Knowledge and Preventive Behaviour among Pregnant Women with Latent Toxoplasmosis in Malaysia

AHMAD N1, OSMAN E2, ABDUL GHANI NA3, LEONG WY2, ARZAEE MZ2, SEOW YY2, DOL BAHAR S2, WAN YAHYA WNN2, MUSTAPHA N2, VADIVEEL Y2

1Department of Community Health, 2Department of Parasitology & Medical Entomology, 3Department of Obstetrics and Gynaecology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia

ABSTRAK

Toxoplasmosis pendam dapat menyebabkan pelbagai gangguan hormon dan tingkah laku dalam hos terjangkit. Kami berhasrat untuk mengkaji sero-prevalensi Toxoplasma gondii (T. gondii) pendam serta hubungan antara jangkitan dengan pengetahuan dan tingkah laku dalam kalangan 400 ibu hamil. Sampel plasma diuji untuk kehadiran antibodi IgG T. gondii dan soal selidik berstruktur digunakan untuk merekodkan ciri-ciri sosio-demografi responden, maklumat umum dan pengetahuan mengenai faktor risiko, gejala, masa jangkitan, pengetahuan pencegahan serta tingkah laku pencegahan toxoplasmosis. Sero-prevalensi toxoplasmosis pendam dalam wanita hamil adalah 31.8%. Kajian menunjukkan, 69.5% daripada mereka mempunyai kurang pengetahuan mengenai toxoplasmosis. Walau bagaimanapun, majoritinya (99.8%) mengamalkan tingkah laku pencegahan. Analisis regresi logistik berganda menunjukkan wanita hamil dengan tahap pendidikan rendah mempunyai hampir dua kali lebih risiko (nisbah ods terlaras: 1.91, 95% SK 1.18, 3.10; p = 0.008) untuk T. gondii IgG seropositif. Wanita hamil yang mempunyai sejarah perubatan lalu mempunyai dua kali lebih kemungkinan (nisbah ods terlaras: 2.32, 95% SK 1.32, 4.06; p = 0.003) untuk T. gondii IgG seropositif. Selain itu, wanita yang tidak pasti mengenai mod penyebaran penyakit melalui pemindahan darah mempunyai empat kali lebih ods (nisbah ods terlaras: 3.93, 95% SK 1.54, 10.01; p = 0.004) untuk sero-prevalensi kronik toxoplasmosis. Wanita yang tidak pasti mengenai keperluan menghindari kucing liar mempunyai nisbah ods terlaras: 0.42 (95% SK 0.24, 0.71, p = 0.001) untuk sero-prevalensi kronik toxoplasmosis. Penterjemahan pengetahuan tentang toxoplasmosis kepada amalan tingkah laku pencegahan...
Latent toxoplasmosis could induce various hormonal and behavioural perturbations in infected hosts. We aimed to study the latent seroprevalence of *Toxoplasma gondii* (*T. gondii*) and the relationship between infection, knowledge and behaviour among 400 pregnant mothers. Plasma samples were tested for the presence of *T. gondii* IgG antibodies while a structured questionnaire was used to record respondents' socio-demographic characteristics, general information and knowledge on plausible risk factors, symptoms, timing of infection, and preventive knowledge and behaviour regarding toxoplasmosis. The seroprevalence of latent toxoplasmosis among respondents was at 31.8%. This study indicated that 69.5% of them had poor knowledge of toxoplasmosis but most of them (99.8%) practised preventive behaviours. Multiple logistic regression analysis showed that pregnant women with low education levels (aOR: 1.91, 95% CI 1.18, 3.10; \( p = 0.008 \)) and past medical history (aOR: 2.32, 95% CI 1.32, 4.06; \( p = 0.003 \)) were both twice as likely to have anti-*T. gondii* IgG seropositivity. Besides, women who were unsure regarding the transmission mode of the disease via blood transfusion were four times more likely (aOR: 3.93, 95% CI 1.54, 10.01; \( p = 0.004 \)) to have chronic toxoplasmosis seroprevalence. Women who were unsure regarding the necessities of avoiding stray cats had aOR of 0.42 (95% CI 0.24, 0.71, \( p = 0.001 \)) for chronic toxoplasmosis seroprevalence. Translating the knowledge on toxoplasmosis into the practice of preventive behaviour via a health education programme is crucial in reducing the risk of disease transmission especially among pregnant women.

**Keywords:** attitude, knowledge, practice, pregnant mother, seroprevalence, toxoplasmosis, *Toxoplasma gondii*

**INTRODUCTION**

Toxoplasmosis is a parasitic zoonosis caused by *Toxoplasma gondii* (*T. gondii*). It has been classified as one of the five Neglected Parasitic Infections (NPI) targeted by the Centres for Disease Control and Prevention in the United States (CDC 2018). Infection with *T. gondii* is acquired by ingestion of raw or undercooked meat which contains viable tissue cysts or by cats that excrete oocyst which could contaminate food or water (Montoya & Liesenfeld 2004). Once infected, *Toxoplasma* develops into tachyzoites.
(active multiplication form) or remains dormant as bradyzoites (in tissue cysts), depending on the host immune status (John & Petri 2006). Besides, the parasite also develops into a tachyzoite form and causes infection in humans upon consumption of the infected animal tissue.

