Review Article

Prevalence of *Toxoplasma gondii* among Iranian Blood Donors: A Narrative Review Article

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**Abstract**

**Background:** Toxoplasmosis is a common parasitic disease. There is likelihood of exposure to *Toxoplasma gondii* in blood donors during the periods of life. Currently, laboratory screening of blood donors for *T. gondii* is not routinely available. The objectives of this review were to study the effects of *T. gondii* on blood safety and to approach for risk reduction in blood recipients.

**Methods:** A literature search was performed using Cochrane library, PubMed, Scopus, Google scholar IranMedex, SID and Magiran without time limitation. All studies, which had reported the prevalence of *T. gondii* in Iranian blood donors in both English and Farsi languages, were evaluated and reviewed. The contents of the transfusion medicine text books related to this issue were reviewed. Searching keywords were "Blood Donors" or "Blood Transfusion" and "Toxoplasmosis", "Toxoplasma gondii", Iran.

**Results:** In order to study the prevalence of *T. gondii* in Iranian blood donors, six studies have been reviewed. IgG and IgM antibodies varied between 12.3% to 52.8% and 0% to 5.47%. Some of these studies have suggested to doing the screening for all blood donors. However, based on parasitological and epidemiological evidences, there is little chance for parasite transmission by blood transfusion.

**Conclusion:** By considering the moderate prevalence, difficulty in the differentiation between recent and past infections, and cost-effectiveness, it is not possible and rational to perform screening of donated blood. To reduce the risk of parasite transmission, leukofiltration method is recommended.
Introduction

Blood transfusion as a lifesaving therapeutic intervention is encountered with several threatening infectious agents including bacteria, viruses, parasite, and fungi. In particular, any agent that may have an intracellular life cycle can be transmitted through blood transfusion. Besides the well-known microbial agents, a number of emerging infections are also a threat to blood safety (1, 2).

Screening tests are performed for some of the known and the most important microbial agents in the blood centers as a routine procedure. According to the standards of the Iranian Blood Transfusion Organization (IBTO) laboratory screening for Hepatitis B virus, Hepatitis C virus, HIV, Treponema pallidum and HTLV-1/2 (in certain geographic areas) are carried out on all donated blood. Furthermore, to reduce the risk of transmission of infectious agents, Leukofiltration method has been suggested and now is accessible in IBTO for multi-transfused patients. By rigorous screening of all donated blood, the prevalence of the above-mentioned infectious agents decline dramatically. However, for various reasons, it is not possible to achieve zero risk.

One of these intracellular microorganisms, which have the potential for transmission through blood components, is Toxoplasma gondii. According to these facts, some researchers are interested in investigating the relationship between T. gondii and blood safety. Following the observation of serological reactions, due to not aware of the details related to the blood banking process and blood safety issues, the researchers concluded that blood centers should routinely undergo screening for T. gondii.

The objectives of this study is describing the impact of the parasite in blood safety and trying to answer the questions "whether there is a necessity for screening of T. gondii in blood donors?", also "whether available diagnostic modalities for T. gondii, are suitable as screening tests on donated blood?" and finally, investigate strategies for risk reduction.

Material and Methods

Search strategy

A literature search was performed in the following electronic databases: Cochrane library, PubMed, Scopus, Google scholar and Iranian databases such as: IranMедex, SID and Magiran. The Keywords were chosen based on MeSH terms and were categorized in three groups as follows: "Blood Donors" OR "Blood Transfusion" AND "Toxoplasma" OR "Toxoplasmosis" And Iran*. In addition, we searched this issue in a number of authoritative books, including textbooks related to transfusion medicine.

Data extraction

All retrieved resources were studied by two investigators. The extracted data from the papers included: location of the study, year of publication, laboratory method, sample size and seroprevalence. For taking more comprehensive insight about the subject, it has been searched and extracted the basic information including, life cycle of the T. gondii, epidemiology and diagnostic evaluation.

