ORIGINAL ARTICLE

Serological and Molecular Detection of Toxoplasmosis among Blood Donors in Tertiary Hospital of Malaysia

Aisha Khodijah Kholib Jati[1], Suharni Mohamad[1], Zeehaida Mohamed[3], Wan Haslindawani Wan Mahmood[4], Wan Muhamad Amir W Ahmad[1], Wan Suriana Wan Ab Rahman[1,2]

[1] School of Dental Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian Kelantan, Malaysia
[2] Hospital Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia
[3] Department of Medical Microbiology & Parasitology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian Kelantan, Malaysia
[4] Department of Haematology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian Kelantan, Malaysia

ABSTRACT

Introduction: This preliminary cross-sectional study aimed to investigate the prevalence of toxoplasmosis among blood donors in Kelantan, Malaysia. **Methods:** A total of 56 blood donors were screened by an enzyme-linked immunosorbent assay (ELISA) for anti-*T. gondii* Immunoglobulin G (IgG) and Immunoglobulin M (IgM) antibodies. Positive *T. gondii* IgG and IgM were further tested for IgG avidity ELISA. All extracted deoxyribonucleic acids (DNAs) from whole blood samples were analyzed for the presence of the *Toxoplasma* B1 gene and the ITS1 region by polymerase chain reaction (PCR). The socio-demographic data of donors was assessed using a data collection form. **Results:** Out of 56 blood donors, 24 (42.86%) donors were IgG+/IgM-, and 2 (3.57%) donors were IgG+/IgM+ with one of them having a high avidity index indicating as past infection for more than 20 weeks and the other with a low avidity index indicating as recent infection within 20 weeks. None of the samples tested positive for the presence of the *Toxoplasma* B1 gene and the ITS1 region. A univariate analysis showed that only employment status was significantly associated with *Toxoplasma* seropositivity. **Conclusion:** The seroprevalence of toxoplasmosis among blood donors in Kelantan, Malaysia, was 46.43%. Nevertheless, direct detection by PCR showed that this parasite was absent in the blood. These results highlight that the blood donors in this study had previously been exposed to *T. gondii* infection. The parasite may still remain in certain tissues but does not freely circulate in the blood.

Keywords: Enzyme-linked immunosorbent assay, Prevalence, PCR, Blood donors, Toxoplasmosis

Corresponding Author:
Wan Suriana Wan Ab Rahman, MD
Email: suriana@usm.my
Tel: +609- 767 5831

INTRODUCTION

Toxoplasmosis is caused by a protozoon parasite, *Toxoplasma gondii*. Its transmission from blood donors to receiving immunocompromised patients has become a concern. It has been highlighted as a public health concern, as one-third of the world population has been infected with *Toxoplasma gondii* (1-4). Ingestion of oocyst-contaminated food or drinks, consumption of raw or undercooked meat containing *T. gondii* cysts, exposure to contaminated soils, and close contact with cats are among the common routes of *T. gondii* transmission (4-11). However, this parasite is rarely transmitted via organ transplant and blood transfusion (4,12). Although uncommon, transfusion-transmitted toxoplasmosis was reported in a leukemic patient after leukocyte transfusion in 1969 (13). Another case recorded a 52 years old woman acquired Toxoplasmic retinochoroiditis after platelet transfusion and the serologic results showed positive for recently acquired toxoplasmosis (14). The American Association of Blood Banks also confirmed four cases of transfusion-transmitted toxoplasmosis of which three were associated with granulocyte transfusion and one involved platelet transfusion (8).

In immunocompetent individuals, the infection is asymptomatic (1,4). Blood donors who have been infected might have mild to no symptoms and could still donate blood, as there is no thorough screening of toxoplasmosis prior to blood transfusion. However, if the contaminated blood were transfused to immunocompromised recipients, it can lead to severe consequences such as encephalitis, chorioretinitis, and myocarditis (15-17). Until 2019, there is only one recent study that was conducted among blood donors in Malaysia, which was in 2002 while the rest of the studies were done before 2000 (18). All these studies reported a toxoplasmosis prevalence of less than 30.0%

among blood donors (18). Thus, this preliminary study was aimed to investigate the current prevalence of toxoplasmosis among blood donors in Malaysia and the sociodemographic risk factors that contribute to the transmission of toxoplasmosis. Therefore, this report can be used as a reference, especially for future blood bank management to avoid any transfusion-transmitted disease.

