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Blood Transfusion-Transmissible Malaria among Blood Donors and its Association with ABO Blood Group Variants in Bahir Dar, Northwest Ethiopia:A Cross-Sectional Study

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BAHIR DAR UNIVERSITY
COLLEGE OF MEDICINE AND HEALTH SCIENCES
SCHOOL OF MEDICAL LABORATORY SCIENCE EDUCATION AND
SERVICE
DEPARTMENT OF CLINICAL HEMATOLOGY AND
IMMUNOHEMATOLOGY

**Blood Transfusion-Transmissible Malaria among Blood Donors and its
Association with ABO Blood Group Variants in Bahir Dar, Northwest
Ethiopia:A Cross-Sectional Study**

By: Belayneh Endaylalu(MSc Candidate)

September 2026

BahirDar, Ethiopia.



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Blood Transfusion-Transmissible Malaria among blood donors and its association with ABO blood group variants in Bahir Dar, Northwest Ethiopia: A comparative cross-sectional study

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A thesis submitted to the Department of Hematology and Immunohematology Education and Service, School of Medical Laboratory Science, College of Medicine and Health Sciences, Bahir Dar University, in partial fulfillment of the requirements for the master's degree in clinical Hematology and Immunohematology.

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BAHIR DAR UNIVERSITY

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This is to certify that a thesis entitled “Blood transfusion-transmissible malaria among blood donors and its association with ABO blood group variants in Bahir Dar, Northwest Ethiopia: A comparative cross-sectional study” has been carried out by Belayneh Endaylalu for partial fulfillment of the requirements for the degree of Master of Science in Clinical Hematology and Immunohematology under my supervision. I confirm that the student has fulfilled the requirements and hence can submit the thesis to the department.

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DECLARATION

I, the undersigned Clinical Hematology and Immunohematology student, declare that this thesis entitled Blood Transfusion-Transmissible Malaria among Blood Donors and its Association with ABO Blood Group Variants in Bahir Dar, Northwest Ethiopia, A Cross-Sectional Study is my original work and has not been presented at any other university, and all sources of material used for this thesis have been properly acknowledged.

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ABBREVIATIONS AND ACRONYM'S

BTTM:Blood Transfusion-Transmitted Malaria

pLDH: pan malaria lactate dehydrogenase

PCR:Polymerase Chain Reaction

pfhrp2:*P. falciparum*-specific histidine-rich protein 2

P. falciparum: *Plasmodium falciparum*

PTM: Post-Transfusion Malaria

qPCR:Quantitative Polymerase Chain Reaction

RDT:Rapid Diagnostic Test

RBC: Red Blood cell

RT-PCR: Real-Time PCR

SnM-PCR: Species-Nested Multiplex PCR

SSA: Sub-Saharan Africa

TTI: Transfusion-Transmissible Infection

TTM: Transfusion-Transmitted Malaria

WHO:World Health Organization

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ABSTRACT

Background: Blood transfusions are a life-saving medical procedure, yet they carry the risk of Transfusion-Transmitted infections, including Transfusion-Transmitted Malaria. This risk is heightened in Sub-Saharan Africa and Ethiopia due to a large pool of asymptomatic, low-parasitemia donors who evade conventional screening. The risk is compounded by the absence of routine malaria screening in Ethiopian blood banks and the limited sensitivity of available diagnostic methods. Examining the link between malaria and host genetic factors, such as ABO blood group, could support the development of targeted, resource-appropriate screening strategies.

Objective: To determine the prevalence of malaria among blood donors and its association with ABO blood group variants using different diagnostic methods in Bahir Dar, Northwest Ethiopia.

Methods: A facility-based cross-sectional study was conducted among 423 blood donors at five mobile blood collection sites in Bahir Dar, Northwest Ethiopia, from January–July 2026, using systematic random sampling. Three milliliters of Blood samples were used for screening *Plasmodium* infection using BIOCREDT pfLDH/pvLDH RDT and microscopy for 423 samples, and PCR (nested PCR and qPCR) on a sub-cohort of 221 samples. Sociodemographic and related data were collected using a structured questionnaire and analyzed using SPSS version 25 and RStudio software with descriptive statistics, Cohen's kappa, chi-square, and logistic regression ($p < 0.05$ significant)

Results: The malaria prevalence was 3.5% (15/423) by microscopy, 4% (17/423) by RDT, 4.5% (19/423) by composite RDT and microscopy, and 4.5% (9/202) by nested and qPCR. RDT and microscopy showed sensitivity of 56% and 52% respectively with a tie overall accuracy of 93.7%. *P. vivax* was predominant, with zero prevalence of *P. malariae* and *P. ovale*. Blood group O was most prevalent (47%; 199/423) and showed significantly higher malaria positivity (10.1%) and odds of infection (AOR=3.08, 95% CI: 1.14–9.11). Rural residence, malaria history, and travel to endemic areas independently increased infection odds, while Rh-positive status and ITN use were protective.

Conclusion and Recommendation: This study revealed a considerable burden of asymptomatic *Plasmodium* infection among blood donors in Bahir Dar. Despite WHO recommendations, malaria screening is not currently integrated into routine blood donation practices. Therefore, incorporating conventional screening methods and more sensitive molecular tools is essential to enhance transfusion safety.

Keywords: Blood donor, *Plasmodium* infection, ABO blood group, Malaria diagnosis.

1. INTRODUCTION

1.1. Background

Blood transfusion is a therapeutic procedure that involves transferring blood or blood components from a donor into a patient's bloodstream to address trauma, surgery, or medical conditions that cause blood abnormalities or deficiencies. Although this process is a life-saving practice, it also poses problems if not well managed, as there is always a risk of immunological adverse reactions and transfusion-transmitted infections(1). Globally, around 92 million people donate blood annually, but around 1.6 million blood units are discarded due to the presence of infectious agents. Historically, malaria is one of the earliest documented pathogens implicated in transfusion-associated infections(2).

Malaria is caused by the *Plasmodium* parasites, which are transmitted to humans through the bite of infected female Anopheles mosquitoes. Five species of *Plasmodium*: *Plasmodium falciparum* (*P. falciparum*), *Plasmodium vivax* (*P. vivax*), *Plasmodium ovale* (*P. ovale*), *Plasmodium malariae* (*P. malariae*), and *Plasmodium knowlesi* (*P. knowlesi*) are responsible for the transmission of malaria in humans(3).

It is also transmitted through blood transfusions, organ transplants, sharing of contaminated needles, and congenital transfer from mother to fetus(4). Transfusion-transmitted malaria (TTM) is an accidental *Plasmodium* infection caused by whole blood or a blood component transfusion from a malaria-infected donor to a recipient. It is characterized by higher rates of severe complications and fatality than natural infection due to the immediate release of parasites into the recipients' bloodstream(5). The prevalence of malaria parasitemia in asymptomatic blood donors in SSA ranges up to 55% in highly endemic regions. This causes the risk of TTM in Sub-Saharan Africa (SSA) to be significant and complicated(6).

Malaria remains a serious and growing public health problem in Ethiopia, despite decades of control efforts. The vast scale of the challenge is underscored by the fact that approximately 75% of the country's landmass is malarious, placing an estimated 84 million inhabitants at risk of infection(7). The disease disproportionately affects vulnerable groups like children, pregnant women, and migrant communities. This problem is compounded by asymptomatic infections due to unscreened blood transfusions in endemic areas, which facilitate silent transmission in

this population group. This burden has been severely aggravated by factors such as ongoing conflict and internal migration(8). The 2024 World Health Organization (WHO) malaria report in Ethiopia shows an alarming surge of malaria from 4.1 million cases and 527 deaths in 2023 to over 7.3 million cases and 1,157 deaths between January and October of 2024. Epidemics in Ethiopia are highly concentrated in the Oromia and Amhara regions, with 31% of the country's burden occurring in Amhara (9).

The Northwestern part of Ethiopia, including Bahir Dar, is characterized by seasonal and unstable malaria transmission, with peak occurrence in the rainy season(10). In these endemic areas, individuals are semi-immunized and persist with low parasitemia without developing signs and symptoms, and they may appear as healthy(11). Blood donor deferral for malaria in blood banks is based on travel or period of residence in a malaria endemic area, history of malaria, and signs and symptoms, thereby increasing the probability of TTM in an endemic area(12). Despite the WHO's recommendation for screening of blood for malaria before transfusion (13) Routine malaria screening is absent from standard blood transfusion safety protocols in Ethiopia, particularly in the Amhara region.

To effectively mitigate TTM, blood should be screened for transfusion. Malaria microscopy is by far the most commonly used method for screening blood for transfusion, but it has limited sensitivity, is time-consuming, is inadequate for examining a large volume of samples, and requires expertise and specialized equipment when fluorescent methods are used(14). The detection of malarial antigens like pan-malarial lactate dehydrogenase (pLDH) and *P. falciparum*-specific histidine-rich protein 2 (*pfhrp2*) with rapid diagnostic tests (RDTs) was originally intended as a more rapid and objective alternative to direct microscopy. Used with minimal training, are field-applicable, and provide results within 10-20 minutes. However, RDTs have low sensitivity when parasitemia falls below 100 parasites/ μ L. It also gives a false negative when the *P. falciparum* parasites are devoid of the *pfhrp2* gene(15), false positives after treatment, and complete recovery(16), and cannot distinguish between the parasite species present, and in addition, it shows cross-reactivity(17).

In comparison, polymerase chain reaction (PCR) is a highly sensitive and specific method and is efficient in the diagnosis of human malaria parasites, particularly in identifying high prevalence of mixed infections(18). However, PCR has its limitations, including high costs and the need

foradequate equipment and trained technicians. The suitability of each method for donor screening needs to be considered in several key criteria, including the prevalence of and immunity to malaria in the donor population, test sensitivity, cost, reliability, speed, and complexity (19).

In addition to parasite factors and the recipient's immune status, host genetic factors, such as ABO blood group polymorphism, play a significant role in susceptibility to malaria infection and disease severity. The most common human blood group, ABO, affects susceptibility to multiple diseases, including malaria, whereby non-O blood groups (A, B, and AB) are associated with an increased risk of severe malaria compared to blood group O. This may occur because RBC surface-expressed non-O blood group antigens mediate binding to *P. falciparum*-infected RBCs to form rosettes that occlude microvascular circulation and cause severe malaria pathology (20). Even though there are several studies in symptomatic patients (21-24). Information on asymptomatic individuals in the context of blood donations, particularly in Bahir Dar, the northwestern part of Ethiopia, is limited.

Blood transfusion is a therapeutic procedure, even though it carries the risk of TTM. In Ethiopia, where approximately 75% of the landmass is malarious, the threat is significant, with the Amhara region bearing a major portion of this burden. Current blood donor screening in the region often relies on health history rather than active parasite testing, creating a gap where semi-immune, asymptomatic donors can transmit the disease. While diagnostic tools like microscopy and RDTs exist, they face limitations in terms of sensitivity; however, PCR is highly accurate, albeit costly. Furthermore, host genetic factors, specifically non-O blood groups, may increase the risk of severe pathology. Despite these risks, there is a critical lack of data regarding asymptomatic malaria prevalence among blood donors in Northwest Ethiopia, particularly in Bahir Dar, highlighting an urgent need for improved screening protocols and localized research.

1.2. Statement of the problem

Malaria remains a persistent challenge in global health, disproportionately affecting populations in endemic regions. According to the WHO's 2024 annual report, there is an increase in global malaria trends, with an estimated 263 million malaria cases globally in 2023, about 11 million more than in 2022, despite control efforts like routine immunizations. The burden remains mainly concentrated in Africa, which accounted for 94% of all global cases and 95% of the estimated 597,000 global deaths(25). Even though blood transfusion is a lifesaving procedure, it poses a potential risk for the transmission of malaria infection, especially in malaria-endemic areas. TTM due to asymptomatic low-density malaria infections is one of the reasons that makes malaria elimination programs unsuccessful(26).

TTM is uniquely severe due to the remarkable resilience and infectivity of the *Plasmodium* parasite in stored blood products, making donor screening critical even in non-endemic areas. *Plasmodium* species can remain viable and infective for an extended period in refrigerated blood units stored at 4°C for more than 28 days, despite a decrease in infectivity after 14 days, and in some species, even the freezing process used for long-term storage does not render the blood non-infective(27). *P. falciparum* infections may persist for up to a decade or longer, with a maximum confirmed 13 years in the blood donors(28). Furthermore, TTM poses a severe risk because it bypasses the liver stage of the parasite life cycle and requires only a low infectious dose of as few as ten infected red blood cells to establish a fatal infection in a non-immune recipient(5). This problem is reinforced by the fact that a low-level parasitemia of just one parasite detected in a microscopic thick blood film corresponds to approximately 10,000 parasites in a standard 450-mL unit of whole blood. Even blood donors with extremely low parasitemia levels, approximately 0.00004 parasite/ μ L, are capable of sufficiently causing TTM, making them a significant threat to blood safety(29).

Due to this high infectivity and parasite resilience, the actual burden of malaria among asymptomatic blood donors remains high across various regions. Several studies(19, 30, 31) have reported the huge burden of malaria in blood donors. Globally, data suggested that the median prevalence of malaria parasitemia among asymptomatic blood donors was 10.54%, 5.36%, and 0.38% by microscopy, polymerase chain reaction, and rapid diagnostic tests, respectively (19). In Africa, malaria prevalence in the blood bag can reach up to 74%(30). In

sub-Saharan Africa, the median prevalence of parasitemia in blood donors was 10.2% (range, 0.7% in Kenya to 55.0% in Nigeria)(29). A 0.5%(32) and 4.1%(33) prevalence of malaria among blood donors was observed in Hawassa and Arba Minch, Ethiopia, respectively. This may have a high prevalence if conducted during the rainy season.

The absence of simultaneously practical, affordable, and sensitive enough diagnostic methods in malaria-endemic areas creates a transfusion safety gap where sub-microscopic, asymptomatic parasitemia remains undetected in donors, and this leads to challenges for controlling the morbidity and mortality related to transfusion-transmitted malaria(34). In addition, these also cause missed treatment for infected patients, and wrongly treating negative patients undermines elimination goals and also causes the development of drug resistance(35). Although the Ethiopian National Blood Bank Service routinely screens donated blood for major bacterial and viral transfusion-transmissible infections, screening for malaria is not performed. Due to these, recipients of blood transfusion, especially children, pregnant women, and immunocompromised patients, remain at risk of malaria through transfusion (36).

Beyond the screening gap, genetic host factors, such as ABO blood group variants, influence individuals' susceptibility and resistance to severe malaria infection. Evidence suggests that individuals with O blood groups were associated with a 66% reduction ($OR=0.34$) in the odds of developing severe malaria compared with the non-O blood groups, potentially making them asymptomatic donors(37). However, this relationship remains inconsistent across populations and has not been examined among blood donors at Bahir Dar, northwestern Ethiopia.

In Bahir Dar, northwestern Ethiopia, an area characterized by endemic and moderate to high malaria transmission, no comprehensive study has been conducted on the burden of transfusion-transmissible malaria, compared diagnostic test performance, and examined the potential association between malaria infection and ABO blood group variants among blood donors. Therefore, this study aimed to address those gaps by assessing the prevalence of asymptomatic malaria among blood donors and determining whether certain ABO blood groups are more susceptible to the infection by using different screening methods in Bahir Dar.

1.3. Significance of the study

This study used to identify the most sensitive, practical, and cost-effective techniques for detecting asymptomatic *Plasmodium* infections in donated blood using diagnostic methods. It is also used to generate information on the association between ABO blood group and malaria infection, inform donor selection criteria, and develop targeted screening strategies for high-risk groups, thereby improving the efficiency and safety of blood transfusion services. The results also benefit both donors and recipients, contribute to malaria control efforts, generate evidence-based recommendations for strengthening safe transfusion medicine, and enhance the overall quality of healthcare delivery in malaria-endemic regions of Ethiopia. Additionally, this study can serve as a basis for further research by future investigators and provide a source of updated information. Furthermore, it is expected to inform policymakers and other relevant bodies for future planning, resource allocation, and the development and revision of guidelines on the prevention of transfusion-transmitted malaria.

2. OBJECTIVES

2.1. General Objective

To determine the prevalence of malaria among blood donors and its association with ABO blood group variants in Bahir Dar, Northwest Ethiopia.

2.2. Specific objectives

-  To determine the prevalence of malaria among blood donors in Bahir Dar, Northwest Ethiopia.
-  To compare the performance of different laboratory diagnostic methods for detecting malaria among asymptomatic blood donors in Bahir Dar, Northwest Ethiopia.
-  To assess the association between malaria infection and ABO blood group variants among blood donors in Bahir Dar, Northwest Ethiopia.
-  To determine factors associated with blood transfusion-transmissible malaria among blood donors in Bahir Dar, Northwest Ethiopia.

