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Review

Parasitic infections and their potential threat to blood safety: a literature review from Iran

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Abstract

Introduction: Transmission of parasitic agents through transfusion can endanger the availability of safe blood and blood components for patients in need. The aim of this research was to describe the current status of transfusion-transmitted parasitic infections (TTPIs) in Iran and propose strategies to minimize their transmission risk.

Methodology: This narrative review included all studies that estimated the prevalence of TTPIs in Iranian blood donors based on parasitological, serological, and molecular techniques. A literature search was conducted for the period between 1960 and 2023 using medical subject headings (MeSH) terms in 11 English and Persian electronic databases. The extracted data were recorded on a pre-prepared checklist, and analyzed using SPSS.

Results: Twenty-nine studies were eligible for inclusion. A total of 12,643 blood donors were examined for malaria, visceral leishmaniasis (VL), and toxoplasmosis in endemic and non-endemic areas. The overall serological prevalence of malaria, *Leishmania infantum*, and *Toxoplasma gondii* infections among blood donors was 9.60%, 1.96%, and 35.75%, respectively. The results of molecular techniques were positive in 0.71%, 39.22% (only seropositive samples), and 8.73% for malaria, VL, and toxoplasmosis, respectively.

Conclusions: Considering the detection of parasitic DNA causing malaria, VL, and toxoplasmosis; and the presence of anti-*T. gondii* IgM antibodies among Iranian blood donors; their transmission through blood and blood components transfusion cannot be ruled out, particularly in endemic areas. Therefore, it is essential to adopt and implement appropriate strategies to minimize the risk of TTPIs and ensure the availability of safe and sufficient blood and blood components for patients in need.

Key words: parasitic infection; blood safety; malaria; leishmaniasis; toxoplasmosis.

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Introduction

Blood and blood components transfusion is sometimes necessary for the survival of patients, but it may also lead to the transmission of infectious agents [1]. Parasitic infections are one of the most significant adverse events related to the transfusion of blood and blood components which should be considered, especially in immunocompromised recipients [2]. Most blood donors infected with these infections are asymptomatic and parasitic infection carriers may possibly be present among donors in endemic and in non-endemic areas.

To date, the transmission of 6 parasitic infections, including 1 helminthic and 5 protozoan infections, through blood and blood components transfusion has been reported: malaria, American trypanosomiasis (Chagas disease), babesiosis, visceral leishmaniasis (VL), toxoplasmosis, and filariasis [3]. Although transfusion-transmitted parasitic infections (TTPIs) are less common than other microbial infections, they can cause life-threatening complications and mortality, particularly in hosts with a suppressed immune system [4]. There are various strategies to reduce the risk of acquisition of TTPIs, including donor selection, laboratory screening of donated blood, and the use of leukodepletion filters and pathogen inactivation technologies (PITs) [5–8].

In Iran, the Iranian blood transfusion organization (IBTO) is responsible for providing safe and sufficient blood and blood components for patients in need. Iranian donors donated about 2.3 million blood units in 2023, which is more than 27 donations per 1,000 population. The first step in the donor selection strategy or epidemiological screening of blood donation volunteers in 31 IBTO blood transfusion centers is an interview by a trained physician (about the history of diseases, traveling, and living conditions) to reduce the risk of transfusion-transmitted infections (TTIs). Furthermore, laboratory screening of the donated blood is performed for human immunodeficiency virus (HIV),

hepatitis B virus (HBV), hepatitis C virus (HCV), and syphilis. In addition, selected centers screen for human T-lymphotropic virus (HTLV) type 1 and type 2 [9]. However, testing of donated blood for TTPIs is not routinely done in Iran. Therefore, this strategy of donor selection is the only way of preventing the TTPIs in both endemic and non-endemic areas [10]. There are 6 transmissible parasitic infections that can be transmitted through the transfusion of blood and blood components; among them, malaria and VL are endemic in some parts of Iran (Figure 1) [11,12], and toxoplasmosis with a high seroprevalence has been reported from all areas of the country [13]. It should be noted that only transfusion-transmitted malaria (TTM) (344 cases) has been reported in Iran [14].

This narrative review was designed to describe the current status of TTPIs in Iran and propose strategies to minimize their transmission risk.

