RESEARCH PAPER

Seroprevalence and Molecular Detection of *Toxoplasma gondii* Among Blood Donors in the cities Duhok and Zakho/Iraq

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Abstract

Toxoplasmosis is a zoonotic infection caused by the parasite *Toxoplasma gondii,* and it is recognized as a public health problem. The transmission of the infection from blood donors to immunocompromised recipients has garnered attention. The aim of the current study was to determine the seroprevalence and molecular detection of anti-*Toxoplasma* IgM and IgG antibodies among people who donated in both Duhok and Zakho cities and its related risk factors. One hundred-eighty-four blood samples were collected from blood donors (92 blood samples from Duhok and 92 blood samples from Zakho). These sera were subsequently analyzed for IgM/IgG using the ELISA techniques. The characteristics of the donors, including age, cat contact, consumption of water and meat, and blood group, were also examined. The seropositivity rates for IgM/IgG, as determined by ELISA, were 4.3% and 53.0%, respectively. A higher prevalence of IgG was noted among donors aged 20–39 years. Factors such as cat contact and the source of drinking water, along with blood group/Rh factors, did not show significant associations with IgM or IgG levels. However, blood group O^+ exhibited the highest IgM levels, while blood group A^+ had the highest IgG levels. Fifteen samples that tested positive for anti-*Toxoplasma* IgM and IgG antibodies (8 for IgM and 42 for IgG) via the ELISA test were selected for amplification of the B1 gene using PCR. Of these fifty samples, three were positive by PCR; specifically, only the three samples that were positive for IgM also tested positive by PCR, while no positive cases for IgG were found. Blood donors in Duhok and Zakho exhibit a high seroprevalence of latent toxoplasmosis infection. To address this significant rate of infection, it is essential to implement effective strategies, including the screening of blood donations in blood banks.

Keywords: Seroprevalence, Toxoplasmosis, PCR, Blood donors, Duhok, Zakho.

How to cite this article: Iman Farsat Yousif (1) * Shameeran Salman Ismael (2). Seroprevalence and Molecular Detection of Toxoplasma gondii Among Blood Donors in the cities Duhok and Zakho/Iraq. Int J Drug Deliv Technol. 2026;16(77s):1012-1020. DOI: 10.25258/ijddt.16.77s.118

.Introduction

The parasite known as *Toxoplasma gondii* is the cause of the zoonotic disease toxoplasmosis. Given that almost one-third of people worldwide become infected with *T. gondii* [1], [2], there are serious public health concerns about the parasite's propensity to spread from blood donors to patients with compromised immunity. Consumption of raw or undercooked meat with *T. gondii* cysts, contact with cats, exposure to contaminated soils, and ingestion of oocyst-infected food or beverages are common ways that *T. gondii* can spread [3]. Rarely, this parasite can spread through blood transfusions and organ transplants [4], [5]. *T. gondii* has been recognized as a public health issue since it has infected one-third of the world's [3], [4]. Rarely, this parasite can spread through blood transfusions and organ transplants [5], [6]. T. gondii has been recognized as a public health issue since it has infected one-third of the world's population [3], [7]. In contrast, blood transfusions and organ transplants are rare methods for the spread of this parasite [8], [9]. In contrast, blood transfusions and organ transplants are rare methods for the spread of this parasite [6], [9].

The life cycle of *Toxoplasma* is complex and has two parts: the asexual part, which replicates and is completed in the intermediate host (humans and other mammals), and the second part, the sexual part, which occurs in the final host (cats) [10]. It's crucial to make food correctly, thoroughly wash fruits and vegetables, maintain excellent hygiene, and stay away from potentially contaminated water in order to reduce the

risk of contracting an infection [11]. In individuals with immunodeficiency or immunosuppressive circumstances, reactivation of latent infection can result in toxoplasmosis [12]. If they are not protected against toxoplasmosis, patients of blood transfusions and organ transplants are at a high risk of developing an acute infection [13].