Inclusion and exclusion criteria

To search for parasite prevalence in Iranian blood donors, resources written in Farsi or English and undertaken in blood donors were our limitations. Due to the small number of studies conducted in Iran, all founded articles were evaluated and reviewed.

Results

After search for evidences relating to the prevalence of the T. gondii in blood donors in Iran, it was determined that six studies have been conducted. There were 4 articles in English, and 2 papers in Farsi. Articles published in Farsi have abstracts in English.

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These studies had been carried out in Gohbad, Zahedan, Fars, Rafsanjan and Tehran. All studies had used ELISA laboratory method by different Toxoplasma detection kits (Pishtaz-Teb Diagnostics, DSI, Trinity Biotech). Only one study has pointed to the sensitivity and specificity of the diagnostic kits, which were 100 and 99 percent respectively. Based on these studies, the prevalence of *T. gondii* IgG and IgM antibodies in Iranian blood donors varied between 12.3% to 52.8% and 0% to 5.47% respectively (Table 1). Molecular methods were used on two studies (Nested PCR and Real-Time PCR). The prevalence of *T. gondii* based on DNA and SAG1 mRNA detection was 1.9% and 6.97% respectively.

Overall, the studies were done on 2890 blood donors. All studies were cross sectional and conducted on Iranian blood donors from 2008–2014. The sample size range was through 235 to 1480. Total numbers of 719 individuals were identified as seropositive blood donors. In other words, in these studies the crude seroprevalence of *T. gondii* in Iranian blood donors could be considered as 25 percent (CI95 % 20% - 26.58%). The seroprevalence of *T. gondii* IgG and IgM antibodies were 21.5% (CI95 % 20% - 23%) and 3.3% (CI95 % 2.65% - 3.95%), respectively. None of the first or correspondent authors had a direct professional relationship with the blood transfusion centers. In order to understand the issue more comprehensive, the results of the search in relation to the life cycle, epidemiologic characteristics and diagnostic methods are presented below.

**The life cycle of *T. gondii* and transmission**

*T. gondii* is an apicomplexan protozoan parasite. It is an obligate intracellular protozoan and a common human pathogen. The parasite's life cycle consists of three different stages; these are oocysts, tachyzoites and bradyzoites (9). Following the activation of humoral and cellular immunity, only intracellular parasites or parasites that are present in the tissue cysts can survive. Therefore, with an effective immune response the numbers of tachyzoites are remarkably reduced in peripheral blood (10). Because we are encounter with an intracellular parasite, there is a possibility of the presence of parasites within white blood cells. Thus, there is potential for transmission by blood transfusion and rare cases of transfusion transmission have been reported (11, 12).

**Prevalence of Infection in general population**

Since blood safety is directly related to the prevalence of an infection in the community, so that, the frequency of infection in the general population is an important factor. It is generally estimated that up to a third of the world’s population have been infected by *T. gondii*. The prevalence rate varies in different geographical areas and different countries (13). Seroprevalence rates of 10-80% have been reported between different countries. North America, South East Asia, Northern Europe, and Sahelian countries of Africa are located in low seroprevalence (10 to 30%) areas. In Central and Southern Europe a moderate prevalences (30 to 50%) have been reported. High seroprevalence, in regions like Latin America and some African countries have been reported (14). According to seroepidemiologic studies obtained from a systematic review, seroprevalence rates in Iranian general population ranged from 18 to 70%. In humid mild northern regions, the highest frequency and the in warm and dry central regions lowest frequency is reported (15). Furthermore, the overall seroprevalence rate of toxoplasmosis among Iranian general populations was 39.3%, therefore, more than one third of Iranian people have been infected with *T. gondii* (16). However, regarding the seroprevalence rate, these studies have been performed in different sample size, sensitivity and specificity of laboratory methods.

**Diagnostic challenges in blood donors**

The most common diagnostic tests for toxoplasmosis are serological methods. However, the differentiation between acute and chronic infection is simply not possible. For this reason, a panel of tests is required for the diagnosis of
acute or chronic infection (17). Commercially available assays in terms of sensitivity and specificity are different (18).