Methodology

Search strategy and databases

A literature search was carried out for literature published between 1960 and 2023 using medical subject headings (MeSH) terms and a combination of several keywords including "malaria", "visceral leishmaniasis", "leishmaniasis", "kala-azar", "*Leishmania*", "*Leishmania infantum*", "toxoplasmosis", "*Toxoplasma*", "*Toxoplasma gondii*", "blood transfusion", "blood donor", "blood donors", "healthy blood donors", "prevalence", "seroprevalence", "screening", and "Iran". A total of 11 electronic databases were searched, including PubMed, Web of Science, Scopus, Google Scholar, Science Direct, Islamic world science citation database (ISC), Elmnet, scientific information database (SID), Magiran, IranMedex, and Irandoc. The searches were limited to the papers published in English and Persian (Farsi).

Figure 1. Map of the geographical location of malarious areas of Iran consisting of Sistan and Baluchestan, Hormozgan and Kerman provinces, as well as the two main foci of visceral leishmaniasis (VL) or kala-azar: Ardabil Province in the Northwest and Fars Province in the South of Iran.

Data collection and analysis

This narrative review included all studies that estimated the prevalence of TTPIs in Iranian blood donors based on parasitological, serological, and molecular techniques. The publications were reviewed twice to eliminate duplicates and non-related studies. Overall, 29 publications were eligible for inclusion. The following data were extracted from the publications: first author's name, year of study, publication year, place (province or city) of study, sample size, testing method, seroprevalence, and molecular prevalence of TTPIs. Finally, the extracted data were recorded on a pre-prepared checklist. The statistical computations and data analysis were done using SPSS version 23.0 (IBM, Armonk, NY, USA).

Results

Six, 2, and 21 studies (out of 29 eligible studies) had reported the prevalence of malaria, VL, and toxoplasmosis, respectively, among blood donors in endemic and non-endemic areas of Iran. Tables 1–3 represent the characteristics and results of the studies included in this review.

Of the 6 studies conducted on 1,235 blood donors for malaria in endemic and non-endemic areas, the results of the testing methods were negative by light microscopic examination of Giemsa-stained peripheral blood smears and rapid diagnostic test (RDT) techniques [15–20]. Furthermore, the restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) and real-time PCR results were positive in 3% (3/100) and 2% (2/100), respectively (Table 1). In the 4 studies where 1,035 blood donors had been tested using indirect immunofluorescence assay (IFA) and enzyme linked immunosorbent assay (ELISA) methods, the results were positive in 13.98% (91/651) and 4.69% (18/384), respectively [16,17,19,20]. The overall serological and molecular prevalence of malaria infection was 9.60% (109/1,135) and 0.71% (5/704) among blood donors by different methods, respectively (Table 1).

Only 2 studies [21,22] had been carried out on 2,603 healthy blood donors in the two main foci of VL,

Table 1. Baseline characteristics of the blood donors from endemic and non-endemic areas of Iran, and the results of the studies included in this narrative review of malaria.

Reference	Year of study	Publication year	Province	City	Sample size	Testing method	% of seroprevalence (No.), method	% of molecular prevalence (No.), method
Hassanpour *et al*. [15]	2010	2011	Hormozgan and Tehran	Bandar Abbas and Tehran	100	ME, RDT, Real-time PCR	0 (0), RDT	2 (2), real-time PCR[a]
Sanei Moghaddam *et al*. [16]	2009	2011	Sistan and Baluchestan	Zahedan	384	ME, RDT, ELISA, PCR	0 (0), RDT; 4.69 (18), ELISA	0 (0), PCR
Moghtadaei *et al*. [17]	2002	2005	Sistan and Baluchestan	Iranshahr	120	ME, IFA, PCR	36.67 (44), IFA	0 (0), PCR
Kazemi *et al*. [18]	2002	2005	Sistan and Baluchestan	Chabahar	100	ME, RFLP-PCR	0	3 (3), RFLP-PCR
Edrissian [19,20]	1973–75	1975, 1985	Gilan and Tehran	Rasht and Tehran	531[b]	ME[c], IFA	8.85 (47), IFA	0
Total					1,235		9.60 (109/1,135)	0.71 (5/704)