MATERIALS AND METHODS

Study design and study population

This preliminary cross-sectional study was carried out from August 2017 to September 2018. Power and Sample Size Calculation (PS) software Dupont and Jr. (1990) was used to determine the required sample size. The parameters required for calculation of sample size using PS Software were; alpha, $\alpha = 0.05$, beta, $\beta = 0.80$, assumed Po = seroprevalence of toxoplasmosis in healthy group in Malaysia was 19.9% and assumed P1 = immunocompromised patients (HIV) was 44.4% respectively (19,20). Fifty-six volunteered blood donor who aged above 18 years old were selected based on the inclusion and exclusion criteria at Transfusion Medicine Unit, Hospital USM. Donors who were deferred from blood donation on a temporary or permanent basis (Refer WHO guideline, 2012) were excluded from this study. The sociodemographic data (gender, ethnicity, marital status, residence area, educational level, employment status) were obtained from the Information System of the Transfusion Medicine Unit, Hospital USM. After consent was obtained, the blood samples were taken and spun at 1500 x g for 10 minutes. The plasma was then stored at -20 °C for further analysis.

Detection of anti-*T. gondii* IgG and IgM antibodies

All plasma samples were screened for specific *T. gondii* IgG and IgM antibodies using Platelia Toxo IgG and Platelia Toxo IgM ELISA kits (BioRad, USA) according to the manufacturer's instructions. A titer of IgG anti-*T. gondii* ≥ 9 IU/ml was considered as positive, and a titer of IgG anti-*T. gondii* < 6 IU/ml was considered as negative. The positive samples indicate latent or pre-existing *T. gondii* infection. For the IgM-ELISA assay, a sample ratio ≥ 1.0 was considered as reactive for the presence of anti-*T. gondii* IgM, while a ratio < 0.8 was considered non-reactive. Subsequently, positive *T. gondii* IgM and IgG samples were further tested for IgG avidity using Platelia Toxo IgG-avidity ELISA (BioRad, USA) to distinguish between recent and past infection. An avidity index (AI) < 0.40 was considered as low avidity, indicating a recent infection of less than 20 weeks; 0.40 ≤ AI < 0.50 considered as avidity intermediate zone; and AI ≥ 0.50 indicate past infection of more than 20 weeks.

Detection of *T. gondii* by triplex PCR

DNA extraction from whole blood was done using a QIAMP DNA Blood Minikit (Qiagen, Germany) as described by the manufacturer. The purity (A260/A280 ratio) and concentration of extracted DNA was determined using a spectrophotometer (Eppendorf, USA). The extracted DNA samples were further tested for the presence of the *Toxoplasma* B1 gene and the ITS1 region by triplex PCR. Briefly, DNA amplification was done in a 20.0 µl reaction mixture consisting of 2 µl of sample, 1.0 µl of each primer, 2.0 µl 10X DreamTaq PCR Buffer, 0.15 µl 1.0 U DreamTaq DNA polymerase, and 0.32 µl 10.0 mM dNTPs. The primer pairs used in this study were B1F1 and B1R1, and ITS1-F and ITS1-R, whereas VHMF and VHA-AS5 were used as the heterologous internal control to detect the presence of the HemM gene in the sample according to a previous study (Table I). A plasmid (100 pg) containing the V. cholerae HemM gene was included in each run, while water was used as non-template control for negative control. The amplification was done using an Applied Biosystem thermal cycler machine with the following cycling conditions: initial denaturation at 94.0 °C for 5 min, denaturation at 94.0 °C for 30 sec, annealing at 59.0 °C for 30 sec, extension at 72.0 °C for 30 sec, and final extension at 72.0 °C for 7 min. All PCR products were visualized on a 1.8% agarose gel using ultraviolet in a 1X TBE buffer stained with a FloroSafe DNA stain (Axon Scientific Sdn. Bhd., Malaysia).

