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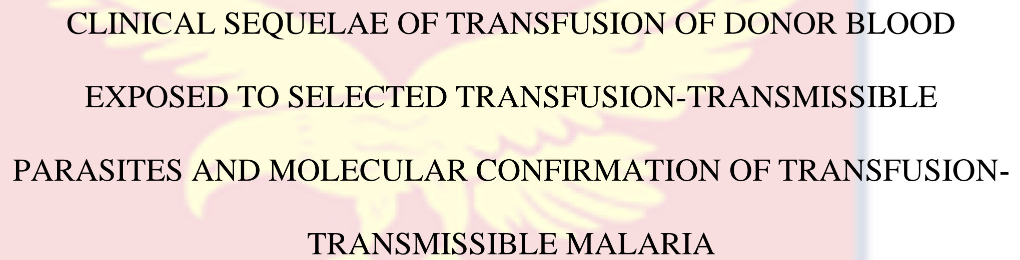

# CLINICAL SEQUELAE OF TRANSFUSION OF DONOR BLOOD EXPOSED TO SELECTED TRANSFUSION-TRANSMISSIBLE PARASITES AND MOLECULAR CONFIRMATION OF TRANSFUSION-TRANSMISSIBLE MALARIA

JULIANA ATTOH

2023

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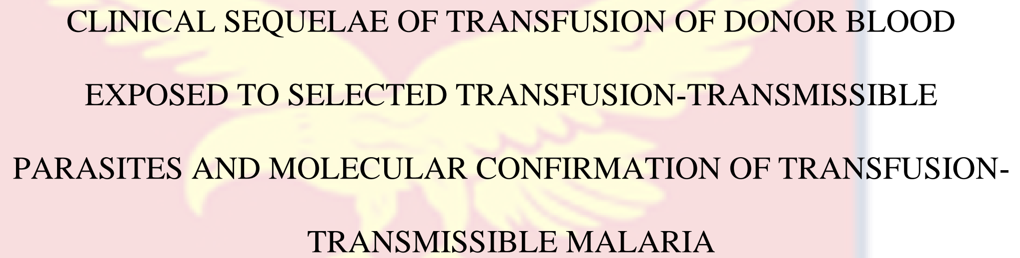

# CLINICAL SEQUELAE OF TRANSFUSION OF DONOR BLOOD EXPOSED TO SELECTED TRANSFUSION-TRANSMISSIBLE PARASITES AND MOLECULAR CONFIRMATION OF TRANSFUSION-TRANSMISSIBLE MALARIA

BY

JULIANA ATTOH

Thesis submitted to the Department of Biomedical Sciences of the School of Allied Health Sciences, University of Cape Coast, in partial fulfillment of the requirements for the award of Doctor of Philosophy degree in Parasitology

June, 2023

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# DECLARATION

## Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: .................................................... Date: ..........................

Name: Juliana Attoh

## Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: ................................... Date: .......................

Name: Prof. Desmond Omane Acheampong

Co-Supervisor's Signature: ......................................... Date: ........................

Name: Dr. Enoch Aninagyei

VERITAS LUMEN NOBIS



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## ABSTRACT

Transfusion-transmitted parasite infections (TTPI) are potentially underreported. However, still regarded as a public health concern, as they might constitute a major threat, particularly in immunocompromised patients. This study was performed at the Nsawam Government Hospital, Ghana and aimed to identify the presence of *Plasmodium falciparum (Pf)*, *Babesia* spp., *Leishmania* spp., and *Toxoplasma gondii* in donor blood and investigate their potential association with acute transfusion responses (ATR). Transfusion-transmissible malaria (TTM) and the associated *Pf* chloroquine resistance transporter (*Pfcrt*), *Pf* multi-drug resistance (*Pfmdr1*), *Pf* dihydropteroate-synthetase (*Pfdhps*), *Pf* dihydrofolate-reductase (*Pfdhfr*) and *Kelch 13* mutant genes were further assessed. Remnants of transfused blood were screened for *Pf* using malaria rapid diagnostic test and microscopy. Enzyme-linked immunosorbent assay was used for the others. Recipients of blood infected with *Pf* were followed up for 35 days. Selective whole genome amplification was used to determine TTM and gene polymorphisms. Approx. 20% (113/571) of recipients were exposed. The prevalence were: *Pf* (12.1%), *Babesia spp.* (1.1%), *Leishmania* spp. (2.8%), and *T. gondii* (3.9%). ATR was experienced by recipients of blood exposed (10.6%, 12/113) and unexposed to parasites (5.8%, 19/327) however, there was no significant difference (p = 0.112) between the two groups. Genomic analysis found mutant haplotypes: *Pfdhps* (14.4%), *Pfmdr*1 (14.4%), *Pfdhfr* (12.9%), *Kelch 13* (9.4%) and *Pfcrt* (5.7%). *Pfcrt* mutant gene, CVINT was identified for the first time in Ghana. Drug-resistant markers were found to be ~20% with a TTM incidence of 7%. Donor blood should be screened for malaria and other haemoparasites and further research to quantify risk of TTM.



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## DEDICATION

In loving memory of my late husband Theophilus Tawia

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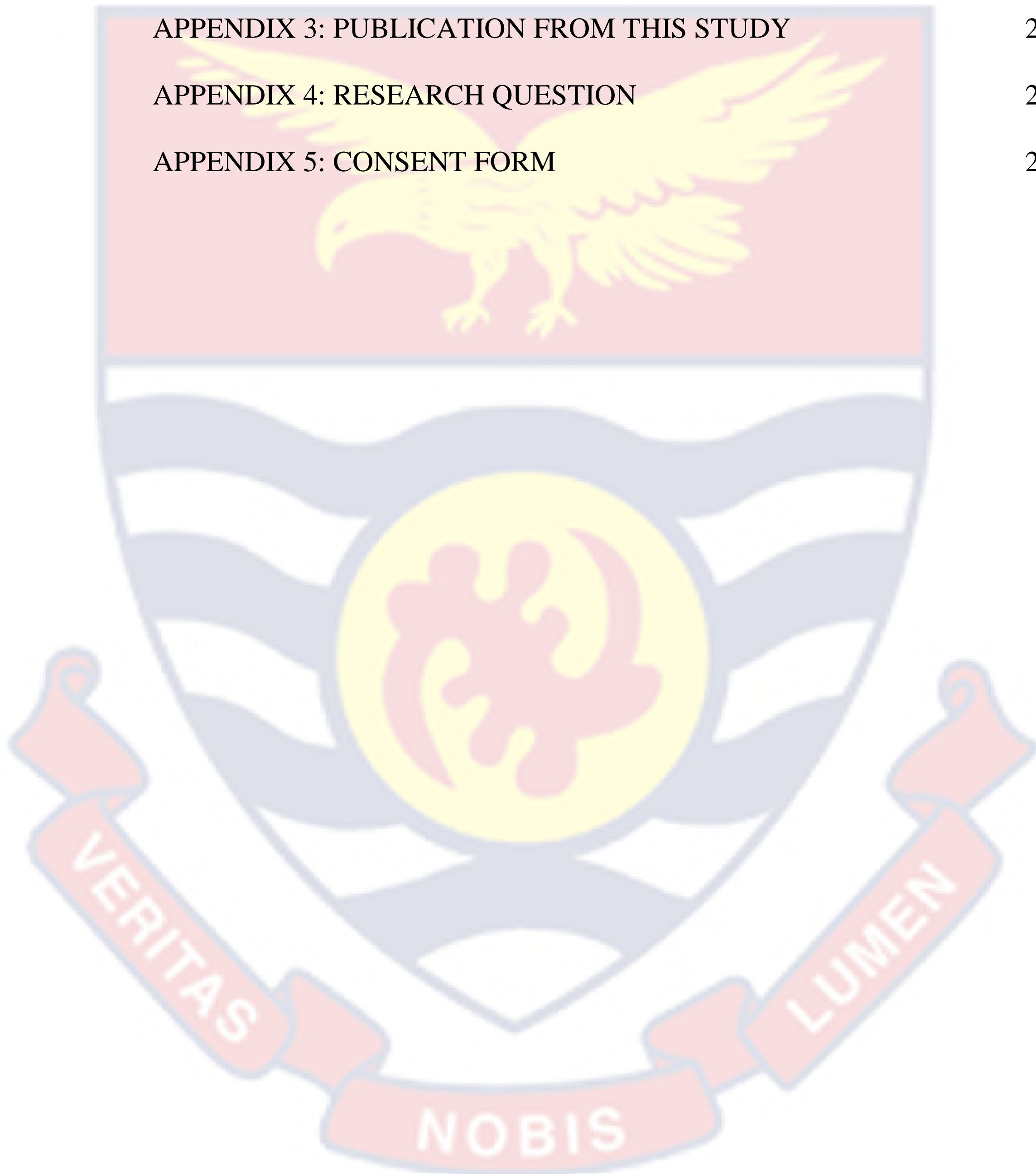




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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ACT | Artemisinin-based Combination Therapy |
| CRBC | Concentrated Red Blood Cell |
| ELISA | Enzyme-linked immunosorbent assay |
| FFP | Fresh Frozen Plasma |
| FNHTR | Febrile Non-Hemolytic Transfusion Reaction |
| GDBS | Global Database on Blood Safety |
| HIV | Human Immunodeficiency Virus |
| IgG | Immunoglobulin G |
| *K13* | Kelch 13 propeller domain on chromosome |
| PCV | Packed Cell Volume |
| *Pfcrt* | *P. falciparum* chloroquine resistance transporter |
| *Pfmdr1* | *P. falciparum* multi-drug resistance |
| *Pfdhfr* | *P. falciparum* dihydrofolate-reductase |
| *Pfdhps* | *P. falciparum* dihydropteroate-synthetase |
| PLT | Platelets |
| PRBC | Packed Red Blood Cell |
| RBC | Red Blood Cells |
| TACO | Transfusion Associated Circulatory Overload |
| TRALI | Transfusion Related Acute Lung Injury |
| TTPIs | Transfusion-Transmitted Parasitic Infections |
| TTPs | Transfusion-Transmitted Parasites |
| TTM | Transfusion-Transmissible Malaria |
| WBC | White Blood Cells |
| WHO | World Health Organization |



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# CHAPTER ONE

# INTRODUCTION

## Background to the Study

Transfusion of blood and blood products is a life-saving therapy that helps millions of individuals suffering from life-threatening conditions. such as anaemia, sickle cell disease, bleeding disorders, or injuries (Blood Transfusion Services, 2017).

In high-income nations, transfusion is primarily focused on supportive care in various medical operations including heart surgery, transplant surgery, major trauma, and treatments for blood-related cancers (Ackfeld et al., 2022) (D'Alessandro et al., 2019; Du Pont-Thibodeau et al., 2014; Stoicea et al., 2017; Wood et al., 2009; WHO, 2002). Blood transfusion is mostly utilized in developing and middle incomes countries to treat pregnancy-related problems and severe childhood anaemia (Stevens et al., 2013; WHO, 2002).

The WHO Global Database on Blood Safety (GDBS) estimated that 118.5 million donor blood units are saved annually on a global scale (WHO, 2018). This underscores the critical role that blood donation and transfusion services play in guaranteeing enough supply of safe and reliable blood for therapeutic use. Approximately 40% of global blood donations are from high-income nations. 76% of transfusions in high-income nations are given to the 60 years. In low-income countries, children under the age of five make up to 54% of all blood transfusions.

Despite the fact that blood transfusions save lives, blood and blood products carry the danger of transferring life-threatening pathogenic agents and adverse immunologic responses. These may cause acute or delayed blood



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transfusion reactions which may be fatal (Fong, 2020; Olaniyi, 2019; Pelletier, 2018). About 0.5–3.0 % of all transfusions result in transfusion reactions (Fatima et al., 2017). This percentage range indicates that a small but notable proportion of blood recipients experience adverse events or complications.

Acute transfusion responses typically manifest within a Twenty-four-hour window following a transfusion of donor blood unit. (Gelaw et al., 2020; Kicklighter & Klein, 2016; Sahu et al., 2014). Depending on the blood product transfused, the reaction may vary (Gelaw et al., 2020). Notable acute adverse reactions following transfusion include fever, urticaria, itching, headache, chills, anaphylaxis (Savage, 2016; Squires, 2011), febrile non-hemolytic transfusion reaction (FNHTR), transfusion-associated circulatory overload (TACO) and transfusion-related acute lung injury (TRALI) (Sharma et al., 2011). The presence of immunoglobulin G (IgG) and complement mediate most acute transfusion reactions because of their capacity to coat donor erythrocytes causing intravascular hemolysis (Vamvakas & Blajchman, 2009a). Additionally, it has also been reported that the accumulation of inflammatory cytokines in donor blood are associated with FNHTR and allergic reaction in recipients (Chang et al., 2018). The mechanism behind FNHTR involves the discharge of an antibody-mediated endogenous pyrogen and the secretion of cytokines (Ackfeld et al., 2022; Addas-Carvalho et al., 2006). When compatibility testing has been accurately done, triggers of transfusion reactions may not be readily known (Sahu et al., 2014). More so, infectious pathogens have been associated with transfusion reactions (Fong, 2020). A variety of biological agents including bacteria, parasites and viruses are most transmitted through this process. According to several studies, Human Immunodeficiency



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Virus (HIV), hepatitis B, hepatitis C, and *Treponema pallidum* are the most often screened pathogens in blood donors (Fiedler et al., 2019; Kaur & Kaur, 2015; Vaillant & Sticco, 2022), however, parasitic infections are not. While transfusion-transmitted parasite infections (TTPIs) are relatively uncommon compared to infections caused by viruses and bacteria, they have the potential outcome resulting in fatal outcomes. Especially in those who have a weakened immune system (Garraud, 2006; Mardani, 2020). Examples of parasites that are associated with blood transfusion transmission are *Plasmodium* spp. (Chiodini et al., 1997; Mardani et al., 2016; A. K. Owusu-Ofori et al., 2013), *Trypanosoma cruzi* (Angheben et al., 2015), *Babesia microti* (Moritz et al., 2016), *Toxoplasma gondii* (Zainodini et al., 2014), *Leishmania donovani* Complex (Dey & Singh, 2006) and *Wuchereria bancrofti* (Bloch et al., 2012). These parasites, after infection, could be sub-clinical, hence capable of contaminating donor blood units.

Throughout the last ten years, transfusion-transmissible malaria (TTM) was documented to frequently occur in low-endemic areas involving the UK, USA and France (Verra et al., 2018a). The situation is however expected to worsen in highly endemic regions, such as sub-Saharan Africa where high asymptomatic carriage of *Plasmodium* parasites exists (Muntaka & Opoku-okrah, 2013).

Although the WHO recommends that blood for transfusion be screened for blood-transmissible pathogens, however, generally malaria-endemic countries including Ghana parasitic infections are not usually carried out. As a result, most clinicians administer anti-malaria drugs as prophylaxis to



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transfused recipients (Freimanis et al., 2013; Owusu-Ofori et al., 2010; Seed et al., 2010).

It has been suggested that the use of antimalarial drugs as prophylaxis may have led to malaria drug resistance. A study in Accra, Ghana reported several suspected anti-malaria drug-resistant markers in blood donors (Aninagyei, Duedu, et al., 2020). The presence of these resistant genes could reduce the potency of the anti-malaria drugs. However, in the Eastern region of Ghana, the *Plasmodium* drug-resistant strains have not been profiled; hence it is unknown if antimalarial prophylaxis would work or not in recipients of infected blood.

Numerous investigations have found variable levels of *Plasmodium* spp infection in potential donors of blood and donor blood units around globe. (Alemu & Mama, 2018; A. Owusu-Ofori et al., 2016). There is also a severe paucity of information regarding the health implications of transfusion-transmitted malaria, impeding rational decision-making.

The following parasites: *Babesia* spp, *Leishmania* spp, *Toxoplasma gondii,* and *P. falciparum* were chosen in this because varying forms of their distribution have been noted in the West African sub-regions. In Ghana in the Oti region, a little over half of the residents have been exposed to *L. donovani* using the leishmania skin test (LST) (Akuffo et al., 2021). In the Volta region, some cases of *L. donovani* were also identified (Kweku et al., 2011). In the Ashanti region of Ghana, a prevalence of 50.3% of *T. gondii* IgG was reported (Agordzo et al., 2020). In a review paper published in 2020, it was indicated that up to about 93% of Ghanaians and up to 64% of some domestic animals are exposed to *T. gondii* (Reynolds, 2020).



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There is limited data regarding human babesiosis in Ghana, however, previous studies have reported the disease in domestic animals in Ghana (Bell-Sakyi et al., 2004; Nagano et al., 2013). Another reason for selecting these parasites is their ability to cause asymptomatic infection in human hosts (Acquah et al., 2020).

## Problem Statement

Blood and blood product transfusion is very essential and lifesaving intervention especially in critically ill and in severe trauma situations. During blood transfusion, the first cause of suspicion of transfusion reaction is associated with ABO blood group and Rhesus incompatibility. When grouping and crossmatching is accurately done, the causes of transfusion reactions may be unknown.

About 118.5 million donor blood units are saved annually (WHO, 2022). However not all are safe for transfusion due to asymptomatic infections among donors. Over the years parasites involved in asymptomatic infections of Ghanaian adults includes *Leishmania* spp which has been described in 98 countries globally with ~350 million at risk of infection. In Ghana (Oti region) prevalence of 41.8% has been reported (Akuffo et al., 2021). *T. gondii* has a global prevalence of 30% however prevalence of 50.3% in Ashanti region (Agordzo et al., 2020). Even though no human babesiosis has been reported till date (Nagano et al., 2013), several cases in domestic animals have however been reported (Addo et al., 2023). Prevalence of *P. falciparum* during high transmission (rainy) season in the forest zone was reported to be 73% (Heinemann et al, 2020) and low transmission (dry) season in the forest zone was 37.8% (Agbana et al, 2022). These haemoparasites in asymptomatic adults



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are considered as public health problem because if present in donor blood can lead to complications in blood recipients. In contemporary practice, the emphasis has been on Human Immune viruses, Hepatitis C viruses and syphilis. However, there is paucity of data with transfused parasitic infection as compared to bacterial infections. One of the reasons is that the parasitic load in infected donors may be very low and that clinical symptoms may not be observed during blood donation. Also, parasites may be latent in donors for years without causing any significant symptoms due to donors' immunity. However, these latent parasites may cause clinical symptoms in susceptible recipients due to weakened immune system or may be critically ill. Despites WHO recommendations that blood should be screen for pathogens before transfusion, Transfused blood is not screened for parasitic infections in most malaria-endemic areas, In Ghana, there is a scarcity of information on TTPs although there is evidence that Transfusion Transmitted Infection exist, its impact may be underestimated and research in such area is essential for developmental goal.

In this study, the clinical effects and exposure rate of *Babesia* spp, *Leishmania* spp, *Toxoplasma gondii,* and *P. falciparum* in donor blood units was carried out. The profiling of *P. falciparum* drug resistant genes in donor blood was assessed, the association of these selected haemoparasites with acute transfusion reactions was determined and the confirmation of transmissibility of transfusion-malaria.



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## Aim of the Study

The aim of this study is to evaluate the exposure and clinical effect of *Babesia* spp., *Leishmania* spp., *Toxoplasma gondii*, and *P. falciparum* in blood donors and recipients, as well as the confirmation of TTM and profiling of *P. falciparum* drug resistant genes in donor blood infected with *P. falciparum.*

## Specific Objectives

The study was specifically designed to:

1. Assess the exposure of *Babesia* spp., *Leishmania* spp., *Toxoplasma gondii,* and *P. falciparum* and their association with acute transfusion reactions.
2. Evaluate the sero and microscopy prevalence of *P. falciparum* in donor blood transfused in Nsawam Government Hospital, Eastern Region, Ghana
3. Sequence and analyse *P. falciparum* homologous gene sequences in parasites detected in transfused blood and in recipients of infected blood units with positive parasitological evaluation.
4. Evaluate the proportions of *P. falciparum* chloroquine resistance transporter (*Pfcrt*), *P. falciparum* multi-drug resistance (*Pfmdr1*), *P. falciparum* dihydropteroate-synthetase (*Pfdhps*), *P. falciparum* dihydrofolate-reductase (*Pfdhfr*) and *Kelch13* genes polymorphisms that confer resistance to malaria parasites in blood donors.



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## Research Questions

1. What is the rate of exposure of donor blood to *Babesia* spp., *Leishmania* spp., *Toxoplasma gondii,* and the *P. falciparum* parasite and is there an association between the exposure of donor blood to these parasites and acute transfusion reactions in recipients?
2. Considering the high asymptomatic carriage of *P. falciparum* among the Ghanaian population, what would be the prevalence of donated blood infected with malaria parasites?
3. Are infecting *Plasmodium* spp. from the donor blood units the same as those isolated in recipients who developed positive parasitemia after transfusion?
4. Does asymptomatic *P. falciparum* infection donor blood harbour anti-malaria drug resistant genes which may cause the spread of resistance to anti-malarial drugs?

## Significance of the Study

Blood for transfusion is not routinely screened for transfusion transmitted parasites in sub-Saharan Africa. Consequently, patients who undergo transfusions may be at risk of exposure to infections, potentially resulting in unfavorable outcomes.

The study sought to show if routine screening of blood will avoid transfused infections by preventing infected blood from being inadvertently transfused to patients.

Transfusion service directors, policymakers, and practitioners across Africa have highlighted important information gaps in the clinical effect of TTM and other transfusion parasitic infections as well as the appropriate malaria



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screening approaches. There is also insufficient evidence to support policies that advocate the screening of donated blood for malaria. The study sought to provide information on TTM as well as other transfusion parasitic infections where data is scarce. It will further inform policymakers to draw guidelines on the practice of treating blood recipients presumptively for malaria and would invariably address the assumption that transfused blood is the source of malaria occurring in post-transfusion.

There is also no data on the association between *Babesia* spp, *Leishmania* spp, *Toxoplasma gondii,* and *P. falciparum* parasite exposed donor blood in relation to acute transfusion reaction in recipients. This study will delineate preliminary data on acute transfusion reactions among recipients that have received blood exposed to these parasites in Ghana. Clinicians have always looked at acute transfusion from one angle without taking into consideration parasitic infections as recorded in high income countries. This will better inform clinicians on the possibilities of ATR being caused by haemo-parasites.

## Delimitation

Donor blood units that were drawn at Nsawam Government Hospital (NGH) for this study were stored at a temperature of 4°C for a minimum of 24 hours. Participation in the study required that a blood recipient be transfused with more than fifty percent of the unit under consideration.

In order to qualify for post-discharge follow-up, blood recipients were required to exhibit negative results for malaria through both mRDT and microscopy testing. Additionally, it was necessary for the donor blood used in the transfusion to contain detectable malaria parasites as determined through microscopy examination of the remaining contents in the blood bags. The



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haemo-parasite exposure rate was established based on this analysis of the donor blood units.

The recipient of malaria-infected donor unit blood was carefully observed on these (2, 7, 14, 21, 28, and 35) selected days. Follow-up was halted at the point where the participant tested positive for *P. falciparum* through mRDT as well as microscopy. Individuals who became infected after receiving blood transfusions were advised to seek care for malaria at the closest healthcare facility.

## Limitation

Polymerase Chain Reaction could not be performed on the selected haemo-parasites, which are believed to be more sensitive than Elisa, due to lack of funds. Furthermore, some of the research participants who received transfusions with donor blood units positive for malaria could not be followed up because they relocated from the location they had previously provided, to family members elsewhere for support. As a result, the number of follow-up samples was reduced, resulting in a smaller sample size for the detection of P. *falciparum* transmission detection.

## Organization of the Study

The present study was structured into five distinct chapters. The first chapter serves as an introduction to the investigation. It also specifies the study's purpose and clearly outlines the goals and objectives. The second chapter evaluated the literature by investigating several selected haemo-parasites and their connection with acute transfusion reactions. It also looked at transfusion transmissible malaria among blood donors. The third chapter covers the study



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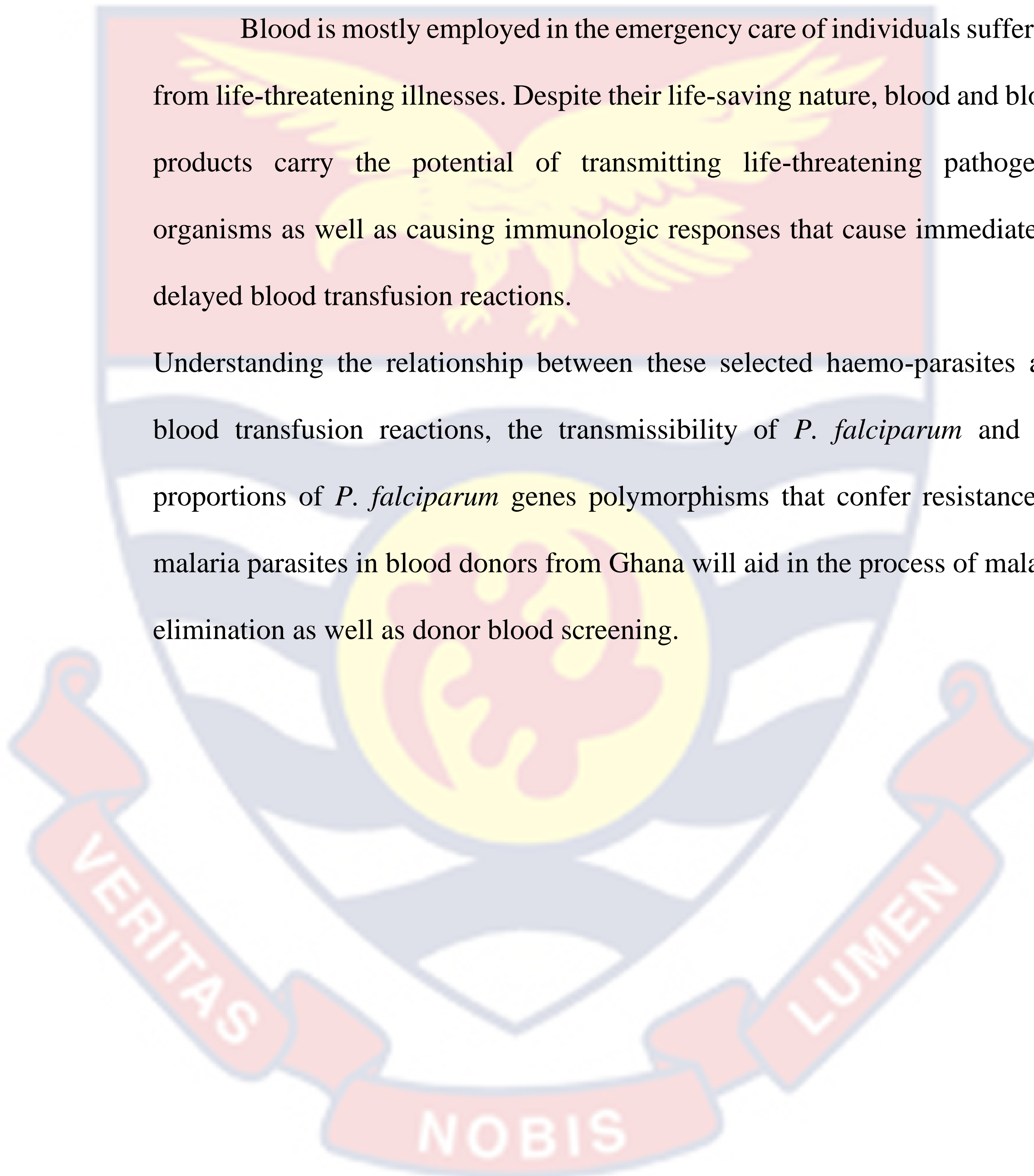
methodologies used to obtain the results, and the last chapter provides the conclusion and recommendations for future research.

## Chapter Summary

Blood is mostly employed in the emergency care of individuals suffering from life-threatening illnesses. Despite their life-saving nature, blood and blood products carry the potential of transmitting life-threatening pathogenic organisms as well as causing immunologic responses that cause immediate or delayed blood transfusion reactions.

Understanding the relationship between these selected haemo-parasites and blood transfusion reactions, the transmissibility of *P. falciparum* and the proportions of *P. falciparum* genes polymorphisms that confer resistance to malaria parasites in blood donors from Ghana will aid in the process of malaria elimination as well as donor blood screening.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# CHAPTER TWO

# LITERATURE REVIEW

This literature review embarks on a comprehensive exploration of *Babesia* spp., *Leishmania* spp., *Toxoplasma gondii,* and *P. falciparum* that have been implicated in blood transfusion-related infections and their association with acute transfusion reaction. It further sheds light on the current state of knowledge, the gaps in understanding, and the implications for blood safety with the aim of providing a foundation for informed decision-making in blood banking practices. It also discusses the *P. falciparum* chloroquine resistance transporter (*Pfcrt*), *P. falciparum* multi-drug resistance (*Pfmdr1*), *P. falciparum* dihydropteroate-synthetase (*Pfdhps*), *P. falciparum* dihydrofolate-reductase (*Pfdhfr*) and *Kelch13* genes polymorphisms that confer resistance to malaria parasites in blood donors.

## Overview of Blood

Blood is a fluid that transports nutrients, gases like oxygen and waste throughout the body. Blood plays a role in defending the body against infections and is responsible for the homeostatic regulation of pH, temperature, and other internal conditions. According to Basu & Kulkarni (2014), blood is made up of cellular elements suspended in plasma which constitutes 90 % of whole blood. The function of blood is to transport oxygen. Hemoglobin is a protein found in red blood cells that binds to oxygen in the lungs and transports it to tissues and organs throughout the body. Blood delivers nutrients from the digestive system to cells for energy and other metabolic functions, including glucose, amino acids, and fatty acids. It also transports carbon dioxide, a waste product of cellular metabolism, from tissues to the lungs.



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Digested nutrients are taken into the blood by capillaries in the villi that line the small intestinal tract. This group of nutrients includes glucose, amino acids, vitamins, minerals, and fatty acids. Some hormones secreted by endocrine glands are also delivered to organs and tissues by blood. Heat is taken up and dispersed through the body by blood. This allows the body to maintain homeostasis by releasing or storing heat. Blood arteries dilate and contract in reaction to external organisms.

These mechanisms facilitate the movement of blood and heat towards or away from the skin's surface, which is where the dissipation of heat occurs (Nelson, 1962). The circulation transports waste substances to the organs that are accountable for their removal and processing before elimination. Renal arteries transport blood into the kidneys, whereas renal veins transport blood away. The kidneys extract urea, uric acid, and creatinine from the blood plasma and transport them through the ureters.

The liver is also involved in the removal of toxins from the bloodstream. It cleanses vitamin-enriched blood during digestion before transferring it to the rest of the body (Wallace, 1998).

Whole blood consists of plasma, red blood cells (RBC), white blood cells (WBC) and platelets (PLT). Historically, whole blood was transfused to patients without separation into its various components. Today, patients are given specific blood components as required, as opposed to whole blood. For instance, if a patient is deficient in red blood cells, they will be transfused with only the red blood cell component.

Component of whole blood can be separated into different components using a centrifuge as they have different densities. When whole blood is



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centrifuged at around 2000 rpm, the red blood cells (RBC) settle at the bottom as they have the highest density (Hardwick 2008; Basu and Kulkarni 2014). The WBC component settles on top of the RBCs with the PLTs above. Each blood component is stored under optimal thermal conditions. The use of preservation solutions and bags used for blood storage that is specially designed for this purpose serves to extend shelf life and enhance the quality of the blood.

## Blood Components

Blood components can be obtained by aphaeresis (Basu & Kulkarni, 2014). When whole blood has been donated, the various components of blood are mainly separated for transfusion. The different blood components are concentrated red blood cell (CRBC) also known as red blood cell concentrates, platelet (PLT), fresh frozen plasma (FFP) and cryoprecipitate (Basu & Kulkarni, 2014) (Figure 1).

Concentrated red blood cells (CRBC), refer to a blood product that has had much of its plasma removed, resulting in a higher concentration of red blood cells. The volume transfused is significantly less than whole blood nevertheless has the same oxygen-carrying capacity (Cywinski, 2017). In a related study, transfused individuals who received one RBC donor unit had their haemoglobin levels rise by 1 g/dL (Roubinian et al., 2019).

Plasma is mostly made up of water with a variety of compounds dissolved or suspended in it, the majority of which are proteins. There are numerous proteins in plasma such as albumin, globulins, and fibrinogens. Albumin, which is produced in the liver forms the majority of the proteins and acts as a carriage medium for fatty acids as well as steroid hormones.



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Alpha, beta, and gamma globulins are the next most frequently encountered proteins. Alpha and beta globulins play roles in transporting iron, lipids, and fat-soluble vitamins to cells, while also regulating osmotic pressure levels.

Gamma globulins are a group of proteins that contribute to the body's immune response. Although the liver produces other proteins that are found in plasma, immunoglobulins are created by plasma cells, which are specialized leukocytes. The least plasma protein is fibrinogen which is vital for blood clotting (Mathew et al., 2022).

Fresh Frozen Plasma (FFP) is plasma frozen within eight hours and contains preservatives and anticoagulants. The clotting factors associated with FFP) make it useful during emergency cases such as trauma resuscitation. Fresh frozen plasma has all clotting factors and can be kept active for 12 months if frozen at 30° C in a blood bank freezer (Wardrop & Brooks, 2001).

Platelets, also known as thrombocytes, are special blood cells which correct bleeding or blood clotting disorders caused by thrombocytopaenia. Platelets, along with red blood cells and most white blood cells, are generated within the bone marrow in adults.

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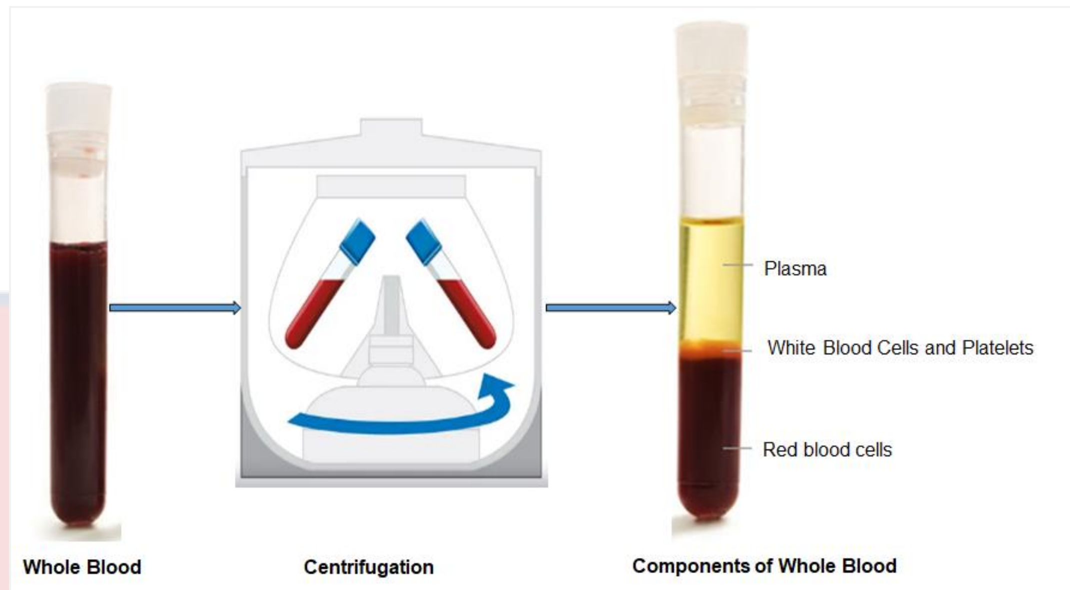


*Figure 1: Whole blood centrifuged into blood components*

## Blood Groups and Compatibility Testing

The ABO blood grouping system discovered by Karl Landsteiner is used to characterize blood. There are 4 main blood groups A, B, AB and O. The presence of Antigen A or B and the presence of antibody A or B is used to define the blood group (Dean, 2005). The H antigen is produced by a specific fucosyltransferase. The H antigen is transformed into either the A antigen, the B antigen, or both antigens depending on a person's ABO blood type. When a person possesses blood group O, the H antigen is unaltered. As a result, the H antigen is most prevalent in blood type O and least prevalent in blood type AB (Dean, 2005). See fig 1. The difference in antigen expression can enhance or reduce a host's susceptibility to a wide range of illnesses. This is because the antigens of the RBCs act as receptors and/or co-receptors for bacteria, parasites, and viruses, therefore blood groups can be associated with particular infections (Cooling, 2015).

