Original Article

Seroprevalence of *Toxoplasmosis* Among Pregnant Women Attending Antenatal Care in Asmara, Eritrea: Preliminary Report

Asmerom Seyoum Tecle¹, Albadawi Abdelbagi Talha², Khalid Abdelsamea Mohamedahmed^{3*}, Adam Dawoud Abakar⁴

¹Department of Parasitology, National Health Laboratory, Asmara, Eritrea

²Department of Clinical Laboratory Sciences, Jouf University of Gezira, Saudi Arabia

³Department of Hematology and Immunology, Faculty of Medical Laboratory Science, University of Gezira, Wad Medani, Sudan

⁴Department of Medical Parasitology, Faculty of Medical Laboratory Science, University of Gezira, Wad Medani, Sudan

Abstract

Introduction: The majority of human infections with *Toxoplasma gondii* produce no symptoms, but in congenitally infected children can cause devastating effects including blindness, brain damage, or miscarriage. Transmission to the fetus occurs predominantly in women who acquire their primary infection during gestation. The study aimed to assess the seroprevalence of toxoplasmosis among pregnant women attending antenatal care (ANC) in different areas of Asmara, Eritrea, and to identify possible risk factors associated with toxoplasmosis among pregnant women attending the ANC centers.

Methods: In this cross-sectional laboratory-based study, the data were collected from 210 pregnant women in four health facilities. Voluntary sampling technique and a structured questionnaire were used to collect the associated data and socio-demographic information. Cobas e411 Analyzer was used to test the blood serum for immunoglobulin G (IgG) and Immunoglobulin M (IgM) antibodies. Epi-Info version 7.0 was used for data entry and SPSS version 20.0 was used for data analysis.

Results: Of the 210 samples, 112 (53.6%) samples were seropositive and 97 (46.4%) samples were seronegative for *T. gondii* specific IgG antibody. Furthermore, 2.9% (6) of the samples were seropositive and 97.1% (203) of the samples were seronegative for *T. gondii*-specific IgM antibodies.

Conclusion: The seroprevalence was considerably high, 53.6% for IgG antibody and 2.9% for IgM antibody, which require attention in order to implement preventive control measures, screening tests, and health education.

Keywords: Seroprevalence, Toxoplasmosis, Risk factors, Pregnant women, Eritrea

Received: November 9, 2020 , Accepted: December 11, 2020 ePublished: December 30, 2020

Introduction

Toxoplasma gondii is an obligate intra-cellular protozoan parasite that can infect most vertebrate animals and causes a disease called Toxoplasmosis (1). It was originally identified in a North African rodent called the gundi, from which it derives its specific name (2,3). Humans can become infected by ingestion of oocysts released from cat feces, consumption of undercooked and raw meat, or drinking of unpasteurized milk containing *T. gondii* tissue cysts (1,4). Transplacental congenital transmission of tachyzoites, blood transfusion, and organ transplantation were very rarely reported (5,6). All stages can be infectious to humans (2). In severely infected women, Toxoplasmosis can be reactivated due to their immuno-compromised state (7). Recently, in immuno-competent individuals, more virulent strains that cause severe symptoms have

been reported (8).

The diagnosis of infection is based on indirect serological tests, direct detection of the parasite, or polymerase chain reaction (PCR). Serologic tests indicating recent or past infection are most effective in immune-competent adults. ECLIA, ELISA, IFA, LA tests, and complement fixation are used to detect immunoglobulin G (IgG) and Immunoglobulin M (IgM) antibodies (9). The presence of a significant IgM titer in the absence of IgG titers indicates early stages of primary infection. The absence of IgM titer is assessed by ruling out recent infection. However, the presence of IgM antibody should be confirmed in a reference laboratory (3,10). Currently, the diagnosis of an active toxoplasmosis of the fetus in utero is made by means of PCR of amniotic fluid (11).

Children above 5 years of age with severe acute

*Corresponding Author: Khalid Abdelsamea Mohamedahmed, Email: khalid.abdelsamea@hotmail.com

infection or active chorioretinitis should be treated with pyrimethamine. Treatments of pregnant women are controversial but treatment with spiramycin is still advocated (12). A recent study confirmed that treating this infection during pregnancy prevents fetal toxoplasmosis (11).