Table I: List of primers used in triplex PCR

Primers (Forward Reverse)	Gene	Sequences (5′ to 3′)	Size (bp)
B1F1 B1R1	B1	ATA GGT TGC AGT CAC TGA CG CTC CTC TTC GCG AAA CCT CA	321
ITS1-F ITS1-R	ITS1	ACA CGT CCT TAT TCT TTA TTA ACC A ATC CCA ACA GAG ACA CGA ATT	234
VHMF* VHA-AS5*	*HemM*	TGG GAG CAG CGT CCA TTG TG CAA TCA CAC CAA GTC ACT C	520

Reference: Rahumatullah et al., 2012 (25)

Statistical analysis

The data was analyzed using Statistical Package for the Social Sciences (IBM SPSS, 22.0 Version) software. Descriptive statistics were used to summarize all the data. The association between risk factors was evaluated using a Chi-square assay and a P-value of less than 0.05 was considered as statistically significant.

Ethical aspects

This study was approved by the Research Ethics Committee (Human), Universiti Sains Malaysia (USM/JEPeM/17030165). All donors were explained verbally about the purpose and procedures of the study and a written informed consent was obtained prior to the blood taking.

RESULTS

Out of 56 blood donors, 26 (42.86%) donors were positive for the *T. gondii* IgG antibody. Two (3.57%) donors, who were positive for both *T. gondii* IgG and IgM antibodies, showed high (AI = 0.76) and low (AI = 0.18) IgG avidity index, respectively. No amplification of the

Toxoplasma B1 gene and the ITSI region was detected in all blood donors using PCR (Fig. 1). The summarized results for ELISA and PCR are presented in Table II.
The majority (59.0%) of our donors were aged between

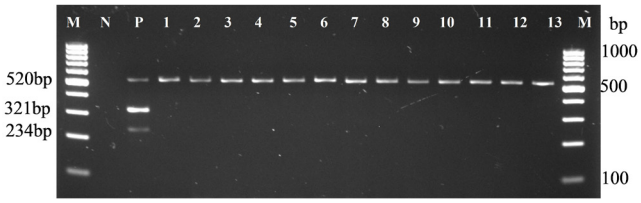

Fig. 1: Triplex PCR analysis of *T. gondii* DNA in blood donors
Lane M: 100bp DNA marker, lane N: Negative control (non-template control), lane P: Positive control (purified DNA of *T. gondii*), lane 1-13: Blood donors samples

Table II: Serological and PCR screening of toxoplasmosis among blood donors

Test	Serological results (n=56) (%)				PCR results (n=56) (%)	
Variables	**IgG (+) IgM (-)**	**IgG (-) IgM (+)**	**IgG (+) IgM (+)**	**IgG (-) IgM (-)**	**(+)**	**(-)**
Samples	24 (42.86)	0 (0.00)	2 (3.57)	30 (53.57)	0 (0.00)	56 (100)
Total	56				56	

***(+) Positive, ** (-) Negative**

18 to 29 years old, males (59.0%), Malay (82.0%), single (55.0%), and employed donors (63.0%). The seropositivity rate was higher in males (61.54%), Malay (82.10%), single (53.85%), and employed donors (80.77%). Nonetheless, only employment factor showed statistically significant with *T. gondii* seropositivity rate (P = 0.009) (Table III). The odd ratios related to *Toxoplasma* seropositivity were calculated based on sociodemographic data. High odd ratios were observed for the older age group (OR = 1.50; 95% CI 0.37-1.19), male (OR = 1.09; 95% CI 0.71-1.68), Malay (OR = 1.06; 95% CI 0.83-1.35), unmarried donors (OR = 1.07; 95% CI 0.59-1.53), and employed donors (OR = 1.73; 95% CI 0.15-0.85).