3. LITERATURE REVIEW

3.1. Burden and Prevalence of Transfusion-Transmitted Malaria

The prevalence of TTM among apparently healthy, asymptomatic blood donors is variable and depends on the area's endemicity and the transmission season. For example, during a high transmission season in a high endemic area, the prevalence of asymptomatic parasitemia was 74.4%, and 42.5% of the population in the wet and dry seasons, respectively, had evidence of *P. falciparum* infection by using a combination of 18S rRNA PCR and microscopy methods(38).

A comprehensive systematic review with in nearly three decades (1989–2017) showed a median malaria parasitemia among asymptomatic blood donors was 10.54% by microscopy, 5.36% by molecular methods (PCR), and 0.38% by RDTs with a pooled prevalence of 7.14% (95% CI, 3.61%–11.74%) among studies conducted before 2010 and 13.61% (95% CI, 9.33%–18.55%) among those conducted after 2010 in which *P. falciparum* was the most frequently identified parasite. TTM was identified as one of the most significant TTIs(2).

In contrast, in low-endemic or pre-elimination settings, the burden is substantially lower but still clinically significant. A five-decade systematic review in India shows a prevalence of *Plasmodium* parasitemia in donors ranged from approximately 0% to 0.87% by light microscopy and 0% to 2.3% by RDTs; however, despite these low initial screening rates, a proportion of post-transfusion malaria (PTM) cases ranged from 0.8% to 6.8% across the reviewed literature, and the risk depends on time and diagnostic method(39).

The asymptomatic reservoir in blood donors depends on donor selection criteria, demographics, and public awareness. A study, which was conducted on health workers in a hospital in Kumasi, Ghana, examined blood samples from 100 blood donors for *Plasmodium* using microscopy and RDT and showed a parasitemia rate of 8% by RDT and 3% by microscopy, and 41% of health workers did not know about the risk of TTM(40). Another Cross-Sectional Study in Cape Coast, Ghana, among 240 voluntary and replacement blood donors (224 males and 16 females) showed an overall prevalence of 2.5% (6/240) *P. falciparum* trophozoites, with all occurrences only in males. Most donors, 45.8% (110/240), were in the age group of 26–35, with the highest prevalence of 1.3% (3/240). All malaria parasites were detected in blood group O individuals, with 2.1% (5/240) parasites detected in Rh-positive individuals(41).

Environmental factors, conflict, and individual preventive behaviors influence the prevalence of TTM. A study conducted in Sana'a, Yemen, showed an increase in TTM from 18 malaria-infected blood donors (2.2%) in 2012 to 814 (97.8%) in 2020, with male donors more frequently infected (99.2%) than female donors (0.8%). The proportion of infected people with *Plasmodium falciparum* (24%) is the highest among other malaria types (4%). The increase in the prevalence of these TTMs was due to war, lack of awareness, migration, and weak economic conditions(42).

In addition, donor type and demographic profile determine the probability of asymptomatic malaria infection. A study in Abuja, Nigeria, the prevalence of asymptomatic malaria parasitemia among 550 blood donors was 45.8%, with replacement donors having a significantly higher prevalence compared to voluntary donors, and a higher prevalence in the age group between 20 and 29 years, without significant differences in infection between males and females(43). This trend was similar to a separate Nigerian cross-sectional study, which showed the prevalence of malaria among the blood donors was 21.1%, with the highest rate among commercial donors (33.3%), followed by family donors (12.9%), then voluntary donors (11.9%), using a method of microscopy(44). Furthermore, a study conducted in two hospitals in Nigeria showed 39.3% (148/377) were malaria-positive. Most of the blood donors were in the age groups 16–25 and 26–35 years, with prevalences of 40.0% and 44.1%, respectively. The prevalence of malaria in both age groups was high compared to the 36–45 years age group (26.7 %). Female blood donors had a higher prevalence of malaria (47.1%) compared to male donors (36.9%); the difference was not statistically significant ($p = 0.057$)(45).

A cross-sectional study in Niger during the rainy season found that 11.6% and 2.4% of the donors were infected with *Plasmodium* by microscopy and RDT diagnostic methods, respectively, with all of the infections being caused by *P. falciparum* and no significant spatial(0.6) and temporal(0.1) difference in prevalence of malaria and there should be pretransfusion screening and posttransfusion therapy to prevent the risk of TTM(46).

Another cross-sectional study conducted at the Lilongwe Branch of the Malawi Blood Transfusion Service showed an overall malaria prevalence of 13.5% (51/377) among blood donors, with 13.4% (9/67) of females and 13.5% (42/310) of males testing positive. Of the positive cases, 85.4% (41/48) were due to *P. falciparum* with highest median parasitemia at approximately 3,000 parasites/ μ L (IQR: 1,500–4,800; range: 200–8,000), 8.3% (4/48) were

mixed infections of *P. falciparum* and *P. malariae* with a median parasitemia of 1,800 parasites/ μ L (IQR: 1,200–2,600; range: 500–3,000, and 6.3% (3/48) were only *P. malariae* with lowest parasitemia of 1,000 parasites/ μ L (IQR: 400–2,600; range: 0–4,000), by using PfHRP2 RDTs for the detection of *P. falciparum* and the microscopy method for the detection of other species. Occupation, residence, blood group, repellent use, and travel history are significantly associated with TTM(47).

In Douala, Cameroon, student blood donors presented an overall asymptomatic prevalence of 10.17%, which accounted for 47.36% of the community's total malaria burden, with a higher occurrence in males than females(48). A descriptive study at the Ebolowa Regional Hospital in South Cameroon showed an alarmingly high prevalence of 73.24% among 512 blood donors, with *Plasmodium falciparum* being the only species identified. The prevalence of malaria parasitemia was statistically higher in males (84.1%) than in females (54.1%), among singles (77.3%) than married (63.3%), and among those not sleeping under a mosquito net (83.61%) than among those sleeping under a mosquito net (13.15%)(49).

According to a cross-sectional study conducted at the Regional Blood Transfusion Center, Nakuru, and Tenwek Mission Hospital in Kenya, of the 594 blood donors who were screened using the SD Bio-line rapid diagnostic test kit and confirmed by microscopy with 10% Giemsa-stained blood films, 0.7% were malaria parasite-positive. This study showed malaria prevalence among apparently healthy blood donors is significant and a public health problem, and should be included in TTI since it poses a serious health risk to non-immune recipients of transfusion(50). Another Kenyan study also showed a parasitemia prevalence of 5/155 (3.2%), 8/155 (5.2 %) and 6/155 (3.9 %) by using microscopy, rapid diagnostic test and quantitative buffy coat, respectively, among 155 voluntary blood donors and concluded that blood and blood products should be screened before transfusion, best with quantitative buffy coat since it showed 100% specificity and negative predictive value with a kappa value of 0.9(51).

A hospital-based cross-sectional study conducted in Sudan showed that, among 100 blood donors, only 1% were infected with malaria parasites when screened using microscopy and RDTs. Both the blood films and the immune chromatography test showed one *P. falciparum* positive result for the donor, with a percentage of 1% blood transfusion malaria(52)

An institution-based cross-sectional study, which was conducted in Arba Minch blood bank, showed that among a total of 416 asymptomatic blood donors, the proportion of infected donors was 4.1% (17/416), which was high compared to the national malaria prevalence, with most of the positive blood films (13/17) showing parasite loads ranging from 100 to 500 parasites/ μ l (33). An institution-based cross-sectional study conducted in Hawassa showed a *P. falciparum* prevalence of 0.5% parasitemia, and this rate might be higher if the study were conducted during high transmission seasons (32).

3.2. Comparative Performance of Screening Tests

The detection of asymptomatic malaria is highly dependent on the specific diagnostic methods used, creating a significant variation in reported prevalence rates worldwide. A comprehensive systematic review and meta-analysis conducted worldwide showed a pooled prevalence of 0.32% (95% CI: 0.25–0.39). The African continent carries the highest burden with a remarkably high pooled prevalence of 15.89% (95% CI: 9.48–22.31). It demonstrated that prevalence is heavily influenced by diagnostic methodology; microscopy identified the highest pooled prevalence at 21.86%, followed by polymerase chain reaction (PCR) at 5.03%, while rapid diagnostic tests (RDTs) detected only 0.21%. This study indicates that using less sensitive screening tools in the endemic African region results in an underestimation of the true risk, and this shows a need for more sensitive screening protocols to ensure a safe blood supply (53).

A meta-analysis study, which was conducted in Asia to evaluate the diagnostic performance of RDTs and microscopy for the detection of asymptomatic malaria, showed a pooled RDT sensitivity of 59% (95% CI: 16–91%) and 51% (95% CI: 7–94%) to detect *P. falciparum* and *P. vivax*, respectively, whereas the microscopy method showed a pooled sensitivity of 55% (95% CI: 25–82%) and 54% (95% CI: 11–92%) to detect *P. falciparum* and *P. vivax*, respectively. This study recommends using PCR-based strategies and Loop-Mediated Isothermal Amplification (LAMP), since RDTs and microscopy have limited sensitivity and are inappropriate for the detection of asymptomatic *Plasmodium* infections (54).

In contrast, a study conducted in Indonesia to compare the diagnostic performance of RDTs, microscopy, and RT-PCR for detecting malaria showed that both RightSign and ScreenPlus RDTs had higher sensitivity (100%) and specificity (98%) than microscopy, although the differences were non-significant. However, a significant difference was observed between the two

models of RDTs and RT-PCR performance (with 100% specificity). The RT-PCR method identified a higher rate of mixed infections and low parasitemia as compared to microscopy and both models of RDTs. This indicated that RT-PCR could serve as an effective tool for confirming malaria diagnoses(55).

A long-term systematic review in Iran (1960–2023) showed that of 1,235 blood donors tested for malaria in endemic and non-endemic areas, all were negative by both microscopic and RDT screening methods. A 3% (3/100) and 2% (2/100) malaria positivity was observed when 100 blood donors were screened by the RFLP-PCR and real-time PCR, respectively. Overall serological and molecular prevalence was 9.60% (109/1,135) and 0.71% (5/704), respectively, among blood donors, as detected using different methods. The detection of malaria-causing parasitic DNA cannot rule out transmission through blood and blood component transfusions. Therefore, it is better to develop and implement appropriate strategies to minimize the risk of TTM and ensure the availability of safe and sufficient blood and blood components for patients in need(56).

In Southwest Nigeria, a comparative study of 163 blood donors, showed 34 (20.9%), 38 (23.3%), 19 (11.7%), 20 (12.3%), 25 (15.3%), 23 (14.1%) parasite positive rate as detected by PCR, microscopy, SD-Bioline™, Micro-point™, CareStart™ and First-Response™ respectively with microscopy had a higher sensitivity and positive predictive value than RDTs and PCR in detecting asymptomatic parasitaemia(57). Conversely, a large-scale study in Ghana among 1040 samples detected malaria parasitaemia prevalence of 17.5%, 24.5%, and 42.1% by microscopy, RDT, and *var*ATS qPCR, respectively. RDT was more sensitive (55.7% vs 39.3%), equally specific (98.2% vs 98.3%), and had higher positive (95.7% vs 94.5%) and negative predictive values (75.3% vs 69.0%) and better diagnostic agreement ($\kappa = 0.571$ vs 0.409) than microscopy when *var*ATS qPCR was the reference method. Even though RDT outperformed microscopy for the diagnosis of *P. falciparum* malaria, both tests missed over 40% of infections that were detected by *var*ATS qPCR (58). These significant variations in the detection of asymptomatic malaria were also observed in Guinea: among the negative samples detected by microscopy and RDT, 335 (19.4%) and 128 (13.3%) were false negatives based on SnM-PCR(59).

3.3. Association between ABO blood group and malaria

Several studies from endemic regions have shown a relationship between the host's ABO blood group and the clinical presentation of malaria, specifically demonstrating that individuals with blood group O are highly predisposed to asymptomatic Plasmodium infections. A study conducted in a malaria hotspot in Bayelsa State, Nigeria, which examined West African sub-populations' ABO antigen polymorphisms, found that 69% of asymptomatic malaria subjects belonged to blood group O(60). This suggests that blood group O serves as the best reservoir for asymptomatic infection. This finding is similar to a study in Onitsha, Nigeria, which reported that individuals with blood group O had the highest overall prevalence of Plasmodium infection at 72.7%, compared with significantly lower rates in groups A (56.5%), AB (36.2%), and B (29.4%)(61).

This trend was also observed at the Arba Minch Blood Bank in southern Ethiopia revealed a significant association between blood group O and asymptomatic malaria, with group O individuals accounting for the highest percentage (51.1%) of asymptomatic parasitemia compared with non-O groups, but no significant association was observed between malaria infection and Rh blood group ($p = 0.104$) (62). Similarly, a study conducted in Yemen reported that donors with blood group O+ were the most infected people (50.4%), whereas donors with AB- were the least infected (0.1%)(63). Furthermore, an evaluation of 250 randomly selected blood donors in Nigeria demonstrated that malaria infections occurred in 42.9% of O+ donors, 41.9% of A+ donors, 33.3% of individuals in a tie between AB+ and A- groups, and 0% of AB- donors, with blood group O consistently displaying both a higher parasite load and overall prevalence(64).

In contrast, another study shows that asymptomatic malaria carriage is not significantly associated with ABO blood group variants. A comprehensive systematic review and meta-analysis of the effect of ABO blood groups on malaria phenotypes showed that no statistically significant association exists between ABO variants and asymptomatic Plasmodium infections. This study from southern Ghana, evaluating the asymptomatic carriage of *P. falciparum* among individuals with variant blood groups and hemoglobin genotypes, found no significant relationship between a donor's ABO phenotype and the likelihood of harboring asymptomatic parasites(65). Similarly, a study analyzing the relationship between ABO/Rh D blood group

phenotypes and malaria among asymptomatic individuals in Kano, Nigeria, found that parasite positivity rates did not significantly differ across blood groups(66).

Another study showed that individuals with blood group O positive donors exhibited the highest prevalence of *P. falciparum* parasitemia. This might be related to the H antigen. The malaria parasite may have a higher affinity for the H-antigen. As the expression of H antigen decreased from 'O' blood group to 'A' and 'B' and then to Bombay blood group, parasite invasion (percent parasitemia) also decreased significantly ($p < 0.01$) and concomitantly, indicating the association of parasite invasion with the amount of H antigen present on the surface of erythrocytes(67).

In Asamankese, blood donors with blood groups A and AB had a higher prevalence of malaria infection (ORs = 1.8 and 15, and $P = 0.432$ and 0.030 , respectively). The Rh-positive blood group was significantly associated with decreased risk of asymptomatic malaria infection (OR = 0.2, $P = 0.020$)(68). On the contrary, another study in this country showed that an overall 2.5% (6/240) had asymptomatic malaria infections, of which 2.1% (5/240) were Rh-positive(69).

The burden of asymptomatic malaria among blood donors is highly variable and affected by the endemic nature of the region, the seasonality of transmission, and the sensitivity of the diagnostic methods used(19). Methods like microscopy and RDTs significantly underestimate the true burden of infection, missing more than 40% of cases identified by high-sensitivity molecular methods (PCR or qPCR). Studies conducted on donor parasitemia rates ranged from zero(70) to as high as 74.4%(71). *P. falciparum* is the most commonly detected species among blood donors. The ABO blood group association compounds this problem. Studies in Yemen(42) showed that Blood Group O is a primary reservoir for asymptomatic infection, whereas other studies, such as in Ghana(68), indicate that non-O blood groups are more susceptible to higher parasite densities. Those conditions create a public health gap, and asymptomatic donors appear healthy during routine screening, which induces risk for TTM. This highlights the need for the development of screening algorithms and post-transfusion prophylaxis in highly endemic areas.

4. CONCEPTUAL FRAMEWORK

A conceptual framework for the study, which shows the major factors associated with transfusion-transmitted malaria.