The seroprevalence of *T. gondii* ranges widely, depending on the region and population. Most countries in South America, the Middle East and other low-income countries have recorded high seropositivity for *Toxoplasma* infection from both normal or immunocompromised host. This includes 59% in Brazil (Ferezin et al. 2013), 84.7% in Congo (Doudou et al. 2014), 83.6% in Ethiopia (Zemene et al. 2012), 75% in India (Chintapalli & Padmaja 2013), and 30.9% in Tanzania (Mwambe et al. 2013). High- and middle-income countries such as Japan, Taiwan and China have a lower incidence as compared to the above-mentioned nations with seroprevalence of 10.3%, 11.8% and 3.98%, respectively (Sakikawa et al. 2012; Chou et al. 2011; Hua et al. 2013). Meanwhile, the seroprevalence rate of *Toxoplasma* infections among pregnant women in South East Asia was reported as 35.2% in Malaysia (Emelia et al. 2014), 23.8% in the Philippines (Salibay et al. 2008) and 28.3% in Thailand (Nissapatorn et al. 2011). In immunocompetent pregnant women, a significant risk of vertical transmission is caused by primary *T. gondii* infection (Remington et al. 2001). Nevertheless, congenital toxoplasmosis resulting from chronically infected immunocompetent pregnant women was attributed to either reinfection or reactivation, which has also been reported previously (Andrade et al. 2010). The strength and profile of the mother’s immune response were suggested as important factors influencing vertical transmission rate and severity of clinical outcome in the congenitally infected foetus (Smereka et al. 2018).

*T. gondii* infection acquired during gestation is preventable through simple precautions taken during pregnancy. Creating awareness would be an appropriate strategy in preventing and reducing the risk of toxoplasmosis exposure in pregnant women. Thus, it is critical to determine their knowledge about toxoplasmosis and the list of potential practices that could prevent infection. Currently, local data available on knowledge and preventive behaviour are scarce. The incidence of toxoplasmosis and its high prevalence in pregnant women in Malaysia has sparked an interest in the current topic. The present study aimed to evaluate the association between the seroprevalence of anti-*Toxoplasma* IgG antibodies as well as the knowledge, preventive behaviours and other recognised risk factors among pregnant mothers.

**MATERIALS AND METHODS**

**Study Design and Data Collection**

A cross-sectional study was conducted at the Antenatal Clinic (ANC) and Antenatal Ward of the Department of Obstetrics and Gynaecology in Universiti Kebangsaan Malaysia.
Medical Centre, Kuala Lumpur, Malaysia. The study was conducted from February until May 2015. A total of 400 pregnant women were enrolled in our study through convenience sampling. Inclusion criteria were: Malaysians, aged ≥ 18 years and above, at any gestational period regardless of parity, able to understand and answer the questionnaires, mentally and physically healthy and could provide written consent. Pregnant women who were in acute emergencies such as eclampsia, cord prolapse and placental abruption were excluded from the study.

Study Tools

All data from the pregnant women were collected using a questionnaire which was divided into two sections. In section 1, the respondents were assessed on sociodemographic characteristics such as age, race, marital status, parity, gestational period, occupation, education level, household income, past medical history and living area. In section 2, knowledge on toxoplasmosis infection was measured using 28 items which included four domains; i) general information and knowledge on toxoplasmosis (six items); ii) risk factors (five items); iii) symptoms and timing of infection (ten items) and iv) preventive knowledge (seven items). Meanwhile, the preventive behaviours were measured by nine items. The knowledge and preventive behaviour against toxoplasmosis infection questionnaire were adapted from previous studies (Jones et al. 2003; Andiappan et al. 2014).

With regard to knowledge on toxoplasmosis and preventive behaviours, each item had a choice of either ‘Yes’ (2 points), ‘Not sure’ (1 point) or ‘No’ (0 point). We considered the mothers who were ‘Not sure’ of certain items to score higher compared to mothers with a firm ‘No’ answer. This is because the pregnant women who chose the ‘not sure’ option might acquire the correct knowledge to a certain extent or preventive behaviours as compared to the mothers who selects ‘No’ who may not be having any preventive behaviours at all.

