (mean + 3 SD of 30 healthy subjects) was taken as positive.

### Statistical analysis

Results were analysed by SPSS<sup>R</sup> program. Differences in proportions were tested with Chi-square test and differences in means by Student's *t*-test. Spearman's correlation regression analysis was used to test for association. A p-value of less than 0.05 was taken as significant.

## RESULTS

A total of 415 serum samples were collected and tested for antitoxoplasma antibodies. Fig 1 shows the frequency distribution of reciprocal antibody titer to *Toxoplasma gondii*. The distribution peak at the titer of 1 : 8. The IFA titer distribution for the three age groups namely children (< 10 years old), young adult (11 - 30 years) and adult (> 30 years) showed similar unimodal distribution. However, there was significantly higher proportion of titers above 1 : 64 in the adult than the children. Higher titers of 1 : 256 and above were only found in adult (Fig 2).

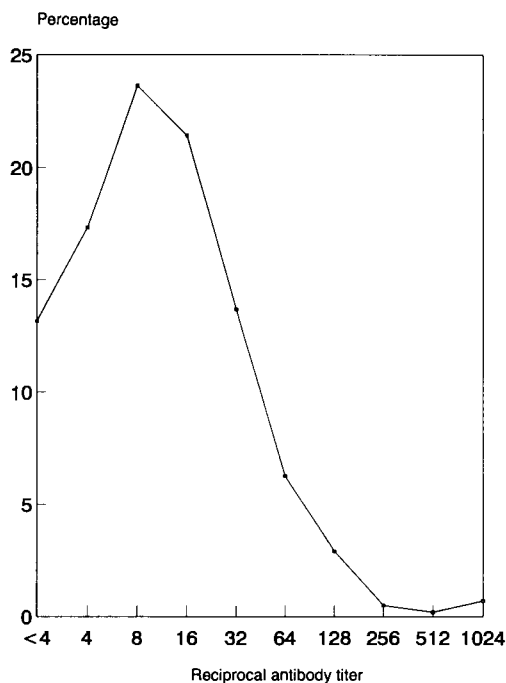


Fig 1—Frequency distribution of reciprocal antibody titer against *Toxoplasma gondii*.

Taking 1 : 64 dilution as the cut-off point, the overall prevalence rate was 10.6%. The rate among female (11.9%) was slightly higher than male (8.9%) but the difference was not statistically significant ( $p = 0.572$ ).

Table 1 shows the prevalence rate by age group where the 31-40 age group recorded the highest rate. There was no significant differences in prevalence rate between the age groups ( $p = 0.445$ ). The proportion of positive cases tend to increase with age from 8.4% (0 - 10) to 10.4% and 15.3% for 11 - 30 and more than 30 years age group respectively but the correlation was not significant ( $r = 0.077$ ,  $p = 0.13$ ).

Stool specimens were available from 124 patients for helminthic infection by direct smear methods. Out of 82 samples negative for helminthic infection, 12 (14.6%) were positive for toxoplasmosis and 16.6% were positive for both ( $p = 0.958$ ). Similarly there was no significant difference in *Toxoplasma* positive cases between toxocariasis positive and negative samples as detected by ELISA.

The mean percent eosinophil for *Toxoplasma* positive cases was  $5.20 \pm 7.22$  as compared to  $5.32 \pm 6.47$  for the negative cases ( $p = 0.460$ ). In contrast, mean

Table 1

Seroprevalence of *Toxoplasma* antibodies by age group.

Age group	No. of specimen	No. positive	% positive
0-4	54	4	7.4
5-10	49	3	6.1
11-20	63	8	12.7
21-30	76	5	6.6
31-40	67	11	16.4
41-50	44	5	11.4
> 50	62	6	9.7

percent eosinophil count were significantly higher in cases with intestinal helminthic infections and in toxocariasis (Table 2).