It is noted that no study on the seroprevalence of toxoplasmosis either in general or specific target groups has been done in Eritrea. Nothing is known on what are the associated risk factors for the spread of the disease as well as its causes. Therefore, the purpose of this study was to assess the seroprevalence of toxoplasmosis and its associated risk factors among Eritrean pregnant women attending antenatal care (ANC) centers in Asmara.

Materials and Methods

Study Area

The study was performed in Asmara, Eritrea, at all the health facilities that provide maternal health services, including delivery, one hospital, and other three health facilities.

Eritrea is located in the horn of Africa bordered by the Sudan, Djibouti, Ethiopia, and the Red Sea with 1212 km seashore. It covers an area of 124000 km² and is divided into three topographic areas (the Western Lowlands, the Central Highlands, and the Coastal Plains). Administratively, it is divided into 6 regions or zones, namely Northern Red Sea Zone, Southern Red Sea Zone, Anseba, Gash-Barka, Maekel and Southern Zone (Central Zone). The total population is about 6.1 million (13). Asmara is the targeted area of the study. It is the capital city of Eritrea and located in the central region (Zoba Maekel).

Study Design, Study Population, and Sample Size

In this cross-sectional laboratory-based study, all pregnant women (16-45 years) living in the catchment areas of Asmara administration were enrolled (14,15).

The data were collected between July and August 2016 from 210 pregnant women (in 4 health facilities) attending their ANC follow-up. Voluntary sampling technique was used after obtaining their consent to participate in the research study.

Data Collection Tools and Techniques

A structured questionnaire was used to collect the pregnant women's socio-demographic information and the associated risk factors. Pregnant women were interviewed by the enumerators who were health professionals and worked in the facilities where the data collection was conducted.

Collection of Sample and Laboratory Investigation

In this study, 3 mL of venous blood was collected from study participants using standard procedures in Lithium

heparin container and stored in freezing condition (-20°C) before analysis. Then, serum was prepared by centrifugation at 2500 rpm for 3 minutes.

The electrochemiluminescence immunoassay (ECLIA) was used for in-vitro quantitative measurement of IgG and IgM antibodies to *T. gondii* in serum of human on Cobas e411 immunoassay analyzer and Elecsys.

Quality Control (QC)

PreciControl was used for quality control. Controls for the various concentration ranges were run every day prior to and following each calibration. Based on the measurements of cal-1 and cal-2, the cut-off was calculated.

Interpretation: IgG: >1 (non-reactive), ≥ 1.0 - > 30 (Intermediate), ≥ 30 (Reactive).

IgM: > 0.8 (non-reactive), ≥ 0.8 - >1.0 (Intermediate), ≥ 1 (Reactive).

Data Entry and Data Analysis

Data was collected using questionnaire then followed by data entry into computers using Epi-Info version 7.0, a free software package developed by CDC. To describe the data in terms of frequency and proportion, SPSS version 20.0 was used (16). To describe the age of participants, which is a continuous variable, mean and standard deviation were used (17). In this study, the chi-squared test was applied to screen for an association between seropositivity of IgG and IgM antibodies and several risk factors included in the study (18).

Ethical Issues, Informed Consent, and Confidentiality

This study was approved by the Ethics Committee of the University of Gezira. The Health Research Proposal Review and Ethical Committees, MOH Eritrea accepted and gave a permission to conduct the research on the specified sites. Ethical Permission was obtained from the Ministry of Health, Zoba Makel.

Participants were briefed about the objectives of this study and requested their willingness to participate in the study. The interview was carried out face-to-face to obtain information and the participants were kindly asked for their consent for enthusiastic participation.

Results

Socio-demographic Characteristics of Study Population

Overall, 210 samples of pregnant women were collected from Edagahmus mini Hospital, Akria Health Center, Vilajo/BMCH Community Hospital, and Godief Health Center, constituting 80 (38%), 50 (24%), 41 (20%) and 39 (19%), respectively. Based on the results, 60% of the pregnant women were in the age group of 25-34 years, 27% were in age groups of 15-24 years, and 13% were in the age group of 35-44 years. The mean and median age was 27 and 26 years, respectively with variation of 5 years by SD.

Only 10% of the women were reported to be unmarried, divorced, widowed, or separated while the remaining 90% were married. Moreover, 98% of the participants were from Tigrigna ethnic group. Additionally, 70% of the pregnant women had secondary education, 16% had elementary education, and 14% had post-secondary education. In addition, 74% were housewives, 14% were daily workers, and 12% had other types of jobs. The majority (42%) of the pregnant women were in their second trimester, 37% were in their third trimester, and 21% were in their first trimester (Table 1).

