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Seroprevalence and risk factors of *Toxoplasma gondii* infection among healthy blood donors in southeast of Iran

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Abstract

This prospective cross-sectional study was aimed to evaluate the prevalence of IgM and IgG anti-*T. gondii* antibodies and the associated risk factors among healthy blood donors in Kerman province, southeastern Iran. Structured questionnaires (before the donors gave blood) were used to obtain information on risk factors for infection. Totally, 500 serum samples from healthy blood donors of Kerman Blood Transfusion Organization (KBTO) at Kerman, Iran, were screened for IgG and IgM anti-*T. gondii* antibodies by enzyme linked immunosorbent assay (ELISA) and Roche Elecsys Toxo IgM assay. Real-Time PCR was used to detect DNA of *T. gondii* in the IgM-positive samples. Seroprevalence of IgG and IgM anti-*T. gondii* antibodies was 28.8% and 3.2%, respectively. In the multiple logistic regression, it could be observed that living in rural regions, having B blood type, being in contact with cats, consuming raw vegetables and raw-milk/egg and doing agricultural activities were independent risk factors for *Toxoplasma* seropositivity. *T. gondii* DNA was also found in one (9.0%) of IgM-positive samples. In this study, it was found that *T. gondii* infection was present among healthy blood donors in southeast of Iran. Therefore, it is suggested to design screening programs for preventing transfusion-transmitted toxoplasmosis.

Keywords: Toxoplasmosis; Blood transfusion; IgG antibody; IgM antibody, Real Time PCR

INTRODUCTION

Toxoplasma gondii is a ubiquitous obligatory intracellular coccidian protozoan organism found throughout the world that infects a wide range of warm-blooded animals and approximately one-third of the world's human population (1). Seroprevalence of this infection varies widely between different countries (10–80%), depending on social and cultural habits,

geographic factors, climate, and transmission routes (2). The majority of toxoplasmosis among immunocompetent people is usually asymptomatic and self-limiting; however, severe diseases and complications can occur in immunocompromised individuals such as transplant recipients and patients with acquired immunodeficiency syndrome and also congenitally infected children (3-6). Humans can be typically infected by three main routes of transmission: [1] ingestion of tissue cysts in raw or undercooked infected meat, [2] ingestion of food or water contaminated with sporulated oocysts shed in the feces of an infected cat, and [3] congenitally, vertical transmission from mother to fetus across the placenta when she is formerly infected through one of the above two routes during pregnancy (7, 8). In addition, *T. gondii* infection can be transmitted by the whole blood or white blood cell transfusions or organ transplantation from seropositive donors to susceptible recipients (9-11). Since a considerable portion of blood donors is at the risk of toxoplasmosis, it is necessary to determine the seroprevalence in donor population so that proper strategies can be adopted to decrease the risk. So far, in various studies, the prevalence of *T. gondii* antibodies among blood donors has been reported in different regions of the globe (12-23). Nevertheless, the prevalence of toxoplasmosis in healthy blood donors of Iran remains uncertain. This cross-sectional study was aimed to evaluate the prevalence of IgM and IgG anti-*T. gondii* antibodies and the associated risk factors among healthy blood donors in Kerman province, southeast of Iran.

MATERIALS AND METHODS

Ethics

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

Study design

A prospective cross-sectional study was performed in the five biggest blood centers of Kerman Blood Transfusion Organization (KBTO) in Kerman province, Iran. This province covers an area of 181714 km² with the population of nearly 2,700,000 and is located in the southeast of Iran (Fig. 1). It is the largest province and includes 11% of the total area of the country. The major cities in this province are Kerman, Sirjan Jiroft, Rafsanjan, and Bam. Its climate varies in different regions, depending on the land relief. Northern, northwestern, and central areas (Kerman, Shahr-e-Babak, Baft, and Sirjan) experience a dry and moderate climate, whereas southern and southeastern (Bam and Jiroft) parts have warm and relatively humid weather.

Sample collection and participants

Totally, 500 serum samples were collected two days in week from apparently healthy blood donors of referred the five biggest blood centers of KBTO in Kerman, Iran, during the period from May to November 2014. All the samples from blood donors were routinely tested in terms of antibodies against human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus surface antigen (HBsAg), and *Treponema pallidum* infection. None of the blood donors were seropositive for HIV, HCV, HBsAg, and *T. pallidum*.