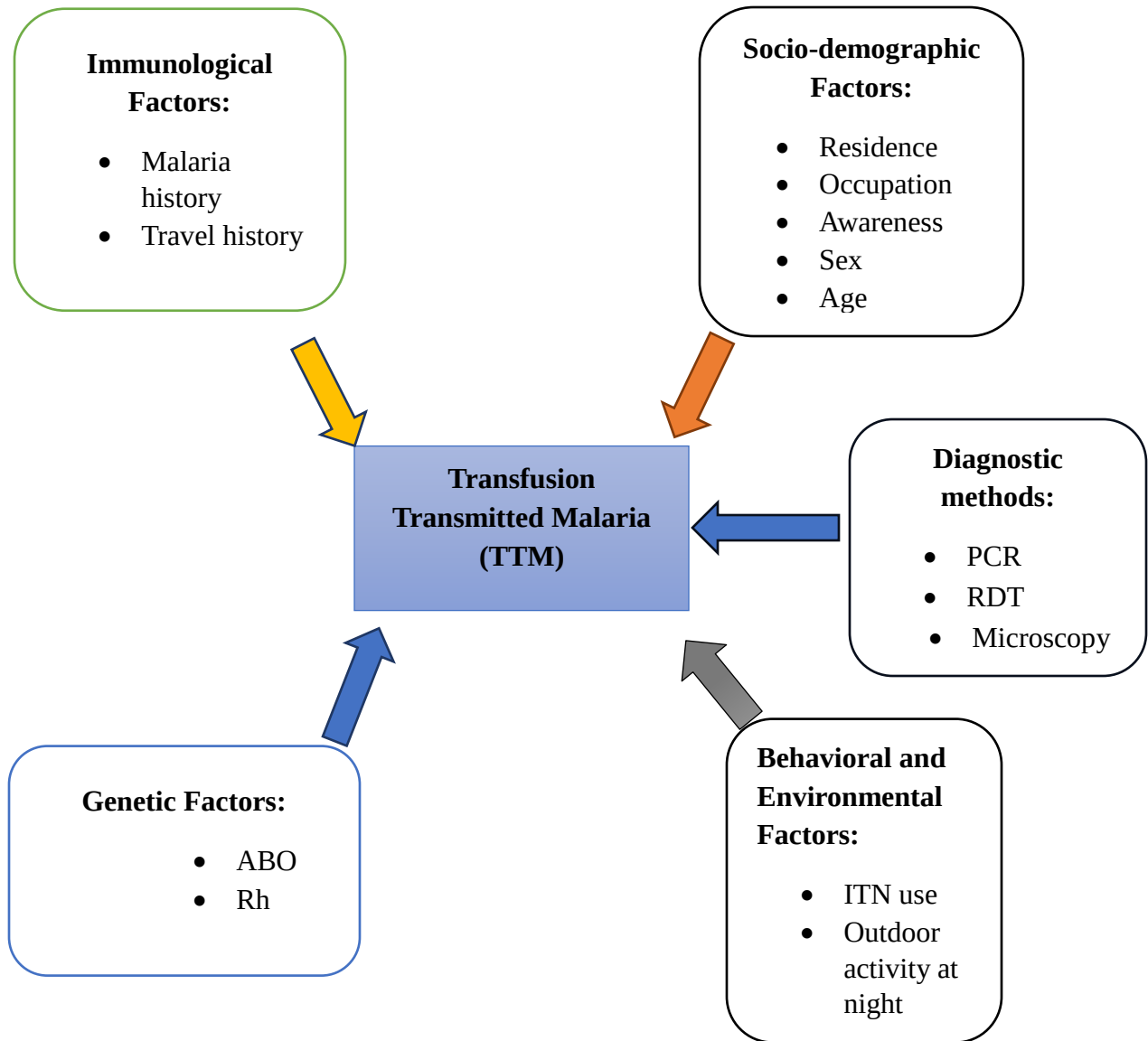


Figure1: Conceptual framework of factors associated with Transfusion-Transmitted Malaria (TTM) among blood donors in Bahir Dar, Northwest Ethiopia, 2026.

5.MATERIALS AND METHODS

5.1. Study area and settings

The study was conducted in Bahir Dar, Northwest Ethiopia, the capital of Amhara Regional State. It is located approximately 565 km Northwest of Addis Ababa at an altitude of about 1,840 m above sea level. The area experiences a tropical climate, characterized by a mean annual rainfall of 1,200–1,600 mm and an average temperature of 17-28°C. Malaria transmission is seasonal, with peaks following the main rainy season in these areas(72).

Bahir Dar Blood Bank serves as the central blood transfusion service for Bahir Dar and its surrounding catchment area, coordinating donor recruitment, blood collection, screening, storage, and distribution to health facilities. In line with the Ethiopian National Blood Bank Service protocol, the blood bank routinely screens donated blood for major transfusion-transmissible bacterial and viral infections.

The study sites comprised health facility sites, including Felege Hiwot Comprehensive Specialized Hospital and Northwest Command Level Three Military Hospital, and educational institution sites included Gish Abay Campus of Bahir Dar University, Tana Haike Secondary School, and Gamby Medical College.

5.2. Study design and period

A facility-based cross-sectional study was conducted from January 9 to July 2026 on five mobile blood collection sites.

5.3. Population

5.3.1. Source population

All blood donors who donated blood at Felege Hiwot Comprehensive Specialized Hospital, Northwest Command Level Three Military Hospital, Gish Abay campus of Bahir Dar University, Tana Hike School, and Gamby Medical College mobile blood collection sites were the source population.

5.3.2. Study population

The study population included individuals who satisfied the standardized donor eligibility criteria established by the National and Regional Blood Bank protocols and all eligible blood donors who donated blood during the study period and provided informed consent.

5.4. Eligibility criteria

5.4.1. Inclusion criteria

All voluntary blood donors who came to Felege Hiwot Comprehensive Specialized Hospital, Northwest Command Level Three Military Hospital, Gish Abay campus of Bahir Dar University, Tana Hike School, and Gamby Medical College mobile blood collection sites, passed the blood bank screening criteria, and provided informed consent were included.

5.4.2 Exclusion criteria

All blood donors who came to mobile blood collection sites from a permanent residence in a non-endemic malaria area and donors on antibiotic treatment within a week before donation were excluded from the study.

5.5. Sample size determination and sampling technique

5.5.1. Sample size determination

The sample size is calculated using a single population proportion formula

$$n = \frac{Z_{\frac{\alpha}{2}}^2 P(1 - P)}{d^2}$$

at 95% confidence level ($Z_{(\alpha/2)} = 1.96$). A 5% margin of error (d) was tolerated, and a proportion (p) of 0.5 was assumed. Substituting the values, the calculated sample size was 384. When 10% non-response is added, the total sample size is 423.

5.5.2. Sampling technique

A systematic random sampling technique was used to select individual study participants at the study sites of Felege Hiwot Comprehensive Specialized Hospital, Gish Abay Campus of Bahir Dar University, Tana Haike School, Gamby Medical College, and Northwest Command Level Three Military Hospital. The sampling frame was determined by reviewing blood donor registries at the blood bank from the past two years during the corresponding study period. The

average number of donors across each study site was determined by tracking the last two years' blood donor flow. A proportional allocation technique was used to ensure the sample is representative of the donor distribution across the different locations. Based on a calculated total sample size of 423, participants were distributed across sites in proportion to each site's donor population.

Data collection date proceeded consecutively by following the specific mobilization dates, which were set by the blood bank for each site in the study period. The sampling interval (Kth) was determined by dividing the total Average Population by the Allocated Sample in each study site. At the start of collection at each location, a random starting point between 1 and Kth was chosen. Then, every Kth eligible blood donor was recruited until the allocated quota for that specific site was fulfilled.

Table 1: Proportional Allocation of the Sample Size and Systematic Sampling Intervals across the Selected Study Sites in Bahir Dar, Northwest Ethiopia, 2026.

Study Site	Average Population	Allocated Sample	Sampling Interval (k)
FelegeHiwot Comprehensive Specialized Hospital	275	66	K=4.17 (4)
Gish Abay Campus	250	60	K=4.17 (4)
Tana Haike School	750	180	K=4.17 (4)
Gamby Medical College	340	81	K=4.20 (4)
Northwest Command Level Three Military Hospital	150	36	K=4.17 (4)
Total	1765	423	K=4.17 (4)

A sequential diagnostic screening approach was implemented based on resource availability. A total of 423 study participants were initially screened using both RDTs and microscopy. Due to logistical and financial constraints, a sub cohort of 221 study participants were selected for high-sensitivity molecular confirmation via nested PCR and qPCR. This sub-selection included all 19 samples that tested positive by RDT and/or microscopy, supplemented by a subset of 202 negative samples chosen via a simple random sampling technique.

5.6. Study variables

5.6.1. Dependent variable

Plasmodium parasite infection

5.6.2. Independent variables

Blood group type, socio-demographic variables including age, sex, occupation, and marital status, frequency of blood transfusions to the individual, history of blood transfusions received, and screening methods.

5.7. Data collection

5.7.1. Sociodemographic data collection

Nurses who were fluent speakers of the local language (Amharic) were selected and trained for data collection. Sociodemographic data were collected using a pretested structured questionnaire administered through face-to-face interviews after it had been translated into the local language (Amharic). The questionnaire covered the respondents' residence, environmental factors, sociodemographic characteristics, and history of previous blood donation.

5.7.2. Blood Sample Collection

Three milliliters of blood were collected from each study participant's blood bag for RDT, blood smear, ABO phenotyping, and dried blood spot (DBS) preparation. Blood grouping, malaria RDT, microscopic smear, and DBS preparation were performed at the blood bank in accordance with the standard operating procedures (SOPs) (Appendix V, Appendix VI, Appendix VII, Appendix VIII), respectively. For DBS preparation, blood samples were spotted onto Whatman filter paper with a minimum of four DBS (~60 µL) per spot for molecular diagnosis. The filter paper was labeled with a unique ID and stored in a clean, dry area, where it was allowed to dry at room temperature. Once dry, it was placed in a filter paper zip-log plastic bag with desiccant. Then it

was shipped to the Amhara Public Health Institute (APHI) in Bahir Dar, Ethiopia, and stored at -20 °C until use.

5.7.3. ABO blood grouping

The ABO/Rhesus blood groupings were performed for each study participant using the slide method. Blood from each sample was placed separately on a clean tile. To each drop of blood, one of the antisera: anti-A, anti-B, or anti-D was added and mixed with brief shaking. The blood groups were determined based on the principle of antigen-antibody reaction, especially agglutination (Appendix V).

5.7.4. Microscopic detection of Plasmodium infection

Both thin and thick blood films from 2µL and 6µL of whole blood, respectively, were prepared on a single slide from a blood bag. The film was labeled with the identification number of participants and the date of preparation. The thin smear was fixed with methanol, and both thin and thick blood film smears were stained with 10% Giemsa staining solution for 10 minutes, after which the slides were arranged in slide boxes. Finally, the dried blood films were examined under a microscope by the investigator, another laboratory technologist, and an independent WHO-certified (Level 1) expert as a tiebreaker who was blinded to the RDT and each other's results with 100x oil immersion (Appendix VI). Parasite quantification was estimated based on the total number of malaria parasites counted in 500 white blood cells and then multiplied by 8000 white blood cells (WBCs). The presence of asexual and/or sexual stages of the parasite indicated a positive diagnosis of *Plasmodium* infection. A minimum of one hundred high-power fields were examined on both thick and thin blood films before a negative result was declared.

5.7.5. Rapid Diagnostic Test by BIOCREREDIT Malaria Ag Pf/Pv (pLDH/pvLDH) RDT

Malaria rapid diagnostic testing was performed using the BIOCREREDIT Malaria Ag Pf/Pv (pLDH/pvLDH) test kit (RapiGEN Inc., Republic of Korea), a one-step immunochromatographic assay that qualitatively detects *P. falciparum*-specific lactate dehydrogenase (PfLDH) and *P. vivax*-specific lactate dehydrogenase (PvLDH) antigens in human whole blood. The test contains two distinct test lines: one line for PfLDH detection and a second line for PvLDH detection, allowing simultaneous differentiation of *P. falciparum* and *P. vivax* infections on a single device. Five microliters (5µl) of the sample were used for the RDT test, and the result was interpreted after 25-35 minutes according to the manufacturer's guidelines.

5.7.6. Molecular Diagnosis of Malaria with Combined Nested PCR and Multiplex qPCR Targeting the 18S rRNA Gene

Molecular diagnosis of malaria in the workflow combined conventional nested PCR and real-time quantitative PCR (qPCR), each applied at a different stage of the diagnostic process and targeting the 18S ribosomal RNA gene. The procedure began with a genus-level nested amplification (N1), after which samples of which N1-positive samples were triaged by species pair: *P. falciparum* and *P. vivax* were screened and quantified by multiplex qPCR, while *P. ovale* and *P. malariae* continued through species-specific nested PCR (N2) followed by gel-based visualization. All nested PCR reactions employ GoTaq Flexi Polymerase from Promega (catalog number M7808), and each master mix, whether for nested PCR or qPCR, is prepared with a pipetting error reserve to ensure sufficient volume across all reactions.

The first stage, the N1 PCR, detects the presence of *Plasmodium* parasites at the genus level, regardless of species, by targeting a conserved region of the 18S gene using the generic primer pair rPLU5 and rPLU6. This reaction amplifies genomic DNA extracted from donors' blood into a large product of approximately 1200 bp, which serves as the template for all downstream species-specific reactions, whether nested PCR or qPCR. Each 25-microliter reaction contains 1X PCR buffer, 2 mM MgCl₂, 0.25 mM each dNTP, 0.25 micromolar each primer, 0.04 U/reaction polymerase, and 5 ng/μL template DNA, with PCR-grade water to complete the volume. Thermocycling begins with an initial denaturation at 95°C for 10 minutes for 1 cycle, followed by 35 cycles of denaturation at 95°C for 60 seconds, annealing at 58°C for 60 seconds, and extension at 72°C for 90 seconds, concluding with a final extension at 72°C for 10 minutes and an indefinite hold at 4°C.

All N1-positive samples are then screened for *P. falciparum* and *P. vivax* using a multiplex TaqMan qPCR assay rather than nested N2 PCR, run on a separate real-time PCR platform. This assay uses TaqMan Fast Advanced Master Mix (Applied Biosystems, 4444964) in a 12 μL final reaction volume with 3 μL of N1 product as input. *P. falciparum*-specific primers and probe (targeting Pf18S_FW/RV with a FAM-labeled MGBNFQ probe) are used at 300 nM and 111 nM, respectively, while *Pv*-specific primers and probe (targeting Pv18S_FW/RV with a Texas Red-labeled BHQ2 probe) are used at 200 nM and 110 nM, respectively, allowing both species to be detected and distinguished by fluorophore in the same multiplex well. Thermocycling consists of a UNG/AmpErase decontamination step at 50°C for 2 minutes, initial denaturation at 95°C for

10 minutes, and 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. Quantification is enabled by running serially diluted standards alongside samples: NF54-cultured parasites diluted from 10⁶ down to 10² parasites/mL for *P. falciparum*, and a Pv 18S plasmid standard diluted from 10⁷ down to 10³ copies/mL for Pv, both prepared fresh on the day of the run.

Table 2: Primers and Probes Used for Multiplex 18S qPCR Detection of Plasmodium falciparum and Plasmodium vivax

Pf18S_FW	GTAATTGGAATGATAGGAATTTACAAGGT
Pf18S_RV	TCAACTACGAACGTTTTAACTGCAAC
Pf18S_probe	FAM-AACAATTGGAGGGCAAG-MGBNFQ
Pv18S_FW	AGCAGCCGCGGTAATTCCA
Pv18S_RV	ATGCGCACAAAGTCGATACGAAG
Pv18Sprobe	TEXASREDAGCAACGCTTCTAGCTTAATCCACAT-BHQ2

In parallel, N1-positive samples are also used as a template for a separate N2 reaction targeting *P. ovale* and *P. malariae*, using the primer pairs rOVA1/rOVA2 and rMAL1/rMAL2, which produce diagnostic amplicons of 749 bp and 144 bp, respectively. This reaction follows a nearly identical master mix formulation to N1, differing mainly in that 2 microliters of N1 product replace the original genomic DNA template, with 23 microliters of master mix aliquoted per tube before template addition. Its thermal profile is similar to that of N1 but with a shortened extension time of 60 seconds and a reduced cycle number of 30. The key distinguishing feature of this reaction is its optimized annealing temperature of 54°C, empirically determined to improve amplification specificity for these two species, while all other cycling parameters remain consistent with the general N2 protocol.

Following amplification, species identification for *P. ovale* and *P. malariae* is achieved through gel electrophoresis of the N2 products. Amplified DNA is separated on a 2% agarose gel prepared in 1X TAE buffer, made by combining 100 milliliters of 1X TAE with 2 grams of agarose and 4 microliters of SYBRTM Safe DNA Gel Stain for visualization under UV light. For each sample, 8 microliters of N2 product are loaded alongside 4 microliters of a 100-bp DNA ladder, chosen because it effectively resolves the expected amplicon sizes of 144 bp and 749 bp.

Electrophoresis is carried out at 120 volts and 400 milliamps for 60 minutes, after which the band pattern is interpreted according to expected sizes: a 144 bp band indicates *P. malariae*, and a 749 bp band indicates *P. ovale*.