DISCUSSION

*Toxoplasma* infection is acquired either by the consumption of raw/undercook meat which contains the cysts or through the ingestion of infectious oocysts from the cat feces or both. Transplacental infection from primarily infected mother may also occur. Differences in consumption patterns and in degree of exposure to cat feces may result in variation in seroprevalence rates. In Malaysia, the Malays who are closely associated with cats were shown to have the

Percentage

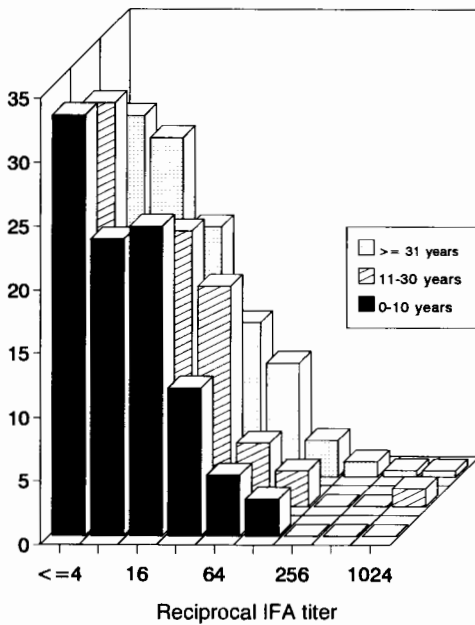


Fig 2-Distribution of IFA titers in three age groups expressed in percentage.

Table 2

Mean percent eosinophil count by type of infection.

	Mean percent eosinophil count $\pm$ SD		
	Negative	Positive	p-value
Toxoplasmosis	$5.32 \pm 7.22$	$5.32 \pm 6.47$	0.46
Helminth infections	$4.87 \pm 5.80$	$9.08 \pm 8.91$	0.04
Toxocariasis	$4.30 \pm 4.74$	$9.82 \pm 9.23$	0.01

highest rates (Sinniah *et al*, 1984). Infected meat animals may be an important source of infection but Malaysians generally cook their food very well thus reducing the risk of infection. This could explain for the lower rate among Malaysian Chinese though they consume a lot of pork which has the highest infection rate among meat animals in this country (Singh *et al*, 1967; Thomas *et al*, 1980). The Aborigines who are not closely associated with cats had the lowest rates (Sinniah *et al*, 1984). The overall prevalence of 10.6% in this study among the Aborigines is very much lower than 16% reported by Dissanaik *et al* (1977) and 17.5% by Sinniah *et al* (1984). This could be a true reduction since the samples collected by Dissanaik were from the same hospital and using the same method.

The frequency distribution of the IFA titers showed a unimodal distribution in contrast to that of bimodal distribution described in other populations (Cross *et al*, 1975; van der Veen and Polak, 1980). The distribution peak at 1 : 8, more or less plateau at 1 : 16 and sharply descended thereafter. A titer of 1 : 16 and lower may actually reflect non-specific reactions or reactions due to cross-reacting antigens although others had suggested that an IFA titer of more than 1 : 8 may be taken as positive (Krahenbuhl and Remington, 1982). *Sarcocystis* antigens may be the cross-reacting antigens where antibodies against this organism was detected as high as 39.4% among the Aborigines in this country (Dissanaik *et al*, 1977). If a titer of 1 : 64 is considered to suggest recent infection then a titer of 1 : 32 probably indicates the presence of residual antibodies to past exposure to *T. gondii*. Taking into consideration that cross-reacting antigens are usually lower in titer than specific reaction and are more transient, 1 : 16 titer could still represented a false negative result. Thus a large majority of this community had actually been exposed to the infection.

What is interesting to note is that high antibody titers (> 1 : 128) occurs mainly in adult (Fig 2) although the proportion of positive cases increasing with age was not significant. Immunoglobulin G antibody against *Toxoplasma* rises rapidly after exposure, persists for a long time but may not be detectable for life with IFA. van Druten *et al* (1990) showed that by using a mathematical model, the mean duration of IFA seropositivity after infection is 40 years. Therefore the higher proportion of high antibody titers in the adult could well be due to continued exposure and reinfection in this age group. Game hunting is the job of the adult in this community and they may go for few days before they come back. Higher consumption of in-

fectured game meat could be an explanation for the continued exposure to infection among the adult. The pattern of infection does not differ significantly from other soil transmitted infections in this study. It seemed that the Aborigines were exposed to both oocysts from the cat as well as cysts in infected meat with the latter playing a greater role in boosting the level of anti-*Toxoplasma* antibodies in adults.

This study shows that toxoplasmosis is not significantly associated with eosinophilia. In a community where polyparasitism is very common, mild eosinophilia is a usual finding.

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