

Original Article

Seroprevalence of Anti-*Toxoplasma Gondii* Antibodies in Healthy Voluntary Blood Donors from Mashhad City, Iran

Mehdi Zarean PhD^{1,2,3}, Reza Shafiei PhD^{4*}, Maryam Gholami MD⁵, Abdolmajid Fata DVM PhD^{2,3}, Mansour Rahmati Balaghaleh MSc², Ashraf Kariminik PhD¹, Farahnaz Tehranian MD⁶, Ali Hasani MD⁶, Arash Akhavan MD⁷

Abstract

Objective: The present investigation aims to evaluate the prevalence of IgM and IgG anti-*T. gondii* antibodies and the associated risk factors among healthy blood donors in Mashhad city, Razavi Khorasan province, Iran.

Methods: We screened a total of 500 serum samples by census method from apparently healthy blood donors of the Mashhad Blood Transfusion Organization (MBTO) for IgG and IgM anti-*T. gondii* antibodies by enzyme linked immunosorbent assay (ELISA).

Results: We found that 29.6%, 25%, 1.4%, and 3.2% of tested donors were positive for anti-*T. gondii* antibodies, only IgG antibody, both IgM and IgG, and IgM antibody alone, respectively. Several risk factors which were significantly related to *T. gondii* seropositivity in the univariate analysis at $P < 0.05$ included female gender (OR = 3.222, $P < 0.001$), age more than 40 years ($P = 0.026$), and sausage/hot dog consumption (OR = 4.472, $P < 0.001$).

Conclusions: The results of this study can be a warning for blood transfusion organizations to pay special attention to toxoplasmosis among blood donors and also design screening programs for prevention of transfusion-transmitted toxoplasmosis.

Keywords: Blood transfusion, IgG antibody, IgM antibody, Mashhad, prevalence, toxoplasmosis

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Introduction

Toxoplasmosis is a worldwide distributed zoonosis, caused by *T. gondii*, an intracellular protozoan parasite belonging to the phylum of Apicomplexa.^{1,2} Humans infection is caused by ingestion of undercooked meat containing tissue cysts, or food and water contaminated by sporulated oocysts, congenitally, from mother to fetus across the placenta, and blood transfusion or organ transplantation from seropositive donors to susceptible recipients.^{3,4} Toxoplasmosis is usually asymptomatic and self-limiting among immunocompetent people; however, it can cause severe diseases such as stillbirth, hydrocephalus, microcephalus, and cerebral calcification in developing fetus and retinochoroiditis, encephalitis, pneumonia, and disseminated systemic disease in immune-compromised patients.^{1,5} Several studies have shown parasitic seroprevalence in Iran.⁶⁻⁸ Seroprevalence of toxoplasmosis varies widely between different countries (10% – 80%), depending on social and cultural habits, geographic factors, climate, and transmission routes.⁹ In Iran,

seroprevalence of toxoplasmosis ranges from about 18% to 70% in various geographical regions; in the humid mild northern, cold and mountainous northwestern and western, dry and mild mountainous western, warm and dry central, and warm and humid southern regions, the seroprevalence has been about 70%, 18% – 38%, 33% – 68%, 39%, and 20% – 35%, respectively.¹⁰⁻¹² These results suggest that a considerable portion of blood donors are at risk of toxoplasmosis; therefore, it is necessary to investigate the seroprevalence of toxoplasmosis in donor population and adopt proper strategies to decrease the risk. Several studies have reported the seroprevalence of *T. gondii* antibodies among blood donors in different regions of the world.¹³⁻¹⁵ However, there are few studies on the seroprevalence of toxoplasmosis in healthy blood donors of Iran.¹³⁻²¹ Thus, the objective of the current cross-sectional study was to investigate the seroprevalence of IgG and IgM anti-*T. gondii* antibodies and the associated risk factors among healthy blood donors in Mashhad, northeast of Iran.

Materials and Methods

Study design

This cross-sectional study by census method was carried out in the blood centers of Mashhad Blood Transfusion Organization (MBTO) in Razavi Khorasan province, Iran. Mashhad is the second most populous city (nearly 3,150,000 people) in Iran and the capital of Razavi Khorasan Province. It is located in the northeast of the country, close to the borders of Turkmenistan and Afghanistan. Mashhad features a steppe climate with hot summers and cool winters.