The use of two distinct PCR platforms in this procedure is driven by practical and resource considerations rather than assay preference alone. First, validated qPCR primers and probes are only available for *P. falciparum* and *P. vivax*; no such reagents currently exist for *P. ovale* or *P. malariae*, so those two species must still be detected through conventional nested N2 PCR and gel-based sizing. Second, running the *Pf/Pv* qPCR directly on N1-positive samples allows simultaneous, same-well detection and relative quantification of both species at an earlier stage, rather than waiting for a separate nested amplification and gel read. The qPCR screen is used specifically to triage samples: by identifying and quantifying *Pf/Pv* positives immediately after N1, only true positives are advanced into further downstream work, sparing negative samples from unnecessary reagent use, technician time, and gel runs. Quantitative PCR is layered in for speed and resource efficiency on the two species for which it is available, while nested PCR is retained as the necessary method for the two species that still lack qPCR-compatible reagents.

Table 3: Nested PCR protocol and *Plasmodium* 18s *rRNA* genes used in this study

Target species	Primer	sequence (5'-3')	Reaction
<i>Plasmodium</i> genus-specific	rPLU6	TTAAAATTGTTGCAGTTAAAACG	Generic/ nested1(N1)
	rPLU5	CCTGTTGTTGCCTTAAACTTC	
<i>Plasmodium</i> species-specific			
	rVIV2	ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA	
<i>Plasmodium malariae</i>	rMAL1	ATA ACA TAG TTG TAC GTT AAG AAT AAC CGC	Nested 2
	rMAL2	AAA ATT CCC ATG CAT AAA AAA TTA TAC AAA	
<i>Plasmodium ovale</i>	rOVA1	ATC TCT TTT GCT ATT TTT TAG TAT TGG AGA	Nested 2
	rOVA2	GGA AAA GGA CAC ATT AAT TGT ATC CTA GTG	

5.8. Data processing and analysis

Data were coded and entered using EpiData and analyzed using SPSS Version 25 and Rstudio software. A descriptive Statistics analysis methods to present prevalence of malaria among blood donors as a percentage, sensitivity, specificity, positive and negative predictive value to compare diagnostic methods and Kappa Statistics to measure the level of agreement between the tests, Chi-square test and Logistic regression to see if there is a significant difference in malaria positivity across blood groups, Odds ratio to quantify the risk and binary logistic regression to test each independent variables associations to malaria was used. The strength of association was interpreted using the odds ratio and 95% confidence interval. A $p\text{-value} < 0.05$ was considered statistically significant in this study. The findings were presented using frequencies, tables, and figures for relevant data.

Parasite density for *P. falciparum* and *P. vivax* was determined from dried blood spot (DBS) samples by qPCR calibrated against species-specific standard curves. The blood volume per 3 mm DBS punch was estimated using $Bv = \pi r^2/3.49$ (2.02 μL), and the dilution factor (DF) was calculated as elution volume (50 μL) divided by Bv, giving $DF = 24.70$. Absolute parasite density was then calculated as $\text{Parasite number/mL} = 10^{((Ct - Y\text{-intercept})/\text{Slope})} \times DF$, using species-specific slope and Y-intercept values derived from standard curves generated by serial dilutions of parasite DNA of known density (NF54 strain for *P. falciparum*; Pv18S standard for *P. vivax*). The *P. falciparum* standard curve yielded a slope of -3.26 and a Y-intercept of 43 , while the *P. vivax* curve yielded a slope of -3.39 and a Y-intercept of 47.7 , both within acceptable amplification efficiency. These values were applied to Ct results from qPCR-positive samples to derive parasite density, expressed as parasites/mL, corrected for dilution factor.

5.9. Data quality assurance

Data were cross-checked for completeness before being entered into Epi Data. To ensure each laboratory analysis followed a scientific protocol, both positive and negative controls were used to ensure data accuracy. Pilot testing was applied to check that the prepared questionnaire was clear and non-confusing.

5.10. Ethical considerations

Ethical clearance was obtained from the Institutional Review Board (IRB) of the College of Medicine and Health Sciences, Bahir Dar University. A support letter was obtained from the College of Medicine and Health Sciences and the Amhara Public Health Institute (APHI). Permission was requested from the Bahir Dar Blood Bank. Study participants received a detailed explanation about their rights, the purpose, procedure, and benefits of the study, and the side effects of the procedure. Written informed consent was also obtained from each study participant. The study participants were informed that they would be treated according to the national treatment guideline if positive for any parasite. Study participants had the right to refuse or to take part in the study, and all participants' data were assured of confidentiality. All biomedical waste generated, including used lancets, needles, Giemsa-stained slides, RDT cassettes, and PCR microtubes, was strictly segregated at the point of generation into color-coded containers and puncture-resistant sharps boxes according to the National Healthcare Waste Management Guidelines of Ethiopia.

5.11. Disseminations of the result

The findings of this study will be shared with the Department of Hematology and Immunohematology, Bahir Dar University, and presented to the Bahir Dar Blood Bank, the Amhara Public Health Institute, and the Armauer Hansen Research Institute to inform malaria screening in blood transfusion services. The results will also be submitted for publication in a peer-reviewed journal and presented at relevant scientific conferences to reach the wider public health and research community.

6. RESULTS

6.1. Socio-demographic characteristics of the study participants

A total of 423 blood donors participated in this study, with a mean age of 29.4 ($\pm$ 9.4) years. More than half of the study participants (55.3%) were within the 18–28-year age group. Most participants were male (61.9%) and had attained a college/university level of education (63.6%). Nearly half of the respondents were students (45.9%), and most were single (59.8%). The majority (75.9%) had donated blood 1–3 times, as shown in **Table 4**.

Table 4: Socio-demographic characteristics of blood donors attending blood collection sites in Bahir Dar, Northwest Ethiopia, January–July 2026 (N = 423).

Variable	Category	N (%)
Age (years)	Mean (SD) / Median (Min-Max)	29.4 (9.4) / 27 (18-57)
Age category	18-28	234 (55.3%)
	29-39	108 (25.5%)
	Greater than or equal to 40	81 (19.1%)
Sex	Female	161 (38.1%)
	Male	262 (61.9%)
Educational status	Unable to read and write	37 (8.7%)
	Primary school	9 (2.1%)
	Secondary school	108 (25.5%)
	College/university	269 (63.6%)
Occupation	Government and private employee	61 (14.4%)
	Merchant	90 (21.3%)
	Student	194 (45.9%)
	Unemployed	41 (9.7%)
	Other	37 (8.7%)

Variable	Category	N (%)
Marital status	Single	253 (59.8%)
	Married	157 (37.1%)
	Divorced	8 (1.9%)
	Widowed	5 (1.2%)
Residence	Urban	256 (60.5%)
	Rural	167 (39.5%)

(SD=standard deviation, Min=minimum, Max=maximum, N=total number across each group)

6.2.Prevalence of Asymptomatic Malaria among Blood Donors

Among the 423 study participants, the overall malaria positivity rates were 4.0% (17/423, 95%CI: 2.52 – 6.34) by BIOCREDT RDT, 3.5% (15/423, 95%CI:2.16 – 5.77) by microscopy, and 4.5% (19/423, 95%CI:2.89 – 6.91) by the composite of BIOCREDT RDT and microscopy. Upon PCR analysis, out of the 221 samples (19 samples composite positive by both RDT and microscopy and 202 negative samples by both methods), 25 (11.3%, 95%CI: 7.8 – 16.2) tested positive. Notably, PCR detected malaria parasites in 9 out of the 202 (4.5%, 95%CI: 2.4% – 8.3%) RDT- and microscopy-negative samples. Malaria positivity varies across socio-demographic categories. Younger donors (18–28 years) showed the highest positivity rates across all three diagnostic methods. Male donors, rural residents, and those with lower educational levels showed higher positivity rates than their counterparts (**Table 5**).

Table 5:Distribution and prevalence of asymptomatic Plasmodium infection by socio-demographic characteristics, detected by RDT and microscopy (N = 423) and PCR (N = 221), among blood donors in Bahir Dar, Northwest Ethiopia, 2026.

Variable	Category	RDT+ n (%)	Microscopy+ n (%)	N_PCR	PCR+ n (%)
Age category	18-28	13 (5.6%)	12 (5.1%)	119	16 (13.4%)
	29-39	1 (0.9%)	1 (0.9%)	60	5 (8.3%)
	greater than or equal to	3 (3.7%)	2 (2.5%)	42	4 (9.5%)

Variable	Category	RDT+ n (%)	Microscopy+ n (%)	N_PCR	PCR+ n (%)
	40				
Sex	Female	6 (3.7%)	5 (3.1%)	90	9 (10%)
	Male	11 (4.2%)	10 (3.8%)	131	16 (12.2%)
Educational status	Unable to read and write	2 (5.4%)	1 (2.7%)	20	3 (15%)
	Primary school	0 (0%)	0 (0%)	6	0 (0%)
	Secondary school	6 (5.6%)	5 (4.6%)	56	9 (16.1%)
	College/university and above	9 (3.3%)	9 (3.3%)	139	13 (9.4%)
Occupation	Govt and private employee	1 (1.6%)	0 (0%)	33	4 (12.1%)
	Merchant	3 (3.3%)	2 (2.2%)	43	3 (7%)
	Student	10 (5.2%)	9 (4.6%)	96	10 (10.4%)
	unemployed	2 (4.9%)	2 (4.9%)	25	5 (20%)
	Other	1 (2.7%)	2 (5.4%)	24	3 (12.5%)
Residence	Urban	6 (2.3%)	6 (2.3%)	129	9 (7%)
	Rural	11 (6.6%)	9 (5.4%)	92	16 (17.4%)

(RDT+=RDT positive, Microscopy+=microscopy positive, PCR+=PCR positive, n=total number of positive in each group, N=total number of participants)

6.3. Malaria Prevalence and *Plasmodium* Species Distribution Across Diagnostic Methods

Across all species, *P. vivax* was the predominant species, followed by *P. falciparum*, while mixed infections (*P. falciparum* + *P. vivax*) were least common, and no *P. malariae* or *P. ovale* were detected at all. PCR detected more *P. falciparum* and mixed infections than RDT or microscopy, indicating its higher sensitivity for identifying low-density and mixed infections. Regarding mixed *Plasmodium* infections, RDT and microscopy detected only 1 mixed *P. falciparum*/*P. vivax* (Pf and Pv) infection each out of 423 samples tested, whereas PCR identified 6 mixed infections among the 221 samples subjected to molecular testing (Figure 1).

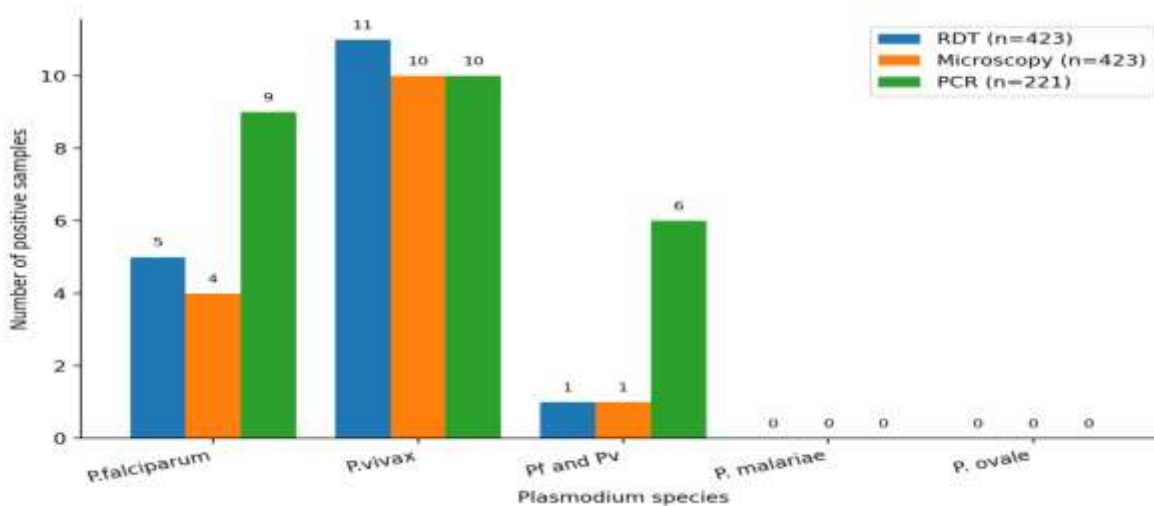


Figure 1: Distribution of asymptomatic *Plasmodium* species detected by RDT, light microscopy, and PCR among blood donors in Bahir Dar, Northwest Ethiopia, January–July 2026

6.4. Comparative performance of diagnostic methods

6.4.1. Agreement Between Diagnostic Methods

Agreement between diagnostic methods was assessed using Cohen's kappa coefficient, with strength of agreement interpreted according to the criteria of Landis and Koch(73). As illustrated in **Table 7**, the strongest agreement was observed between RDT and microscopy ($\kappa = 0.805$, 95% CI: 0.65–0.96), indicating almost perfect agreement. Agreement between both RDT and microscopy with PCR was substantial, with kappa values of 0.633 (95% CI: 0.447–0.819) for RDT against PCR and 0.618 (95% CI: 0.424–0.811) for microscopy against PCR.

Table 6: Agreement between RDT, microscopy, and PCR malaria screening methods among blood donors in Bahir Dar, Northwest Ethiopia, 2026, assessed using Cohen's kappa coefficient.

Method Pair	Kappa	95% CI	Agreement Level
RDT versus Microscopy (n=423)	0.805	0.65-0.96	Almost perfect
RDT versus PCR (n=221)	0.633	0.447-0.819	Substantial
Microscopy versus PCR (n=221)	0.618	0.424-0.811	Substantial

Of the 28 samples positive by at least one method, PCR detected the highest number of positives overall, including 9 (32.1%, 95%CI: 17.9 – 50.7) unique cases missed by both RDT and microscopy, while microscopy alone detected none. Full concordance across all three methods was observed in 11 samples (39.3%, 95%CI: 23.6 – 57.6), the largest single subgroup, whereas BIOCREDIT RDT detected only 1 unique positive (3.6%, 95%CI: 0.6 – 17.7) not confirmed by the other methods, as shown in **Figure 2**.

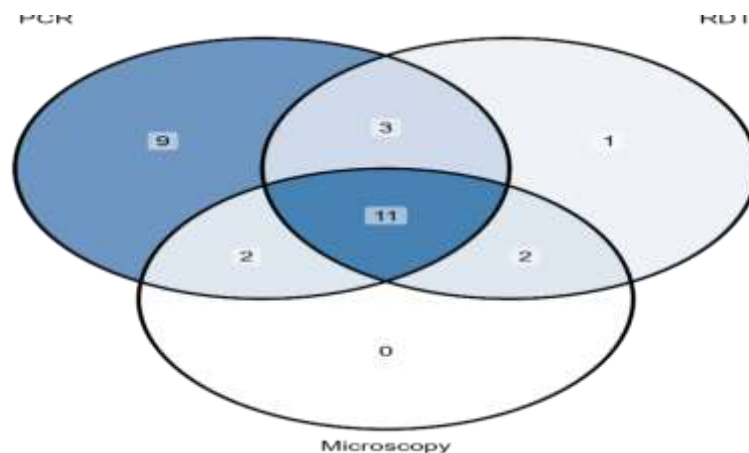


Figure 2: Diagnostic overlap and concordance of malaria positivity across PCR, RDT, and microscopy among blood donors in Bahir Dar, Northwest Ethiopia, 2026.

6.4.2. Diagnostic Performance Metrics (Sensitivity, Specificity, PPV, NPV)

Compared with PCR as the reference standard, both RDT and microscopy showed specificity (98.5% and 99%, respectively). Overall accuracy was 93.7% for both, but only sensitivity of 56% and 52% for RDT and microscopy, respectively. Both methods correctly identified almost half of

PCR-confirmed malaria cases. As indicated in **Table 8**, both methods performed similarly to each other, with microscopy showing a higher positive predictive value (86.7%) than RDT (82.4%).

Table 7: Diagnostic performance of RDT and light microscopy against PCR as the reference standard among blood donors in Bahir Dar, Northwest Ethiopia (N = 221).