In a patient who has clinical manifestations of toxoplasmosis, identification of Anti-Toxoplasma IgM antibody, while Anti-Toxoplasma IgG antibody is negative, most likely indicates an acute infection. Subsequently, the appearance of IgG antibody confirms the diagnosis of acute infection. If the titers of IgM antibodies are still high in repeated samples, without the appearance of IgG antibodies, probably this is a false positive reaction. Therefore, only seroconversion or the presence of IgM antibodies may be indicative of a recent infection.

In many cases, a positive result for IgM and IgG antibodies are reported simultaneously. In such cases, IgM antibodies could persist for a long time after primary infection. For this reason, based on only IgM antibody positivity, it could not be interpreted as acute infection. Therefore, the presence or absence of clinical findings is important. Even if there is a clinical suspicion of acute *Toxoplasma* infection, the diagnosis should be confirmed by another assay such as IgM capture ELISA and IgG avidity test as alternative diagnostic methods. Moreover, the IgG avidity test can be used for the differentiation between recent and past infections. In addition, the rising pattern of IgG could also be helpful.

False positive serologic results should be considered in the serologic test interpretation. A false positive IgM result may be related to non-specific binding, presence of rheumatoid factor and antinuclear antibodies (19, 17).

Molecular methods such as PCR are valuable to identify *Toxoplasma* infection in immunocompromised patients. Sensitivity of PCR varies widely and it is rarely performed in patients who are immunocompetent. The sensitivity of PCR on whole blood or Buffy coat ranged from 15% to 85% (20). However it should be mentioned that the detection of *Toxoplasma* DNA not necessarily indicates the presence of live parasites (11, 20, 21).

Histopathology is another diagnostic method. Histopathology assay can be done on blood, certain body fluids and tissue specimens. However, due to complexity of method, it is not implemented as a screening test. Blood cultures or cultures of certain body fluids and inoculated into mice or tissue culture also help in the diagnosis. However, these methods are not available as usual and routinely performed (22).

Considering above-mentioned facts, laboratory tests results should be interpreted precisely by clinical evidences, especially in blood donors.

### Table 1: The studies referred to the prevalence of *T. gondii* in Iranian blood donors

| Ref.                     | City-yr of publication | Laboratory Method | Total No. | Overall seroprevalence |
|--------------------------|------------------------|-------------------|-----------|------------------------|
| Ferdowsi et al. (3)      | Gonabad 2011           | ELISA             | 48 (16)   | 55 (18.3)              |
| Jafari Modrek et al. (4) | Zahedan 2014           | ELISA             | 94 (25)   | 94 (25)                |
| Sarkari et al. (5)       | Fars 2014              | ELISA PCR         | 182 (12.3)| 286(19.3)              |
| Ormazdi et al. (6)       | Tehran 2008            | ELISA             | 132 (52.8)| 141(56.4)              |
| Shaddel et al. (7)       | Shiraz 2014            | ELISA             | 58 (23.2)| 59(23.6)              |
| Zainodini et al. (8)     | Rafsanjan 2014         | ELISA PCR         | 80 (34.04)| 84(35.8)              |

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Discussion

There are special conditions in conjunction with the implementation of new laboratory tests and management for transfusion-transmissible infections. Therefore, any recommendations for laboratory screening in blood transfusion centers should be reinforced by specific technical evidence related to the screening process. Otherwise, recommending any advice based on the results of non-specialized research, will lead to a large amount of challenges. To give a more comprehensive insight about the subject, basic concepts are discussed first, and then the results of the desired articles would be noticed.

The characteristics of microbial agents to be considered as transfusion threat include: 1) the presence of the microorganism within host blood circulation in the course of disease 2) the presence of asymptomatic stages in the course of infection 3) the ability of the microorganism to survive under blood product storage conditions and 4) the ability of microorganism to affect the recipients’ health.