Authors' affiliations: ¹Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran, ²Department of Parasitology and Mycology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, ³Cutaneous Leishmaniasis Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, ⁴Vector-borne Diseases Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran, ⁵Student Research Committee, Sabzevar University of Medical Sciences, Sabzevar, Iran, ⁶Research Center of Iranian Blood Transfusion Organization, Khorasan Razavi, Mashhad, Iran, ⁷Student Research Committee, Golestan University of Medical Sciences, Gorgan, Iran.

***Corresponding author and reprints:** Reza Shafiei PhD, Vector-borne Diseases Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran. Tel: +98-915-7029331, E-mail: R.Shafiei@nkums.ac.ir, reza_shafi@yahoo.com. Accepted for publication: 14 June 2017

Ethics

This study was approved by the Ethics Committee of Islamic Azad University of Medical Sciences, Kerman Branch. In addition, written informed consent was obtained from all participants before blood sampling.

Sample collection and participants

A total of 500 serum samples were collected from apparently healthy blood donors who referred to the blood centers of MBTO in Mashhad, Iran, from May to November 2013. None of the blood donors were seropositive for human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus surface antigen (HBsAg), or *Treponema pallidum* infection.

Questionnaire

Before collection of blood samples, a questionnaire was applied to inquire about demographic data including age, gender, education, residence, and blood group. Moreover, possible risk factors, such as animal contacts (cats), raw/half-cooked meat consumption (fish, lamb, and beef), sausage/hot dog consumption, raw-milk/egg consumption and blood transfusion were also assessed.

Enzyme-linked immunosorbent (ELISA) test

To detect the anti-*T. gondii* antibodies, serum samples were transported to Parasitology Laboratory, Department of

Parasitology and Mycology, Jundishapur University of Medical Sciences (Ahvaz, Iran) and stored at -20°C until tested. All serum samples were tested using the commercially available ELISA kit (Radim, Italy). Analyses were performed following the manufacturer's instructions. According to the ELISA kit, positive results for IgG and IgM were defined as index values of ≥ 0.55 and ≥ 0.3 , respectively. The range of equivocal results was index values between $0.45 - 0.55$ and $0.2 - 0.3$ for IgG and IgM antibodies, respectively. Also, negative results were defined as index values of < 0.45 and < 0.2 for IgG and IgM, respectively.

Statistical analyses

Statistical analysis was carried out using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Chi-square and Fisher exact test were used to compare the seroprevalence values based on the characteristics of the subjects. In this survey, $P < 0.05$ was considered to be statistically significant.

Results

Participants

Totally, 500 blood donors were included in this investigation; the participants' mean age was 29.7 years (ranging from 18 to 61 years). Most participants were male ($n = 448$, 89.6%), living in urban areas, who had college education or above.

Table 1. Correlation between demographic characteristics and IgG anti *T.gondii* antibodies among healthy blood donors in Mashhad, Razavi Khorasan province, Iran

Risk factor	Seropositive antibodies		P	OR	95% CI	
	No	Yes			Lower	Upper
Sex			< 0.001	3.222	1.79	5.799
Male	348 (%77.7)	100 (%22.3)				
Female	27 (%51.9)	25 (%48.1)*				
Age groups (yr)						
< 20	79 (%78.2)	22 (%21.8)*	0.018	2.873	1.175	7.024
21-40	281 (%75.5)	91 (%24.5)*	0.026	2.47	1.115	5.471
> 40 (Reference)	15 (%55.6)	12 (%44.4)				
Residential place						
Urban	305 (%75.7)	98 (%24.3)	1.2	0.473	0.729	1.977
Rural	70 (%72.2)	27 (%27.8)				
Education						
Less than diploma	198 (%71.2)	80 (%28.8)*	0.030	1.589	1.047	2.413
Diploma and above	177 (%79.7)	45 (%20.3)				
Contact with cat						
Yes	44 (%73.3)	16 (%26.7)	0.751	1.104	0.599	2.036
No	331 (%75.2)	109 (%24.8)				
Raw/half-cooked meat consumption						
Yes	37 (%74)	13 (%26)	0.863	1.06	0.544	2.066
No	338 (%75.6)	112 (%24.4)				
Sausage/hot dog consumption						
Yes	18 (%43.9)	23 (%56.1)*	<0.001	4.472	2.322	8.609
No	357 (%77.8)	102 (%22.2)				
Raw-milk/egg consumption						
Yes	19 (%73.1)	7 (%26.9)	0.816	1.112	0.456	2.710
No	356 (%75.1)	118 (%24.9)				
Blood transfusion						
Yes	4 (%66.7)	2 (%33.3)	0.643	1.508	0.273	8.335
No	371 (%75.1)	123 (%24.9)				