Questionnaire

The applied questionnaire (before the donors gave blood) was based on 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), consumption of raw vegetables and raw egg/milk, gardening or agriculture activity, and blood transfusion were also evaluated.

Serologic tests

Enzyme-linked immunosorbent (ELISA) test

To evaluate the anti-*T.gondii* antibodies, serum samples were transported from the five blood centers to Parasitology Laboratory, Department of Parasitology and Mycology, Kerman University of Medical Sciences (Kerman, Iran) and stored at -20°C until being tested. All the serum samples were tested using the commercially available ELISA kit (Dia.Pro, Italy). Analyses were performed following the manufacturer's instructions. On the basis of the ELISA kit, positive results for IgG and IgM were defined as values of ≥ 500 international units (IU)/ml and index values of ≥ 0.6 , respectively. Range of equivocal results was from 250 to 500 IU/ml and index values of 0.5 to 0.6 were assumed for IgG and IgM, respectively. Also, negative results were defined as < 250 IU/ml and index values of < 0.5 were considered for IgG and IgM, respectively.

Roche Elecsys Toxo IgM assay

Considering low specificity of IgM in the ELISA test, all IgM-positive samples were analyzed by Roche Elecsys Toxo IgM assay (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's recommendations. This automated system is based on an electrochemiluminescence immunoassay and is intended for use on Roche Elecsys and Roche “cobas e” immunoassay analyzers for the in vitro quantitative determination of IgM antibodies to *T. gondii* in human serum. Results are expressed as a ratio (cutoff index—signal sample/cutoff) in the IgM assay. Interpretation of Roche results was based on the manufacturer's criteria which were classified as follows: < 0.8 as nonreactive, ≥ 0.8 to < 1.0 as indeterminate, and ≥ 1.0 as reactive.

Molecular diagnosis

Real-Time PCR was used to detect DNA of *T. gondii* in the IgM-positive samples. DNA from blood samples was isolated using a high Pure PCR Template Preparation Kit (Roche Applied Sciences, Germany) according to the manufacture's protocol. Real-time PCR was performed using the iQ5 real-time PCR detection system (Bio-Rad, Hercules, CA) and SYBR green was used to detect amplification products. The primer used (Invitrogen) amplified a 126 bp B1 gene region. The forward and reverse sequences were 5'-GGAGGACTGGCAACCTGGTGTTCG -3' and 5'-TTGTTTCACCCGGACCGTTTAGCAG -3', respectively. As positive control, *T. gondii* genomic DNA, serially 10 fold diluted and ranging from 5000 to 0.5 parasites per microliter (TIB MolBiol), and a negative control, prepared by the substitution of template DNA with distilled water, were used in each Real-Time PCR run. Finally, Data analysis was performed using iQTM5 optical system software (Bio-Rad).

Statistical analyses

Analytical and descriptive statistics was carried out using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were reported in terms of percent (for categorical) and mean (SD) (for continuous) variables. Chi-square test was applied to access the univariate association between independent variables and outcome. Multifactorial Logistic regression models were used to evaluate association between *T. gondii* seropositivity and the potential risk factors.

RESULTS

Participants

A total of 500 blood donors were included in the present study; the mean age of the participants was 28.9 years old (ranging from 18 to 57 years old). Most participants were male (91.0%), aged 18-25 years old, living in urban areas, who had college education or above.

