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Prevalence and risk factors of *Toxoplasma* infection – an update in Malaysian pregnant women

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Abstract. *Toxoplasma gondii* is a protozoan parasite that is capable of causing a zoonotic disease, known as toxoplasmosis. Vertical transmission of *T. gondii* from the mother to the fetus, during pregnancy may cause severe complications to the developing fetus. This current study aimed to determine the seroprevalence and investigate the associated risk factors of *Toxoplasma* infection in pregnant women (n=219) visiting the antenatal clinic at UMMC. While the elevated level of anti-*Toxoplasma* IgG and IgM antibodies indicates the presence of infection, it fails to differentiate between a past and a recent infection. Thus, the study also demonstrates the usefulness of IgG avidity in validating the timing of infection. The serum samples were tested for the presence of anti-*Toxoplasma* IgG and IgM antibodies by ELISA test, and the seropositive samples for both anti-*Toxoplasma* IgG and IgM antibodies were further evaluated by IgG avidity. The results showed that the overall prevalence of *T. gondii* seropositivity was 34.7%. Of these, 30.6% (67/219) were positive for anti-*Toxoplasma* IgG antibody only, 2.3% (5/219) were positive for anti-*Toxoplasma* IgM only, and the remaining 1.8% (4/219) was positive for both anti-*Toxoplasma* IgG and IgM antibodies. All of the pregnant women who were positive for both anti-*Toxoplasma* IgG and IgM antibody were found to have past infection when evaluated by IgG avidity. In this study, Malay ethnicity and the number of existing previous children were significantly associated with *T. gondii* seropositivity ($p<0.05$). Based on these findings, information and education on the transmission and prevention of congenital toxoplasmosis are very crucial as a public health effort towards a healthier society.

INTRODUCTION

Toxoplasmosis is a world-wide zoonotic disease caused by *Toxoplasma gondii*. Both wild and domestic cats are the only known definitive hosts for *T. gondii*, whereas other warm-blooded animals, including humans are the intermediate hosts (Tenter *et al.*, 2000). Two groups of individuals take high risks for acquisition of *Toxoplasma* infections including pregnant women and immunocompromised subjects. While 90% of the infected mothers are asymptomatic, some may develop symptoms such as low-grade fever, malaise and lymphadenopathy (Paquet *et al.*, 2013). Toxoplasmosis may cause severe complications in immuno-compromised persons such as those who are receiving immunosuppressive remedy and patients with neoplastic diseases. In such

occasions, toxoplasmosis induces severe sequelae including encephalitis, pneumonitis and ocular diseases (Machala *et al*., 2015). The most potential routes of acquiring the infection by humans are ingestion of tissue cyst in raw or undercooked meat, or contact with sand or soil contaminated with oocyst-infected cat feces, or congenitally from the mother to the fetus when pregnant women got the first infection during pregnancy (Elsheikha, 2008; Paquet *et al*., 2013).

Various serologic approaches have been described for the detection of *T. gondii* infection such as dye test (DT), modified agglutination test (MAT), indirect fluorescent antibody test (IFAT), indirect hemagglutination test (IHA), enzyme-linked immunosorbent assay (ELISA) as well as immunosorbent agglutination assay (ISAGA) (Montoya, 2002). Among these, ELISA test is the most widely applied in the setting of clinical (non-reference) laboratory by principally demonstrating the presence of specific antibodies to *T. gondii* (Villard *et al*., 2016). Generally, seropositive for anti-*Toxoplasma* IgM antibody test results do not necessarily signify a recent infection since IgM can be detected for months or even years following the primary infection (Igbal and Khalid, 2007). An avidity test has been widely used to discriminate between recently acquired infections and chronic infections acquired in the distant past, and it is of particular prominence for pregnant mothers (Candolfi *et al.*, 2007). For instance, high avidity test result can be used to rule out maternal *Toxoplasma* infection during the first trimester of gestation. Since the infection was acquired before the time of gestation, the fetus is not at risk of attaining congenital toxoplasmosis (Remington *et al*., 2001). Nonetheless, avidity testing is a part of confirmatory testing and should not be used solely for the diagnosis of *Toxoplasma* infection (Liesenfeld *et al*., 2001; Tanyuksel *et al*., 2004). The serious consequences of motherly *Toxoplasma* infection on unborn babies such as intracranial calcifications, hydrocephalus and hepatosplenomegaly are relied on the duration of conception. The higher risks of transplacental transmission occur during the third trimester, but the severity of developing diseases in the infected fetus is higher earlier in the gestation (Wallon *et al*., 2013). Therefore, the precise confirmation of acute *Toxoplasma* infection in the pregnant mothers is essential to the efficient prevention of maternal to fetal transmission and thus allowing prompt initiation of appropriate therapeutic approach. Although, previous studies on seroprevalence of *Toxoplasma* infection in pregnant women in Malaysia were 49% and 42.5% (Nissapatorn *et al*., 2003; Andiappan *et al*., 2014), the country does not perform maternal serum screening programs for the detection of acute *Toxoplasma* infection in pregnant women. The aim of this study was therefore to determine the seroprevalence of anti-*Toxoplasma* antibodies in infected pregnant women and to identify the risk factors associated with *Toxoplasma* infection. We also confirm the necessary of avidity test in diagnosis of recently acquired *Toxoplasma* infections in the first serum samples with seropositive for anti-*Toxoplasma* IgG and IgM antibodies.