Detection of IgG and IgM Antibodies among Study

Table 1. The Socio-demographic Information of Study Population

Characteristics		Number	Percent
Health facility	Edagahamus mini Hospital	80	38
	Akria Health Center	50	24
	BMCH Community Hospital	41	20
	Godaif Health Center	39	19
Age group	15–24 years	57	27
	25–34 years	125	60
	35–44 years	28	13
Marital status	Married	189	90
	Not married	21	10
Education	Primary	33	16
	Secondary	147	70
	University	30	14
Pregnancy stage	1st trimester	77	36.7
	2nd trimester	88	41.9
	3rd trimester	45	21.4
Occupation	Housewife	155	74
	Daily worker	29	14
	Others	26	12
Ethnic groups	Tigrigna	205	98
	Tigre	2	1
	Saho	1	0.5
	Hidarb	1	0.5

Table 2. Association between IgG and IgM Antibodies

			IgM Status		Total
			Negative (-ve)	Positive (+ve)	
IgG Status	Negative (-ve)	Cases	94	3	97
		Percent	45.0	1.45	46.4
	Positive (+ve)	Cases	109	3	112
		Percent	52.1	1.45	53.6
Total	Cases		203	6	209
	Percent		97.1	2.9	100.0

Population

Of the total 209 pregnant women included in the study, 112 (53.6%) were seropositive and 97 cases (46.4%) were seronegative for *T. gondii* specific IgG antibody. Similarly, 6 samples (2.9%) were seropositive and 203 samples (97.1%) were seronegative (of which 2 samples were on the borderline) for *T. gondii* specific IgM antibody. Out of the total 112 IgG seropositive samples, 3 were also seropositive for IgM, and out of the 97 IgG seronegative samples, 3 were seropositive for IgM (Table 2).

Distribution of IgG and IgM Seropositive Cases Among the Socio-demographic Characteristics and Risk Factors for Toxoplasma

Tables 3 and 4 indicate the prevalence of seropositivity for *T. gondii* in socio-demographic categories (place, age, educational level, marital status, occupation, and stage of pregnancy) using IgG and IgM antibodies. Chi-square test was used to assess the association between *T. gondii* seropositivity and the factors, but no significant association was observed.

Distribution of IgG and IgM Seropositive Cases Among Risk Factors for Toxoplasma Gondii

Based on a different research study, possible risk factors were identified and included in this research study. These factors include source of water, use of raw meat, contact with soil, availability of domestic animals in general, and presence of a cat in particular. In addition to them (3), possible health factors such as history of abortion, HIV status, and presence of mentally abnormal child were also included (Tables 5 and 6). Chi-square test showed some significant associations between *T. gondii* seropositivity and contact with soil, but no significant association was observed for other factors.

Discussion

Several research studies have noted that toxoplasmosis is a food-borne infectious disease which is the third leading cause of death after salmonellosis and listeriosis (19). Though it is highly neglected, congenital *T. gondii* infection particularly is a common problem in communities with a high prevalence of infection. Epidemiological studies

Table 3. Distribution of IgG Antibody Among the Different Characteristics

Socio-demographic Characteristics		IgG Status				Total (N)
		Negative (-ve)		Positive (+ve)		
		No.	%	No.	%	
Health Facility	Edagahamus	45	56.2	35	43.8	80
	Akria	20	40.0	30	60.0	50
	BMCH	16	40.0	24	60.0	41
	Godaif	16	41.0	23	59.0	39
Age group	15–24	32	56.1	25	43.9	57
	25–34	56	44.8	69	55.2	125
	35–44	9	33.3	18	66.7	28
Marital status	Married	84	44.4	105	55.6	189
	Not married	13	65.0	7	35.0	21
Education	Primary	16	50.0	16	50.0	33
	Secondary	68	46.3	79	53.7	147
	Above	13	43.3	17	56.7	30
Occupation	Housewife	68	43.9	87	56.1	155
	Daily worker	17	58.6	12	41.4	29
	Other	12	48.0	13	52.0	26
Pregnancy stage	1st trimester	21	46.7	24	53.3	45
	2nd trimester	43	48.9	45	51.1	88
	3rd trimester	33	43.4	43	56.6	77
Total		97	46.4	112	53.6	210