Understanding the distribution of ABO and Rh blood groups aids in ensuring an adequate supply of compatible blood for patients in need (Doku et al., 2019,



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2022; Tiruneh et al., 2020; Woldu et al., 2022). Blood group O happens to be the most common in some populations. In Saudi Arabia blood group O was found to be the most prominent ABO blood group, whereas AB was the least (Belali, 2022).

According to a similar study in Uganda, blood group O (50.3%) was the majority, followed by A (24.6%), B (20.7%), and AB (4.5%). Rhesus (D) positive and negative proportions were 98 and 2%, respectively (Apecu et al., 2016).

Doku et al., 2019, also reported the ABO blood group distribution in Ghana. He observed that O+ blood was the most prevalent blood group in the region. For blood group O+(46.3%), A+(18.9%), B+(24.4%), AB+(3.1%), O-(4.4%), for A-(1.7%), B-(1.3%), and AB-(0.1%) respectively.

Numerous studies have linked blood groups to various disease conditions across the globe (Alemu & Mama, 2016; Rattanapan et al., 2023; Tonen-Wolyec & Batina-Agasa, 2021).

Susceptibility to *P. falciparum* malaria parasite varies according to blood group. The relationship between the ABO blood group system and susceptibility to malaria has been well elucidated by several studies (Beiguelman et al., 2003; Cserti & Dzik, 2007; Obisike, 2020; Onanuga & Lamikanra, 2016b; Tazebew et al., 2021). Fischer & Boone (1998) reported that persons with blood group A are at greatest risk of cerebral malaria from *P. falciparum* whereas those with blood group O tend to have milder disease outcomes. Rattanapan et al. (2023) investigated the link between ABO blood groups and the risk of *P. falciparum* malaria infection in a Thai community. The study found that those with blood type O had a decreased risk of infection than those with other blood types.



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Persons with blood group A, on the other hand, were more susceptible to infection. Multiple studies have provided evidence suggesting that individuals with blood group A have a higher susceptibility to *P. falciparum* infection when compared to individuals with blood group O (Afoakwah, Aubyn, Prah, Nwaefuna, and. Boampong 2016; Panda et al., 2012). In a similar study, Tonen-Wolyec & Batina-Agasa (2021) observations revealed that individuals with blood group A had a higher likelihood of contracting severe malaria in comparison to those with blood group O. Subsequent studies did not identify a correlation between ABO blood groups and malaria. (Igbeneghu et al., 2012; Onanuga & Lamikanra, 2016a, 2016b; X. Zhang et al., 2017).

The variations between blood group phenotypes and the vulnerability and intensity of *P. falciparum* malaria infection have been attributed to the phenomena of rosetting and cytoadherence exhibited by infected erythrocytes. (Rowe et al., 2007). The formation of rosettes in *P. falciparum* malaria impedes the flow of blood in the microvasculature, thus playing a significant role in the development of severe malaria pathogenesis (Kaul et al., 1991; Rowe et al., 2007). Various studies have been conducted to investigate the occurrence of rosetting in *P. falciparum* malaria among different blood groups. In laboratory studies, it has been demonstrated that the rosettes are comparatively smaller, less robust and lower in the O blood group phenotype compared to A, B, and AB (Barragan et al., 2000; Carlson & Wahlgren, 1992; Rowe et al., 2007). These findings are consistent with other studies on malaria pathogenesis. (Barragan et al., 2000; Carlson & Wahlgren, 1992; Rowe et al., 2007).

Pre-transfusion compatibility testing is done to verify whether a specific blood group type may be safely transfused to a patient to help prevent hemolytic



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transfusion reactions (Armstrong et al., 2008; Davenport & Bluth, 2021). The compatibility test involves verification of a crossmatch between the ABO-Rh blood type of the donor to that of the recipient serum and the absence of unexpected antibodies. Typically, immune-mediated transfusion responses develop as a result of a mismatch or incompatibility between the transfused product and the recipient. They include antibodies that are normally present in the blood recipient (such as anti-A and anti-B antibodies, which are frequently responsible for severe hemolytic transfusion responses) as well as antibodies that are produced in response to foreign antigens (alloantibodies). Many reactions are caused by alloantibodies, including mild allergy, febrile non-hemolytic, acute hemolytic, and anaphylactic reactions. Antibodies found in blood donors can also trigger responses and are suspected of being implicated in transfusion-associated lung injury (TRALI) (Aubron et al., 2018; Erony et al., 2018; Olaniyi, 2019).

Cross-matching is a straightforward procedure, however, if done incorrectly, can lead to fatalities.

Table 1: ABO Blood Grouping System

| Blood Group | Antigen Present on Red Blood Cell | Antibodies Present in Serum/Plasma |
|---|---|---|
| **A** | A Antigen | B Antibody |
| **B** | B Antigen | A Antibody |
| **AB** | A and B Antigen | None |
| **O** | None | A and B Antibody |



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## Blood Transfusion

Blood transfusion is a medical process in which blood is extracted from a donor's circulatory system and transferred to a recipient for therapeutic purposes (Learoyd, 2012). The importance of blood transfusion in saving a patient's life cannot be overemphasized. Maternal haemorrhage fatalities in hospitals are linked to a shortage of blood (Kolin et al., 2020; S. C. Murphy & Breman, 2001). A considerable loss of blood through haemorrhage can lead to dangerous haemoglobin levels and oxygen deprivation, both of which can injure the body's organs (Gutierrez et al., 2004). If the haemorrhage, the body's platelet and plasma stores are depleted, leading blood clotting to be disrupted and bleeding to continue. Blood can, however, be transfused to prevent certain bleeding disorders. More so, blood transfusions are used to replace blood or blood components in people who have suffered serious injuries in car accidents or natural disasters, those undergoing major surgical procedure that will necessitate blood transfusions to replace any blood lost during the procedure or have other disorders that affect the blood or its components such as anaemia, leukaemia or kidney disease (Orish et al., 2016; Tiruneh et al., 2020; White, 2018). Anaemia is termed as a reduction in the number of circulating RBCs, a decrease in haemoglobin concentration, or a decrease in HCT and due to that, the supply of oxygen to the body's organs is reduced (Turner et al., 2022). According to the WHO, anaemia is described as haemoglobin level of <13 g/dL or an HCT of less than 41% in males and < 12 g/dL or an HCT <36 % in women (WHO,2018). Anaemia has been also classified into moderate anaemia hemoglobin level 8 – 11 g/dL and severe anaemia < 8 g/dL(Tesema et al., 2021).



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Anaemia affects about one-third of the world's population. Each year, 1 million infants under the age of 5 die from severe anaemia, and 26 percent of maternal haemorrhage fatalities in hospitals are linked to a shortage of blood (Bates et al., 2008; Murphy & Breman, 2001; Safiri et al., 2021). Here in Ghana, blood transfusion is equally indispensable and of public health importance. There are a few haemovigilance systems in Sub-Saharan Africa, and data on transfusion-related adverse events are scarce. The creation of the National Blood Transfusion Service (NBTS) in Ghana is vital for the country's long-term supply of high-quality blood products. The NBTS has the responsibility to manage, organise and monitor blood transfusion-related activities to reduce the transmission of unsafe blood to the recipient. Although WHO recommends that blood for transfusion should be screened for transmissible pathogens, routine screening is not performed in most malaria-endemic countries (Abdullah & Karunamoorthi, 2016; Aninagyei et al., 2020; Tsehay et al., 2020). This is partly due to the lack of a universally accepted screening procedure, the affordability of screening tools and the sensitivity of the kits (Owusu-Ofori, Parry, & Bates, 2010; Owusu-Ofori et al., 2013).

In high malaria endemic countries in Sub-Saharan Africa, *Plasmodium* parasites are carried asymptomatically with dire outcomes (Muntaka & Opoku-Okrah, 2013). In Ghana, the asymptomatic transfused malaria parasite has been estimated to be between 25 % and 40 % (Acquah et al., 2020; Bereczky et al., 2004). However, Aninagyei et al. (2020) reported a much lower prevalence of 11.8 %.

Donor recruiting, selection, and screening for TTI are all part of the blood donation procedure in Ghana (WHO, 2012). The stages for choosing



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include pre-donation information and the completion of a medical history questionnaire.

In addition, interviews are conducted to determine health risks followed by counseling. The final stage is to obtain the donor's permission. Physical examination, assessment of vital indicators such as blood pressure and pulse, and evaluation of veins for easy venipuncture are all part of the pre-donation screening.

Commercial donors, volunteer donors, family replacement donors, and autologous donors are the most common sources of blood (Yambasu et al., 2018). In line with this, healthy donors are carefully selected to provide safe blood for transfusion. It must be processed using trustworthy test procedures, particularly during the manufacturing of the blood components, storage, and transportation processes.

## Blood Transfusion Reactions

Adverse reactions arising from the transfusion of blood products are known as blood transfusion reactions (Sahu et al., 2014; Suddock & Crookston, 2022). This is the most common side effect of blood transfusion (Davenport & Bluth, 2021; Kicklighter & Klein, 2016). Transfusion reactions have been shown to occur in about 1% of all transfusions (Learoyd, 2012). However, Olaniyi (2019) reported transfusion reactions of approximately 0.5-3.0%. Transfusion reactions can range from minor to fatal. The risk of fatal responses varies between 1 in 0.6 - 2.3 million people (Vamvakas & Blajchman, 2009b).



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The two types of transfusion reactions are acute and delayed. Acute transfusion reactions develop within 24 hours of receiving blood and the response varies depending on the blood product transfused (Gelaw et al., 2020).

Notable acute adverse reactions following transfusion include fever, urticaria, itching, headache, chills, anaphylactic (Savage, 2016; Squires, 2011), febrile non-hemolytic transfusion reaction (FNHTR), transfusion associated circulatory overload (TACO) and transfusion related acute lung injury (TRALI). Fever, chills, urticaria (hives), and itching are the most prevalent signs and symptoms (Figure 1).

Some symptoms resolve on their own or with minimal or no treatment. A more severe response includes respiratory pain, a high temperature, hypotension (low blood pressure), and crimson urine (hemoglobinuria). The severity and frequency of transfusion responses differ based on the type of reaction, the prevalence of sickness in the donor community, and the patient's quality of follow-up care. The hazards and fatalities connected with blood transfusions are decreasing as a result of progress in donor screening, improved testing methods, and the implementation of automated data systems (Jacquot & Delaney, 2018; Suddock & Crookston, 2022).

The presence of Immunoglobulin G (IgG) and complement gives rise to most acute transfusion reactions because of their capacity to coat donor erythrocytes leading to intravascular hemolysis (Vamvakas & Blajchman, 2009a).

In Uganda, Waiswa et al., (2014) reported an ATR frequency of 9.6% of the 507 recipients of which the most prevalent transfusion reactions were fever (49 %) and allergic reactions (14 %).



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Similarly, In a study by Gwaram et al.,(2012), a low prevalence of 3.6 % (11/302) of acute transfusion reactions was observed. Of the ATRs, FNHTRs accounted for 3.3 % and allergy 0.3 %. However, Negi et al. (2015) indicated that allergic reactions and febrile non-hemolytic transfusion reactions were the prevailing types of transfusion reactions observed. Similarly, Kaur et al., (2013), discovered that allergic reactions were the most frequently encountered side effects of blood transfusions (65.6 %).

In Ghana, there is scarcity of data on the prevalence and risk factors of transfusion reactions. Also, there are discrepancies in the published data on the level of ATRs. In a study in Ghana by Owusu-Ofori et al. (2017) to determine the incidence and pattern of ATRs, an unusually high level of ATRs was reported contrary to the other studies. The overall ATRs incidence rate was found to be 21.3 % (92/432). This high level of transfusion reaction is in stark contrast to the 3 % reported by Vamvakas & Blajchman, (2009b).

Several mechanisms may lead to acute transfusion reactions. The presence of immunoglobulin G (IgG) and complement mediate most acute transfusion reactions because of their ability to coat donor red blood cells leading to intravascular hemolysis (Vamvakas & Blajchman, 2009a). Additionally, it has also been reported that accumulation of inflammatory cytokines in donor blood are associated with ATR such as FNHTR and allergic reaction in recipients (Chang et al., 2018). It is aided by the release of endogenous pyrogens and cytokines, with antibody-mediated mechanisms involved. Interleukin-1, interleukin-6, interleukin-8, and tumor necrosis factor are among the usual cytokines associated with acute transfusion reactions (ATRs) (Addas-Carvalho et al., 2006).



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The primary reason for immune-mediated transfusion reactions stems from a mismatch or incompatibility between the blood or serum of the recipient and the transfused product. (Davenport & Bluth, 2021; Kicklighter & Klein, 2016). The incompatibility is brought about by antibodies being naturally produced in response to foreign antigens in the recipients' blood (Jasinski & Glasser, 2019). Complement activation, intravascular hemolysis, volume overload, and infections are all caused by the incompatibility of blood products that cause antibody-antigen responses (Namikawa et al., 2018; Strobel, 2008).

Bacterial contaminated donor blood has been linked to acute transfusion reactions (Apriastini & Ariawati, 2017). It is the most common cause of transfusion-related morbidity and mortality (Bihl et al., 2007; S. B. Harris & Hillyer, 2007). Bacterial contamination can occur due to insufficient cleaning of the donor's arm or presence of germs in the donor's blood, or inappropriate product handling (Agzie et al., 2019; Kahigwa et al., 2002).

Parasites have also been implicated in blood transfusion reactions, however, there is limited data on some organisms of public health importance such as *P. falciparum, Babesia, T. gondii,* and *Leishmania*. This is due to their ability to cause asymptomatic infections (Harris & Hillyer, 2007). Reactions can manifest with nonspecific symptoms that often overlap, posing challenges in their identification.



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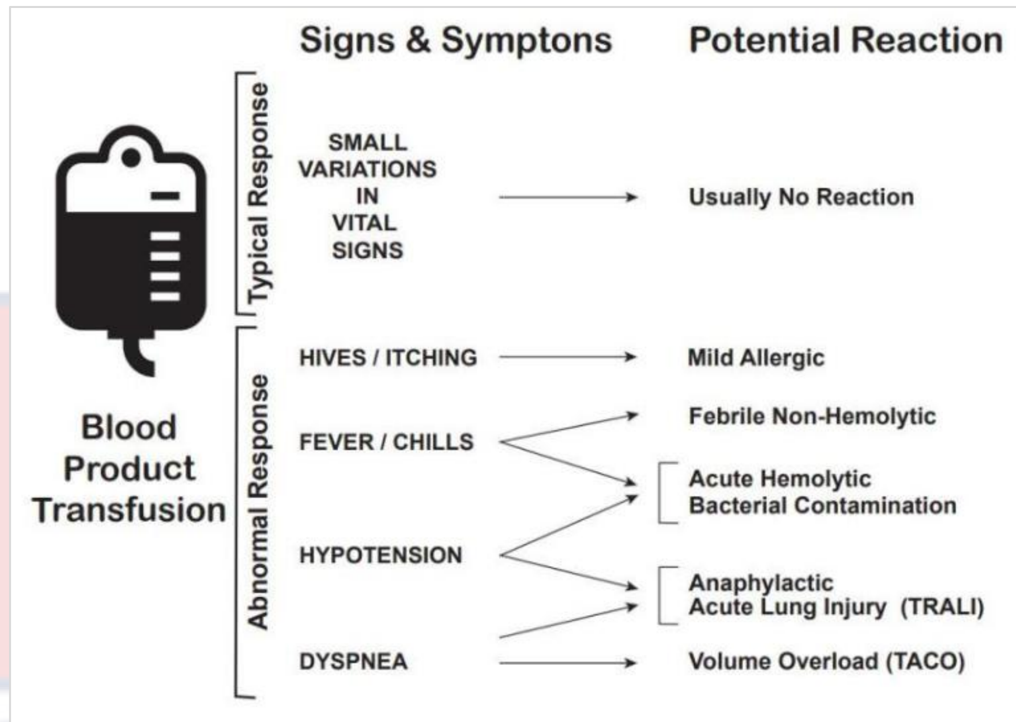


*Figure 2: Clinical signs and symptoms associated with ATRs*

Source: NCBI, 2023

## Blood Transfusion Reaction Risk Factors

Transfusion reactions are danger to patients (Ackfeld, Schmutz, Guechi & Terrier, 2022). Transfusion risk factors include the patient's age, blood product type and age, and the previous patient's transfusion history (Apriastini & Ariawati, 2017; Pedrosa et al., 2013).

Other risk factors are allergic reactions to allergens and antibodies, as well as anaphylactic reactions to antibodies against plasma donor proteins (immunoglobulin-IgA).

In research by Apriastini & Ariawati (2017) using 3251 blood samples, transfusion reactions were most common in male children and those over the age of 12 months, accounting for 2 % and 4.11 % of all transfusions respectively. The highest incidence of transfusion reactions was seen in blood



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type O (1.97 %) and thrombocyte concentrate (TC) among the blood types and components (1.88 %).

## Transfusion Transmissible Infections (TTIs)

Previously patients and clinicians regarded the danger of transfusion-transmitted infections (TTI) as unavoidable. However, the present blood screening initiatives and techniques have made a significant contribution to lowering TTIs (Barla et al., 2018). The main factors that make transfusion-transmitted infections (TTIs) a significant risk to blood safety are their ability to persist even after processing and storage, and the potential for donors to carry these infections without showing any symptoms during the infectious phase (Fong, 2020).

Donor exclusion has been in place since the beginning of blood donation because of infections such as hepatitis. Donor assessment, laboratory screening tests, and pathogen inactivation techniques are now regarded as critical instruments for reducing TTI risk, although they do not completely eliminate the risk (Bihl et al., 2007; Busch et al., 2019). Significant progress has been made in the field of pathogen reduction (PR) of blood components over the past two decades. In therapeutic settings, pathogen-free fresh-frozen plasma and platelet concentrates are now used (Allain & Goodrich, 2017; Bihl et al., 2007; Busch et al., 2019; Domanović et al., 2019).

More than 118 million units of blood are donated worldwide each year, with 18 million of them not being screened for transfusion-transmissible illnesses. (TTIs) (Adu-poku et al., 2020; Chaudhary et al., 2014; Tiruneh et al., 2020). Common TTIs are caused by bacteria, viruses, parasites, and prions. It is



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expected that over 354 million people globally have chronic infections with either hepatitis B or hepatitis C viruses. According to this data, this lead to in about 1.1 million deaths globally in 2015 (Peliganga et al., 2021; World Health Organization., 2017).

Ensuring the safety of blood for transfusion and preventing transfusion-transmitted infections (TTIs) requires screening of potential blood donors for infections such as hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and syphilis (Peliganga et al., 2021; World Health Organization., 2017).

Given the high prevalence of transfusion-transmitted infections (TTIs), the risk of transmission to patients can be significant if proper screening procedures are not in place. It is worth noting that the population at high risk for contracting TTIs is also the same population that donates blood for transfusion.

This creates a particularly challenging situation for minimizing the risk of TTIs. Several studies have reported varying frequencies of TTIs among blood donors. In a study conducted on a North Indian population, it was found that the overall prevalence of TTIs was 1.07% (116 out of 10,797). Among the specific infections, the seroprevalence rates of HIV, HBV, HCV, malaria, and syphilis were 0.03%, 0.49%, 0.50%, 0.009%, and 0.05%, respectively (Cheema et al., 2022), Another similar survey was carried out in Yemen to assess the prevalence of HCV, HIV, syphilis, and malaria among blood donors. The results showed a remarkably high prevalence of anti-Treponema pallidum (3.1%) among manual workers. The distribution of anti-malaria antibodies was found to be significantly different based on residency and age groups. The prevalence of HCV, HIV, syphilis, and malaria among the donors was observed to be 2.0%,



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0.2%, 2.4%, and 0.7%, respectively, according to the study findings (Alharazi et al., 2022). The high prevalence of these infections among blood donors in the above studies highlights the need for improved donor selection.

Regions such as Southeast Asia, Oceania, and the Middle East are prone to malaria transmission. In addition to malaria, other parasitic organisms such as *Trypanosoma cruzi, Babesia microti, Toxoplasma gondii*, and *Leishmania* species have been implicated in transfusion-transmitted infections (Barla et al., 2018). *Babesia microti*, the etiologic agent of babesiosis, have also been linked to transfusion-associated transmission. Ixodes ticks carry *Babesia microti*, which can cause serious disease conditions such as hemolytic anaemia, thrombocytopenia, and mortality, especially in immunocompromised or asplenic people have been recorded (Zimmer & Simonsen, 2022).
Although there are few studies on transfusion-transmitted babesiosis, more than 60 cases have been reported in the United States from tourists visiting Sub-Saharan Africa (Lobo, Cursino-Santos, Alhassan & Rodrigues, 2013).

Within Sub-Saharan Africa, blood transfusions account for approximately 5-10% of HIV transmission cases and 12.5% of hepatitis infections (Bartonjo et al., 2019). It is worth noting that contaminated blood transfusions are responsible for as many as 16 million new cases of Hepatitis B infections and 5 million new cases of Hepatitis C infections. (Zaheer & Waheed, 2014).

In Africa, the prevalence of TTIs varies by country and region. A research study conducted in Nigeria focused on prospective blood donors to examine the prevalence of specific transfusion-transmissible infections (TTIs)



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discovered an overall prevalence of 14.96%, with HBV, HCV, HIV and being 4.1%, 3.6% 4.2 and 3.1%respectively (Okoroiwu et al., 2018).

A separate study in Ethiopia found a total seroprevalence rate of 6.25% among blood donors, with specific rates for HBV, HCV, HIV, and syphilis of 4.2%, 0%, 0.26%, and 1.82%, respectively (Kebede et al., 2020).

The risk factors that contribute to TTI transmission in Africa include inadequate screening procedures for blood donors, a shortage of safe blood products, and poor infection control practices in healthcare settings. Several measures are being taken to prevent the transmission of TTIs in Africa. One of the most crucial processes is the establishment of national blood transfusion services and blood donor recruitment programs.

TTI is an ongoing problem in Ghana, particularly among blood donors. In urban areas, the frequency of Hepatitis B infection between blood donors varies widely. from 9.6 % to 12.0 %, while in rural areas, it can reach 21 %. Among blood donors, the seroprevalence of Hepatitis C has been documented to range between 1.3 percent and 8.4 percent, while the prevalence of HIV ranges from 1.5 percent to 3.8 percent. (Arshad et al., 2016; Walana et al., 2014).

The prevalence of transfusion-transmissible infections (TTIs) in Ghana has been recorded and document in several studies. A study conducted by Allain et al. (2010) found that the overall prevalence of TTIs in blood donors in Ghana was 6.2%. The most common infections found were hepatitis B virus (HBV) (4.4%) and syphilis (1.3%). A different investigation, conducted by Owusu-Ofori et al. (2016), discovered a prevalence of 6.7% for HBV, 2.2% for hepatitis C virus (HCV), and 0.3% for human immunodeficiency virus (HIV) among



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blood donors in Ghana's Ashanti area. The level of TT prevalence in these studies is consistent with that reported above for the wider African region.

**Asymptomatic Infections Among Donors**

The majority of infections are asymptomatic and might linger weeks or months. Around 170 million people are infected with the hepatitis C virus (HCV), more than 350 million with the hepatitis B virus (HBV), and 38 million with the human immunodeficiency virus (HIV). Lokpo et al. (2017), in a retrospective study, described a high asymptomatic blood-borne pathogen burden among the adult population in Ghana. This population were most vulnerable to HBV, HCV, and Syphilis infections.

Asymptomatic infections (AIs) among donors are a threat to blood transfusion practices are of great public health importance globally (Arshad et al., 2016). Asymptomatic infection is a challenge for blood bank services because of the occurrence of the risks it poses to blood recipients (Lima et al., 2018). As recommended by the WHO, donor samples must be screened to avoid transmission of infectious pathogens to recipient (WHO, 2002). There have been several instances confirming asymptomatic infections as parasites and bacteria have been identified in donors' blood (Dobroszycki et al., 1999; Lindblade et al., 2013).

Asymptomatic parasitemia tends to occur regardless of age. Infections that are asymptomatic might persist for a long time (Ashley & White, 2014). *P. falciparum* infections can linger up to 13 months according to reports in the literature (Lindblade et al., 2013). Using an all-age cohort in Ghana — an area with significant *P. falciparum* transmission – a statistical modelling method



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paired with very sensitive genetic tools found that untreated asymptomatic infections had a mean duration of 194 days (95 % CI: 191–196) (Lindblade et al., 2013).

## Parasites Associated with Blood Transfusion

Parasites predominantly associated with blood transfusions include *Babesia spp., Leishmania* spp., *T. gondii and P. falciparum* can cause serious illness (Amoo et al., 2020; Transfusion-Transmitted Parasitic Infections, 2010).

### *Babesia* spp.

Babesiosis is a parasitic infection transmitted by *Babesia* parasites.

It's a zoonotic disease which is transmitted to humans through the bite of ticks belonging to the Ixodidae family, which is known for spreading the infection from animals to humans. This parasitic organism infects individuals and resides within red blood cells, causing symptoms that resemble those of malaria (Editor & Bronze, 2021; Zhou et al., 2014). Onyiche et al (2021) found that zoonosis spread of babesiosis has been found in numerous regions of Europe, Asia, Africa, Canada and South America (Moritz et al., 2016; Tonnetti, O'Brien, et al., 2019; Tonnetti, Townsend, et al., 2019; E. Vannier & Krause, 2012). Babesiosis is unaffected by gender or race. Although babesiosis can affect people of any age, clinically ill patients with intact spleens are often 50 years or older, indicating that age plays a role in illness severity (Hildebrandt et al., 2021; Vannier & Krause, 2009).

There have been many documented incidences of *Babesia* infection through blood donations from donors who lived in or travelled to an endemic region (Leiby, 2011; Van & Civen, 2009). Transfusion-associated *Babesia*



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illness appears to have a 6-to-9-week incubation period. In endemic locations, the chance of getting babesiosis from a unit of packed RBCs is 1 in 600-1800. People who receive blood products are frequently immunocompromised or possess preexisting medical issues. The implications of transfusion-transmitted babesiosis are frequently serious with approximately one-fifth of cases resulting in mortality (Gubernot et al., 2009; Herwaldt et al., 2011; Tonnetti et al., 2009). *Babesia* and *Plasmodium spp*. possess similar clinical and diagnostic features which may contribute to underreporting and/or misinterpretation, especially in locations where both diseases are widespread (Zhou et al., 2013).

Infections with *Plasmodium* and *Babesia* are known to cause substantial morbidity in equally people and animals (Djokic et al., 2021; Zhou et al., 2013). Both organisms have evolutionary relationships and have similar life cycles and immunological responses, resulting in illness presentations that are similar according to Frolich et al. (2012). When bitten by nymph or adult ticks, humans become unintentional *Babesia* hosts Ticks transfer *Babesia* organisms from a vertebrate reservoir to humans (humans are often dead-end hosts), resulting in human babesiosis (Vannier et al., 2015; Zhou et al., 2014). *Babesia microti*, a common mouse pathogen, causes the majority of infections. *B. duncani, B. divergens, B. venatorum,* and *B. crassa* occur among the other species reported to infect people.

The infection of *Babesia spp.* can cause fever, hemolytic anaemia, and hemoglobinuria. RBC fragmentation, like malaria, can produce capillary occlusion or microvascular stasis, which could explain the involvement of the liver, spleen, kidneys, and central nervous system (Van & Civen, 2009; Zimmer



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& Simonsen, 2022). Increased cytoadherence of infected RBCs has been established in animal experiments to be a possible cause of vascular obstructions (Vannier et al., 2015).

The reticuloendothelial system (RES) in the spleen removes injured or damaged RBC fragments during malaria. Hemolytic anaemia occurs when RBCs are destroyed and the majority of transfusion-related cases were involved red blood cell components (Herwaldt et al., 2011). Though the origin is unknown, the quantity of hemolysis seems to be unrelated to the intensity of parasitemia (Hildebrandt et al., 2021). However, most *Babesia* infections are asymptomatic in healthy people. Patients who are immunocompromised are the most seriously affected (Vannier et al., 2015; Vannier & Krause, 2009). Treatment is not always necessary for asymptomatic individuals and the choice to treat should be made on an individual basis. Patients should also be told to avoid tick bites and not donate blood for at least two years after receiving a positive *Babesia* nucleic acid test (Bloch et al., 2021).

Diagnosing babesiosis can be challenging. Patients with babesiosis have few, if any, localizing indications that imply the disease, even though the index of suspicion should be high in locations where *Babesia* infection is widespread. The degree of parasitemia, as well as the knowledge and experience of laboratory professionals, all play a role in the confirmation of the diagnosis (Editor & Bronze, 2021). Another reason why diagnosing babesiosis is challenging is because its lifecycle is similar to that of malaria (Acosta et al., 2013). Unlike malarial Plasmodia, which is spread by female anopheles mosquitoes, *Babesia spp*. is spread by ticks. Babesia and *Plasmodium* have similar lifecycles, including asexual reproduction in vertebrate host



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erythrocytes and sexual reproduction in the midgut lumen of the arthropod vector serving as the ultimate host (Florin-Christensen & Schnittger, 2009). Babesia exhibits significant differences in their intraerythrocytic cycles when compared to Plasmodia which causes malaria. These differences include the absence of an intraerythrocytic life cycle, the absence of schizogony, and distinct multiplication processes.(Sevilla et al., 2018).

***Leishmania* spp.**

Leishmaniasis is an infectious disease that is caused by an intracellular protozoan parasite from the *Leishmania donovani* complex (Arenas et al., 2017). It is a substantial infectious disease that primarily impacts impoverished regions and vulnerable populations around the world. It is a parasitic disease caused by intracellular protozoan parasites belonging to the genus *Leishmania* and transmitted by tropical vectors. (Arenas et al., 2017; Rodrigues et al., 2016; Terefe et al., 2015).

The Leishmanin Skin Test (LST) is employed for identifying exposure to and immunity against the Leishmania parasite. The LST has been an essential tool in epidemiological research, aiding in the determination of the prevalence and distribution of Leishmania infections (Carstens-Kass et al., 2021).

The initial instances of leishmaniasis in Ghana were documented in the Ho municipality of the Volta region, where the identification of Leishmania amastigotes was conducted on skin lesion biopsy samples obtained from patients. Microscopic examination was employed to examine these samples (Kweku et al., 2011). Another research on Leishmania exposure was conducted in the Oti Region, where 3,071 individuals from three communities, namely Ashiabre, Keri, and Sibi Hilltop, underwent the Leishmanin Skin Test (LST).



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The findings demonstrated an overall prevalence of Leishmania infection exposure at 41.8%. Specifically, the community prevalence rates were determined to be 39.4% for Ashiabre, 55.1% for Keri, and 34.2% for Sibi Hilltop, respectively (Akuffo et al., 2021).

Accurate diagnosis of the specific species involved in a Leishmania infection is crucial due to the presence of over 20 species within the genus. Consequently, the Leishmanin Skin Test (LST) continues to be of paramount importance in understanding the epidemiology of Leishmania infections and evaluating the efficacy of control measures (Georgiadou et al., 2015).

The transmission of the disease occurs through the bite of female sandflies belonging to the *Phlebotomus* or *Lutzomyia* species, which become infected with the pathogen (Arenas et al., 2017). Two main categories of leishmaniasis occur, depending on where the parasite is found in mammalian tissues: visceral. and cutaneous. Cutaneous leishmaniasis causes skin lesions, and it is the most frequent. Visceral leishmaniasis commonly affects various internal organs, such as the spleen, liver, and bone marrow (Arenas et al., 2017; Spickler, 2017). If left untreated, can be fatal (Hajj et al., 2018).

During the life cycle of *Leishmania*, a parasitic flagellated form called promastigote is transmitted by sand flies to various mammalian hosts, including humans. Upon entering the bloodstream of the host, the promastigotes undergo a transformation and become amastigotes. (Scott & Novais, 2016) Consequently, these amastigotes can infect fresh phagocytic cells, thereby successfully establishing an infection in the recipients of the infected blood. Upon the presence of promastigotes in the bloodstream, the immune response is triggered, leading to the production of proinflammatory cytokines that



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activate additional immune cells. Consequently, the infected host cells generate elevated levels of cytokines, reactive nitrogen species, and reactive oxygen species (Scott & Novais, 2016).

Co-infections of malaria and leishmaniasis are widespread in African nations where both diseases are endemic (van den Bogaart et al., 2012). Due to clinical overlap, malaria and other febrile splenomegalies are frequently included in the differential diagnosis of leishmaniasis. Multiple studies have emphasized the significance of conducting malaria screening among individuals infected with Leishmania and residing in regions where malaria is prevalent, with particular attention to patients under the age of five (Ferede et al., 2017). Leishmaniasis and malaria are recognized as two of the most critical diseases on the Tropical Disease Research list compiled by the World Health Organization. Each year, approximately 2 million new cases of Leishmaniasis are diagnosed globally (Arenas et al., 2017).

Men are at higher risk of contracting infection (Altamimi et al., 2020; Bunders & Altfeld, 2020; Klein, 2000; Migliore et al., 2021). Contrary to this Ferede et al. (2017) reported that males were no more likely to contract Leishmaniasis or malaria (Bucheton et al., 2002; van den Bogaart et al., 2012). It is postulated that because men are more involved in outdoor activities, they are subject to infectious mosquito and sandfly bites. On the other hand, women are predominantly at home and so protected from bites.

***Toxoplasma gondii***

Toxoplasmosis is a major zoonotic illness which caused by *Toxoplasma gondii*, a protozoan parasite that infects nearly all warm-blooded animals, as well as man (Gupta et al., 2012; Portes et al., 2020). One-third of the population



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of the globe is affected by the disease, making it one of the most prevalent parasitic infections worldwide toxoplasmosis (Dubey & Jones, 2008; Tenter et al., 2000). Toxoplasmosis is an illness that can cause serious problems, including death in immune-compromised people (Robert-Gangneux & Dion, 2020; Zhou et al., 2014).

Numerous investigations have found that *T. gondii* infection has a negative impact during pregnancy. This is because the developing fetus is affected. This disease have been associated with complications including chorioretinitis, hydrocephalus, stillbirth, and mortality. (Agordzo et al., 2020; Ayi et al., 2016; McAuley, 2014). Therefore, it is important for pregnant women to take measures to reduce their risk of *T. gondii* infection and for healthcare professionals to provide appropriate screening and treatment when necessary. Persons with acute infection or reactivation of latent toxoplasmosis may experience encephalitis, brain abscess, and myocarditis as a consequence (Liu et al., 2015).

*T. gondii* has the potential to be transmitted through the process of blood transfusion and organ transplant, according to many investigations (Alvarado-Esquivel et al., 2018; Ayi et al., 2010; Karimi et al., 2014). The primary methods of transmission involve the ingestion of water contaminated with oocysts, consuming raw or undercooked meat containing tissue cysts, and transmission from mother to child during pregnancy (congenital transmission). Toxoplasma gondii, a protozoan parasite, is responsible for causing toxoplasmosis in humans and various animal species by following a complex life cycle. Its life cycle involves both definitive and intermediate hosts, with cats being the definitive host. The parasite undergoes sexual reproduction within the small intestine of



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felines, resulting in the production of oocysts that are excreted in the feces. Intermediate hosts, such as rodents, birds, or humans, can become infected by taking in oocysts through contaminated food, water, soil, or surfaces. Once inside the host cells, the parasite replicates asexually to produce tachyzoites, which can spread throughout the body and cause tissue damage. Eventually, the tachyzoites transform into bradyzoites and form cysts, usually in the brain and muscle tissues. The intermediate host can be ingested by a cat, and the cysts rupture in the small intestine to release bradyzoites that reproduce sexually and produce oocysts, which are shed in the faeces. Humans can acquire the infection by consuming contaminated meat, water, or soil, or by coming into contact with cat feces.

Toxoplasmosis is usually asymptomatic in immune-competent people, but it can be fatal in immune-compromised people such as transplant recipients, HIV-positive people, and cancer patients (Zhou et al., 2013).