Many tests, including the latex agglutination test, Sabin-Feldman dye test, Western blot, indirect fluorescent antibody test, enzyme-linked immunosorbent assay, and molecular approaches, are used in the laboratory to identify the *Toxoplasma* parasite or *Toxoplasma* antibodies [14]. According to Foroutan-Rad et al. [4] blood samples from healthy donors included *T. gondii* DNA, indicating that a sizable portion of recipients of blood transfusions may be at risk for toxoplasmosis. The infection shows no clear symptoms in immunocompetent individuals [3], [5]. Because comprehensive screening for toxoplasmosis prior to blood transfusions is lacking, infected blood donors may present few to no symptoms while still being eligible to donate blood. The current study aims to investigate the seroprevalence and molecular detection of toxoplasmosis among blood donors in the cities of Duhok and Zakho, Iraq. Additionally, to assess the potential risk factors for blood transfusion-transmitted toxoplasmosis

1. Materials and Methods

1.1. Study design and sample collection

This study was cross-sectional and conducted among people who donated blood in both Duhok and Zakho cities, Iraq, between the periods of September and December of 2025. One hundred eighty-four blood samples were collected from donors (92 blood samples from Duhok and 92 blood samples from Zakho). Five milliliters of blood were collected from each donor and put into two tubes: One contain gel and second is EDTA tubes, and all tubes were stored at -20°C until used. All volunteers who agreed to participate in this study signed an Informed Consent Form

1.2. Detection of *T. gondii*

1.2.1. Serological detection of *Toxoplasma* IgM/IgG by ELISA test

In the current study, *Toxoplasma* IgM/IgG (Biovantion Inc., China) kits were used for the detection of *Toxoplasma* IgM and IgG antibodies, and all blood samples were tested for both *Toxoplasma* IgM/IgG antibodies.

1.2.2. Molecular detection of *Toxoplasma*

DNA was extracted from blood samples with using of Qiagen DNeasy extraction kit Qiagen, Germany). The extracted DNA samples were stored at -20°C until they were needed for molecular analysis. The concentration and purity of the extracted DNA were assessed using a NanoDrop spectrophotometer.The primers used in this study targeted the *Toxoplasma* B1 gene [15], as detailed in Table 1. Amplification of the B1 gene was conducted using conventional PCR techniques. The total volume for the PCR reaction was set at 25 µl. The reaction mixture consisted of 12.5 µl of master mix, 1 µl of each forward and reverse primer, 2 µl of DNA template, and 8.5 µl of RNase-free water, bringing the total volume to 25 µl. The PCR cycler conditions were configured as outlined in Table 2. Finally, 10 µl of the PCR product was electrophoresed on a 1% agarose gel and visualized under UV light [16], [17].

Table 1. Primers were used in the current study

Primer	Gene	Sequences (5' to 3')	Size (bp)
Forward	B1	CGCTGCAGGGAGGAAGACGAAAGTTG	529
Reverse		CGCTGCAGACACAGTGCATCTGGATT	

Table 2. PCR cycling protocol was used in the current study

Steps	Temperature ºC	Duration	Cycles (No.)
Initial denaturation	95	3 minutes	1
Denaturation	95	60 seconds	35
Annealing	56	60 seconds	
Extension	72	90 seconds	
Final extension	72	3 minutes	1
Hold	4	∞ 1	1

2.3. Data Analysis

Chi-Square tests were utilized to compare the seropositivity of the parasite with various case features. The Statistical Package for the Social Sciences, commonly known as SPSS, was used for the statistical calculations and it considered a significant at p-value of 0.05. and used the fisher test for relation between age and IgM.

3. Results

3.3. ELISA Results

According to Fig. 1, out of one hundred eighty-four serum samples tested for anti-*Toxoplasma* (IgM) antibodies by ELISA test, 8 (4.3%) were seropositive and 176 (95.7%) were seronegative. As illustrated in Fig. 2, out of one hundred eighty-four serum samples tested for anti-*Toxoplasma* (IgG) antibodies, 98 (53.0%) were seropositive and 86 (47.0%) were seronegative.

