Toxoplasmosis among Blood Donors: Unsafe Blood Transfusion in Ibadan, Southwest Nigeria

Abimbola Amoo1,2, Kariuki Njaanake2, Dada-Adegbola O. H1, Gloria Omosa-Manyonyi2

Abstract:
BACKGROUND: In Nigeria, there is paucity of data on transfusion-transmissible parasitic infections that can cause post-transfusion illness, especially in immunocompromised and transfusion-dependent patients. This study was designed to bridge the gap by screening for Toxoplasma gondii which can be transmitted by blood transfusion.

OBJECTIVES: The main objective of the study is to employ serology methods to screen blood donor’s serum for T. gondii in Blood Bank Transfusion Service Centre, Southwest Nigeria.

MATERIALS AND METHODS: This is a cross-sectional study. Donor sera were tested for T. gondii infection using IgG and IgM enzyme-linked immunosorbent assay test kits.

RESULTS: A total of 248 donated blood sera were tested for T. gondii infection after storage. The seroprevalence of T. gondii IgG and IgM was 19.8% and 42.7%, respectively. There was a significant difference in anti-T. gondii IgM seroprevalence between vegetarians and generalists (3.2% vs. 40.8%; P < 0.002).

CONCLUSIONS: The anti-T. gondii IgM prevalence was relatively higher compared to anti-T. gondii IgG, implying the majority of seropositive donors had an acute or recent infection, might seroconvert to chronic infection. There was a lower seropositivity of T. gondii among vegetarians.

Keywords: Transfusion-transmissible infection, transfusion-transmitted toxoplasmosis, haemoparasite

Introduction
A transfusion-transmissible infection (TTI) is an infection transmitted from one person to another through blood transfusion.[1] Infectious pathogens that cause TTI include bacteria, viruses, and parasites. Some of these pathogens also can be transmitted by other means including intravenous drug use, vector, and sexual intercourse, but as transfusion is an important mode of transmission of this pathogenic microorganism, there is a need to screen blood before transfusion.[1-2]

Current transfusion blood screening services in Sub-Saharan Africa have focused mainly on viruses, such as human immunodeficiency virus (HIV), Hepatitis B and C, and bacteria such as Treponema pallidum.[1] However, there is a need to consider other important pathogens such as Plasmodium spp. and Toxoplasma gondii which have been implicated in TTI.[3]

The epidemiologic characteristics of most of the developing tropical countries such as Nigeria are characterized by the occurrence of endemic parasitic infections and diseases, which are either absent or rare in developed countries. Another major difference is lack of or inadequate capacity, both in terms of workforce and infrastructure, to diagnose and manage the diseases.[4] In the developed countries, especially in Europe and the United States, there is continuous...
improvement of screening procedures with the relatively recent development of advanced serological and nucleic acid amplification test.[6] These have resulted in decreased residual risk of TTI over the past three decades.[6] Since the incidence of blood transfusion-transmitted parasitic (TTP) infections is lower in nonendemic regions, most blood banks do not screen donor blood samples for potentially pathogenic hemoparasites. However, in endemic regions, if the same practice is copied, it poses a risk to those requiring blood transfusion.

This risk is higher in multiple blood recipients, neonates, pregnant women, and immunocompromised patients, who have less ability to mount an immune response against the parasites.[2] The WHO recommends quality-assured screening of all donated blood for transfusion-transmissible pathogens such as HIV, hepatitis B, hepatitis C, T. pallidum (syphilis), Trypanosoma cruzi, and Plasmodium spp. in some countries.[5] In spite of this, blood screening for parasites is not routinely carried out in many countries such as Nigeria.[8] The concern about HIV and transfusion-transmitted hepatitis infection has overshadowed the fact that other diseases, particularly parasites like T. gondii can be spread by transfusion and cause severe infection, especially in immunocompromised patients.

