



Prevalence of *Toxoplasma gondii* Infection in Healthy Volunteer Blood Donors Using Serological and Molecular Methods from Chaharmahal and Bakhtiari Province, Southwest Iran

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Abstract

Background: Toxoplasmosis is a ubiquitous zoonotic disease caused by *Toxoplasma gondii*. Blood and blood products can be a probable route of *T. gondii* transmission, especially in patients undergoing multiple transfusions.

Objectives: The aim of this cross-sectional study was to determine the prevalence of *T. gondii* infection among healthy blood donors in Chaharmahal and Bakhtiari province.

Methods: We collected 385 blood samples from blood donors referring to the three biggest Blood Transfusion Organization Centers in the province during the period from January to June 2017. IgG and IgM antibodies against *T. gondii* were examined using enzyme-linked immunosorbent assay. Moreover, all samples were tested by loop-mediated isothermal amplification (LAMP) method for the detection of DNA of *T. gondii*.

Results: The seroprevalence rates of IgG and IgM anti-*T. gondii* antibodies were 37.9% and 1.56%, respectively. With the LAMP method, 1.56% of the samples were positive for the DNA of *T. gondii*. Of these, four (1.04%) were seropositive for both IgG and IgM and two (0.52%) were positive only for IgG. Moreover, statistical analyses indicated that several risk factors including gender, age, contact with cats, and consumption of undercooked meat were significantly related to *T. gondii* seropositivity in blood donors.

Conclusions: This study indicated that anti-*T. gondii* antibodies were highly prevalent among apparently healthy blood donors in Southwest Iran. There was a possibility of infection transmission by blood transfusion while there is a lack of screening tests for Toxoplasma infection in the Blood Transfusion Organizations. It is strongly suggested that appropriate and sensitive screening programs be designed by the LAMP method for the detection of *T. gondii* in blood donors.

Keywords: Blood Donors, Toxoplasmosis, Seroprevalence, Loop-Mediated Isothermal Amplification, Chaharmahal and Bakhtiari Province

1. Background

Toxoplasmosis is one of the widespread serious zoonotic parasitic diseases in humans and animals worldwide caused by a single-cell obligate intracellular opportunistic protozoan belonging to the phylum *Apicomplexa* (1). Human toxoplasmosis occurs postnatally (horizontally) through consuming or handling viable tissue cysts of undercooked or raw meat, consuming contaminated food (unwashed raw fruits and vegetables), and drinking water contaminated with sporulated oocysts or unpasteurized milk containing viable tachyzoites. Moreover, tachyzoites in the blood may be crossed to the fetus

via the placenta when an uninfected woman acquires the infection during pregnancy (2, 3). Another way of infection transmission is through organ transplantation or blood transfusion from infected donors to susceptible recipients (*Toxoplasma*-seronegative patients) (4-8).

Epidemiological surveys demonstrate that toxoplasmosis has a global distribution with a highly variable prevalence from 1% to 90% in various areas of different countries. It is estimated that approximately 25% to 30% of the world's population is chronically infected with *Toxoplasma gondii*, especially in developing countries (4, 9). Human toxoplasmosis in healthy adults with normal im-

immune systems is usually an asymptomatic chronic disease without clinical symptoms or with self-limited symptoms. However, in the fetus and newborns who are congenitally infected, it can cause congenital anomalies such as stillbirth, microcephaly, hydrocephalus, mental retardation, abortion, cerebral calcification in the developing fetus, and fetal death (10). Moreover, in immunosuppressed patients, such as AIDS patients, patients undergoing therapies for malignancies, recipients of solid-organ transplants, and people on hemodialysis, it causes irreversible complications including encephalitis, pneumonia, and disseminated systemic disease (11, 12).

Another risk group consists of patients with aplastic anemia, sickle cell anemia, thalassemia, and lymphoproliferative disorders who receive blood transfusion regularly or frequently for survival. The transfusion of infected blood in these patients due to their immunodeficiency disorders would cause serious disease (13, 14). *Toxoplasma gondii* is one of the intracellular microorganisms that can stay alive for up to 50 days in the citrated blood and the buffy coat at 5°C (15). Therefore, this parasite is potent for transmission via blood and blood components such as leukocytes. However, many countries do not perform rigorous screening on blood as a routine procedure before blood transfusion. Therefore, scientific surveys have been carried out to investigate the relationship between *T. gondii* and blood safety in blood donors and blood bags by different techniques such as serological and molecular methods. Many investigations have indicated that the seroprevalence of toxoplasmosis varies widely from 0.96% to 73.26% in healthy volunteer blood donors in different countries. In Iran, the seroprevalence of toxoplasmosis in healthy volunteer blood donors is reported from 16.8% to 56.3% in different geographic areas (6, 8, 16-19).

