Seroprevalence and Sources of Toxoplasmosis among Orang Asli (Indigenous) Communities in Peninsular Malaysia

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Abstract. This study aims to evaluate the current seroprevalence of toxoplasmosis among indigenous communities in Peninsular Malaysia and relate its association with epidemiological data. Overall seroprevalence of *Toxoplasma gondii* was 37.0% with 31.0% immunoglobulin (Ig) G, 1.8% IgM, and 4.2% seropositivity for both anti-*Toxoplasma* antibodies. Multivariate analysis showed that age above 12 years (odds ratio [OR] = 2.70, 95% confidence interval [CI] = 1.75–4.04, $P < 0.001$), using untreated river and mountain water supplies (OR = 1.50, 95% CI = 1.01–2.40, $P = 0.050$), and close proximity with cats (OR = 1.40, 95% CI = 1.10–1.76, $P = 0.010$) were factors associated with toxoplasmosis. Given the high seroprevalence of toxoplasmosis among these communities who live in poor socioeconomic conditions, a comprehensive health surveillance program and screening should be initiated among women of childbearing age and pregnant women during the antenatal period for early diagnosis and treatment. The role of domestic cats and environmental contamination with oocyst in soil and water has to be highlighted and addressed in future prevention strategies for these communities.

INTRODUCTION

Human toxoplasmosis is a zoonotic infection caused by the obligate intracellular protozoan parasite *Toxoplasma gondii*. Approximately one-third of the world population is estimated to be infected with *T. gondii*.¹ There are various ways in which humans acquire toxoplasmosis, and these ways include ingestion of contaminated raw meats with tissue cysts of *Toxoplasma*, ingestion of vegetables or water contaminated with infected cat feces, blood transfusion, organ transplantation, and congenital acquisition of infection. Acquired toxoplasmosis is generally asymptomatic, whereas clinical toxoplasmosis may present in different forms. The most common manifestation of acute acquired toxoplasmosis is lymphadenopathy, and the illness may resemble the flu and is self-limited. Besides these factors, ocular toxoplasmosis may cause chorioretinitis, and this usually follows congenital infection. Another form primarily involves the brain and is usually seen in immunocompromised populations, particularly acquired immunodeficiency syndrome (AIDS) patients. Significantly, toxoplasmosis may cause serious complications in fetuses of infected pregnant mothers² and in immunocompromised patients who have undergone solid organ transplants³ or have human immunodeficiency virus (HIV)/AIDS.⁴

In Malaysia, the prevalence rate of toxoplasmosis in the general population was reported to be at 28.1% in 2002.⁵ Seroprevalence of toxoplasmosis among HIV/AIDS patients was higher, with rates recorded at 51.2% ($N = 406$)⁶ and 44.8% ($N = 505$).⁷ However, information on toxoplasmosis among the indigenous groups in Peninsular Malaysia has been very limited. The last available information on the distribution of anti-*Toxoplasma* antibodies among the indigenous people (Orang Asli) in Malaysia was in 1994.⁸ Of 415 serum samples collected and tested by indirect fluorescent antibody (IFA) test, 10.6% were seropositive.⁸ Given that the indigenous communities are unique compared with the general population because of their distinct living lifestyles, educational backgrounds, and environ-

mental characteristics, therefore, this study was carried out to determine the current seroprevalence of toxoplasmosis among the indigenous communities as well as to assess the association between the risk factors with *Toxoplasma* seropositivity. This study is significant in providing the most current epidemiological data of toxoplasmosis among these minority communities besides enhancing the understanding of potential risk to which they are exposed. With improved knowledge, effective preventive measures can be instituted and implemented.

