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Toxoplasma gondii infection among selected indigenous community in Sarawak, East Malaysia

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Abstract. *Toxoplasma gondii* is an obligate intracellular protozoan parasite that causes toxoplasmosis in humans. To date, little is known about *T. gondii* infection among the indigenous community, particularly in East Malaysia. This study was conducted to determine the status of *T. gondii* infection and to investigate associated risk factors among the indigenous community of Sarawak, East Malaysia. The sociodemographic data was obtained using a pre-tested questionnaire. A serological test was done to detect the presence of specific IgM and IgG antibodies against *T. gondii* in serum samples. A nested polymerase chain reaction (PCR) was used to determine acute infection among seropositive individuals. The overall seroprevalence of *T. gondii* infection was 50% (95% CI = 43.3 – 56.7). From this subset, 40.1%, 5.7%, and 4.2% were positive for anti-*T. Gondii* IgG antibodies, IgM, and both IgG and IgM, respectively. Four seropositive samples were amplified through PCR. None of the pregnant women tested positive for *T. gondii* infection based on the serological and PCR assays. A significant association was found between age, low monthly household income, unemployment, usage of untreated water and close contact with *T. gondii* seropositive cats. These results provide basic information on *T. gondii* infection and may be useful for policymakers to initiate prevention and control programs, especially amongst pregnant women and women of childbearing age in the indigenous community.

INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by the obligate intracellular protozoa parasite, *Toxoplasma gondii*. About one-third of the world population is estimated to be infected with this parasite (Flegr *et al.*, 2014). *T. gondii* is capable of infecting most mammals, including birds. Human can acquire toxoplasmosis via ingestion of *T. gondii* oocyst from contaminated food and water, consumption of raw or undercooked meat containing tissue cyst, congenital transmission, blood transfusion, and organ transplantation (Dubey & Jones, 2008). Humans also can be infected by consumption of unpasteurized milk (Boughattas, 2015) and uncooked vegetables (Hussain *et al.*, 2017).

T. gondii infection in immunocompetent individuals is generally asymptomatic, and they only display some self-limiting flu-like symptoms (Taila *et al.*, 2011; Akturk *et al.*, 2017). However, *T. gondii* infection can be fatal in high-risk groups, including acquired immunodeficiency syndrome (AIDS) patients (Wang *et al.*, 2017), organ transplant recipients, pregnant women and newborn children (Gashout *et al.*, 2014; McAuley, 2014; Nimri *et al.*, 2014; Olariu *et al.*, 2014; El Bissati *et al.*, 2018). In pregnant women, the congenital infection may cause stillbirth, abortion, or defects to the newborn, such as

microcephaly (McAuley, 2014), intellectual disability (Olariu *et al.*, 2014), and visual and hearing impairment (Garweg *et al.*, 1996; El Bissati *et al.*, 2018).

To date, several seroepidemiologic studies of *T. gondii* infection have been conducted among immunocompromised (Shamilah *et al.*, 2001; Nissapatorn *et al.*, 2002, 2003; Mohamed *et al.*, 2016) and immunocompetent groups (Ngui *et al.*, 2011) in Malaysia. The most recent study showed a seropositivity rate of 57.4% among migrant workers in Peninsular Malaysia (Sahimin *et al.*, 2017). However, information on toxoplasmosis among the indigenous communities is still limited. The last available data on anti-*Toxoplasma* antibodies among the indigenous group in Peninsular Malaysia showed a sero-prevalence rate of 37% (Ngui *et al.*, 2011). There is no community-based study of *T. gondii* among the East Malaysia community. Moreover, the previous studies were based on the detection of *Toxoplasma*-specific immunoglobulin IgG and IgM in the serum. Molecular testing had not been implemented, and information about 'true-positive' *T. gondii* infection and combining risk factors is scarce in a community-based setting. This study was, therefore conducted to investigate the status of *T. gondii* infection and risk factors associated with toxoplasmosis in selected indigenous people in Sarawak, East Malaysia. This is the first community-based sero-molecular epidemiology study of *T. gondii* in Malaysia.

MATERIALS AND METHODS

Study area and population

Sarawak, the largest state of Malaysia, is located in the north-west of Borneo. According to the latest national census obtained from the Department of Statistics, Malaysia, 28 indigenous groups in Sarawak make up 71.2% of their overall population. The *Ibans*, who were referred to as Sea Dayaks during the colonial period, is the largest indigenous ethnic group, comprising about 28.8% of the population.