The risk of transfusion-transmitted toxoplasmosis persists for patients who receive blood transfusions, especially those with compromised immune systems, highlighting the ongoing importance of vigilance in blood screening and donor selection. (Jafari Modrek et al., 2014; Siransy et al., 2016). Given that *T. gondii* infection typically persists throughout a person's lifetime, it is noteworthy that the majority of infected individuals do not exhibit any symptoms, blood donors must be tested for toxoplasmosis (Abu-Madi et al., 2010; Flegr et al., 2014).

***Plasmodium falciparum***

*Plasmodium falciparum, P. vivax, P. ovale, P. malariae,* and *P. knowlesi* are the species that cause human malaria (Verra et al., 2018b). *P. falciparum* is



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usually spread when an infected female anopheles mosquito bite a person during its blood meal (Cator et al., 2014; Cox, 2010).

Transmission of *P. falciparum* through transfusion of contaminated donor blood is thought to be a likely mode of infection (Antwi-Baffour et al., 2019; Faruk et al., 2017). To ensure safety, blood banks in regions where malaria is prevalent have implemented mandatory measures. This requires screening of blood donors for the presence of *P. falciparum*, as a preventive measure. This parasite can remain viable for a period of up to 14 days when stored under refrigeration at a temperature of 4 ° The freezing process of donor blood infected *with P. falciparum* has not been observed to effectively eliminate the presence of the parasite, indicating that the unit of blood may not achieve sterility through freezing alone (Aninagyei et al., 2018; Chattopadhyay et al., 2011; Seed et al., 2005).

A research study conducted by Antwi-Baffour et al. (2017) revealed that the survival of Plasmodium spp., when stored at a temperature of −4°C, extended up to 18 days in both whole blood and plasma donor units. Furthermore, detectable parasites were found to persist for up to 28 days following the freezing process.(Antwi-Baffour et al., 2017).

Furthermore, under normal storage circumstances, red blood cells (RBCs) have a lifespan of about 120 days while circulating in the body before being broken down by the hematopoietic system. However, when RBCs become infected with *Plasmodium* spp., the potential for survival of the cells is diminished due to parasite damage inside the RBCs. The level of RBC destruction is determined by the individual species of *Plasmodium* and, as a result, influences the overall RBC profile (Paul et al., 2015). Adherence to the



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World Health Organization's (WHO) recommendations regarding the screening of all donor blood for infectious pathogens is not consistently followed. This may explain the likelihood of transfusion-transmitted malaria.

The morphology of the *Plasmodium* species can typically be recognized. The high amount of parasitemia and the banana shape of *P. falciparum's* gametocytes separate it from the other plasmodia.

The incubation period for *Plasmodium* species varies. *P. falciparum* infection normally develops after one month of exposure, implying that antimalarial treatment should be continued for four weeks after returning from an endemic location to a low (Orish et al., 2016).

In rare cases, *P. falciparum* has been observed to cause initial infection up to a year later. On the other hand, *P. vivax and P. ovale* infections may manifest weeks to months after the primary infection. These two species have a dormant form called hypnozoite, which can persist in the liver for an extended period before reactivating and causing a relapse of the original infection. The hypnozoite form is absent if transmitted by transfusion (Voorberg-van der Wel et al., 2021).

Malaria transmission is primarily observed in tropical and subtropical regions. around the world. Studies have identified certain regions as having low malaria transmission. These areas often include countries or specific regions within countries where the malaria burden is relatively low. For example, some European countries have achieved malaria elimination and are now considered low transmission areas. Studies in low transmission areas have focused on surveillance, prevention of reintroduction, and effective case management to maintain the low transmission status (Cambodia, Thailand, Haiti, Solomon



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Islands and Sri Lanka). Moderate malaria transmission areas are characterized by an intermediate level of malaria incidence. These regions typically experience a consistent number of malaria cases throughout the year, but the disease burden is not as high as in high transmission areas (Uganda, Zambia, Brazil, Senegal and Congo). The prevalence of asymptomatic malaria infections falls within the range of 3.0% to 20%. (Hsiang et al., 2010; Koukouikila-Koussounda et al., 2012; Ladeia-Andrade et al., 2009; Stresman et al., 2010; Vafa et al., 2008) and High transmission areas are known for their intense malaria transmission and significant disease burden. These regions, primarily found in sub-Saharan Africa, have the highest number of malaria cases and related deaths (Tanzania, Gabon, Ghana, São Tome and Kenya) with asymptomatic infections above 20% (Bereczky et al., 2004; Crookston et al., 2010; Dal-Bianco et al., 2007; Gahutu et al., 2011; Heinemann et al., 2020; Lindblade et al., 2013; Pinto et al., 2000).

Certain regions in Ghana have been identified as having low transmission to moderate areas (Abukari et al., 2019; Atelu et al., 2016). These areas may include parts of southern Ghana, urban centers, and areas with effective malaria control interventions in place. However those in the northern and central parts experience high malaria transmission (Owusu-Agyei et al., 2002; Tiedje et al., 2017).

Asymptomatic malaria is widespread in Ghana, particularly in the forest zone, and especially around the season of rainfall, when malaria transmission is at its peak. The forest zone encompasses areas with dense vegetation, abundant water bodies, and favorable conditions for the proliferation of malaria vectors. Malaria transmission is sustained throughout the year but exhibits seasonal



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variations, with a peak during the rainy season (Browne et al., 2000; Heinemann et al., 2020).The rainy season plays a substantial role in breeding of the mosquitoes leading to their transmission.

The malaria parasite undergoes a life cycle that involves two hosts: humans and female Anopheles mosquitoes. The female Anopheles mosquito bites an infected human and injects sporozoites into their bloodstream (Dover & Schultz, 1971; Verra et al., 2018b). Upon infection, the sporozoites of the malaria parasite multiply and release merozoites, which invade and infect red blood cells. The replication of the parasite within the bloodstream leads to the manifestation of malaria symptoms.

Sporozoites invade liver cells, forming the liver schizont. The sporozoites reproduce and produce merozoites, which infect red blood cells. Malaria symptoms are caused by the parasite multiplying in the blood. Some parasites undergo development into sexual stages called gametocytes, which are acquired by mosquitoes when they feed on an infected individual. Within the mosquito, the gametocytes differentiate into male and female gametes. These gametes then combine, forming a zygote that subsequently transforms into a mobile ookinete. After the ookinete penetrates the wall of the mosquito's midgut, it develops into an oocyst. Inside the oocyst, sporozoites are produced. These sporozoites then migrate to the salivary glands of the mosquito. When the mosquito subsequently bites another human, the sporozoites are injected into the bloodstream, thus perpetuating the malaria cycle. In the case of *P. vivax and P. ovale*, the manifestation of symptoms may occur several weeks to months after the initial infection. These two species possess a dormant form known as hypnozoites, which can persist in the liver for an extended period before



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reactivating and triggering a recurrence of the infection subsequent to the initial episode. The hypnozoite form is absent if transmitted by transfusion (Voorberg-van der Wel et al., 2021).

## Transfusion Transmissible Malaria (TTM) among Blood Donors

Transfusion-transmitted malaria (TTM) is the accidental transfer of the malaria *Plasmodium* parasites through blood transfusion of either whole blood or component of blood to recipient (Pulvirenti et al., 2021). TTM is an important public health concern worldwide, particularly in malaria endemic areas. However, this can pose a challenge to blood transfusion services in non-endemic areas, where the prevalence of malaria is low, and screening and testing may not be routine.

Several studies have investigated the prevalence of TTM in non-endemic areas. A study in Italy reported that the prevalence of TTM amongst blood donors was low, at 0.5 cases per 10,000 donors, nevertheless, the concern regarding the risk of malaria has arisen due to the growing population of immigrants originating from malaria-endemic regions (Scaramozzino et al., 2017). Another research in Japan found that the prevalence of TTM was even much low at 0.06 cases per 10,000 donors. Screening methods employed were deemed effective in detecting infected donors.

Even though TTM is expected to be low in non-endemic countries. a study conducted in the United States found that TTM was higher in donors who had recently moved to endemic areas, particularly in sub-Saharan Africa (Niederhauser & Galel, 2022). Another study conducted in Australia found that the risk of TTM was higher in donors who had immigrated from malaria-



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endemic regions and in donors who had a history of travel to these regions (Polizzotto et al., 2013).

Efforts to reduce the incidence of TTM in non-endemic areas include improving screening and testing methods for blood donors and promoting awareness of the risk of TTM among healthcare workers and the public.

In Africa, several studies have investigated the prevalence of TTM in different regions. One study which was performed in Nigeria found that the prevalence of TTM was 2.7%, with the majority of cases caused by *Plasmodium falciparum* (Oladeinde et al., 2014). Other regions have reported a relatively low prevalence of TTM. A study performed in Tanzania found that the prevalence of TTM was 0. 4% and that most cases were asymptomatic (Sundelin et al., 2019). Other studies have investigated the risk factors for transfusion-transmitted malaria (TTM). A study in Uganda found that, the risk of TTM was higher in donors who had recently traveled to malaria-endemic areas and had a higher parasite density (Batista et al., 2019).

The reinfection of new clones of the malaria parasites in asymptomatic blood donors can cause infection in recipients. This is due to the increase in parasite density in the donor. This may cause the clinical symptoms related to malaria, such as fever, chills, fatigue, and other manifestations of the disease in the recipient. This phenomenon is particularly relevant in areas where multiple strains of the malaria parasite coexist, leading to a higher risk of reinfection with genetically distinct clones.

According to Kun et al. (2002), It has been hypothesized that the occurrence of symptoms and an increase in parasite density could result from reinfection with new strains of malaria parasites, to which an individual has not



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been previously exposed. The study suggests that when an individual encounters a new strain or clone of the malaria parasite, the immune system may not have developed sufficient immunity or defense mechanisms against it.

TTM can cause serious illnesses and fatalities, especially in susceptible groups such as children and pregnant women. Several studies have investigated the prevalence of TTM among blood donors in Ghana. Research conducted in Ashanti region of Ghana discovered a high prevalence (4.3%) of TTM within blood donors and that a significant proportion of the infected donors were asymptomatic (Nsobya et al., 2004); Asare et al., 2021).

TTM risk factors in Ghana include a high prevalence of malaria in the population as a whole and poor screening and testing of blood donors, and inadequate resources for malaria control programs. Efforts to reduce the incidence of TTM in Ghana is the same as that for non-endemic countries, that is to improve screening and testing methods for blood donors and promote awareness of the risk of TTM.

Several studies have investigated the effectiveness of different screening methods for detecting TTM among blood donors in Ghana. A study conducted in Kumasi found that the use of rapid diagnostic tests (RDTs) was an effective method for detecting asymptomatic malaria infections among blood donors (Opoku Afriyie et al., 2023). Another study discovered that RDTs based on malaria antigen (whole blood) are as specific as traditional microscopy and appear to be more sensitive than microscopy (Azikiwe et al., 2012).



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**Prevalence of asymptomatic *P. falciparum* infections among blood donors**

Infection with *Plasmodium* sp. is prevalent among blood donors and donor blood units, particularly among those from malaria-endemic areas (Antwi-Baffour et al., 2019; Murphy et al., 2020; Schindler et al., 2019). While clinical malaria is detected and treated, asymptomatic infections are becoming increasingly crucial in stopping the spread of malaria. *Plasmodium* species have separate locations where they are primarily endemic, while there may be regional similarities in their distribution. Malaria poses a risk to persons living in various regions including Central America, South America, Hispaniola, Sub-Saharan Africa, the Indian subcontinent, Southeast Asia, the Middle East, and Oceania. Sub-Saharan Africa has the highest percentage of *P. falciparum* transmission to tourists from the United States among these areas (Delaney et al., 2016; Franco-Paredes & Santos-Preciado, 2006). Asymptomatic *Plasmodium* infections vary in prevalence from one geographic location to the next. Asymptomatic malaria was reported to be 37.5, 82.2, and 92 % in Brazil, the Solomon Islands, and Cambodia, respectively (Da Silva-Nunes & Ferreira, 2007; I. Harris et al., 2010; Hoyer et al., 2012). Asymptomatic malaria was reported to be present in 10.0 percent of African blood donors in Ghana (Owusu-Ofori et al., 2016). Asymptomatic malaria parasitaemia in blood donors was reported to be 27.54 % (Mogtomo et al., 2009), 65.3 % (Diop et al., 2009), > 30 % (Kinde-Gazard et al., 2000), and 40 % (Oladeinde et al., 2014) in Cameroon, Senegal, Benin, and Nigeria, respectively.

## Screening of Blood Donors for Malaria

*Plasmodium* species identification is critical in malaria-endemic areas where species of the malaria parasite are found. It is used not just to select the



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best therapeutic regimen, but also to facilitate the adoption of highly successful malaria control programs. The incorrect identification of *Plasmodium* species may result in serious public health problems due to ineffective medications, which could lead to recurrence and drug resistance (Tseha, 2021). The techniques listed below are used in Ghana and across the world to identify malaria parasites.

**Malaria Rapid Diagnostic Tests (RDTs)**

The immunochromatography assay, also known as the lateral flow test, is an analytical technique employed in malaria diagnosis that identifies the existence or nonexistence of malaria parasite in blood samples, and the predominant form is the use of a rapid diagnostic test kit called rapid diagnostic tests (RDTs).

Rapid diagnostic tests (RDTs) are critical in the detection of malaria because they identify the existence of malaria parasites in the blood of individuals. RDTs offer an alternative to clinical or microscope-based diagnosis, mainly in regions where trained personnel for microscopy is unavailable in portions of Sub-Saharan Africa (Kozycki et al., 2017). In Africa, rapid diagnostic tests (RDTs) are extensively utilized as the fundamental tool for parasitological diagnosis and confirmation of malaria. In fact, RDTs accounted for 75% of diagnostic testing for suspected malaria in public healthcare facilities in 2017. Malaria rapid diagnostic tests (RDTs) commonly rely on the detection of specific markers produced by the malaria parasite during its erythrocytic cycle. These markers include parasite histidine-rich protein II (HRP2), *Plasmodium* lactate dehydrogenase (pLDH), and p-aldolase. These components are utilized in RDTs as they allow for the accurate identification of



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malaria infection (Benito et al 1994). Up to 100 parasites per microlitre can be detected with RDTs (Moody, 2002). The main limitations of RDTs are false positives, which occur because HRP2 persists in the blood for many days after infection clearance (Berhane et al., 2017), and false negatives, which have recently been shown for HRP2 in field isolates from Eritrea (Berhane et al., 2018). Non-*P falciparum* infections are also thought to be unreliable in these tests (Romay-Barja et al., 2016).

RDTs for *Plasmodium falciparum* are potentially useful tools for parasite-based malaria diagnosis and therapy. RDT was used in a research to analyze the incidence of malaria in high risk foci in Egypt and to investigate the usefulness of quick diagnostic tests in diagnosis to help in malaria control (Kamel et al., 2016). It was stated that rapid diagnostic testing found three positive instances out of 600 study participants. According to another study, rapid diagnostic tests for malaria are easy and effective for implementing control measures in various locations. Another study in Ghana used malaria RDTs and microscopy to collect baseline data on TTM. Antigenemia and parasitemia in *P. falciparum* donors were found to be 12.1 % and 8.4 %, respectively, whereas parasitemia in blood recipients was determined to be 3.2 % (Attoh et al., 2022). Although RDT may be used alone in locations where microscopy is unavailable, it is intended to supplement rather than replace microscopy, which is the gold standard for diagnosis.

**Microscopy**

In malaria-endemic countries, microscopy is usually regarded as the "gold standard" for identifying malaria based on parasite detection. It is widely used in large health clinics and hospitals to accurately diagnose malaria



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infections. The sensitivity of this method typically ranges from 50 to 500 parasites per microliter (μL) (Moody 2002). Whilst microscopy remains the gold standard for malaria detection, the quality of microscopy-based diagnosis is typically insufficient to assure good sensitivity and specificity, significantly compromising medical outcomes and the usage of resources (WHO, 2015). Malaria microscopy allows for detection and identification of several species of malaria-causing parasites (*Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale,* and *Plasmodium knowlesi*), It also identifies other parasite stages, such as gametocytes, and quantifies parasite density in order to monitor therapy effectiveness (Feleke et al., 2017; WHO, 2015). Microscopy is the preferred approach for investigating malaria therapy failures. Giemsa is the recommended dye for malaria microscopy, and diagnosis requires the evaluation of both thin and thick films from the same patient. Light microscopy has historically served as the gold standard against which other diagnostic procedures have been measured.

**Molecular testing**

The WHO advises that nucleic acid amplification assays should be employed exclusively in epidemiological studies and for mapping sub-microscopic infections for molecular determination of malaria. The use of molecular approaches as diagnostic procedures is problematic in Sub-Saharan Africa due to the equipment required, reagent upkeep, and qualified staff needed. In malaria-endemic locations, a sensitive diagnostic technique that is both easy to perform and compatible with blood banks is necessary to detect low parasitaemia associated with asymptomatic malaria infections.



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Polymerase chain reaction (PCR), the most sensitive method, identifies parasitemia at levels as low as 2-5 parasites per liter. However, because it is an expensive and time-consuming operation, it is not suitable for use in the field. Aninagyei et al. (2019) investigated the sensitivity of microscopy, the rapid diagnostic test (RDT), the loop-mediated isothermal amplification (LAMP) assay, and the selective whole genome amplification (sWGA) approach in identifying *P. falciparum*. PCR-based molecular tests were shown to be the most accurate method for identifying malaria parasites (Amoah et al., 2019; Aninagyei, Tetteh, et al., 2020; Tetteh et al., 2023)

**Selective Whole Genome Amplification (SWGA)**

The isolation and amplification of DNA sequences is a basic prerequisite for molecular biology. Over this past decade, immense advances in whole genome sequencing technology have contributed to a better understanding of parasite population structure, intra- and inter-population genomic diversity, alongside the identification of genomic regions under selective pressure, such as drug resistance associated genes. Selective whole genome amplification (SWGA) is a technology that was designed to amplify just a specific target genome of pathogens from complete genomic extracts generated from target and host DNA. Thus, SWGA is similar to Polymerase chain reaction (PCR) in that it amplifies a specific section of the DNA in a sample. Amplifying a full genome by SWGA varies from amplifying a specific gene region by PCR in terms of primer selection and amplification method. PCR is also used to amplify specific sequences; however, it can introduce sequence mistakes and is restricted to the amplification of short DNA segments.



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In SWGA the primers used bind to DNA sequence motifs that are frequent in the target genome but uncommon in the genomes of other species in the environmental sample. The SWGA approach uses the inherent variances in the frequency of sequence motifs among species to develop a set of pathogen-specific primers that are unique to a target species (Dean et al., 2001, 2002). DNA amplification for the target genome genomes is carried out using the highly processive, strand-displacing phi29 DNA polymerase and the set of pathogen-specific primers that target short (6 to 12 nucleotide) motifs that are prevalent in the pathogen genome but uncommon in the host genome (Fuller et al., 2009).

The strand displacement function of phi29 leads to preferential amplification of genomic areas where primers bind often, resulting in preferential amplification of genomes with frequent primer-binding sites. The technique of selective whole genome amplification (SWGA) has been utilized to raise sub-microscopic DNA levels of *Plasmodium* parasites in infected blood samples (Larremore et al., 2015; Sundararaman et al., 2016) and *P. falciparum* genomes from dried blood spots (Guggisberg et al., 2016).

In a study to assess the sensitivities of microscopy, rapid diagnostic test (RDT), loop-mediated isothermal amplification (LAMP) assay and selective whole genome amplification (SWGA) technique among asymptomatic blood donors infected in detecting *P. falciparum* infections, it was reported that PCR based molecular assays remain the most reliable technique for detecting malaria parasites. This was as a result of SWGA being detected in most infections followed by the two LAMP assays (Aninagyei et al., 2019).



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Whole-genome sequencing (WGS) of *Plasmodium* parasites from clinical samples has yielded crucial insights into the biology, epidemiology, and drug resistance mechanisms of malaria (Aninagyei, Duedu, et al., 2020; Borrmann et al., 2013; Manske et al., 2012; Miotto et al., 2013; Winter et al., 2015). Aninagyei et al., (2020) conducted the first study in Ghana to employ Whole-genome sequencing (WGS) to identify the incidence of possible anti-malaria drug resistance indicators in blood donors. The aim of the study was to assess the potential risk associated with the transmission of suspected anti-malaria medication resistance biomarkers through blood transfusion. The findings indicated that the presence of certain mutations, such as the C580Y Kelch 13 mutation, the YFN triple mutation haplotype in the *Pfmdr1* gene, and the quadruple mutations in the *Pfdhfr/Pfdhps* genes (*Pfdhfr*: N51I, C59R, S108N resulting in IRNI haplotype and *Pfdhps*: A437G, K540E resulting in AGESS haplotype), could potentially contribute to treatment failure with artemisinin-based combination therapy (ACT) and reduced efficacy of sulphadoxine-pyrimethamine (SP) in individuals receiving transfusions of blood infected with malaria.

## Transmission of anti-malaria resistant strains

The development and distribution of anti-malarial drug-resistant strains of *Plasmodium* is a major concern, as it limits the effectiveness of current control measures. The transmission of anti-malarial drug-resistant strains of *Plasmodium* is complex and influenced by various factors, including the parasite's biology, human behavior, and efficiency of surveillance measures. A key factor in the transmission of anti-malarial drug-resistant strains is the capability of the parasite to persevere in the human host, as well as its ability to



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infect mosquitoes, which serve as vectors for transmission to other individuals (Nair et al., 2016). The Transmission of drug resistant *Plasmodium falciparum* is a significant concern, as it can reduce the efficacy of antimalarial treatments and impact to the extent of malaria spread.

The capacity of *Plasmodium falciparum* to elude the host immune system and produce resistance to anti-malarial drugs is a complex phenomenon that has been attributed to its genetic diversity This relationship has been widely investigated and has imperative consequences for the development of effective malaria interventions. Specifically, the genetic heterogeneity of *P. falciparum* has been shown to play an important role in its pathogenesis, and its ability to adapt to changing environmental pressures, such as exposure to anti-malarial drugs (Duah et al., 2016; Manske et al., 2012b; Sondo et al., 2019; Ekland & Fidock, 2007).

***P. falciparum* Drug Resistance Markers**

Managing malaria is a complex task because there has been a rise in drug resistance to various anti-malarial medications, such as chloroquine (CQ), sulfadoxine-pyrimethamine, mefloquine, and more recently, artemisinin (Ndong Ngomo et al., 2023; Plowe, 2022). There are several known drug resistance markers associated with *P. falciparum.* These genetic mutations are as a result of Single nucleotide polymorphisms (SNP) associated with anti-malaria drug resistance. This review focuses on *Plasmodium falciparum* Chloroquine resistance marker (*Pfcrt*), *P. falciparum* multidrug resistance-1 *(Pfmdr1)*, *P. falciparum* dihydrofolate reductase *(Pfdhfr), P. falciparum* dihydropteroate synthase *(Pfdhps)* and *Kelch 13* propeller genes(K13).



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***P. falciparum* chloroquine resistance transporter gene (*Pfcrt*)**

Chloroquine was formerly used extensively as the first line treatment for uncomplicated *Plasmodium falciparum* malaria. Chloroquine resistance in *P. falciparum* is linked to mutations in the chloroquine resistance transporter gene (*Pfcrt*) which encodes a transmembrane protein localized to the parasite digestive vacuole membrane occurring in codons 72– 76 (Foguim et al., 2020; Pacheco et al., 2020; Ross et al., 2018). There are three major haplotypes: wild-type CVMNK, mutant CVIET, and SVMNT. CVMNK is primarily based in China. CVIET confers the highest degree of resistance, and it is the most common in Africa. The mutant SVMNT haplotype, is more common in South American isolates and confers a lesser resistance than the CVIET haplotype (Djimdé et al., 2001; Djurković-Djaković et al., 2019; Fidock et al., 2000; Wellems & Plowe, 2001). Also, a single nucleotide polymorphism in the *Pfcrt* gene at position 76 resulting in a change in coding from lysine to threonine K76T has been linked to chloroquine resistance and has been utilized as a genetic marker in epidemiological research to assess chloroquine resistance. Several studies around the globe have reported variations in the K76T mutations. In South-East Asia, notably in Thailand and Cambodia, a higher level of K76T mutation was quite frequent (98.2%) (Srimuang et al., 2016). Similarly, a study in India reported a prevalence (87%) of K76T mutation (Sharma et al., 2011). A slightly lower prevalence was found in other parts of Africa. Zambia (69.1%) and Nigeria (69.6%) had somewhat lower prevalence rates (Mulenga et al., 2021) (Soniran et al., 2017) however the K76T extremely (2.3%) was reported in Mozambique. (H. Gupta et al., 2018). In a study conducted in 2017-2018 in Bo, Sierra Leone, Leski et al., (2022) discovered that



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the prevalence of malaria resistance-associated mutations in *Plasmodium falciparum* was 22% among the investigated samples. The researchers discovered mutations in the *Pfcrt* gene at positions 74, 75, and 76, namely M74I, N75E, and K76T.

In Ghana, the incidence of K76T has been reported in several studies (Afoakwah et al., 2014; Aninagyei, Duedu, et al., 2020). A study conducted ten years after the withdrawal of Chloroquine found that the prevalence of the *Pfcrt* 76T had decreased from 92.7% to 71.8 (Asare et al., 2021). These suggest that the prevalence of *Pfcrt* mutations associated with Chloroquine resistance in Ghanaian *P. falciparum* isolates have decreased over time. However, the *Pfcrt* K76T mutation is still present at a relatively high frequency in Ghanaian *P. falciparum* populations and is associated with reduced susceptibility to Chloroquine and other anti-malarial drugs. In Ghana, Artemisinin-based combination therapy (ACT) has replaced chloroquine as the primary treatment for malaria (Roux et al., 2021). Artemisinin-based combination therapy, which combines an artemisinin derivative with a partner drug, has proven to be highly effective against malaria parasites, including those carrying the K76T mutation. These combinations work by targeting different stages of the malaria parasite's life cycle, ensuring a more comprehensive and potent treatment approach. The possibility of re-introducing chloroquine arises from observations indicating a reversal of chloroquine resistance after a period of withdrawal.

A study in Saudi Arabia, found the *pfcrt* 76T molecular marker for CQ resistance in 66.4% (156/235) of the isolates, while the K76 CQ-sensitive wild type was found in 33.6%. The CVIET triple-allele (56.2%), SVMET double-allele (1.7%), and CVMNT single-allele (8.5%) mutant haplotypes were also



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discovered as well as the CVMNK wild haplotype (33.6%). The data supports the potential re-emergence of CQ-susceptible *P. falciparum* strains in the Jazan region more than a decade after CQ termination (Madkhali et al., 2021). Laufer et al. (2006) conducted a study in Malawi, where chloroquine had been largely abandoned due to resistance. After a decade of chloroquine withdrawal, they found out that the drug exhibited renewed efficacy against malaria parasites.

In Africa, other mutations such as CVIET and CVINT have also been linked to chloroquine drug resistance (Djimde et al., 2001; Chen et al., 2015; Patel et al., 2017; Vinayak et al., 2010; Nsobya et al., 2004). In a study conducted in Niger, a triple mutation of *Pfcrt* CVINT and CVIET was detected for the first time. In 2014, a study conducted in Niger to investigate the prevalence of *Pfcrt* mutations in *Plasmodium falciparum* isolates detected the presence of a triple mutant *Pfcrt* CVINT/CVIET haplotype. This triple mutation may have originated from the neighboring country Burkina Faso, where similar mutations have been reported.

Another mutation conferring resistance to Chloroquine is the N86Y allele of *pfmdr- 1*. There has been a study on the role of *pfmdr-1* mutations, including N86Y, Y184F, S1034C, and D1246Y, in the development of drug resistance in malaria parasites. The study found that the N86Y mutation is strongly associated with resistance to chloroquine as well as the Y184F mutation which is also associated with changes in parasite susceptibility to various drugs, including ACT.

***P. falciparum multidrug resistance 1 gene (Pfmdr1)***

The multi-drug resistance (MDR) of *P. falciparum*, particularly to artemisinin-based combination therapies (ACTs), is a major challenge to



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malaria control and elimination of efforts. *P. falciparum* multi-drug resistance gene 1 *(Pfmdr1)* has been identified as a key molecular marker of MDR in *P. falciparum*(Ward et al., 2022)(Dhorda et al., 2021)(Gil & Krishna, 2017).

The *Pfmdr1* gene, located on chromosome 5 of *P. falciparum*, encodes that a protein plays a critical role in the transporting of drugs across the parasite plasma membrane(Ikegbunam et al., 2019). *Pfmdr1* gene amplification and point mutations have been associated with reduced sensitivity to a range of anti-malarial drugs, including chloroquine, mefloquine, and ACTs. The global distribution of *Pfmdr1* polymorphisms is not uniform, and regional differences have been reported (Wurtz et al., 2014). SNPs at codons 86 (A86Y), 184 (S184F), and 1246 (D1246Y) in the gene have also been implicated in modulating the susceptibility or resistance to a range of antimalarial drugs. A combination of the SNPs, with other genetic variations, can affect the parasite's response to drugs like amodiaquine, lumefantrine, and quinine (Duraisingh et al., 1997; Reed et al., 2000).

Several studies have reported on the prevalence and distribution of *Pfmdr1* gene mutations in different regions of the world. In Southeast Asia, the *Pfmdr1* gene has been extensively studied due to the emergence and spread of artemisinin resistance in the region.

In a study conducted, it was found that the *Pfmdr1* N86Y and Y184F mutations were associated with artemisinin resistance (Li et al., 2014). Another study conducted in Thailand found that the *Pfmdr1* N86Y and Y184F mutations were associated with increased resistance to mefloquine and artemisinin-based combination therapies (Price et al., 2014). However, another study reported contrasting finding that, N86Y increases parasite susceptibility to the partner



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drugs lumefantrine and mefloquine, and to the active artemisinin metabolite dihydroartemisinin as well. (Veiga et al., 2016a; Venkatesan et al., 2014). Southeast Asia has been identified as a hot spot for *Pfmdr1*-mediated MDR, particularly in Cambodia and Thailand. In Cambodia, high levels of *Pfmdr1* gene amplification and point mutations have been associated with treatment failures with ACTs.

The *Plasmodium falciparum* multidrug resistance 1 *(Pfmdr1)* gene is known to play a key role in the resistance of the malaria parasite to a wide range of antimalarial drugs in Africa.

Numerous studies have reported the association between certain alleles and reduced sensitivity of artemisinin-based combination therapies (ACTs) and other anti-malaria monotherapies in Ghana. These alleles are often found in the *dhfr* and *dhps* genes, which are known to confer resistance to sulfadoxine-pyrimethamine (SP) (Abugri et al., 2018).

A study conducted in Ghana found a high prevalence of the *Pfmdr1* N86Y mutation, which is associated with increased resistance to mefloquine and lumefantrine (Abugri et al., 2018). Another study conducted in Senegal found that the *Pfmdr1* N86Y mutation was associated with increased resistance to artemether-lumefantrine (Hastings & Ward, 2005; Diop et al., 2009).

In East Africa, a study conducted in Ethiopia found a high prevalence of the *Pfmdr1* N86Y mutation, which is associated with increased resistance to mefloquine and lumefantrine (Tadesse et al., 2018). Another study conducted in Tanzania found that the *Pfmdr1* N86Y and Y184F mutations were associated with increased resistance to artemether-lumefantrine (Mwaiswelo et al., 2019).



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In addition, several studies have investigated the impact of *Pfmdr1* gene mutations on the efficacy of antimalarial drugs in African populations. A study conducted in Kenya found that the *Pfmdr1* N86Y mutation was associated with the reduced efficacy of mefloquine (Murebwayire et al., 2018). Another study conducted in Malawi found that the *Pfmdr1* N86Y and Y184F mutations were associated with reduced efficacy of lumefantrine (Kublin et al., 2003).

Overall, these studies suggest that *Pfmdr1* gene mutations are widespread and play a significant role in the development of antimalarial drug resistance in *P. falciparum* populations in Africa. Continued surveillance of *Pfmdr1* gene mutations and their association with antimalarial drug resistance is essential for the effective control and elimination of malaria in Africa.

***P. falciparum dihydrofolate reductase (Pfdhfr)***

Another study by Amato et al. (2018) used genomic data from *P. falciparum* isolates collected from 88 countries worldwide to investigate the global distribution of *pfdhfr* mutations. The *P. falciparum* dihydrofolate-reductase *(Pfdhfr)* gene has been extensively studied worldwide. This is due to its role in the development of antifolate drug resistance, such as sulfadoxine-pyrimethamine (SP). This is the only anti-malarial drug formulation approved for use in intermittent preventive treatment in pregnancy (IPTp) and intermittent preventive treatment in infants (IPTp) (Pacheco et al., 2020),

*Pfdhfr* is an essential enzyme in the folic acid pathway which plays a crucial role in the synthesis of DNA and RNA. *Pfdhfr* is the target for several antimalarial drugs, including pyrimethamine and cycloguanil. However, the emergence and spread of drug resistance have limited their efficacy. (Shibeshi et al., 2020)



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Resistance to antimalarial drugs targeting *pfdhfr* has a significant impact on malaria control efforts. A study by (Yan et al. (2021) investigated the impact of pfdhfr drug resistance on the efficacy of SP for intermittent preventive treatment in infants (IPTi) in Tanzania. The study found that the presence of *pfdhfr* mutations was associated with reduced efficacy of SP for IPTi. The study also found that the prevalence of *pfdhfr* mutations was high in areas with high levels of SP use.

There are three types of SP-resistant parasites: "partially resistant," "fully resistant," and "super resistant." The parasites are classed depending on the mix of mutations in the two genes (*dhfr* and *dhps*) that they carry (Naidoo & Roper, 2013).

Point mutations in *Pfdhfr* result in amino acid substitutions that alter the protein structure and reduce the affinity of the enzyme for antimalarial drugs (Yan et al., 2021). The most common mutations associated with resistance include S108N, C59R, and I164L. These mutations lead to weaker drug binding, allowing the parasite to evade the inhibitory effects of pyrimethamine and cycloguanil.(Pacheco et al., 2020). The molecular basis of resistance in *Pfdhfr* is similar to that which was observed in another enzyme of the folic acid pathway, *Pfdhps* (*P. falciparum* dihydropteroate synthase.

These four-point mutations in *Pfdhfr P. falciparum* are responsible for the conversion of dihydrofolate (DHF) to tetrahydrofolate (THF). THF is a crucial cofactor involved in the synthesis of purines and pyrimidines, which are necessary for DNA and RNA production in the parasite.(Abdullah & Karunamoorthi, 2016; Chaianantakul et al., 2013) Thus, inhibition of *Pfdhfr*



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disrupts folate metabolism, leading to impaired nucleic acid synthesis and parasite death.

A study conducted in South-East Asia reported high levels of the *Pfdhfr* triple mutation (N51I, C59R, S108N) in *P. falciparum* isolates, and it was also found to lower the efficacy of SP (Chaianantakul et al., 2013). Another study in Cambodia found that the *Pfdhfr* triple mutation was highly prevalent in isolates from patients with uncomplicated malaria, and also reduced the efficacy of SP (Madkhali et al., 2020).

Study in Uganda, investigated the distribution of *Pfdhfr* and *P. falciparum* dihydropteroate synthase *(Pfdhps)* gene mutations and their association with the efficacy of SP treatment. The study found that the *Pfdhfr* triple mutation was associated with reduced efficacy of SP, while the *Pfdhps* double mutation (A437G and K540E) was associated with high-level resistance to SP (Kateera et al., 2016; Bouyou-Akotet et al., 2015). In Africa, studies have demonstrated high genetic diversity in *Pfdhfr among P. falciparum* isolates. Multiple point mutations in the *Pfdhfr* gene have been identified, including the most commonly reported mutations at codons 51, 59, 108, and 164. Notably, the prevalence of these mutations varies geographically, suggesting distinct patterns of drug resistance in different areas.