ME: light microscopic examination of Giemsa-stained thick and thin blood smears; RDT: rapid diagnostic test; PCR: polymerase chain reaction; ELISA: enzyme linked immunosorbent assay; IFA: indirect immunofluorescence assay; RFLP: restriction fragment length polymorphism. [a]Two cases of 50 blood donors belonging to Bandar Abbas city. [b]These were paid donors. [c]This test was performed only in IFA positive donors.

including Ardabil and Fars provinces (Figure 1 and Table 2). The seroprevalence of *Leishmania infantum* (*L. infantum*) infection was 1.96% (51/2,603) by the direct agglutination test (DAT). Among the seropositive blood donors, 3.57% (1/28) and 82.61% (19/23) were positive by PCR and kinetoplastid deoxyribonucleic acid (kDNA) PCR techniques, respectively. The overall molecular prevalence of *L. infantum* infection was 39.22% (20/51 DAT-positive samples) (Table 2).

A total of 21 studies [23-43] have been reported (up to December 2023) on 8,805 healthy blood donors for toxoplasmosis in 14 provinces of Iran (Table 3). The overall seroprevalence of *Toxoplasma gondii* (*T. gondii*) infection was 35.75% (3,148/8,805), mainly (90.48%) detected by the ELISA method. The lowest and highest seroprevalence was observed in the Fars (15.25%) and Mazandaran (77.50%) provinces, respectively. The prevalence of anti-*T. gondii* IgG and IgM antibodies was 32.55% (2,741/8,420) and 2.18% (177/8,135) among Iranian healthy blood donors, respectively. The overall molecular prevalence of *T. gondii* infection was 8.73% (117/1,340) in the 10 studies performed by different methods (Table 3).

Discussion

Several strategies are used to prevent TTPIs in endemic and non-endemic areas, including donor selection, testing of donated blood, and the use of leukoreduction filters and PITs [5–8]. In Iran, the strategy of donor selection is the only way for risk mitigation of TTPIs [10].

The current status of transmissible endemic parasitic infections via blood and blood components transfusion, as well as the strategies for prevention of TTPIs and their challenges on the availability of blood safety in Iran are discussed separately in the following sections.

Malaria

Malaria is a life-threatening disease in humans caused by 5 species of the *Plasmodium* parasite [44]. In 2022, there were 249 million cases of malaria in 85 countries with 608,000 deaths [45]. The transfusion of blood and its components is one of the potential routes of malaria transmission [46]. Overall, more than 3,000 cases of TTM have been reported in the world [14]. According to a global systematic review and meta-analysis study, the median worldwide prevalence of malaria parasitemia among healthy blood donors was 10.54%, 5.36%, and 0.38%, identified by microscopy examination, molecular methods, and rapid diagnostic tests (RDTs), respectively [47]. Two major strategies, including donor selection and the testing of donated blood, are being implemented in endemic and non-endemic areas of malaria to reduce the risk of TTM

Table 2. Baseline characteristics of the blood donors from two main foci of Iran, and the results of the studies included in this narrative review of visceral leishmaniasis (VL).

Reference	Year of study	Publication year	Province	Sample size	% of seroprevalence (No.), Method	% of molecular prevalence (No.), Method
Asfaram *et al*. [21]	2016	2017	Ardabil	600	3.8 (23), DAT	82.61 (19 of 23 DAT-positive), kDNA PCR
Sarkari *et al.* [22]	NR	2015	Fars	2,003	1.4 (28), DAT	3.57 (1 of 28 DAT-positive), PCR
Total				2,603	1.96 (51/2,603)	39.22 (20/51)

DAT: direct agglutination test; kDNA: kinetoplastic deoxyribonucleic acid; PCR: polymerase chain reaction; NR: not reported.

[46]. Moreover, PITs and leukoreduction by filtration of blood and blood components could be efficient and safe techniques to prevent TTM [48–50].

Malaria is endemic in the southeastern parts of Iran, consisting of Sistan and Baluchestan, Hormozgan, and Kerman provinces (Figure 1) [11]. In 2022, 5,677

Table 3. Baseline characteristics of the blood donors from different parts of Iran, and the results of the studies included in this narrative review of toxoplasmosis.