A total score of 50% (median of total score) was used as a cut-off point during the evaluation of overall scores. For the assessment of knowledge on toxoplasmosis, women who scored less than 28 were considered as having poor knowledge and those who scored 28 and above were considered as having good knowledge. Besides that, in the assessment of preventive behaviour against toxoplasmosis, women who scored less than 9 were considered as not practising preventive behaviour, whereas those who scored 9 and above were considered to be practising preventive behaviours. For the multivariable analysis, each item in this questionnaire was analysed individually in association with anti-Toxoplasma IgG antibodies seropositivity.

Collection and Serological Analysis of Blood Samples

Approximately 3 ml of blood samples were collected from each pregnant
| Variables                      | Category                    | Positive n (%) | Negative n (%) |
|-------------------------------|-----------------------------|----------------|----------------|
| Age (years)                   | 20-29                       | 30 (24.2)      | 94 (75.8)      |
|                               | 30-39                       | 91 (35.3)      | 167 (64.7)     |
|                               | ≥40                         | 6 (33.3)       | 12 (66.7)      |
| Race                          | Malay                       | 110 (34.9)     | 205 (65.1)     |
|                               | Chinese                     | 10 (19.6)      | 41 (80.4)      |
|                               | Indian                      | 2 (7.7)        | 24 (92.3)      |
|                               | Others                      | 5 (62.5)       | 3 (37.5)       |
| Occupational Status           | Government Employee        | 50 (41.0)      | 72 (59.0)      |
|                               | Semi Government Employee   | 12 (19.0)      | 51 (81.0)      |
|                               | Private Employee            | 34 (27.0)      | 92 (73.0)      |
|                               | Self Employed              | 23 (34.3)      | 44 (65.7)      |
|                               | Unpaid Worker              | 8 (36.4)       | 14 (63.6)      |
| Level of Education            | Low education               | 56 (37.6)      | 93 (62.4)      |
|                               | High education              | 71 (28.3)      | 180 (71.7)     |
| Marital Status                | Married                     | 126 (31.6)     | 273 (68.4)     |
|                               | Un-married                  | 1 (100.0)      | 0              |
| Household Income (MYR) Median (IQR): | Low (<RM1500)              | 6 (46.2)       | 7 (53.8)       |
|                               | Middle (RM1500- RM8000)    | 112 (31.1)     | 248 (68.9)     |
|                               | High (≥RM 8000)             | 9 (33.3)       | 18 (66.7)      |
| Living Area                   | Urban                       | 118 (31.6)     | 256 (68.4)     |
|                               | Rural                       | 1 (25)         | 3 (75)         |
|                               | Sub urban                   | 8 (36.4)       | 14 (63.2)      |
| Medical history               | Hypertension                | 4 (36.4)       | 7 (63.6)       |
|                               | Diabetes                    | 14 (51.9)      | 13 (48.1)      |
|                               | Thyrotoxicosis              | 2 (28.6)       | 5 (71.4)       |
|                               | Cardiac                     | 0              | 1 (100)        |
|                               | Others                      | 12 (46.2)      | 14 (53.8)      |
|                               | None                        | 95 (31.8)      | 273 (68.2)     |
| Parity                        | 0                           | 35 (26.7)      | 96 (73.3)      |
|                               | 1                           | 27 (23.9)      | 86 (76.1)      |
|                               | >1                          | 65 (41.7)      | 91 (58.3)      |
| Gestational Period (weeks)    | <16 weeks                   | 20 (36.4)      | 35 (63.6)      |
|                               | 16-27 weeks                 | 38 (36.9)      | 65 (63.1)      |
|                               | ≥28 weeks                   | 69 (28.5)      | 173 (71.5)     |
| Soil Contact                  | Yes                         | 1 (12.5)       | 7 (87.5)       |
|                               | No                          | 126 (32.1)     | 266 (67.9)     |
woman by venepuncture under sterile conditions. The collected samples were centrifuged at 3000 g for 15 minutes to obtain the plasma and stored at a temperature of -20°C before further serological test. The serum samples were tested using the Platelia Toxo IgG commercial ELISA kit (BioRad, USA), following the manufacturer’s instructions. Samples with anti-Toxoplasma IgG antibodies were grouped into three categories based on the titre value: negative (<6 IU/ml), positive (≥9 IU/ml) and equivocal (9 IU/ml <titre ≤6 IU/ml).

Ethical Aspect

Ethical approval for the study was obtained from the Institutional Research Ethics Committee. Written consent was also obtained from each participating pregnant woman before engaging in the study.

Statistical Analysis

Categorical data were presented in frequency (n) and percentage (%). The normally distributed continuous data were presented as mean and standard deviation (sd). The association between the independent factors and anti-Toxoplasma IgG antibody seropositivity was analysed using simple logistic regression analysis to obtain the crude odds ratio (cOR) and its 95% confidence interval (CI). Independent variables with p<0.25 (Bursac et al. 2008) were used in the adjusted analyses to obtain the adjusted OR (aOR). A few variables were recategorised for multiple logistic regression analysis. Statistical analyses were performed using Statistical Package for Social Science (SPSS) version 22.0.