***P. falciparum dihydropteroate synthetase (Pfdhps) genes***

The evolution of drug resistance in *P. falciparum* has become a major public health challenge in the management of malaria. One of the mechanisms of resistance is mutations in the dihydropteroate synthetase (*Pfdhp*s) enzyme, encoded by the *Pfdhp*s gene, which is a target of sulfadoxine-pyrimethamine (SP), a widely used antimalarial drug (Delaney et al., 2016 A single amino acid



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residue change at codon 437 of the *Pfdhps* gene is the main mutation linked with sulfadoxine resistance in *Plasmodium falciparum*. The mutation specifically involves alanine (A) to glycine (G) substitution at that codon (Braun et al., 2015). The A437G mutation in the *Pfdhps* gene has previously been observed as a selection by sulfadoxine-pyrimethamine (SP) during intermittent preventive therapy for infants (IPTi). IPTi is a malaria control approach that involves delivering a course of antimalarial medications to newborns in areas with high malaria transmission in order to prevent malaria infection and its accompanying consequences (Rupérez et al., 2016). Sulfadoxine-pyrimethamine (SP) is employed in numerous antimalarial preventative treatments in Africa, particularly IPTp (Intermittent preventative Treatment in Pregnant Women), IPTi (Intermittent Preventive Treatment in Infants), and SMC (Seasonal Malaria Chemoprevention). In locations where *Plasmodium falciparum* resistance to SP is high, the efficacy of SP-based preventive therapies is jeopardized. The frequency of mutations in the dihydropteroate synthase gene (*pfdhps*) can be utilized to track the efficacy of SP (Flegg et al., 2022).

In Southeast Asia, where *P. falciparum* is also prevalent, *Pfdhps* mutations have also been reported. A study conducted in Myanmar showed a high prevalence of the double mutant haplotype (437G+540E) and the triple mutant haplotype (437G+540E+581G) in the *Pfdhps* gene (Zhao et al., 2019). Another study conducted in Saudi Arabia reported that the prevalence of the triple mutant haplotype was significantly high (Madkhali et al., 2020). The emergence of resistance to SP highlights the importance of monitoring the



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prevalence of *Pfdhps* mutations in *P. falciparum* to guide malaria treatment and control strategies.

Antimalarial drugs have been used to control and treat Malaria, but the emergence and spread of drug-resistant parasites have challenged malaria control efforts. Several studies have reported high levels of *Pfdhps* mutations in Africa. A study conducted in Kenya showed a high prevalence of the triple mutant haplotype (437G+540E+581G) in the *Pfdhps* gene, which confers high-level resistance to SP. The study also reported a significant association between the presence of the triple mutant haplotype and treatment failure with SP (Gikunju et al., 2020). Again, a study conducted in Tanzania reported a high prevalence of the triple mutant haplotype in *P. falciparum* isolates collected from patients with clinical malaria.

Overall, the prevalence of *Pfdhps* mutations in Africa is high, and the emergence of new mutations highlights the urgent need for alternative antimalarial drugs and the importance of monitoring the prevalence of *Pfdhps* mutations to guide malaria treatment and control strategies.

In Ghana, several studies have reported high levels of *Pfdhps* mutations in Ghana. A study conducted in 2004 in southern Ghana reported a high prevalence of *Pfdhps* mutations, with the triple mutant haplotype (437G+540E+581G) being the most common (Adegbola *et al.,* 2023). The study also reported a significant association between the presence of *Pfdhps* mutations and treatment failure with SP. Another study conducted in 2014 in northern Ghana reported a high prevalence of the triple mutant haplotype in *P. falciparum* isolates collected from patients with uncomplicated malaria (Madkhali et al., 2020).



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***Kelch 13 (K13)***

Mutations in *K13* remained to be the key predictor of ART resistance (Bwire *et al.,* 2020) and have been associated with resistance to artemisinin-based combination therapies, which are currently the first-line treatment for uncomplicated *P. falciparum* malaria in many countries (Ndwiga *et al.,* 2021). *K13* encodes a protein that plays a role in the regulation of parasite growth and development. The most common *K13* mutations associated with artemisinin resistance are C580Y, R539T, and Y493H; C580Y being the most dominant and stronger molecular marker for artemisinin-based resistance. The emergence and spread of artemisinin-resistant *P. falciparum* strains are a major concern in malaria control efforts. The prevalence of these mutations varies across regions, with high levels reported in South-East Asia and low levels in some African countries (Nydahl *et al.,* 2021). Studies have reported the presence of *K13* mutations in *P. falciparum* isolates collected from different regions of Ghana. A study conducted on the polymorphisms in the Kelch propeller domain of Ghanaian malaria parasites from three different ecological zones at several time periods were assessed. The study reports 16.0% mutants Kelch 13 isolates (Amoah et al 2019).

A study conducted in 2019 *on P. falciparum* isolates collected from three regions in Ghana (Ashanti, Brong-Ahafo, and Upper West) reported the presence of *K13* mutations in 25% of the isolates (Quashie et al.,2019). Another study conducted in 2021 on *P. falciparum* isolates collected from asymptomatic blood donors in Ghana reported the presence of *K13* mutations in 16.6%.% of the isolates (Aninagyei, Duedu, et al., 2020).



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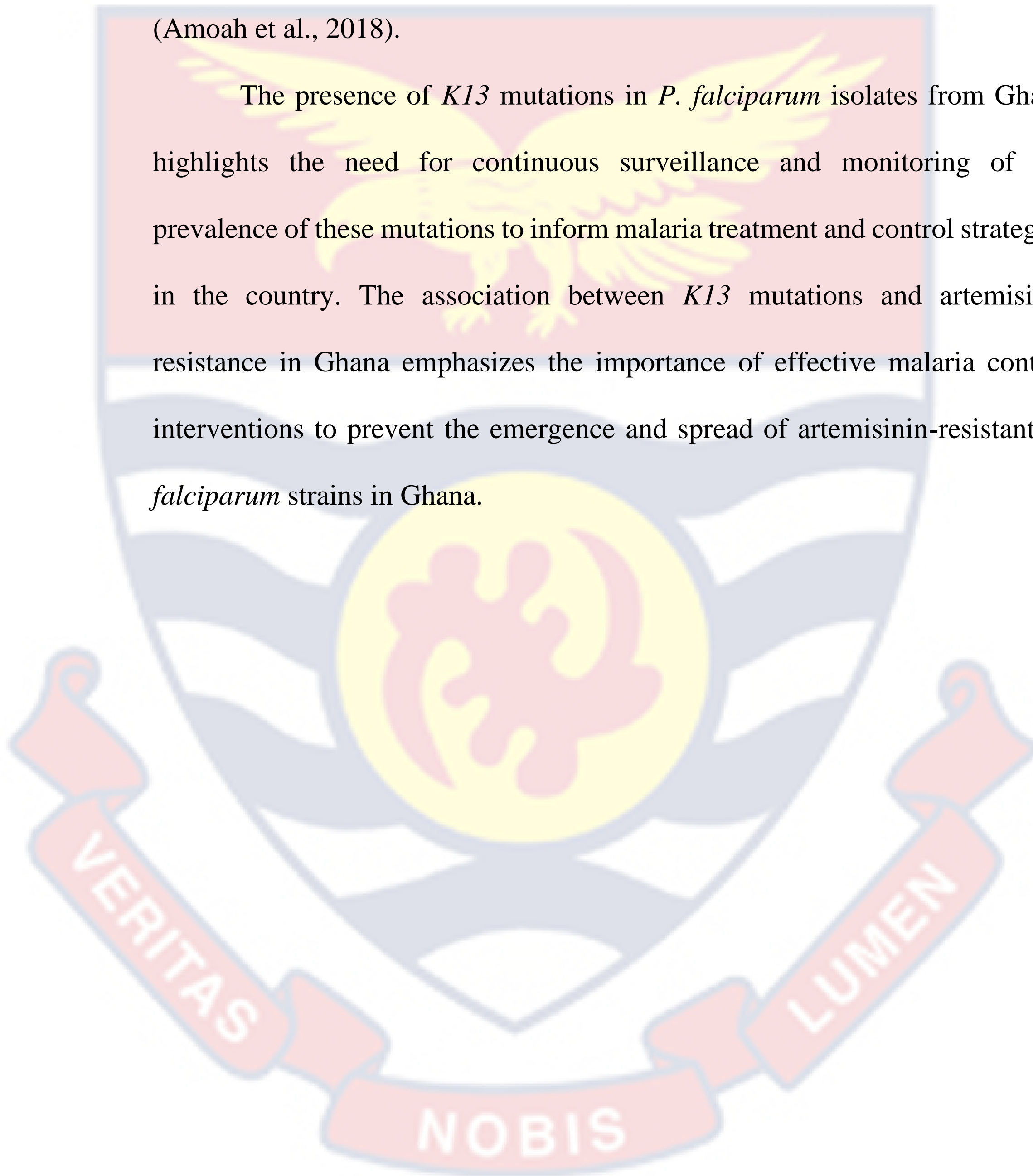
Several studies have also investigated the association between *K13* mutations and artemisinin resistance in Ghana. A study conducted in 2018 in the Ashanti region of Ghana reported an association between the presence of *K13* mutations and delayed parasite clearance following artemether-lumefantrine treatment (Amoah et al., 2018).




The presence of *K13* mutations in *P. falciparum* isolates from Ghana highlights the need for continuous surveillance and monitoring of the prevalence of these mutations to inform malaria treatment and control strategies in the country. The association between *K13* mutations and artemisinin resistance in Ghana emphasizes the importance of effective malaria control interventions to prevent the emergence and spread of artemisinin-resistant *P. falciparum* strains in Ghana.



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# CHAPTER THREE

# RESEARCH METHODS

## Introduction

Blood transfusion is still used routinely in critical care and surgical settings to save millions of lives however it carries a risk of transmitting virulent pathogens and causing immunological reactions. Despite WHO recommendations to screen donor blood for transmissible infections, most malaria-endemic countries do not consistently do so. Blood donors can be asymptomatic consequently; these infections can inadvertently be transmitted to the recipient making recipients more vulnerable to TTIs.

When compatibility testing is done accurately, other possible causes of transfusion reactions may be attributable to TTIs. This emphasizes the importance of accurate compatibility testing but acknowledges that, in some cases, transfusion reactions may be caused by factors such as TTIs, highlighting the need for comprehensive screening and testing procedures in blood donation and transfusion processes.

Infectious pathogens are known to elicit immunological and inflammatory responses in the host. According to several studies, parasitic infections have been implicated in blood transfusion (Aninagyei et al., 2019; Mardani, 2020; Niederhauser & Galel, 2022; Verra et al., 2018a). However, the relationship between parasitic infection exposure and transfusion reactions has not been explored. Therefore, this study aimed to assess the endemicity of *Babesia* spp., *Leishmania* spp., *Toxoplasma gondii,* and *P. falciparum* in the study site and determine how their endemicity could impact on donor seroprevalence as well as their association with acute transfusion reactions. The



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study further sought to determine the transmissibility of *P. falciparum* in haemo-transfusion.

This chapter provides an overview of the different methodologies utilized in the study. Consent was sought from each patient or guardian of the prospective blood recipient. Figure 3 shows the general workflow for the study. Samples used included left-over blood from the donor's blood bag and fresh venous blood from patients who were yet to be transfused. Blood samples were also taken from follow up patients who had received positive donor units and showed positivity. 3 ml of blood samples were drawn from the follow up patients for the study on days 0, 2, 7, 14, 21, 28, and 35 respectively (Fig 3). In situations where the patient was less than 5 years, 1 ml of blood was collected, grouping and cross matching was performed as well as malaria RDT and microscopy. Patients were monitored within 24 hours for possible acute transfusion reactions. At least 3 ml of the remaining blood in the blood bag was drained into a plain tube. Samples were centrifuged and serum kept at -20 $^{o}$C for ELISA screening for *Babesia* spp., *Leishmania* spp., and *T. gondii* IgG antibodies. In addition to this, malaria parasites were detected using both malaria rapid diagnostic test kit (mRDT) and microscopy, in post-transfusion donor blood. A patient that tested negative for malaria by both microscopy and mRDT but received malaria parasite-infected donor blood was followed up to 35 days' post-transfusion (days 2, 7, 14, 21, 28, and 35). In all cases, both mRDT and microscopy was performed. All malaria positive blood samples were sent to WELCOME SANGER for genotyping.



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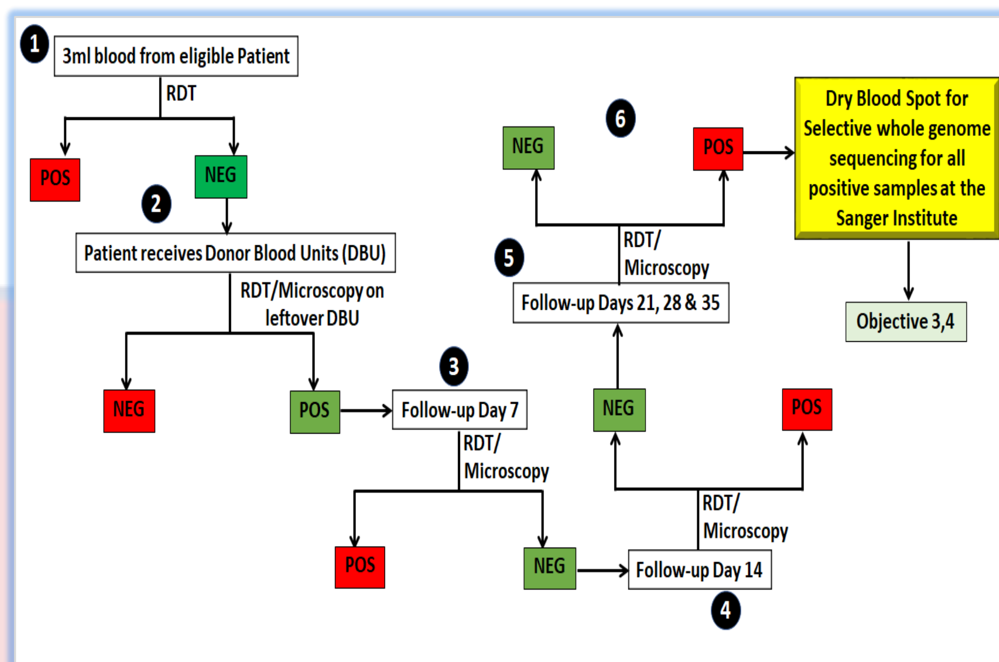


*Figure 3: General workflow for this study*

## Study Design

This was both a cross-sectional and cohort study, with the recruitment of in-patients who had received blood transfusions. The study experimented with the transmissibility of malaria parasites through unintentional receipt of *P. falciparum*-infected donor blood to patients and the clinical sequelae of some selected haemoparasites to patients admitted at the Nsawam Government Hospital (NGH) in the Eastern Region of Ghana. All blood samples were collected by an in-house blood donation team. Most of the blood donors were family replacement donors with few voluntary donors. The majority of the transfused blood was in the form of whole blood while concentrated red cell transfusions were in the minority. Blood donors were screened for hepatitis B virus, hepatitis C virus, HIV, and *Treponema pallidum*. As the practice in Ghana, blood donors and donor blood units were not screened for malaria parasites.



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## Study Area

The study was conducted at the Nsawam Government Hospital (NGH) which is one of the renowned district hospitals in the Eastern Region of Ghana, located in the Akuapim South Municipality. It is on the main railway line connecting Accra and Kumasi, as well as the highway to Kumasi. It shares a boundary with Adoagyirie via the Densu River.

NGH is a 135-bed capacity hospital with 24-h service delivery. The hospital has all the vital clinical units, namely, diagnostic units, wards, theatres, and public health units. The hospital performs an average of 823 blood transfusions per annum. It is noted as one of the best in the area of quality healthcare delivery in the eastern region.

## Study Population

Nsawam has a population of about 44,522 people. In this community, the Akans form the largest ethnic group, followed by the Gas and Ewes. The study population comprised prospective blood recipients who had been hospitalized for more than a day. They included patients from the pediatric obstetrics' and gynaecology unit as well as male and female wards. Females formed the majority.

## Sampling Procedure

### Ethical approval

Ethical approval for the study was granted by the Ghana Health Service Ethics Review Committee (approval ID: GHS-ERC-004/12/19). Written consent was obtained from all participating blood recipients. Informed consent was taken from parents or guardians for children less than five years of age who met the



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inclusion criteria. All laboratory results were communicated to study participants and the appropriate actions taken promptly.

**Donor blood sample size determination**

Due to the unavailability of the specific prevalence of malaria parasites infection rate in the Eastern Region of Ghana, the prevalence was estimated as 50 %. Using the formula, $n = \frac{z^2 p(1-p)}{d^2}$, where n = sample size, p = proportion of blood donors with malaria, 1-p = proportion of blood donors without malaria, z = confidence level at 95 % (standard value of 1.96), d = margin of error at 5 % (standard value of 0.05), the minimum sample size was estimated as 384. A prevalence of 50% in sample size calculation is often done when the true prevalence of a condition or characteristic in the population is unknown or when researchers want to be conservative in their estimates. This choice is made because, in the absence of any prior information, a prevalence of 50% maximizes the required sample size, ensuring that the study is adequately powered to detect differences or associations.

**Inclusion criteria**

Donor blood units included in this study were blood units that were bled at NGH and had been stored at 4 °C for not less than 24 hrs. All donor blood units collected outside NGH were excluded from this study. To be included in this study, a blood recipient or an accompanying relative must give consent. A blood recipient needed to be transfused with more than half of the unit to be qualified to be included in the study. To be qualified to be followed up after discharge, a blood recipient must test negative for malaria by both mRDT and microscopy and must have received donor blood with microscopy detectable malaria parasites.



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**Exclusion criteria**

Patients excluded from this study were patients considered too ill (unconscious, in shock or delirious). Patients were excluded if they were to be transfused in the operating theatre because they were not accessible for monitoring. Patients who were positive for malaria parasites by microscopy prior to their transfusion or had taken antimalarials within 5 days prior to transfusion were also excluded. Excluding individuals with antimalarial exposure ensures a more homogeneous study population, which can enhance the internal validity of the study. This allows for a more focused investigation into specific genetic aspects of drug resistance within a more uniform group. Additionally, a recipient who was unwilling to provide accurate details of place of residence, as well as demographic and clinical information was excluded. Finally, a blood recipient whose residence was further than 30 km from Nsawam Township was excluded from the study.

**Withdrawal criteria**

Participation in the study was voluntary and participants had the right to withdraw from the study at any time without penalty and without having to give any reasons.

**Sources of Donor Blood**

All donor blood used in this study was collected by the in-house static blood bank from August 2020 to May 2021.

**Weight-to-Volume Conversion of Donor Blood**

The weight of each donor's blood was taken with the blood bank scale (Lasany, India, sensitivity = 0.1 g). Weight in grams (g) was converted to volume millilitres (mL) assuming the density of blood was approximately 1



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g/mL. This estimated P, falciparum parasitaemia per blood unit of blood was calculated based on the blood that was left.

## Data Collection Instruments and Procedures

Prospective blood recipients were recruited into the study after consent had been sought from them or their guardians. A standard questionnaire was designed and used to collect both demographic and clinical data which included: age, gender, marital status, employment status, accurate description of the place of residence (including Ghana Post address, landmarks, telephone number of the recipient and at least one relative) and clinical history necessitating the transfusion. Body temperatures were also taken before final discharge (Owusu-Ofori et al., 2013).

Patients' blood transfusion information records were obtained (whether client had received a blood transfusion before, number of blood units transfused, blood group and rhesus group). Pre, during and post transfusion vitals (temperature, blood pressure) were taken. Body temperature was recorded using digital infrared non-contact thermometers (Kinlee, Guangdong), and the blood pressure with digital blood pressure meters (Omron, Kyoto-Japan).

### Residence verification of the infected blood recipient

On the discharge day, a member of the research team accompanied the recipient home. The digital address of the place was taken using the Ghana Post digital address application. Additionally, Google map coordinates were also taken. All immovable landmarks were taken note of as well as the telephone numbers of relatives of the recipients. This follow-up strategy has previously been used by (Aninagyei et al., 2020).



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### Follow-Ups on Recipients of Malaria-Infected Donor Blood

Recipients of malaria-infected donor blood were followed up on days 2, 7, 14, 21, 28, and 35. On each day of follow-up, a member of the research team visited the residence of the recipient. Using aseptic means and ensuring maximum protection against SARS-CoV-2, at most 100 µL whole blood was collected into pediatric EDTA tubes. Temperatures were taken using a noncontact infra-red thermometer. Drawn recipients' blood samples were immediately screened by both mRDT and microscopy for the detection of *P. falciparum*. Follow-ups ceased when a recipient turned out positive for *P. falciparum* infection. All infected post-transfusion individuals were referred to the nearest health facility for malaria management.

### Blood phlebotomy for Recipient/Patient

Recipient/Patient whole blood was collected into a plain tube and labelled with a recipient identification number (RID). A vein of a good size that was visible, straight and clean, located in the antecubital fossa region was selected for venipuncture. A tourniquet was applied about 4–5 finger widths above the venipuncture site and the vein was re-examined to ensure that it was firm and prominent. Prior to venipuncture, hand hygiene was performed and non-sterile gloves were used. The site to be punctured and its surrounding skin surface were disinfected with a 70% alcohol swab for 30 seconds and allowed to dry completely (30 seconds). The vein was anchored by holding the patient's arm and placing a thumb below the venipuncture site and asking the patient to form a fist, so the veins were more prominent. Venipuncture was performed by a smooth entry of the vein at a 30-degree angle or less. The donor was asked to open and close their fist slowly every 10–12 seconds during collection. Once



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3ml of blood had been drawn, the tourniquet was released before withdrawing the needle. The needle was gently withdrawn, and pressure was gently applied to the site with a clean gauze or dry cotton wool ball and covered by a phlebotomy plaster.

## Laboratory Analysis

### ABO blood grouping

The slide or tile method of blood grouping test was used in this study. Three (3) ml of the recipient's blood was collected into a plain tube and labelled with blood recipients' details. One drop of antiserum A, antiserum B, and antiserum AB was placed into each cavity as shown in Figure 4. Each blood drop and the antiserum were mixed in an area of approximately 15 cm using a fresh applicator stick. The tile was maintained at room temperature while agglutination in the form of fine red granules was observed within 2 mins.

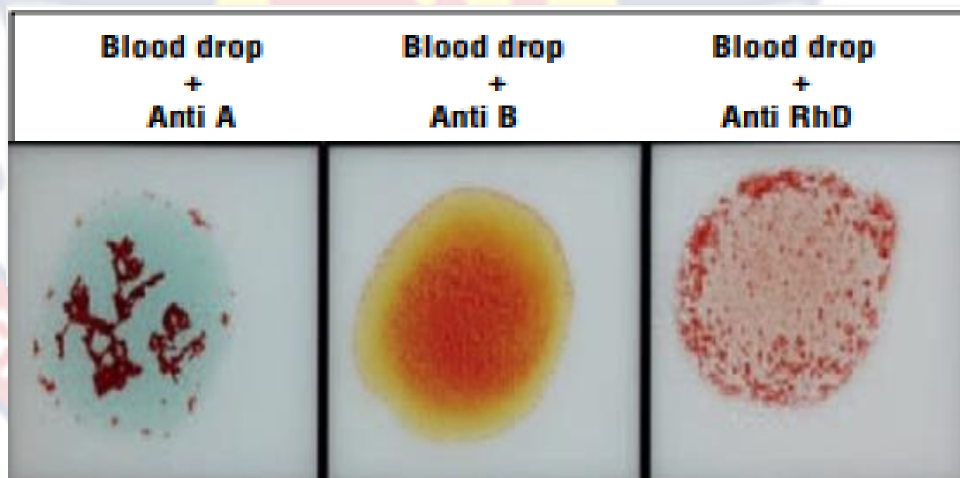


*Figure 4: Blood Group Typing*

### Major cross matching test

Cross matching is a method used prior to the transfusion of blood or its related products to detect any serological incompatibility in the donor and recipients' blood. Before transfusing a donor's blood into a recipient, there



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should be no antigens or antibodies that would react with each other and cause a transfusion reaction. Cross matching was based on the principle of serological identification of any clinically significant irregular/unexpected antibodies in either the donor or recipient's blood. The Major Cross Match approach was employed in this study. It entailed comparing the donor's red cells to the recipient's serum to detect the presence of any antibodies that may induce haemolysis or agglutination of the donor's red cells.

Blood recipients' requisition forms were checked for completeness prior to blood specimen collection. Crossmatch worksheets were created for accurate recording of findings. Blood specimens were centrifuged at 1500 rpm for 1 minute to separate plasma from red blood cells (RBCs) and serum was transferred to clean test tubes. An ABO blood group screening was performed on each patient's sample to be cross matched to aid in the selection of an acceptable donor unit from the blood bank fridge. Details of selected donor unit number(s) were recorded onto the crossmatch worksheet. Segments from the donor red cell unit were removed and the donor unit(s) returned to the fridge. Blood from the donor unit segments was placed into labelled test tubes. The donor cells from the test tubes were washed with isotonic saline and a 2-5% red cell suspension(s) was prepared (1 drop RBC: 20 drops saline) in corresponding labelled tubes and labelled donor RBC. In another tube labelled tube A, two (2) drops of recipient plasma and one (1) drop of the donor RBC suspension was added and mixed. The mixture was incubated at 37°C for approximately 60 minutes and centrifuged after which the mixture was observed macroscopically for hemolysis and agglutination and results were recorded. Red cells were washed three to four times with saline and supernatant completely decanted.



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Two (2) drops of polyspecific Anti-Human Globulin (AHG) was added to the red cell and mixed, allowed to stand for approximately 5 minutes and then centrifuged. Macroscopic observation for agglutination was performed. When macroscopic agglutination was not observed, a small amount was transferred onto a glass slide and examined for microscopic agglutination. The agglutination was graded, and results were recorded on crossmatch worksheets. Results were reported as follows:

- No agglutination or haemolysis indicating the donor unit(s) was compatible.
- The presence of agglutination or haemolysis indicating the donor unit(s) was incompatible.

Confirmation of the validity of negative results was done by adding IgG-coated red cells termed "check cells". After check cells were added and centrifuged, agglutination presence confirmed the validity of a negative result. The absence of agglutination of check cells invalidated crossmatch results, hence crossmatch testing was repeated.

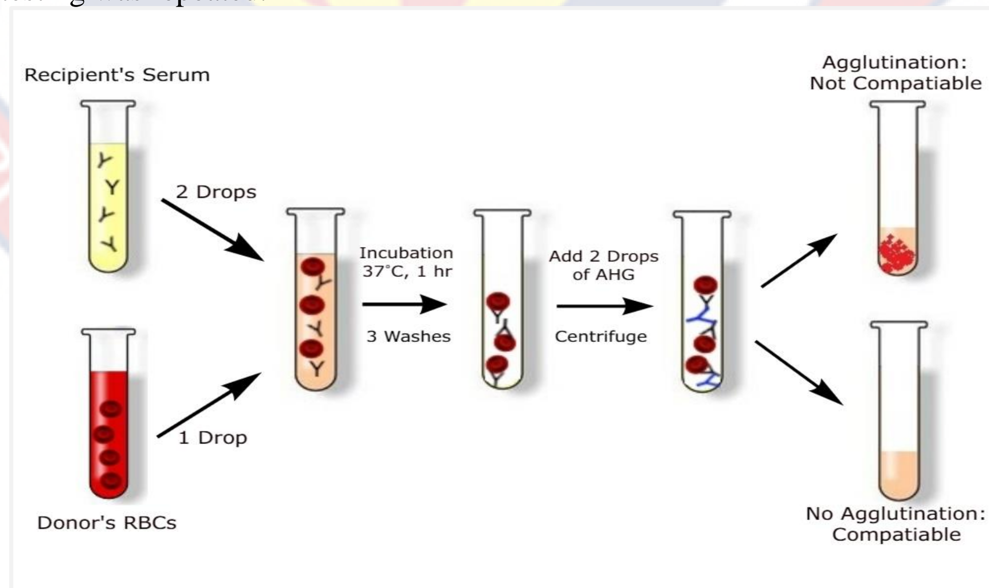


*Figure 5: Workflow for Cross Matching Test*
(Source: http://laboratorytests.org/wpcontent/uploads/2019/07/Major-Cross-Matching.jpg)



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**Post-transfusion reaction**

The National Blood Service of Ghana guidelines for monitoring transfusion recipients was adopted Appendix 3. The patient's vital signs (temperature, pulse, respirations, and blood pressure) were recorded shortly before transfusion. After the transfusion was initiated, the rate of flow was observed and regulated, according to the physician's orders. During the transfusion, the patient was observed periodically, especially during the first 10 to 15 minutes, for signs and symptoms of transfusion reaction. The time frame for experiencing these acute transfusion reaction were recorded within 15minutes after blood transfusion. In this transfusion setting, a fever was defined as a temperature elevation of 1° C or 2° F beyond the average body temperature of (37 ° C) 98.6 ° F. Prior to transfusion the patient was given instructions on potential problems following transfusion and was instructed to alert a health care provider of any discomfort or unusual sensations.

Upon completion of the transfusion, the patient's vital signs temperature, pulse, Oxygen saturation ($SpO_2$), and blood pressure were recorded, and compared with the baseline values to detect acute transfusion reactions that may need immediate or further investigation and treatment. Temperature, mean pulse, $SpO_2$, blood pressure, fever, urticaria rash, respiratory distress and sweating were recorded as part of the adverse transfusion reaction.

**Detection of *P. falciparum* and other Haemo-parasites**

There are several methods used in the laboratory for the detection of *P. falciparum*. These methods are microscopy, immunochromatography also called rapid diagnostic test - RDT, and advanced molecular techniques



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(selective Whole Genome Amplification – sWGA). In all, microscopy remains the gold standard for malaria diagnosis, as it provides information on both parasite species and density (Moody 2002). In this study, other haemo-parasites (*Babesia, Toxoplasma, Leishmania*) were detected using enzyme-linked immunosorbent assay (ELISA).

**Immunochromatographic screening of *Plasmodium* infections**

In this study, *Plasmodium falciparum* malaria parasites were detected using CareStart mRDT (Access Bio, Somer- set, USA). The CareStart™ mRDT is an in vitro immunochromatographic test kit which consists of a membrane strip pre-coated with a monoclonal antibody as a single line across a test strip coupled to a signal that detects the histidine–rich protein 2 (HRP2) of the *Plasmodium falciparum* antigen. The sensitivity and specificity of the Carestart mRDT was 98.2% (95% CI: 95.9, 99.4). mRDTs are used to detect current malaria infections. They primarily indicate the presence of malaria parasites in the bloodstream at the time of testing.

Parasite testing and reporting were performed according to the manufacturer's protocol. Test kits were labelled with the donor or recipient (patient) identification number and date. Testing was done on both transfused donor blood units and pre- transfusion recipient blood. Approximately 5.0 µL of whole blood sample was added onto the test device window using the sample applicator provided with the kit (Fig 6). Two drops of assay diluent was dispensed into the assay diluent well and incubated at room temperature for 20 mins after which test results were recorded.

Positive result for *P. falciparum* was recorded when two bands were visible (one band in the control area and the other band in the test area). Negative



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*P. falciparum* result was recorded only if one band appeared on the control line (Figure 6). A photograph of the RDT was taken at the time of reading.

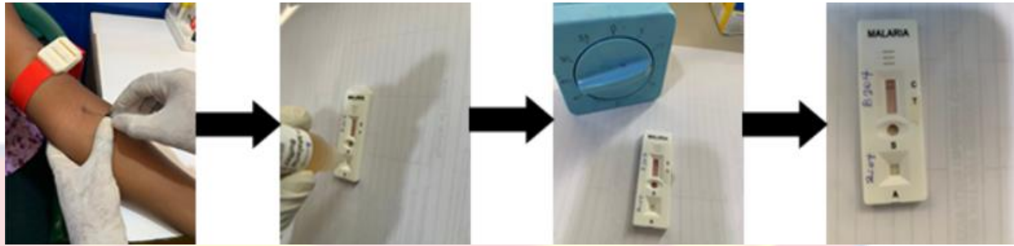

*Figure 6: Systematic stages involved in rapid detection for diagnosis of malaria*

**Detection and quantification of *P. falciparum* using microscopy**

The direct microscopic visualization of malarial parasites has been the gold standard for malaria diagnosis. It is usually the most practical, cost-effective, and widely accessible technique. This diagnostic technique involves a series of steps including (i) the preparation of blood smear, (ii) staining, (iii) detection of the parasite as well as the species, and (iv) the determination of the parasite density.

The parasite that causes malaria, *Plasmodium,* has five species: *Plasmodium falciparum, Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium knowlesi* (Amoah et al., 2022; Menkin-Smith & Winders, 2021) Additionally, each *Plasmodium species* has four life cycle phases, including the ring, trophozoite, schizont, and gametocyte stages (Capone et al., 2013; Das, Mukherjee, et al., 2015a).

The purpose of the underlying study was to use light microscopy techniques (thin and thick blood smears) to identify infected and non-infected RBCs carefully and accurately to determine the *Plasmodium falciparum* parasitemia, a crucial measure of the severity of the infection. Regarding their intended use,



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the two techniques are different. Thick blood smears are best for identifying the presence of *Plasmodium* parasites, while thin blood smears are helpful in identifying *Plasmodium species* (Bharti et al., 2007).

**Thin blood smears preparation**

As indicated above, the thin blood film is used for identification the of the *Plasmodium species*. In this study, thin and thick blood smears were prepared as previously described by Aninagyei et al. (2019) with slight modifications.

The frosted end of a glass slide was labelled with the patient's details such as study number and date. The thin blood film was prepared by placing a slide on a slide preparation template and a 2.0 µl drop of blood was placed on the slide using a micropipette. With the slide holding the blood resting thin on a flat, firm surface, the small drop of blood was touched with the edge of a spreader (another clean slide). The blood was allowed to run right along the smooth edge of the spreader (World Health Organization 2010), and quickly smeared forward on the slide surface (Fig 7).

**Thick blood smears preparation**

For the thick blood smear preparation, a 6.0 µl of blood was dropped 1 cm apart from the thin film on the other half of the same glass slide where the thin film was prepared. The blood spot was then agitated in a circular motion with the corner edge of another slide for the thick blood film spread (Figure 7). The blood smears were left to air dry before the thin film was fixed with methanol. Contact between the thick film and methanol was avoided since methanol and its vapour can quickly fix the thick film, which does not stain properly.



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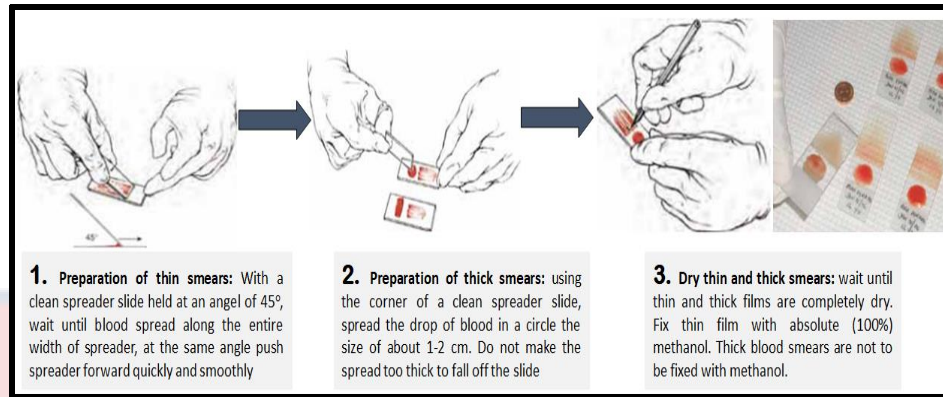


*Figure 7: Thin and Thick Blood Smears Preparation*

Image adapted and modified from *Basic malaria microscopy – 2nd edition. Contents: - Part 1: Learner's guide- Part 2: Tutor's guide*

## Staining of Prepared Slides

Malaria parasitaemia was evaluated in this study by using 10% Giemsa working solution produced in pH 7.2 buffered water. Giemsa staining is the gold standard staining method used to test blood for malaria parasites on both thin and thick smears. A freshly prepared working solution of Giemsa, made from well-prepared stock and diluted with water buffered to pH 7.2 is recommended. Each slide was stained with approximately 3 ml of Giemsa dye. The slides were stained for 10 minutes before being carefully rinsed with clean water to prevent smears from washing off and metallic-green surface scum from clinging to the film. After washing, the slide was placed in a slide drying rack and left to dry naturally.