DISCUSSION

Toxoplasmosis is a transfusion-transmissible infection that can lead to severe complications including mortality in immunocompromised individuals. Even though safe and sufficient blood supply is always under strict regulations and surveillance, transfusion-transmitted infection remains a concern (8). It has been reported that *T. gondii* was transmitted through blood transfusion as the dissemination of *T. gondii* at very early stage of infection occurs via blood flow to other large organs (4,12,21). This preliminary study determined the prevalence of toxoplasmosis among blood donors using serological and molecular methods. Specific *Toxoplasma* IgM, IgG and IgG avidity were detected using ELISA, while the *Toxoplasma* B1 gene and the ITSI region were detected

Table III: Sociodemographic characteristics of blood donors

Characteristics	Frequency (%)	Serology		*P* value	OR
		Positive IgG n (%)	Negative IgG n (%)		
Age (years)					
18 – 29	59	13 (50.0)	20 (66.7)	0.206	0.75
≥ 30	41	13 (50.0)	10 (33.3)		1.50
Gender					
Male	59	16 (61.5)	17 (56.7)	0.71	1.09
Female	41	10 (38.5)	13 (43.3)		0.89
Ethnicity					
Malay	82	22 (84.6)	24 (80.0)	0.73	1.06
Others**	18	4 (15.4)	6 (20.0)		0.77
Marital status					
Married	45	12 (53.8)	13 (43.3)	0.83	0.95
Unmarried	55	14 (46.2)	17 (56.7)		1.07
Employment status					
Unemployed	37	5 (19.2)	16 (53.3)	0.009*	0.36
Employed	63	21 (80.8)	14 (46.7)		1.73

*P <0.05 indicate as significant association between employment status with toxoplasmosis seropositivity rate
** Others includes Chinese, Indian and Siamese

using triplex PCR.

The data on the seroprevalence of toxoplasmosis in Malaysia is scarce, particularly among blood donors. This study found that the seroprevalence of toxoplasmosis among blood donors in Malaysia was 28.1% (57/203), which was detected by *Toxoplasma* IgG antibody (18). Our study showed a high seroprevalence of *T. gondii* infection among blood donors (46.43%). Globally, the high seroprevalence of toxoplasmosis among blood donors has been reported in France, Africa, North India, New Zealand, the Czech Republic, and Egypt with some record up to 50% seropositivity for *T. gondii* infection. Meanwhile, a lower rate of *T. gondii* infection has been reported in Thailand (9.6%), Taiwan (9.3%), China (4.83%), Mexico (7.4%), Turkey (19.5%), and Iran (19.3%) (4,8,12,22). This variation in findings could be due to many factors such as environmental factors, climatic conditions, geographical status, disease control and treatments, regional and ethnic customs, and human activities (23). Furthermore, changes in social and population demographic characteristics also play a role in the risk of transmission.

In this study, one donor showed a high avidity index, indicating as past infection of more than 20 weeks. Meanwhile, another donor showed a low avidity index, indicating as recent infection of less than 20 weeks. However, both blood donors were negative for the presence of *T. gondii* DNA in their blood. The *Toxoplasma* B1 gene and ITS region are highly specific and well conserved for all *T. gondii* strains including from the samples isolated from AIDS patients (24-27). Primers used were tested for specificity in the BLASTN database (http://www.ncbi.nlm.nih.gov/BLAST) and were predicted to be 100% for the *Toxoplasma* B1 gene. *T. gondii* DNA were detected in blood by PCR only when

the infection is in early acute phase with the highest antibody level (28). This could explain the negative result in all the blood donors in this study, which could be due to unknown kinetics of parasitemia in infected people where the short duration of parasitemia or the number of tachyzoites of *T. gondii* circulating in peripheral blood is too low (29). Based on the primer used in this study, its limit of detection (LoD) was as little as 10 pg *T. gondii* DNA (29). In addition, during chronic infection, the tachyzoites reside in tissues as tissue cyst, and thus cannot be freely found in blood. This tissue cyst is commonly found in the central nervous system, eyes, skeletal or cardiac muscles of the host, and causing an inflammatory lesions (permanent damage) in immunocompromised patients or in the fetus (30). The tissue cyst may remain dormant in normal individuals, but it can be reactivated in immunocompromised patients (31).

Based on sociodemographic risk factors, only employment showed a significant association with the toxoplasmosis seropositivity rate. *T. gondii* seropositivity rate was found to be highly significant among employed donors, possibly due to the increased exposure to *T. gondii* by occupation, as our donors worked as butchers, planters, fishermen, and veterinarian officers. However, this finding contradicts from other studies that found no significant difference between employment status and toxoplasmosis seropositivity rate (32-34). Employed donors (OR = 1.73) showed a greater risk of getting infected with *T. gondii* compared to unemployed donors (OR = 0.36). In male-dominated jobs, gardeners and butchers, for example, are exposed for longer periods to contaminated meats and environments (35).