This study was conducted in 2015 among the *Iban* tribe in the Pakan sub-district (1.88333°N latitude, 111.68333°E longitude), a small town located in Sarawak. Three longhouses were selected using the convenience sampling method, based on willingness to participate, road accessibility and village entry approval by the district health officer. Each longhouse comprises of 120 to 200 occupants, and is separated by a door with up to 40 doors within the longhouse. The primary occupation of the inhabitants of this community is farming, and domesticated animals such as cats and dogs are common here. The community often shares a close relationship with these animals, especially dogs, as the dogs often assist the men in the hunting fields. These animals roam freely and allowing them into the houses is a common practice among the community.

Data and sample collection

The study protocol was approved by the Ethics Committee of the University Malaya Medical Centre (UMMC) (MEC ID: 201401-0672). Before the commencement of this study, meetings were sought with various local government agencies and community leaders to clarify the objectives and protocol of this study. A pre-tested questionnaire was designed to collect the necessary information on the biological and socio-demographic risk factors associated with toxoplasmosis and clinical history/presentation of *T. gondii* infection (if any). The questionnaire was designed in English, and translated to *Bahasa Malaysia*, to ensure that the questions could be understood by the participants.

Before sample collection, the parents and their children were briefed orally by trained field assistants on the objectives and procedures of the study. The participants were informed that their participation was voluntary and that they could withdraw at any time without needing to provide any reason. If they agreed to participate, literate participants signed an informed consent form, while illiterate participant, gave their consent by placing their thumbprint on the consent form. For children, consent was obtained from their parents or guardians who acted as head of the family.

Approximately 2–5 mL of venous blood was obtained from each participant with the assistance of medical officers and nurses. The blood was collected in plain and EDTA tubes, and transported in a standard ice-packed storage box to Sarikei District Hospital laboratory. The blood samples in the plain tubes were then centrifuged at 1,500 rpm for 10 minutes to obtain sera, then stored at -20°C until future use. Both sera and EDTA tubes were couriered by air to the Department of Parasitology, Faculty of Medicine, the University of Malaya for further analysis.

Detection of immunoglobulin G and M antibodies to *T. gondii* using enzyme-linked immunosorbent assay (ELISA) test

Sera were analyzed for the presence of IgM and IgG antibodies against *T. gondii* using a commercialized ELISA kit (Trinity Biotech Co., New York, USA). The specificity and sensitivity of the IgG ELISA kit is 95.3% and 100%, respectively. For IgM, the kit has 100% sensitivity and 97.4% specificity. Briefly, a series of dilutions were carried out on the sera samples, then added to the ELISA plates coated with specific antigens of *T. gondii*. Positive control, negative control, calibrator and blank were also included. Immune Status Ratio (ISR) value was calculated according to the manufacturer's protocol. The sample was considered positive if the ISR value was more than or equal to 1.10 (>51 IU/mL anti-*Toxoplasma* IgG and IgM antibody). Positive IgG indicated a latent or pre-existing *Toxoplasma* infection, while positive IgM indicated a recent infection. All positive sera were further tested with polymerase chain reaction (PCR) to detect the presence of *Toxoplasma* DNA to confirm an active infection.

DNA extraction and amplification

Briefly, genomic DNA was extracted from whole blood samples in EDTA tube using NucleoSpin® Blood DNA Isolation Kit (MACHEREY-NAGEL GmbH & Co., Duren, Germany) according to the manufacturer's protocol. The extracted DNA was stored at -20°C until future use. A nested PCR which specifically amplified the B1 gene of *T. gondii* was used (Burg *et al.*, 1989). The PCR assay's sensitivity and specificity are 99% and 100%, respectively (Burg *et al.*, 1989). Human glyceraldehyde-3-phosphate dehydrogenase was used as an internal control. Four sets of primers were used, including outer primers B1F1 (5'-CCG TTG GTT CCG CCT CCT TC-3') and B1R1 (5'-GCA AAA CAG CGG CAG CGT CT-3') and inner primers, B1F2 (5'-CCG CCT CCT TCG TCC GTC GT-3') and B1R2 (5'-GTG GGG GCG GAC CTC TCT TG-3'). Amplified PCR products were stained with SYBR Safe DNA stain (Invitrogen, USA) and electrophoresed in 2% agarose gels at 100V for 40 minutes.