RESULTS

The overall seroprevalence of anti-Toxoplasma IgG antibodies of these pregnant women ranging from different age group was 31.83% (127/400). Table 1 shows that the prevalence of anti-Toxoplasma IgG antibody was higher in Malays (34.9%), government employee (41.0%), and pregnant women with low education (37.6%) and low household income (46.2%). The anti-Toxoplasma IgG antibodies seropositivity prevalence were 36.4% for women from the sub-urban living area, 41.7% among those with

| Variable                          | Category | n (%)  |
|-----------------------------------|----------|--------|
| Knowledge on toxoplasmosis mean score: 25.22 (sd 24.0) | Good     | 278 (69.5) |
|                                   | Poor     | 122 (30.5)   |
| Practised preventive Behaviour mean score: 14.88 (sd 2.16) | Yes      | 399 (99.8)   |
|                                   | No       | 1 (0.2)      |

Note:
Knowledge: good: ≥28, poor: <28.
Practised preventive Behaviour: Yes: ≥9, No: <9.
| Items                                                                 | Yes         | Not Sure    | No          |
|---------------------------------------------------------------------|-------------|-------------|-------------|
| **Knowledge:**                                                      |             |             |             |
| General information and knowledge                                   |             |             |             |
| Have you ever read, heard or seen any information about toxoplasmosis? | 162 (40.5)  | 192 (48.0)  | 46 (11.5)   |
| Have you ever been tested for toxoplasmosis?                        | 10 (2.5)    | 372 (93.0)  | 18 (4.5)    |
| Is toxoplasmosis caused by an infection?                             | 125 (31.3)  | 226 (56.5)  | 49 (12.3)   |
| Is toxoplasmosis caused by a poison?                                 | 129 (32.3)  | 254 (63.5)  | 17 (4.3)    |
| Is *T. gondii* shed in the faeces of infected cats?                  | 121 (30.3)  | 257 (64.3)  | 22 (5.5)    |
| Is *T. gondii* sometimes found in raw or undercooked meat?          | 59 (14.8)   | 297 (74.3)  | 44 (11.0)   |
| **Risk factors**                                                     |             |             |             |
| Can people get toxoplasmosis by changing cat litter?                | 103 (25.8)  | 254 (63.5)  | 43 (10.8)   |
| Can people get toxoplasmosis by eating undercooked meat?            | 66 (16.5)   | 272 (68.0)  | 62 (15.5)   |
| Can people get toxoplasmosis by receiving blood transfusion?        | 41 (10.3)   | 279 (69.8)  | 80 (20.0)   |
| Can people get toxoplasmosis by drinking untreated water, e.g., rain, tap, or unboiled water? | 53 (13.3)   | 267 (66.8)  | 80 (20.0)   |
| Can people get toxoplasmosis by gardening without gloves?           | 112 (28.0)  | 240 (60.0)  | 48 (12.0)   |
| **Symptoms and timing of infection**                                 |             |             |             |
| Can pregnant women develop serious complications after infection with toxoplasmosis (*T. gondii*)? | 160 (40.0)  | 225 (56.3)  | 15 (3.8)    |
| Can unborn and/or newborn children develop serious complications after infection with toxoplasmosis (*T. gondii*)? | 156 (3.90)  | 232 (58.0)  | 12 (3.0)    |
| Can toxoplasmosis in a pregnant woman cause fever and feeling like you have ‘flu’? | 105 (26.3)  | 277 (69.3)  | 18 (4.5)    |
| Can toxoplasmosis in a pregnant woman cause swollen glands (lymph nodes)? | 48 (12.0)   | 341 (85.3)  | 11 (2.8)    |
| Can toxoplasmosis in a pregnant woman cause no symptoms?            | 44 (11.0)   | 314 (78.5)  | 42 (10.5)   |
| Toxoplasmosis (*T. gondii*) can only be passed from a pregnant woman to her foetus if she is newly infected during that pregnancy. | 98 (24.5)   | 282 (70.5)  | 20 (5.0)    |
| Toxoplasmosis (*T. gondii*) is rarely passed from a pregnant woman to her fetus if she was infected before becoming pregnant. | 30 (7.5)    | 340 (85.0)  | 30 (7.5)    |
| A baby with toxoplasmosis may have no signs of illness at birth but develops illness later. | 57 (14.3)   | 323 (80.8)  | 20 (5.0)    |
| A baby with toxoplasmosis may have vision problems.                 | 43 (10.8)   | 340 (85.0)  | 17 (4.3)    |
| A baby with toxoplasmosis may be treated with medicine.             | 70 (17.5)   | 313 (78.3)  | 17 (4.3)    |
pregnant women chose the ‘not sure’ option for 27 items on knowledge regarding toxoplasmosis with these women practising preventive behaviours including routine washing of hands after gardening, handling raw meat and raw vegetables, as depicted by Table 3.