## Investigation of Blood Films for Malaria Parasites

### Thin film microscopic examination

*Plasmodium falciparum* was identified based on specific morphological characteristics observed under the microscope: trophozoite stage, schizont stage



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and gametocyte Stage. The thin film is used in the identification of the *plasmodium* species.

The slide was placed on the stage, with the x100 oil immersion objective positioned over the edge of the middle of the thin film. A drop of immersion oil was placed on the edge of the middle of the film, and the mechanical stage was raised until the objective lens came into contact with the immersion oil. The blood film was viewed in the manner depicted in Figure 8, travelling along the edge of the film, then inwards by one field, returning in a lateral movement, and so on. Before declaring the slide negative, it was examined until the presence of malaria parasites was discovered, or up to 800 fields as previously published (Aninagyei et al., 2019).

**Thick film microscopic examination**

All slides to be examined were placed on the microscope's mechanical stage, with the thick film aligned with the objective lens. A drop of immersion oil was applied to the thick film and spread. A field with malaria parasites and an equal distribution of white blood cells was chosen using paired x10 oculars and an x40 objective lens. The x100 oil immersion objective lens was rotated over the specified area of the thick film by raising the spinning nosepiece away from the stage until the objective lens made gentle contact with the immersion oil. The field was fine-tuned using the fine adjustment. The field was carefully evaluated field by field, going to each contiguous field, as in the pattern examination of a thick film (Fig 8A) which was based on the assessment of 100 fields. It took approximately 10 minutes to examine 100 oil immersion microscopic fields. This was done by following the techniques for thick and thin films microscopic examination algorithm (WHO, 2010). The trophozoite stage often called the



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ring stage varies from small to quite large within the host cell. Usually, there is one chromatin dot; two are common *in P. falciparum*.

For each positive slide, the species were identified, parasite density was also calculated, and the findings were documented.

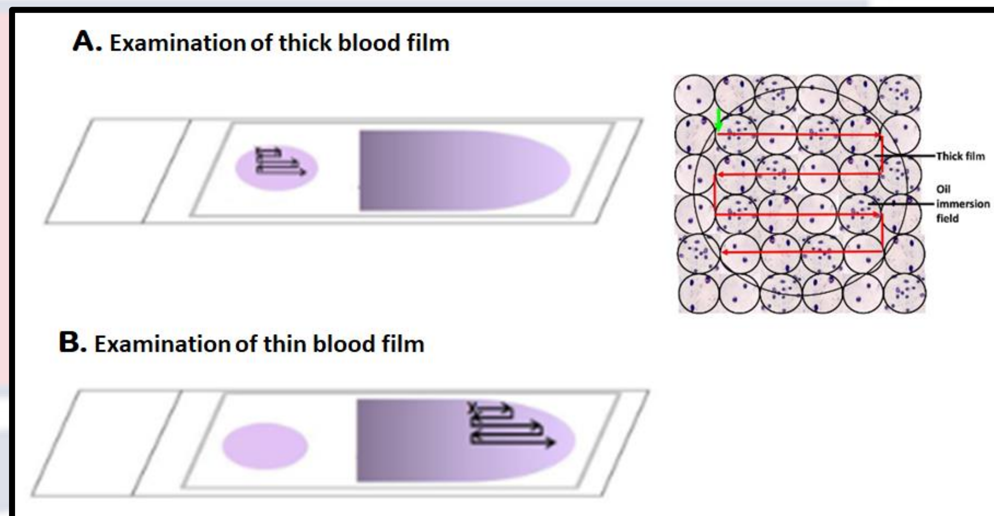


*Figure 8: Techniques for thick and thin films microscopic examination.*

**Determination of malarial parasite density**

The number of parasites spotted was counted on one tally counter and the amount of white blood cells on the other. After counting 200 white blood cells and determining the number of parasites, the findings were written on a form in terms of parasites per 200 white blood cells. When the number of parasites was 99 or less after counting 200 white blood cells, counting was extended to 500 white blood cells. When all of the parasites had been counted, the number of parasites relative to the number of leukocytes was computed and represented as 'parasites' per microlitre of blood. From the simple mathematical formula:

$$\frac{(\textit{Number of parasites counted})}{\textit{Number of leukocytes}} \; x\, 8000$$

$$= \textit{parasites per microlitre}$$



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## Detection of *Babesia* spp., *Leishmania* spp., and *T. gondii* IgG Antibodies

### Enzyme linked immunosorbent assay (ELISA) Principle

The ELISA performed was based on the qualitative enzyme immunoassay technique. Each microplate is pre-coated with an antigen specific to *Babesia*, *Leishmania* and *T. gondii* IgG antibodies. The antigen is immobilized on a solid surface during the test. This is accomplished by the use of a surface-immobilized capture antibody. After that, the antigen is complexed with a detection antibody coupled with a detectable molecule, and the optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The use of sample pooling was used because it has been documented in earlier research for ELISA where the use of pooled serum was reported to be an effective technique for monitoring infectious diseases (Bloch et al., 2021; Hernandez-Medrano et al., 2021).

ELISAs are extremely simple to perform, inexpensive, widely available, and have been validated for a wide range of diseases utilizing samples such as plasma and serum. When analyzing a large number of samples, the expense of analyzing individual samples can be too expensive; thus, sample pooling may be a more suitable strategy.

## Sample Preparation

Immediately after the transfusion of blood, at least 3.0 ml of the left-over blood in the blood bag was drained into a plain tube. *P. falciparum* HRP2 antigens were detected before plasma was separated from packed cells. Plasma was immediately frozen at -20 ºC until ready to be used.



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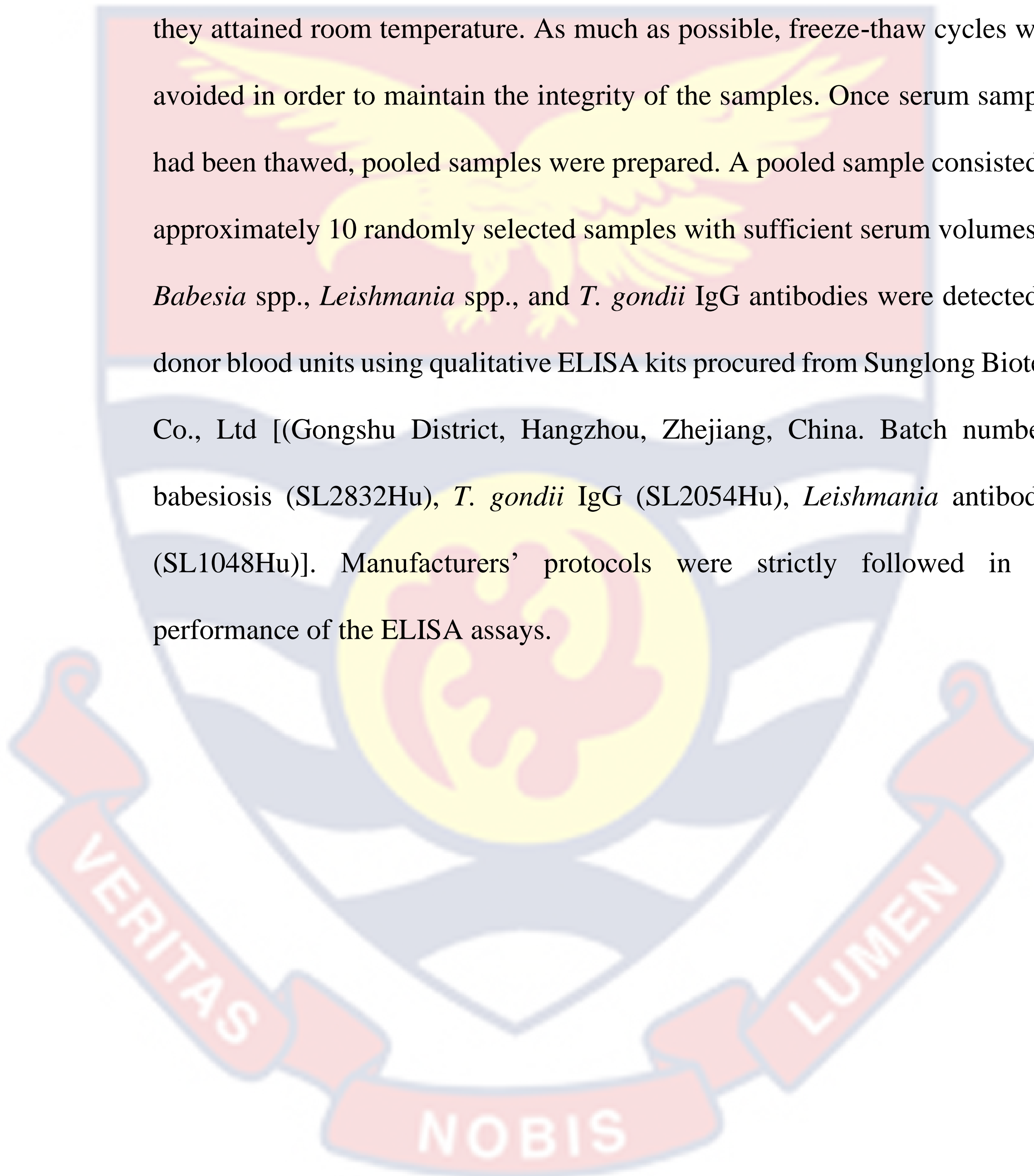
**Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of *Babesia* spp., *Leishmania* spp., and *T. gondii* IgG antibodies**

Before sero-detection of *Babesia* spp.*,* *Leishmania* spp., and *T. gondii* IgG, stored blood samples were retrieved and allowed to thaw on the bench until they attained room temperature. As much as possible, freeze-thaw cycles were avoided in order to maintain the integrity of the samples. Once serum samples had been thawed, pooled samples were prepared. A pooled sample consisted of approximately 10 randomly selected samples with sufficient serum volumes. *Babesia* spp., *Leishmania* spp., and *T. gondii* IgG antibodies were detected in donor blood units using qualitative ELISA kits procured from Sunglong Biotech Co., Ltd [(Gongshu District, Hangzhou, Zhejiang, China. Batch numbers: babesiosis (SL2832Hu), *T. gondii* IgG (SL2054Hu), *Leishmania* antibodies (SL1048Hu)]. Manufacturers' protocols were strictly followed in the performance of the ELISA assays.



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Briefly, corresponding micro wells in the microplate were labelled in succession, and five wells were labeled and set as controls: two wells as negative controls, two wells as positive controls, and one empty well as blank control. Ten microliters of each pooled sample wa sadded to the appropriate labelled well while 50.0 µL of negative and positive controls were also added to the control wells. Forty microliters (dilution factor is 5) of sample dilution buffer was added to the samples well and gently mixed. The microplate was then covered with a closure plate membrane and incubated at 37 °C for 30 mins. Subsequently, the wells were washed by filling the wells with the wash buffer solution for 30 secs before discarding them. This step was repeated five times after which 50 µL HRP-conjugate reagent was added to each well except the blank control well, incubated at 37 °C for 30 mins and washed as previously described above. Fifty microliters of chromogen solution A and chromogen solution B were added to each well, gently shook in order for a homogenous mixture to be formed and incubated at 37 °C for 15 mins. The plate was placed in the cupboard to avoid light during this process. The reaction was terminated with the addition of 50 µL stop solution to each well. Colour changed from blue to yellow and optical densities (ODs) were read at an absorbance of 450 nm using a microtiter plate reader within 15 mins of stopping the reaction.

**Interpretation of ELISA Results**

Test effectiveness: the average value of positive control ≥1.00; the average value of negative control ≤ 0.10.

- The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15
- Negative judgement: if the OD value < CUT OFF, the sample is negative



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- Positive judgement: if the OD value ≥ CUT OFF, the sample is positive

ELISA was rerun on individual samples following the above-described protocol for pools that turned out positive.

**Selective Whole Genome Sequencing of detected *Plasmodium* Parasites**

The *P. falciparum* Amplicon Toolkit protocols provided by the MalariaGEN Genomic Epidemiology Network were employed in this study. Figure 9 provides an overview of the processes followed.

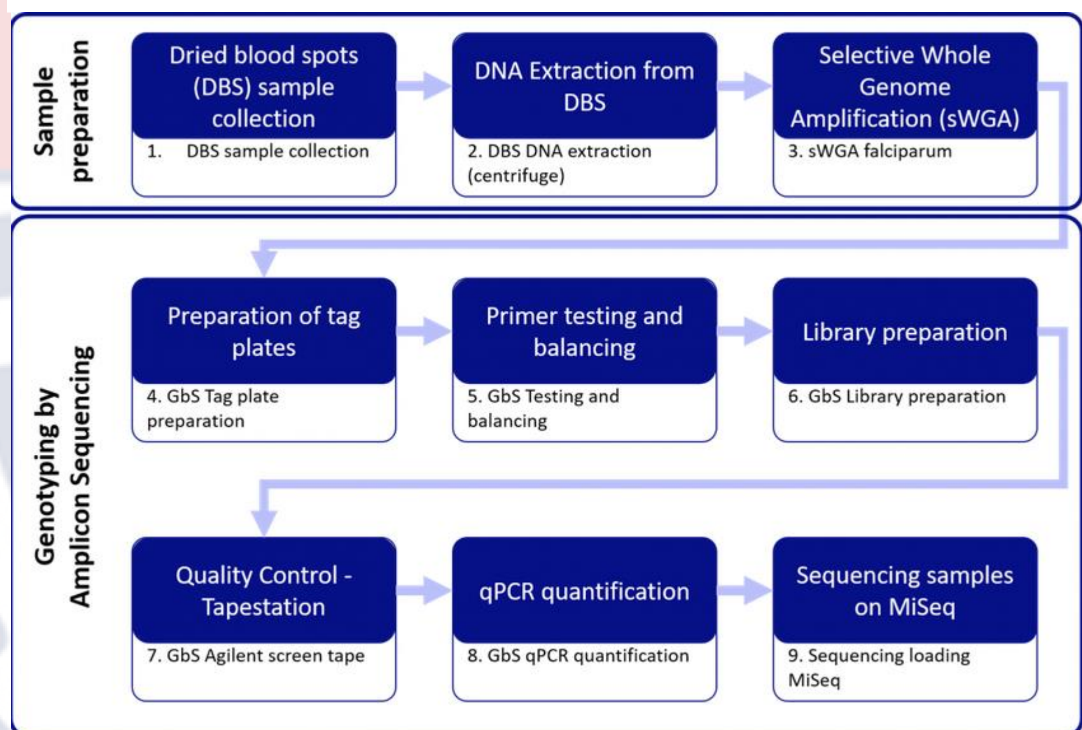


*Figure 9: Overview of steps involved in Plasmodium spp. genotyping by amplicon sequencing: (MalariaGen, 2023)*

**Preparation of Dry Blood Spots (DBS)**

Dried blood spots are a relatively inexpensive source of nucleic acids and are easy to collect, transport, and store in large-scale field surveys, especially in resource-limited settings. DBS is also a robust sample type for



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genomic analysis in large population-based studies, providing excellent WGS data.

Dried blood spots were prepared for both donor and recipient *Plasmodium* spp. positive blood specimens. Each paper card for sample preparation was labelled with a unique barcode, and the same barcode was placed on a sample information sheet that accompanied the samples to the Sanger Genome Centre, UK.

Approximately 50 µl of whole blood was carefully and slowly released onto each labelled paper card to make a spot. For each paper card, two blood spots were prepared (Figure 10). The blood spots were air dried and placed in a sealable plastic bag with a desiccant for shipment.

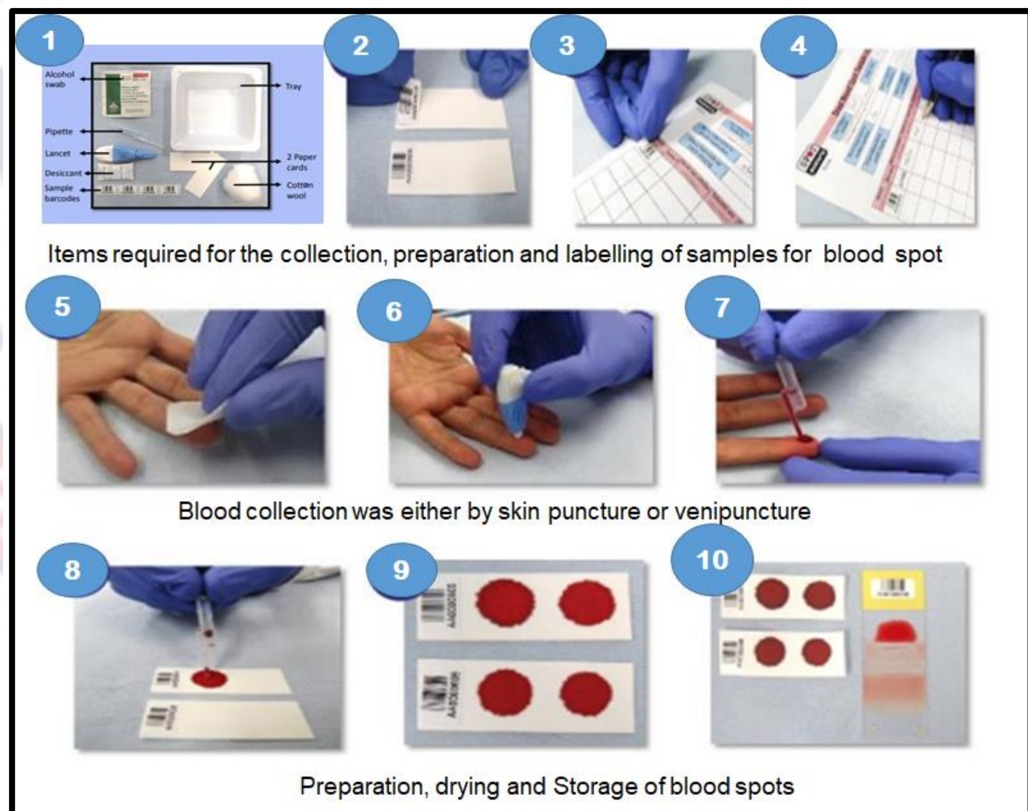


*Figure 10: Preparation, Labelling and Storage of Dried Blood Spots (DBS): Adapted and modified from: (MalariaGen, 2021a)*



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**Extraction and purification of DNA from Dried Blood Spot**

DNA was extracted from dried blood spot samples using the QIAamp DNA Kit (QIAGEN, Germantown, MD, USA). This kit combines the selective binding properties of a silica-based membrane with the speed of micro-spin to generate high-quality purified DNA. The QIAamp DNA isolation was done manually following the manufacturer's instructions.

Briefly, dried blood spot samples were grouped into batches of 94 and punched using a BSD 600PLUS robotic dried blood spot puncher (Microelectronic System, Brendale, Australia) into a labelled 1.5ml micro-centrifuge tube (EppendorfTM DeepwellTM). Three hundred microliters (300 µL) of lysis buffer (Buffer ATL) and 30 µL of Proteinase was added to each tube, pulse vortexed and incubated in a thermomixer at 56 °C with shaking at 600 rpm for 17 hours. The sample tubes were pulse-spanned at 1000 rpm to remove drops from the inside of the lids. At this point, samples were divided into batches of 24 and stored at -20 °C until ready to be processed further. Taking the first batch of 24 samples and to guarantee efficient lysis, 300 µL Buffer AL was added to each sample and mixed vigorously to obtain a homogenous solution. The tubes were reinserted into the thermomixer, and incubated at 70 °C with shaking at 900 rpm for 10 minutes. The sample tubes were pulse-spanned at 1000 rpm to remove drops from the inside of the lids. If solid white particles were still visible, the samples were spanned at 8000 rpm for 1 minute. The supernatant of each sample was carefully transferred into new 1.5ml Eppendorf tubes, 150 µL of absolute ethanol was added and contents were mixed thoroughly by pulse vortexing for 15 seconds. To purify the lysed parasite DNA, the vacuum pump was set up and the required number of



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QIAamp MinElute columns were labelled and placed onto the manifold. The lysate solution was carefully applied onto the QIAamp MinElute column, and the vacuum pump was started to run until all the sample lysates had been ran through the column. To wash away contaminants and inhibitors, 500 µL of wash buffer AW1 was run through each QIAamp MinElute columns. Seven hundred microliters (700 µL) of buffer AW2 was then ran through the MinElute columns after which 700 µL of absolute ethanol was added to each column. The vacuum pump was kept running for approximately 10 minutes to allow the membranes of the columns to completely dry. The QIAamp MinElute columns were placed onto clean 1.5 ml eppendorf tubes, and the lids opened and incubated at room temperature for another 10 mins. Once the membranes were dry, 100 µL of buffer ATE was applied to the center of the membrane and incubated at room temperature for 5 minutes. The columns were then centrifuged at 14 000 rpm for 2 minutes. For each sample, the total volume of elute was transferred into a 96 well plate. The plate was sealed with a Microseal "A" seal once all the wells in the plate had been filled. A note was made of the sample IDs, location and volumes. The samples were stored at -20 °C until ready to be used.

**Selective Whole Genome Amplification (sWGA) of extracted parasite DNA.**

DNA extracted from DBS filter papers usually have low parasite DNA yield and an enormous human DNA contamination often posing severe limitations to downstream genetic analyses. Selective whole genome amplification (sWGA) using short oligonucleotide probes as primers (Table 2) that preferentially bind to the target genome was employed. Amplification was



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performed in a 0.2 ml 96-well PCR plate. A 20 µl sWGA master mix containing: 0.5 µl 100X BSA, 5.0 µl 10 mM dNTPs, 0.5 µl 2.5 µM of each amplification primer, 5.0 µl of 10X Phi29, 6.0 µl nuclease free water and 3.0 µl of 30 units Phi enzyme was prepared and added to each reaction well of the PCR plate containing 30 µl of template DNA (total volume, 50 µl). The plate was sealed with a microseal "A" seal, centrifuged briefly and placed in a thermocycler programmed for a stepdown PCR protocol of 35 °C for 5 mins, 34 °C for 10 mins, 33 °C for 15 mins, 32 °C for 20 mins, 31 °C for 30 mins, 30 °C for 16 hrs, followed by heating at 65°C to inactivate the Phi29 enzyme and cooling at 4 °C. The sWGA plate(s) were wrapped in Parafilm and stored at -20 °C for future downstream assays.

Table 2: Primers used for the sWGA of Plasmodium falciparum

| Primer Name | Primer Sequence* | Primer Quantity |
|---|---|---|
| Pf1 | ATATATATAT*A | 250 nmol |
| Pf2 | TATATATATAT*T | 250 nmol |
| Pf3 | TATATATATA*A | 250 nmol |
| Pf4 | TAATATATA*T | 250 nmol |
| Pf5 | TATATATATT*T | 250 nmol |
| Pf6 | ATTATTATTA*T | 250 nmol |
| Pf7 | TAATAATAAT*A | 250 nmol |
| Pf8 | AAAAAAAAAAA*A | 250 nmol |
| Pf9 | AATAATAATA*A | 250 nmol |
| Pf10 | TATTATATA*T | 250 nmol |

*Indicates that the 3' base is joined through a phosphorothioate bond to inhibit the exonuclease activity of Phi29



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## Genotyping by amplicon Sequencing (GbS)

The GbS process is important for the generation of "tag" plates and the normalization of library yields. Genomic DNA libraries were constructed for sequencing using the KAPA Library Preparation Kit (Kapa Biosystems, Woburn, MA). Five hundred nanograms (500 ng) was fragmented with the Covaris E210 bp. Libraries were prepared using the protocol as described by the MalariaGen Genomic Epidemiology Network ((MalariaGen, 2021b).

The DNA was purified between enzymatic reactions and library size selection was performed with AMPure XT beads. Generated libraries were assessed for concentration and fragment size using the DNA High Sensitivity Assay on the LabChip GX (Perkin Elmer, Waltham, MA). Library concentrations were also assessed by qPCR using the KAPA Library Quantification Kit. Libraries were pooled and sequenced on a 150 bp paired-end Illumina HiSeq 2500 run DNA sequencer (Illumina, San Diego, CA).

**Tag plate preparation**

PCR 1 was a restricted cycle PCR in which a low concentration of seeding primers precisely annealed to the genomic template (obtained during sWGA), while PCR 2 was set up to anneal indexed tagged primers to and extending from the common 5'-tails added during PCR 1.

**Pre-amplification of DNA samples**

A frame star well plate was stamped with 1.0 µL of DNA and 1.0 µl of T0.1E as a negative control. The MJ thermocycler processed the plates in a single batch.



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**First genotype by sequencing PCR (GbS PCR 1)**

A plate of pre amplified DNA samples (1µl per well) was pulse centrifuged to make sure all liquid was at the bottom of the plate. PCR master mix was prepared to the number of plates being processed as shown in Table 3. To ensure that the complete volume of the primer panel was transferred into the master mix, it was crucial to apply the reflex pipetting technique. The master mix was gently mixed by vortexing and followed by pulse centrifugation to get all contents to the bottom of the tube. Each well of a plate received approximately 4.5 µl of master mix.

Using a Biorad heat sealer set to 175 °C for 5 seconds, the plate(s) were sealed. To accomplish this, a foil pierceable plate seal was placed on top of the plate with the red stripe pointing upwards. The wells were sealed by pressing the seal button on the Biorad heat sealer. The plates were centrifuged at 1000g for 20 seconds. The lid was moved to a genotyping thermocycler, and the appropriate block was flashing as the lid was fingertip tightened. Cycling conditions were performed as follows: Initial denaturation at 95 °C for 15 mins followed by 49 cycles of denaturation at 95 °C for 20 secs, annealing at 51 °C for 40 mins, extension at 60 °C for 3 min and a final holding at 4 °C. To avoid the development of non-specific products, the plates were immediately placed on ice and for PCR 2 to be immediately carried out.

**Second genotyping by sequencing PCR (GbS PCR 2)**

After PCR 1, no additional cleanup was required for PCR 2 to be carried out. A proportion of amplicons from PCR 1 were transferred into a dual dual-indexed plate that had been dried down. These indexed tag primers stretched



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from the 5'-tails added during PCR 1 and annealed to them, making it possible to multiplex up to 1536 samples for each batch of sequencing.

Briefly, 10 µl of amplicons from PCR 1 were transferred into the corresponding wells of the PCR 2 tagged 96 well plates, and the mixture was thoroughly mixed by reflex pipetting to ensure the plate's dried tag primers were completely re-suspended. The red strip facing upwards was attached to a foil pierceable plate seal that was placed on top of the plate. The lid was heated to 175 °C and held there for five seconds using a plate sealer. The prepared PCR 2 tag plate was placed, centrifuged at 4 °C, (2000 g) for 1 minute and sent to the post-PCR room for the next step. PCR 2 was run on the MJ thermocycler programme at 31 cycles of denaturation at 95°C for 20 secs; 4 cycles of annealing at 68 °C for 15 secs, and extension at 60 °C for 15 secs; a final extension at 60 °C for 3 mins and a final holding at 4 °C.

Table 3: Mastermix Preparation for GbS PCR 1 Reaction

| **Reagents** | | **Number of required Plates** | | | | |
|---|---|---|---|---|---|---|
| | | **½ Plate** | **1 Plate** | **2 Plate** | **3 Plate** | **4 Plate** |
| Number of samples | **x1** | **x250** | **x484** | **x968** | **x1452** | **X1936** |
| Nuclease free water (µl) | 1.71 | 427.50 | 828.00 | 1655.00 | 2483.00 | 3311.00 |
| 2x qiagen mastermix (µl) | 2.75 | 687.50 | 1331.00 | 2662.00 | 3993.00 | 5324.00 |
| 0.4nM Primer Panel (µl) | 0.04 13 | 10.30 | 20.00 | 40.00 | 60.00 | 80.00 |
| **Total (µl)** | **4.50** | **1125.30** | **2179.00** | **4357.00** | **6536.00** | **8715.00** |



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**Creation of library of DNA by pooling PCR 2 products**

The amplicons used in the two-step PCR protocol were made to capture a specific size range (190-250 bp inclusive of the priming sites), which complements the sequencing length of the MiSeq v2 300 kit (Illumina, San Diego, CA), while also allowing effective Ampure XP beads size selection away from contaminating smaller off-target amplification products. According to testing and rebalancing done by GbS, primers are adjusted. By doing this, the multiplex's coverage is as even as possible across all amplicons.

After PCR 2, plates were removed from the thermocycler and set on ice. Two new Vblok200 250ml V-bottom reservoirs and one 2ml lo-bind Eppendorf tube were labelled. Through upside-down centrifugation at 2000 g for 1 minute, the full reaction volume, or 5µL of PCR 2 products was pooled into the Vblok200 reservoir. To ensure even recovery of all samples, the 384 plate was withdrawn from the Vblok200 and 4 ml of elution buffer (EB)was pipetted over the reservoir's surfaces. The PCR 2 plate was then thrown out. The Vblok200 had a maximum volume of 6ml (384x5l plus 4ml EB), which was completely mixed by swaying the reservoir back and forth and side to side ten times. In the 2 ml lo-bind Eppendorf tube, roughly 1.7 ml of the pool was transferred. The vblok200 was discarded together with the liquid that was left over. An analysis on a DNA1000 bioanalyser chip was used to view the DNA pool's size profile.

**DNA pool size selection and clean-up**

Using a 2 x 0.75X solid phase reversible immobilization (SPRI), DNA pool size selection and cleanup were carried out. The pooled PCR product was properly transferred from the 2 ml lo-bind Eppendorf tube into the labelled 1.5 ml lo-bind Eppendorf tube after the Ampure XP beads were vortexed to re-



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suspend them. After that, 75 µl of Ampure XP beads was added and vortexed to combine with the combined DNA. The mixture of pooled DNA and Ampure beads was pulse spun down to collect all liquid at the bottom of the tube without affecting the bead suspension, and it was then placed at room temperature for five minutes on a non-magnetic rack. The supernatant was removed and disposed of while leaving the beads in place once they had become clear and all of the beads had been caught on the tube's side. 700 µl of 75% ethanol was added and left on the beads for 30 seconds to wash them. The ethanol was then thrown away. The beads underwent two washings. For about 2 minutes, the tube's lid was left ajar until the beads seemed to be "wet paint". To release the target product in the to solution, the beads were re-suspended in 105 µl of elution buffer by pipetting up and down. A magnetic rack was used to hold the low-bind Eppendorf tube with the cleaned beads inside. Once the supernatant was clear and the beads had been attracted to the magnet, 100 µl of the supernatant, which contained the size-selected PCR products that had been purified, were placed into a second, branded 1.5 ml Eppendorf tube. Pipetting up and down was used to re-suspend the beads in 22 µl of elution buffer.

The tube was moved to a rack without a magnet and incubated there for two minutes. One 1.5 ml lo-bind Eppendorf tube was used to contain the tube's contents. 20 µL of the supernatant containing the purified size chosen PCR products were transferred to another 1.5 ml Eppendorf tube once the supernatant had become clear and the beads had been secured on the magnet.

**Amplicon sequencing on MiSeq DNA sequencer**

DNA libraries were sequenced using the MiSeq within a 24-hour period. DNA was diluted to 4 nM in a 1.5 ml Eppendorf tube using a KAPA SYBR



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FAST qPCR complete kit in order to prepare the sample for sequencing using Miseq. The diluted denatured DNA library was loaded into the reagent cartridge by piercing the foil and adding the total volume of the f diluted denatured DNA library.

Guidelines are specific to the version of software installed on the MiSeq were followed for pre-run checks, flow cell setup and loading, reagent loading and running of the sequencer.

## Data Processing and Analysis

### Statistical analysis

Data were entered into Microsoft Excel and statistical analyses were performed using Excel graphs and SPSS 25.0 software (IBM Corp., Armonk, NY, USA).The prevalence of exposure to each of the four transfusion-transmitted parasites was calculated by dividing the number of exposed donor units by the number of donor blood units analysed (Noordzij et al., 2010). The chi-square test was used to test the associations between demographic data, clinical characteristics and *Plasmodium falciparum* gene alleles as well as the association between donor blood attributes with exposure status. Significance was fixed at $P<0.05$. Bivariate and multiple logistic regression analysis were done to determine risk factors of malaria infection. Also, parasitaemia in blood units was calculated. Fishers' exact test of independence was used to determine associations in parasite exposed donor blood.

The assumptions that underlined the calculations of the parasitaemia in blood units are based on confirmed study that the measured density of blood to be similar to water (Vitello et al., 2015). If the density of a liquid equals the mass



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of the liquid divided by its volume; D = m/v. (density of water is 1 gram per cubic centimeter) then density of blood equals to one. If density of blood equals to one, then the volume of blood is equivalent to the weight or mass of blood.

The frequency of each gene allele and transmissibility of the parasite was determined by analyzing for greater than 95% homology of *Plasmodium* genes.

## Bioinformatics Analysis

DNA fragments from DNA library were sequenced collectively during multiplex sequencing. The connected barcode (sample marker) sequences were used to distinguish the reads during a subsequent de-multiplexing phase**.** Sequence reads were demultiplexed and fastq data files generated downstream automatically using the onboard PC. Sequence reads were trimmed of low-quality bases from their ends using BioEdit v7.2 after sequence data for each sample has been subjected to standard Illumina QC procedures. Reads per sample were subjected to detailed analysis for enrichment, quality, content, and coverage. Each dataset was analysed independently by mapping sequence reads to the reference genome. Undiscovered and heterogeneous genes were given the letters "X" and "N," respectively.



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# CHAPTER FOUR

# RESULTS AND DISCUSSION

## Characteristics of Donor Blood Units

In this study, a total of 571 remaining transfused donors blood units from the Nsawam Government Hospital in the Eastern Region of Ghana were used. Of these donors' units, 304 (53.0%) were of blood group O, 123 (21.5%) blood group A, 129 (22.6%) blood group B, and 15 (2.6%) for blood group AB (Table 4). In all, 528 (92.5%) was whole blood (WB) and 43 (7.5%) was concentrated red blood cells (CRBC). Majority 452 (79.2%) of the blood was stored for just a day, 83 (14.5%) between days two and five, 15 (2.6%) between days six and nine, 10 (1.8 %) between days ten and thirteen and 11 (1.6%) for more than thirteen days.

Table 4: Characteristics of donor blood units

| Characteristics | N (%) |
|---|---|
| ***Blood group*** | |
| O | 304 (53%) |
| A | 123 (21.5%) |
| B | 129 (22.6%) |
| AB | 15 (2.6%) |
| ***Type of product*** | |
| Whole blood | 528 (92.5%) |
| CRBC | 43 (7.5%) |
| ***Days in storage*** | |
| 1 | 452 (79.2%) |
| 2 – 5 | 83 (14.5%) |
| 6 – 9 | 15 (2.6%) |
| 10 – 13 | 10 (1.8%) |
| >13 | 11 (1.9%) |



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**Exposure rates of transfusion-transmitted parasites (TTPs) in blood donors**

Out of the 571 donor blood samples analyzed, 106 (18.6%) were either exposed to *P. falciparum, Babesia* spp., *Leishmania* spp., or *Toxoplasma. gondii.* Separately, 69 (12%) of the donor blood samples contained *P. falciparum* histidine-rich protein 2 (PfHRP2) antigens, while exposure rates of *Babesia* spp., *Leishmania* spp. and *T. gondii* were 6 (1.1%), 16 (2.8%) and 22 (3.9%) respectively.

In the transfusion-transmitted parasites (TTPs) studied, frequencies of exposure were recorded highest in blood group AB 5/15 (33.3%), followed by blood group O 64/304 (21.0%), then blood group B 25/129(19.4%) with the least in blood group A 19/123(15.4%). AB>O>B>A.

With regard to *P. falciparum* among the blood groups. frequencies of exposure were recorded highest in blood group AB 5/15 (33.3%), followed by blood group O 39/304 (12.8%), then blood group B 13/129(10.1%) with the least in blood group A 12/123(9.7%). AB>O>B>A.

With regard to *Babesia* spp., among the blood groups. frequencies of exposure were recorded highest in blood group O 4/304 (1.3%), followed by blood group O 1/304 (0.8%) and blood group B 1/129 (0.8%) with the none in blood group AB.

*Leishmania* spp., was higher in blood group B 7/129 (5.4%), and least in blood group A 2/123(1.6%). *Toxoplasma. gondii* was higher in blood group O 14(4.6%) and least in blood group B 4/129(3.1%) whereas *none for Babesia* spp., *Leishmania* spp., and *Toxoplasma gondii* for blood group AB.