MATERIALS AND METHODS

Study population. The study was carried out from November 2007 to October 2010 among various Orang Asli (indigenous) subgroups living in Peninsular Malaysia. The Orang Asli constitutes the minority people who live in the closest possible association with tropical forest of the lowlands and hills of Peninsular Malaysia. Despite their small number, they are not homogenous, because each group has its own language and culture; most importantly, they perceive themselves as different from the others. There are three major groups (Negrito, Senoi, and Proto-Malay), and each group is also divided into six subgroups, totaling 18 distinct cultural linguistic groups. Their population grew from 54,033 in 1969 to 92,529 in 1994 at a rate of almost 2.3% per year.⁹ In 2004, according to the latest available records of the Department of Orang Asli Affairs, there were a total of 147,412 Orang Asli, representing a mere 0.6% of the national population, living in 869 villages.⁹ Of these, seven subgroups, including Temuan, Selangor, Semai, Perak, Semai Pahang, Mah Meri, Semelai, Temiar, and Orang Kuala, from five different states have voluntarily participated in this study (Figure 1). The study included 495 eligible Orang Asli individuals who gave informed consent before this study. The villages and the selection of the study participants have been described in a different study.¹⁰

Information collection. Information on sources of infection and environmental characteristics was collected by means of a questionnaire together with blood samples. A pre-coded and pre-tested questionnaire designed to detect sociodemographic and biologically plausible risk factors associated with toxoplasmosis, clinical history, and presenting signs and symptoms related to toxoplasmosis (if any) was used

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FIGURE 1. Location of the study areas in Peninsular Malaysia.

to elicit information by trained field assistants. An operational definition was used for risk factors. Sources of drinking water were defined as water from a clean source, such as mineral, boiled, filtered, or government municipal water supply types, whereas unclean source included water from rivers, wells, or rain. A person in contact with pets was defined as a person who kept cats as personal pets in his/her home environment or had close contact with stray cats or a person who kept dogs or other warm-blooded animals as pets. Meanwhile, eating with hands was defined as persons with the habit of eating food using hands, and the method of washing hands could not be warranted. The questionnaire survey was designed in Bahasa Malaysia (national language for Malaysia), which was well-understood by the participants. For children and older participants, the questionnaire was completed by their parents, guardians, or heads of the family who signed the consent form.

Blood sample collection. Approximately 5 mL venous blood were drawn by trained medical assistants and nurses using disposable syringes into a plain tube (without anticoagulant) from each qualified subject who fulfilled the specific criteria. The blood samples were kept in a standard ice-packed storage box and were transported back to the Department of Parasitology, Faculty of Medicine, University of Malaya for analysis. Blood samples were spun at 1,500 rpm for 10 minutes, and the sera were kept at -20°C until use.

Detection of immunoglobulin G and M antibodies to *T. gondii*. Toxoplasmosis was screened using a standard enzyme-linked immunosorbent assay (ELISA) commercial kit (immunoglobulin G [IgG] and IgM; Trinity Biotech, New York, NY) in accordance with the manufacturer's instructions

and was performed at the Department of Parasitology, Faculty of Medicine, University of Malaya. A series of dilutions were prepared from serum samples and then added to the ELISA plates coated with specific antigens of *T. gondii*. A result of > 51 IU/mL anti-*Toxoplasma* IgG antibody was regarded as positive, indicating latent or pre-existing *Toxoplasma* infection, and a result of > 51 IU/mL anti-*Toxoplasma* IgM antibody was regarded as positive, indicating a recently acquired *Toxoplasma* infection. Subsequently, all samples positive for both anti-*Toxoplasma* IgG and anti-*Toxoplasma* IgM antibodies were also tested for IgG avidity using a standard ELISA commercial kit (IgG; NovaLisa, Dietzenbach, Germany) according to the manufacturer's instructions; high avidity ($> 40\%$) indicates past infections, and low avidity ($< 40\%$) indicates recently acquired infections.¹¹