MATERIALS AND METHODS

Study site and sample collection

This cross-sectional study was conducted at the Department of Parasitology, Faculty of Medicine, University of Malaya during October 2016 to March 2017. The inclusion criteria for the study participants were 1) pregnant women with > 18 years old, a random selection approach was used on eligible pregnant women visiting the antenatal clinic (ANC) of University Malaya Medical Centre (UMMC); 2) pregnant women with a gestational age ranging from 5 to 38 weeks. Written informed consent was obtained from all participants before the commencement of this study. Approximately 5 mL of venous blood was drawn from each participant to collect serum samples by centrifuging the whole blood at 2,000 rpm (rotation per minute) for 10 minutes, and stored at -20°C until further serology test. Along with the blood collection, each participant are required to complete a questionnaire form consisting of questions to assess their

demographic profile, their awareness of *Toxoplasma* infection during pregnancy as well as the related risk factors to toxoplasmosis.

Ethical consideration

This study was approved by the ethical review committee of the University of Malaya Medical Centre (Ref No: 20166-2605).

Questionnaires

The interviewer-administered questionnaire was filled up by conducting a structured interview with each consenting pregnant women, from which the following information were obtained: (1) Socio-demographic profiles include age, area of residency, educational level, occupation, gestation period and parity; (2) Awareness of *Toxoplasma* infection during pregnancy; and (3) Related risk factors to toxoplasmosis. The risk factors included were bad obstetrics history, presence of pet cat at home, presence of stray cats around the neighborhood area, consumption of raw or undercooked meat, drinking tap or untreated water and washing hands before and after eating. Bad obstetrics history was defined as a pregnant women having miscarriage, still birth, premature labor, low birth weight, congenital anomalies or prenatal death in their previous pregnancy. The questionnaire was formatted according to literature reviews and was a modified version (Nissapatorn *et al.*, 2011).

Detection of anti-Toxoplasma antibodies and avidity test

All sera were tested for the presence of antibodies against *T. gondii* by using IgG ELISA (Novalisa, Dietzenbach, Germany), and the obtained results were interpreted based on manufacturer's instructions. A result of > 35 IU/mL was interpreted as reactive, a result of 30–35 IU/mL was interpreted as equivocal, and a result of < 30 IU/mL was interpreted as non-reactive. The presence of IgM against *T. gondii* was also determined by using IgM μ-capture ELISA (Novalisa, Dietzenbach, Germany), wherein a result of > 11 NTU was interpreted as positive, a result of 9 to 11 NTU was interpreted as equivocal, and a result of < 9 NTU was interpreted as negative. Seropositive samples for both anti-*Toxoplasma* IgG and IgM antibodies were further measured with *T. gondii* IgG Avidity ELISA (Novalisa, Dietzenbach, Germany), and the results were interpreted based on the manufacturer's instructions. The avidity index (AI) with >40% indicates the presence of high avidity toxoplasmosis antibody which translates to past infection. Contrarily, AI ≤40% indicates the presence of low avidity toxoplasmosis antibody which translates to a more recent infection.

Statistical analysis

The data analysis was conducted using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). The prevalence rate of *Toxoplasma* infection was calculated as the proportion of seropositive samples among all number of samples tested. The Chi-square test was applied to assess the association between the patient's characteristics and *T. gondii* seropositivity. A p value ≤ 0.05 was considered statistically significant.