2. Objectives

The aim of this cross-sectional study was to evaluate the prevalence of *T. gondii* infection using serological and molecular methods and determine the associated risk factors among healthy blood donors in Chaharmahal and Bakhtiari province, Southwest Iran.

3. Methods

3.1. Study Design

This cross-sectional study was carried out at the three biggest Blood Transfusion Organization centers of Chaharmahal and Bakhtiari province, Iran. The province is located between 31° 09' North latitude and 32° 48' East longitude in the mountainous areas of Southwestern Iran, covering an area of 16,332 square kilometers, with a population of about 895,263. It is characterized by various climates from cold, snowy, and rainy weather especially in fall

and winter to hot weather in summer. Animal husbandry, farming, and gardening are common in this province and the diet of most people contains meat, milk, and local vegetables.

3.2. Sample Collection and Participants

In this study, according to statistical calculations, the sample size was determined to be 385 cases based on the prevalence of 50% as reported in some studies from Iran (17, 20, 21), the confidence level of 95%, and the error of 0.05. Blood samples were collected from apparently healthy blood donors from seven counties of the province, Shahrekord, Lordegan, Boroujen, Farsan, Kiar, Ardal, and Kohrang. The donors referred to the three biggest Blood Transfusion Organization centers in the province during the period from January to June 2017.

Before collecting the blood samples, a questionnaire was administered to inquire about socio-demographic information of the donors including age, sex, job, residence, education level, and blood group. Moreover, other possible risk factors were recorded such as contact with animals (cats), raw/half-cooked meat consumption, raw milk/egg consumption, raw vegetable consumption, and history of blood transfusion. Then, 5 mL of venous blood was aseptically taken from each apparently healthy donor and divided into two parts. One part was transferred into plastic tubes containing K2EDTA as an anticoagulant for isolating buffy coat for molecular assay and the other part was poured into a disposable tube free of anti-coagulants to isolate sera for serological assay.

The specimen was left for 15 - 30 minutes or put in the water bath at 37°C. Then, it was centrifuged at 3000 rpm for 5 minutes to separate clear serum. Moreover, all donated blood samples were assayed to determine blood groups and Rh factors. All the blood samples 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 was seropositive for the infection with these microorganisms. After being collected, the samples were transported to the Parasitology Laboratory, Department of Parasitology and Mycology, Shahrekord University of Medical Sciences, and kept at -20°C until the day of the experiment.

3.3. Serological Assay

IgM and IgG antibodies against *T. gondii* were tested in all serum samples using the commercially available enzyme-linked immunosorbent assay (ELISA) kit (Pishtaz Teb Co. Tehran, Iran) according to the instructions of the manufacturer. The IgG antibody titer was considered positive if the values were ≥ 10 mL/IU and negative if they were < 10 mL/IU. In addition, anti-*T. gondii* IgM antibody levels of

≥ 1.1 mL/IU and < 0.9 mL/IU were defined as positive and negative, respectively.

3.4. Molecular Diagnosis

Loop-mediated isothermal amplification (LAMP) is one of the new easy-to-perform molecular methods for detection of DNA of different microorganisms by nucleic acid amplification with extremely high sensitivity and specificity to discriminate single nucleotides. The technique was performed by a DNA polymerase with strand displacement activity, a set of two specially designed inner primers (FIP and BIP), and two outer primers (F3 and B3) that amplified DNA with high specificity, efficiency, and rapidity under isothermal conditions (22). Therefore, we used LAMP to detect DNA of *T. gondii* in blood samples of donors.