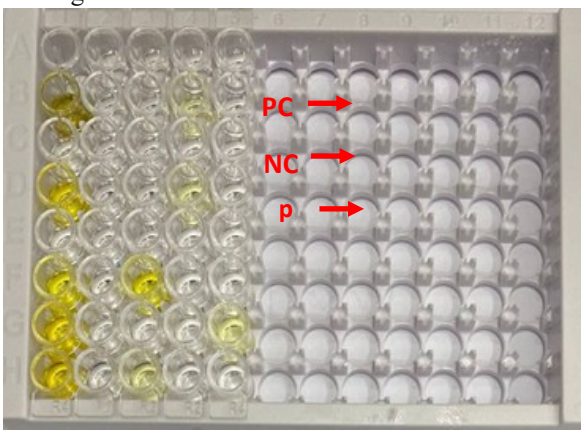

Figure 1. ELISA test results for detection of anti-*Toxoplasma* IgM among blood donor, NC: Negative control, and PC: Positive control

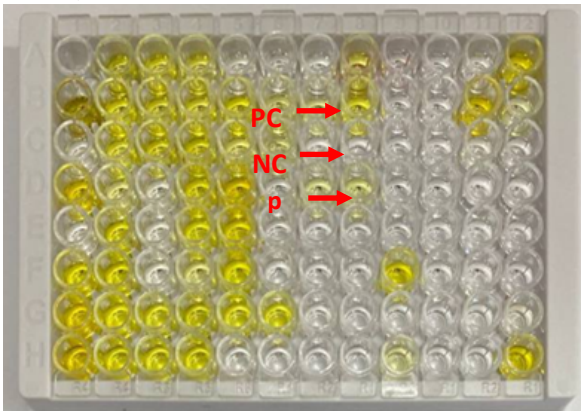

Figure 2. ELISA test results for detection of anti-*Toxoplasma* IgG among blood donor, NC: Negative control, and PC: Positive control

Table 3. illustrated the seroprevalence of IgM anti-*Toxoplasma* among blood donors according to age group. The highest rate was found in the 30-39-year-old age group, which was 6 (8.7%), and in the 20-29-year-old age group, it was 2 (3.4%), and this is statistically significant at p-value < 0.05. The seroprevalence of IgG anti-Toxoplasma among blood donors was high in the 30-39-year-old and 20-29-year-old age groups, which were 41 (59.4%) and 34 (57.6%), followed by the 40-49-year-old age group, which was 19 (52.8%), and the 50-59-year-old age group, which was 4 (20.0%); and this is statistically significant at p-value < 0.05. IgM positivity did not significantly differ between age groups, according to Fisher's test (p = 0.108).

Table 3. The seroprevalence of *Toxoplasma gondii (*IgM/IgG) among people who donated blood in Duhok City (n=184) by ELISA test according to age groups

Years	Total examined samples	IgM		IgG	
		Positive cases	Negative cases	Positive cases	Negative cases
20-29	59	2(3.4%)	57(96.6)	34(57.6%)	25(42.4%)
30-39	69	6(8.7%)	63(91.3%)	41(59.4%)	28(40.6%)
40-49	36	0(0.05)	36(100.0%)	19(52.8%)	17(47.2%)
50-59	20	0(0.0%)	20(100.0%)	4(20.0%)	16(80.0%)
Total	184	8(4.3%)	176(95.7%)	98(53.0%	86(47.0%)

Fisher test; p = 0.108 (IgM) $\chi^2 = 12.67$, *p = 0.005 (IgG)*

Table 4 shows that those who had contact with cats had the highest seroprevalence of IgM, 5 (6.7%), whereas blood donors who had no contact with cats had the lowest seroprevalence, 3 (2.8%). At a p-value > 0.05, these differences between the two groups were not statistically significant. Additionally, participants who had contact with cats had a higher seroprevalence of IgG than those who did not: 42 (56.0%) and 56 (51.4%), respectively. When the p-value is greater than 0.05, there is no statistical correlation. Given that cats are the primary source of infection for humans and all other mammals, this is linked to social media movements that favor the rights of domestic pet animals.