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**Blood storage days**

Day greater than 13 stored blood had a higher prevalence of *P. falciparum* 3/11 (23.1%), *and Toxoplasma gondii* 1 (7.6%).

Day 2-9 stored blood ha high prevalence of *P. falciparum* (13.3 %). Table 5

Day 1 stored blood had a moderately high prevalence of *P. falciparum* 52/452 (11.5%) and the least for *Babesia* spp. 6/452(1.3).

***Type of product (whole blood vs CRBC)***

It was also observed that high frequencies 7/43(16.8%) of *P. falciparum* antigens were detected in concentrated red blood cell (CRBC) donor products, whereas in whole blood it was slightly lower 52/452(11.5%). Of the antibodies that were detected, *Babesia* and *Leishmania* spp. antibodies were undetected in CRBC however they were detected in the whole blood at varying percentages.

Table 5: Prevalence of P.falciparum PfHRP2 antigen, Babesia spp., human Leishmania spp. and T. gondii antibody

| **Donor blood characteristics** | **Positive for PfHRP2 antigen** | **Positive for *Babesia* spp. antibody** | **Positive for human *Leishmania* spp. antibody** | **Positive for *T. gondii* IgG** |
|---|---|---|---|---|
| *Blood group* | | | | |
| *Sub total* | (n=69) | (n=6) | (n=16) | (n=22) |
| O (n=304) | 39 (12.8%) | 4 (1.3%) | 7 (2.3.%) | 14 (4.6%) |
| A (n=123) | 12 (9.7.%) | 1 (0.8%) | 2 (1.6%) | 4 (3.3%) |
| B (n=129) | 13 (10.1%) | 1 (0.8%) | 7 (5,4%) | 4 (3.1%) |
| AB (n=15) | 5 (33.3%) | 0 (0%) | 0 (0%) | 0 (0%) |
| *Type of product* | | | | |
| Whole blood (n=528) | 62 (11.8%) | 6 (1.1%) | 16 (3.0%) | 20 (3.8%) |
| CRBC (n=43) | 7 (16.3%) | 0 (0%) | 0 (%) | 2 (4.7%) |
| *Days in storage* | | | | |
| 1 (n=452) | 52 (11.5%) | 6 (1.3%) | 14 (3.1%) | 19 (4.2%) |
| 2 – 5 (n=83) | 11 (13.3%) | 0 (0%) | 0 (%) | 1 (1.2%) |
| 6 – 9 (n=15) | 2 (13.3%) | 0 (0%) | 2 (13.3%) | 1 (6.7%) |
| 10 – 13 (n=10) | 1 (10.0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| >13 (n=11) | 3 (23.1%) | 0 (0%) | 0 (%) | 1 (7.6%) |

mRDT – malaria rapid diagnostic testing, CRBC – Concentrated red blood cells.



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## Multiple infections in donor blood units

Few multiple infections were encountered in this study. Only 6 (8.7%) of 69 multiple infections co-existed with PfHRP2 antigenemia. All 6 *Babesia* spp. antibodies were found to co-exist with PfHRP2 antigenemia whereas only 1 of the 22 (4.5%) *T. gondii* IgG co-existed with PfHRP2 antigenemia (Table 6).

Table 6: Multiple Infections of donor blood

| Multiple infections | **Positive for PfHRP2 antigen** | **Positive for *Babesia* spp. antibody** | **Positive for human *Leishmania* spp. antibody** | **Positive for *T. gondii* IgG** |
|---|---|---|---|---|
| | n=69 | n=6 | n=16 | n=22 |
| PfHRP2 antigen | - | 6 (100%) | 0 (0%) | 1 (4.5%) |
| *Babesia* spp. antibody | - | - | 0 (0%) | 0 (0%) |
| *Leishmania* spp. antibody | - | 0 (0%) | - | 0 (0%) |
| *T. gondii* IgG | - | 0 (0%) | 0 (0%) | - |

## Demographic and clinical characteristics of recipients of donor units with parasite antigens and antibodies

A total of 440 patients were transfused with 571 donor blood units in this study. Most of the recipients of donor blood exposed to *P. falciparum, Babesia* spp., *Leishmania* spp. and *T. gondii* were over 19 years old. Similarly, more females received donor blood exposed to *P. falciparum, Babesia* spp, *Leishmania* spp. or *T. gondii* 61 (88.4%),6 (100%),12 (75%),19 (86.4%) *than males* 8 (11.6%),0 (0%),4 (25%),3 (13.6%) respectively.

Furthermore, the majority of recipients of exposed donor blood to TTPs were blood group O and anaemic. Notably, the mean body temperature of the four cohorts of blood recipients before transfusion was statistically similar (F =



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1.15, p = 0.33). Additionally, over 50% (60.9%, 66.7%, 50%, and 54.5%) of the blood recipients that received blood donors exposed to *P. falciparum, Babesia* spp., *Leishmania* spp. and *T. gondii* respectively, were married. Finally, majority of the recipients of exposed TTPs blood were informal sector employees. Finally, almost all the recipients were normothermic (mean temperature = 36.54%)

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Table 7: Distributions of sero-positivity of TTPs among variables of recipients of infected donor blood

| **Blood recipients' characteristics** | **Blood recipients' (n=571)** | **Positive for PfHRP2 antigen (n=6)** | **Positive for *Babesia* spp. Antibody (n=6)** | **Positive for *Leishmania* spp. Antibody (n=16)** | **Positive for *T. gondii* IgG (n=22)** |
|---|---|---|---|---|---|
| *Age range (years)* | | | | | |
| **0 – 9** | 54(12.7%) | 3 (4.3%) | 1 (16.7%) | 5 (31.3%) | 2 (9.1%) |
| **10 – 19** | 39(8.9%) | 7 (10.1%) | 0 (0%) | 1 (6.3%) | 3 (13.6%) |
| **20 – 29** | 139(31.6%) | 24 (34.8%) | 2 (33.3%) | 2 (12.5%) | 10 (45.5%) |
| **> 29** | 208(47.2%) | 35 (50.7%) | 3 (50%) | 8 (50%) | 7 (31.8%) |
| *Gender* | Gender | | | | |
| **Male** | 63(14.3%) | 8 (11.6%) | 0 (0%) | 4 (25%) | 3 (13.6%) |
| **Female** | 377(85.7%) | 61 (88.4%) | 6 (100%) | 12 (75%) | 19 (86.4%) |
| *Marital status* | | | | | |
| **Under marital age (<18years[2]** | 56(28.9%) | 3 (4.3%) | 0 (0%) | 6 (37.5%) | 2 (9.1%) |
| **Single** | 127(28.9%) | 21 (30.4%) | 2 (33.3%) | 1 (6.3%) | 8 (36.4%) |
| **Married** | 251(57.0%) | 42 (60.9%) | 4 (66.7%) | 8 (50%) | 12 (54.5%) |
| **Divorced** | 6(1.4%) | 3 (4.3%) | 0 (0%) | 1 (6.3%) | 0 (0%) |
| *Employment category* | | | | | |
| **Below employable age** | 47(10.7%) | 3 (4.3%) | 1 (16.7%) | 6 (37.5%) | 3 (13.6%) |
| **Formal sector** | 22(5.0%) | 2 (2.9%) | 0 (0%) | 1 (6.3%) | 0 (0%) |
| **Informal sector** | 242(55.0%) | 43 (62.3%) | 5 (83.3%) | 6 (37.5%) | 12 (54.5%) |
| **Student** | 5(4.3%) | 1 (1.4%) | 0 (0%) | 1 (6.3%) | 0 (%) |
| **Unemployed** | 124(1.1%) | 20 (%) | 0 (0%) | 2 (12.5%) | 7 (31.8%) |

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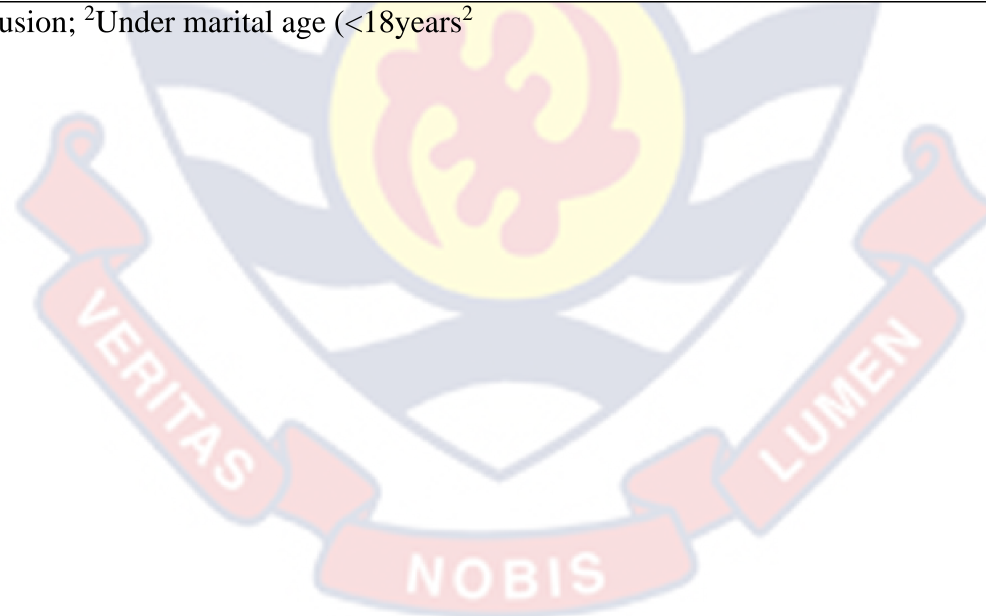
Table 7: Cont'd

| *Blood group* | **Blood group** | | | | |
|---|---|---|---|---|---|
| **O** | 219(49.8%) | 31 (45%) | 3 (50%) | 7 (43.7%) | 13 (59.1%) |
| **A** | 100(22.7%) | 16 (23.2%) | 1 (16.7%) | 4 (25%) | 5 (22.7%) |
| **B** | 103(23.4%) | 16 (23.2%) | 2 (33.3%) | 5 (31.3%) | 3 (13.6%) |
| **AB** | 18(4.1%) | 6 (8.7%) | 0 (0%) | 0 (0%) | 1 (4.5%) |
| ***Medical condition*** | Medical condition | | | | |
| **Anaemia** | 379(86.1%) | 57 (82.6%) | 6 (100%) | 14 (87.5%) | 19 (86.3%) |
| **Fetal distress** | 13(3.0%) | 1 (1.4%) | 0 (0%) | 0 (0%) | 1 (4.5%) |
| **Haemorrhage** | 48(10.9%) | 11 (15.9%) | 0 (0%) | 2 (12.5%) | 2 (9.1%) |
| **Mean[1] temperature (ºC)** | | 36.4±0.9 | 36.1±0.4 | 36.1±1.8 | 36.7±0.6 |

[1] Mean temperature before transfusion; [2]Under marital age (<18years[2]



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**Post-transfusion reaction**

Upon completion of the transfusion, the patient's vital signs temperature, pulse, Oxygen saturation ($SpO_2$), and blood pressure were recorded, and compared with the baseline values to detect acute transfusion reactions that may need immediate or further investigation and treatment. Fever, hematuria, urticaria, rash and sweating were also recorded as part of the post transfusion reaction. Haematuria refers to the presence of blood in the urine

The post-transfusion temperature readings of the recipients transfused with blood collected from donors exposed to TTPs were significantly higher than recipients of unexposed blood (37.5 vs. 37.2, t=6.3, p = 0.0035). Tukey multiple comparative tests indicated that the mean temperature readings of recipients of *P. falciparum*, *Babesia* spp. and *Leishmania* spp. exposed blood was higher than in recipients of unexposed blood while the reverse was observed in recipients of *T. gondii* exposed blood. Additionally, mean pulse readings were significantly higher in recipients of *P. falciparum* (83±3 vs. 87±5, $p<0.001$), *Leishmania* spp. (83±3 vs. 87±5, $p<0.001$) and *T. gondii* (83±3 vs. 89±4, $p<0.001$) compared to recipients of unexposed blood. Notwithstanding the foregoing, mean $SpO_2$ and blood pressure readings did not differ significantly across the blood recipient groups.

In this study 113 of the 440 blood recipients received TTPs exposed blood of which 9.7% (11/113) had blood transfusion reactions as opposed to unexposed group 12/113 given *that Babesia spp*. is known to co-exist with *P. falciparum* The observed differences were not significant (exposed vs. unexposed; p = 0.112). Among the 440 blood recipients, 327 received



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unexposed blood of which transfusion reactions were observed in 5.8% (19/327).

The transfusion reactions recorded were fever, hematuria, urticaria rash, respiratory distress and sweating. Both fever and sweating were associated with recipients of *P. falciparum* exposed blood ($x^2$ =4.2, p = 0.038) while sweating alone was associated with *Leishmania* spp. exposed blood ($x^2$ = 7.5, p = 0.006). Finally, urticaria rash was associated with a recipient of *Babesia* spp. exposed blood ($x^2$ = 14.4, p = 0.0014). None of the transfusion reaction variables were associated with recipients of *T. gondii* while none of the blood recipients experienced respiratory distress and haematuria, but three recipients of unexposed blood developed transient haematuria (Table 8)

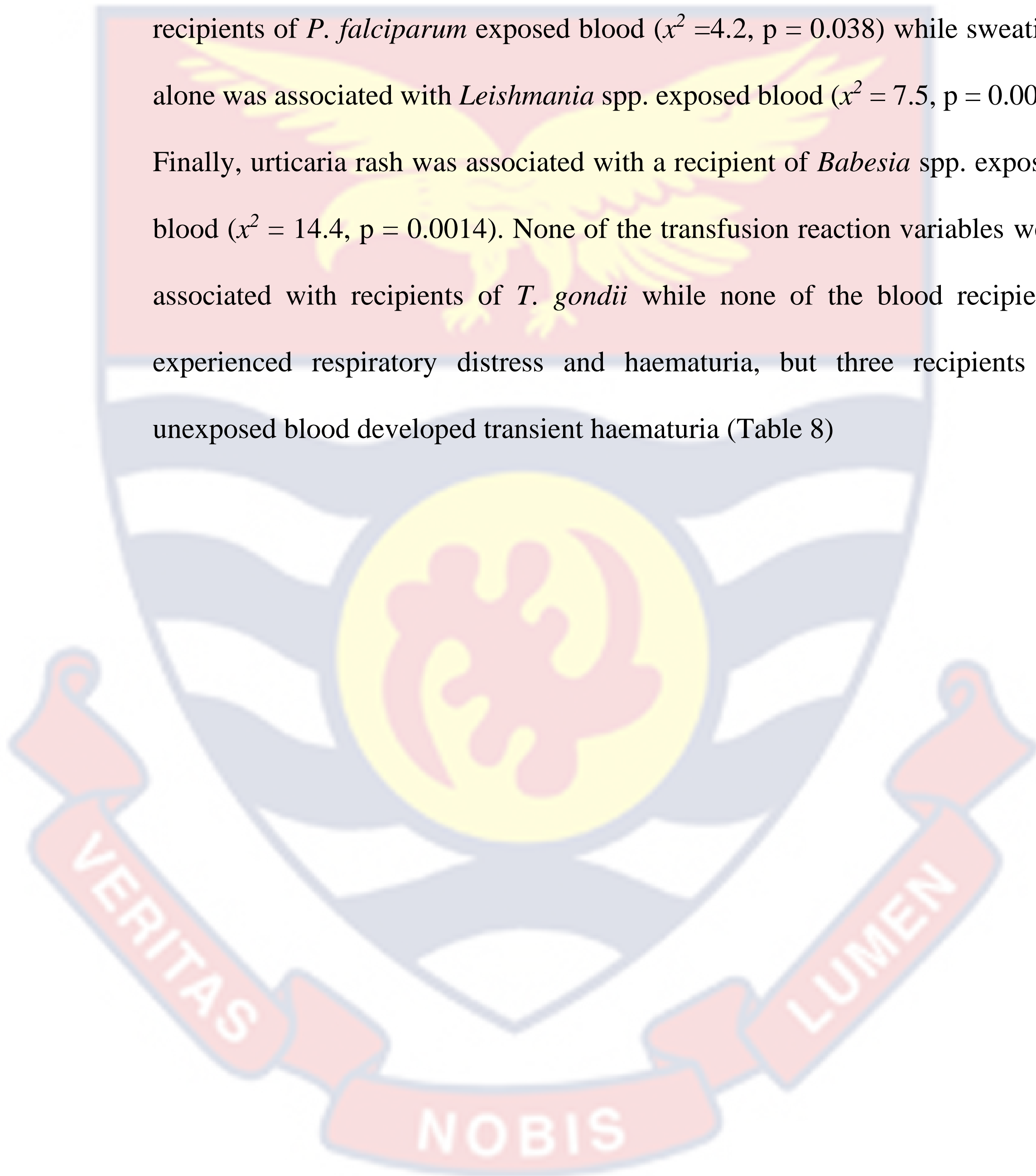



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Table 8: Variables associated with transfusion reactions within 24 hours.

| Variables | Recipients of TTPs unexposed blood (n=327) | Recipients of TTPs exposed blood (n=113) | | | | |
|---|---|---|---|---|---|---|
| | | *P. falciparum* (n=69) | *Babesia spp./ P. falciparum* (n=6) | *Leishmania* spp. (n=16) | *T. gondii* (n=22) | Statistical analysis |
| Temperature (ºC) (n=440) | 37.2±1.2 | 37.8±1.1* | 37.3±0.7 | 37.9±1.7* | 36.8±1.3* | 12.2 (<0.001)1 |
| Mean Pulse (bpm) (n=440) | 83±3 | 87±5* | 84±3 | 87±5* | 89±4* | 8.97 (<0.001)1 |
| $SpO_2$ (%) (n=440) | 96.8±3.2 | 95.1±2.7 | 97.4±3.0 | 95.4±2.0 | 96.1±2.2 | 2.47 (0.408)1 |
| Blood pressure | | | | | | |
| Diastolic (mmHg) (n=347) | 89.6±7.7 | 86.1±4.6 | 85.9±4.9 | 91.6±5.7 | 94.7±4.3 | 1.30 (0.889)1 |
| Systolic (90=120) (n=347) | 139.7±8.1 | 134.6±5.1 | 135.3±4.4 | 137.1±3.8 | 140.7±2.5 | 3.74 (0.067)1 |
| **Sub total** | 19 | 7 | 1 | 4 | 0 | |
| Fever (n=424) | 3 (0.9%) | 3 (4.3%)** | 0 (0%) | 1 (6.3%) | 0 (0%) | |
| Hematuria (n=406) | 3 (0.9%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | |
| Urticaria rash (n=440) | 2 (0.6%) | 1 (1.4%) | 1 (16.7%)** | 0 (0%) | 0 (0%) | |
| Respiratory distress (n=440) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | |
| Sweating (n=440) | 11 (3.4%) | 3 (4.3%)** | 0 (0%) | 3 (18.8%)** | 0 (0%) | |

Presented as F-ratio (p-value); * Mean values significantly different from unexposed blood mean values (Post Hoc Tukey test); ** Variable significantly associated parasite exposed donor blood using the Fishers' exact test of independence.



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**Comparison between the prevalence of *P. falciparum* antigenemia and parasitemia in donor blood by mRDT and microscopy**

This section focuses only on the malaria parasite (*P. falciparum*) in donor blood determined by mRDT and microscopy as presented in Table 9.

*P. falciparum* detection was found to be higher by mRDT than by microscopy. Out of the 571 donor blood units, 69 (12.1%) exhibited *P. falciparum* positive for HRP2 mRDT whereas 48(8.4%) exhibited positive parasitemia by microscopy.

*P. falciparum* antigenemia and parasitemia were observed in all blood types, though at varying frequencies, except blood type A negative and AB negative. In all cases where *P. falciparum* parasite was detected by microscopy, it was observed that *P. falciparum* HRP2 antigenemia was also identified.

The analysis by blood group type showed that all positive cases identified by microscopy were also identified by mRDT irrespective of the blood group.

For blood group A positive, 9 of the 12 samples found to be positive by RDT, were all identified by microscopy.

24 (8.2%) of the 35 donor blood units of blood group O positive blood type were positive for *P. falciparum* by microscopy. Of the 12 donor units with P. falciparum antigenemia, 9 (7.8%) were found to be positive for blood group A positive. Similarly, seven (5.8%) of the 12 of the donor blood type B positive with *P. falciparum* antigenemia was positive. All the number of donor blood units with blood groups O negative and B negative with *P. falciparum* antigenemia were positive microscopically.



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When comparing C to concentrated red blood cells, the level of *P. falciparum* antigenemia was found to be higher in concentrated red blood cells compared to that in concentrated red blood cells. In whole blood, *P. falciparum* antigenemia by mRDT was recorded in 62 (11.7%) donor blood units compared to 7 (16.2%) in CRBC. For *P. falciparum* determined by microscopy 45(8.7%) positive identification were recorded for whole blood compared to 3(7.0%) in CRBC.

Out of the donor units tested, 52 (11.5%) were positive for *P. falciparum* antigenemia, while 37 (8.2%) were positive for microscopy after one day of storage. (Table 9).

Table 9: Prevalence of *Plasmodium falciparum* infection in donor blood by mRDT and microscopy

| | **mRDT** | | **Microscopy** | |
|---|---|---|---|---|
| | Positive n =69 (12.1%) | Negative n = 502 (87.9%) | Positive n = 48 (8.4%) | Negative n = 523 (91.6%) |
| ***Blood group*** | | | | |
| O Rh D positive (n=294) | 35 (11.9%) | 259 (88.1%) | C | 270 (91.8%) |
| O Rh D negative (n=10) | 4 (40.0%) | 6 (60.0%) | 4 (40.0%) | 6 (6.0%) |
| A Rh D positive (n=115) | 12 (10.4%) | 103 (89.6%) | 9 (7.8%) | 106 (92.2%) |
| A Rh D negative (n=8) | 0 (0.0%) | 8 (100.0%) | 0 (0.0%) | 8 (100%) |
| B Rh D positive (n=120) | 12 (10.0%) | 108 (90.0%) | 7 (5.8%) | 113 (94.2%) |
| B Rh D negative (n=9) | 1 (11.1%) | 8 (88.9%) | 1 (11.1%) | 8 (88.9%) |
| AB Rh D positive (n=15) | 5 (33.3%) | 10 (66.7%) | 3 (20.0%) | 12 (80.0%) |
| AB Rh D negative (n=0) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| ***Type of product*** | | | | |
| Whole blood (n=528) | 62 (11.7%) | 466 (88.3%) | 45 (8.5%) | 483 (91.5%) |
| CRBC (n=43) | 7 (16.2%) | 36 (83.7%) | 3 (7.0%) | 40 (93.0%) |
| ***Days in storage*** | | | | |
| 1 (n=452) | 52 (11.5%) | 400 (88.5%) | 37 (8.2%) | 415 (91.8%) |
| 2 – 5 (n=83) | 11 (13.3%) | 72 (86.7%) | 7 (8.4%) | 76 (91.6%) |
| 6 – 9 (n=15) | 2 (13.3%) | 13 (2.3%) | 2 (13.3%) | 13 (86.7%) |
| 10 – 13 (n=10) | 1 (10.0%) | 9 (90.0%) | 1 (10.0%) | 9 (90.0%) |
| 14 – 17 (n=4) | 2 (50.0%) | 2 (50.0%) | 1 (25.0%) | 3 (75.5%) |
| > 18 (n=7) | 1 (14.3%) | 6 (85.7%) | 0 (0.0%) | 7 (100.0%) |

mRDT – malaria rapid diagnostic testing, CRBC – Concentrated red blood cells



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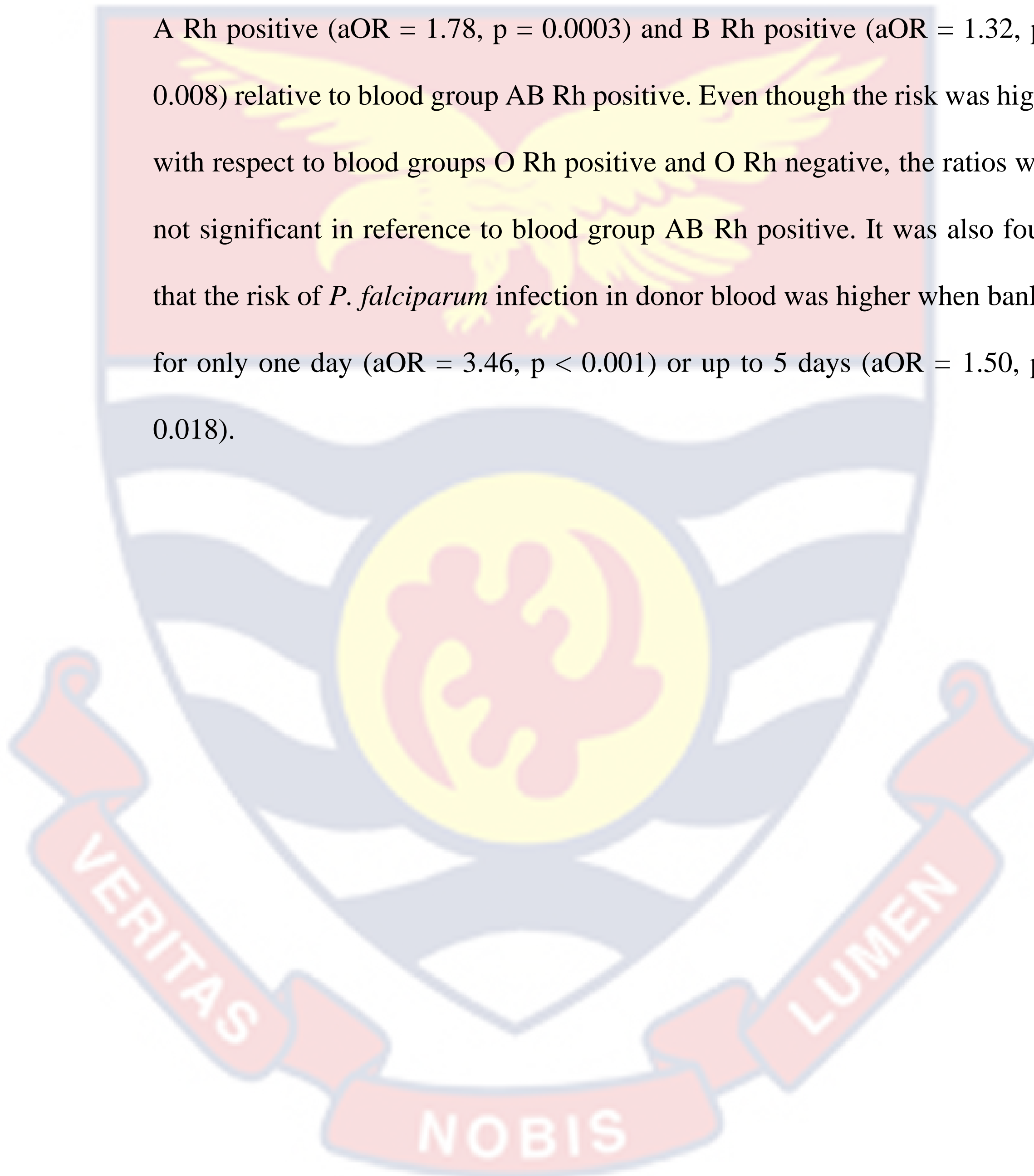

**Distribution of *P. falciparum* parasitemia in blood donors**

Distribution of *P. falciparum* parasitemia in donor blood units are presented in (Table 9). From the table, it is observed that the risk of having donor blood infected with *P. falciparum* is significantly higher in blood groups A Rh positive (aOR = 1.78, p = 0.0003) and B Rh positive (aOR = 1.32, p = 0.008) relative to blood group AB Rh positive. Even though the risk was higher with respect to blood groups O Rh positive and O Rh negative, the ratios were not significant in reference to blood group AB Rh positive. It was also found that the risk of *P. falciparum* infection in donor blood was higher when banked for only one day (aOR = 3.46, p < 0.001) or up to 5 days (aOR = 1.50, p = 0.018).



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Table 10: Distribution of *P. falciparum* parasitemia in blood donors

| | **Sub Total (n)** | **Positive** | **Negative** | ***aOR (95% CI)** | **p-value** |
|---|---|---|---|---|---|
| ***Total*** | 571 | 48 (8.4%) | 523 (91.6%) | | |
| ***Blood group*** | | | | | |
| O Rh D positive | 294 | 24 (8.2%) | 270 (91.8%) | 1.23 (0.35-4.27) | 0.067 |
| O Rh D negative | 10 | 4 (40.0%) | 6 (60.0%) | 1.01 (0.62-1.64) | 0.122 |
| A Rh D positive | 115 | 9 (7.8%) | 106 (92.2%) | 1.78 (1.02-3.13) | 0.0003 |
| A Rh D negative | 8 | 0 (0.0%) | 8 (100.0%) | 0.71 (0.42-1.19) | 0.413 |
| B Rh D positive | 120 | 7 (5.8%) | 113 (94.2%) | 1.32 (0.80-2.17) | 0.008 |
| B Rh D negative | 9 | 1 (11.1%) | 8 (88.9%) | 0.71 (0.42-1.19) | 0.331 |
| AB Rh D positive | 15 | 3 (20.0%) | 12 (80.0%) | Reference value | |
| ***Type of product*** | | | | | |
| Whole blood | 528 | 45 (8.5%) | 483 (91.5%) | 12.4 (0.37-4.18 | 0.725 |
| CRBC | 43 | 3 (7.0%) | 40 (93.0%) | Reference value | |
| ***Days in storage*** | | | | | |
| 1 | 452 | 37 (8.2%) | 415 (91.8%) | 3.46 (1.23-8.52) | <0.001 |
| 2 – 5 | 83 | 7 (8.4%) | 76 (91.6%) | 1.50 (1.08-2.09) | 0.018 |
| 6 – 9 | 15 | 2 (13.3%) | 13 (86.7%) | 0.17 (0.13-0.24) | 0.410 |
| 10 – 13 | 10 | 1 (10.0%) | 9 (90.0%) | 0.12 (0.08-0.18) | 0.233 |
| 14 – 17 | 4 | 1 (25.0%) | 3 (75.0%) | 1.07 (0.58-2.79) | 0.07 |
| > 18 | 7 | 0 (0.0%) | 7 (100.0%) | 1.06 (0.12-2.14) | 0.091 |

***Adjusted odds ratio** is an odds ratio that has been adjusted to account for other predictor variables in a model. It helps understand how a predictor variable affects the odds of an event, *after* adjusting for the effect of other predictor variables.



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**Characteristics of donor blood units with microscopy detectable malaria parasitemia**

Infected blood group A positive carried the highest number of malaria parasites with an estimated mean parasitemia of 1,058,374,028 parasites / unit of donor blood with average volume of 388.8 mL, as shown in Table 10. This was followed by blood group O positive with an estimated parasitemia of 764,867,704 parasites / unit of donor blood with average volume of 410.2 mL. The rest of the blood group contained between 250 – 500 million parasites/unit of donor blood while blood group AB positive contained the least parasitemia with an estimated count of 209,666,067 parasites / unit of donor blood (Table 11).

Table 11: Estimated *P. falciparum* parasitemia per unit of blood transfused

| Blood group | Number of donor units | Mean weight (g) of donor blood | Mean estimated volume (µL) of donor blood transfused[1] | Mean parasitemia (/µL) | Total mean estimated malaria parasite load per unit of blood |
|---|---|---|---|---|---|
| O Rh D positive | 24 | 410.2 | 410229.1 | 1858 | 764,867,704 |
| O Rh D negative | 4 | 415.2 | 415175.3 | 1123 | 472,942,025 |
| A Rh D positive | 9 | 388.8 | 388822.2 | 2722 | 1,058,374,028 |
| B Rh D positive | 7 | 370.5 | 370499.9 | 962 | 376,359,514 |
| B Rh D negative | 1 | 385.1 | 385199.8 | 580 | 223,358,000 |
| AB Rh D positive | 3 | 274.9 | 274933.0 | 750 | 209,666,067 |

Weight-to-volume conversion is based on the specific gravity of blood being approximately 1gm/mL.



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**Distribution of blood recipients in Nsawam Government Hospital**

In all, 440 blood recipients received 571 donor blood units. Of these blood recipients, 366 (83.2%), 50 (11.4%), 17 (3.9%), 5 (1.1%), and 2 (0.4%) received one, two, three, four and five unit(s) of blood respectively. The age range of the blood recipient was 1 month 4 days to 83 years. The modal age range was 21 – 30 years (Figure 11a). Female blood recipients were disproportionally high (85.68%) (Figure11b). More than half of the recipients were married (56.59%) while 12.5% of the blood recipients were below marital age (≤ 18 years). Additionally, majority of the recipients were petty traders (32.72%) while pensioners were in the minority (0.45%)). The blood recipients received blood for various reasons; 379 (86.14%), 55 (12.5%) and 6 (1.36%) were transfused on account of anaemia, haemorrhage and fetal distress respectively. The blood recipients were hospitalized between 1 – 21 days; a little over 92 % of them were hospitalized between 1 – 3 days while the rest (approx. 8%) were hospitalized up for to 21 days. Furthermore, majority of the recipients were blood group O positive (47.95%) whereas only one (0.23%) of the recipients was blood group AB negative (Table 12).