Comparison	Sensitivity	Specificity	PPV	NPV	Overall accuracy	TP	FP	FN	TN
Microscopy	52.0% (31.3-72.2)	99.0% (96.4-99.9)	86.7% (59.5-98.3)	94.2% (90-97)	93.7% (89.6-96.5)	13	2	12	194
RDT	56% (34.9-75.6)	98.5% (95.6-99.7)	82.4% (56.6-96.2)	94.6% (90.6-97.3)	93.7% (89.6-96.5)	14	3	11	193

(PPV=Positive Predictive Value, NPV=Negative Predictive Value, TP=True Positive, FP=False Positive, FN=False Negative, TN=True Negative)

6.4.3. Comparison Between Microscopy and qPCR for Parasite Density Determination

Parasite density among blood donors varies by species, detection method, and blood type of the donor, as shown in **Table 9** and **Table 10**. Among microscopy-positive samples, the median density was 91.1 (IQR: 74.5–111.2) for *P. falciparum* and 233 (IQR: 189.5–298.9) for *P. vivax*. Among PCR-positive samples, the median density was 45.7 (IQR: 13.3–110.4) for *P. falciparum* and 3045 (IQR: 241–5132.5) for *P. vivax*.

Table 8: Parasite density and detection rate of Plasmodium falciparum and P. vivax by microscopy and PCR among blood donors in Bahir Dar, Northwest Ethiopia, 2026.

Method	Species	N Positive	Median Density (IQR)
Microscopy (n=423)	<i>P. falciparum</i>	5	91.1 (74.5-111.2)
Microscopy (n=423)	<i>P. vivax</i>	11	233 (189.5-298.9)
PCR (n=221)	<i>P. falciparum</i>	15	45.7 (13.3-110.4)
PCR (n=221)	<i>P. vivax</i>	16	3045 (241-5132.5)

(Density units: parasites/uL for microscopy and PCR-Pf; gene copies/uL for PCR-Pv)

6.5. Association Between Malaria Infection and ABO Blood Group Variants among Blood Donors

6.5.1. Distribution of ABO blood group and Rh factor among blood donors

Among the 423 blood donors, blood group O was the most prevalent, accounting for nearly half of all participants (47%). With respect to the Rh factor, most donors were Rh-positive (96%). As shown in **Figure 3**.

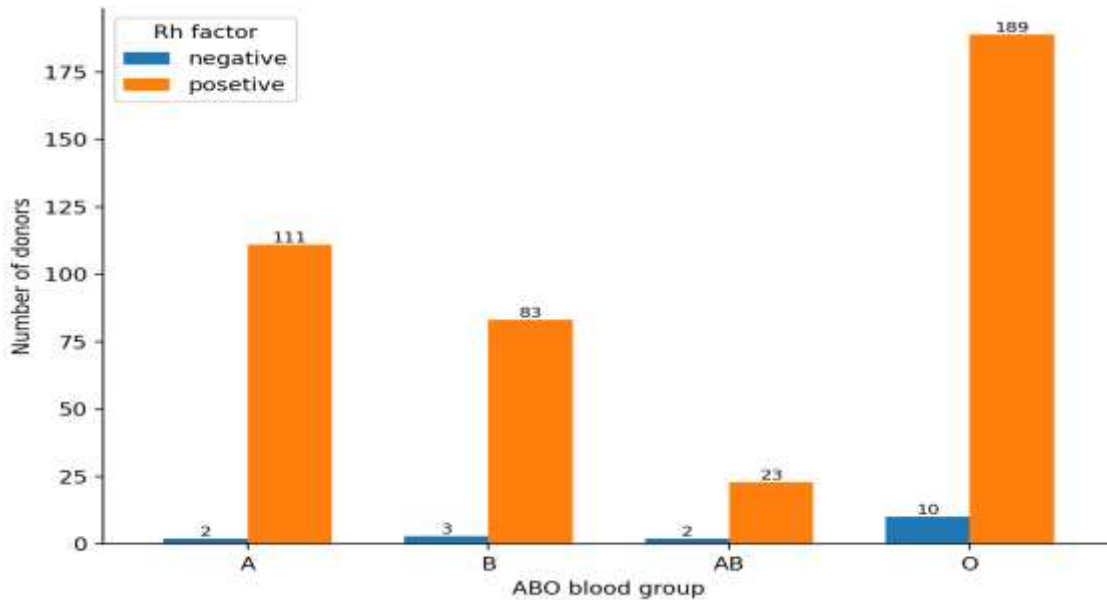


Figure 3: Distribution of ABO blood groups and Rh factor among blood donors in Bahir Dar, Northwest Ethiopia, January–July 2026 (N = 423).

6.5.2. Prevalence of asymptomatic malaria across ABO blood groups among blood donors

Malaria positivity varied by ABO blood group, with group O having the highest rate (10.1%), followed by group A (4.4%) and group B (3.5%). At the same time, no positive cases were detected in blood group AB (**Table 10**). Parasite density also varied by blood group, with blood group O showing the highest microscopy-detected *P. falciparum* density and blood group B the highest *P. vivax* density. On PCR, *P. vivax* density was highest in blood group O, while *P. falciparum* density was highest in blood group A. Across all groups, PCR-detected densities were consistently higher than those detected by microscopy.

Table 9: Malaria positivity and median parasite density by ABO blood group phenotype among blood donors in Bahir Dar, Northwest Ethiopia, 2026.

ABO Blood Group	N	TTM Positive, n (%)	Microscopy Median <i>P. f</i> Density (IQR)	Microscopy Median <i>P. v</i> Density (IQR)	PCR Median <i>P. f</i> Density (IQR)	PCR Median <i>P. v</i> Density (IQR)
A	113	5 (4.4%)	63.9 (63.9–63.9)	145.4 (69.5–223.3)	136.4 (103.0–169.7)	252.0 (226.6–3131.0)
B	86	3 (3.5%)	N/A	323.2 (283.6–362.7)	6.8 (5.5–8.2)	3260.0 (1643.8–4635.0)
AB	25	0 (0.0%)	N/A	N/A	N/A	N/A
O	199	20 (10.1%)	104.2 (91.1–118.2)	233.0 (199.3–284.7)	45.7 (21.7–108.8)	3400.0 (900.5–4815.0)
Total	423	28 (6.6%)	91.1 (74.5–111.2)	233.0 (189.5–298.9)	45.7 (13.3–110.4)	3045.0 (241.0–5132.5)

(Chi-square $p = 0.078$), Density units: parasites/uL for microscopy and PCR-Pf; gene copies/uL for PCR-Pv. TTM = transfusion-transmitted malaria, *P.f.* = *Plasmodium falciparum*, *P.v.* = *Plasmodium vivax*, N=total study participants across each blood group, IQR=interquartile range.

6.5.3. Associations of ABO blood group and transfusion-transmitted malaria among blood donors

Blood group O showed a statistically significant association with asymptomatic malaria positivity in both crude (OR = 2.55, 95% CI: 1.15–6.04, $p = 0.024$) and adjusted models (AOR = 3.08, 95% CI: 1.14–9.11, $p = 0.031$). Individuals with blood group O had approximately three times the odds of asymptomatic malaria positivity compared with non-O individuals, even after adjusting for covariates shown in the footnote of **Table 11**. No significant associations were found for blood groups A or B compared with their non-group counterparts (Group A: crude OR = 1.06, $p = 0.888$; adjusted AOR = 0.76, $p = 0.599$; Group B: crude OR = 0.63, $p = 0.399$; adjusted AOR = 0.7, $p = 0.61$). Similarly, when compared directly with group O, both group A (crude OR = 0.41, $p = 0.087$; adjusted AOR = 0.32, $p = 0.079$) and group B (crude OR = 0.32, $p = 0.075$; adjusted AOR = 0.34, $p = 0.197$) showed lower odds of asymptomatic malaria positivity, with statistically non-significant results.

Table 10: Crude and adjusted odds ratios for the association between ABO blood group and asymptomatic malaria positivity among blood donors in Bahir Dar, Northwest Ethiopia, 2026.

Comparison	Crude OR (95% CI)	COR p-value	Adjusted OR (95% CI)	AOR p-value
A versus non-A*	1.06 (0.43-2.4)	0.888	0.76 (0.25-2.07)	0.599
B versus non-B*	0.63 (0.18-1.68)	0.399	0.7 (0.15-2.45)	0.61
AB versus non-AB*	0 (0-Inf)	0.985	0 (0-Inf)	0.992
O versus non-O*	2.55 (1.15-6.04)	0.024	3.08 (1.14-9.11)	0.031
A versus O*	0.41 (0.13-1.06)	0.087	0.32 (0.08-1.06)	0.079
B versus O*	0.32 (0.07-0.98)	0.075	0.34 (0.05-1.53)	0.197
AB versus O*	0 (Inf-Inf)	0.99	0 (Inf-Inf)	0.994

* = reference category. Vs=versus. Inf=infinity. COR=crude odds ratio. AOR=adjusted odds ratio. AOR adjusted for: Age category; Occupation; Marital status; Residence; Donation count category; Malaria history in the past year; Awareness about transfusion-transmitted malaria; Rhesus (Rh) factor; Travel history to malaria-endemic area; Outdoor activity at night; ITN use (variables with bivariate $p < 0.25$ against transfusion-transmitted malaria).

The association between ABO blood group and PCR-quantified parasite density was assessed separately for *P. falciparum* and *P. vivax* infections (**Figure 4**). The independent-samples Kruskal-Wallis test showed no statistically significant difference in *P. falciparum* and *P. vivax* density across ABO blood groups ($\chi^2 = 4.149$, $df = 2$, $p = 0.126$), and ($\chi^2 = 0.361$, $df = 2$, $p = 0.835$), respectively.

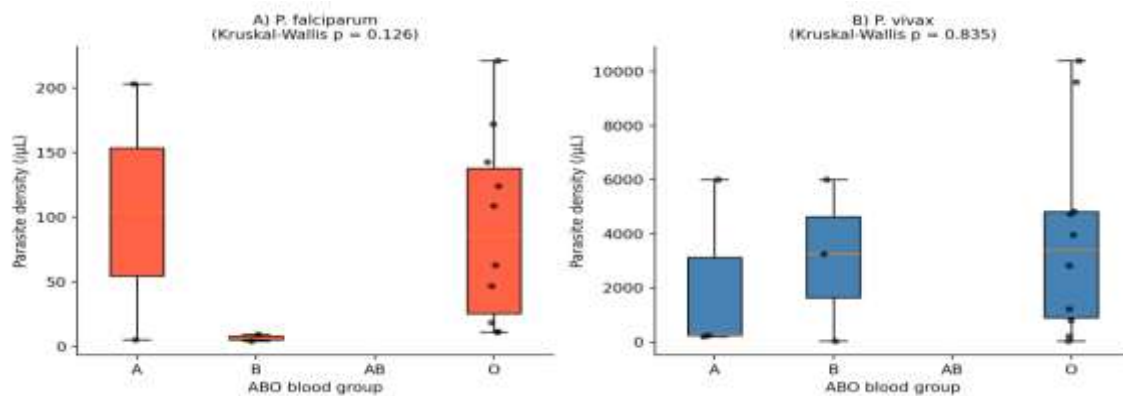


Figure 4: Association Between ABO Blood Group and PCR-Quantified Parasite Density of *P. falciparum* (A) and *P. vivax* (B)

6.6. Factors Associated with Transfusion-transmitted Malaria

Variables with $p < 0.25$ in the bivariate analysis were eligible for multivariable analysis. After adjusting for potential confounders, rural residence (AOR = 2.73, 95% CI: 1.16–6.89, $p = 0.026$), history of malaria (AOR = 3.42, 95% CI: 1.54–7.90, $p = 0.003$), history of travel to a malaria-endemic area within the past six months (AOR = 4.96, 95% CI: 2.20–11.19, $p < 0.001$) were significantly associated with malaria infection. Conversely, being Rh-positive was associated with significantly reduced odds of malaria infection (AOR = 0.16, 95% CI: 0.05–0.54, $p = 0.002$), as was regular use of insecticide-treated nets (AOR = 0.24, 95% CI: 0.07–0.66, $p = 0.011$). Age category, occupation, awareness about transfusion-transmitted malaria, and outdoor activity at night were not significantly associated (**Table 13**).

Table 11: Bivariate and multivariable logistic regression of socio-demographic, clinical, and behavioral factors associated with transfusion-transmitted malaria among blood donors in Bahir Dar, Northwest Ethiopia, 2026.

Variable	Level	COR (95% CI)	COR p-value	AOR (95% CI)	AOR p-value
Age category	18-28*	1.00 (Reference)		1.00 (Reference)	
	29-39	0.55 (0.18-1.41)	0.246	0.43 (0.12-1.34)	0.163
	greater than or equal to 40	0.59 (0.17-1.62)	0.348	0.61 (0.15-2.13)	0.457
Sex	Female*	1.00 (Reference)			
	Male	1.11 (0.51-2.57)	0.791		
Educational status	unable to read and write*	1.00 (Reference)			
	Primary school	0 (0-Inf)	0.986		
	Secondary school	1.29 (0.37-5.93)	0.713		
	College/university and above	0.62 (0.19-2.8)	0.473		
	Govt and private employee*	1.00 (Reference)		1.00 (Reference)	
	Merchant	0.49 (0.09-2.31)	0.364	0.45 (0.08-	0.34

Occupation				2.34)	
	Student	0.94 (0.31-3.46)	0.917	0.58 (0.15-2.63)	0.447
	Unemployed	2.44 (0.65-10.13)	0.189	1.29 (0.31-5.87)	0.726
	Other	1.26 (0.24-6.03)	0.773	0.87 (0.15-4.54)	0.866
Residence	urban*	1.00 (Reference)		1.00 (Reference)	
	Rural	2.97 (1.36-6.85)	0.008	2.73 (1.16-6.89)	0.026
Awareness about transfusion-transmitted malaria	no*	1.00 (Reference)		1.00 (Reference)	
	yes	0.37 (0.12-0.91)	0.047	0.4 (0.13-1.01)	0.073
Ever received blood transfusion	no*	1.00 (Reference)			
	yes	1.01 (0.33-2.55)	0.986		
Malaria history in the past year	no*	1.00 (Reference)		1.00 (Reference)	
	yes	3.42 (1.57-7.73)	0.002	3.42 (1.54-7.9)	0.003
Rhesus (Rh) factor	negative*	1.00 (Reference)		1.00 (Reference)	
	positive	0.14 (0.05-0.48)	<0.001	0.16 (0.05-0.54)	0.002
Travel history to a malaria-endemic area within the past 6 months	NO*	1.00 (Reference)		1.00 (Reference)	
	yes	5.81 (2.62-12.93)	<0.001	4.96 (2.2-11.19)	<0.001

Outdoor activity at night	no*	1.00 (Reference)		1.00 (Reference)	
	yes	2.02 (0.71-4.96)	0.148	1.77 (0.6-4.6)	0.263
ITN use	No*	1.00 (Reference)		1.00 (Reference)	
	yes	0.2 (0.06-0.53)	0.003	0.24 (0.07-0.66)	0.011
Stagnant water near home	no*	1.00 (Reference)			
	yes	0.74 (0.27-1.78)	0.532		
* = reference category (OR fixed at 1.00). AOR shown only for variables retained in the multivariable model (bivariate p<0.25); ITN = insecticide-treated net.					

7. DISCUSSION

Transfusion-transmitted malaria remains a critical but often overlooked challenge to blood transfusion safety in malaria-endemic regions(74). This study evaluated the prevalence of asymptomatic *Plasmodium* infection among eligible and volunteer blood donors in Bahir Dar.

The overall prevalence of asymptomatic *Plasmodium* infection among blood donors in this study varied by diagnostic method: 3.5% (15/423, 95%CI: 2.16 – 5.77) by microscopy, 4% (17/423, 95%CI: 2.52 – 6.34) by RDT, and 4.5% (19/423, 95%CI: 2.89 – 6.91) composite prevalence of microscopy and RDT. This study contradicts the higher rates reported in comparable blood-donor studies across sub-Saharan Africa(75, 76). This is lower than the study reported in OAUTHC, Ile-Ife, Nigeria: 21.1% by microscopy(44),45.8% by microscopy in Abuja, Nigeria(77),73.2% by RDT and /or microscopy in Ebolowa, Cameroon(78),74.4% in Bongo District, Ghana(71)by microscopy and/or PCR. This discrepancy might be due to the study settings differing sharply in baseline transmission intensity: Bongo District, Ghana, and Ebolowa, Cameroon,were explicitly described as hyperendemic with stable, near-perennial transmission(71),whereas Bahir Dar lies within a comparatively lower and more seasonally restricted transmission, or itmight be due to donor selection differences: both studies at Abuja, Nigeria and OAUTHC, Ile-Ife, Nigeria found voluntary donors to consistently have the lowest prevalence of all donor categories (31.6% and 11.9%, respectively) compared with family/replacement (47.2%, 12.9%) and commercial donors (33.3%), but the present study sampled exclusively voluntary donors while the comparator studies included higher-risk replacement/commercial donors.