The toxoplasmosis seropositivity rate was found to be equal in both age groups; however, older aged donors showed greater risk to get infected with toxoplasmosis (OR = 1.50). This result could be due to the donors' longer exposure time to the *T. gondii*, which make them more vulnerable to the infection (32). Furthermore, with a weakened immune system, older aged donors are more prone to infection. However, age was not significantly associated with seropositivity rate in this study, which was similar to previous studies (3,6,33,36-39). Male donors showed a greater risk of infection (OR = 1.09) possibly because this group is more active in outdoor activities, sports, or work in which they are exposed to contaminated soil (19). In regard to ethnicity, the highest seropositivity rate was found in Malays and this finding is similar with previous study (19). This could be due to the Malay habits and preferences of keeping cats as pets. Moreover, Malay neighborhood areas are full of stray cats, further exposing the community to *T. gondii* infection (19). Nevertheless, no significant difference between ethnicity and the seropositivity rate of toxoplasmosis was found in this study.

However our study only involves a small sample size. It is good and recommended to have a bigger sample size and a wide range of blood donor from the whole Malaysia to represent blood donors from Malaysia, including Sabah and Sarawak.

CONCLUSION

In conclusion, the preliminary study of seroprevalence rate of toxoplasmosis among blood donors in Kelantan, Malaysia, was 46.43%. However, no active parasitemia was detected in their blood by PCR. This high seropositivity rate indicated that our blood donors had previously been exposed to *T. gondii* infection. The parasites may still remain but in the form of tissue cysts, which reside in certain tissues such as the brain but are not freely circulating in the blood. Therefore, bigger population representing blood donors in Malaysia is recommended and implementing an awareness or education programme among blood donors on the possible transfusion-transmitted diseases needs to be considered in the future.

ACKNOWLEDGEMENTS

This study was funded by the Universiti Sains Malaysia Short-Term Grant (304/PPSG/6315008). We would like to thank the staff of the Central Research and Craniofacial Science Laboratory, staff nurses and doctors of Universiti Sains Malaysia and Hospital USM for their guidance and technical assistance.

REFERENCES

1. Angal L, Ngui R, Amir A, Kamarulzaman A. Toxoplasmosis in HIV and non HIV prisoners in Malaysia. Tropical biomedicine. 2016;33(1):159-69.
2. Berredjem H, Aouras H, Benlaifa M, Becheker I, Djebar MR. Contribution of IgG avidity and PCR for the early diagnosis of toxoplasmosis in pregnant women from the North-Eastern region of Algeria. African Health Science. 2017;17(3):647-57.
3. Sahimin N, Lim YAL, Ariffin F, Behnke JM, Bas6cez M-G, Walker M, et al. Socio-demographic determinants of *Toxoplasma gondii* seroprevalence in migrant workers of Peninsular Malaysia. Parasites & Vectors. 2017;10(238):1-8.
4. Sarkari B, Shafiei R, Zare M, Sohrabpour S, Kasraian L. Seroprevalence and molecular diagnosis of *Toxoplasma gondii* infection among blood donors in southern Iran. Journal of Infection in Developing Countries. 2014;8(4):543-7.
5. Abamecha F, Awel H. Seroprevalence and risk factors of *Toxoplasma gondii* infection in pregnant women following antenatal care at Mizan Aman General Hospital, Bench Maji Zone (BMZ), Ethiopia. BMC Infectious Diseases. 2016;2016(16):1-8.
6. Davami MH, Pourahmad M, Baharlou R, Jahromi AS, Vasmejani AA, Solhjoo K, et al.