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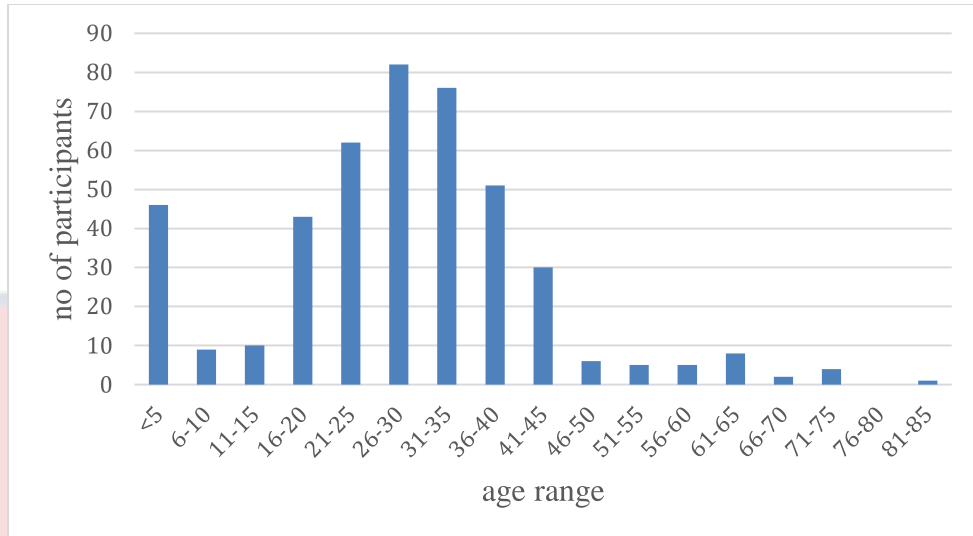


*Figure 11a: Age groups (years) of the blood transfusion recipients*

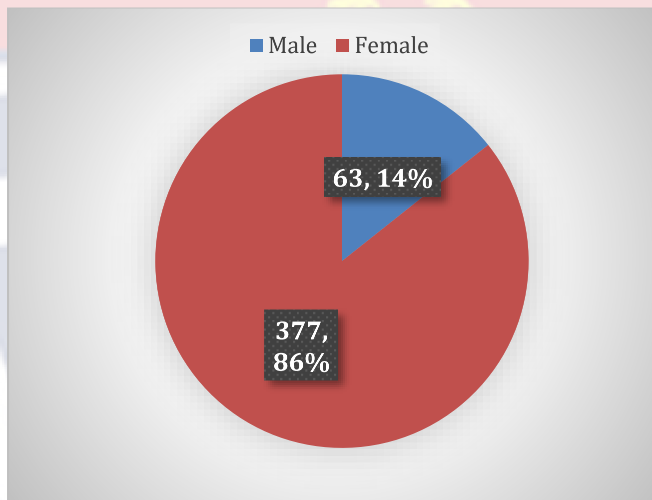


*Figure 11b: Gender of the blood transfusion recipients*

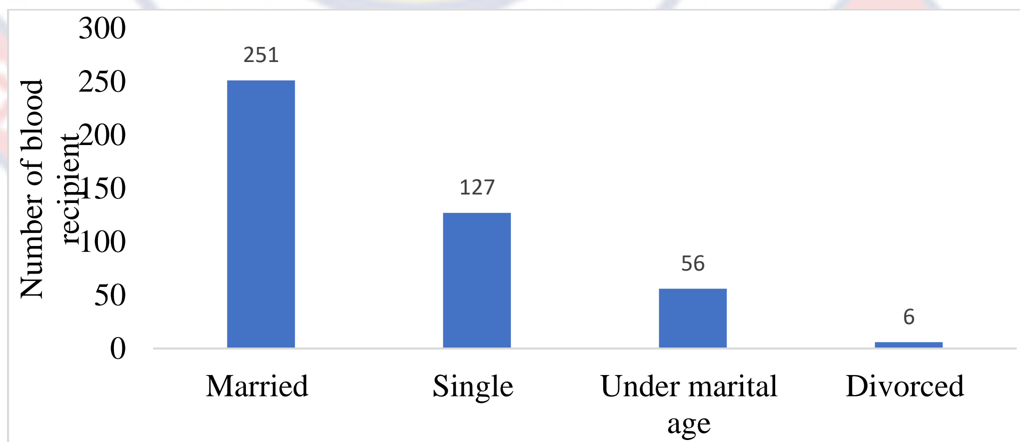


*Figure 11c: Marital status among blood transfusion recipient*



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Table 12: Clinical characteristics of the recipients of donor blood

| Variables | Number of blood recipients (n =440) | Percentage |
|---|---|---|
| ***Clinical condition classification*** | | |
| Anaemia[1] | 379 | 86.1 |
| Haemorrhage[2] | 55 | 12..5 |
| Fetal distress[3] | 6 | 1.4 |
| ***Number of days on admission*** | | |
| 1 – 3 | 405 | 92.1 |
| 4 – 6 | 15 | 3..4 |
| 7- 9 | 4 | 0.9 |
| > 10 | 16 | 3.6 |
| ***Mean temperature ($^o$ C)*** | 36.54 | |
| ***Blood group*** | | |
| O Rh D positive | 211 | 48.0 |
| O Rh D negative | 7 | 1.6 |
| A Rh D positive | 93 | 21.1 |
| A Rh D negative | 7 | 1.6 |
| B Rh D positive | 97 | 22.1 |
| B Rh D negative | 7 | 1.6 |
| AB Rh D positive | 17 | 3.9 |
| AB Rh D negative | 1 | 0.2 |

[1] Anaemia [(moderate anaemia hemoglobin level 8 – 11 g/dL (n=330); severe anaemia < 8 g/dL (n=49)]; [2] Haemorrhage (cesarian section n=21, myomectomy n=19, ectopic n=12, hernia surgery n=1, pile bleeding n=1, prolonged labor n=1); [3] Fetal distress (oligohydramnios n=3, cord prolapse n=2, intra-uterine fetal death n=1)

### *P. falciparum* parasite density transfused to patients with various clinical conditions

Forty-eight blood recipients received donor blood with microscopically detected *P. falciparum*. Of this number, only one received malaria parasites less than 99 million parasites per donor unit. This blood was transfused to a 2-month-old baby with anaemia with blood group AB. Additionally, 17 (35.4%) received an estimated 100 – 299 million parasites per unit of blood. These recipients were females with majority being of blood group O with over 70% of them being anaemic.  Furthermore, 14 (29.2%) of the recipients received donor blood of parasitemia between 300 – 499 million parasites per donor blood. Both



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males (21.4%) and females (78.6%), with a mean age of 29 years of which most were anemic (64.3%), received such blood. Six recipients received blood with parasitemia 500 – 699 million parasites, with most of them being females (83.3%), blood group O (50%) and anemic (83.3%). Two males and one female were transfused with the blood of parasitemia 700 – 899 million parasites. Transfusion was on account of anaemia (66.7%). Finally, seven female recipients received the blood of parasitemia > 900 million parasites. Most of these recipients were blood group A on account of anaemia (100%) (Table 13)

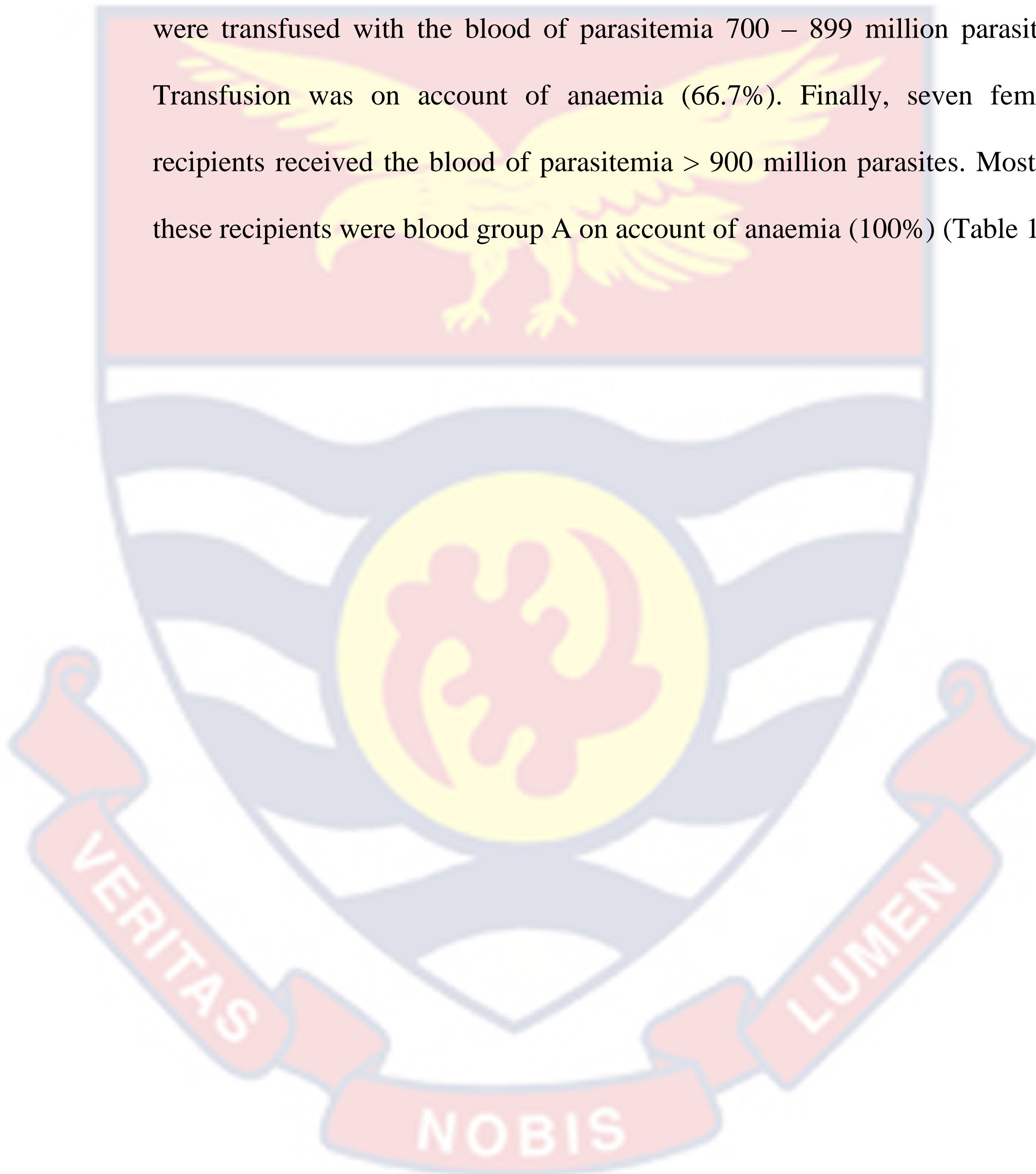




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Table 13: *P. falciparum* parasite density transfused to patients with various clinical conditions

| **Parasite load** | | | **Gender** | | | **Blood group** | | | | **Clinical conditions** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **x10$^6$ /unit of blood** | **Number of infected blood recipients (n=48)** | **Mean age (yrs)** | **Male** | **Female** | **Mean temp ($^o$ C)** | **O -/+** | **A -/+** | **B -/+** | **AB -/+** | **Anaemia (n=36)** | **Fetal distress (n=1)** | **Haemorrhage (n=11)** |
| < 99 | 1 (2.1%) | < 1 | 1 (100%) | 0 (0%) | 34.2 | 0 (0%) | 0 (0%) | 0 (0%) | 1 (100%) | 1 (100%) | 0 (0%) | 0 (0%) |
| 100 – 299 | 17 (35.4%) | 34 | 0 (0%) | 17 (100%) | 36.3 | 9 (53%) | 3 (17.6%) | 3 (17.6%) | 2 (11.8%) | 12 (70.6%) | 0 (0%) | 5 (29.4%) |
| 300 – 499 | 14 (29.2%) | 29 | 3 (21.4%) | 11 (78.6%) | 36.0 | 8 (57.1%) | 2 (14.3%) | 3 (21.4%) | 1 (7.1%) | 9 (64.3%) | 0 (0%) | 5 (35.7%) |
| 500 – 699 | 6 (12.5%) | 35 | 1 (16.7%) | 5 (83.3%) | 36.1 | 3 (50%) | 2 (33.3%) | 1 (16.7%) | 0 (0%) | 5 (83.3%) | 1 (16.7%) | 0 (0%) |
| 700 – 899 | 3 (6.3%) | 40 | 1 (33.3%) | 2 (66.7%) | 36.5 | 1 (33.3%) | 1 (33.3%) | 1 (33.3%) | 0 (0%) | 2 (66.7%) | 0 (0%) | 1 (33.3%) |
| > 900 | 7 (14.6%) | 30 | 0 (0%) | 7 (100%) | 37.1 | 1 (14.3%) | 5 (71.4%) | 1 (14.3%) | 0 (0%) | 7 (100%) | 0 (0%) | 0 (0%) |



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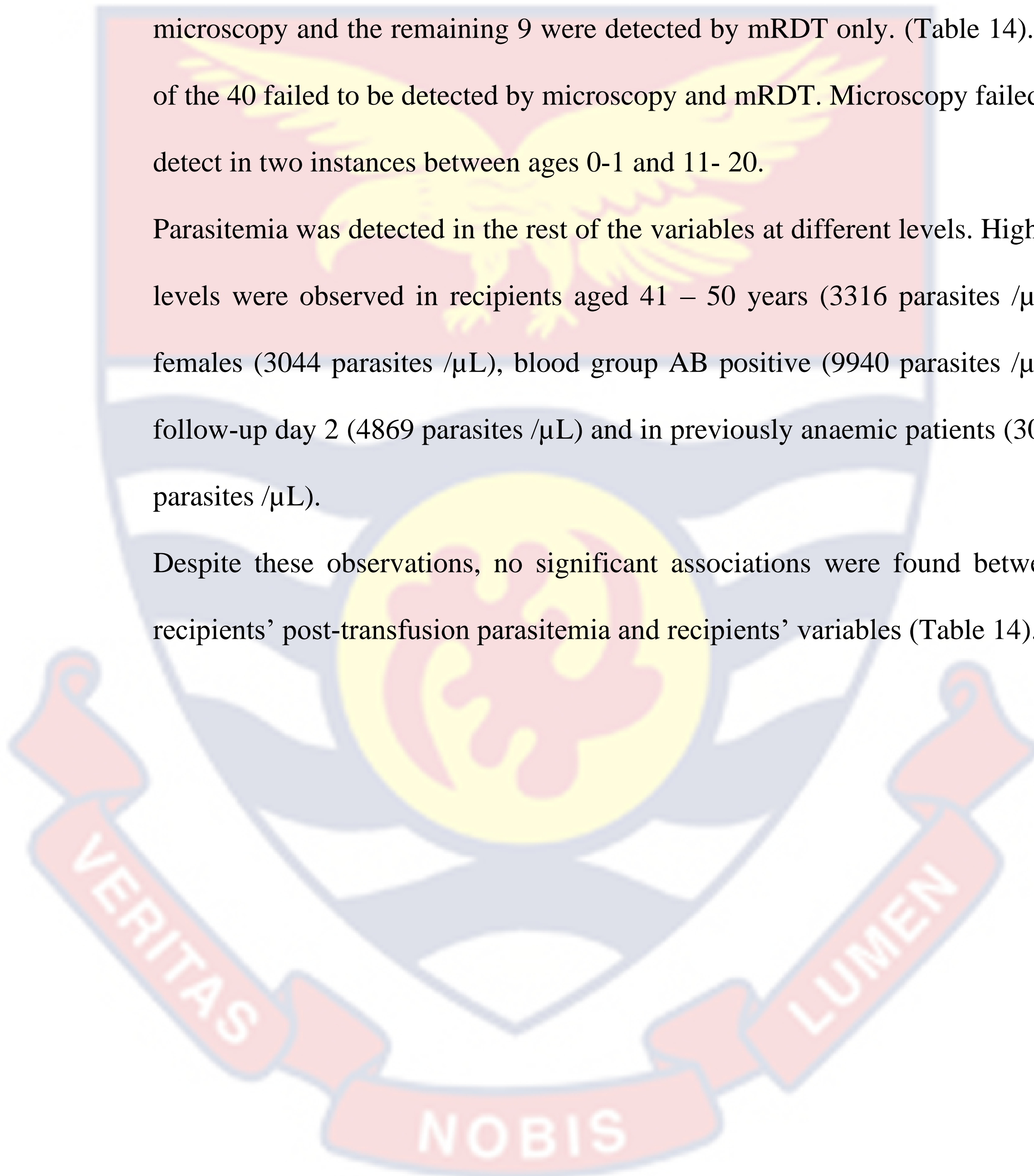

**Parasitological outcome of follow-up of recipients of infected blood**

There were 48 recipients of microscopically detected *P. falciparum* in the donor blood unit of which follow-up was completed for 40 (83.3%). Of these 23 out of the 40 were positively identified. 14 were detected by both mRDT and microscopy and the remaining 9 were detected by mRDT only. (Table 14). 17 of the 40 failed to be detected by microscopy and mRDT. Microscopy failed to detect in two instances between ages 0-1 and 11- 20.

Parasitemia was detected in the rest of the variables at different levels. Highest levels were observed in recipients aged 41 – 50 years (3316 parasites /µL), females (3044 parasites /µL), blood group AB positive (9940 parasites /µL), follow-up day 2 (4869 parasites /µL) and in previously anaemic patients (3075 parasites /µL).

Despite these observations, no significant associations were found between recipients' post-transfusion parasitemia and recipients' variables (Table 14).



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Table 14: Parasitological outcome of recipients' follow-up

| Variables | Negative n=17 | Positive by mRDT only n=23 | Positive by mRDT and microscopy n=14 | Mean Parasitemia (/µL) |
|---|---|---|---|---|
| ***Age range (year)***[1] | | | | |
| 0 – 1 | 0 (0%) | 1 (4.3%) | 0 (0%) | Not applicable |
| 2 – 5 | 0 (0%) | 0 (0%) | 0 (0%) | Not applicable |
| 6 – 10 | 0 (0%) | 1 (4.3%) | 1 (7.1%) | 960 |
| 11 – 20 | 2 (11.8%) | 1 (4.3%) | 0 (0%) | Not applicable |
| 21 – 30 | 6 (35.3%) | 12 (52.2%) | 5 (35.7%) | 3316 |
| 31 – 40 | 6 (35.3%) | 2 (8.7%) | 2 (14.3%) | 1854 |
| 41 – 50 | 2 (11.8%) | 4 (17.4%) | 4 (28.6%) | 3867 |
| 51 – 60 | 1 (5.9%) | 0 (0%) | 0 (0%) | Not applicable |
| > 60 | 0 (0%) | 2 (8.7%) | 2 (14.3%) | 1231 |
| ***Gender***[2] | | | | |
| Male | 2 (11.8%) | 4 (17.4%) | 3 (21.4%) | 1991 |
| Female | 15 (88.2%) | 19 (82.6%) | 11 (78.6%) | 3044 |
| ***Blood group***[3] | | | | |
| O -/+ | 9 (52.9%) | 6 (26.1%) | 5 (35.7%) | 1002 |
| A -/+ | 5 (29.4%) | 8 (34.8%) | 5 (35.7%) | 2624 |
| B -/+ | 2 (11.8%) | 5 (21.7%) | 3 (21.4%) | 3605 |
| AB + | 1 (5.9%) | 4 (17.4%) | 1 (7.1%) | 9940 |
| ***Follow ups*** | | | | |
| Day 2 | | 1 (4.3%) | 1 (7.1%) | 4869 |
| Day 7 | | 6 (26.1%) | 3 (21.4%) | 2308 |
| Day 14 | | 5 (21.7%) | 3 (21.4%) | 4238 |
| Day 21 | | 4 (17.4%) | 2 (14.3%) | 2138 |
| Day 28 | | 3 (13.0%) | 2 (14.3%) | 3752 |
| Day 35 | | 4 (17.4%) | 3 (21.4%) | 818 |
| ***Medical condition necessitating the blood transfusion***[4] | | | | |
| Anaemia | 11 (64/7%) | 21 (91.3%) | 12 (85.7%) | 3075 |
| Fetal distress | 0 (0%) | 1 (4.3%) | 1 (7.1%) | 996 |
| Haemorrhage | 6 (35.3%) | 1 (4.3%) | 1 (7.1%) | 1521 |

[1] Suspected TTM was not associated with age ($x^2$ = 1.61, p=0.656)

[2] Suspected TTM was not associated with gender ($x^2$ – 0.31, p=0.587)

[3] Suspected TTM was not associated with blood group ($x^2$ – 0.39, p=0.941)

[4] Suspected TTM was not associated with medical condition necessitating blood transfusion ($x^2$ – 1.39, p=0.497)



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**Attributes of infected donor blood units that resulted in recipients' P. falciparum parasitemia**

Table 15 describes the 48 transfused pf positive donor blood units. In this study 10 (71.4%) of the donor blood that have been stored for a day was positive for *Plasmodium falciparum*, those of blood group O positive (50%) and units with mean total parasitemia between 100 – 299 million parasites per unit of blood. On the other hand, relatively high recipient parasitemia was observed in recipients that were transfused with blood stored for over 5 days (5344 parasites/µL), infected blood of type AB positive (9940 parasites/µL) and donor blood of total parasitemia more than 900 million parasites (5308 parasites/µL). Notwithstanding the foregoing, the number of donor blood storage days, donor blood group and infected donor blood total parasitemia did not associate with frequency of detecting malaria parasitemia in infected blood recipients. All 14(100%) of the donor blood units were whole blood (Table 15).



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Table 15: *P. falciparum* parasitemia in donor blood and recipient blood on follow up

| Parameter | Mean total parasitemia in transfused blood | Number of recipients with malaria parasitemia n = 14 | $x^2$ (p-value) | Mean recipient parasite count (/µL) on follow-up testing |
|---|---|---|---|---|
| ***Storage days*** | | | 0.44 (0.801) | |
| 1 (n=37) | 845,929,080 | 10 (71.4%) | | 2694 |
| 2 – 5 (n=7) | 939,542,300 | 2 (14.3%) | | 2272 |
| > 5 (n=4) | 296,303,200 | 2 (14.3%) | | 5344 |
| ***Blood group*** [a] | | | 0.07 (0.999) | |
| O + (n=24) | 1,062,909,957 | 7 (50%) | | 2068 |
| O - (n=4) | 715,022,100 | 1 (7.1%) | | 1521 |
| A + (n=9) | 597,028,267 | 3 (21.4%) | | 3859 |
| B + (n=7) | 322,101,200 | 2 (14.3%) | | 2080 |
| AB + (n=3) | 340,300,000 | 1 (7.1%) | | 9940 |
| ***Parasite count range*** [b] | | | 1.67 (0.795) | |
| 100 – 299 (n=17) | 230,005,840 | 5 (35.7%) | | 1653 |
| 300 – 499 (n=14) | 437,165,867 | 3 (21.4%) | | 4797 |
| 500 – 699 (n=6) | 547,063,200 | 1 (7.1%) | | 996 |
| 700 – 899 (n=3) | 758,429,050 | 2 (14.3%) | | 1299 |
| > 900 (n=7) | 2,135,176,967 | 3 (21.4%) | | 5308 |

[a] The blood groups summed up to 47 because B negative did not result in any recipient parasitemia; [b] frequency of parasite count range did not add 48 because parasitemia less than 100 million did not result in recipient follow-up up parasitemia.



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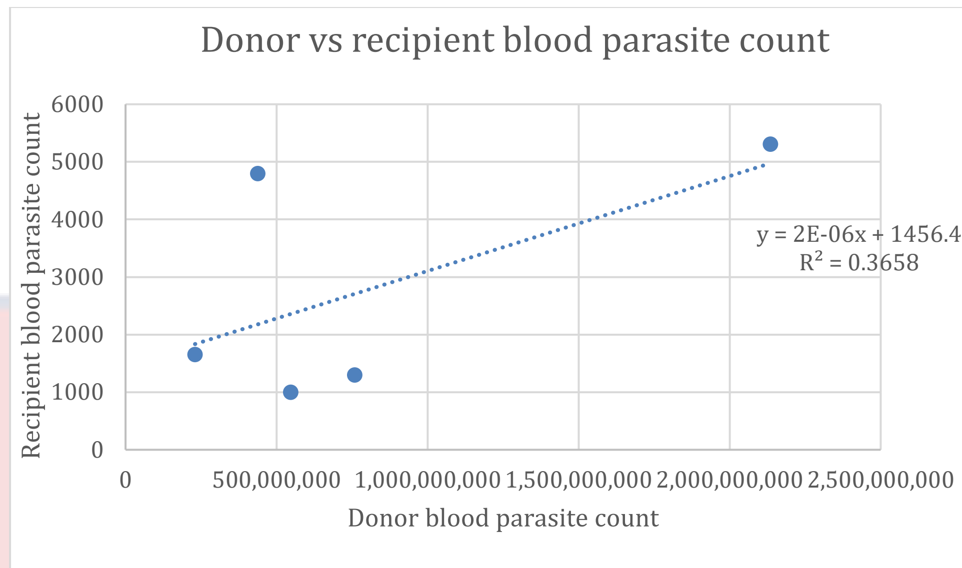


*Figure 12: Donor vs recipient blood parasitic count*

**Parasitological outcome of *Plasmodium* infected recipients**

A total of 14 patients were followed up through home visits. The level of parasitemia was analysed by age range, gender, blood group, follow-up days and recipients with medical conditions.

In terms of age range, it was observed that were observed in recipients aged 41 – 50 years (3316 parasites /µL), in females (3044 parasites /µL) and blood group AB positive (9940 parasites /µL). With regards to follow up days and recipients with medical conditions necessitating the blood transfusion the highest levels of parasitemia were found in follow-up day 2 (4869 parasites /µL) and in previously anaemic patients (3075 parasites /µL). Despite these observations, no significant associations were found between post-transfusion parasitemia and recipients' subcategories as the p values were high (Table)



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Table 16: Parasitological outcome of *Plasmodium* infected recipients' follow-up

| Variables | Positive by microscopy n=14 | Mean Parasitemia (/µL) |
|---|---|---|
| **Age range (year)** | | |
| **1 – 10** | 1 (7.1%) | 960 |
| **11 – 20** | 0 (0%) | Not applicable |
| **21 – 30** | 5 (35.7%) | 3316 |
| **31 – 40** | 2 (14.3%) | 1854 |
| **41 – 50** | 4 (28.6%) | 3867 |
| **51 – 60** | 0 (0%) | Not applicable |
| **> 60** | 2 (14.3%) | 1231 |
| **Gender** | | |
| **Male** | 3 (21.4%) | 1991 |
| **Female** | 11 (78.6%) | 3044 |
| **Blood group** | | |
| **O -/+** | 5 (35.7%) | 1002 |
| **A -/+** | 5 (35.7%) | 2624 |
| **B -/+** | 3 (21.4%) | 3605 |
| **AB +** | 1 (7.1%) | 9940 |
| **AB -** | 0 (0%) | |
| **Follow up day** | | |
| **Day 2** | 1 (7.1%) | 4869 |
| **Day 7** | 3 (21.4%) | 2308 |
| **Day 14** | 3 (21.4%) | 4238 |
| **Day 21** | 2 (14.3%) | 2138 |
| **Day 28** | 2 (14.3%) | 3752 |
| **Day 35** | 3 (21.4%) | 818 |
| **Medical condition necessitating the blood transfusion** | | |
| **Anaemia** | 12 (85.7%) | 3075 |
| **Fetal distress** | 1 (7.1%) | 996 |
| **Haemorrhage** | 1 (7.1%) | 1521 |



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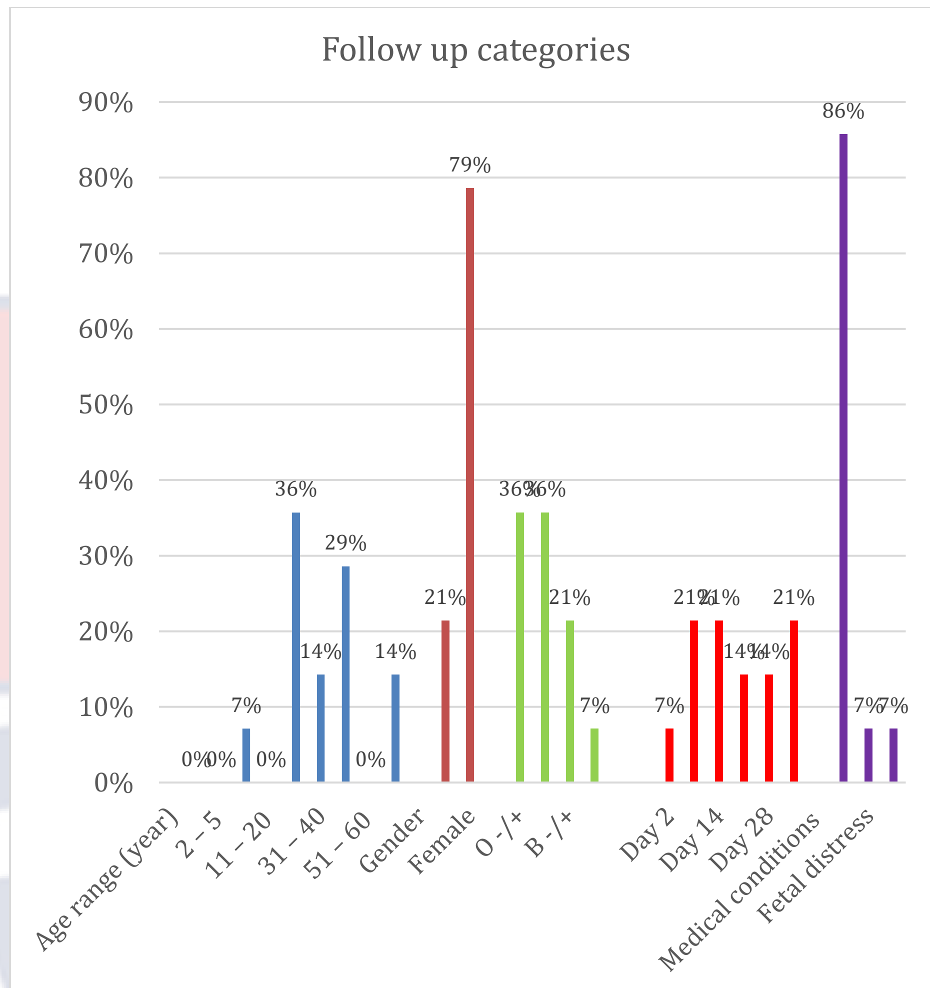


*Figure 13: Follow-up by categories*

There was no parasitemia recorded within the ages 0-5,11-10 and ages 51-60.yrs. The age group of 21-30 had the highest incidence of parasitemia (36%), followed by the age group of 41-50 (29%). The numbers for the age groups of 31-40 and those over 60 were similar. As depicted in Figure 14, females accounted for 76% of the recorded parasitemia cases during the follow-up, while males accounted for 21%. Among blood groups, the prevalence of blood groups O and A was the same (36%), followed by blood group B (28%) and blood group AB (7%). Possible cases of transfusion-transmitted malaria were recorded for all days, with the highest incidence (21%) on days 7, 14, and 35, while day 2 had the lowest incidence.



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**Analysis of *P. falciparum* homologous gene sequences**

In this study, the 28 samples that were microscopically positive for malaria parasites and mRDT (14 each from the donor blood and corresponding blood recipient) were sent for genotyping and were evaluated for transfusion-transmitted malaria (Table 17). None of these 14 recipients had malaria parasitemia prior to transfusion.

For Sample ID 120, the amino acid sequences of the parasite isolated from the donor blood was closely related to the parasite isolated from the corresponding blood recipient. However, at amino acid position 86, the parasite isolated from the recipient was tyrosine (Y) while asparagine (N) was observed in the parasite isolated from the donor blood. This does not make it a TTM. For Sample IDs, 237, ID 276 and ID 182 the percentage sequence identity was 78%, 70% and 74% respectively. These percentages were due to variations in the amino acid sequence of the donor and recipient at different amino acid positions as shown in Table 17. The same was observed for Sample IDs 157 (80%), 83 (83%), 92 (83%), 128 (83%), 150 (87%), 240 (83%) and 116 (87%) even though the percentages of homology were over 80%, several amino acid differences were observed in their sequences. It was observed that in Sample ID 122, the amino acid sequences observed in the parasite isolated from the donor blood unit were the same as was found in the corresponding blood recipient. It was intriguing to observe that the Kelch 13 mutation in both sets of parasites were the same (P413L). With this observation, TTM could be said to have occurred.



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Table 17: Molecular determination of transfusion transmitted malaria (TTM)

| Study No. | Amino acid positions | 51 | 59 | 72 | 73 | 74 | 75 | 76 | 86 | 108 | 127 | 164 | 184 | 193 | 326 | 356 | 415 | 436 | 437 | 484 | 540 | 581 | 613 | 1246 | K13 | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 157 | Pf_Donor | N | R | C | V | M | N | K | N | N | V | I | Y | D | N | I | E | S | A | T | K | A | A | D | WT | 82% |
| | Pf_Recipient | N | C | C | V | M | N | K | N | S | V | I | Y | D | N | I | E | A | G | T | K | * | * | N | V534A | |
| 202 | Pf_Donor | N | C | C | V | M | N | K | Y | S | V | I | F | D | N | I | E | F | G | T | K | A | S | N | A578V | 91% |
| | Pf_Recipient | N | C | C | V | M | N | K | N | S | V | I | Y | D | N | I | E | * | G | T | K | A | * | N | K420R | |
| 237 | Pf_Donor | N | R | C | V | M | N | K | N | N | V | I | Y | D | N | I | E | A | G | T | K | S | S | D | WT | 78% |
| | Pf_Recipient | N | C | C | V | M | N | K | Y | S | V | I | Y | D | N | I | E | F | G | T | K | A | S | D | WT | |
| 276 | Pf_Donor | I | R | C | V | M | N | K | N | N | V | I | Y | D | N | I | E | S | A | T | K | A | A | D | WT | 70% |
| | Pf_Recipient | N | R | C | V | M/I | N/E | K/T | N | N | V | I | Y | D | N | I | E | A | G | T | K | S/A | A | D | A578S, L429S | |
| 120 | Pf_Donor | N | C | C | V | I | E | T | N | S | V | I | Y | D | N | I | E | A | G | T | K | S | A | Y | F614S | 96% |
| | Pf_Recipient | N | C | C | V | I | E | T | Y | S | V | I | Y | D | N | I | E | A | G | T | K | S | A | Y | WT | |
| 83 | Pf_Donor | N | C | C | V | M | N | K | Y | N | V | I | F | D | N | I | E | * | * | T | K | A | S | N | WT | 83% |
| | Pf_Recipient | N | R | C | V | M | N | K | Y | N | V | I | Y | D | N | I | E | S | G | T | K | A | A | D | WT | |
| 86 | Pf_Donor | N | C | C | V | M | N | K | N | S | V | I | Y | D | N | I | E | A | G | T | K | A | S | D | WT | 87% |
| | Pf_Recipient | N | R | C | V | M | N | K | N | S | V | I | Y | D | N | I | E | S | G | T | K | A | A | D | WT | |
| 92 | Pf_Donor | N | C | C | V | M | N | K | N | S | V | I | Y | D | N | I | E | * | G | T | K | A | S | Y | R513H | 83% |
| | Pf_Recipient | N | C | C | V | M | N | K | N | N | V | I | Y | D | N | I | E | F | G | T | K | S | A | D | A621D A578V | |
| 128 | Pf_Donor | I | R | C | V | I | E | T | N | N | V | I | Y | D | N | I | E | S | * | T | K | A | A | D | WT | 83% |
| | Pf_Recipient | N | R | C | V | M | N | K | N | N | V | I | Y | D | N | I | E | S | A | T | K | A | A | D | WT | |
| 150 | Pf_Donor | I | R | C | V | M | N | K | N | N | V | I | Y | D | N | I | E | F | G | T | K | * | * | N | F506L | 87% |
| | Pf_Recipient | N | C | C | V | M | N | K | N | N | V | I | Y | D | N | I | E | A | G | T | K | A | S | N | WT | |
| 122 | Pf_Donor | N | R | C | V | I | N/E | T | Y | N | V | I | F | D | N | I | E | A | G/A | T | E | S | S | D/N | P413L | 100% |
| | Pf_Recipient | N | R | C | V | I | N/E | T | Y | N | V | I | F | D | N | I | E | A | G/* | T | E | S | S | D/* | P413L | |
| 240 | Pf_Donor | N | R | C | V | M | N | K | N | N | V | I | Y | D | N | I | E | * | G | T | K | A | A | Y | WT | 83% |
| | Pf_Recipient | N | R | C | V | M | N | K | Y | S | V | I | Y | D | N | I | E | F | G | T | K | A | S | D | F395S K390R | |
| 182 | Pf_Donor | N | C | C | V | M | N | K | Y | S | V | * | Y | D | N | I | E | A | G | T | E | S | S | D | V568A | 74% |
| | Pf_Recipient | N | R | C | V | M | N | K | Y | N | V | I | Y | D | N | I | E | S | G | T | K | A | A | D | WT | |
| 116 | Pf_Donor | N | R | C | V | M | N | K | Y | N | V | I | Y | D | N | I | E | * | * | T | K | * | * | D | WT | 87% |
| | Pf_Recipient | N | C/R | C | V | M | N | K | Y | S | V | I | F | D | N | I | E | A | G | T | K | S | S/A | D | G449A | |



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## Donor and recipient attributes of the confirmed TTM

Only one (7%) sample out of the 14 donor recipient pair had 100% amino acid sequence identity. Table 18 shows the characteristics of the donor blood unit and that of the recipient that resulted in the TTM. The patient was a ten-year-old female who had visited the clinic with moderate anaemia. She was admitted for three days and the initial temperature before transfusion was 35.8 $^{o}$C and the temperature during the follow up visit was 37.2 $^{o}$C. The patient received infected blood of B positive type with a parasitemia of 164,044,800 parasites/µL of blood that was banked for only a day. Incidentally, the donor was also of blood type B positive.

Table 18: Donor and recipient characteristics of the conformed TTM

| **Characteristics of donor blood unit** | |
|---|---|
| Donor blood group | B+ |
| Storage days | 1 |
| Parasite count | 576 |
| Estimated total parasite count (/µL) | 164,044,800 |
| **Characteristics of the patient that developed TTM** | |
| Age (Yrs) | 10 |
| Gender | Female |
| Days Admitted | 3 |
| Current medical condition | Moderate anaemia |
| Recipient pre transfusion temperature ($^{o}$C) | 35.8 |
| Recipient blood group | B+ |
| Follow up temperature ($^{o}$C) | 37.2 |
| Follow-up microscopy | 960 |
| Follow-up positivity day | 35 |



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**Characterization of *P. falciparum* resistant markers in infected donor blood and blood of recipients of infected blood**

A total of 28 dried blood spot (DBS) samples were amplicon sequenced. Of this number 14 were *P. falciparum* microscopy positive samples from leftover donor units and 14 were recipients' follow-up *P. falciparum* microscopy positive samples. Amplicon sequencing of the target genes of four (4) anti-malaria drug targets was sequenced. They were *P. falciparum* chloroquine transporter gene (*Pfcrt*), *P. falciparum* dihydrofolate reductase gene (*Pfdhfr*), *P. falciparum* dihydropteroate synthetase gene (*Pfdhps*), *P. falciparum* multi-drug resistance gene 1 (*Pfmdr1*), and *Kelch 13* propeller gene.