Simple logistic regression analysis (Table 4) indicated that five socio-demographic characteristics, five items regarding knowledge on toxoplasmosis and four items regarding preventive

| Preventive knowledge                                                                | Yes (%) | No (%) | Don’t Know (%) |
|--------------------------------------------------------------------------------------|---------|--------|---------------|
| Is feeding your cat dry or commercial cat food and not letting it kill and eat rodents can prevent from getting Toxoplasmosis. | 70 (17.5) 272 (68.0) 58 (14.5) |
| Is avoiding stray cats can prevent pregnant mother from getting Toxoplasmosis?       | 124 (31.0) 240 (60.0) 36 (9.0) |
| Is letting someone else change the cat’s litter box can prevent pregnant mother from getting Toxoplasmosis? | 71 (17.8) 251 (62.8) 78 (19.5) |
| Toxoplasmosis can be prevented by making sure the cat’s litter box is changed daily. | 183 (45.8) 206 (51.5) 11 (2.8) |
| Toxoplasmosis can be prevented by cooking meat well until no pink is seen and the juices run clear. | 124 (31.0) 254 (63.5) 22 (5.5) |
| Toxoplasmosis can be prevented by thoroughly washing and/or peeling all fruits and vegetables before eating them. | 135 (33.8) 238 (59.5) 27 (6.8) |
| Toxoplasmosis can be prevented by cleaning all cutting boards and utensils thoroughly after each use. | 159 (39.8) 18 (4.5) 223 (55.8) |

| Preventive Behaviour                                                              | Yes (%) | No (%) | Don’t Know (%) |
|------------------------------------------------------------------------------------|---------|--------|---------------|
| Do you do gardening without glove?                                                | 107 (26.8) 139 (34.8) 154 (38.5) |
| Do you routinely wash your hands after gardening?                                 | 267 (66.8) 126 (31.5) 7 (1.8) |
| Do you do change cat litter without glove?                                        | 44 (11.0) 212 (53.0) 144 (36.0) |
| Do you routinely wash your hands after changing cat litter?                       | 180 (45.0) 209 (52.3) 11 (2.8) |
| Do you routinely wash your hands after handling raw meat?                         | 355 (88.8) 37 (9.3) 8 (2.0) |
| Do you eat raw meat?                                                              | 21 (5.3) 40 (10.0) 339 (84.8) |
| Do you routinely wash your hands after handling raw vegetables?                   | 370 (92.5) 19 (4.8) 11 (2.8) |
| Do you eat unwashed raw vegetables?                                               | 1 (0.3) 18 (4.5) 381 (95.3) |
| Do you eat unwashed fruits?                                                        | 6 (1.5) 18 (4.5) 376 (94.0) |

higher parity (>1) and 32.1% among participants with no soil contact.

Table 2 shows the total means score and proportion of knowledge regarding toxoplasmosis and preventive behaviour practices. A total of 69.5% of these women had poor knowledge regarding toxoplasmosis with the mean total knowledge score of 25.22 (sd 24.0). Meanwhile, the proportion of good preventive behaviour was high (99.8%) with a total mean score of 14.88 (sd 2.16). A majority of the pregnant women chose the ‘not sure’ option for 27 items on knowledge regarding toxoplasmosis with these women practising preventive behaviours including routine washing of hands after gardening, handling raw meat and raw vegetables, as depicted by Table 3.

Simple logistic regression analysis (Table 4) indicated that five socio-demographic characteristics, five items regarding knowledge on toxoplasmosis and four items regarding preventive
behaviour had \( p \)-value <0.25. Adjusted analysis showed that pregnant women had twice the risk for anti-\textit{Toxoplasma} IgG antibodies seropositivity, specifically those with low education [aOR: 1.91 (95% CI: 1.18, 3.10), \( p = 0.008 \)] and those with a past medical history [aOR: 2.32 (95% CI: 1.32, 4.06), \( p = 0.003 \)]. For the knowledge on risk factor for toxoplasmosis, pregnant women who were not sure regarding the risk of acquiring the disease through blood transfusion had fourfold higher risk [aOR: 3.93 (95% CI: 1.54, 10.01), \( p = 0.004 \)] for anti-\textit{Toxoplasma} IgG antibodies seropositivity as compared to women who were aware. Besides, women who were not sure regarding the need to avoid stray cats had aOR of 0.42 (95% CI: 0.24, 0.71), \( p = 0.001 \) for anti-\textit{Toxoplasma} IgG antibodies seropositivity compared to those who responded yes.