* $P < 0.05$, difference is statistically significant; OR: Odds Ratio

Seroprevalence of anti-*T. gondii* antibodies

A total of 148 samples (29.6%, CI: 0.26 – 0.34) tested seropositive for anti-*T. gondii* antibodies; 125 donors (25%, CI: 0.2 – 0.3) tested seropositive for only IgG antibody, 7 (1.4%, CI: 0.004 – 0.024) tested seropositive for both IgM and IgG and 16 (3.2%, CI: 0.017 – 0.047) were positive for IgM antibody alone. Regarding gender, the seroprevalence of anti-*T. gondii* IgG antibody in male donors was significantly higher than female donors. Twenty-five (20%) female donors were positive for IgG anti-*T. gondii* antibodies compared to 100 (80%) male donors ($P < 0.05$) (Table 1). However, there is no significant difference in the prevalence of IgM anti-*T. gondii* among the female and male donors ($P = 0.678$) (Table 2). Considering age, the Chi-square test for trend showed a significant difference in the prevalence of IgG ($P = 0.026$) and IgM ($P = 0.426$) anti-*T. gondii* antibodies among different age groups (Table 1 and 2).

Risk factors of anti-*T. gondii* antibodies

Several risk factors which were significantly related to *T. gondii* seropositivity in the univariate analysis at $P < 0.05$ included female gender ($P < 0.001$), age above 40 years ($P = 0.026$), and sausage/hot dog consumption ($P < 0.001$). However, other demographic and risk factors of the blood donors did not show any association with *T. gondii* seropositivity (Table 1).

Discussion

Toxoplasma gondii infection is considered one of the neglected parasitic infections—a group of five parasitic diseases that have been targeted by CDC for public health action. Toxoplasmosis, as a transfusion transmissible disease, can lead to serious clinical symptoms in immunocompromised patients, blood recipients, especially in multiply transfused patients, pregnant women, fetus, etc.¹² Considering the fact that existing therapies not fully effective and there is no effective vaccine available, and also considering the almost high rate of *T. gondii* in the Iranian general population and the fact that screening laboratory test are not performed on healthy volunteer blood donor population, it is crucial to make efforts for reducing toxoplasmosis transmission to decrease its severe complication. The ELISA method is the best method for detection of anti-*T. gondii* antibodies in most studies.

The findings of the present study show that 31.6%, 25%, 1.6%, and 5% of donors were positive for anti-*T. gondii* antibodies, only IgG antibody, both IgM and IgG, and IgM antibody alone, respectively. In another study in Iran, Ferdowsi, et al.¹⁶ showed 2.3% of donors with IgM antibody positivity in Gonabad, Jafari Modrek, et al.¹⁸ reported 25% of donors with IgG antibody positivity in Zahedan, Shaddel, et al.²⁰ and Sarkari, et al.¹⁵ indicated 23.2% and 12.3% with IgG positivity and 0.4% and 5.47% with IgM positivity in Shiraz, Ormazdi, et al.¹⁹ showed that 52.8% and

Table 2. Correlation between demographic characteristics and IgM anti *T.gondii* antibodies among healthy blood donors in Mashhad, Razavi Khorasan province, Iran