In contrast, this prevalence rate in the present study is higher than the results reported from Sudan 1%(79),Kenya 0.7%(80), and India(0.05%(81)and 0.024%(82)).This difference might be due to differences in baseline endemicity: the Kenyan study attributes its low prevalence was due to sampling outside the April–October rainy season and lower malaria endemicity, while India is classified as a pre-eliminationarea for malaria, and the Sudanese study's smaller sample size (n=100) was responsible for the lower prevalence than the present study.

Regarding species distribution, across all three diagnostic methods, *P. vivax* was the predominant species detected in blood donors, followed by *P. falciparum* and mixed *P. falciparum*–*P. vivax* infections, while no *P. ovale* and *P. malariae* were detected (Figure 1). This result contradicts the

P. falciparum dominance reported in Cape Coast, Ghana; 100% *P. falciparum*(41); in Niamey, Niger, 100% *P. falciparum* alone(83); and in Lilongwe, Malawi, *P. falciparum* accounted for 85.4% of positive donors, with the remainder comprising 6.3% *P. malariae* mono-infections, 8.3% *P. falciparum* and *P. malariae* mixed infections; no *P. vivax* was identified at all(47). These differences might be due to West and Central Africa (where Nigerian, Ghanaian, Nigerien, and Malawian populations are located) having an almost lack of *P. vivax* receptor Duffy-negative antigens, whereas Ethiopia has a genetically heterogeneous population with a substantial Duffy-positive fraction alongside Duffy-negative individuals(84). This may also be due to methods of screening; Niamey, Niger used the SD Bioline RDT, which detects HRP-2, a protein that only *P. falciparum* expresses; Lilongwe, Malawi used the ParaHIT f RDT, which also targets HRP-2. Again, this antigen simply doesn't exist in *P. vivax*, so their RDT screen was structurally a falciparum-only assay; in contrast, our study used the BIOCREREDIT pLDH/pvLDH RDT, which is specific for *P. falciparum* and *P. vivax* and is 100% sensitive to *P. vivax* (source: manufacturers' leaflets). These discordances might also be due to climate change, relapse(85), and the expansion of *P. vivax* populations that could infect both Duffy-positive and Duffy-negative individuals in sub-Saharan Africa, including Ethiopia(86)

This study is in line with a study from Arba Minch, southern Ethiopia, which found an almost equal rate between the two species, with *P. vivax* (9/17) slightly outnumbering *P. falciparum* (8/17) among infected donors(85). This might be due to the predominant distribution of the *Anopheles arabiensis* vector in both Bahir Dar and Arba Minch(87).

This present study showed a relatively higher sensitivity of RDT than microscopy (56% (95%CI: 37.07 – 73.33) vs 52%(95%CI: 33.50 – 69.97); these findings contradict that microscopy showed higher sensitivity than RDT in Ogun State, southwest Nigeria 65.3% vs 51.4%)(88), in Korhogo, Northern Côte d'Ivoire 20% vs 17.8%)(89), and Edo State, Nigeria (99.2% vs 55.2%)(90). These contradictions might occur because, unlike the HRP2-based RDTs used in those comparator studies, the pLDH-based BIOCREREDIT RDT used here is unaffected by *pfhrp2/3* gene deletions, which have been documented in comparator studies(86, 91, 92) and likely contributed to false-negative RDT results. These also might be due to comparator studies examining fresh capillary blood films immediately on-site; blood in the present study was stored for a minimum of 14 hours before microscopy smear preparation, likely causing parasite morphological degradation

and might lower microscopy's sensitivity independent of true parasite prevalence. Notably, the Nigerian study's authors themselves recommended that RDTs targeting alternative antigens, such as lactate dehydrogenase, could improve diagnostic performance over HRP2-based tests(86).

In our study, one sample was reactive by BIOCREREDIT pfLDH/pvLDH RDT alone, but negative by both microscopy and PCR. This is in line with another study in Ethiopia(93):These might be due to non-specific cross-reactivity in the BIOCREREDIT RDT with rheumatoid factors, Leishmania, Toxoplasma, and M. tuberculosis. (*source: manufacturers' leaflets*),which are prevalent in these study areas(94, 95). Two samples were tested positive by both RDT and microscopy but werenegative by PCR;this discordance might be due to CPDA in Stored blood samples suppressing Taq polymerase activity, or it might also be due to Human serum protein immunoglobulin G (IgG) binding to single-stranded DNA templates or interfering with *Taq* polymerase activity(96).It might also be due to target gene deletions(97).One sample that was detected as *P. Falciparum*by RDT and confirmed positive by PCR was missed by microscopy; this might be due to the adherence of infected erythrocytes to the endothelial cells, despite Parasite lactate dehydrogenase continuing to enter the circulation even when parasites are sequestered and absent from peripheral blood(93).

Upon PCR analysis, out of the 221 samples (19 samples composite positive by both RDT and microscopy and 202 negative samples by both methods), 25 (11.3%) tested positive in addition, PCR detected malaria parasites in 9 out of the 202 RDT- and microscopy-negative samples. corresponding to a false-negative rate of 4.5% for the conventional methods. This finding carries significant implications for blood safety in this endemic setting: nearly one-third of all PCR-confirmed malaria infections among these asymptomatic blood donors would have passed conventional screening undetected. Their combined use as a dual screening strategy would still fail to capture a proportion of low-density, submicroscopic infections typical of asymptomatic carriers. This finding aligns with a study at Ashanti, Ghana(58)using highly sensitive qPCR as the gold standard, which found that RDT and microscopy missed 44.3% and 60.7% of qPCR-positive cases, respectively. Similarly, another study in Lagos, Nigeria(98)reported that *var*ATS qPCR detected 35.4% prevalence compared to 24.7% by microscopy and 31.3% by RDT, with 30.1% of PCR-positive individuals testing negative on both conventional tests. This consistent pattern across studies showed that relying only on RDT or microscopy for donor screening

leaves preventable but fatal asymptomatic malaria infections. This leaves donors harboring undetected parasitemia able to pass routine screening and enter the blood supply, exposing recipients, especially children, pregnant women, and immunocompromised patients, to transfusion-transmitted malaria.

Regarding the level of parasite density detection, our study's PCR-positive samples showed markedly lower median parasite densities than microscopy-positive samples: 45.7 (IQR: 13.3–110.4) parasites/μL for *P. falciparum* by PCR versus 91.1 (IQR: 74.5–111.2) parasites/μL by microscopy. This shows PCR captured a broader range of parasite densities, including low-density infections below microscopy's detection threshold. This is consistent with known diagnostic limits: microscopy detects roughly 5–100 parasites/μl depending on microscopist skill, while molecular methods can detect as low as 0.1–2 parasites/μl(93), making them valuable for identifying asymptomatic, sub-microscopic infections. These findings show that microscopy alone misses the very low-density, PCR-detectable infections that pose the greatest transfusion risk to blood recipients.

Regarding ABO blood groups, our study showed Blood group O donors had three times higher odds of asymptomatic malaria positivity than non-O donors (AOR 3.035, 95% CI 1.14–9.11, $p=0.031$), while Neither group A (crude OR = 1.06, $p = 0.888$; AOR = 0.76, $p = 0.599$) nor group B (crude OR = 0.63, $p = 0.399$; AOR = 0.7, $p = 0.61$) showed significant association, and AB had zero positive cases. These findings are in line with studies in Arba Minch, southern Ethiopia(85) Bayelsa State, Nigeria(60) and Onitsha, Nigeria(61). These significant associations of O blood group against non-O with asymptomatic malaria might be due to different properties of the O erythrocyte; group O erythrocytes express the highest levels of H antigen among the ABO phenotypes, and parasite invasion efficiency has been shown to fall progressively as H-antigen expression declines from group O to A and B and finally to the Bombay phenotype(99). Human macrophages have higher avidity for *P. falciparum*-infected O erythrocytes due to increased hemichrome deposition and band 3 aggregation on infected O cells(100), and it might also be due to the rosettes formed by *P. falciparum* in group O blood are smaller and more mechanically and immunologically fragile than those formed in A, B, or AB blood, because O erythrocytes lack the A- and B-specific surface sugars that stabilize rosette architecture(101).

In contrast to the present finding, studies in southern Ghana(65)and Kano, Nigeria(66),found no statistically significant association between ABO variants and asymptomatic malaria. Another study from Asamankese, Ghana(102),reported an inverse pattern, with groups A and AB showing elevated odds of malaria positivity (OR = 1.8 and 15, respectively), though only the AB estimate reached significance ($p = 0.030$), rather than ABO group O, emerging as the significant protective factor against asymptomatic infection (OR = 0.2, $p = 0.020$). These discordant findings may reflect variable transmission intensity and differences in diagnostic sensitivity, as conventional studies can underestimate low-density carriage relative to highly sensitive molecular tests. It may hide true ABO effects, and it might also be due to constraints of sample size, particularly for the AB group, which is frequently too small in donor cohorts to generate stable estimates.

The present study showed that parasite density did not differ significantly across ABO blood groups for either *Plasmodium* species (Kruskal-Wallis $\chi^2 = 4.149$, $df = 2$, $p = 0.126$) for *P. falciparum*, and ($\chi^2 = 0.361$, $df = 2$, $p = 0.835$) for *P. vivax*. These findings are in line with a study in Asamankess(102),Lilongwe, Malawi(47),and Jose, Nigeria(103),which found no significant association between ABO blood group and parasite density, even though a significant association was found between blood group O and asymptomatic malaria. In contrast to our findings, studies at Arba Minch(85)found a significant association between ABO blood group and parasitemia density among asymptomatic donors, with blood group O donors showing both the highest positivity and the greatest proportion of higher-density infections than other groups. This might occur due to methodological differences between the two studies. Unlike our study, in which donors were screened and excluded for other underlying diseases before enrollment, the Arba Minch study did not apply such screening. Since several non-malarial diseases have themselves been linked to specific ABO blood groups(104),their co-occurrence with malaria infection in unscreened donors may have contributed to elevated parasite densities(105)within that blood group, thereby confounding the observed ABOparasite density association.

Regarding socio-demographic factors, rural residence was an independent predictor of asymptomatic malaria, with rural dwellers having significantly higher odds than their urban counterparts (AOR=2.73, 95% CI:1.16-6.98, $p=0.026$). This study is consistent withAccra,Ghana (AOR=9.1, 95% CI:3.5-27.3, $P=0.001$)(106)and Burkina Faso (106), where rural donors had

more than double the odds of malaria positivity compared with urban donors (OR 2.40, $p < 0.001$). This might be due to a combination of higher environmental vector exposure, housing conditions, occupational hazards, and acquired semi-immunity. In contrast to the present study, studies at the Lilongwe Blood Transfusion Centre in Malawi showed that residence was not significantly associated with TTM. This discrepancy might be due to Lilongwe's near-uniformly rural donor base (80.4% rural)(107), which introduced collinearity with other demographic variables and limited the statistical power needed to detect an independent effect. In contrast, in the present study, Bahir Dar's more balanced and heterogeneous urban-rural donor mix (60.5% urban vs. 39.5% rural) provided sufficient contrast and exposure variance across groups to isolate rural residency as a strong, independent predictor.

Regarding clinical factors, the present study found that self-reported malaria within the past year and Rh blood group emerged as independent predictors of transfusion-transmitted malaria, with donors reporting a recent malaria history having over three times higher odds of TTM (AOR = 3.42, 95% CI: 1.54–7.9, $p = 0.003$). This is consistent with reports among Nigerian blood donors(108), where individuals with a prior history of malaria infection showed a higher malaria positivity rate than those without such a history. This might be due to incomplete clearance, relapsing (as with *P. vivax* hypnozoites), or may be due to renewed local exposure reactivating low-density parasitemia detectable at the time of blood donation. In contrast, a case-control study among Ghanaian blood donors(106) found that a clinical malaria episode within the past year was not significantly associated with TTM (OR = 1.3, 95% CI: 0.45–4.2, $p = 0.09$). This discrepancy might be due to differences in transmission intensity: in hyperendemic Ghanaian communities sampled, all donors remain under continuous reinfection pressure regardless of history, which could dilute the discriminatory value of a one-year recall window, whereas Bahir Dar is relatively characterized by seasonal transmission.

Regarding Rh blood group, our study showed Rh-positive donors had significantly lower odds of TTM than Rh-negative donors (AOR = 0.16, 95% CI: 0.05–0.54, $p = 0.002$). In contrast, a study in Kano, Nigeria, found the opposite pattern: Rh -positive individuals had significantly higher odds of malaria infection than Rh -negative individuals, with all confirmed malaria cases (100%) occurring exclusively among Rh D-positive participants ($\chi^2 = 4.171$, $p = 0.041$)(66). This discrepancy might be due to the very small number of Rh-negative individuals in both

comparator studies, which limits the precision and stability of the estimated associations, and it may also be due to variation in the distribution of Rh antigen subtypes and their differential effect on erythrocyte susceptibility to *Plasmodium* invasion.

In our study, travel history to a malaria-endemic area within the preceding six months was a strong independent predictor of TTM (AOR = 4.96, 95% CI: 2.2–11.19, $p < 0.001$). This is consistent with a systematic review and meta-analysis of 22 studies across sub-Saharan Africa, which found that travel was a significant risk factor for malaria infection (with a pooled OR of 3.77 (95% CI: 2.49–5.70)(109). This concordance supports the behavioral plausibility that donors moving into malaria-endemic areas face greater exposure to infective mosquito bites than those remaining within lower-transmission areas such as Bahir Dar.

ITN use was a significant protective factor against transfusion-transmitted malaria among blood donors in this study, with users showing markedly lower odds of infection than non-users (AOR = 0.24, 95% CI: 0.07–0.66, $p = 0.011$). This is consistent with a study in Makurdi, Nigeria, which found significantly higher *P. falciparum* prevalence among non-users of ITNs (61.0%) compared with users (13.7%) ($\chi^2 = 38.83$, $P = 0.001$)(110)

8. STRENGTHS AND LIMITATIONS

Strength

- ✚ Using three complementary methods gave a fuller picture of asymptomatic malaria burden than any single test could.

Limitations

- ✚ For PCR screening, all RDT/microscopy-positive samples were purposively included, and only a portion of negative samples were selected at random; these may likely inflate relative to what a fully random PCR sample would show, since test-positive donors were deliberately over-represented.
- ✚ With only 25 PCR-confirmed positives, subgroup estimates like zero cases in blood group AB limit statistical power.
- ✚ Data collection took place during a low-transmission season, which may underestimate the true burden of asymptomatic malaria and TTM risk among blood donors during peak transmission months.

9. CONCLUSIONS

Asymptomatic Plasmodium infection is present among blood donors in Bahir Dar at a level that poses a real risk to transfusion safety. Of the 221 PCR-confirmed samples, RDT or microscopy missed a large proportion of positive samples; this causes recipients to be at risk of TTM. Blood group O, rural residence, recent malaria history, and travel to endemic areas were independently associated with higher odds of infection, while Rh-positive status and ITN use were protective. Because PCR testing was enriched for test-positive samples rather than fully randomized, the PCR-based prevalence should be read as an upper-bound signal of the diagnostic gap rather than a precise population estimate.

10.RECOMMENDATIONS

- ✚ The regional blood bank should establish routine malaria screening within its standard pre-transfusion donor screening panel, like major bacterial and viral screening, to safeguard blood safety
- ✚ Although RDT and microscopy demonstrated only substantial sensitivity compared with even sub cohort PCR-confirmed samples, their use is strongly recommended over the current practice of no malaria screening at all.
- ✚ A risk-based selective screening or deferral strategy should be considered, prioritizing donors who are blood group O, rural residents, have recent travel history to malaria-endemic areas, have a malaria history in the past year, or do not use ITNs, when there is a shortage of resources, as these subgroups showed significantly elevated odds of infection in this study.
- ✚ A stratified or quota sampling approach should be followed to deliberately oversample AB blood group individuals relative to their natural population frequency, to ensure sufficient subgroup sample size for meaningful statistical comparison

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APPENDICES

Appendix I: English version of information sheet

1. Introduction

You are being asked to participate in a research study being conducted by a postgraduate student from Bahir Dar University. The purpose of this consent form is to provide you with information about the study and to help you decide whether you wish to participate or not. Please read this form carefully and ask the researcher any questions you may have.

2. Purpose of the Study

The general objective of this study is to determine the prevalence of blood transfusion-transmissible malaria among blood donors and to assess its association with ABO blood group variants in Bahir Dar, Ethiopia. The study will also compare the performance of various laboratory tests, including microscopy, RDT, and PCR, for detecting malaria in donated blood.