Data analysis

Data entry and analysis were conducted using the Statistical Package for Social Sciences (SPSS) software version 21 (SPSS, Chicago, IL, USA). Qualitative data was expressed as frequencies and percentages. Pearson's Chi-square test was used to assess the association between *T. gondii* infection and the variables of interest. A univariate analysis was used to determine any potential risk factors associated with *T. gondii* infection. Significant factors, as determined by the univariate analysis, were further analyzed using multivariate analysis to identify the significant predictor factors. In addition, any variables with significance levels between 0.10 and 0.25 were also included in the multivariate analysis to ensure that potentially important factors were not excluded because of low number of predictor variables. A p-value of less than 0.05 was set as the level of statistical significance. The odds ratios (OR) and 95% confidence interval (95% CI) were computed for each test.

RESULTS

General characteristics of the study population

A total of 212 participants enrolled in this study, comprising of 102 (48.1%) males and 110 (51.9%) females. Of these, two female participants were pregnant at the time of sample collection. The majority of the participants were teenagers and adults

(189; 89.2%) while only 23 (10.8%) were children. Almost all of the families (95.3%) received a monthly household income of less than RM 750, below the national poverty line. Most of the adults (69.8%) were involved in small-scale agricultural activities, including paddy and black pepper cultivation, without a stable monthly income. More than half of the participants had no formal education. Likewise, almost every family (98.6%) used water supplies from natural and untreated sources including river, well and rainwater for their daily chores. Only a few households (1.4%) used treated water from the government municipal water supply. Close contact with domestic animals and eating with hands were common practices among the community.

***Toxoplasma gondii* infection**

The overall seroprevalence of *Toxoplasma gondii* infection was 50% (95% CI = 43.3 – 56.7). Of these, 40.1% (95% CI = 33.7 – 46.9) were positive for anti-*T. gondii* IgG antibodies, 5.7% (95% CI = 3.3 – 46.9) were positive for IgM and 4.2% (95% CI = 2.3 – 7.9) were positive for both IgG and IgM (Table 1). There was no significant difference in the overall seroprevalence of *T. gondii* infection between gender (Table 2). In addition, no significant difference was reported in the specific anti-IgG, anti-IgM and both anti-*T. gondii* antibodies between males and females. The overall prevalence of *T. gondii* infection was significantly higher among those aged 13 years and above (53.9%) compared to participants aged 12 years and below (17.4%) ($p<0.001$). A similar observation was seen in the seroprevalence of specific anti-*T. gondii* IgG antibodies, where the seroprevalence was significantly higher among individuals aged 13 years and above (43.4%) than participants aged 12 years and below (8.7%) ($p<0.001$). None of the pregnant women were seropositive for IgG and IgM.

Genomic DNA was extracted from all the seropositive samples. These included 85 samples that were positive for anti-IgG, twelve anti-IgM and nine which were positive for both anti-*T. gondii* antibodies. The molecular analysis revealed that four IgM-seropositive samples were successfully amplified with PCR. All PCR positive samples were from females aged 13 years and above. No PCR amplification was seen in the IgG and both IgG/IgM-positive serum.

Table 1. Seroprevalence of specific anti-*Toxoplasma* IgG and IgM antibodies among the selected indigenous community in Sarawak (N=212)

Anti-*Toxoplasma* antibodies	n	%	95% CI
Anti-IgG (+)	85	40.1	33.7 – 46.9
Anti-IgM (+)	12	5.7	3.3 – 9.6
Both (IgG & IgM) (+)	9	4.2	2.3 – 7.9
Total	106	50.0	43.3 – 56.7

n: Number of seropositive samples; %: Percentage.