Table 4 The seroprevalence of *Toxoplasma (*IgM/IgG) among people who donated blood by ELISA test according have contact with cats or not (n=184)

Contact with cats	Total examined samples	IgM		IgG	
		Positive cases	Negative cases	Positive cases	Negative cases

Yes	75	5(6.7%)	70(93.3%)	42(56.0%)	33(44.0%)
No	109	3(2.8%)	106(97.2%)	56(51.4%)	53(48.6%)
Total	184	8(4.3%)	176(95.7%)	98(53.3%)	86(46.7%)

$\chi^2 \approx 1.48$, $p \approx 0.224$ (IgM) *$\chi^2 \approx 0.39$, $p \approx 0.531$ (IgG)*

It is clear that consuming tap water was associated with both IgM and IgG seroprevalence; this is because the cities of Duhok and Zakho have contaminated drinking water. This was statistically significant between both groups for IgM/IgG at a p-value < 0.05. As seen in Table 5.

Table 5. The seroprevalence of anti-*Toxoplasma* IgM/IgG among people who donate blood by ELISA according to source of drinking water (n=184)

		IgM		IgG	
Source of drinking water	**Total samples examined**	**Positive cases**	**Negative cases**	**Positive cases**	**Negative cases**
Tap water	111	6(5.4%)	105(94.6%)	85(76.6%)	26(23.4%)
Distilled water	73	2(2.7%)	71(97.32-%)	13(17.8%)	60(82.2%)
Total	184	8(4.3%)	176(95.7%)	98(53.3%)	86(46.7%)

$\chi^2 \approx 3.38$, $p \approx 0.066$ *(IgM)* *$\chi^2 \approx 65.4$, $p < 0.001$ (IgG)*

Table 6: Blood groups, Rh factor, and IgM/IgG did not significantly correlate with a p-value > 0.05. The O+ blood group had the highest seroprevalence of IgM (4/64, or 6.25%), whereas the O- blood group had the lowest. Furthermore, the rate of IgG across all blood groups and Rh factors did not change significantly. There were no distinctions between blood types; the A+ blood type had the highest incidence of IgG, the AB blood group the lowest, and the remaining blood groups were either low or nonexistent. This was not significant with a p-value > 0.05.

Table 6. The seroprevalence of anti-*Toxoplasma* IgM/IgG by ELISA test according blood groups and Rh factor (n=184)

		IgM		IgG	
Blood group & Rh	**Total samples examined**	**Positive cases**	**Negative cases**	**Positive cases**	**Negative cases**
A+	68	2(2.9%)	66(97.1%)	43(63.2%)	25(36.7%)
A-	4	0(0.0%)	4(100.0%)	1(25.0%)	3(75.0%)
B+	30	1(3.3%)	29(96.7%)	12(40.0%)	18(60.0%)
B-	4	0(0.0%)	4(100.0%)	1(25.0%)	3(75.0%)
AB+	6	1(16.7%)	5(83.3%)	2(33.3%)	4(66.7%)
AB-	5	0(0.0%)	5(100.0%)	0(0.0%)	5(100.0%)
O+	64	4(6.25%)	60(93.75%)	39(60.9%)	25(39.1%)
O-	3	0(0.0%)	3(100.0%)	0(0.0%)	3(100.0)
Total	184	8(4.3%)	176(95.7%)	98(53.3%)	86(46.7%)

$\chi^2 \approx 5.12$, $p \approx 0.40$ (IgM) *$\chi^2 \approx 9.28$, $p \approx 0.16$ (IgG)*

3.4. Molecular Results

Fifty positive samples for anti-*T. gondii* IgM and IgG antibodies (8 for IgM and 42 for IgG) by ELISA test were selected for amplification of gene B1 by PCR. Out of fifty samples, three samples were positive for IgM and were also positive for PCR), and there were no positive cases for IgG, as seen in a clear band at 529 bp in Fig. 3. The reason is that *T. gondii* trophozoites do not stay in the blood circulation; instead, they migrate to vital organs.