First, DNA was extracted from buffy coat of all samples by a commercial DNA extraction kit DNG-Plus™ (DN8118C, Cinnacolon Co., Iran) according to the manufacturer's instructions. Genomic DNA was extracted from the *T. gondii* tachyzoites of RH strain provided by the Toxoplasmosis Research Center, Mazandaran University of Medical Sciences (Sari, Iran), as a positive control. Then, the concentration and quality of DNA were determined using an ND-1000 spectrophotometer (Nanodrop Technologies, USA). Each *T. gondii* one 80-Mbp genome was equal to about 80 fg DNA. In the LAMP assay, the designed primers were used for 529 bp repeated elements of *T. gondii* (23).

The LAMP assay was carried out in a reaction mixture (25 μ L) containing 1 μ L of template DNA, 40 pmol of each of FIP and BIP primers, 20 pmol of each of LF and LB primers, 5 pmol of each of F3 and B3 primers, 8 U of Bst DNA polymerase (New England Biolabs, USA), 1.4 mM of deoxynucleoside triphosphates (dNTP), and 2 \times reaction buffer (1.6 M betaine (Sigma-Aldrich), 40 mM of Tris-HCl (pH 8.8), 20 mM of KCl, 20 mM of $(\text{NH}_4)_2\text{SO}_4$, 16 mM of MgSO_4 , and 0.2% Tween 20). In order to determine the optimal time and temperature conditions for the LAMP assay, the reactions including positive control (*T. gondii* RH-strain DNA) and negative control (double distilled water) were carried out at 60 to 67°C for 30, 45, 60, and 75 minutes (24). Loop-mediated isothermal amplification products were visualized by 1.5% agarose gel electrophoresis stained with ethidium bromide solution (1 μ g/mL). In addition, LAMP amplicons in the reaction tube were evaluated by visual inspection that was performed by adding 1 μ L of 1:10 dilution of SYBR green I (invitrogen lot: 49743A, USA). After the reaction, fluorescent signals of the solutions were observed under UV light.

3.5. Statistical Analyses

SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used for all descriptive and inferential statistics. The chi-square

test was applied to access the univariate association between independent variables and outcome. The association between *T. gondii* seropositivity and the potential risk factors were evaluated using multifactorial logistic regression model. In this survey, $P < 0.05$ was considered statistically significant.

4. Results

4.1. Participants

Blood samples in this survey belonged to 385 healthy blood donors, including 365 (94.8%) men and 20 (5.2%) women. The participants' mean age \pm SD was 37.2 ± 9.81 years, with the youngest and oldest being 20 and 71 years, respectively. Most participants were aged 26 - 35 years, living in urban areas, and had a college education or above.

4.2. Seroprevalence of Anti-Toxoplasma gondii Antibodies

The serological tests indicated that anti-*T. gondii* antibodies were detected in 154 (40%) serum samples of the 385 blood donors. Moreover, 146 (37.9%) cases were seropositive for IgG antibody, six (1.56%) for both IgM and IgG, and four (1.03%) for IgM antibody alone. Therefore, the prevalence rate of IgG and IgM antibodies against *T. gondii* was 37.9% and 1.03%, respectively. Regarding geographical location, as shown by the ELISA test, the seroprevalence rates of anti-*T. gondii* IgG antibody were 71.2%, 5.5%, 5.5%, 4.8%, 4.1%, 4.1%, 2.3%, 2.3%, and 0.7% in Shahrekord, Saman, Kiar, Farsan, Ardal, Ben, Lordegan, and Koohrang, respectively. In terms of gender, the seroprevalence rate of anti-*T. gondii* IgG antibody was significantly higher in female donors than in male donors. The rates of anti-*T. gondii* IgG antibody in females and males were 65% and 36.4%, respectively.

4.3. Risk Factors of Anti-Toxoplasma gondii Antibodies

Several risk factors, including gender, age, residence place, education level, blood type, diet, and contact with cats, which were possibly associated with toxoplasmosis, were evaluated by statistical methods including descriptive and inferential statistics. The results demonstrated that four variables, including gender, age, contact with cats, and consumption of undercooked meat, were significantly related to *T. gondii* seropositivity in blood donors. However, other risk factors and demographic features of blood donors did not show any statistical association with the acquisition of toxoplasmosis (Tables 1 and 2).

4.4. Detection of Toxoplasma gondii DNA by LAMP

All blood samples were tested using the LAMP assay for the presence of *T. gondii* DNA. The DNA was found in six (1.56%) samples, four of which had IgG and IgM anti-*T. gondii* antibodies and two had only IgG antibody in the serological assay (Figures 1 and 2).