Table 2. *Toxoplasma gondii* infections according to age group and gender as determined by ELISA and PCR

Characteristics	N	Anti-*Toxoplasma* antibodies			Total ELISA	*PCR n (%)
		Anti-IgG n (%)	Anti-IgM n (%)	Both n (%)		
Gender						
Male	102	43 (42.2)	4 (3.9)	7 (6.9)	54 (52.9)	0 (0)
Female	110	42 (38.2)	8 (0.1)	2 (1.8)	52 (47.3)	2 (25.0)
Age groups						
(≤12 years)	23	2 (8.7)	1 (4.3)	1(4.3)	4 (17.4)	0 (0)
(>12 years)	189	83 (43.4)	11 (5.8)	8 (4.2)	102 (53.9)	2 (18.2)
Total	222	85 (40.1)	12 (5.7)	9 (4.2)	106 (50.0)	4 (33.3)

N: number of examined; n: number of seropositive; %: Percentage.
* PCR were conducted on all IgM positive sera.

Table 3. Logistic regression analysis of the potential risk factors associated with *Toxoplasma gondii* infection among the selected indigenous community in Sarawak (N=212)

Variables	N	Seropositive n (%)	OR (95% CI)	*p* value
Gender				
Male	102	54 (52.94)	1.26 (0.73, 2.15)	0.410
Female	110	52 (47.27)	1	
Age				
Adults (>12 years)	189	102 (53.97)	1.18 (1.06, 1.29)	<0.001*
Children (≤12 years)	23	4 (17.39)	1	
Education attainment				
Uneducated	106	54 (50.94)	1.08 (0.63,1.85)	0.780
Formal education (went to school)	106	52 (49.06)	1	
Household income				
<RM750	202	97 (48.02)	1.43 (0.91, 2.24)	0.010*
≥RM750	10	9 (90)	1	
Employment status				
Not working (housewife, student)	64	46 (71.88)	1.77 (1.28, 2.44)	<0.001*
Working	148	60 (40.54)	1	
Source of water supply				
Others (river, rainwater, well)	209	105 (49.8)	2.02 (1.18, 22.6)	0.040*
Government pipe	3	1 (33.3)	1	
Presence of cats at home				
Yes	205	104 (50.7)	1.90 (1.41, 2.94)	0.040*
No	7	2 (28.6)	1	
Eating with hands				
Yes	209	104 (49.76)	0.49 (0.04, 5.55)	0.560
No	3	2 (66.67)	1	

N: Number of samples; n: Number of positive samples; %: Percentage; CI: Confidence Interval.
OR: Odd Ratio: Reference group refer as 1.
*: Significant association as potential risk factors (P<0.05).

In addition, all samples of pregnant women were also subjected to PCR, although they were determined to be seronegative through serological testing. No amplification was reported in these samples.

Risk factors of *Toxoplasma gondii* infection

The risk factors analysis showed that the seroprevalence of age groups, household income, employment status, source of water supply and close contact with cats were positively associated with *T. gondii* infection. The analysis demonstrated that those aged 12 years and above (OR = 1.18, 95% CI = 1.06 – 1.29, p<0.001), had a low monthly household income (OR = 1.43, 95% CI = 0.91 – 2.24, p=0.01), unemployed (OR = 1.77, 95% CI = 1.28 – 2.24, p<0.001), used untreated water sources (OR = 1.9, 95% CI = 1.41 – 2.94, p=0.04) and had close contact with cats (OR = 1.9, 95% CI = 1.41 – 2.94, p=0.04) were significantly associated with *T. gondii* infections (Table 3). Multivariate analysis demonstrated that the participants aged 12 years and above, unemployed participants and low monthly household income were 3.9 times (95% CI = 1.24 – 12.18, p=0.02), 2.1 times (95% CI = 1.10 – 3.61, p=0.022) and 3.7 times (95% CI = 2.7 – 7.37, p=0.010) more likely to acquire *T. gondii* infection, respectively.

DISCUSSION

This study demonstrated that about half of the indigenous longhouse community had latent or past exposure to *T. gondii* infection. The seroprevalence rate reported in this

study was slightly higher compared to previous studies in Malaysia (Shamilah *et al.*, 2001; Nissapatorn *et al.*, 2002; Mohamed *et al.*, 2016). In contrast, the recent study among migrant workers in Peninsular Malaysia showed a much higher prevalence rate of anti-*Toxoplasma* immunoglobulin compared to this study (Sahimin *et al.*, 2017). Another local study among indigenous people in Peninsular Malaysia showed a relatively low seroprevalence rate (Ngui *et al.*, 2011). Comparing these findings with indigenous groups from other countries, this study showed that the seroprevalence rate was comparatively similar to Taiwanese (Fan *et al.*, 2002), and Thailand (Fan *et al.*, 2003) indigenous groups. However, the seroprevalence rate reported in this study was much higher compared to minority groups in other countries, including Nigeria (Sowemimo *et al.*, 2018), and China (Xin *et al.*, 2015).