In Nigeria, screening for T. gondii and other endemic hemoparasites are not included in the current National Blood Transfusion Guidelines. This is because transmission of parasitic microorganisms through blood transfusion is generally not regarded as a serious problem in an adult whose level of immunity is thought to be sufficiently effective in combating TTP infection.[9] High level of occurrence of blood transfusion demanding health conditions increases the possibility of transmission of bloodborne parasitic diseases.[9]

**Literature review**

T. gondii are considered as threats to blood recipients’ health, especially immunocompromised and multiple units blood recipients. Seroprevalence of 20.8% was reported for T. gondii infection in a study among healthy individuals in Nigeria Ishaku et al.,[10] in Zaria, Northwest and Deji-Agboola et al.,[11] in Lagos, Southwest Nigeria revealed 29.9% and 40.8%, respectively.[9] These two-parasite species are transmissible via blood and blood product transfusions mainly due to: (1) the presence of asymptomatic stages in the course of parasitic infection; (2) the presence of an infective form of parasites within donor blood circulation, which can cause severe illness when transfused to immunosuppressed recipient in high concentration; (3) the ability of the parasite to survive in blood sample or blood product under storage conditions.[6]

The WHO recommends that donated blood should be screened for endemic parasites and other pathogenic organisms prior to transfusion.[9] Unfortunately, this is not done in most countries.[12] In Nigeria, the national blood transfusion service policy recommends that all donated blood be screened for HIV, hepatitis B and C, and syphilis,[13] thereby laying emphasis on screening for the viruses and syphilis and less or no attention is paid to the effect of transfusion-transmitted Plasmodium falciparum and T. gondii despite their endemcity and public health importance.

Toxoplasmosis is an opportunistic infection caused by the obligate intracellular blood protozoan called T. gondii, first discovered by Nicolle and Manceaux in 1908 in a small North African rodent called Ctenodactylus gundi. It is an important zoonotic infection, in which feline is the definitive hosts. The parasite infects about one-third of the world’s population.[14] Two forms of toxoplasmosis are found in humans, the actively proliferating tachyzoites, usually seen in the initial, more acute phase of the infection and the slowly dividing bradyzoites, which form cysts in brain and skeletal muscle, as a result of the immune response from the host.[15] Routes of T. gondii infection in humans include (1) ingesting food or water that is contaminated with oocysts shed by cats, in the soil or in garbage; (2) eating undercooked or raw meat containing tissue cysts; (3) contamination of open wounds, arthropod bites, transplantation, and blood[6] transfusion; and (4) congenitally, from infected mothers to their babies[15] and; (5) sexual transmission.[16]

Following infection, the parasite invades human tissue and cells,[17] multiply in all nucleated cells by internal budding (endodyogeny). They proliferate within the host cell during the acute stage Toxoplasma infection and form pseudocyst. These asexual stages of T. gondii give rise to merozoites, which enter the blood circulation and form cysts in tissue.[18] Toxoplasmosis acquired via blood or blood product transfusion is the main cause of ocular Toxoplasma infection, which occurs as result of a chronic proliferation of tachyzoites in the retina or as result of hypersensitivity response to ruptured tissue cyst.[19‑21]

This may present as uveitis, thalassemia, who need frequent and regular blood transfusion sourced from different donors for survival.[21] As a result, infected asymptomatic blood donors may transmit T. gondii.[22]

Studies have shown a high prevalence of anti-T. gondii antibodies among blood donors in Sub-Saharan Africa, but still screening of blood for T. gondii is not routinely done.[23] Similarly, studies on the epidemiology of T. gondii infection among groups of patients and healthy individuals have been carried out, but only a few studies have been conducted among blood donors in
T. gondii transmitted from healthy blood donors to recipients has become a major concern in transfusion medicine, especially among groups of recipients with an impaired immune system.\(^\text{24}\) Screening for T. gondii infection among blood donors is mainly by serological tests, which includes Sabin–Feldman test, indirect hemagglutination test, direct hemagglutination, indirect immunofluorescence test, and enzyme-linked immunosorbent assay detection of anti-Toxoplasma IgG/IgM antibodies in serum. Molecular screening, immunoblotting, and tissue biopsy have also been used for the detection of active T. gondii infections.\(^\text{25,26}\) However, serological diagnosis is difficult in immunocompromised patients, because reactivation of T. gondii or chronic phase of infection does not promote changes or may fail to detect any changes in the antibody level,\(^\text{26}\) especially in AIDS patients, also in some chronic cases of toxoplasmosis, the IgM antibodies persist, thus confusing the interpretation of serological results.\(^\text{6,26}\)