Table 1. The Prevalence of Toxoplasmosis Based on Demographic Characteristics Detected by Different Methods Among Healthy Blood Donors in Chaharmahal and Bakhtiari Province, Southwest Iran^a

Variable	No. (%)	Seropositive Antibodies		LAMP Positivity
		Positive (%)	Positive IgM (%)	
Gender				
Male	365 (94.8)	133 (36.4)	3 (0.82)	5 (1.37)
Female	20 (5.2)	13 (65)	1 (5)	1 (5)
Age group, y				
< 25	42 (10.9)	10 (23.8)		
26 - 35	146 (37.9)	43 (29.6)	3 (2.1)	3 (2.1)
36 - 45	118 (30.6)	55 (46.6)	1 (0.85)	2 (1.7)
> 45	79 (20.5)	38 (48.1)		1 (1.27)
Residence place				
Urban areas	329 (85.5)	127 (38.6)	4 (1.2)	6 (1.8)
Rural areas	56 (14.5)	19 (33.9)		
Education				
Illiterate	4 (1)	3 (75)		
Primary school	35 (9.1)	14 (40)		1 (2.86)
Middle school	66 (17.1)	29 (43.9)	1 (1.5)	2 (3)
High school	147 (38.2)	51 (34.7)	1 (0.68)	1 (0.7)
Graduate	133 (34.5)	49 (36.8)	2 (1.5)	2 (1.5)
Blood type				
A ⁺	115 (29.9)	39 (33.9)	2 (1.74)	3 (2.6)
A ⁻	15 (3.9)	6 (40)		1 (6.7)
B ⁺	66 (17.1)	35 (53)		
B ⁻	6 (1.6)	3 (50)	1 (50)	1 (16.7)
AB ⁺	24 (6.2)	10 (41.7)		
AB ⁻	7 (1.8)	3 (42.9)		
O ⁺	53 (13.8)	13 (24.5)		
O ⁻	99 (25.7)	37 (37.4)	1 (1.01)	1 (1.01)
Contact with cats				
Yes	49 (12.7)	28 (57.1)		1 (2)
No	336 (87.3)	118 (35.1)	4 (1.2)	5 (1.5)
Raw/half-cooked meat consumption				
Yes	25 (6.5)	11 (44)		1 (4)
No	360 (93.5)	135 (37.5)	4 (1.1)	5 (1.4)
Raw milk/egg consumption				
Yes	223 (57.9)	85 (38.1)	1 (0.45)	2 (0.9)
No	162 (42.1)	61 (37.7)	3 (1.9)	4 (2.5)
Unwashed vegetables consumption				
Yes	14 (3.6)	4 (28)	1 (7.1)	3 (21.4)
No	371 (94.4)	90 (24.3)	3 (0.8)	3 (0.8)
Blood transfusion				
Yes	0 (0)			
No	385 (100)	146 (37.9)	4 (1.03)	6 (1.6)

^aValues are expressed as No. (%).

5. Discussion

Organ transplantation and blood transfusion can be of the potential transmission routes of *T. gondii*. These routes of transmission are less common; however, if donors

are infected, it will cause acute toxoplasmosis because the recipients are usually immunocompromised patients, with significant clinical consequences such as encephalitis, brain abscess, myocarditis, and chorioretinitis (10). Despite the serious complications of this parasitic disease, es-

Table 2. Correlation Between Demographic Characteristics and IgG Anti-*Toxoplasma gondii* Antibodies Among Healthy Blood Donors in Chaharmahal and Bakhtiari Province, Southwest Iran^a