In this study, there was no significant association between sero-positivity and gender, which is in agreement with previous studies (Fan *et al.*, 2003; Ngui *et al.*, 2011; Cañón-Franco *et al.*, 2014; Sowemimo *et al.*, 2018). In contrast, a study among hospitalized patients in Malaysia showed that the *T. gondii* infection in females was significantly higher than male patients (Mohamed *et al.*, 2016). One of the possible reasons could be that people acquire *T. gondii* infection through foodborne, zoonotic, continental transmission and through an organ transplant or blood transfusion, regardless of their gender. The seroprevalence rate in this study indicated significant heterogeneity among age groups where the infection rate increases with age. This finding is in agreement with a study among indigenous populations in Peninsular Malaysia (Ngui *et al.*, 2011), Thailand (Fan *et al.*, 2003), Brazil (Sobral *et al.*, 2005), Taiwan (Fan *et al.*, 2002), and Colombia (Cañón-Franco *et al.*, 2014). This is probably because there is a positive correlation between age and exposure to infection. However, the result might differ in a larger sample size.

Low household income is also a significant risk factor for *T. gondii*. Previous studies showed that poverty is associated with the likelihood that an individual will be infected with *T. gondii* (Alvarado-Esquivel *et al.*, 2006). Additionally, previous studies showed that unemployed/unskilled workers influence the likelihood that a person will be infected with *T. gondii* (Nissapatorn *et al.*, 2003; Ngui *et al.*, 2011), consistent with the result of this study. In contrast, the most recent study in Nigeria reported no significant association between occupation/employment status and infection (Sowemimo *et al.*, 2018). Generally, a participant whose occupation is categorized as unemployed/unskilled worker tends to be infected more frequently, due to their poor knowledge of personal hygiene. In this study, most of the unemployed participants were housewives who stayed at home and had close contact with domestic pets. Since cats and dogs are the most common animals in this community, the participants might have accidentally acquired the infection by ingestion of *T. gondii* oocyst in contaminated food and water, or after handling or playing with their pets.

The findings of this study further support this observation, where there was a significant association between high seroprevalence of *T. gondii* infection and those who had close contact with domestic animals. Previous studies also showed that close contact with domestic pets, especially cats, is a significant risk factor for *T. gondii* infection among the other indigenous communities in Malaysia (Ngui *et al.*, 2011), Taiwan (Lin *et al.*, 2008; Chiang *et al.*, 2014), and South America countries (Cañón-Franco *et al.*, 2014). Based on our observation, dogs and cats shared food from the same plate with their owners. Some cats also defecated indoors, especially near the kitchen. These cats may shed millions of oocysts in their faeces, thus spreading oocysts to the environment. This situation is further aggravated by the type of kitchen flooring, which is traditionally made from mixed soil and sand, providing an ideal condition for the development, survival, and transmission of the *Toxoplasma* oocyst. *Toxoplasma* oocysts may remain infectious for months in this environment. Moreover, most of the domestic cats in this study roam freely. The cats may

also acquire an infection while hunting and foraging wild prey that acts as intermediate hosts, such as mice, rats, birds. A recent study in Japan showed that cats that roam freely and greater chances to hunt for prey subsequently showed higher seropositivity than indoor cats (Salman *et al.*, 2018).

Other than that, water supply from natural sources such as a river, rainwater and well are also significant risk factors for toxoplasmosis in this study. Similar results were seen among indigenous and marginalized communities, including in Malaysia (Ngui *et al.*, 2011), Taiwan (Chiang *et al.*, 2014) and Mexico (Alvarado-Esquivel *et al.*, 2006). As reported in the socio-demographic profiles of the participants, most of the community still use untreated water from natural resources for domestic needs, including drinking, cooking, bathing, and washing clothes. Thus, they may have accidentally ingested *T. gondii* oocysts if the water was contaminated with oocysts from infected wild animals that drank or defecate from the same river banks or wells. An outbreak due to *T. gondii* infection associated with drinking water contaminated with parasite oocysts has been reported in several studies (de Moura *et al.*, 2006; Vieira *et al.*, 2015). This situation is further aggravated by the habit of drinking improperly boiled water among the community. Thus, the community, especially pregnant women, should be educated on the hazard of drinking improperly boiled water.