Statistical analysis. Data obtained from both the questionnaire and laboratory tests were entered, edited, and analyzed using the statistical software SPSS version 10 (SPSS Inc., Chicago, IL). Before each analysis, initial data entry was cross-checked regularly to be sure that data were entered correctly and consistently. The data with quantitative variables were expressed as means ($\pm$ standard deviation [SD]) and ranges, whereas qualitative variables were estimated and presented as frequencies and percentages. A Pearson's χ^2 test on proportion was used to test associations between variables. A univariate statistical model was used to assess potential associations between toxoplasmosis seropositive (outcome of interest) and the characteristics of potential risk factors. To make sure that the potentially important predictors are not excluded and also, because of a low number of predictor variables, all variables with or without lower significance levels between 0.10 and 0.25 were included in the multivariate analysis using both backward and forward stepwise selection to produce the subset for final model to sequentially determine significant differences in demographics and confounding risk factors among studied participants. A significance level of $P < 0.05$ according to the odds ratios (OR) and 95% confidence interval (95% CI) was used for all tests to explore the strength of the association between *T. gondii* seropositivity and the variable of interest.

Ethical consideration. The study was approved by the Medical Ethics Committee of the University Malaya Medical Center (UMMC; Medical Ethics Committee reference number 824.11). Before sample and data collection, the study group members introduced themselves, and an oral briefing to describe the objective and methodology of the study was given to the participants. They were also informed of the potential risk of used procedures and given assurance that their identity and personal particulars would be kept confidential and anonymous. Participations were voluntary, and they could be withdrawn from the study at any time without giving any reason. Agreement of participation was indicated by the signing of a consent form for the literate participants and the imprinting (thumb print) of the thumb for the illiterate participants. For children, consent was obtained from their parents or guardians in a similar manner.

RESULTS

Demographic and baseline characteristics of the study group.

A total of 495 individuals from seven subgroups, including 22.4% (111) Semelai, 18.6% (92) Semai Pahang, 18.0% (89) Orang Kuala, 15.6% (77) Mah Meri, 13.1% (65) Semai Perak,

9.1% (45) Temuan, and 3.2% (16) Temiar, participated in this study. Their ages ranged from 1 to 82 years, with a mean $\pm$ SD of 16.92 ± 13.92 and proportions of 1.2%, 2.2%, 69.7%, 3.5%, and 23.4% for the age groups 1–4, 5–6, 7–12, 13–17, and above 18 years, respectively. The majority of participants were female (282, 57.0%), were in the age group of less than 12 years (362, 73.1%), had at least primary level education (369, 73.1%), were unemployed (430, 87.0%), and received a household income of less than Ringgit Malaysia (RM) 500 (US \$166.7) per month (331, 67.0%). A large number of this community drank water from sources other than the government municipal water supply (302, 61.0%), had closed contact with pets (432, 87.3%), and had the habit of eating with their hands (487, 98.4%) (Table 1).

Seroprevalence of toxoplasmosis. The overall seroprevalence of toxoplasmosis in these Orang Asli communities was 37.0% (183/495; 95% CI = 32.7–41.2%); 31.0% (153/495; 95% CI = 26.9–35.1%) were seropositive for anti-*Toxoplasma* IgG, 1.8% (9/495; 95% CI = 0.6–3.0%) were seropositive for IgM, and 4.2% (21/495; 95% CI = 2.5–6.0%) were seropositive for both IgG and IgM antibodies (Table 2). Additional analysis according to gender and specific age groups indicated that males (39.0%) had slightly higher seroprevalence of toxoplas-

TABLE 1

Demographic and baseline characteristics compositions of the survey population ($N = 495$)