3. Procedures

If you agree to participate in this study, the following procedures will be followed: You will be asked to complete a brief questionnaire (approximately 10-15 minutes) that will gather your socio-demographic information, travel history, and previous malaria exposure. The blood sample that is routinely collected for blood donation screening will be used for this study. No additional blood will be drawn beyond the standard amount required for your donation, and the sample will be tested for the presence of malaria using three different laboratory methods: Malaria Rapid Diagnostic Test (RDT), Microscopy (Thick and Thin Blood Smear), and Molecular Test (Polymerase Chain Reaction - PCR). In addition, your standard ABO/Rh blood group determination will also be determined using a monoclonal antibody.

4. Risks and Discomforts

Since the study utilizes blood samples already collected for donation, there are no additional physical risks beyond those associated with the standard blood donation process. Your laboratory results will be kept confidential.

5. Benefits of the Study

You will not receive immediate personal benefit from participating in the study, but your participation will help the blood bank and the regional health authority to understand the actual burden of malaria among donors. This information is crucial for improving blood screening policies, enhancing transfusion safety, and contributing to public health knowledge in the region.

6. Confidentiality

All information collected from you will be kept strictly confidential; Your identity will be protected by assigning a unique code to your data, and the results will be reported in group formats (statistics) only, and no individual participant will be identified in any publication or presentation.

7. Voluntary Participation and Withdrawal

You are free to choose not to participate, and your decision will not affect your relationship with anyone.

8. Declaration by the Participant

I have read the information (it has been clearly read to me in a language I understand). I have had the opportunity to ask questions, and they have been answered to my satisfaction. I hereby voluntarily agree to participate in this research study.

Participant's Name Signature Date (DD/MM/YYYY)

Declaration by the Investigator

I have fully explained the nature and purpose of this study to the participants. I confirm that the participant was allowed to ask questions and that all questions were answered accurately. I confirm that the participant did not feel bad.

InvestigatorName Signature Date (DD/MM/YYYY)

Appendix II: English version of the Informed Consent form for blood donors

Title: Blood transfusion-transmissible malaria among blood donors and its associations with ABO blood group variants in Bahir Dar, Northwest Ethiopia, 2026.

I have been given enough information on the proposed study and understand that all information collected will be kept confidential. I have been allowed to ask questions and discuss all aspects of the study with the investigators or their deputies. I have understood the advice and information provided. I also authorized the investigator to disclose the results of my participation in the study, but not my name. I understand that I have the autonomy to withdraw from participating at any time. Therefore, I consented to participate voluntarily in the research project.

Name: ----- Signature: ----- Date: -----

I confirm that I have fully explained the purpose of the study and what is involved

Name: ----- Signature: ----- Date: -----

Name and signature of data collector _____ Date _____

Date Name and signature of Investigator _____ Date _____

I have given the above-named copy of this form together with the information sheet.

Appendix III:English version of questionnaire

General Instructions: This questionnaire is part of a research study aimed at understanding the prevalence, risk factors, and associations of malaria with the ABO blood group among blood donors. The information you provide will be kept strictly confidential and used only for academic purposes. Thank you for your time and honest response.

PartI: Socio-Demographic Information

- | | | | | |
|--|------------------------|------------------------|---------------------------------|---------------------------|
| 1. Age (in years) | ----- | | | |
| 2. Sex | A. Male | B. Female | | |
| 3. Educational Status | A. Illiterate | B. Read and write only | C. Primary School | |
| | D. Secondary School | | E. College/University and above | |
| 4. Occupation | A. Government Employee | | B. Private Sector Employee | |
| | C. Student | D. Unemployed | E. Merchant | F. Other (Specify: _____) |
| 5. Marital Status | A. Single | B. Married | C. Divorced | D. Widowed |
| 6. Residence | A. Urban | | B. Rural | |
| 7. Have you received a blood transfusion before? | A. Yes | | B. No | |
| 8. Did you know about transfusion-transmitted malaria? | A. Yes | | B. No | |

PartII: Malaria-Related Risk Factors and Exposure

1. Did you have malaria history before a year? A. Yes B. No
2. Did you have a travel history to a known malaria-endemic area within the last 6 months?
A. Yes B. No
3. Do you use an Insecticide-Treated Bed Net (ITN) at night?
A. Yes, regularly B. Yes, sometimes C. No

Appendix IV: Amharic version of the information sheet

1. መግቢያ

እርስዎ ጥያቄ አድረገው ለሰዎች ለወባባሪ ዳርድር ሽርሰቲያ ድህረ ምረቃ ተማሪ በሆኑ ካሂደው ጥናት ምርምር ላይ እንዲሳተፉ ወ።

የዚህ የስምምነት ቅጽ አላማ እርስዎ ለጥናቱ መረጃ ማቅረብ እና በጥናቱ ላይ መሳተፍ ለሰዎች ፍፍላጎት ለመወሰን እንዲረዳዎት ወ።

እባክዎን ይህንን ቅጽ በጥንቃቄ ንብብር እና እርስዎ ሊኖሩዎት የሚችሉትን ማንኛውንም ጭንቀት መረጃ ማስቀመጥ ይጠበቃል።

2. የጥናቱ ስሜት

የዚህ ጥናት ስሜት ስም አላማ እርስዎ በደም ጋሾች መከላከል የሚገኝ ነፃነት ወባባሪ ፋፋትን መወሰን እና ከኤቢአ (ABO) የደም ቡድን ልዩነት ትንተና ለወንግን ኑህ ትመጣ ምግ ምግ ወ።

ጥናቱ በተለገ ሰደምወስ ጥያቄ ወባባሪ ስታን ለመላ የትምህርት ስኬት፤

የወባፈጣን ምርመራ (RDT) እና ሞላኪወላ ዊሙራ (PCR)ን ጨምሮ የተለያዩ የላብራቶሪ ምርመራዎችን ብቃትና አፈጻጸም ንፃሕን ወዳድራል።

3. ሂደቶች

በዚህ ጥናት ላይ መሳተፍ ከተስማሙ ማስተላለፍ ሂደቶች ይከተላሉ። ይኸውም በረሀው -ስነ-ህዝብ መረጃ ጽ/ቤት፤

የጉዞ ታሪክዎን እና የቀድሞ ወባባሪ መግቢያ ጽ/ቤት ትዎን የሚያስሰሉ ጽ/ቤት መጠይቆችን (በግምት ከ 10-15 ደቂቃ የሚቆይ) እንዲያጠናቁ ይጠበቃል።

ለደም ሳምፍ መራብ መደብኛት ትዎ ማስቀመጥ ወይም ደም መጥፋት ለዚህ ጥናት ጥቅም አይደለም።

ደም ማሳገጥ ስለማይቻል ስራዎን ያመደብኛቸው ደም መጠን በላይ ምንም ዓይነት ምርመራ ደም ይወስድም እንዲሁም ማዕዘን ደም መጥፋት ወባባሪ በሽታ መኖሩን ለመለየት በሶስት የተለያዩ የላብራቶሪ ደም ማሳገጥ ትምህርት ወባፈጣን ምርመራ (RDT), ማክሮስኮፒክ (ወፍራም እና ቀጭን የደም ማሳገጥ) እና ሞላኪወላ ዊሙራ (PCR) ይሰራል።

በተጨማሪም እርስዎ መደብኛቸው ኤቢአ/አርኤች (ABO/Rh) የደም ቡድን ወሳኔ የሚወሰን ወሞኖክሎፍ ልፀረ እንግዳካላ ትንበ መጠየቅ ወ።

4. አደጋዎች እና ምቹት ማጣት

ጥናቱ ቀደም ሲል ለሚገኝ ስያሜ ስለሚጠቀም መደበኛ ወይም ደምልገሳ ሂደት ጋር ከተያያዙት በላይ ምን ምትጮራ ይኖራል እንደሚታወቅ ለሁሉም የእርስዎ የላብራቶሪ ወጤቶች በሚገኝ ጥርጣሬ ሊታሉ።

የላብራቶሪ ወጤቶች ወጣ መኖርን የሚያመለክት ከሆነ መልክት ይደርስዎታል እንዲሁም በጠፍ እንደሚከሰቱ ቢታወቅም ጥተኛ ቢወጥር መሆኑንና ህክምና እንዲያደርጉ ይመከራል።

5. የ ጥናቱ ጥቅሞች

በጥናቱ ውስጥ ሚና ተጫውቶ ሲገኝ ግልጽ ጥቅም ያላቸው ፕሮጀክቶች ገናኝ ጥናት የእርስዎ ትኩረት ያደግጋል እና የክልሉ ጠፍባለስልጣንን በሰጋ ሾች መካከል ወጣ ስር ጭነት እንዲረዳ ይረዳል። ይህ መረጃ የደምግር መራገጫዎችን ለማሻሻል፤

የደምግር ወደ ደህንነት ትንሳኤ ልበት እና በክልሉ ውስጥ ህዝብ ጠፍ እንዲቀንስ ስተዋፅዖ ለማድረግ ወሳኝነት ወ።

6. ምስጢራዊነት

ከእርስዎ የተሰበሰቡ ሁሉም መረጃዎች ምስጢራዊ ይሆናሉ።

ማንነትዎን ምስጢራዊ ለማድረግ ምልዩ የሆነ ኮድ በመስጠት መረጃዎች ይቆያሉ።

እንዲሁም ወጤቶች ሪፖርት የሚደረጉት በቡድን ብቻ ነው።

እና በማንኛውም ትመት ወይም አቀራረብ ውስጥ ምትሳታፊ አይታወቅም።

7. በፈቃደኝነት ትተሳትፎ እና መውጣት

ላለ መሳተፍ የመጋረጥ ጥንቃቄ ትክክለኛ ሆኖ እና ወሳኔ ሆኖ ከማንም ጋር ያለ ህሽን ግንኙነት አይካሄድም።

8. በተሳታፊዎች የተሰጠ መግለጫ

መረጃዎች እንዲሰጡ (መረጃ ትክክለኛነት ወይም ተቀባይነት) ። ጥያቄዎችን የሚጠቁ ቅጂዎች ማሻሻል ሲቻል፤

እና ምስጢራዊነት ታላቅ ተስማሚነት ሲሰጥ። በዚህ የምርምር ጥናት ላይ ለመሳተፍ በፈቃደኝነት ትተስ ማምቻላሁ።

የተሳታፊነት ምዕራፍ ማየት ድረስ (ቀ/ወ/ዓ. ም)

ጥናቱን በሚገልጽበት ሂደት የተሰጠ መግለጫ

የዚህን ጥናት ምንነት እና አላማዎችን መላክ ብራርቻለሁ።

ተሳታፊዎችን እንዲጠይቅ እንደተፈቀደለት እና ሁሉም ጥያቄዎችን ትክክል መመለሳቸውን አረጋግጣለሁ።

ተሳታፊዎችን መጽኑን ዳልተሰማወክረሁ ለጋጣለሁ።

ጥናቱን የሚሰራው በሰዎች ስምዬር ማቅን (ቀ/ወ/ዓ. ም)

Appendix V. Amharic version of informed consent

ርዕስ፤ ደም በሚወሰድ ሊኖሩ የሚችሉ ፍጥነት ያላቸው የደም ብረት (ABO)

የደም ብረትን ለመለየት የሚያስፈልጉትን መረጃዎች ለወጥሶ ለሰጠሁ ለዚህ ምክር ቤቱ ምስጋና ያሳውቃለሁ።

በተጨማሪም ትላይብ ቁጥር ማስተላለፍ እና ሁሉም ተሰባሳቢ መረጃዎችን ማስተላለፍ እንደሚጠበቅብኩት ይታወቃል።

በሁሉም ጥናቶች ላይ ተሳታፊዎችን እንዲጠይቅ እና ጥናቱን ከሚሰራው በሰዎች ስምዬር ጋር እንደወያይተዋል ይታወቃል።

በዚህ ምክር ቤቱ ትላይብ ቁጥር ማስተላለፍ እና መረጃዎችን ይታወቃል።

በጥናቱ ውስጥ ተሳታፊነትን ወጥሶ ለሰጠሁ ለዚህ ምክር ቤቱ ምስጋና ያሳውቃለሁ። ግን ስሜ አይደለም።

በማንኛውም ሁኔታ ተጨማሪ መረጃዎች ለሰጠሁ ለዚህ ምክር ቤቱ ምስጋና ያሳውቃለሁ።

ስለዚህ በምር ምርጫ ጥያቄዎችን ተጨማሪ መረጃዎችን ይታወቃል።

ስም: ----- ፊርማ ----- ቀን -----

የመረጃ ሰጪው ሰው ስም እና ፊርማ _____ ቀን _____

ከላይ ተሰየመው የዚህን ቅጽ ማረጋገጫ መረጃ ወረቀቱ ጋር ስትያስተላለፉ።

Appendix VI: Amharic version of questionnaire

አጠቃላይ መመሪያዎች፡ ይህ መጠይቅ የወባስር ጭነትን፤

መንስኤዎችን እና ከኤቢአ (ABO) የደም ቡድን ጋር በደምላጋሾች መካከል ያለውን ግንኙነት ትለመረዳት ያለመ ምርምር ጥናት አካል ነው። የመግቢያ ቅጽ በጥብቅ መላክ ጥራዊ ሆኖ ለትምህርት ዓላማዎች ብቻ ጥቅም ላይ ይውላል።

ለጊዜዎ እና ለታማኝነት ሽያጭ ማስገባት ለን።

ክፍል አንድ፡ ማህበራዊ-ሰነድ አዝብ መረጃ

1. ዕድሜ (በአመታት) -----

2. ጾታህ . ወንድ ለ . ሴት

3. የትምህርት ሁኔታህ . ምን ምትምህርት ያልተማረህ . ማን ብብ እና መጻፍት ማቻል

ሐ. አንደኛ ደረጃ ትምህርት ቤት መሆኑ . ሁለተኛ ደረጃ ትምህርት ቤት

ሰ. ኮሌጅ/ዩኒቨርሲቲ እና ከዚያ በላይ

4. ሥራህ . የመንግስት ስራ ተኛለ . የግል ዘርፍ ስራ ተኛ

ሐ. ተማሪ መሆኑ ሥራ አጥሰ . ነጋዴ . ሌላ (ይግለጹ፡)

5. የጋብቻ ሁኔታህ . ያላገባለ . ያገባህ ተፋታመ መባለት

6. መኖሪያህ . ከተማ . ገጠር

7. የሰጠህ ሳወቅ ደረጃ ትምህርት . በፈቃደኝነት ት/ያልተከፈለህ . ለመተካት

ሐ. አወቅሎጂካል መሆኑ ሌላ (ይግለጹ)

8. ደም ለገ ሱትምን ያህል ጊዜ ነ ወ? -----

9. ከዚህ በፊት ደምወስ ደዋል? ሀ. አዎ ለ. አይደለም

10. በደም ዘወር የ ማህላ ለ ፍ የ ወባ በሽታ ያወቃሉ? ሀ. አዎ ለ. የለም

ክፍል ሁለት፡ ከወባጋር የተያያዙ ስኬቶች እና ተጋላጭ ት

1. ባለፈው 1 አመት የ ወባ ህመም እንዳለብዎታዎታል? ሀ. አዎ ለ. የለም

2. ባለፉት 6 ወራት ውስጥ የ ወባ ስር ጭነት እንደ አለበት የ ማታወቅ አካባቢ ተጉዘዋል? ሀ. አዎ ለ. የለም

3. በምሽት ፀረ -ተባይ መድሃኒት የ ታከመ አልጋ መረብ ይጠቀማሉ?

ሀ. አዎ፣ በመደበኛነት ስለ. አዎ፣ አንዳንድ ጊዜ የለም

4. ከቤት ዎ/መኖሪያ ዎ አጠብቀው ታቆረ ወሃ ወይም ቁጥጥጦች ይገኛሉ? ሀ. አዎ ለ. የለም

5. ማታመታከቤት ወጭ መቆየት ወይም መስራት ልምድ አለዎት?

ሀ. አዎ አለኝ ለ. የለኝም

ክፍል ሶስት፡ የላብራቶሪ መረጃ

1. የደም ቡድን ሀ. ኤለ. ቢሒኤቢ. አ

2. አቸ ኤችዓይነት ሀ. አዎን ታዊለ. አሉታዊ

3. የ ወባ ፈጣን የ ምርመራ (RDT) ውጤት ሀ. አዎን ታዊለ. አሉታዊ

4. ወፍራም/ቀጭን የ ደም ስሜ ውጤት ሀ. አዎን ታዊለ. አሉታዊ

5. አዎን ታዊ ካሉ ለጥያቄ ቁጥር 4፣ ዝርያዎችን የትኞቹ እንደሆኑ ይምረጡ?