Serological assays, such as enzyme-linked immunosorbent assay (ELISA) is still routinely used to diagnose *T. gondii* infections. The detection of IgG antibodies indicates *T. gondii* infection, but provide no information on the time of infection, while the presence of anti-*T. gondii* IgM is not an accurate marker of acute infection (Robert-Gangneux & Darde, 2012). The IgG avidity test is commonly used to differentiate between acute and chronic *T. gondii* infections (Hedman *et al.*, 1989). Molecular methods such as PCR can be used in addition to conventional serological methods to diagnose *T. gondii* infection. There is no doubt that serological methods are usually accurate, but the information can be limited in prenatal cases or immunocompromised patients. For example, a pregnant woman may be diagnosed accurately by serology tests that she has an infection during pregnancy, so her baby may be at risk of congenital infection. While the serology results cannot confirm whether the parasite has been transferred to the baby, PCR assays can do so. The small number of parasites that might have been released from the tissue into subclinical level can be detected with PCR (Garweg *et al.*, 1996).

In this study, PCR was done on the IgM and IgM/IgG seropositive samples, and two seronegative samples of pregnant women to detect the presence of *T. gondii* DNA. Of these, four IgM seropositive samples of women aged 13 and above showed evidence of infection by PCR. This confirms the sensitivity and specificity of PCR for detecting recent infections, especially among high-risk groups, such as pregnant women and women of childbearing age. This is in agreement with previous reports that PCR is recommended over serological assays for diagnosis of toxoplasmosis in high-risk groups (Nimri *et al.*, 2014; Gashout *et al.*, 2014).

However, we acknowledge that the result should be interpreted with caution. The results of this study relied on a serological assay to detect IgG and IgM antibodies against *T. gondii* without IgG avidity testing. The IgG avidity test allows for distinction between a recent infection/acute phase (more than three months ago, high avidity) and infection acquired a long time ago/ chronic phase (less than three months ago, low avidity). Though PCR is used world-wide to detect *T. gondii* DNA, there could also be false-positives, subsequently, leading to misdiagnosis when conducted among immunocompetent patients with no evidence of clinical signs and symptoms. Therefore, it is essential to include the IgG avidity assay to discriminate between acute and chronic *T. gondii* infections in future studies.

In conclusion, this study showed a relatively high seroprevalence rate of *T. gondii* infection among the indigenous community in East Malaysia. The risk factor

analysis indicated that those aged 12-years-old and above, low monthly household income, unemployment, usage of untreated water, and close contact with cats were more likely to acquire *T. gondii* infection. Thus, there is a need for prevention measures, including a public health education program and surveillance against *T. gondii* infection in this community. Findings of this study may be useful for policymakers to initiate the prevention programs, especially among high-risk groups, such as pregnant and women of childbearing age.

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None declared.

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REFERENCES

Akturk, H.K., Sotello, D., Ameri, A., Abuzaid, A.S., Rivas, A.M. & Vashisht, P. (2017). *Toxoplasma* infection in an immuno-competent host: possible risk of living with multiple cats. *Cureus* **9**: e1103.

Alvarado-Esquivel, C., Sifuentes-Álvarez, A., Narro-Duarte, S.G., Estrada-Martínez, S., Díaz-García, J.H., Liesenfeld, O., Martínez-García, S.A. & Canales-Molina, A. (2006). Seroepidemiology of *Toxoplasma gondii* infection in pregnant women in a public hospital in northern Mexico. *BMC Infectious Diseases* **6**: 113.

Boughattas, S. (2015). Commentary on: "Detection of *Toxoplasma gondii* in raw caprine, ovine, buffalo, bovine, and camel milk using cell cultivation, cat bioassay, capture ELISA, and PCR methods in Iran". *Frontiers in Microbiology* **6**: 215.

Burg, J.L., Grover, C.M., Pouletty, P. & Boothroyd, J. (1989). Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii* by polymerase chain reaction. *Journal of Clinical Microbiology* **27**: 1787-1792.