Variables	Number examined	Percent examined
Age (years)		
Range	1–82	
Mean ($\pm$ SD)	16.92 ($\pm$ 13.92)	
Age groups (years)		
1–4	6	1.2
5–6	11	2.2
7–12	345	69.7
13–17	17	3.5
≥ 18	116	23.4
Age groups (years)		
≤ 12	362	73.1
> 12	133	26.9
Ethnic groups		
Orang Kuala	111	22.4
Semai Pahang	92	18.6
Temiar	89	18.0
Temuan	77	15.6
Semai Perak	65	13.1
Semelai	45	9.1
Mah Meri	16	3.2
Level of education		
No formal education	126	25.4
Formal education (at least primary school)	369	74.6
Occupation categories		
Working	65	13.1
Not working	430	86.9
Household income (RM/month)		
< RM 500 (< US \$166.7)	331	67.0
> RM 500 (> US \$166.7)	164	33.0
Water supply categories		
River and mountain pipe water	276	55.8
Others (government or rain water)	219	44.2
Close contact with pets		
Yes	432	87.3
No	63	12.7
Eating with hands		
Yes	487	98.4
No	8	1.6

TABLE 2

Seroprevalence of IgG and IgM antibodies to *T. gondii* among the survey population as assessed by the ELISA test ($N = 495$)

Antibodies	<i>T. gondii</i> seropositive ($N = 495$ participants)		
	Number seropositive	Percent seropositive	95% CI
IgG+	153	31.0	26.9–35.1
IgM+	9	1.8	0.6–3.0
IgG+IgM+*	21	4.2	2.5–6.0
Total	183	37.0	32.7–41.2

*Twenty-one IgG+IgM+ also have low IgG avidity, indicating recently acquired infection.

mosis compared with females (35.3%) (Table 3). With regards to specific age groups, seroprevalence showed significant age-dependent relationships, with the highest seroprevalence seen among those participants aged 13–17 years (64.7%) followed by 18 years and above (50.9%), 7–12 years (31.9%), and 5–6 years (27.3%) ($\chi^2 = 23.01$, $P < 0.001$) (Table 3). None of the participant aged 1–4 years old was seropositive with toxoplasmosis. Among the Orang Asli subgroups, seropositivity of *Toxoplasma* infection was found to be highest among the Mah Meri (95.0%, 12/16) followed by Temiar (71.4%, 55/77), Orang Kuala (39.3%, 35/89), Semelai (35.1%, 39/111), Temuan (33.3%, 15/45), Semai Pahang (19.6%, 18/92), and Semai Perak (13.8%, 9/65; data not shown).

A total of 21 samples positive for both IgG and IgM antibodies were also tested for IgG avidity, and all samples were < 40% (low avidity), suggesting recently acquired infections. It is interesting to note that 15 of these low-avidity samples were found in females: 12 samples among those participants aged 7–12 years, 2 samples from those participants aged 13–17 years old, and 1 sample from those participants aged 18 years and above. The remaining six samples were among males aged between 7 and 12 years old (data not shown).

Seroprevalence of toxoplasmosis in relation to demographic characteristics and risk factors. Univariate analysis indicated that age above 12 years (OR = 1.47, 95% CI = 1.21–1.78, $P < 0.001$), lack of education (OR = 1.82, 95% CI = 1.21–2.74, $P = 0.004$), employment (OR = 2.32, 95% CI = 1.35–3.96, $P < 0.001$), use of untreated river and mountain water supplies for daily chores (OR = 1.48, 95% CI = 1.02–2.14, $P = 0.040$), and finally, close contact with pets (OR = 2.50, 95% CI = 1.32–4.73, $P = 0.004$) were significantly associated with seropositivity of *T. gondii* (Table 4). Multivariate analysis also confirmed that participants who were above 12 years old, using untreated river and mountain water supplies for daily chores, and having close contact with pets were 2.7 times (95% CI = 1.75–4.04, $P < 0.001$), 1.5 times (95% CI = 1.01–2.40, $P = 0.050$), and 1.4

TABLE 3

Seroprevalence of *T. gondii* among the survey population by gender and age groups