Reference	Year of study	Publication year	Province	Sample size	% of seroprevalence and method					% of molecular prevalence (No.), Method
					Total (No.)	IgG (No.)	IgM (No.)	IgG & IgM (No.)	Testing method	
Asfaram *et al*. [23]	2017	2021	Ardabil	462	36 (166)	32.5 (150)	1.5 (7)	1.9 (9)	ELISA	18 (30 of 166 seropositive samples), PCR
Hosseini *et al*. [24]	2014	2020	Mazandaran	400	77.50 (310)	73.5 (294)	2.2 (9)	1.8 (7)	ELISA	2.38 (7 of 294 seropositive samples), nested PCR
Manouchehri Naeini *et al*. [25]	2017	2019	Chaharmahal and Bakhtiari	385	40 (154)	37.9 (146)	1.56 (4)	1.03 (6)	ELISA	1.56 (6), LAMP
Saki *et al.* [26]	2015	2019	Khuzestan	380	37.9 (144)	34.47 (131)	0.5 (2)	2.9 (11)	ELISA	40 (20 of 50 seropositive samples), nested PCR
Kalantari *et al.* [27]	2016–2017	2018	Mazandaran	500	63.8 (319)	63.2 (316)	0.95 (3)	0	ELISA	31.8 (21of 66 IgG-positive samples), PCR
Moshfe *et al.* [28]	2015	2018	Kohgiluyeh and Boyer-Ahmad	285	16.8 (48)	16.30 (46)	0	0.7 (2)	ELISA	0 (0 of 48 seropositive samples), PCR
Sadooghian *et al.* [29]	2014-2015	2017	Khorasan Razavi	491	40.7 (200)	37.5 (184)	1.6 (8)	1.6 (8)	ELISA	100 (16 only IgM-positive and both IgG and IgM samples), nested PCR
Bahhaj *et al.* [30]	2014	2017	East Azerbaijan	194	39.69 (77)	38.66 (75)	1.03 (2)	0	CLIA	0
Hazrati Tappeh *et al.* [31]	2013	2017	West Azerbaijan	270	37.8 (102)	37.8 (102)	0 (0)	0	ELISA	0
Zarean *et al.* [32]	2013	2017	Khorasan Razavi	500	29.6 (148)	25 (125)	3.2 (16)	1.4 (7)	ELISA	0
Mahmoudvand *et al.* [33]	2014	2015	Kerman	500	32 (160)	28.8 (144)	2.2 (11)	1 (5)	ELISA	9 (1 of 11 only IgM-positive samples), real-time PCR
Gholami *et al.* [34]	2013	2015	Hamedan	540	56.3 (304)	54.4 (294)	1.9 (10)	0	ELISA	0
Davami *et al*. [35]	2010–2011	2015	Fars	400	15.25 (61)	13.5 (54)	1.75 (7)	0	ELISA	0
Zainodini *et al*. [36]	2013	2014	Kerman	235	35.7 (84)	34.04 (80)	1.71 (4)	0	ELISA	6.97 (14 of 200 samples), real-time PCR
Shaddel *et al*. [37]	2013	2014	Fars	250	23.6 (59)	23.2 (58)	0.4 (1)	0	ELISA	0
Sarkari *et al.* [38]	2012–2013	2014	Fars	1,480	19.3 (286)	12.3 (182)	5.47 (81)	1.6 (23)	EIA	1.9 (2 of 104 only IgM-positive and both IgG and IgM positive samples)), nested PCR
Shaddel *et al*. [39]	2012	2014	Tehran	223	39 (87)	38.6 (86)	0.45 (1)	0	ELISA	0
Jafari Modrek *et al.* [40]	NR	2014	Sistan and Baluchestan	375	25 (94)	25 (94)	0 (0)	0 (0)	ELISA	0
Ferdowsi *et al.* [41]	2011	2013	Khorasan Razavi	300	18.33 (55)	16 (48)	0.6 (2)	1.6 (5)	ELISA	0
Ormazdi *et al.* [42]	2008	2010	Tehran	250	56.4 (141)	52.8 (132)	3.6 (9)	0	ELISA	0
Jalayer *et al.* [43]	1996	NR	Isfahan	385	38.7 (149)	0	0	0	IFA	0
Total				8,805	35.75 (3,148/8,805)	32.55 (2,741/8,420)	2.18 (177/8,135)			8.73 (117/1,340)