**DISCUSSION**

The congenital transmission of \textit{T. gondii} has long been considered to occur only if the mother is primarily infected during pregnancy. However, in other reported instances of vertical transmission to the fetus, toxoplasmosis can be reactivated in chronically-infected mothers while in a transiently immunosuppressed state (Desmonts et al. 2004; Wong & Remington 1994). Furthermore, the reactivation of latent infection could also be induced by pregnancy-associated changes in cellular immunity during pregnancy (Kodjikian et al. 2004; Szekeres-Bartho 2002). Therefore, exposure to toxoplasmosis and awareness of its related preventive behaviour is crucial in an attempt to reduce the infection rate, especially in pregnant women. The current study indicated that the seroprevalence of chronic toxoplasmosis among Malaysian pregnant women was 31.83%. This finding was consistent with the previous toxoplasmosis seroprevalence rate of 33.5% among Malaysian pregnant women, as reported by Emelia et al (2014). The percentage of anti-\textit{Toxoplasma} IgG antibody seropositivity was also reported in other Asian countries including Thailand (28.3%), Philippines (23.8%), Pakistan (24.8%), China (7.01%) and Japan (10.3%) (Salibay et al. 2008; Nissapatorn et al. 2011; Sadiqui et al. 2019; Duan et al. 2012). However, these percentage rates of incidence were lower than the seroprevalence reported in Malaysia. The variation in seroprevalence of toxoplasmosis among the countries or different ethnicities in a country could be due to factors such as dietary habits, socioeconomic status, cultural habits, quality of water, and sanitation coverage (Fan et al. 2012; Pappas et al. 2009). For instance, the high seroprevalence among pregnant women in Malaysia may be attributable to the cultural etiquette among Malaysians, which includes the practice of eating with hands. Thus, failing to maintain hand hygiene may pose a source of infection especially after contact with soil or cat faeces (Yan et al. 2018).

The present study showed that the majority of pregnant women had poor knowledge of toxoplasmosis. On the contrary, a study reported that almost
### Table 4: Socio-demographic, knowledge and preventive behaviour regarding toxoplasmosis and association with anti-Toxoplasma IgG antibodies seropositivity

| Variable                  | Category | Seropositivity | Seropositivity | cOR (95% CI) | p value | aOR (95% CI) | p value |
|---------------------------|----------|----------------|----------------|--------------|---------|--------------|---------|
| **Socio-demographic**     |          |                |                |              |         |              |         |
| Age group                 | ≥ 35 years | 41 (38.0)      | 67 (62.0)      | 1            |         |              |         |
|                           | < 35 years | 86 (29.5)      | 206 (70.5)     | 0.68 (0.43, 1.08) | 0.105  | -            | -       |
| Race                      | Non-Malay | 17 (20.0)      | 68 (80.0)      | 1            |         |              |         |
|                           | Malay     | 110 (34.9)     | 205 (65.1)     | 2.15 (1.20, 3.83) | 0.010  | -            | -       |
| Level of Education        | High      | 71 (28.3)      | 180 (71.7)     | 1            | 1       |              |         |
|                           | Low       | 56 (37.6)      | 93 (62.4)      | 1.53 (0.99, 2.35) | 0.054  | 1.91 (1.18, 3.10) | - |
| Past medical history      | No        | 95 (29.0)      | 233 (71.0)     | 1            | 1       |              |         |
|                           | Yes       | 32 (44.4)      | 40 (55.6)      | 1.96 (1.16, 3.31) | 0.011  | 2.32 (1.32, 4.06) | 0.003* |
| Parity                    | 0         | 35 (26.7)      | 96 (73.3)      | 1            |         |              |         |
|                           | ≥ 1       | 27 (23.9)      | 86 (76.1)      | 0.86 (0.48, 1.54) | 0.614  | 0.61 (0.36, 1.03) | 0.066  |
|                           | >1        | 65 (41.7)      | 91 (58.3)      | 1.96 (1.19, 3.23) | 0.009  | 0.52 (0.29, 0.92) | 0.024  |
| **Knowledge**             |          |                |                |              |         |              |         |
| *General knowledge and information* |      |                |                |              |         |              |         |
| Have you ever been tested for toxoplasmosis? | Yes | 3 (30.0)       | 7 (70.0)       | 1            |         |              |         |
|                           | Not Sure  | 2 (11.1)       | 16 (88.9)      | 0.29 (0.04, 2.15) | 0.227  | -            | -       |
|                           | No        | 122 (32.8)     | 250 (67.2)     | 1.14 (0.29, 4.48) | 0.853  | -            | -       |
| Is toxoplasmosis caused by a poison? | Yes | 36 (27.9)      | 93 (72.1)      | 1            |         |              |         |
|                           | Not Sure  | 88 (34.6)      | 166 (65.4)     | 1.34 (0.86, 2.18) | 0.184  | -            | -       |
|                           | No        | 3 (17.6)       | 14 (82.4)      | 0.55 (0.15, 2.04) | 0.374  | -            | -       |
| **Risk factors**          |          |                |                |              |         |              |         |
| Can people get toxoplasmosis by receiving blood transfusion? | Yes | 7 (17.1)       | 34 (82.9)      | 1            |         |              |         |
|                           | Not Sure  | 95 (34.1)      | 184 (65.9)     | 2.51 (1.07, 5.87) | 0.340  | 3.93 (1.54, 10.01) | 0.004* |
|                           | No        | 25 (31.2)      | 55 (68.8)      | 2.21 (0.86, 5.66) | 0.990  | 2.62 (0.97, 7.09) | 0.057  |
**Preventive knowledge**