Toxoplasmosis is self-limited, especially in immunocompetent individuals, hence treatment is not necessary. However, treatment is appropriate for toxoplasmosis in pregnancy because the risk of congenital infection can be reduced. Furthermore, appropriate therapy is necessary in patient with persistent, severe, or visceral T. gondii infection.\(^\text{6}\)

The choice of treatment for T. gondii infection is combination of sulfadiazine (1–1.5 g orally four times daily) and pyrimethamine (200 mg loading dose, then 50–70 mg [1 mg/kg] orally once daily) with folinic acid (10–20 mg orally once daily), these drugs have effect against acute stage or active tachyzoites of T. gondii but less beneficial in active multiplication of T. gondii and do not eradicate infection.\(^\text{24}\)

Pyrimethamine and sulfadiazine are active against multiplying tachyzoites. However, because of the serious side effects such as bone marrow suppression, leukocytopenia, and thrombocytopenia, alternative regimens are possible such as clindamycin. Other possible alternatives are cotrimoxazole or combination of pyrimethamine with clarithromycin, atovaquone, dapsone, or azithromycin.

The standard regimen for the treatment of acute T. gondii infection during pregnancy is spiramycin (1 g orally three times daily until delivery) because pyrimethamine causes teratogenic effects during pregnancy. Furthermore, doses of the standard regimen can be used in severe immunosuppressed cases as prophylaxis due to increased risk of myelotoxicity and encephalitis.\(^\text{24}\)

### Materials and Methods

The study population consisted of blood donors, at the Oyo state National Blood Transfusion Service Centre, Ibadan, Southwest Nigeria, majority of whom were members of voluntary nonremunerated blood donors. The study was a cross-sequential study among blood donors in Oyo state National Blood Transfusion Service Centre conducted between January and August 2018. Only blood donors that were fit for blood donation and gave their consent were recruited into the study. Their venous blood samples were taken from the cord of the blood bags and examined for T. gondii infection.

A random sampling technique was used to select all eligible and fit participants for the study. From the blood bank, 248 consenting donors aged between 18 and 59 years were randomly recruited following their informed consent, excluding those that have being on cotrimoxazole in last 1 month.

### Data and specimen collection of Toxoplasma gondii infection

Structured closed-ended self-administrated questionnaire was used. Section A covered the sociodemographic characteristics of blood donors such as age, race, marital status, and determinants socioeconomic status, such as level of education and occupation. Section B explored the risk factors determining the susceptibility of donors to T. gondii infection such as open and stagnant drainage system, and raring cats.

About 2.5 ml of each sample was aliquoted; a universal bottle labeled. The blood in bottles labeled f0 and was stored at 4°C in the refrigerators at the Department of Medical Microbiology, University College Hospital, Ibadan, then used to screen for T. gondii after storage. Anti-T. gondii IgG and IgM blood samples were detected by using qualitative anti-T gondii enzyme-linked immunosorbent assay (ELISA) kits.\(^\text{6}\)

Anti-T. gondii IgM and IgG were detected using commercially available anti-Toxoplasma antibody ELISA kits, according to the manufacturer’s instructions.

The donors stored serum samples were diluted and put in the wells precoated with T. gondii antigens. Anti-T. gondii IgM or IgG in test sera are bound to the antigens to form an immune complex and enzyme-conjugated antihuman globulin detects and binds any such complex.