Seroepidemiology of *Toxoplasma* infection in blood donors in Jahrom District, Southern Iran. Asian Pacific Journal of Tropical BioMedicine. 2015;5(12):1060-4
7. Dubey JP. Toxoplasmosis of Animals and Humans, Second Edition. Boca Raton: CRC Press; 2016.
8. Foroutan-Rad M, Majidiani H, Dalvand S, Daryani A, Kooti W, Saki J, et al. Toxoplasmosis in Blood Donors: A Systematic Review and Meta-Analysis. Transfusion Medicine Review. 2016;30(3):116-22.
9. Hussain MA, Stitt V, Szabo EA, Nelan B. *Toxoplasma gondii* in the Food Supply. Pathogens. 2017;6(21):1-7.
10. Lass A, Pietkiewicz H, Szostakowska B, Myjak P. The first detection of *Toxoplasma gondii* DNA in environmental fruits and vegetables samples. European Journal of Clinical Microbiology & Infectious Diseases 2012;2012(31):1101-8.
11. Moshfe A, Arefkhah N, Sarkari B, Kazemi S, Mardani A. *Toxoplasma gondii* in Blood Donors: A Study in Boyer-Ahmad County, Southwest Iran. Interdisciplinary Perspectives on Infectious Diseases. 2018;2018:3813612.
12. Alvarado-Esquivel C, Rascon-Careaga A, Hernandez-Tinoco J, Corella-Madueno MA, Sanchez-Anguiano LF, Aldana-Madrid ML, et al. Seroprevalence and Associated Risk Factors for *Toxoplasma gondii* Infection in Healthy Blood Donors: A Cross-Sectional Study in Sonora, Mexico. BioMed Research International. 2016;2016:9597276.
13. Siegel SE, Lunde MN, Gelderman AH, Halterman RH, Brown JA, Levine AS, et al. Transmission of Toxoplasmosis by Leukocyte Transfusion. Blood Journal. 1971;37(4):388-94.
14. Nelson J, Kauffmann D, Ciavarella D, Senisi W. Acquired toxoplasmic retinochoroiditis after platelet transfusions. Annals of Ophthalmology. 1989;21(7):253-4.
15. Arefkhah N, Hosseini SA, Karimzade R, Moshfe A, Hadinia F, Abbasi Larki R, et al. Seroprevalence and risk factors of *Toxoplasma gondii* infection among Cancer and Hemodialysis Patients in southwest Iran. Clinical Epidemiology and Global Health. 2019.
16. Ben-Harari RR, Connolly MP. High burden and low awareness of toxoplasmosis in the United States. Postgraduate Medicine Journal. 2019;131(2):103-8.
17. Ibrahim HM, Mohamed AH, El-Sharaawy AA, El-Shqanqery HE. Molecular and serological prevalence of *Toxoplasma gondii* in pregnant women and sheep in Egypt. Asian Pacific Journal of Tropical Biomedicine. 2017;10(10):996-1001.
18. Nissapatorn V, Kamarulzaman A, Init I, Tan LH, Rohela M, Norliza A, et al. Seroepidemiology of Toxoplasmosis Among HIV-Infected Patients and Healthy Blood Donors. Medical Journal of Malaysia. 2002;57(3).
19. Brandon-Mong GJ, Che Mat Seri NA, Sharma RS, Andiappan H, Tan TC, Lim YA, et al. Seroepidemiology of Toxoplasmosis among People Having Close Contact with Animals. Frontiers in Immunology. 2015;6:143.
20. Nissapatorn V, Lim Y, Jamaiah I, Agnes L, Amyliana K, Wen CC, et al. Parasitic Infections in Malaysia: Changing and Challenges. Southeast Asian Journal of Tropical Medicine and Public Health. 2005;36(4):50-9.
21. Harker KS, Ueno N, Lodoen MB. *Toxoplasma gondii* Dissemination: A Parasite's Journey through the Infected Host. Parasite Immunology. 2015;37(3):141-9.
22. Siransy L, Dasse SR, Dou Gonat SP, Legbedji A, N'Guessan K, Kouacou PA, et al. Immunity Status of Blood Donors Regarding *Toxoplasma gondii* Infection in a Low-Income District of Abidjan, Cote d'Ivoire, West Africa. Journal of Immunology Research. 2016;2016:6830895.
23. Yan C, Liang L-J, Zheng K-Y, Zhu X-Q. Impact of environmental factors on the emergence, transmission and distribution of *Toxoplasma gondii*. Parasites & Vectors. 2016;9(137).
24. Burg JL, Grover CM, Pouletty P, Boothroyd JC. Direct and Sensitive Detection of a Pathogenic Protozoan, *Toxoplasma gondii*, by Polymerase Chain Reaction. American Society for Microbiology. 1989;27(8):1787-92
25. Mesquita RT, Vidal JE, Pereira VL, Chioccola. Molecular diagnosis of cerebral toxoplasmosis: comparing markers that determine *Toxoplasma gondii* by PCR in peripheral blood from HIV-infected patients The Brazilian Journal of Infectious Diseases. 2010;14(4):346-50.
26. Rostami A, Karanis P, Fallahi S. Advances in serological, imaging techniques and molecular diagnosis of *Toxoplasma gondii* infection. Journal of Infection 2018;46(3):303-15.
27. Rahumatullah A, Khoo BY, Noordin R. Triplex PCR using new primers for the detection of *Toxoplasma gondii*. Experimental Parasitology. 2012;131(2):231-8.
28. Rostami A, Kehsavarz H, Shojaee S, Mohebali M, Meamar AR. Frequency of *Toxoplasma gondii* in HIV Positive Patients from West of Iran by ELISA and PCR. Iranian Journal of Parasitology. 2014;9(4):474-81.
29. Ghoneim NH, Shalaby SI, Hassanain NA, Zeedan GSG, Soliman YA, Abdalhamed AM. Comparative Study Between Serological and Molecular Methods for Diagnosis of Toxoplasmosis in Women and Small Ruminants in Egypt. Foodborne Pathogens and Disease. 2010;7(1):17-21.
30. Dunay IR, Gajurel K, Dhakal R, Liesenfeld O, Montoyae JG. Treatment of Toxoplasmosis: Historical Perspective, Animal Models, and Current Clinical Practice. American Society for Microbiology. 2018;31(4):e00057-17.