| Prevention                                      | Yes    | No    | Odds Ratio (95% CI) | p-value | *p*-value |
|-------------------------------------------------|--------|-------|---------------------|---------|-----------|
| Is avoiding stray cats can prevent pregnant mother from getting toxoplasmosis? | Yes    | 47 (37.9) | 77 (62.1) | 1 | 0.63 (0.40, 1.01) | 0.052 | 0.42 (0.24, 0.71) | 0.001* |
| Not Sure                                        | 67 (27.9) | 173 (72.1) | 1 | 0.93 (0.43, 2.00) | 0.845 | 0.89 (0.39, 2.03) | 0.784 |
| No                                              | 13 (36.1) | 23 (63.9) | 1 |                      |       |                       |       |
| Toxoplasmosis can be prevented by thoroughly washing and/or peeling all fruits and vegetables before eating them. | Yes    | 50 (37.0) | 85 (63.0) | 1 | 0.72 (0.46, 1.13) | 0.154 | - | - |
| Not Sure                                        | 71 (29.8) | 167 (70.2) | 1 |                      |       |                       |       |
| No                                              | 6 (22.2) | 21 (77.8) | 1 | 0.48 (0.18, 1.28) | 0.145 | - | - |
| Preventive                                       |        |        |                    |         |           |
| Do you routinely wash your hands after gardening? | Yes    | 86 (32.2) | 181 (67.8) | 1 |                      |       |                       |       |
| Not Sure                                        | 36 (28.6) | 90 (71.4) | 1 | 0.84 (0.53, 1.34) | 0.467 | - | - |
| No                                              | 5 (71.4) | 2 (28.6) | 1 | 5.26 (1.00, 27.67) | 0.050 | - | - |
| Do you routinely wash your hands after changing cat litter? | Yes    | 59 (32.8) | 121 (67.2) | 1 |                      |       |                       |       |
| Not Sure                                        | 62 (29.7) | 147 (70.3) | 1 | 0.87 (0.56, 1.33) | 0.509 | - | - |
| No                                              | 6 (54.5) | 5 (45.5) | 1 | 2.46 (0.72, 8.39) | 0.150 | - | - |
| Do you eat raw meat?                            | Yes    | 113 (33.3) | 226 (66.7) | 1 |                      |       |                       |       |
| Not Sure                                        | 10 (25.0) | 30 (75.0) | 1 | 0.67 (0.32, 1.41) | 0.290 | - | - |
| No                                              | 4 (19.0) | 17 (81.0) | 1 | 0.47 (1.56, 1.43) | 0.471 | - | - |
| Do you routinely wash your hands after handling raw vegetables? | Yes    | 122 (33.0) | 248 (67.0) | 1 |                      |       |                       |       |
| Not Sure                                        | 2 (10.5) | 17 (89.5) | 1 | 0.24 (0.05, 1.05) | 0.058 | - | - |
| No                                              | 3 (27.3) | 8 (72.7) | 1 | 0.76 (0.20, 2.92) | 0.692 | - | - |

*a* simple logistic regression, *b* backward LR multiple logistic regression, *c* significant at *p*<0.01

Half (45.3%) of the pregnant women in Poland had good knowledge of toxoplasmosis (Pawlowski et al. 2001). Meanwhile, research in Saudi Arabia (Amin et al. 2013) and the Netherlands (Pereboom et al. 2013) have reported that only 25.1% and 9.4% of the pregnant women were classified as knowledgeable about this disease, respectively. These
inconsistent findings could be due to the differences in study tools and cut-off points used to measure knowledge regarding toxoplasmosis.

Interestingly, despite having poor knowledge of toxoplasmosis, the majority of pregnant women in the present study had routinely practised preventive behaviours. This is probably due to awareness about other zoonotic infections among pregnant women, despite lacking specific knowledge of toxoplasmosis. Similarly, studies conducted by Pawlowski et al. (2001) and Andiappan et al. (2014), also showed that most participants engaged in their study had limited knowledge about toxoplasmosis, yet had routinely practised preventive behaviours. Our current finding also indicated that pregnant women routinely washed their hands before or after handling food. However, this study did not assess how these women wash their hands. A study by Shabnam (2010) demonstrated that verbal response about handwashing behaviour does not provide an accurate picture of the actual practice. Most respondents were found to practice handwashing with only water, without using soap before eating as it seemed sufficient cleanliness for them. Proper handwashing could help in reducing food borne infection rate as the World Health Organization has reported approximately half of toxoplasmosis cases were food borne (WHO 2015).