Variables	N	<i>T. gondii</i> seropositive	
		Number	Percent
Sex			
Male	213	83	39.0
Female	282	100	35.3
Age groups (years)			
1–4	6	0	0
5–6	11	3	27.3
7–12	345	110	31.9
13–17	17	11	64.7
18 and above	116	59	50.9

TABLE 4
Potential risk factors associated with seropositivity of *T. gondii* among the studied population (univariate analysis; *N* = 495)

Variables/characteristics	<i>N</i>	<i>T. gondii</i> seropositive		OR (95% CI)	<i>P</i> value
		Number	Percent		
Sex					
Male	213	83	39.0	1.16 (0.80–1.68)	0.424
Female	282	100	35.5	1	
Age* (years)					
> 12	133	70	52.6	1.47 (1.21–1.78)	< 0.001
≤ 12	362	113	31.2	1	
Level of education					
No formal education	126	60	47.6	1.82 (1.21–2.74)	0.004
Formal education	369	123	33.3	1	
Occupation					
Working	65	35	53.8	2.32 (1.35–3.96)	< 0.001
Not working	430	148	34.4	1	
Household income (RM/month)					
< RM 500	331	115	34.7	0.75 (0.51–1.11)	0.146
> RM 500	164	68	41.5	1	
Water supply*					
River and mountain pipe water	276	113	40.9	1.48 (1.02–2.14)	0.040
Others (government pipe or rain water)	219	70	32.0	1	
Close contact with pets*					
Yes	432	170	39.4	2.50 (1.32–4.73)	0.004
No	63	13	20.6	1	
Eating with hands					
Yes	487	182	37.4	4.18 (0.51–34.22)	0.148
No	8	1	12.5	1	

Reference group marked as OR = 1. Confidence interval (CI) significant association set at *P* < 0.05.

*Variables were confirmed by multivariate analysis as significant predictors of *T. gondii*.

times (95% CI = 1.10–1.76, *P* = 0.010) more likely to acquire toxoplasmosis, respectively.

DISCUSSION

This study focused on the investigation of *T. gondii* infection among indigenous communities in Malaysia using standard commercial kits to detect their seropositive titer specific for anti-*Toxoplasma* IgG and IgM antibodies and determine the current seroprevalence of *T. gondii* infection and risk predictors. About one-third of the Orang Asli had latent toxoplasmosis, which indicated that they had previous exposure to this infection. To our surprise, this prevalent rate (37.0%) was comparatively much higher than the previous studies carried out in Malaysia, which varied from 10.6% to 17.5%.^{8,11,12,13} Certain factors such as sample size, locations, and differences in serodiagnostic tests may have contributed to a sharp rise in the prevalence of toxoplasmosis in these indigenous populations.

The seroprevalence rate of toxoplasmosis in Malaysian indigenous people is comparable with that of indigenous people in Taiwan (40.6%),¹ Croatia (38.1%),¹⁴ and Thailand (37.9%).¹⁵ However, other studies among Taiwan indigenous people have reported a lower seroprevalence of 19.4% to 21.8% in Atayal,¹⁶ 26.7% in Paiwan,¹⁷ and 9.1% in Akka¹⁷ subgroups. In contrast, seroprevalence rates among indigenous people in Brazil¹⁸ and Venezuela^{19–23} have been relatively high: 73.5% and up to 88.0%, respectively. This global status on *T. gondii* infection indicates that, although these minority people stay in different geographical locations, they still experience similar poor sanitary living conditions. Epidemiological surveillances are, therefore, essential to investigate the trend of *T. gondii* infection in these marginalized communities.

Apart from the prevalence of latent/chronic *T. gondii* infection, the present study also found that, by using IgG avidity,

21 (4.2%) samples positive for both IgG and IgM antibodies were also confirmed as recently acquired infection. IgG avidity has recently been tested in a single serum sample and was found to be effective for diagnosis of acute toxoplasmosis in pregnant women and other adults.^{24,25} In this present study, there were no pregnant women involved. Additional study is, therefore, recommended to be carried out on a larger scale, particularly in screening women of reproductive age, to prevent the risk of congenital transmission.