ሀ. ፕላዝሞዲየም ፋልሲፓሪ ምላ. ፕላዝሞዲየም ቫይቫክስ

ሐ. የተቀላቀለ መ. ሌላ ይግለጹ

6. አዎንታዊነት ለጥያቄ ቁጥር 4፤ የጥገኛ ህዋሱን መጠን ይግለጹ።

7. የጥንካሬ ዊሽካራ (PCR) ወጠቃህ. አዎንታዊ ለ. አሉታዊ

8. አዎንታዊነት ለጥያቄ ቁጥር 7፤ ዝርያዎችን የትኞቹ እንደሆኑ ይመረጡ?

ሀ. ፕላዝሞዲያ ምፋልሲፓሪ ምላ. ፕላዝሞዲያ ምቫይቫክስሐያ ተቀላቀለ መ.

Appendix VII: Standard operating procedure for ABO blood grouping

1. Principle: Detects A and B antigens on the surface of the patient's red blood cells (RBCs) using known commercial antisera (Anti-A and Anti-B) by using the principle of agglutination

2. Specimen requirements: blood collected in a blood bag

3. Procedure in forward grouping.

- Prepare a clean glass slide and divide it into three sections labeled A, B, and D
- One drop of Anti-A, Anti-B, and Anti-D is placed in their respective sections
- One drop of the donor's whole blood is added next to each antiserum drop
- The mix of blood and antisera is mixed using separate applicator sticks
- Observe the presence or absence of macroscopic agglutination

4. Interpretation of Results

Agglutination indicates the presence of an antigen, whereas no agglutination indicates the absence of an antigen (the Landsteiner rule is applied)

Appendix VIII: Standard operating procedure for the microscopic examination of malaria

Slide preparation:

1. One thick and one thin blood films were prepared on clean, grease-free glass slides.
2. For the thick smear, approximately 6–10 μL of blood, forming a spot 7 mm in diameter, was placed at the center of the slide and spread gently in a circular motion to produce an even, uniformly thick area, and it was allowed to air-dry and was not fixed.
3. For the thin smear, approximately 2–3 μL of blood, forming a spot 2–3 mm in diameter, was placed about 1 cm from one end of the slide. Using a clean spreader slide held at a 30–45° angle, the blood was spread forward to create a thin film with a uniform monolayer of red blood cells and a well-defined feathered edge. It was air-dried and subsequently fixed with absolute methanol.

Fixation and staining:

1. Thin smears were air-dried completely and fixed with absolute methanol for about two seconds.
2. Thick smears were not fixed to allow lysis of red blood cells during staining. Both thick and thin smears were stained using freshly prepared 10% Giemsa solution buffered to pH 7.2 for 10 minutes.
3. After staining, the slides were gently rinsed with clean buffered water and allowed to dry vertically in a dust-free environment.

Microscopic examination:

All slides were examined under oil immersion with a 100 $\times$ objective lens. Thick smears were screened first to determine the presence or absence of malaria parasites, as they provided a concentrated view of parasitized cells. At least 100 high-power fields were examined before declaring a slide negative, in accordance with WHO recommendations. When parasites were

detected, quantification was performed by counting the number of parasites per 200 white blood cells (WBCs), and parasite density was calculated as parasites per microliter of blood using standard conversion formulas.

Species identification:

It was performed by examining the thin blood smear for species confirmation. The thin film preserved red blood cell morphology and allowed differentiation of *Plasmodium* species. Species were identified based on parasite morphology and red blood cell characteristics. *P. falciparum* typically showed small ring forms and Maurer's clefts in infected cells; *P. vivax* and *P. ovale* showed enlarged infected RBCs with Schüffner's stippling; *P. malariae* often showed band-form trophozoites. The parasite species was recorded, and parasitemia was estimated as the percentage of parasites per μL according to standard practice. The presence of an asexual and/or sexual stage of the parasite indicated a positive diagnosis of *Plasmodium* infection.

Appendix IX: Standard operating procedure for RDT examination of malaria

Malaria rapid diagnostic testing was performed for all eligible blood donors using a WHO-prequalified BIOCREREDIT Malaria Ag Pf/Pv (pLDH/pLDH) malaria RDT in accordance with the manufacturer's instructions. The RDT device used in the study contained a membrane precoated with two monoclonal antibodies, one in each of two separate lines across the test membrane: one *Plasmodium* Lactate Dehydrogenase (pLDH) monoclonal antibody, which is specific to *P. falciparum* (Pf-pLDH), and another pLDH monoclonal antibody, which is specific to *P. vivax* (Pv-pLDH). In both test lines, the Rabbit IgG gold conjugate is coated. The Control Line is precoated with anti-Rabbit IgG antibody. Approximately 5 µL of blood was applied to the test cassette, followed by the addition of the supplied buffer. Test results were read after 25–35 minutes and interpreted based on the presence of control and test lines as positive, negative, or invalid. All RDT results were recorded on structured data collection forms and used for immediate clinical management, as well as for comparison with microscopy and molecular diagnostic findings.

Appendix X: Standard operating procedure for molecular examination of malaria

Dry blood spot preparation

1. A Whatman 3MM chromatography paper (903 TM protein saver card) was labeled with the subject ID.
2. Approximately 50–100 μL of blood was applied per circle to fill from 2–3 adjacent circles. The blood was not smearing, and it was allowed to soak evenly through the paper.
3. The card was air-dried horizontally at room temperature for 3 hours. Care was taken to ensure spots did not touch each other or other surfaces during drying.
4. Once fully dry, each card was placed into a separate sealable plastic bag or envelope with a desiccant pack. The samples were sealed and stored in a cool, dry, dark place (at $-20\text{ }^{\circ}\text{C}$ for long-term storage) until processed.

Sample elution

1. Using a clean 5 mm sterile puncher (single-hole Harris punch), three disks were removed from each DBS and placed into a 1.5 mL Eppendorf tube. A punch was sterilized between samples to prevent cross-contamination.
2. To each tube containing the three disks, 200 μL of Qiagen Buffer ATL (tissue lysis buffer) was added. The mixture was vortexed briefly to wet the disks completely.
3. The tube was sealed and incubated at $85\text{ }^{\circ}\text{C}$ for 10 minutes to facilitate the release of cellular contents from the dried blood matrix. After heating, the tube was briefly centrifuged to collect condensate.

4. Approximately 20 μL of Qiagen Proteinase K was added to each tube. The solution was vortexed to mix, then incubated at 56 °C for 1 hour, followed by a brief centrifugation step.
5. Buffer AL (Qiagen lysis buffer, 200 μL) was added to each tube, vortexed thoroughly, and incubated at 70 °C for 10 minutes.
6. Absolute ethanol (200 μL) was added to each tube, and the mixture was vortexed again to mix. Where a white precipitate (DNA/protein aggregate) formed, care was taken to ensure it remained suspended in the solution. Finally, the tubes were briefly centrifuged to collect drops from the lids.

DNA extraction

1. The entire lysate (including any precipitate) was carefully transferred onto a QIAamp Mini spin column placed in a 2 mL collection tube. The column cap was closed, and the assembly was centrifuged at 8,000 rpm for 1 minute, after which the flow-through and the collection tube were discarded.
2. The spin column was placed in a new 2 mL collection tube. Approximately 500 μL of Buffer AW1 (wash buffer) was added to the column, the cap was closed, and the column was centrifuged at 8,000 rpm for 1 minute. The filtration and collection tube was subsequently discarded.
3. The column was transferred to a new 2 mL collection tube. Buffer AW2 (500 μL) was added to the column, and the assembly was centrifuged at full speed (14,000 rpm) for 3 minutes. The flow-through and tube were discarded. A secondary centrifugation step was performed at 14,000 rpm for 3 minutes (dry run) to ensure the complete removal of residual Buffer AW2.
4. The column was transferred to a clean 1.5 mL Eppendorf tube. For elution, 50 μL of Buffer AE (elution buffer, pre-warmed to room temperature (70 °C)) was applied directly to the center of the column membrane. The column was allowed to stand for 3 minutes at room temperature and was then centrifuged at 8,000 rpm for 1 minute to collect the purified DNA.
5. The extracted DNA eluate was placed in a freezer until further molecular analysis.

DNA amplification and detection by Nested PCR

1. First-Round PCR Amplification

A. Reagents & Workspace Preparation

1. Performed all pre-PCR handling in a dedicated sterile laminar hood.
2. Wiped down the laminar flow hood and pipettes with 70% ethanol and 10% bleach, followed by 15 minutes of UV irradiation.
3. Thawed 5X Buffer, MgCl_2 , dNTPs, and primer mixes on ice.
4. Vortexed each reagent briefly and spun down in a microcentrifuge.
5. Kept the GoTaq Polymerase on a chilled block or ice at all times.
6. Labeled a sterile 5 mL Eppendorf tube as **N1-MM (96x)**.

B. Combining master mixed components into a 5 mL tube for 96 samples:

1. Pipetted 1,259.3 μL of PCR-grade water into the 5 mL tube.
2. Added 528.0 μL of 5X GoTaq Flexi Buffer.
3. Added 211.2 μL of 25 mM MgCl_2 solution.
4. Added 26.4 μL of 25 mM dNTP mix.
5. Added 66.0 μL of primer pool (rPLU6 + rPLU5).
6. Inverted or vortexed the tube gently to combine the buffer components.
7. Added 21.1 μL of GoTaq Polymerase last.
8. Mixed the entire 2.11 mL batch gently by pipetting up and down 10 times to avoid creating excess foam or air bubbles

C. Master Mix Aliquoting & Template Addition

1. Dispensed 20.0 μL of Master Mix into every well of a standard 96-well PCR plate using a standard Eppendorf repeater
3. Reserved well H12 for the No Template Control (NTC) and added 5.0 μL of negative DNA extraction control and H10 and H11 for positive control of pf/pv and po/pm, respectively

4. Added 5.0 μL of Qiagen-extracted DNA template to each designated sample well using new aerosol-barrier tips for every addition.
5. Sealed the 96-well plate securely with an optical adhesive film using a plate roller to ensure a complete seal.
6. Centrifuged the plate at $1,000 \times g$ for 1 minute to collect all liquid at the bottom of the wells

D. Thermocycling conditions

1. Loaded the sealed plate into the thermal cycler.
2. Programmed and executed the run under the following thermocycling parameters:

- ✚ Initial Denaturation: 95°C for 10 minutes (1 cycle)

- ✚ Amplification (35 cycles):

 - ✓ Denaturation: 95°C for 60 seconds

 - ✓ Annealing: 58°C for 60 seconds

 - ✓ Extension: 72°C for 90 seconds

- ✚ Final Extension: 72°C for 10 minutes (1 cycle)

- ✚ Hold: 4°C indefinitely

E. Gel Electrophoresis Detection (2% Agarose Gel Preparation and Target Band Analysis)

1. Weighed 2.0 grams of agarose powder on an analytical balance and added it to 100 mL of 1X TAE running buffer in a 250 mL Erlenmeyer flask.
2. Microwaved the solution in short 30-second intervals, swirling gently between pulses, until the agarose was completely melted and clear, and allowed the solution to cool on the bench
3. Added 4.0 μL of SYBR Safe DNA Gel Stain into the cooled solution and swirled gently to mix without forming bubbles.
4. Poured the gel solution into a casting tray fitted with appropriate 96-well comb sets, and inserted combs carefully.
5. Allowed the gel to solidify completely at room temperature for approximately 30 minutes
6. Removed combs carefully and placed the gel tray into the electrophoresis tank filled with 1X TAE running buffer until the gel was submerged.

7. Loaded 4.0 μL of 100 bp DNA Ladder into the corner wells of the gel and 8.0 μL of amplified PCR product directly into each corresponding well
8. Connected electrical leads: red to positive, black to negative, and the power supply was adjusted to 120 V and 400 mA current limit, running for 60 minutes.
9. Removed the gel after the run and imaged it under a Blue-Light Transilluminator, and N1 primary product (rPLU6/rPLU5) was identified as a generic parasite band at $\sim 1,200$ bp

2. Second-Round PCR Amplification

1. Similar to N1, the hood and pipettes were decontaminated with ethanol, bleach, and UV light; thaw buffers/primers on ice; keep GoTaq Polymerase on ice. Labeled a sterile 5 mL Eppendorf tube as N2-MM.
2. Pipetted 1,576.1 μL of PCR-grade water into the 5 mL tube.
3. Added 528.0 μL of 5X GoTaq Flexi Buffer, 211.2 μL of 25 mM MgCl_2 , 26.4 μL of dNTP mix, and 66.0 μL of species-specific primer mix (rOVA1/rOVA2 for *P. ovale* OR rMAL1/rMAL2 for *P. malariae*).
4. Added 21.1 μL of GoTaq Polymerase last.
5. Mixed gently by pipetting up and down 10 times.
6. Dispensed 23.0 μL of N2 Master Mix into every well of a standard 96-well PCR plate (*differs from N1's 20.0 μL*).
7. Added 2.0 μL of the diluted N1 PCR product as template into each designated well (*differs from N1's 5.0 μL DNA extract*).
8. NTCs were added, the plate was sealed with adhesive film using a roller, and pulse-centrifuged at $1,000 \times g$ for 1 minute.

9. Loaded the sealed plate into the thermal cycler and ran the program with the following parameters:

✚ Initial Denaturation: 95°C for 10 minutes (1 cycle)

✚ Amplification (30 cycles — Denaturation: 95°C for 60 seconds

✚ Annealing: 54°C for 60 seconds

10. Extension: 72°C for 60 seconds. Final Extension: 72°C for 10 minutes (1 cycle). Hold: 4°C indefinitely. Similar to N1 (prepared a 2% agarose gel in 1X TAE with SYBR Safe, loaded 4.0 µL of 100 bp ladder and 8.0 µL of N2 PCR product per well, and run at 120 V / 400 mA for 60 minutes).

11. Target Band Analysis (Species Specificity):

✚ For Plasmodium ovale (Po) and Plasmodium malariae, no band was observed at 749 and 144.

Quality Control

1. DNA known to contain the target sequence was included in each nested PCR assay. A clear, distinct band at the expected molecular weight was successfully observed for both first- and second-round amplification products. Negative controls were included.
2. Only first-round PCR products alone were processed in parallel to confirm that unamplified first-round products were not detectable without second-round enrichment amplification.

Interpretation of Results

Positive Result: A clear, distinct DNA band appears on the gel at the expected molecular weight for the nested PCR product (typically 100-300 bp), confirmed by comparison to the DNA ladder. The positive control shows the expected band.

Negative Result: No visible band appeared in the sample lane, while positive controls show the expected band, indicating the target DNA is absent for both *P. malariae* and *P. ovale*

Standard Operating Procedure for Multiplex 18S qPCR Detection and Quantification of *P. falciparum* and *P. vivax*

1. All reagents, including the master mix, primers, probes, standards, and samples, were thawed on ice and gently mixed and briefly centrifuged.
2. Ten-fold serial dilutions of the standards were prepared: the NF54-cultured *P. falciparum* standard from 10^6 to 10^2 parasites/mL, and the *P. vivax* 18S plasmid standard from 10^7 to 10^3 copies/mL; one full dilution series was included in each run.
3. The multiplex master mix (MMX) was prepared by combining TaqMan Fast Advanced Master Mix (2X) with Pf18S primers and probe, Pv18S primers and probe, and nuclease-free water, to a final volume of 9 μ L per reaction (300 nM primer and 111 nM probe for Pf; 200 nM primer and 110 nM probe for Pv). The mix was gently combined and briefly centrifuged,
4. Nine microliters of the prepared master mix were dispensed into each well of a 96-well optical qPCR plate, followed by the addition of 3 μ L of template giving a total reaction volume of 12 μ L per well. The complete standard curve series and a no-template control (NTC) were included on every plate. The plate was sealed with optical film and briefly centrifuged ($1,000 \times g$ for 1 minute) to remove air bubbles.

5. The plate was then loaded onto a real-time PCR instrument and amplified under the following conditions: an initial UNG activation step at 50°C for 2 minutes, followed by polymerase activation at 95°C for 10 minutes, then 45 cycles of denaturation at 95°C for 15 seconds and annealing/extension with fluorescence acquisition at 60°C for 1 minute (FAM channel for Pf, Texas Red channel for Pv).
6. Following amplification, the NTC was checked to confirm the absence of amplification, and the standard curve was evaluated for acceptable amplification efficiency (approximately 90–110%) and linearity ($R^2 \geq 0.98$). Parasite density (parasites/ μ L) for *P. falciparum* and gene copy number (copies/ μ L) for *P. vivax* were determined for each sample by interpolating the Ct value against the corresponding standard curve.

Appendix XI:Declaration

I, the undersigned, declare that this is my original work, has never been presented in this or any other University, and that all the resources and materials used for the research have been fully acknowledged.

Principal investigator Name: _____

Signature: _____

Date: _____

Advisor Name: _____

Signature: _____

Date: _____