Seroprevalence of anti-*T. gondii* antibodies

Out of the 500 blood donors, 160 (32.0%) tested seropositive for anti-*T. gondii* antibodies; 144 (28.8%) donors tested seropositive for only IgG antibody, 5 (1.0%) tested seropositive for both IgM and IgG and 11 (2.2%) were positive for IgM antibody alone. Thus, the prevalence rates of IgG and IgM anti-*T. gondii* antibodies were 28.8% and 3.2%, respectively. In terms of geographical region, seroprevalence of anti-*T. gondii* IgG antibody in five blood centers including Kerman, Sirjan, Rafsanjan, Bam, and Jiroft were 32.6%, 29.2%, 27.7%, 20.9%, and 31.7%, respectively. For gender, seroprevalence of anti-*T. gondii* IgG antibody was significantly higher among female donors. Twenty (44.4%) female donors were positive in terms of IgG anti-*T. gondii* antibodies compared to 124 (27.2%) male donors ($p < 0.05$). In contrast, there was no significant difference in the prevalence of IgM anti-*T. gondii* among the female (4.4%) and male (3.0%) donors ($p = 0.811$). Furthermore, Chi square test for trend revealed that seroprevalence of IgG and IgM anti-*T. gondii* antibodies increased with age ($p = 0.04$) (Table 1).

Roche Elecsys Toxo IgM assay

In order to low specificity IgM antibody in the ELISA test, Roche Elecsys Toxo IgM assay with higher sensitivity and specificity was used in the 16 (3.2%) IgM-positive samples. Results showed that 1 (0.2%) sample tested with Elecsys assay were nonreactive, while 15 (3.0%) samples tested were reactive.

Risk factors of being anti-*T. gondii* antibodies

Table 2 shows the associations between risk factors, status of anti-*T. gondii* IgG antibodies in the univariate analysis (crude OR), and also multiple logistic regression (adjusted OR). Variables used in the adjusted model were age, gender, education, residence, blood group, animal contacts, raw/half-cooked meat consumption, consumption of raw vegetables and raw egg/milk, gardening or agriculture activity, and blood transfusion. Several risk factors which were significantly related to *T. gondii* seropositivity (at least one of the IgG and IgM being positive) in the univariate analysis at $p < 0.05$ included gender of female ($p = 0.021$), age of more than 45 years old ($p = 0.032$), living in rural regions ($p < 0.001$), having B blood type ($p = 0.005$), contact with cats ($p < 0.001$), raw/half-cooked meat consumption ($p = 0.015$), consumption of raw vegetables ($p = 0.015$), and agricultural activities ($p < 0.001$). However, other demographic and risk factors of the blood donors did not show any association with *T. gondii* seropositivity (Table 2). In multiple logistic regression, it could be observed that living in rural regions, having B blood type, being in contact with cats, consuming raw vegetables and raw-milk/egg, and doing agricultural activities were independent risk factors for *Toxoplasma* seropositivity.

Real-time PCR

All IgM-positive samples were tested using the real-time PCR assays for the presence of *T. gondii* DNA. *T. gondii* DNA was found in one (9.0%) of IgM-positive samples. However, correlation between IgM positivity and PCR results was not statistically significant ($p>0.05$).

DISCUSSION

Toxoplasma gondii infection as a blood-borne disease can lead to significant clinical consequences among blood recipients, especially in immunocompromised, multiply transfused patients, pregnant women, fetus, etc. (3-8). Since the existing therapies are not fully effective and no safe and efficacious vaccines are available, it is crucial to make efforts for reducing toxoplasmosis transmission to diminish the severe complication of toxoplasmosis. In the present study, it was found that 32.0%, 28.8%, 1.0%, and 2.2% of donors tested were positive for anti-*T. gondii* antibodies, only IgG antibody, both IgM and IgG, and IgM antibody alone. However, due to low specificity of IgM in the ELISA test, all IgM-positive samples were analyzed by Roche Elecsys Toxo IgM assay based on an electrochemiluminescence immunoassay. The results obtained in this assay demonstrated that 1 (0.2%) sample tested with Elecsys assay were nonreactive, while 15 (3.0%) samples tested were reactive. In line with these findings Prusa et al (2010) reported that Elecsys assay is a useful tool as a first-line screening method to detect *Toxoplasma* infections with high sensitivity and specificity of 97.1% and 100.0%, respectively (24).