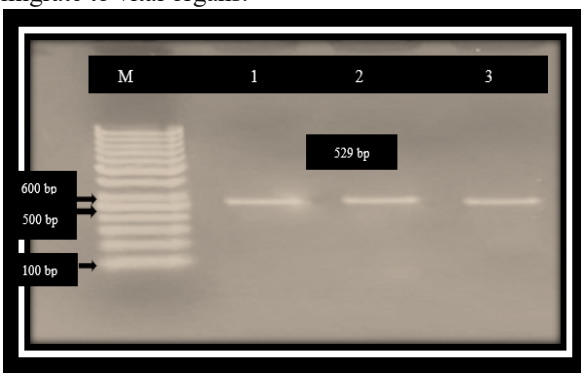

Figure 3: PCR products of *T. gondii* on 1% agarose. M: Marker 100 bp, and lanes 1-3 positive samples (band 529 bp)

4. Discussion

Toxoplasmosis is a transfusion-transmissible infection that can lead to severe complications, including mortality, particularly in immunocompromised individuals. Although the supply of blood is maintained under strict regulations and surveillance to ensure safety, the risk of transfusion-transmitted infections continues to be a concern [4]. Several studies indicate that *T. gondii* can be transmitted through blood transfusions, as the organism can spread to other large organs via blood flow during the early stages of infection [6], [18]. This study reported the seroprevalence of toxoplasmosis by ELISA and PCR.

This study reported IgM at 4.3% and IgG at 53.0% in 184 donors by ELISA, indicating low acute but high past exposure to *T. gondii* among blood donors, raising questions about transfusion safety and comparison with other regions. The presence of IgM and/or low-avidity IgG suggests recent infection and possible parasitemia. Several studies involving blood donors have identified *T. gondii* DNA in a subset of samples that tested positive for IgM [14], [19]. A high prevalence of IgG indicates that many donors have experienced past infections; however, serological testing alone cannot differentiate between infectious and non-infectious donations [20]. ELISA-based study in Al-Najaf province reported 33.7% IgG and 5.0% IgM positivity, reinforcing that overall IgG seropositivity in Iraq typically ranges around 30–40%, with IgM usually below 10% [21]. There is difference in rate of *T. gondii* IgM/IgG regionally and globally and this is depend on the geographical condition and associated risk factors for infection [1]

Regarding age pattern, the seroprevalence of *T. gondii* IgM/IgG in this study was increased with age, and this is compatible with cumulative acquisition of infection in early and middle adulthood, widely observed in blood donors and general populations [22]. As in studies from Romania by Lupu et al. [23], Iran by Hamdi et al. [24], and the United States by Jones et al. [22], rising seroprevalence with age reflects ongoing exposure to contaminated meat, water, soil, and other sources over time. Many population studies show that IgG seroprevalence rises with age and often peaks in middle or later adulthood, while IgM remains low and fairly stable, broadly aligning with your description, though details vary by setting. In Baghdad, IgG seropositivity by ELISA reached 90.2% in 30–39-year-olds, compared with 25.1% in <20-year-olds, clearly increasing with age [25]. In Zakho women, IgG seroprevalence peaked in 36–45 and 46–55 years (41.7% and 47.7%) and was lowest in 56–79 years; IgM was highest in 18–25 years but with no significant age trend, supporting an age-related IgG rise with relatively low IgM [26]. All these studies support that in Iraq and globally, *T. gondii* IgG seroprevalence generally increases from youth to middle age or later, then may plateau or decline slightly, while IgM remains low and relatively stable, with only modest elevations in some younger groups. A higher IgM in 20–39 years could indicate more recent infections in younger adults, similar to reports where infection risk correlated with younger age and specific behaviors (e.g., dietary habits, cat/soil contact [22]. Similar age-associated increases were seen in Romania (32.6% at 18–25 up to 67.6% at 56–63) and Serbia, where age was a strong independent risk factor [23], [24]. The age trend, which shows increased IgG seropositivity in donors between the ages of 20 and 39, is consistent with cumulative infection acquisition in early and middle adulthood, which is frequently seen in both general populations and blood donors [28]. Rising seroprevalence with age is indicative of continuous exposure to contaminated meat, water, soil, and other sources throughout time [27], [29].