A chromogenic substrate was added to produce yellow color whose intensity depends on the amount of antibody in the complex. The absorbance of the microwell contents was read by a spectrophotometer with a filter of 450 nm. The calibrators and controls
are utilized in internal quality control and in the qualitative interpretation of the assay. A sample that turned positive for anti- \textit{T. gondii} IgG and negative for IgM was interpreted to mean a chronic \textit{T. gondii} infection. A sample with both anti- \textit{T. gondii} IgG and IgM \textit{T. gondii} were considered to be a presence of acute on chronic \textit{T. gondii} infection. A positive IgM anti-\textit{T. gondii} only sample was considered to be the presence of acute and recent \textit{T. gondii} infection. Data were entered into Microsoft Excel Spreadsheet and double checked for accuracy. Data on the prevalence of \textit{T. gondii} infection and sociodemographic risk factors were analyzed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Categorical variables such as gender and occupation were presented in frequencies and percentages. Comparative statistics were carried out using Student’s t-test for continuous variables and Chi-square test for categorical variables. Correlation between parasite positive samples with sociodemographic characteristics was tested using the Pearson’s correlation coefficient. For all tests of significance, confidence level was set at 95% and \( P < 0.05 \) was considered as statistically significant.

**Ethical considerations**

Ethical review and approval for the study were obtained from the Oyo State ethical review committee in Oyo state, Ibadan, Nigeria. Written informed consent was obtained from each study participant before enrolment into the study. The participation was totally voluntary. Confidentiality was maintained at all times during and after the data collection. Specimen and data were stripped of all personal identifiers.

**Results**

A total of 248 asymptomatic blood donors aged between 18 and 59 years were enrolled in the study. Eighteen (7.2%) of the participants were female and 230 (93%) were male, with 246 (99.2%) voluntary nonremunerated blood donors, while 2 (0.8%) were commercial blood donors. Two hundred and thirty-two (94%) participants were residents of Ibadan and 194 (78.2%) were natives of Southwest Nigeria. About 42.7% were between aged 29 and 39 years, and 58% of the participants were higher institution graduates.

Of the 249 blood donors, 49 (19.8%) tested positive for \textit{T. gondii}. With regard to age, the highest prevalence of anti-\textit{T. gondii} (58%) was found in the age group 29–39. The difference between age group and presence of anti-\textit{T. gondii} IgG antibodies was not statistically significant (Chi-square test; \( P = 0.148 \)). The difference between age group and presence of anti-\textit{T. gondii} IgM antibodies was not statistically significant (Chi-square test; \( P = 0.470 \)).

ELISA qualitative test was done for each sample following the manufacturers’ instructions, independent of the ELISA anti-\textit{T. gondii} IgG antibody results. Out of 248 stored blood samples tested, \textit{T. gondii} IgM antibody were detected in 106 samples, corresponding to 42.7% IgM seropositive sera. Chi-square was used to determine the association between \textit{T. gondii} infection and the sociodemographic characteristics as shown in the Table 1.

The highest prevalence (30%) and (43.3%) of anti-\textit{T. gondii} IgM positivity was reported in the 19–29 and 29–39 years age group, respectively, and the lowest (0.01%) was found in the <19 years age group. The difference between age groups in the prevalence of anti-\textit{T. gondii} IgM antibodies was not statistically significant (Chi-square test; \( P = 0.917 \)).

There was significant association between seroprevalence of \textit{T. gondii} IgM antibodies in vegetarians (20%) and (47%) nonvegetarians \( (P < 0.01) \). There was no significant association between anti-\textit{T. gondii} IgM antibodies in drinking unpasteurized milk (Chi-square test; \( P = 0.051 \)). There was no significant association between seroprevalence of anti-\textit{T. gondii} IgM rearing cats \( (P = 0.470) \), eating rodents \( (P = 0.659) \), education \( (P = 0.705) \), previous blood transfusion \( (P = 0.470) \), or eating undercooked meat \( (P = 0.498) \), as shown in Table 1.