Table 4. Distribution of IgM Antibody Among the Different Characteristics

Socio-demographic Characteristics		IgM Status				Total (N)
		Negative (-ve)		Positive (+ve)		
		No.	%	No.	%	
Health Facility	Edagahamus	77	96.2	3	3.8	80
	Akria	48	96.0	2	4.0	50
	BMCH	41	100.0	0	0.0	41
	Godaif	38	97.4	1	2.6	39
Age group	15–24	57	100.0	0	0.0	57
	25–34	119	95.2	6	4.8	125
	35–44	28	100.0	0	0.0	28
Marital status	Married	185	97.9	4	2.1	189
	Not married	19	90.5	2	9.5	21
Education	Primary	31	93.9	2	6.1	33
	Secondary	143	97.3	4	2.7	147
	Above	30	100.0	0	0.0	30
Occupation	Housewife	151	97.4	4	2.6	155
	Daily worker	29	100.0	0	0.0	29
	Other	24	92.3	2	7.7	26
Pregnancy stage	1st trimester	43	95.6	2	4.4	45
	2nd trimester	86	97.7	2	2.3	88
	3rd trimester	75	97.4	2	2.6	77
Total		204	97.1	6	2.9	210

Table 5. Distribution of IgG Antibody Among the Different Risk Factors of *Toxoplasma Gondii*

Risk factors		IgG Status				Total	
		Negative (-ve)		Positive (+ve)			
		N	%	N	%	N	%
Source of water	Pipe water	70	46.7	80	53.3	150	71.4
	Well	5	62.5	3	37.5	9	4.3
	Other	22	43.1	29	56.9	51	24.3
Use of raw meat	No	92	46.9	104	53.1	197	93.8
	Yes	5	38.5	8	61.5	13	6.2
Availability of cat	No	71	45.5	85	54.5	157	74.8
	Yes	26	49.1	27	50.9	53	25.2
Availability of other domestic animals	No	73	48.7	77	51.3	150	71.4
	Yes	24	40.7	35	59.3	60	28.6
Contact with soil	No	94	48.2	101	51.8	196	93.3
	Yes	3	21.4	11	78.6	14	6.7
HIV status	No	97	100.	111	99.1	209	99.5
	Yes	0	0.0	1	0.9	1	0.5
Abortion status	No	87	49.2	90	50.8	178	84.8
	Yes	10	31.2	22	68.8	32	15.2
Child with a mental problem	No	91	46.7	104	53.3	195	92.9
	Yes	6	42.9	8	57.1	15	7.1
Total		97	46.4	112	53.6	210	100.0

Table 6. Distribution of IgM Antibody among the Different Risk Factors of *Toxoplasma Gondii*

Risk factors		IgM Status				Total	
		Negative (-ve)		Positive (+ve)			
		No.	%	No.	%	No.	%
Source of water	Pipe water	148	98.7	2	1.3	150	71.4
	Well	9	100.0	0	0.0	9	4.3
	Other	47	92.2	4	7.8	51	24.3
Use of raw meat	No	192	97.5	5	2.5	197	93.8
	Yes	12	92.3	1	7.7	13	6.2
Availability of cat	No	152	96.8	5	3.2	157	74.8
	Yes	52	98.1	1	1.9	53	25.2
Availability of other domestic animals	No	147	98.0	3	2.0	150	71.4
	Yes	57	95.0	3	5.0	60	28.6
Contact with soil	No	190	96.9	6	3.1	196	93.3
	Yes	14	100.0	0	0.0	14	6.7
HIV status	No	203	99.5	6	100.0	209	99.5
	Yes	1	0.5	0	0.0	1	0.5
Abortion status	No	173	97.2	5	2.8	178	84.8
	Yes	31	96.9	1	3.1	32	15.2
Child with a mental problem	No	190	97.4	5	2.6	195	92.9
	Yes	14	93.3	1	6.7	15	7.1
Total		204	97.1	6	2.9	210	100.0

reporting the prevalence of toxoplasmosis in pregnant women across the world suggest considerable variation between areas, ranging between 9% and 67% in Europe and reaching

as high as 92.5% in African countries (6). Therefore, conducting a study on the seroepidemiology of *T. gondii* infection among women of childbearing age

may give appropriate information which could help to design preventive measures (3).