31. Saki J, Tavakoli S, Pedram M. Seroprevalence and molecular evaluation of toxoplasmosis in children with cancer in Khuzestan province, Southwest of Iran. Journal of Parasitic Diseases. 2017;41(4):947-51.
32. Avelar JB, Silva MGD, Rezende HHA, Storchilo HR, Amaral WND, Xavier IR, et al. Epidemiological factors associated with *Toxoplasma gondii* infection in postpartum women treated in the public healthcare system of Goiania, State of Goias, Brazil. The Journal of the Brazilian Society of Tropical Medicine 2018;51(1):57-62.
33. Tegegne D, Abdurahaman M, Mosissa T, Yohannes M. Anti-Toxoplasma antibodies prevalence and associated risk factors among HIV patients. Asian Pacific Journal of Tropical Biomedicine. 2016;9(5):460-4.
34. Zemene E, Yewhalaw D, Abera S, Belay T, Samuel A, Zeynudin A. Seroprevalence of *Toxoplasma gondii* and associated risk factors among pregnant women in Jimma town, Southwestern Ethiopia. BMC Infectious Diseases. 2012;12(337).
35. Retmanasari A, Widartono BS, Wijayanti MA, Artama WT. Prevalence and Risk Factors for Toxoplasmosis in Middle Java, Indonesia. Ecohealth. 2017;14(1):162-70.
36. Chiang T-Y, Hsieh H-H, Kuo M-C, Chiu K-T, Lin W-C, Fan C-K, et al. Seroepidemiology of *Toxoplasma gondii* Infection among Healthy Blood Donors in Taiwan. PLoS ONE. 2012;7(10):e48139.
37. Daryani A, Sharif M, Meigouni M. Seroprevalence of IgG and IgM anti-Toxoplasma antibodies in HIV/AIDS patients, northern Iran. Asian Pacific Journal of Tropical Biomedicine. 2011;2011:271-4.
38. Elhence P, Agarwal P, Prasad KN, Chaudhary RK. Seroprevalence of *Toxoplasma gondii* antibodies in North Indian blood donors: implications for transfusion transmissible toxoplasmosis. Transfusion and Apheresis Science. 2010;43(1):37-40.
39. Minbaeva G, Schweiger A, Bodosheva A, Kuttubaev O, Hehl AB, Tanner I, et al. *Toxoplasma gondii* infection in Kyrgyzstan: seroprevalence, risk factor analysis, and estimate of congenital and AIDS-related toxoplasmosis. PLoS Negl Trop Dis. 2013;7(2):e2043.