Making decision in establishment of a screening laboratory test on donated blood is a complex issue. Some of the important factors, which could be interfering, are the sensitivity and specificity of the test, the prevalence of infection in the donor population, and the likelihood of transmission of infection to recipients. In this regard, interpretation and implement of test as a screening on healthy low risk population like blood donors is completely different to use a test as diagnostic tool on a limited population of patients.

Regarding the blood safety layers, volunteers are selected as eligible blood donors by donor selection process that is made of interview and physical examination, but still it is likely that the apparently healthy donors, with dormant contamination of certain types of microorganisms enter into the donation cycle. Given the high rate of T. gondii seroreactivity in Iranian general population, it could be expected that substantial percentage of seroreactivity in blood donors would be detected.

T. gondii can survive in citrated blood at 4 °C for as long as 50 days (23). Thus, theoretically, T. gondii has the ability to transfer from the blood transfusion. Probably, considering these characteristics, a number of researchers are interested in the study of T. gondii transmission by blood transfusion. As a result, they have investigated the prevalence of T. gondii antibodies in blood donors.

The seroreactivity rate of toxoplasmosis in blood donors ranged between 18.3 to 56.4 percent (Table 1). In all of these studies in order to identify either IgG or IgM antibodies, the ELISA method by different commercial diagnostic kits has been used. In the materials and methods section of studies except one, details of sensitivity and specificity of the kits were not indicated. Ferdowsi et al. (3) just based on IgM antibody positivity, without considering other interfering factors have suggested that 2.3% of blood donors were infected with acute toxoplasmosis. Jafari Modrek et al. (4) also based on the positive results of IgG ELISA, concluded that 25% of donors were chronically infected with Toxoplasma. Shaddel et al, based on seroprevalence, which were obtained by ELISA, without adjusting by other circumstances, suggested that there is an acute or chronic infection in blood donors (24), and accordingly, recommended that performing screening tests should be considered. In all of these studies the diagnosis of chronic or acute infection is made only based on IgG or IgM ELISA test results. While, according to the previously mentioned details on the diagnostic evaluation section a serological test alone cannot define acute or chronic infection.

Moreover, in one study, in addition to ELISA, Nested PCR method was used. Among IgM antibody-positive donors, two PCR positive cases were found (5), in Zainodini investigation (8) testing was performed on 200 samples by Real-Time PCR, and have reported a high prevalence of SAG1 mRNA (6.97%)
positivity. In Sarkari et al. study, the Buffy coat samples were used to perform PCR, but Zainodini et al. has used the serum sample. Detection of mRNA could be interpreted as the presence of living microorganism or they may be associated with dead microorganism (25). Therefore, identification of viable parasites especially in serum samples, at this rate (about 7%) in blood donors with no clinical symptoms and signs, can be ambiguous and require further investigation. Regarding to the above-mentioned advantages and limitations of the PCR method, the results of these studies are considerable.

To investigate the seroprevalence of T. gondii in blood donors, numerous studies have been conducted in different countries. Totally frequency of anti-T. gondii IgG antibody of 4.1% to 75%, and IgM antibody of 0.28% to 5% have been recorded (Table 2). The results are approximately similar to the results of studies conducted in Iran.

Another important issue besides the effect of organism on recipients’ safety which should be considered to carry out some screening test on donated blood is the impact of screening test on blood supply, in the six mentioned studies, if the criterion for rejecting or accepting blood donors was the serologic test result, in the study population of 2890, 719 (25%) blood donor would be removed from the blood donation cycle, that means the loss of a large percentage of valuable resources.

Given the widespread presence of antibodies in the general population and the difficulty in the differentiation between recent from past infection, and since seropositive donors at all are not parasitemic and threat for recipient, so that in practice it is not possible and logic to implement screening on donated blood.