The present findings revealed that both contact with cats and consumption of raw/half-cooked meat are significant risk factors for *T. gondii* seropositivity, which exhibits that, among the blood donors in this study, both infection routes, the ingestion of oocysts (animal to human transmission) and that of tissue cysts in meat (food-borne transmission), existed,

similar to the infection routes reported in other studies (23,25,26). Prevalence of *T. gondii* seropositivity was similar to the seroprevalence reported in Czech Republic (17) and Mexico (20) and higher than those reported previously in Turkey (19), Mexico (22), India (12), northeast of Thailand (21), and Taiwan (15). However, it was less than the one reported among blood donors in central Iran (26), male blood donors in northeast of Brazil (27), north of India (16), and Egypt (13), where the seroprevalence of *T. gondii* has varied from 50 to 75.0%. These variations in the prevalence of *T. gondii* among the blood donors might be related to sociocultural habits, geographical and environmental factors, and transmission routes in the studied population (13, 22). In the present study, similar to the work conducted by Elhence et al. (16), seroprevalence of *T. gondii* in female donors was significantly higher than that in males. But, it was in contrast to the results of Sundar et al. (12) and Ormazdi et al. (26). Such significant difference could be attributed to greater exposure of females to oocysts and tissue cysts during their daily activities. However, due to the small number of female donors in the present study, the results need to be confirmed on a larger sample size. This investigation exhibited that rate of seropositivity increased with age as a consequence of increased opportunity for exposure; such a finding was in agreement with those observed in other studies (13, 22, 27). No difference was found between education, raw-milk/egg consumption, and blood transfusion on the one hand seroprevalence of anti-*T. gondii* antibodies on the other. Interestingly, the present study for the first time demonstrated a significant correlation between *T. gondii* seropositivity and blood group B ($P= 0.008$). Since this correlation may be accidental or accounted for by biased sampling, further studies are required to elucidate this correlation in more depth.

In addition, it was observed that risk of *T. gondii* was higher in the donors living in rural ($p<0.001$) than those living in urban regions. This significant difference could be attributed to occupational activities related to contact with animals and having lower socioeconomic and

lower hygienic lifestyle levels as described elsewhere (16). In this investigation, similar to the previous studies, it was identified contact with cats, raw/half-cooked meat consumption, consumption of raw vegetables, and agricultural activities (as potential risk factors for acquiring toxoplasmosis) were associated with the seropositivity of *T. gondii* (16, 22, 23). Our findings also demonstrated that *T. gondii* DNA was found in one (9.0%) of IgM-positive samples, therefore presence of parasitemia confirmed by PCR in IgM-positive healthy blood donors ensures the likelihood of transmission of *Toxoplasma* through blood transfusion. However, some factors such as the short duration of parasitemia and the low numbers of trophozoites circulating in peripheral blood, could lead to a sampling error that will produce false negative results in such cases (28).

To conclude, in this study, it was found that *T. gondii* infection was prevalent among healthy blood donors in the southeast of Iran with the overall seroprevalence rate of 32%. These results also showed that both infection routes (ingestion of oocysts and that of tissue cysts) existed in the blood donors of this region. Moreover, it was observed that anti-*T. gondii* IgM seroprevalence among blood donors included in the present study had a high considerable rate. Regarding the presence of immunosuppressed and organ transplant patients who are hospitalized in the studied area and their constant need for blood and blood products; therefore, the results of this study can be a warning for blood transfusion organizations in order to pay special attention to toxoplasmosis among blood donors and also design screening programs for prevention transfusion-transmitted toxoplasmosis.

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Figure caption:

Figure 1. Geographical locations where this study was carried out

Table 1 demographic characteristics and *T.gondii* sero-prevalence among healthy blood donors in Kerman