A high prevalence of IgM was shown among blood donors, who may have a latent infection, as tested by an ELISA IgG rate of 53.0%. Blood donors in Iran (32.9%), Romania (45.9%), Turkey (24–26%), and Ghana (49%) have comparable or somewhat lower IgG prevalence [23], [30], [31], [22]. This indicates a severe environmental and/or foodborne exposure in

this area and places the donor population in Duhok and Zakho at the top end of the global range.

On the other hand, a number of studies and meta-analyses among those who donated blood that either indicate a weak or no independent effect of these parameters after adjustment are consistent with the lack of a substantial connection with cat contact, source of drinking water, canned meat, and blood group/Rh [23], [30]. While some big datasets indicate that cat interaction raises risk [32], others reveal no discernible correlation in blood donors, indicating that food pathways and ambient contamination may be more significant than direct contact with pet in many situations [33]. The lack of a blood group or Rh effect is also in line with worldwide analyses and Iranian donor data, which show that ABO and Rh variables often have little effect on infection risk [3], [9].

From the standpoint of transfusion safety, the combination of extremely high IgG and low IgM is comparable to trends in other endemic areas and suggests a sizable pool of donors who are chronically sick, with a smaller percentage possibly having just contracted the virus [30], [33]. Despite the seeming rarity of transfusion-transmitted toxoplasmosis, recorded instances and serological evidence in transfusion recipients demonstrate a real, although minimal, risk, especially for immunocompromised patients and pregnant women [6], [9]. These findings encourage the consideration of risk-based strategies to lower environmental acquisition and potential transfusion-related transmission, such as targeted screening of donations intended for high-risk recipients or enhanced food and hygiene education, given the high background exposure observed in Duhok and Zakho cities.

The PCR results (3/8 IgM+ PCR+, 0/42 IgG+ PCR+) indicate that blood PCR is most useful around acute/early infection and has low sensitivity in chronic IgG-only cases; this is within the line of Esboei *et al.* [34]. Experimental and clinical work shows that during chronic infection, *T. gondii* rapidly leaves the bloodstream and localizes in tissues (brain, muscle, lungs, and liver [35]. In a mouse model, blood in the chronic phase was PCR-negative, while brain tissue remained strongly positive [36]. Reviews of dissemination emphasize that parasites use leukocytes and then accumulate in organs, not persist in free circulation. This explains why IgG-positive, chronically infected patients often lack detectable DNA in peripheral blood [37]. Rezavand et al. [37] support the current study and the obtained results for ELISA *T. gondii* IgM/IgG and for *T. gondii* DNA by PCR as follows: 60.0% and 6.0%.

5. Conclusion

In conclusion, this study assessed the seroprevalence rate of toxoplasmosis among blood donors in Duhok and Zakho cities. Using ELISA, the seropositivity rates for IgM and IgG were found to be 4.3% and 53.0%, respectively. The seropositivity rates indicate that blood donors had previously been exposed to toxoplasmosis. Of the fifty samples tested, three were positive by PCR; notably, these three samples were also positive for IgM, while there were no positive results for IgG. Consequently, there is a need to consider implementing an awareness or education program for blood donors regarding potential transfusion-transmitted diseases in the future.

Declarations

Acknowledgments:

The authors gratefully acknowledge all researchers who study toxoplasmosis and blood donation.

Ethics approval and consent to participate:

The present study was approved by the ethical committee at General Duhok Director of Health in Duhok City with reference No. (15102025-8-4).

Consent for publication: I agree

Availability of Data and Materials:

Data will be provided upon receiving a valid request.

Conflicts of interest:

The authors declare that there is no conflict of interest

Conflict of Interest:

The authors declare that there is no conflict of interests.

Funding:

No funds.

Authors' contributions

First Author.: Methodology, Writing - review & editing.

Second Author: Conceptualization, Data curation, Methodology, Writing - original draft.

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