A recent study showed a seroprevalence of 53.6% among 210 pregnant women in Asmara. Even though these pregnant women were not at risk of getting *T. gondii* infection, because they have already developed IgG antibodies, the remaining 46.4% were at risk of getting the infection. Additionally, the seroprevalence of toxoplasmosis was noted to be 2.9%, which is lower compared with many countries. In Saudi Arabia, the prevalence of *T. gondii* IgG and IgM antibodies during pregnancy was 32.5% and 6.4%, respectively (14).

As it was indicated, the study was done on pregnant women in Asmara. Asmara is the capital city of Eritrea where the majority of its population have better access to different social services such as access to clean water for drinking, sanitation and hygiene, health, and education (15). Had it been conducted in other rural parts of the countries where access to social services is relatively low, the seroprevalence would have been increased.

In this study, the seroprevalence of IgG antibody among the pregnant women was 53.6%, which is higher compared to others studies done in other countries. For instance, the prevalence was 30.9% in Tanzania (20), 15.2% in Eastern China (6), 27% in Sudan (9), and 43.8% in France (21) and lower compared to other countries such as Cameroon with 77.1% in 1992, Ethiopia with 80.0% in 1998, Argentina with 72% in 2001, Indonesia with 58% in 2000 (22) and Kinshasa, D.R of Congo with 80.3% (23).

Though, the result revealed that there was no association between seropositivity of *T. gondii* and different age groups, the seropositivity rate was obviously higher in the age group of 35-44 years compared to other two groups. Despite small variation in age groups, the result is in line with studies from other countries (20,24). However, a comparative study done on pregnant women in Malaysian and Myanmar has identified that *T. gondii* seropositivity was found to have an association with the age group of 30 years and above (18). The study conducted among the pregnant women in Malaysia has also identified that mothers' education, gestational age, gravida, awareness of disease, obstetric history, having contact with soil or cats, consumption of undercooked meat, use of untreated water, and consumption of unpasteurized milk have a relationship with toxoplasmosis. Though this is the first study ever done on toxoplasmosis in the country, the result showed that the seroprevalence of toxoplasmosis was considerably high (53.6%) using IgG antibody. When IgM antibody was considered, the disease prevalence was noted to be 2.9%. It has been noted that the areas of the study were located in Asmara where the exposure to risk factors is relatively minimal. Therefore, even considering this, the result was higher which requires attention. The results also showed that except for contact with soil, none of the risk factors included in the study had any significant

association with the disease pattern.

Conclusion

The absence of a statistically significant association between the prevalence of toxoplasmosis and many of the studied risk factors does not necessarily mean that these factors have no relationship with the transmission of *T. gondii* infection. This could be due to the test method which demands a higher number of disease cases. Probably a larger sample size would have revealed significant relationships.

Conflict of Interests

In this study, we declare that we have no conflict of interests.

Ethical Issues

In this study, ethical considerations have been fully observed.

Acknowledgment

We would like to acknowledge the staff of Antenatal Care (ANC) in Asmara City, Eritrea and all the patients who participated in the study.

Authors' Contribution

AST and AAT designed the study and did data collection. AST, AAT and KAM did writing of the original draft. KAM and ADA did editing and reviewing of the original article.

Funding

No financial support was received for the original article from any funding agencies.

References

1. Gilot-Fromont E, Lélou M, Dardé ML, et al. The Life Cycle of *Toxoplasma gondii* in the Natural Environment. IntechOpen; 2012. doi:10.5772/48233
2. John DT, Petri WA. Markell and Vogle's Medical Parasitology. 9th ed. St. Louis: Elsevier Inc; 2006.
3. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 1. 2nd ed. Cambridge University Press; 2009:300-302.
4. Food Standards Agency. Annual Report and Consolidated Accounts 2012/13. Food Standards Agency; 2013.
5. Dubey JP, Jones JL. *Toxoplasma gondii* infection in humans and animals in the United States. Int J Parasitol. 2008;38(11):1257-1278. doi:10.1016/j.ijpara.2008.03.007
6. Cong W, Dong XY, Meng QF, et al. *Toxoplasma gondii* infection in pregnant women: a seroprevalence and case-control study in Eastern China. Biomed Res Int. 2015;2015:170278. doi:10.1155/2015/170278
7. Montoya JG, Remington JS. Management of *Toxoplasma gondii* infection during pregnancy. Clin Infect Dis. 2008;47(4):554-566. doi:10.1086/590149
8. Tekkesin N. Diagnosis of toxoplasmosis in pregnancy: a review. HOAJ Biol. 2012;1(1):1-8. doi:10.7243/2050-0874-1-9
9. Mustafa M, Fathy F, Mirghani A, et al. Prevalence and risk factors profile of seropositive *Toxoplasmosis gondii* infection among apparently immunocompetent Sudanese