ELISA: enzyme-linked immunosorbent assay; PCR: polymerase chain reaction; RFLP: restriction fragment length polymorphism; LAMP: loop-mediated isothermal amplification; CLIA: chemiluminescence immunoassay; EIA: enzyme immunoassay; NR: not reported; IFA: indirect immunofluorescence assay.

malaria cases were reported, of which 4,238 (74.65%) were imported cases [45]. The donor selection strategy is the only step in the prevention of TTPIs in both endemic and non-endemic areas in Iran. Based on this strategy, the potential blood donors with a past history of malaria are permanently deferred from donating blood. Furthermore, blood donation volunteers with a history of residence in, or travel to, malarious regions are deferred for 3 years and 1 year, respectively [10].

In this study, the serological and molecular prevalence of malaria infection among blood donors was 9.60% (109/1,135) and 0.71% (5/704), respectively (Table 1). The results demonstrate that the transmission risk of malaria via blood transfusion still exists in the endemic and non-endemic areas of Iran. Furthermore, the donor selection strategy alone is not sufficient to prevent TTM and cannot guarantee the availability of safe blood and blood components, especially in endemic areas. Therefore, in addition to the strict and complete implementation of donor selection strategy in the 3 malarious provinces (Figure 1), all donated blood should be screened with an antigen-based detection test or a molecular method (universal screening). The donor selection strategy in non-endemic areas of malaria must be fully implemented and suspected cases screened with an antibody-based detection test (selective screening). It has been found that the incidence of TTM decreases in parallel with the increasing use of leukoreduction filters [50]. Therefore, this strategy is recommended to minimize the risk of TTM in all blood transfusion centers of the IBTO at the time of blood collection (pre-storage leukoreduction) or hospitals at the patient's bedside during the blood transfusion (post-storage leukoreduction). In Iran, 53.63% of packed red blood cells (PRBCs) were leukoreduced in blood transfusion centers of the IBTO in 2023. It is notable that all or part of donated blood is tested for malaria in 51 countries using microscopy examination of peripheral blood smears and immunodiagnostic (antibody or antigen) techniques [51].

Visceral leishmaniasis (VL)

The etiologic agents of VL or kala-azar, the most severe clinical form of leishmaniasis, are *Leishmania donovani* (*L. donovani*) and *L. infantum* [52,53]. It is estimated that 50,000 to 90,000 new cases of VL occur worldwide every year, but only 25–45% of cases are reported to the World Health Organization (WHO) [52]. Although transfusion-transmitted leishmaniasis (TTL) is relatively rare [3] and only 14 controversial cases have been reported in the world [54], *Leishmania*-infected blood donors could be a potential source of infection transmission for the high-risk groups such as immunocompromised patients, pregnant women, and infants [3,54]. The seroprevalence of VL was 7% among blood donors based on studies in different regions of the world [55]. There is a lack of approved tests [56] and the laboratory screening of blood donations for VL is not routinely performed in blood transfusion centers worldwide [54]. Moreover, the majority of *L. donovani* and *L. infantum* infections are without clinical symptoms in healthy hosts such as blood donors [57], as well as a positive serologic result does not demonstrate active infection [58]. Therefore, reducing the risk of TTL is not possible by only implementing donor selection and serological screening strategies. Since *Leishmania* parasites are obligate intracellular pathogens of host macrophages [59], filtration of leukocytes seems to be the most efficient strategy to reduce TTL [57,58]. The use of PITs has indicated different results in decreasing the parasite load of *Leishmania* in various blood components [56,60–62].