The present study also showed that there are certain factors contributing to *T. gondii* infection in this community. It is interesting to note that a high seroprevalence rate was found among females of this community. There were 33 IgG seropositive and 7 IgG/IgM seropositive in 55 women aged above 12 years. However, there was no significance with gender, suggesting that the rate of infection for *T. gondii* is not sex related or that the differences observed are not crucial. Moreover, it has to be highlighted that activities performed by men and women among these communities are different and that most of them had varying degrees of frequent contact with risk factors. Although there was no pregnant woman who participated in this study, toxoplasmosis is an important parasitic disease, which can be congenitally transmitted to the fetus from a primary infected pregnant mother. Therefore, we recommend additional study to include the investigation of seroprevalence and associated risk factors of toxoplasmosis among pregnant women in these communities. Studies have shown that women are susceptible to acute *T. gondii* infection during the childbearing years and therefore, increasing risk of congenital toxoplasmosis.²⁶

Results also showed that the seroprevalence rate was comparatively higher in those participants above 12 years old compared with those participants below 12 years. In addition, the seroprevalence rate also showed significant heterogeneity

among age groups in which infection rate increases with age. A recent study among Brazilian indigenous groups has also indicated that the frequency of infection with *T. gondii* increases with age.²⁷ This finding is most probably because of the fact that, with an increase of age, the probability that an individual may be associated with at least one of the mechanisms of transmission also increases.^{28,29}

Nonetheless, the high frequency of toxoplasmosis among young age groups suggests that these children have already been exposed to the risk for acquiring the infection. They may acquire infection from unhygienic habits, such as putting contaminated hands into their mouths. During the visit to each village, children were noted playing with soil and sand outside their houses. Because of their young age, many children are still not fully aware of personal hygiene and good cleanliness practices or realize the significance of exposing themselves to pathogenic organisms. This condition is also aggravated by some degree of negligence and lack of parental supervision in personal hygiene and cleanliness, such as in cases where both parents were working and not at home to supervise the children. They were frequently exposed to infection and reinfection because of lack of proper parental guidance. Therefore, educational efforts targeting mothers are needed so that they can, in turn, teach children about cleanliness and personal hygiene.

In the present study, the villagers' most common livelihood is small-scale animal husbandry (e.g., pigs, chickens, and ducks). The villagers also keep dogs, cats, monkeys, rabbits, and birds, and the majority of these domestic animals are left to roam freely. Occasionally, dogs would be observed following their owners into the jungle to hunt. The villagers have very close contact with dogs and cats. These animals were observed sharing food from the same plate with their owners and sleeping on the clothing racks. They defecate outdoors promiscuously, and some cats also defecate indoors, especially near the fire wood in the kitchen area.

This situation is exacerbated by the type of kitchen flooring, which is usually made of mixed soil and sand, providing an ideal condition for the development, survival, and transmission of the *Toxoplasma* oocyst. Some of the domestic and stray cats that live in close proximity to these people may acquire infection while hunting and foraging (especially those cats eating wild prey that act as intermediate hosts, such as mice, rats, and birds). They may also defecate in the kitchen, and they are capable of shedding millions of oocysts in their feces during primary infection, thus spreading oocysts inside the house. These oocysts remain infectious for months in the tropic soil environment.^{30,31} In addition, roaches and flies can also serve as transport hosts, because they have access to cat feces in the soil or kitchen area, which was shown experimentally.³² Nevertheless, these potential transport hosts were not examined in the present study and should be considered in the future study.