Table 2: The results related to the prevalence of T. gondii in blood donors

| Ref No. | Country        | Seroprevalence (%) | Laboratory Method |
|---------|----------------|--------------------|-------------------|
| 28      | India          | 20.5 3.6           | ELISA             |
| 29      | India          | 51.8 5             | ELISA             |
| 30      | Malaysia       | 28.1 -             | ELISA             |
| 31      | Iraq           | 30.25 2.50         | ELISA             |
|         |                | 34                | Latex agglutination |
| 32      | Mexico         | 7.4 1.9            | ELISA             |
| 33      | Brazil         | 75                | ELISA             |
| 34      | Egypt          | 59.6 -             | ELISA             |
| 35      | New Zealand    | 42.9 -             | Latex agglutination |
| 36      | Taiwan         | 9.3 0.28           | ELISA             |
| 37      | Turkey         | 20.25 2.33         | ELISA             |
| 38      | Saudi Arabia   | 52.1 -             | IFAT              |
| 39      | Czech Republic | 32.1 2.4           | IFAT              |
| 40      | North East Thailand | 4.1 4.3    | ELISA             |

Thus, by considering that there is a slight residual risk of T. gondii, the question arises: what is the solution? The first solution, which may be proposed, is doing an antibody screening in donated blood. However, as mentioned on the diagnostic evaluation procedures, each laboratory diagnostic method has their own limitations and is not suitable for screening. Even if blood centers decide to implement molecular tests, or a combination of serological and molecular tests, due to difficulties in interpretation of the results, need to a lot of time and large cost, carrying out of these methods are not possible in practice in blood transfusion centers. Therefore, it is always important that the cost-effectiveness for employing a screening
test have been considered before any recommendation.

Another choice would be selection of healthy blood donors. Regarding the process of donor selection which is included interviewing by physician and taking a medical history and physical examination. However, the majority of the people infected with parasites does not show any clinical symptoms or have distinguished history. Therefore, despite the selection process, reach to zero risk is not accessible.

Another strategy that can reduce the risk of transmission of intracellular microorganism is leukofiltration procedure. Leukoreduction of blood components can be done either at the time of collection (pre-storage) or by the side of the patient (bedside). By implementing this strategy, it is expected to reach residual leukocyte counts below 5 x 10^6 to 1 x 10^6 based on the type of filter (39). This intervention could reduce the leukocyte related immunologic adverse reactions, in conjunction with significant reduction in transmission of intracellular microorganisms such as *T. gondii*. Currently, IBTO provides the pre-storage leukoreduced products for a significant percentage of multi-transfused patients (thalassemia) to reduce the risk of alloimmunization and infectious adverse reaction.

The review of a number of reference books in the field of transfusion medicine is indicative of the fact that there is no recommendation for routine laboratory screening. Recommended interventions by the authors are leukofiltration of blood products and the selection of a limited number of seronegative blood donors for the special cases of the recipients (Table 3).

### Table 3: Recommendations and Interventions to improve blood safety in reference books

| References                        | Recommendation for routine serologic screening | Recommended Action                  |
|-----------------------------------|-----------------------------------------------|-------------------------------------|
| Rossi’s Principles of transfusion Medicine (12) | NO                                            | Leukocyte filtration                |
| Practical Transfusion Medicine (40)       | NO                                            | Leukocyte filtration                |
| Transfusion Microbiology (41)           | NO                                            | Selective donor screening            |
| Mollison's Blood Transfusion in Clinical Medicine (42) | NO                                            | Leukocyte filtration                |

Due to a high rate of exposure to the parasite in the general population, it would be possible that many of the blood recipients are already exposed and resistant to reinfection. However, if they have a serious disorder in the immune system, they may be at risk. Therefore, because major cases of acute and severe toxoplasmosis may occur in people with immunodeficiency, the other strategy might be identification and establishment a set of seronegative blood donors to blood donation for high-risk recipients.

### Conclusion

Considering the parasitological characteristics, specific conditions related to the process of selecting and screening of blood donors, laboratory limitations, and conditions related to blood recipients, remains a slight residual risk associated with *T. gondii*. Currently, laboratory screening of blood donors is not routinely available. The use of other methods for further reducing the residual risk, such as the leukofiltration system can provide more effective blood safety.

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