- women. BMC Res Notes. 2019;12(1):279. doi:10.1186/s13104-019-4314-0
10. Agmas B, Tesfaye R, Koye DN. Seroprevalence of *Toxoplasma gondii* infection and associated risk factors among pregnant women in Debre Tabor, Northwest Ethiopia. BMC Res Notes. 2015;8:107. doi:10.1186/s13104-015-1083-2
11. Paquet C, Yudin MH. Toxoplasmosis in pregnancy: prevention, screening, and treatment. J Obstet Gynaecol Can. 2013;35(1):78-81. doi:10.1016/s1701-2163(15)31053-7
12. Satoskar AR, Simon GL, Hotez PJ, Tsuji M. Medical Parasitology. Austin, Texas: Landes Bioscience; 2009:129-135.
13. WHO, UNICEF, UNFPA, World Bank Group and United Nations Population Division Maternal Mortality Estimation Inter-Agency Group (2015) Maternal mortality in 1990-2015: Eritrea. Available at http://www.who.int/gho/maternal_health/countries/eri.pdf?ua=1. Accessed May 16, 2017.
14. Alghamdi J, Elamin MH, Alhabib S. Prevalence and genotyping of *Toxoplasma gondii* among Saudi pregnant women in Saudi Arabia. Saudi Pharm J. 2016;24(6):645-651. doi:10.1016/j.jsps.2015.05.001
15. National Statistics and Evaluation Office (NSEO) (Eritrea) and ORC Macro (2003) Eritrea Demographic and Health Survey 2002, p.197. Calverton, Maryland, USA: National Statistics and Evaluation Office and ORC Macro. Available at <http://dhsprogram.com/what-we-do/survey/survey-display-170.cfm>. Accessed May 22, 2017.
16. Bruce N, Pope D, Stanistreet D. Quantitative Methods for Health Research: A Practical Interactive Guide to Epidemiology and Statistics. 2nd ed. Chichester, UK: John Wiley & Sons Ltd; 2018.
17. Petrie A, Sabin C. Medical Statistics at a Glance. 4th ed. USA: Wiley-Blackwell; 2019.
18. Steward A. Basic Statistics and Epidemiology: A Practical Guide. 4th ed. United Kingdom: Radcliffe Medical Press Ltd; 2016.
19. Mehlhorn H. Human Parasites: Diagnosis, Treatment, Prevention. Switzerland: Springer International Publishing; 2016. doi:10.1007/978-3-319-32802-7
20. Mwambe B, Mshana SE, Kidenya BR, et al. Sero-prevalence and factors associated with *Toxoplasma gondii* infection among pregnant women attending antenatal care in Mwanza, Tanzania. Parasit Vectors. 2013;6:222. doi:10.1186/1756-3305-6-222
21. Ghazaei C. Serological survey of antibodies to *Toxoplasma gondii*. Afr J Health Sci. 2006;13(1-2):131-134.
22. Murebwayire E, Njanaake K, Ngabonziza JC, Jaoko W, Njunwa KJ. Seroprevalence and risk factors of *Toxoplasma gondii* infection among pregnant women attending antenatal care in Kigali, Rwanda. Tanzan J Health Res. 2017;19(1):1-8. doi:10.4314/thrb.v19i1.2
23. Doudou Y, Renaud P, Coralie L, et al. Toxoplasmosis among pregnant women: high seroprevalence and risk factors in Kinshasa, Democratic Republic of Congo. Asian Pac J Trop Biomed. 2014;4(1):69-74. doi:10.1016/s2221-1691(14)60211-2
24. Kolbekova P, Kourbatova E, Novotna M, Kodym P, Flegr J. New and old risk-factors for *Toxoplasma gondii* infection: prospective cross-sectional study among military personnel in the Czech Republic. Clin Microbiol Infect. 2007;13(10):1012-1017. doi:10.1111/j.1469-0691.2007.01771.x

© 2020 The Author(s); This is an open-access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.