Thus, it was not surprising that our results showed that close contact with pets, especially cats, played a significant role in facilitating the transmission of *T. gondii*. It has also been reported in a previous local study that high prevalence of toxoplasmosis is significantly associated with close contact with cats among the Peninsular Malaysia indigenous community.¹² This finding was also consistent with other studies reported from Taiwan¹ and Venezuela.^{21,23} Based on the overall results obtained, these indigenous communities were possibly exposed to the infection through *T. gondii* oocysts excreted

from infected cats that contaminate soil. Therefore, it is imperative to promote awareness of this parasitic infection through educating this community on the importance of collecting cats' feces in litter boxes, discarding them properly, spaying cats to reduce overpopulation, and most importantly, washing hands before eating, after gardening, and after contact with soil.

Water-borne toxoplasmosis associated with drinking water contaminated with *T. gondii* oocysts has since been reported in some large-scale outbreaks.³³⁻⁴⁰ In the present study, using untreated river and mountain water for domestic needs (i.e., drinking, cooking, bathing, and washing clothes) was also identified as a risk factor for acquiring the infection. The majority of the indigenous communities, especially those people living in rural and remote areas, totally rely on rivers located adjacent to the village as their main source of water. A possible source of infection could be the consumption of water contaminated with *T. gondii* oocysts from feces of infected wild or domestic felines, because they may come to the river bank to drink and at the same time, defecate in or near the river. In support of the present study, similar findings were found among indigenous women in Taiwan¹ and in a multiethnic Indian community in Brazil.¹⁸ In addition, from our personal communication with the villagers, drinking of unboiled (especially mountain water) or improperly boiled water is a common practice in this community.

Significant correlation between consumption of unboiled water and the seropositivity of toxoplasmosis has been noted in many studies, particularly among disadvantaged and indigenous communities living in rural and remote areas.^{38,40-42} Therefore, these communities, especially women of childbearing age and particularly pregnant women, should be given health education on the danger of drinking unboiled or improperly boiled water. Given the significant role of water in the transmission of toxoplasmosis as shown in this study, other ways of transmission by contaminated water, such as washing of vegetables with untreated water before eating, should be further investigated in the future, because it has been shown to be a significant risk factor of infection in one recent study.⁴²

The lack of a significant association with eating with hands is rather surprising. However, this finding could be because of the fact that eating with hands is a general practice among these communities: almost all of the participants (98.4%) eat using their hands. In fact, eating with hands without proper washing could be the source of *T. gondii* infection through accidental ingestion of oocysts, especially after contact with contaminated soil or cleaning up cat's feces.

In general, this indigenous population lives near or within the forest, and game hunting is a common practice for the adults in most of these subgroups. Although data on meat consumption were not collected in this study because of the traditional cultural taboo, beliefs, and sensitivity in some subgroups, it is believed that eating raw or undercooked meats, especially hunted wild animals (i.e., wild feline and canine, which also can be definitive and intermediate hosts, respectively for *T. gondii*), is a common practice among these indigenous populations. This practice could be an explanation for the continued exposure to infection because of the higher consumption of infected game meat, particularly among the adults. A previous study among these indigenous communities also reported that adults who were involved in hunting activities were more likely to acquire toxoplasmosis compared with other family members.⁸ Thus, these communities should also be advised on

the importance of eating well-cooked meat, especially hunted wild prey, to avoid acquiring toxoplasmosis from tissue cysts in raw or improperly cooked meat.

In conclusion, this study showed a high seroprevalence rate of *T. gondii* infection among Peninsular Malaysia indigenous people. Increased age, use of untreated river and mountain water for daily chores, and close contact with pets (i.e., cats) were significant risk factors for acquiring *Toxoplasma* infection in these communities. This epidemiological study has provided baseline data for future studies to be carried out among other minority groups living in different parts of Peninsular Malaysia. A health surveillance program on toxoplasmosis and effective preventive measures should be initiated, especially among pregnant women during their antenatal visit, to facilitate early diagnosis and treatment. Educating these communities on the various ways of acquiring toxoplasmosis is also crucial, and they should be provided with the proper knowledge to effectively prevent the transmission of this disease in their communities.

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