RESULTS

Seroprevalence of Toxoplasma infection

In this study, 76 out of 219 pregnant women who showed seropositivity for *Toxoplasma* infection represented an overall seroprevalence of 34.7%, as shown in Table 1. Of this, 30.6% were positive for anti-*Toxoplasma* IgG antibody only, which implies that 67 out of 219 pregnant women has been previously infected with *T. gondii* and usually for more than 6 months. While, 2.3% were positive for anti-*Toxoplasma* IgM only which their second serological follow-up were also done three weeks later to repeat testing of rising levels of IgG antibodies. The results showed that no seroconversion of anti-*Toxoplasma* IgG antibodies was detected in these samples, and therefore the false positive IgM reaction has occurred in the initial result. The remaining 1.8% was positive for both anti-*Toxoplasma* IgG and IgM antibodies, which also denotes a possible acute *Toxoplasma* infection within 12 months or a false-positive IgM reaction. All of the four seropositive samples for both anti-*Toxoplasma* specific

Table 1. Seroprevalence of *T. gondii* infection in pregnant women as evaluated by ELISA test

Anti-*Toxoplasma* antibodies	Number of *Toxoplasma* seropositive pregnant (%)	95% CI	Avidity index (%)
IgG	67 (30.6%)	0.24 – 0.37	NT*
IgM	5 (2.3%)	0.02 – 0.10	NT
IgG & IgM	4 (1.8%)	0.00 – 0.40	> 40
Total	76 (34.7%)		

* NT; not tested.

IgG and IgM antibodies have been evaluated by IgG avidity and it was found that all of them showed high avidity with avidity percentage of more than 40%. This indicates that these seropositive pregnant women have past infection.

Sociodemographic profiles and risk factors associated with T. gondii seropositivity

A total of 219 pregnant women recruited in this study had gestation period ranging from 13 to 37 weeks and aged between 22 to 43 years with a mean of 31.1±4.8 years. The majority of the participants were in the age group of 30 to 39 years (52.1%), the Malays (73.5%), lived in the urban areas (62.1%), had tertiary education (70.3%), employed in private sector (41.1%), in their second trimester of pregnancy (52.1%), had one or more child (60.2%) and had no experience of miscarriage or bad obstetrics history (75.8%), as shown in Table 2. Similarly, most of the study subjects (82.6%) were unaware of the existence of *Toxoplasma* infection and did not know that this specified infection is related to cats. A greater number of the pregnant women (85.8%), did not own pet cat at home and never had close contact with the stray cats around their neighborhood areas. Undoubtedly, all of the participants (100%) practice good hand hygiene in terms of washing their hand after gardening, after changing cat litter box, after handling raw meat as well as after eating. Only 8 (3.7%) of them had consumed raw or undercooked meat during the course of pregnancy while 2 (0.9%) of them had drink untreated tap water.

This study revealed that racial group and the number of existing previous children were found to be significantly associated with *T. gondii* seropositivity with p-value 0.026 and 0.020 ($p<0.05$) respectively. A higher seroprevalence was seen among women with one or more child compared to women without children. Other socio-demographic profiles such as age group ($p=0.455$), area of residency ($p=0.382$) and education level ($p=0.262$) were not statistically significant with *Toxoplasma* infection. Similarly, risk associations of trimester of pregnancy ($p=0.442$), bad obstetrics history ($p=0.621$), knowledge on toxoplasmosis ($p=0.711$), cat ownership ($p=0.223$), and consumption of undercooked meat ($p=0.267$) and untreated tap water ($p=1.000$) were not significant with the presence of *Toxoplasma* infection ($p > 0.05$).

DISCUSSION

This study found that the seroprevalence of *Toxoplasma* infection in these pregnant women was 34.7%, and that the racial group and the number of existing previous children were significantly associated with *Toxoplasma* infection. The seroprevalence from this study is however considerably low when compared to the previous studies showed 42.5% and 49% seroprevalence, respectively (Nissapatorn *et al.*, 2003; Andiappan *et al.*, 2014). Among the Southeast Asian countries, high seroprevalence with more than 60% were reported in Indonesia (Terazawa *et al.*, 2003) but significantly lower rates were observed in the Philippines (Salibay, 2008), Thailand (Naissapatorn *et al.*, 2011) and Vietnam (Buchy *et al.*, 2003) with seroprevalence ranging from 23.8%, 28.3% and 11.2%, respectively. Likewise, few studies from the East Asia countries showed

Table 2. Sociodemographic profiles of the pregnant women and risk factors associated with *Toxoplasma* seropositivity