Risk factor	Seropositive antibodies		P	OR	95% CI	
	No	Yes			Lower	Upper
Sex						
Male	434 (%96.9)	14 (%3.1)	0.678	1.24	0.274	5.614
Female	50 (%96.2)	2 (%3.8)				
Age groups (year)						
< 20	96 (%95.1)	5 (%4.9)	1	1.354	0.152	12.104
21–40	366 (%98.4)	6 (%1.6)	0.39	0.426	0.049	3.674
> 40 (Reference)	26 (%96.3)	1 (%3.7)				
Residential place						
Urban	390 (%96.8)	13 (%3.2)	1	1.044	0.292	3.739
Rural	94 (%96.9)	3 (%3.1)				
Education						
Less than diploma	270 (%97.1)	8 (%2.9)	0.647	0.793	0.293	2.146
Diploma and above	214 (%96.4)	8 (%3.6)				
Contact with cat						
Yes	50 (%83.3)	10 (%16.7)*	<0.001	14.467	5.044	41.49
No	434 (%98.4)	6 (%1.4)				
Raw/half-cooked meat consumption						
Yes	47 (%94)	3 (%6)	0.209	2.146	0.59	7.802
No	437 (%97.1)	13 (%2.9)				
Sausage/hot dog consumption						
Yes	36 (%87.8)	5 (%12.2)	0.06	5.657	1.864	17.169
No	448 (%97.6)	11 (%2.4)				
Raw-milk/egg consumption						
Yes	22 (%84.6)	4 (%15.4)	0.07	7	2.088	23.468
No	462 (%97.5)	12 (%2.5)				
Blood transfusion						
Yes	6 (%100)	0	0.999	0	0	0
No	478 (%96.8)	16 (%3.2)				

* $P < 0.05$, difference is statistically significant; OR: Odds Ratio

3.6% of donors in Tehran had IgG and IgM positivity, Zainodini, et al.²¹ showed that 34.04% and 1.71% of donors in Rafsanjan had IgG and IgM positivity, and Mahmoudvand, et al.¹⁴ showed that 28.8% and 3.2% of donors in Kerman had IgG and IgM anti-*T. gondii* antibodies. Our results revealed that the seroprevalence of *T. gondii* was similar to those reported in Czech Republic²² and Mexico;²³ while it is higher than those reported previously in Turkey,²⁴ Mexico,²⁵ India,²⁶ northeast of Thailand,²⁷ and Taiwan.²⁸ Interestingly, this seroprevalence was less than the one reported among blood donors in northeast of Brazil,²⁹ north of India,³⁰ and Egypt,³¹ where the seroprevalence of *T. gondii* has varied from 50% to 75%. Reviews have reported that this difference in the seroprevalence of *T. gondii* among blood donors might be related to some factors such as sociocultural habits, geographical and environmental factors, transmission routes in the studied population, sample size, and methodology.^{25,31}

The obtained findings revealed that the seroprevalence of *T. gondii* in male donors was significantly higher than females. Similarly, Elhence, et al.³⁰ have reported similar results; however, Sundar, et al.²⁶ and Ormazdi, et al.¹⁹ have reported that the seroprevalence of *T. gondii* in male donors was significantly higher than females. This significant difference could be attributed to greater exposure of females to oocysts and tissue cysts during their daily activities. Our results show that the rate of seropositivity increased with age as a consequence of increased opportunity for exposure; this finding was in agreement with those reported in other studies.¹⁴

The obtained results suggest that sausage/hot dog consumption is a significant risk factor for *T. gondii* seropositivity; among the blood donors in the present investigation, the ingestion of tissue cysts in meat (food-borne transmission) seems to be the main route of infection, similar to the infection routes reported in other studies.^{14,32,33} In the present study, no difference was found between individuals based on their education, raw-milk/egg consumption, contact with cats, consumption of raw vegetables, and agricultural activities. Here, in line with previous investigations, we identified that sausage/hot dog consumption and raw/half-cooked meat consumption were associated with the seropositivity of *T. gondii*.^{25,34}

In conclusion, our findings indicate that *T. gondii* infection was prevalent among healthy blood donors in Mashhad, Iran. The obtained findings also showed that ingestion of tissue cysts is the main route of infection in the blood donors of this region. Therefore, toxoplasmosis may be a significant blood transfusion risk in Mashhad, Iran. The results of this study can be a warning for blood transfusion organizations to pay special attention to toxoplasmosis among blood donors and also design screening programs for prevention of transfusion-transmitted toxoplasmosis.

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