Variable	No. (%)	Seropositive Antibodies IgG (%)	P Value	OR	95% CI
Gender					
Male	365 (94.8)	133 (36.4)	0.015 ^b	3.240	1.261 - 8.320
Female	20 (5.2)	13 (65)	-	1	
Age group, y					
< 25	42 (10.9)	10 (23.8)	0.011	2.966	1.285 - 6.843
26 - 35	146 (37.9)	43 (29.6)	0.006 ^b	2.220	1.259 - 3.914
36 - 45	118 (30.6)	55 (46.6)	0.837	1.062	0.60 - 1.878
> 45	79 (20.5)	38 (48.1)	-	1	
Residence place					
Urban areas	329 (85.5)	127 (38.6)	0.506	0.817	0.450 - 1.482
Rural areas	56 (14.5)	19 (33.9)	-	1	
Education					
Illiterate	4 (1)	3 (75)	0.161	.194	0.020 - 1.921
Primary school school	35 (9.1)	14 (40)	0.731	0.875	0.408 - 1.876
Middle school school school	66 (17.1)	29 (43.9)	0.335	0.744	0.408 - 1.357
High school	147 (38.2)	51 (34.7)	0.708	1.098	0.673 - 1.791
Graduate	133 (34.5)	49 (36.8)	-	1	
Blood type					
A ⁺	115 (29.9)	39 (33.9)	0.598	1.163	0.664 - 2.038
A ⁻	15 (3.9)	6 (40)	0.845	0.895	0.295 - 2.717
B ⁺	66 (17.1)	35 (53)	0.048	0.529	0.281 - 0.994
B ⁻	6 (1.6)	3 (50)	0.540	0.597	0.114 - 3.111
AB ⁺	24 (6.2)	10 (41.7)	0.698	0.835	0.337 - 2.071
AB ⁻	7 (1.8)	3 (42.9)	0.773	0.796	0.169 - 3.754
O ⁺	53 (13.8)	13 (24.5)	0.111	1.836	0.870 - 3.874
O ⁻	99 (25.7)	37 (37.4)	-	1	
Contact with cats					
Yes	49 (12.7)	28 (57.1)	0.004 ^b	0.406	0.221 - 0.746
No	336 (87.3)	118 (35.1)	-	1	
Raw/half-cooked meat consumption					
Yes	25 (6.5)	11 (44)	0.004 ^b	0.406	0.221 - 0.746
No	360 (93.5)	135 (37.5)	-	1	
Raw milk/egg consumption					
Yes	223 (57.9)	85 (38.1)	0.926	0.981	0.646 - 1.488
No	162 (42.1)	61 (37.7)	-	1	
Unwashed vegetables consumption					
Yes	14 (3.6)	4 (28)	0.826	1.049	0.686 - 1.603
No	371 (94.4)	90 (24.3)	-	1	
Blood transfusion					
Yes	0 (0)				
No	385 (100)	146 (37.9)			

Abbreviation: OR: odds ratio.

^aValues are expressed as No. (%).^bP < 0.05, statistically significant difference.

pecially in immunocompromised patients, available treatments are not fully effective; moreover, safe and efficacious vaccines are not available; thus, the prevention of parasite transmission is one of the effective strategies for re-

ducing toxoplasmosis (25). Detecting the source of infection such as infected blood and organs for recipient candidates, especially patients undergoing multiple transfusions or those requiring blood transfusion for transplan-

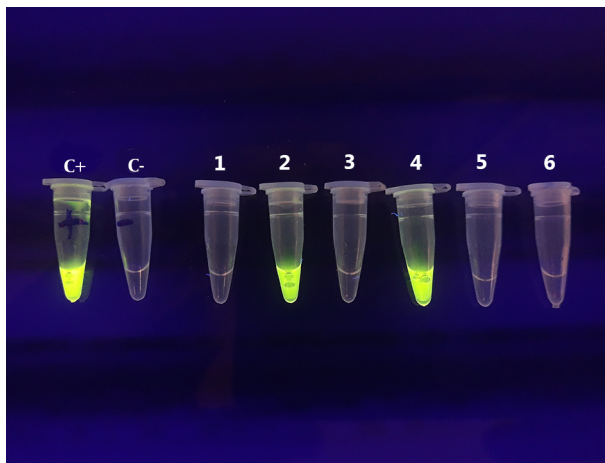


Figure 1. Amplification of *Toxoplasma gondii* DNA in blood samples using the LAMP assay; positive reactions turned green after the addition of SYBR green I while the negative reactions remained uncolored; C+, positive control; C-, negative control; tubes 1-6, randomly selected samples from patients.