Cañón-Franco, W.A., López-Orozco, N., Gómez-Marín, J.E. & Dubey, J.P. (2014). An overview of seventy years of research (1944-2014) on toxoplasmosis in Colombia, South America. *Parasites & Vectors* **7**: 427.

Chiang, T.Y., Kuo, M.C., Chen, C.H., Yang, J.Y., Kao, C.F., Ji, D.D. & Fang, C.T. (2014). Risk factors for acute *Toxoplasma gondii* diseases in Taiwan: a population-based case-control study. *PLos One* **9**: e90880.

de Moura, L., Bahia-Oliveira, L.M., Wada, M.Y., Jones, J.L., Tuboi, S.H., Carmo, E.H., Ramalho, W.M., Camargo, N.J., Trevisan, R., Graça, R.M., da Silva, A.J., Moura, I., Dubey, J.P. & Garrett, D.O. (2006). Waterborne toxoplasmosis, Brazil, from field to gene. *Emerging Infectious Diseases* **12**: 326-329.

Dubey, J. & Jones, J. (2008). *Toxoplasma gondii* infection in humans and animals in the United States. *International Journal of Parasitology* **38**: 1257-1278.

El Bissati, K., Levigne, P., Lykins, J., Adlaouj, E.B., Berraho, A., Laboudi, M., El Mansouri, B., Ibrahimi, A., Rhaiaoui, M., Quinn, F., Murugesan, M., Seghrouchni, F., Gomez-Marin, J.E., Pevron, F. & McLeod, R. (2018). Global initiative for congenital toxoplasmosis: an observational and international comparative clinical analysis. *Emerging Microbes and Infections* **7**: 165.

Fan, C.K., Liao, C.W., Wu, M.S., Su, K.E. & Han, B.C. (2003). Seroepidemiology of *Toxoplasma gondii* infection among Chinese aboriginal and Han people residing in mountainous areas of northern Thailand. *Journal of Parasitology* **89**: 1239-1242.

Fan, C.K., Su, K.E., Wu, G.H. & Chiou, H.Y. (2002). Seroepidemiology of *Toxoplasma gondii* infection among two mountain aboriginal populations and Southeast Asian laborers in Taiwan. *Journal of Parasitology* **88**: 411- 414.

Flegr, J., Prandota, J., Sovièková, M. & Israili, Z.H. (2014). Toxoplasmosis – a global threat. Correlation of latent toxoplasmosis with specific disease burden in a set of 88 countries. *PLoS One* **9**: e90203

Garweg, J., Boehnke, M. & Koerner, F. (1996). Restricted applicability of the polymerase chain reaction for the diagnosis of ocular toxoplasmosis. *German Journal of Ophthalmology* **5**: 104-108.

Gashout, A., Amro, A., Erhuma, M., Al-Dwibe, H., Elmaihub, E., Babba, H., Nattah, N. & Abudher, A. (2016). Molecular diagnosis of *Toxoplasma gondii* infection in Libya. *BMC Infectious Diseases* **16**: 157.

Hedman, K., Lappalainen, M., Seppala, I. & Makela, O. (1989). Recent primary Toxoplasma infection indicated by a low avidity of specific IgG. *The Journal of Infectious Diseases* **159**: 736-740.

Hussain, M.A., Stitt, V., Szabo, E.A. & Nelan, B. (2017). *Toxoplasma gondii* in the Food Supply. *Pathogens* **6**: 21.

Lin, Y.L., Liao, Y.S., Liao, L.R., Chen, F.N., Kuo, H.M. & He, S. (2008). Seroprevalence and sources of *Toxoplasma* infection among indigenous and immigrant pregnant women in Taiwan. *Parasitology Research* **103**: 67-74.

McAuley, J.B. (2014). Congenital Toxoplasmosis. *Journal of the Pediatric Infectious Disease Society* **3**: S30-S35.

Mohamed, Z. & Hajissa, K. (2016). Seroprevalence of *Toxoplasma gondii* infection among patients in Hospital Universiti Sains Malaysia. *Tropical Biomedicine* **33**: 78-83.

Ngui, R., Lim, Y.A., Amir, N.F.H., Nissapatorn, V. & Rohela, M. (2011). Seroprevalence and sources of toxoplasmosis among Orang Asli (indigenous) communities in Peninsular Malaysia. *The American Journal of Tropical Medicine and Hygiene* **85**: 660-666.