Variables	province, Iran.			
	No. (%)	IgG positive	IgM positive	At least one positive
Gender				
Male	455 (91.0)	124 (27.3)	14 (3.1)	135 (29.7)
Female	45 (9.0)	21 (46.7)	2 (4.4)	21 (46.7)
Age groups				
<25 yrs	217 (43.4)	57(26.3)	5(2.3)	60(27.6)
26-35	180 (36)	51(28.3)	6(3.3)	57(31.7)
36-45	76 (15.2)	24(31.6)	4(5.3)	26(34.2)
>45yrs	27 (5.4)	13(48.1)	1(3.7)	13(48.1)
Residential place				
Urban	388 (77.6)	91(23.5)	13(3.4)	101(26)
Rural	112 (22.4)	54(48.2)	3(2.7)	55(49.1)
Education				
Less than diploma	254 (50.8)	79(31.1)	8(3.1)	85(33.5)
Diploma and above	246 (49.2)	66(26.8)	8(3.3)	71(28.9)
Blood type				
A	154 (30.8)	41(26.6)	4(2.6)	43(27.9)
B	36 (7.2)	18(50.0)	1(2.8)	19(52.8)
AB	83 (16.6)	28(33.7)	3(3.6)	29(34.9)
O	227 (45.4)	58(25.6)	8(3.5)	65(28.6)
Being in contact with cat				
No	311 (62.2)	66(21.2)	6(1.9)	71(22.8)
Yes	189 (37.8)	79(41.8)	10(5.3)	85(45)
Raw/half-cooked meat consumption				
No	440 (88.0)	118(26.8)	13(3.0)	129(29.3)
Yes	60 (12.0)	27(45.0)	3(5.0)	27(45.0)
Eating uncooked vegetables				
No	100 (20.0)	19(19.0)	2(2.0)	21(21.0)
Yes	400 (80.0)	126(31.5)	14(3.5)	135(33.8)
Gardening or agriculture				
No	407 (81.4)	97(23.8)	11(2.7)	105(25.8)
Yes	93 (18.6)	48(51.6)	5(5.4)	51(54.8)
Raw-milk/egg consumption				
No	444 (88.8)	123(27.7)	12(2.7)	131(29.5)
Yes	56 (11.2)	22(39.3)	4(7.1)	25(44.6)
Blood transfusion				
No	488 (97.6)	143 (29.3)	16 (3.27)	155 (31.8)
Yes	12 (2.4)	2 (16.6)	0 (0.0)	2 (16.6)

Table 2 Univariate (crude OR) and multiple (adjusted OR) logistic regression analysis of the potential factors associated with *T. gondii* IgG sero-prevalence among healthy blood donors in Kerman province, Iran.

Variables	IgG Positive			
	Crude OR (95% CI)	P value	Adjusted OR (95% CI)	P value
Gender				
Male	1	-	1	-
Female	2.3 (1.3, 4.3)	0.007*	2.1(1.1,4.2)	0.034*
Age groups				
<25 yrs	1	-	1	-
26-35	1.1 (0.7, 1.7)	0.64	1.0(0.6,1.6)	0.95
36-45	1.3 (0.7, 2.3)	0.37	0.9(0.5,1.7)	0.68
>45yrs	2.6 (1.1, 5.8)	0.02*	2.0(0.8,4.8)	0.14
Residential place				
Urban	1	-	1	-
Rural	3.0 (1.9, 4.7)	<0.001*	2.0(1.2,3.3)	0.006*
Education				
Diploma and above	1	-	1	-
Less than diploma	1.2 (0.8, 1.8)	0.29	1.2(0.8,1.9)	0.38
Blood type				
A	1	-	1	-
B	2.7 (1.3, 5.8)	0.008*	2.3(1.0,5.3)	0.044*
AB	1.4 (0.78, 2.5)	0.25	1.4(0.7,2.6)	0.29
O	0.94 (0.59, 1.5)	0.81	0.8(0.5,1.3)	0.40
Being in contact with cat				
No	1	-	1	-
Yes	2.6 (1.8, 3.9)	<0.001*	2.3(1.5,3.6)	<0.001*
Raw/half-cooked meat consumption				
No	1	-	1	-
Yes	2.2 (1.3, 3.8)	0.004*	1.7(0.9,3.1)	0.10
Eating uncooked vegetables				
No	1	-	1	-
Yes	1.96 (1.1, 3.3)	0.015*	1.8(1.0,3.3)	0.038*
Gardening or agriculture				
No	1	-	1	-
Yes	3.4 (2.1, 5.4)	<0.001*	2.2(1.3,3.8)	0.003*
Raw-milk/egg consumption				
No	1	-	1	-
Yes	1.7 (0.95, 3.1)	0.074	1.5(0.8,2.9)	0.18
Blood transfusion				
No	1	-	1	-
Yes	1.4 (1.0,2.2)	0.35	1.40 (1.1,2.3)	0.4