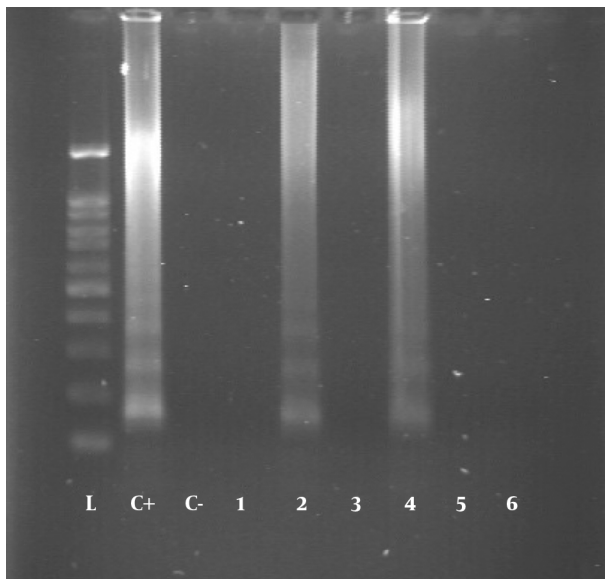


Figure 2. Amplification of *T. gondii* DNA in blood samples using the LAMP assay on 1.5% agarose gel staining with ethidium bromide. L, 100 bp molecular weight marker; C+, positive control; C-, negative control; lanes 1-6, randomly selected samples from patients.

tation management, can be crucial to control and prevent toxoplasmosis (16). Currently, *T. gondii* screening in blood and blood products of blood donors is not routinely considered in worldwide therefore certainly do not assay the relationship between *T. gondii* and blood safety in blood transfusion centers.

In this study, 385 blood samples were collected from

healthy blood donors from Chaharmahal and Bakhtiari province, Iran, and were evaluated by serological and molecular methods for *T. gondii* infection. The results indicated that 154 (40%) samples were seropositive for anti-*T. gondii* antibodies while 37.9% and 1.56% were positive for IgG and IgM anti-*T. gondii* antibodies, respectively. These findings showed a considerable prevalence rate of toxoplasmosis in this population. Although seropositivity of IgG (latent infection) was high, the rate of anti-*T. gondii* IgM antibodies (acute infection) was low.

Previous investigations conducted in Iran showed that the seroprevalence of *T. gondii* in volunteer blood donors was reported to be 34.4% (95% CI: 24.9% - 43.9%), ranging from 18.3% in Gonabad city (26) to 56.4% in Hamedan province (20). However, different rates were reported for various regions by serological methods for IgG and IgM antibodies respectively as 25% and 0% in Zahedan (Southeast Iran) (27), 25% and 3.2% in Mashhad (Northeast Iran) (28), 37.8% and 0% in Urmia (Northwest Iran) (29), 38.66% and 1.03% in Tabriz (Northwest Iran) (21), 16.30% and 0.7% in Boyer-Ahmad county (Southwest Iran), 34.47% and 0.5% in Ahvaz (Southwest Iran) (17), and 28.8% and 3.2% in Kerman (Southeast Iran) (30), which may be related to living conditions, geographical and environmental factors, sanitary conditions, economic and social situation of blood donors, sample size, and sensitivity of detection methods.

The overall seroepidemiological studies of *T. gondii* on blood donors in different regions of the world indicated very different prevalence rates for IgG and IgM antibodies from 0.64% and 0% to 70.29% and 5.81%, respectively (8). The highest seroprevalence rates were from Brazil (75%) (31) and Ethiopia (73%) (32) while low seroprevalence rates of toxoplasmosis in blood donors were observed in Namibia (0.96%) (33), Thailand (9.6%), Taiwan (9.3%), and Mexico (7.4%) (17).

In the present study, the obtained results showed that the seroprevalence of *T. gondii* was significantly higher in female than in male donors, which may be due to sampling bias because most blood donors were males. Similarly, Mahmoudvand et al. (30) and Sundar et al. (34) reported that the seroprevalence of *T. gondii* in female donors was significantly higher than that in male donors. However, some studies reported contradictory results (28, 35).

According to a recently published systematic review of toxoplasmosis in Iranian blood donors, the difference between men and women was not statistically significant (36). Regarding age groups, IgG positivity was more frequent in donors aged > 45 years that was similar to other studies indicating that the rate of seropositivity increased with age as a consequence of the increased opportunity of acquiring the infection. The obtained findings disclosed that blood donors with blood group B+ had more *Toxoplasma* seropositivity than other blood groups; however,

no correlation was found between *T. gondii* seropositivity and blood groups. Shaapan *et al.* reported that blood group A (48.7%) had the most frequent *Toxoplasma* seropositivity among blood donors from Egypt (37). However, Mahmoudvand *et al.*, (30) Shaddel *et al.*, (38) and Elsheikha *et al.* (19) indicated a significant correlation between toxoplasmosis and blood groups AB, A, and O, respectively. Due to the small number of reports of the correlation between toxoplasmosis and blood groups, this hypothesis needs to be confirmed in further investigations.