Nimri, L., Pelloux, H. & Elkhatib, L. (2004). Detection of *Toxoplasma gondii* DNA and specific antibodies in high-risk pregnant women. *The American Journal of Tropical Medicine and Hygiene* **71**: 831-835.

Nissapatorn, V., Kamarulzaman, A., Init, I., Tan, L.H., Rohela, M., Norloza, A., Chan, L.L., Latt, H.M., Anuar, A.K. & Quek, K.F. (2002). Seroepidemiology of toxoplasmosis among HIV-infected patients and healthy blood donors. *Medical Journal of Malaysia* **57**: 304-310.

Nissapatorn, V., Lee, C.K., Cho, S.M., Rohela, M., Anuar, A.K., Quek, K.F. & Latt, H.M. (2003). Toxoplasmosis in HIV/AIDS patients in Malaysia. *The Southeast Asian Journal of Tropical Medicine and Public Health* **57**: 18-19.

Olariu, T.R., Remington, J.S. & Montoya, J.G. (2014). Polymerase chain reaction in cerebrospinal fluid for the diagnosis of congenital toxoplasmosis. *The Pediatric Infectious Disease Journal* **33**: 566-570.

Robert-Gangneux, M.L. & Darde. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical Microbiology Reviews* **25**: 264-296.

Sahimin, N., Lim, Y.A.L., Ariffin, F., Behnke, J.M., Basáñez, M.G., Walker, M., Lewis, J.W., Noordin, R., Abdullah, K.A. & Zain, S.N.M. (2017). Socio-demographic determinants of *Toxoplasma gondii* seroprevalence in migrant workers of Peninsular Malaysia. *Parasites & Vectors* **10**: 1-8.

Salman, D., Pumidonming, W., Oohashi, E. & Iqarashi, M. (2018). Prevalence of *Toxoplasma gondii* and other intestinal parasites in cats in Tokachi subprefecture, Japan. *Journal of Veterinary Medical Science* **80**: 960-967.

Shamilah, H., Lokman, H.S., Noor Azain, M.Y., Malkith, K. & Yusri, M.Y. (2001). Seroprevalence of *Toxoplasma gondii* antibodies in HIV positive and negative patients using the immunofluorescence antibody test (IFAT) methods. *Tropical Biomedicine* **18**: 137-141.

Sobral, C.A., Amendoeira, M.R., Teva, A., Patel, B.N. & Klein, C.H. (2005). Seroprevalence of infection with *Toxoplasma gondii* in indigenous Brazilian populations. *The American Journal of Tropical Medicine and Hygiene* **72**: 37-41.

Sowemimo, O.A., Wu, T.H., Lee, Y.L., Asaolu, S.O., Chuang, T.W., Akinwale, O.P., Badeioko, B.O., Gyang, V.P., Nwafor, T., Henry, E. & Fan, C.K. (2018). *Toxoplasma gondii*: seroprevalence and associated risk factors among preschool-aged children in Osun State, Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **112**: 486-491.

Taila, A.K., Hingwe, A.S. & Johnson, L.E. (2011). Toxoplasmosis in a patient who was immunocompetent: a case report. *Journal of Medical Case Reports* **5**: 16.

Vieira, F.P., Alves, M.G., Martins, L.M., Rangel, A.L.P., Dubey, J.P., Hill, D. & Bahia-Oliveira, L.M.G. (2015). Waterborne toxoplasmosis investigated and analysed under hydrogeological assessment: new data and perspectives for further research. *Memyrias do Instituto Oswaldo Cruz* **110**: 929-935.

Wang, Z.D., Liu, H.H., Ma, Z.X., Ma, H.Y., Li, Z.Y., Yang, Z.B., Zhu, X.Q., Xu, B., Wei, F. & Liu, Q. (2017). *Toxoplasma gondii* infection in immunocompromised patients: a systematic review and meta-analysis. *Frontiers in Microbiology* **8**: 389.

Xin, K.S., Liu, H., Wang, H.B. & Yao, Z.L. (2015). Seroprevalence of *Toxoplasma gondii* among primary school children in Shandong Province, China. *The Korean Journal of Parasitology* **53**: 489-492.