Patient's characteristics	N = 219 Total (%)	*Toxoplasma* seropositivity = 76 (34.7%)	P-value
Age group			0.455
20 – 29	94 (42.9)	32 (34.0)	
30 – 39	114 (52.1)	42 (36.8)	
≥ 40	11 (5.0)	2 (18.2)	
Race			0.026*
Malay	161 (73.5)	65 (40.4)	
Chinese	28 (12.8)	5 (17.9)	
Indian	27 (12.3)	6 (22.2)	
Others**	3 (1.4)	0 (0)	
Area of residency			0.382
Urban	136 (62.1)	44 (32.4)	
Suburban	83 (37.9)	32 (38.6)	
Education			0.262
Primary	1 (0.5)	0 (0)	
Secondary	64 (29.2)	27 (42.2)	
Tertiary	154 (70.3)	49 (31.8)	
Occupation			0.247
Public	69 (31.5)	21 (30.4)	
Private	90 (41.1)	28 (31.1)	
Statutory body	31 (14.2)	13 (41.9)	
Unemployed	29 (13.2)	14 (48.3)	
Trimester of pregnancy			0.442
First	17 (7.8)	4 (23.5)	
Second	114 (52.1)	38 (33.3)	
Third	88 (40.2)	34 (38.6)	
Parity			0.020*
None	87 (39.7)	25 (28.7)	
One	71 (32.4)	21 (29.6)	
Two or more	61 (27.9)	30 (49.2)	
Miscarriage			0.621
Yes	53 (24.2)	20 (37.7)	
No	166 (75.8)	56 (33.7)	
Awareness			0.711
Yes	38 (17.4)	12 (31.6)	
No	181 (82.6)	64 (35.4)	
Cat ownership			0.223
Yes	31 (14.2)	14 (45.2)	
No	188 (85.8)	62 (33.0)	
Hygienic practice			–
Yes	219 (100)	76 (34.7)	
No	0 (0)	0 (0)	
Undercooked meat			0.267
Yes	8 (3.7)	1 (12.5)	
No	211 (96.3)	75 (35.6)	
Untreated tap water			1.000
Yes	2 (0.9)	1 (50.0)	
No	217 (99.1)	75 (34.6)	

* Significant difference p value < 0.05.
**Others are indigenous minorities.

low seroprevalence with *T. gondii* ranging from 0.79% in South Korea (Song *et al.*, 2005) to 10.3% in Japan (Sakikawa *et al.*, 2012). Nevertheless, the seroprevalence of *Toxoplasma* infection varies in different parts of the world such as 17.3% in London (Flatt & Shetty, 2013), 24.1% in Saudi Arabia (Aqeely *et al.*, 2014), 30.9% in Tanzania (Mwambe *et al.*, 2013), 68.4% in Brazil (Gontijo da Silva *et al.*, 2015), and 80.3% in Democratic Republic of Congo (Doudou *et al.*, 2014). These differences might be due to various factors such as geographical location and distribution, standard of living, risk factors and serological diagnostic methods.

In our study, the racial group showed significant association with *T. gondii* seropositivity with $p<0.05$. Given a fact that Malays are the major ethnic group in Malaysia, a higher seroprevalence with *T. gondii* was observed among Malays when compared to others. The Malays were known to keep cats as pet at home and a large proportion of them also live in close association with the stray cats around their neighborhood areas. Although this significant level may get influenced from the vast difference in sample size between four ethnic groups, the significant association has been documented in other previous studies (Cheah *et al.*, 1975; Nissapatorn *et al.*, 2003). Also, it was observed that the significant association was found with the parity or the number of existing previous children with *p*-value 0.02 ($p < 0.05$), whereby a greater seroprevalence was seen among women with one or more child compared to women with no child. Possible explanation is that the mother with children tends to have more outdoor activities in terms of playing with their kids in the sand or accidentally eating contaminated food or undercooked meat. Maternal age may serve as a confounding factor since increasing parity is usually correlated with increasing age. Our finding is in consistent with related studies from Sweden (Jenum *et al.*, 1998) and Norway (Birgisdottir *et al.*, 2006) also reported proportional correlation between the number of children and *T. gondii* seropositivity.