VL caused by *L. infantum* is endemic in at least 8 provinces in Iran with about 100–300 new cases per year [12,63]. All blood transfusion centers of the IBTO have adopted the permanent deferral of blood donation volunteers with a history of VL as the only strategy to minimize the risk of TTL [10]. In the present study, the overall serological and molecular prevalence of *L. infantum* infection was relatively high among healthy blood donors; 1.96% (51/2,603) and 39.22% (20/51 DAT-positive samples), respectively (Table 2). These results indicate that the risk of TTL still remains in endemic and non-endemic areas of Iran, and the current strategy is not sufficient and there is a need for more effective approaches to reduce the transmission risk of *Leishmania* parasites via blood transfusion. Therefore, in addition to the donor selection strategy, filtration of blood and blood components for removing or reducing leukocytes is recommended as the most efficient strategy to prevent the TTL in high-risk groups, especially in VL-endemic areas.

Toxoplasmosis

An obligate intracellular zoonotic protozoan named *T. gondii* is the causative agent of toxoplasmosis in warm-blooded hosts [64,65]. The seroprevalence of *T. gondii* infection has been reported to be between less than 10% and more than 90% in different parts of the world [65]. This infection is often asymptomatic in individuals with normal immune systems [66]. Pregnant women, neonates and children, and immunocompromised hosts are the high-risk groups for

toxoplasmosis [65,67–69]. The occurrence of transfusion-transmitted toxoplasmosis (TTT) is rare and only 5 cases have been reported, including 4 definite cases and 1 possible case through granulocyte concentrates and platelet transfusions, respectively [70,71]. The overall prevalence of anti-*Toxoplasma* antibodies was 33% among healthy blood donors in different countries, based on a systematic review and meta-analysis study [72]. Preventing or reducing the risk of transmitting toxoplasmosis through blood and blood components transfusion is not feasible by donor selection and serological testing strategies due to the following reasons [73]: (1) high seroprevalence of *T. gondii* infection among blood donors [72]; (2) lack of licensed laboratory test for *T. gondii* infection screening in blood donors [9]; and (3) a positive serologic result does not mean active *T. gondii* infection [72]. The *T. gondii* parasite has the ability to survive and multiply within leukocytes [73]. Therefore, the use of leukodepletion filters can be effective in reducing the risk of TTT [74]. It should be noted that discarding donated blood based on positive serological results severely endangers blood and blood components supply for patients in need, especially in countries with a high prevalence of *T. gondii* infection [73].

The only strategy adopted by the IBTO for preventing TTT is up to 6 months deferral of blood donation volunteers with clinical toxoplasmosis after potential treatment and complete recovery [10]. In the current study, the overall serological and molecular prevalence of *T. gondii* infection among healthy blood donors was 35.75% (3,148/8,805) and 8.73% (117/1,340), respectively (Table 3). On the other hand, the transmission risk of toxoplasmosis by the transfusion of blood and blood components still exists due to its relatively high prevalence. Moreover, the current strategy alone is not enough, and other existing, applicable, and effective strategies should be used for mitigating the risk of TTT. Regarding the ability of the *T. gondii* parasite to survive and multiply within leukocytes [73], in addition to the donor selection strategy, the filtration of blood and blood components is strongly recommended, particularly for groups at high-risk of toxoplasmosis.

To date, there is no documented report of Chagas disease (CD), human babesiosis, and filariasis; as well as their transmission by the transfusion of blood and blood components in Iran. Although the blood donation volunteers with a history of CD and babesiosis are permanently deferred in blood transfusion centers of the IBTO, no strategies are implemented to prevent the transfusion-transmitted filariasis (TTF). Given the increasing travels to areas endemic to CD, babesiosis, and filariasis, an efficient strategy should be adopted for preventing their transmission via blood and blood components transfusion in Iran.

Conclusions

Adopting and implementing appropriate strategies plays a key role in the supply of safe blood and blood components, especially for immunocompromised patients in need.

Given the detection of parasitic DNA causing malaria, VL, and toxoplasmosis, and the presence of anti-*T. gondii* IgM antibody among Iranian blood donors, their transmission through blood and blood components transfusion cannot be ruled out, particularly in endemic areas. Furthermore, the availability of parasite-free blood and blood components is not possible by implementing the current policy of donor selection alone. Therefore, in addition to the donor selection strategy, screening of all blood donations is strongly recommended in malarious areas with a test based on antigen detection or molecular method. Moreover, the filtration of blood and blood components is recommended, especially for high-risk groups of malaria, VL, and toxoplasmosis in endemic areas.

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Conflict of interest

No conflict of interest is declared.

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