**Discussion**

Screening of blood donors is important for prevention and control of transfusion-transmissible pathogens to the blood recipients. Serological screening tools such as ELISA are essential for detection of parasite antigen and antibodies. The 19.8% prevalence of anti-\textit{T. gondii} IgG found in this study is low compared to 32% seroprevalence reported among healthy individuals in northern part of Nigeria, but similar to findings in the other countries of same socioeconomic status such as China, Costa Rica, South Africa, Turkey, Mexico, and Chile.

| Sociodemographic characteristics | Anti-Toxoplasma IgM | Chi-square test | \( P \) |
|---------------------------------|----------------------|-----------------|------|
| Vegetarian                      |                      |                 |      |
| Yes                             | 8                    | 32              | 10.0789 | 0.002* |
| No                              | 98                   | 110             |       |
| Eating undercooked meat         |                      |                 |      |
| Yes                             | 65                   | 81              | 0.4589 | 0.498 |
| No                              | 41                   | 61              |       |
| Contact with cat                |                      |                 |      |
| Yes                             | 3                    | 1               | 0.5229 | 0.470 |
| No                              | 103                  | 141             |       |

*Statistically significant at level of <0.05
as Karnataka Indian.[27] However, the findings were discordant to seroprevalence among blood donors in Iran[28] and Thailand,[29] with lower results of 12.3% and 4.1%, respectively. This result suggests that there was less chronic *T. gondii* infection among blood donors in Ibadan, Southwest Nigeria. The seroprevalence of anti-*T. gondii* IgM in this study was 43%, this was high compared to the prevalence of 11.3%, 5.5%, and 0.3% among blood donors in Abidjan, Côte d’Ivoire,[21] Southern Iran[40] and Taiwan,[24] respectively. This implies that many of the blood donors will seroconvert from acute infection to chronic toxoplasmosis, which may lead to retinochoroiditis, encephalitis, or epilepsy later in life.[29]

These variations in the seroprevalence of anti-*T. gondii* antibodies among blood donors might be due to difference in sex, ages, food habits, residences, level of education, environmental hygiene, contact with cat, and socioeconomic status[30] in the study area. Moreover, the types of antibodies for detection, the types of test kit, as well as sensitivity and specificity of the test kit also varies.[24]

In addition, the high seroprevalence rate of 12.9% and 18.5% were found in age group 19–29 years and 29–39 years, respectively. This has been reported from another study among blood donors in Northeast Thailand,[28] although contrary to the seroprevalence of anti-*T. gondii* IgM reported among similar age groups (5.4%) in the Southern Iran.[31] This indicates an increase in the prevalence with increase in age; however, acute or recent infection was observed in younger age group blood donors. The reason might be due to increased risk to exposure to infection source with age.[31] Seropositivity of *T. gondii* IgM antibodies was higher in nonvegetarian (47%) than in vegetarian (20%) as similarly reported in 98% to 2% in Karnataka India, and 96% to 4% in Southwest Iran,[32] among blood donors as well as in nationwide study in Germany.[24] The reason may be due to frequent consumption meat infected by *T. gondii* that is not well cooked.

**Conclusions**

The seroprevalence of *T. gondii* was high among blood donors from the Southwest region of Nigeria. Anti-*T. gondii* IgM prevalence was relatively higher compared anti-*T. gondii* IgG, implying the majority of seropositive donors had an acute or recent infection, might seroconvert to chronic infection. There was a lower seropositivity of *T. gondii* among vegetarian, suggesting that eating meat may pose a major risk of exposure to *T. gondii* infection in the region. Age was negatively correlated with risk of *T. gondii* infections among the donors. Mass screening should be considered to identify asymptomatic voluntary blood donors that are at risk of transmitting via blood transfusion. Further study on molecular diagnosis is needed to determine other parasitic infection that can be transmitted via blood and to reduce false-positive diagnosis. There is a need to determine the incidence of transfusion-transmitted *T. gondii* infection pre- and posttransfusion among immunocompromised blood recipients.

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**Conflicts of interest**

There are no conflicts of interest.

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