This study showed that the seroprevalence of *Toxoplasma* infection was higher among pregnant women within the age-group of 30 to 39 years (36.8%), owing to the fact that individuals are more exposed to *Toxoplasma* infection as they get older. However, this correlation is not statistically significant with *T. gondii* seropositivity ($p>0.05$). There was also a positive trend between maternal age and the presence of anti-*Toxoplasma* antibodies in a study in Turkey, whereby the prevalence was higher (55.1%) in the age-group 30 to 40 years (Ertug *et al.*, 2005). Despite the fact that felines are the only known definitive hosts for *T. gondii*, this study did not find any significant association between cat ownership and *T. gondii* seropositivity. This finding is in agreement with studies established in London (Flatt & Shetty, 2013) and Tanzania (Mwambe *et al.*, 2013), by which they observed that contact with the infected cats is not a significant source of the infection with *T. gondii*. According to a previous study demonstrated that cat ownership is not a consistent risk factor to toxoplasmosis, but the likely mechanism of transmission is the exposure to feces from cat that is shedding oocysts (Jones *et al.*, 2003). Furthermore, domestic cats that are kept indoor and are not fed raw or undercooked meat are unlikely to obtain *Toxoplasma* infection and thus do not contribute to zoonotic transmission of toxoplasmosis. Again, the risk of infection is not related to direct contact with the cats, but more on the ways of handling the contaminated cat litter trays. Yet, a sero-epidemiological study in Ghana reported a significant association between direct contact with cats and increased *T. gondii* seropositivity (Ayi *et al.*, 2009).

In addition, significant association of *T. gondii* seropositivity with eating undercooked meat, drinking untreated tap water and poor hygienic practice were not established in this study. Supporting to this, a previous study on the seroprevalence with *Toxoplasma* infection among African pregnant women reported similar results, whereby no significant correlation was found between *Toxoplasma* infection and consumption of contaminated undercooked meat (Frimpong *et al.*, 2017). Yet, another studies showed a contrasting results, having

found significant association between *T. gondii* seropositivity and undercooked meat consumption (Kolbekova *et al.*, 2007; Elsheikha *et al.*, 2009). The differences in the finding could be due to variations in human preferences and local cultures, which at the same time affect the types of meat being consumed as well as different circumstances under which the meat is handled.

Anti-*Toxoplasma* specific IgG and IgM antibodies were positive in 67/219 cases (30.6%) and 5/219 (2.3%), respectively. Four pregnant women (1.8%) were positive for both anti-*Toxoplasma* IgG and IgM antibodies. A negative IgM with a positive IgG indicates the infection acquired in the distant past. In a different scenario, a positive IgM result may point out more recent infection or a possible false-positive reaction. The presence of elevated levels of anti-*Toxoplasma* antibodies implies that the infection has occurred but does not distinguish between recent and past infection. Hence, IgG avidity is applied to validate the timing of infection. Initially, following primary infection, the functional affinity of specific IgG antibodies is low and it rises over the succeeding weeks and months due to antigen-driven B cell selection. Therefore, a low IgG avidity result indicates a recent infection, whereas a high avidity indicates a past infection. The seropositive samples for both anti-*Toxoplasma* specific IgG and IgM antibodies were then evaluated by IgG avidity, and it was fortunately found that all of them had past infections.

Concerning the limitations of this project, the *Toxoplasma* seroprevalence established in this study cannot be used to represent the entire population of pregnant women in Kuala Lumpur, Malaysia due to the small sample size obtained. Likewise, the sample size of the pregnant women who were in their first trimester of pregnancy is too small in comparison to those who were in their second and third trimesters. This shortcoming was then shown against the proper risk associations between trimester of pregnancy and *T. gondii* seropositivity. Furthermore, only a single serum sample was obtained per study subjects and this circumstance might complicate the process for testing seroconversion and thus the inability to rule out false positive IgM reaction. In spite of the absence of infection, false positive reaction could arise when the host's natural IgM antibody reacts with *T. gondii* antigen.

CONCLUSION

From this study, it was found that the seroprevalence of *Toxoplasma* infection in pregnant women who were visiting the antenatal clinic at UMMC was 34.7%, and that the racial group and the number of existing previous children were significantly associated with *Toxoplasma* infection. Screening for anti-*Toxoplasma* specific IgG and IgM antibodies in the first trimester of all antenatal women is strongly recommended for a monitoring and preventive purpose. In pregnant women who are negative for both antibodies, an approach towards education regarding the transmission and prevention of toxoplasmosis is strongly recommended. While, the evaluation of IgG avidity on pregnant women who are positive for both anti-*Toxoplasma* IgG and IgM antibodies will essentially help to rule out recent infection and thus prevent unnecessary follow-up tests and treatment.

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Conflict of interest

The authors declare that they have no conflict of interests.

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