The present study also showed that pregnant women with lower levels of education were at higher risk for anti-\textit{Toxoplasma} IgG seropositivity. A previous study revealed that poor hand hygiene and poor personal health practices amongst individuals with low educational level may contribute to \textit{T. gondii} infection (Yan et al. 2018). Moreover, the study by Andiappan et al. (2014) revealed that pregnant women in Malaysia and Thailand undertook better precaution or preventive practice against \textit{Toxoplasma} infection, compared to their Filipino counterparts. This is because pregnant Malaysian and Thai women had higher levels of education which may be indicative of higher knowledge of toxoplasmosis. Lower levels of education is associated with lower socioeconomic status, which could be related to employment in sectors with greater soil exposure (Jones et al. 2001). Another study conducted in Brazil showed that a low level of education increases the possibility of consuming contaminated food (Avelino et al. 2004). The study also revealed that having a medical history could be a factor that was significantly associated with anti-\textit{Toxoplasma} IgG antibodies seropositivity. Among them, diabetes mellitus contributed the highest percentage of anti-\textit{Toxoplasma} IgG seropositivity in pregnant women. The hyperglycemic condition favours immune dysfunction, leading to lower immunity levels, thus increasing the odds for contracting toxoplasmosis.

In the study, pregnant women who were unsure whether the transmission could take place by receiving blood transfusion were found to be significantly associated with anti-\textit{Toxoplasma} IgG antibodies seropositivity. The previous study by Alvarado-Esquivel et al. (2018) showed
that a history of blood transfusion was a risk factor for *T. gondii* infection. Furthermore, a study in southwest Iran demonstrated that more than 16% of healthy blood donors have *anti-* *T. gondii* antibodies in their sera (Moshfe et al. 2018). Although screening for *T. gondii* infection is not yet performed in blood banks (WHO 2010), knowledge of potential toxoplasmosis via blood transfusion may prevent the occurrence of the disease.

Stray cats infected with *T. gondii* could be a vital source that contributes to human and animal toxoplasmosis. Research done by Torrey & Yolken (2013) reported that approximately 100 oocytes were found in 7-13 mg of soil collected from the nails of a cat after digging in the dirt. This suggests that an individual who has come in contact with infected cats had a high potential for indirectly contracting toxoplasmosis. Interestingly, the present study showed that pregnant women who were not sure of the need to avoid stray cats had lower odds of acquiring the disease. This could be due to their handwashing preventive behaviour especially when dealing with food. This explains why a person is less likely to be infected with this parasite if hand hygiene is maintained despite having direct physical contact with cats. Therefore, it is important to instil health education in pregnant women, especially on the mode of transmission of *T. gondii* without needing to avoid stray cats.

General information regarding toxoplasmosis and proper guidance on health practises should be continuously provided to pregnant women. Few studies have underscored the importance of health education on toxoplasmosis. A study in Belgium demonstrated that health education was associated with a reduction in *T. gondii* seroconversion by 34% amongst pregnant women (Foulon et al. 1994). In addition to that, a study in Poland demonstrated that toxoplasmosis-related education in pregnant women has increased their level of knowledge about the disease and preventive measures within 4 years (Pawlowski et al. 2001). Therefore, the Malaysian Health Authority should provide adequate health education programmes on toxoplasmosis to pregnant women, especially to those who were not been infected with toxoplasmosis, to prevent the occurrence of congenital toxoplasmosis.

The design of the cross-sectional study could not determine if the exposure or outcome occurred first (cause and effect) due to the lack of temporality. The sampling method applied hindered the generalisability to pregnant women outside of the study site. The assumption pregnant women who choose the ‘not sure’ option might have the correct knowledge to a certain extent or preventive behaviours, thus given higher score as compared to the mothers who selects ‘No’ warrants further investigation. The association between the items that measured knowledge and preventive behaviour with anti-*Toxoplasma* IgG antibodies seropositivity should be interpreted with caution as Bonferroni correction was not applied to correct the multiple testing. This correction...
was not conducted, so that the study findings at the set significance level could be used to generate hypothesis for further studies.

CONCLUSION
The seroprevalence of latent toxoplasmosis was high in the present study with a notable proportion of respondents having inadequate knowledge regarding *T. gondii*. Although they practised routine preventive behaviours, lack of proper knowledge may cause a certain aspect of the disease transmission to be overlooked, thus exposing them to the infection. Low levels of education, presence of past medical history and lack of knowledge regarding mode of transmission through blood transfusion pose a greater risk of latent toxoplasmosis seroprevalence. The importance of knowledge on toxoplasmosis and its complications should be emphasized in healthcare settings. This could potentially reduce disease transmission and the incidence of clinical outcomes such as having newborns with toxoplasmosis.

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