Although cats are the definitive host of this protozoon and infected cats can infect the environment by defecated oocysts, conflicting findings have been reported concerning the transmission of *T. gondii* via contact with cats. However, our study suggested that contact with cats is a potential risk factor for acquiring toxoplasmosis. Concerning food habits, the study exhibited that the seroprevalence rate of toxoplasmosis was higher in blood donors who consumed raw/half-cooked meat than in other blood donors. Therefore, ingesting meat containing tissue cysts seems to be the main route of infection among the other routes. Moreover, some studies such as Mahmoudvand *et al.* study confirmed this finding (30). In this study, no significant relationships were found between the consumption of raw vegetables, occupational activities, education level, residence place, blood transfusion, and raw milk/egg consumption (as potential risk factors for acquiring toxoplasmosis) and seropositivity of *T. gondii*.

Despite this fact that serological methods such as ELISA are used in many medical diagnostic laboratories for detecting antibodies against *T. gondii*, these methods have false-positive results and cannot differentiate past and present infections; therefore, the diagnosis of toxoplasmosis remains unsatisfactory with serological methods. This is while molecular methods can obviate this limitation (39, 40). Many researchers have used molecular methods such as PCR and real-time PCR for detecting *T. gondii* in blood donors. Studies carried out based on the detection of DNA of *T. gondii* in blood donors in Iran indicated that 0.9% of IgM-positive samples in Kerman (30), 6.97% samples in Rafsanjan (18), 40% of seropositive samples in Ahvaz (17), and 100% of IgM-positive samples in Razavi Khorasan (41) contained the DNA of the parasite. Nevertheless, by the PCR method, *T. gondii* DNA was not detected in the buffy coat of any of the seropositive cases in Boyer-Ahmad County, Southwest Iran (42).

Our findings according to the molecular assay demonstrated that six (1.56%) samples had the DNA of *T. gondii*. Of these, four (1.04%) were seropositive for both IgG and IgM antibodies and two (0.52%) were positive only for IgG antibody. In line with these findings, Fallahi *et al.* reported that LAMP is a useful tool for the diagnosis of toxoplasmosis in children with leukemia with high sensitivity and

specificity (24). Loop-mediated isothermal amplification is an attractive DNA amplification method compared to other molecular methods such as PCR because it requires a minimal DNA sample such as a single tachyzoite or 10 copies of recombinant plasmid whereas the PCR method in toxoplasmosis diagnosis needs much more DNA sample (24, 39). Furthermore, the LAMP assay was very simple, as the reaction would be carried out in a single tube under one isothermal condition and at one-hour reaction time. Therefore, the LAMP method has the advantages of specificity, rapidity, sensitivity, and easiness for the clinical diagnosis of *T. gondii* (43).

5.1. Conclusions

In conclusion, the results demonstrated that the prevalence of *T. gondii* infection is high among apparently healthy blood donors in Southwest Iran. Thus, there is the possibility of transmission of infection by blood transfusion while screening tests are not used to detect *Toxoplasma* infection in Blood Transfusion Organizations. Furthermore, serological methods have potential weaknesses such as time-intensiveness and false-positive results. Therefore, it is strongly suggested that appropriate and sensitivity programs be designed for screening of toxoplasmosis by the LAMP method to detect *T. gondii* DNA in blood donors and prevent the transmission of *T. gondii* to at-risk people, especially multi-transfused patients.

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Footnotes

Authors' Contribution: Rahman Abdizade, Kourosh Manouchehri Naeini, and Gharib Karimi conceived and designed the study. Ehsan Heidari Soureshjani helped to samples collected. Mahnaz Jafari and Shahrbanou Parchami performed laboratory works. Rahman Abdizade and Kourosh Manouchehri Naeini drafted the paper and revised the manuscript for important intellectual content.

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Patient Consent: Written informed consent was obtained from all participants before blood sampling.

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