**Undetected haplotypes and alleles**

The respective number of undetected haplotypes and alleles are presented in Table 19. Entire gene sequencing was not achieved due to the missing of the entire haplotype or undetected one or more alleles in all 28 samples. In the *Pfcrt* gene, none of the wild types of haplotypes was missing in both donor and recipient samples. In the *Pfdhfr* gene only one (3.6%) of the donors had one amino acid of the wild type of haplotype missing at position (I164), however, the missing amino acid is unknown. Also, in the *Pfdhps* wild type haplotype is (S436, A437, K540, A581, A613) however the following genes were missing at codon or positions S436(16.7%), A437(10.7%), A581(7.4%), A613(7.4%), for donor and S436 (3.6%), A437(3.6%), A581(3.6. %), A613(7.4%), for the recipient. Likewise, the *Pfmdr1* gene, Asp1264 was missing in the donor sample and none missing in the recipient respectively.



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### Genetic mutations in *P. falciparum* genome

Mutations were identified in *Pfcrt, Pfdhfr, Pfdhps, Pfmdr1* and *Kelch 13.* A total of 139 haplotypes were characterized from both donor and recipient samples. Out of that 60(43.0%) haplotypes were the wild type of *Pfcrt, Pfdhfr, Pfdhps, Pfmdr1* and *Kelch* 13. resistance genes and 79 (57%) were identified in Table 19.

Table 19: Overall Percentage gene characterization

| Haplotype | *Pfcrt* | *Pfdhfr* | *Pfdhps* | *Pfmdr1* | *Kelch13* |
|---|---|---|---|---|---|
| Wildtype | 23 (16.5%) | 10 (7.5%) | 3 (2.15%) | 9 (2.5%) | 15 (10.8%) |
| Mutant Haplotype | 8 (5.7%) | 18 (12.9%) | 20 (14.4%) | 20 (14.4%) | 13 (9.4%) |
| Total | 31 (22.3) | 28 (20.1) | 23 (16.5) | 29 (20.8) | 28 (20.1) |

### Putative drug resistant haplotypes of medical importance in genotyped samples

*Pfcrt, Pfdhfr, Pfdhps Pfmdr1and Kelch 13* genes were successfully sequenced. Table 20 gives the list of resistance genes. *Pfcrt* mutations CVIET were found to be present in both the donor and recipient's blood, however, CVINT was recorded in only the recipient's sample. Single nucleotide polymorphism (SNP at K76T) in the *Pfcrt* was present in both the donor and recipient samples.



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In the *Pfdhfr,* IRNI mutants were found in only the donors'. S108N was found in the donor. SNP of N51**I** and/or C59**R** was present in the donors' blood. *Pfdhps* AGESS was present only in the donors' blood and not the recipient's. *Pfmdr1* 1246Y was present in the donors' sample only. YYY was found in the recipients. NYY was found in the donor and not the recipient N86 was present in both the donor and recipient and the haplotypes. 86Y was found in both donor and recipient samples.

Table 20: Putative drug resistant haplotypes identified in this study

| | | Source | |
|---|---|---|---|
| **Gene** | Haplotype | Donor blood | Follow-up cases |
| ***Pfcrt*** | CVIET | Yes | Yes |
| | CVINT | No | Yes |
| | K76T | Yes | Yes |
| ***Pfdhfr*** | IRNI | Yes | No |
| | S108N | Yes | No |
| | N51I and /or C59R | Yes | No |
| ***Pfdhps*** | AGESS | Yes | No |
| ***Pfmdr1*** | 1246Y | Yes | No |
| | YYY | No | Yes |
| | NYY | Yes | No |
| | N86 | Yes | Yes |
| | 86Y | Yes | Yes |



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**Drug resistance profile of the confirmed TTM**

Out of the 14 donor recipient pair samples sequenced only one confirmed transfusion-transmitted malaria (TTM) case was recorded. Table 21 shows the drug resistance profile of the confirmed TTM. All the five drug resistance genes in this study were present. For the *P. falciparum* chloroquine transporter gene (*Pfcrt*), the wildtype CVMNK and two mutant genes CVINT and CVIET were observed.

For the *P. falciparum* dihydrofolate reductase gene (*Pfdhfr*), the wildtype NCSI and NRNI were observed. For the *P. falciparum* dihydropteroate synthetase gene (*Pfdhps*), the wildtype SAKAA and the mutant AGESS and AAESS were reported. Also, regarding the *P. falciparum* multi-drug resistance gene 1 (*Pfmdr1*), the wildtype NYD and two of the mutant genes YFD and YFN. Finally, *Kelch 13* propeller gene was reported to have a P413L mutation.

Table 21: Drug resistance profile of the confirmed TTM

| Gene | WT | Mutations |
|---|---|---|
| *Pfcrt* | CVMNK | CVINT |
| | | CVIET |
| *Pfdhfr* | NCSI | NRNI |
| *Pfdhps* | SAKAA | AGESS |
| | | AAESS |
| *Pfmdr1* | NYD | YFD |
| | | YFN |
| *Kelch 13* | WT | P413L |



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**Frequencies of putative drug resistance haplotype isolated from donor blood unit**

The frequencies of potential drug resistance strains in donor blood units infected with *P. falciparum* transfused to the recipient without evidence of transfusion-transmitted malaria are shown in Table 22.

Out of the 15 haplotypes in the *Pfcrt* gene, 11 (73.0%) were of the wild type CVMNK. There were two categories of mutations, a double and a triple. However, there was no single mutation. This double mutation was as a result of changes in amino acid positions 74 and 76, 74 (methionine → isoleucine) and 76 (lysine → threonine) 2(4.8%) of the resultant haplotype CVINT as recorded. Also, the triple mutation was found in positions 74, 75 and 76, 74 (methionine → isoleucine), 75 (asparagine → glutamic acid) and 76 (lysine → threonine). 2 (4.8%) of the resultant haplotype CVIET were recorded in the donor's sample.

In the *Pfdhfr* gene, out of 12 characterized haplotype genes, 4 (33.3%) were of the wild type and 7 (66.7%) were mutated. Two categories of mutations, double and triple mutations were observed. The wildtype haplotype for *Pfdhfr* is NCSI. The most conserved amino acid position was isoleucine at position 108 (Ser108) (Table 22). There were no single mutations found however, one double mutation 5 (41.6%) was identified at position 59 and position 108; (NCSI to NRNI) from 59 (cysteine → arginine) and at position 108 (serine → asparagine). Also, one triple mutation was observed in positions 51, 59 and 108, from 51 (asparagine →isoleucine), 59 (cysteine → arginine), and 108 (serine → asparagine). This resulted in IRNI 3 (25.0%) haplotype (Table 22).

Out of the 10 haplotypes, 3(30%) were of the wildtype SAKAA and 7 (66.7%) form the mutant genes. There were three categories of mutation, the triple,



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quadruple and quintuple. Three (30%) triple mutations occurred at different positions. The first (mutation (FGKAS) occurred at positions 436 (serine → phenylalanine), 437 (alanine → glycine) and 613 (alanine → serine). The second mutation (AGKAS) occurred at positions 436 (serine → alanine), 437(alanine → glycine), and 613 (alanine → serine). The last triple mutation (AGKSA) occurred at positions 436 (serine → alanine), 437 (alanine → glycine) and 581(alanine → serine). Two quadruple mutations identified were observed at position 436 (serine → alanine), 437 (alanine → glycine), 540 (alanine → serine) and 581 (alanine → serine) yielding AGKSS (10%). Another quadruple mutation occurred at positions 436 (serine → alanine), 540 (lysine →glutamate) 581(alanine → serine) and 613 (alanine → serine) resulting in AAESS (20%) haplotype. Only one quintuple mutation resulting in AGESS (10%) at positions 436(serine → alanine), 437 (alanine → glycine), 540 (lysine →glutamate), 581 (alanine → serine) and 613 (alanine → serine) was observed in the donor's sample (Table 22). The *P. falciparum* multi-drug resistance gene 1 (*Pfmdr1*) was reported to have three different mutations of the NYD wild type. These mutations were the single mutant NYY (20.0%), YYD (13.3%), and NYN (6.7%), which involved changes in amino acid positions 1264 (aspartate → tyrosine), 86 (asparagine → tyrosine), and 1246 (aspartate →asparagine), respectively.

The double mutation was observed in YFD (6.7%) with changes in amino acid position 86 (asparagine → tyrosine) and 184 (tyrosine → phenylalanine). Triple and complete mutation YFN (20.0%) was also reported.



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Finally, six different mutations of equal frequencies were found in the *Kelch 13 propeller gene*. The mutations were A578V, F614S, R513H, F506L, P413L, and V568A.

Table 22: Frequency of putative drug resistance haplotypes in donor blood unit

| Gene polymorphs | Frequency | Percentage |
|---|---|---|
| *Pfcrt (n=15)* | | |
| CVMNK (WT) | 11 | 73.0% |
| CVIET | 2 | 4.8% |
| CVINT | 2 | 4.8% |
| *Pfdhfr (n=12)* | | |
| NCSI (WT) | 4 | 33.3% |
| NRNI | 5 | 41.6% |
| IRNI | 3 | 25.0% |
| *Pfdhps (n=10)* | | |
| SAKAA (WT) | 3 | 30.0% |
| FGKAS | 1 | 10.0% |
| AGKAS | 1 | 10.0% |
| AGKSA | 1 | 10.0% |
| AGKSS | 1 | 10.0% |
| AAESS | 2 | 20.0% |
| **AGESS** | 1 | 10.0% |
| *Pfmdr1 (n=15)* | | |
| NYD (WT) | 5 | 33.3% |
| NYY | 3 | 20.0% |
| YYD | 2 | 13.3% |
| NYN | 1 | 6.7% |
| YFD | 1 | 6.7% |
| YFN | 3 | 20.0% |
| *Kelch 13 propeller gene (n=6)* | | |
| A578V | 1 | 16.7% |
| F614S | 1 | 16.7% |
| R513H | 1 | 16.7% |
| F506L | 1 | 16.7% |
| P413L | 1 | 16.7% |
| V568A | 1 | 16.7% |

**Putative drug resistance profiling in the donor blood**

Table 23 shows the frequencies of putative drug resistance strains in study patients that were transfused with donor blood infected with P. falciparum but did not develop transfusion-transmitted malaria (TTM). Sequencing



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analysis of the Plasmodium falciparum chloroquine resistance transporter (Pfcrt) gene revealed that 75.0% of the parasites harbored the wild type haplotype CVMNK which has the amino acids cysteine, methionine, asparagine and lysine at positions 72, 73, 74, 75 and 76. Four mutant haplotypes were identified; CVINT (6.2%), a double mutant haplotype with amino acid changes at positions 74 (methionine → isoleucine) and 76 (lysine → threonine) and CVIET (18.8%), a triple mutant haplotype with amino acid changes at positions 74 (methionine → isoleucine), 75 (asparagine → glutamic acid) and 76 (lysine → threonine).

During the investigation, it was discovered that 37.5% of the parasites analyzed had the wild-type (NCSI) P. falciparum dihydrofolate reductase gene (Pfdhfr), which is characterized by the presence of asparagine, cysteine, serine, and isoleucine at positions 51, 59, 108, and 164, respectively.

Three different mutant haplotypes were identified, namely NCNI (serine → asparagine at amino acid position 108), NRSI (cysteine → arginine at amino acid position 59) and NRNI (cysteine → arginine at amino acid position 59; serine → asparagine) at 12.5%, 18.85 and 31.2% proportions.

Regarding the P. falciparum dihydropteroate synthetase (Pfdhps) gene, nine different haplotypes were found, with the wild type being one of the minority haplotypes; SAKAA (7.1%), where alanine occupies amino acid positions 437, 581 and 613 with serine and lysine occupying positions 437 and 540 respectively. Two of the mutant haplotypes prevailed at 21.4% each. They were SGKAA (alanine → glycine at amino acid position 437) and AGKSA (serine → alanine, alanine → glycine, alanine → serine at amino acid positions 436, 437 and 581 respectively). Asfrom ide FGKAS triple mutant (serine →



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phenylalanine, alanine → glycine and serine at positions 436, 437 and 613 respectively) that occurred in two samples, the rest of the mutant haplotypes occurred in only one sample each.

Regarding the P. falciparum multi-drug resistance gene 1 (Pfmdr1), four different haplotypes were identified, including the wild type (NYD with asparagine, tyrosine and aspartic acid at amino acid positions 86, 184 and 1246 respectively). The wild type and one single mutant haplotype YYD (asparagine → tyrosine at amino acid position 86) were found in 30.8% of the parasites sequenced. Another single mutant haplotype, NYN (aspartic acid → asparagine at position 1246) was found in 23.1% of the parasites with a double mutant haplotype, YFD (86 tyrosine and 184 phenylalanine) found in 15.4% of the parasites.

Finally, ten different mutations of equal frequencies were found in the Kelch 13 propeller gene. The mutations were V534A, K420R, L429S, A621D, P413L, F395S, K390R and G449A except A578S which was present on 20.0% of the parasites.



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Table 23: Distribution of putative drug resistance haplotypes in the donor

| Gene polymorphs | Frequency | Percentage |
|---|---|---|
| *Pfcrt (n=16)* | | |
| CVMNK (WT) | 12 | 75.0% |
| CVIET | 3 | 18.8% |
| CVINT | 1 | 6.2% |
| *Pfdhfr (n=16)* | | |
| NCSI (WT) | 6 | 37.5% |
| NCNI | 2 | 12.5% |
| NRSI | 3 | 18.8% |
| NRNI | 5 | 31.2% |
| *Pfdhps (n=14)* | | |
| SAKAA (WT) | 1 | 7.1% |
| SGKAA | 3 | 21.4% |
| AGKAA | 1 | 7.1% |
| FGKSA | 1 | 7.1% |
| FGKAS | 2 | 14.3% |
| AGKAS | 1 | 7.1% |
| AGKSA | 3 | 21.4% |
| AGKSS | 1 | 7.1% |
| AGESS | 1 | 7.1% |
| *Pfmdr1 (n=14)* | | |
| NYD (WT) | 4 | 28.6% |
| YYD | 4 | 28.6% |
| NYN | 3 | 21.4% |
| YFD | 2 | 14.2% |
| YYY | 1 | 7.1% |
| *Kelch 13 propeller gene (n=10)* | | |
| A578S | 2 | 20.0% |
| K420R | 1 | 10.0% |
| V534A | 1 | 10.0% |
| L429S | 1 | 10.0% |
| A621D | 1 | 10.0% |
| P413L | 1 | 10.0% |
| F395S | 1 | 10.0% |
| K390R | 1 | 10.0% |
| G449A | 1 | 10.0% |



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# DISCUSSION

Blood transfusion is a lifesaving procedure however, transfused blood or its components can produce significant side effects such as immunological responses and transfusion transmissible infections (TTIs) (Fong, 2020; Olaniyi, 2019; Pelletier, 2018).

Severe impairments or even death have been reported from parasitic infections (Fong, 2020; Pelletier, 2018). About 0.5–3.0% of all transfusions result in transfusion reactions (Fatima et al., 2017). In most of the post-transfusion reactions, investigations have revealed that compatibility testing was accurately done (Kumar et al., 2013). Therefore, it is suspected that there may be another root cause for the post-transfusion reaction. The study sought to establish a relationship between parasitic infected donor blood and the post transfusion reactions in the recipient.

In this study, 113 recipients were exposed to antigens of *P. falciparum*, and IgG of *Babesia* spp., *Leishmania* spp. or *T. gondii* with about 10% developing transfusion reactions such as chills, urticaria rash and sweating. It was inferred that the immune system of recipients were reacting to the certain biomolecules from the donor blood units (Ackfeld et al., 2022). Recipients of donor blood units exposed to *P. falciparum* and *Leishmania* spp. had a significant increase in body temperature. This observation was considered due to increased levels of pyrogenic cytokines such as TNF-α and IL-12 in infected donor blood as has been previously published (Aninagyei, et al., 2020).

Parasite screening in blood donors is essential due to help avoid transfusion reactions. This study found out that, 18.6% of blood donors living in Ghana were exposed to either *P. falciparum*, *Babesia* spp., *Leishmania* spp. or *T.*



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*gondii*. Previous studies reported various prevalence's of *P. falciparum* in Ghanaian blood donors (Adusei et al., 2018; A. Owusu-Ofori et al., 2016). Previous studies did not find *Babesia* spp. in Ghanaian blood donors. However, *Babesia* spp has been identified in some donor blood products in other countries, namely Canada (Tonnetti, O'Brien, et al., 2019), United States of America (Moritz et al., 2016; Tonnetti, Townsend, et al., 2019) and Poland (Tonnetti, Townsend, et al., 2019), albeit at very low prevalence (< 1%).

In this study, *Babesia* spp. antibodies were detected in 6/571 (approx. 1%) donor blood samples. Notably, all the six donor samples that had *Babesia* spp. antibodies also contained *P. falciparum* HRP2 antigens. Considering the similarity of *Babesia* spp. to *P. falciparum* in terms of biology and lifecycle (Acosta et al., 2013), the previous studies that did not find *Babesia* may have identified it as *P. falciparum*. Note, the observed enzymatic amplification in the ELISA assay could be non-specific.

In this present study, 2.8% out of 571 of blood donors were found to be exposed to the IgG antibodies of *Leishmania* parasite. The ELISA kit used in this study did not differentiate between the type of antibody being detected (i.e., IgG or IgM), therefore it was not possible to determine if the antibodies were in the acute or chronic phase.

*Leishmania* spp. has been detected in the Oti region of Ghana using the *Leishmania* skin test (Akuffo et al., 2021;Carstens-Kass et al., 2021). Almost 42% of this regional population was found to be exposed to *Leishmania* spp. The *Leishmanin* skin test is useful to determine previous exposure to *Leishmania*.



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Majority of blood transfusions in sub-Saharan Africa are in the form of whole blood (Allain & Goodrich, 2017), therefore, the blood units are not leukodepleted (i.e. blood from which the white cells have been removed). In view of this, mononuclear phagocytic cells may contain the promastigotes of the *Leishmania* parasites which could be transfused to recipients of such units of blood. In the host's bloodstream, promastigotes are transformed into amastigotes (Scott & Novais, 2016). These are capable of infecting new phagocytic cells therefore establishing infection in the blood recipients. In this study, 14/452 (3.1%) of donor blood stored for a day were exposed to the *Leishmania* parasites. Therefore, the parasite could be viable during this short storage period to infect blood recipients. In the instance where the recipient has had a past *Leishmania* infection, cytokines storm could be inimical to blood recipients. Immunologically, promastigotes have been shown to induce proinflammatory cytokines that activate other immune cells and these infected host cells produce more cytokines and reactive nitrogen and oxygen species (Scott & Novais, 2016).

It is not surprising to find out that 22/571 (approx. 4%) of the donor blood units in this study were exposed to *T. gondii* IgG. Of this 17/22 (77%) were pregnant women. Agordzo et al. (2020); Kwofie et al. (2016) reported similar findings.

When a pregnant woman becomes infected with *T. gondii* for the first time during pregnancy, there is a risk of transmitting the infection to the fetus. This leads to acute *T. gondii* infection congenital toxoplasmosis which can cause serious complications for the developing fetus. This includes neurological disorders, eye damage, hearing loss, developmental issues and stillbirth



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(Agordzo et al., 2020; Ayi et al., 2016; McAuley, 2014). The severity of these complications can vary depending on the timing of infection during pregnancy. It is important to screen for *T. gondii* infection in blood donors since it is possible some donors could harbour the parasite which can be transfused to the recipient.

Malaria parasite screening was not done on the donor blood at the study facility consequently a number of recipients (48) received *Plasmodium* infected blood. The majority of recipients were transfused with blood that had been stored for a day 452/571, 79.2%). Infections in whole blood units were very high (9/43, 23.2%).

Several studies have linked ABO blood groups to the susceptibility, resistance, and severity of *P. falciparum* malaria infection (Alemu & Mama, 2016; Rattanapan et al., 2023; Tonen-Wolyec & Batina-Agasa, 2021).

The present study showed significantly varied degrees of malaria infection among the ABO blood groups using both mRDT and microscopy. In this study, the AB blood group had the highest prevalence of *P. falciparum* infection (5/15, 33.3%), followed by blood group O (39/304,12.8)., then blood group B (13/129, 10.1%) and blood group A being the least (12/123,9.7) - Table 9. However, this study was contrary to a study which aimed to assess the association between malaria infection in adult patients with ABO blood groups in Ethiopia. The highest (35.8%) malaria infection was observed among individuals with A (35.8%), followed by B (30.3%), and O (20.5%) blood groups, while individuals with AB (13.4%) blood groups were the least affected (Asmerom et al., 2023). These findings are also different from those conducted in Kenya which assessed the burden of malaria infection among individuals with varying



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blood groups seeking treatment at selected hospitals in Kenya .It was reported that blood group $O^+$ was the most prevalent among the enrolled individuals (46.50%), followed by $A^+$ (27.71%), $B^+$ (21.02%) and $AB^+$ (4.78%) respectively (Yeda et al., 2022). This discrepancy in results could due to mutations in the genetic mechanisms (A, B, and H carbohydrate antigens), which can regulate protein activities during infection and antibodies against the malaria parasite.

Blood group O (24/294, 12.8%) was next after blood group AB being the blood group with the highest prevalence of *P. falciparum* infections. Even though blood group O is the majority in several populations however, this is not always the case due to variations in ethnic differences (Doku et al., 2019, 2022; Tiruneh et al., 2020; Woldu et al., 2022).

In a previous similar study, high parasitaemia levels were recorded (Alemu & Mama, 2016). An association between susceptibility and blood group O could not be established (Beiguelman et al., 2003). However, the variations in susceptibility and severity of *P. falciparum* malaria infection among blood groups have been ascribed to the rosetting and cytoadherence of parasitized red blood cells (Rowe et al., 2007). Rosetting obstructs microvascular blood flow, which contributes to the pathogenesis of severe malaria (Kaul et al., 1991; Rowe et al., 2007). Studies have shown that the occurrence of rosetting is lower in "O" blood group red blood cells compared to those with non-O blood group (A, B, and AB) in laboratory studies of *P. falciparum*. (Carlson & Wahlgren, 1992) and field isolates. Compared to blood group O, fewer number of blood units in blood group A were found to be infected with the parasites. However, the parasitemia in blood group A was about 1.7 times that of blood group O.



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Nevertheless, the reverse was observed in blood group B, where the estimated mean total parasitemia was unexpectedly less than that of blood group O (299,858,757 vs. 618,904,865 parasites per pint of blood; about 3-fold decrease). In cases of asymptomatic malaria, the host immunity could prevent massive infection of uninfected red cells, and for that matter, the mechanism of rosetting-mediated unchecked invasion of the parasites in uninfected red blood cells may not be observed. It's been observed that blood group O cells can still form rosettes, although they tend to be smaller and less sturdy than those seen in non-O blood groups. (Barragan et al., 2000; Carlson & Wahlgren, 1992; Rowe et al., 2007). As a result, it is "believed that" having "O" blood type might offer some protection against severe malaria. (Rowe et al., 2007), but this may not be the case in asymptomatic malaria, as has been shown in this study.

Blood group AB was found to have a high frequency of *P. falciparum* infection incidence followed by blood group O. However, the highest mean parasitemia was in blood group A (3859 parasites/µL) as compared to blood group O (2068 parasites/µL). This explains the susceptibility of blood group A to *P. falciparum* infection.

This study found that female recipients (61/69, 88.4%) were the majority of suspected TTM cases. One plausible explanation for this observation is the disproportionate number of female (61) to male (8) recipients in the study. In contrast to the findings in this study, males have been reported to be more susceptible to infectious diseases than females (Altamimi et al., 2020; Bunders & Altfeld, 2020; Klein, 2000; Migliore et al., 2021).

The highest post transfusion malaria parasitemia was among anaemic patients as compared to temperature, pulse, and blood pressure. This observation may



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be due to compromised immunity associated with anaemia. Hassan et al. (2016) reported significantly lower IgG levels, IL-6, and phagocytic activity in anaemic patients. Similarly, it has also been established that anaemia, especially iron deficient anaemia, increases the susceptibility of individuals to infectious diseases (Beard, 2018).

Another objective of this study was to assess whether donor blood infected with *P. falciparum* could be transmitted through transfusion to the recipient. In view of this, this study was carried out to assess malaria parasitemia in recipients of *P. falciparum* infected donor blood. Fourteen (14) of the 48 recipients of infected donor blood with malaria parasites detected by microscopy developed malaria parasitemia. Though the recipients received various quantities of malaria parasites, an analysis of the parasite count level in the donor blood and that of the recipient shows that there is no correlation. This is confirmed by the low correlation coefficient ($R^2$=0.37) in Figure 12, In other words, there is no need to determine the parasite count level of the infected blood to be transfused.

The data in Table 16, shows that up to day 5, the parasite count level was significantly high (>848 million). Generally, after day 5, there was a reduction in the parasite count (>296 million). *P. falciparum* has been reported to remain intact for up to 28 days, but viability is lost after 14 days of storage at 4 °C (Chattopadhyay et al., 2011). Therefore, it is not surprising that in this study *P. falciparum* was present in the donor blood after storage day 5.

Post-transfusion parasitemia was reported on all follow-up days, with the majority of transfusion infections on day 7, 14, and 35. This could mean that *P. falciparum* established infection as soon as transfusion was completed while



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others delayed until after a month. This observation could be possible since, unlike *P. falciparum* transmission through mosquito bites, infected blood transfusions directly release the malaria parasite in the recipient's bloodstream (Garraud, 2006) and within 8 – 29 days later, which is the incubation period for TTM (Dover & Schultz, 1971; Verra et al., 2018b), TTM could occur. For this reason, the detection of malaria parasites for up to 35 days is not impossible. (Verra et al., 2018b). Except for donor blood group B negative post-transfusion parasitemia was observed in all donor blood variables (blood group type, number of days of storage, and levels of parasite contamination). Similarly, in all age groups, gender, blood groups, and clinical conditions of the recipients, TTM was suspected. This implies that donor *P. falciparum* contamination could potentially be transmitted to all categories of blood recipients. It was also observed that TTM was not suspected in all infected blood recipients, therefore, it is important to identify factors that render some blood recipients susceptible to TTM.

Throughout the follow-up period, none of the infected blood recipients developed body temperatures above 37.1 °C (range: 34.2 – 37.1 °C). The mean parasitemia recorded in the infected blood donor transfused to the recipients was 3012 parasites/µL of blood. On questioning the recipients, none reported any symptoms associated with malaria. As these recipients are asymptomatic, they were immediately referred to the nearest health facility for further assessment and subsequent management. It must be emphasized that the *P. falciparum* infections detected in infected blood recipients may not necessarily be due to the parasites present in the donor blood units. Once the blood recipients live in a malaria endemic district new infections and/or recrudescence of previous



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infections is a possibility. Even though all infected blood recipients were negative by mRDT, the technique has been found not to be able to detect *P. falciparum* antigens in infections with parasitemia less than 50 parasites/µL of blood. Therefore, it is important to determine the genetic relatedness of the pre- and post-transfusion parasites to confirm whether or not TTM has occurred.

The genetic relatedness of the parasite in the donor blood and the parasite in their corresponding blood recipients were assessed. The amino acid sequences of the parasite pair reveal that the amino acids were identical in only 74% to 100% positions. In samples numbers 92, 116, 120, 150, 157, 182, 202, 240, and 276, despite the differences in some amino acid positions, the *Kelch 13* genes were all different. In other samples (samples 83, 86, 128 and 237), even though the *Kelch 13* genes were the same (wild-type genes), in several positions, the amino acids were different. There was only one sample (sample 122) that the amino acids were the same at each locus together with the same *Kelch 13* gene. With this observation, TTM was confirmed in 1/14 samples representing 7.1%. Blood transfusion therapy saves life; however, it is a vehicle for transmission of infections such as malaria and other haemo parasites. In this study, there was one case of confirmed TTM from transfused whole blood in a 10-year-old girl. Although this was the only case presented, it could be postulated that infected blood transfused could result in TTM. This hypothesis is yet to be verified as data is limited, hence future studies are required.

When malaria is transmitted through a blood transfusion to a non-immune recipient, it can be rapidly fatal. Majority of recipients of blood transfusions living in malaria-endemic areas in SSA are semi-immune to malaria but the degree of protection that this immunity confers against



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transfusion-transmitted malaria is unknown (Antwi-Baffour et al., 2019; Tetteh et al., 2023). Young infants in areas where malaria is endemic who have not had repeated exposure to the parasite may be regarded as a non-immune recipient. Therefore, they may be as susceptible to transfusion-transmitted malaria as a non-immune person who lives in a non–malaria-endemic country. The clinical severity of transfusion-transmitted malaria is likely to differ in countries where the disease is endemic from non-endemic countries. Because of their immunity to malaria, blood donors in SSA are able to harbour low levels of parasites without developing clinical symptoms (Adusei et al., 2018; Aninagyei et al., 2019; Attoh et al., 2022). The incubation period for TTM varies (Dover & Schultz, 1971; Verra et al., 2018a), however, for *P. falciparum*, it ranges from 7 to 27 days. This variability may depend on factors such as inoculum size and the intrinsic factors of the recipient such as age, level of immunity, presence of protective red blood cell abnormalities such as sickle cell etc. Recent observations from TTM cases indicate that the incubation period may last several months (Verra et al., 2018b) (Mali et al., 2011). The determination of incubation periods is based on the ability of the patient to recollect exactly when the symptoms started and knowing the date of the implicated transfusion. This is not always known. In an observational study of non-immune subjects in Yugoslavia, 3 children who received blood from the same source had varying incubation periods of 7, 15 and 23 days. Assuming that they each received the same dose of parasites then, it was the recipient's immunity that played the major role in determining the incubation period. The inoculum size in a transfused unit of blood can be huge. When one parasite/µL



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is found, that translates to about 500,000 parasites in a unit of blood (Owusu-Ofori et al., 2016).

The 13 other blood recipients were infected with a different parasite. This could either be the reactivation of dormant parasites or new infections in the community. Ashley & White, (2014) reported that *P. falciparum* can persist in the human host for up to 8 years. Considering the fact that the study site is a malaria endemic district with a prevalence of malaria of about 55% (Amoah et al., 2019), reinfection could also be a possibility. The blood recipients with non-TTM malaria were referred to the nearest health centre for treatment. This needed to be done because, in 2007, it was suggested that a proportion of people with asymptomatic blood-stage malaria infection may become symptomatic. There are several global studies to confirm this. In Brazil, 93 asymptomatic infections in persons aged 5 years and older were followed for 2 months after their infection was identified. Ten (10) of them became symptomatic during that period (Da Silva-Nunes & Ferreira, 2007). However, the background transmission rate would suggest that eight of those cases could have been the result of new infections. In a cohort study of asymptomatic parasitaemia in Tanzanian children aged 1–5 years who were followed over 31 days, 55.9% (19 out of 34) of the children eventually developed fever, which was associated with spikes in parasite density (Magesa et al., 2002). In another study in Tanzania, it was found that the risk of developing clinical malaria with previous asymptomatic infections is high. The study followed 265 parasitaemic but asymptomatic residents for 40 weeks and observed that 21 (7.9%) of them developed fever in conjunction with parasite density >400 parasites/μL (Bereczky et al., 2004). Sub-patent asymptomatic infections may be less likely



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to become symptomatic than infections detectable by microscopy. In Uganda, 25 out of 63 (39%) children aged 6 months to 5 years with sub-patent infections who were asymptomatic developed symptoms within 20 weeks of observation compared with 43 out of 53 (82%) children with patent infections (Nsobya et al., 2004). These findings suggest that low-density malaria infections may persist for long periods without causing symptoms if not treated. It is not clear what triggers the appearance of symptoms in individuals who have been parasitaemic but remained asymptomatic for some time. It has been suggested that reinfection with new clones (to which the individual had not previously been exposed) could trigger an increase in parasite density and the development of symptoms (Kun et al., 2002), but other studies have demonstrated that symptoms can appear without any change in clonal diversity (Nsobya et al., 2004).

The amino acid sequences on which the TTM was assessed were based on the amino acid haplotypes for the determination of putative drug resistant parasites present in both the donor blood and the recipients. The amino acid sequences made it possible to determine either the wild type or the mutant haplotypes for *Plasmodium falciparum* chloroquine resistance transporter (*Pfcrt*), *P. falciparum* multi-drug resistance (*Pfmdr1*), *P. falciparum* dihydropteroate-synthetase (*Pfdhps*), *P. falciparum* dihydrofolate-reductase (*Pfdhfr*) and Kelch 13 propeller genes.

In this study a total of 139 haplotypes were characterized from both the donor and the recipient samples. Sixty (43.0%) were of the wild type gene, while 79 (57%) were of mutant genes. Mutant genes are known to confer drug resistance.



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Chloroquine (CQ) is one of the drugs that have been used to treat and control malaria over the years until its resistance was first reported in southeast Asia which later spread through malaria endemic countries (Ross et al., 2018). Mutations in the *Pfcrt* have been identified to confer chloroquine drug resistance. A decrease in the mutant *Pfcrt* was observed as compared to the wild type in this study. This may be due to the withdrawal of CQ to Artemisinin-based Combination Therapy (the combination of artesunate plus aminoquinoline). Other studies have reported a similar trend in the other part of Ghana (Aninagyei, Duedu, et al., 2020) with a decrease in the prevalence of the mutant *Pfcrt* allele. In the parasites from both the donor blood and the blood recipients, the prevalence of *Pfcrt* haplotypes that confer resistance to chloroquine was found to be 4.8-18.8%. Similar or higher prevalence rates have been reported globally (Afoakwah et al., 2014; Foguim et al., 2020). The K76T mutation is present in all clinical samples of *P. falciparum* that are resistant to CQ. The K76T mutation is highly predictive for Chloroquine (CQ) and amodiaquine (AQ) resistance and higher prevalence has been cited by several studies in East Africa; the prevalence of K76T throughout West Africa has been estimated to range from 20 to 80% (Afoakwah et al., 2014; Frosch et al., 2011; Hassen et al., 2022; Kublin et al., 2002; A. Mohammed et al., 2013; T. Zhang et al., 2018); This finding is similar to a study to determine the prevalence of malaria resistance-associated mutations in *Plasmodium falciparum* circulating in 2017–2018, Bo, Sierra Leone where the proportion of *Pfcrt mutations at* positions 74, 75 and 76 (M74I, N75E and K76T) were observed to be 22% of the studied samples (Leski et al., 2022). In Ghana, the *Pfcrt* gene was found to contain three different amino acids at positions 74, 75 and 76 resulting in M74I,



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N75E and K76T (Aninagyei, Duedu, et al., 2020). and, the incidence of K76T has been reported in several studies (Afoakwah et al., 2014; Aninagyei, Duedu, et al., 2020), however, K76T mutation may not complicate TTM, since chloroquine is no longer the drug of choice for the treatment of malaria.

Triple mutation yielding CVINT and CVIET was found in both the recipient and donor samples in this study. The existence of the CVINT haplotype was predicted by evolutionary analyses of *Pfcrt* sequences, but this is the first time that this form has been detected in conditions of natural transmission in Ghana. This study, although limited, is the first to describe *Pfcrt* haplotypes at codon 56–118 in isolates from Ghana. This is similar to a study conducted in Niger where for the first time CVINT mutant was detected (Salissou et al., 2014). Even though the findings suggest that there has been a significant decline in CQ-resistant genotypes since the previous estimates for Niger were obtained however, there was emergence of a new genotype, *Pfcrt* CVINT, possibly selected under the pressure of the new treatments combining artesunate and AQ introduced into Niger in 2008 (Salissou et al., 2014). In Ghana as in other sub-Saharan African countries, chloroquine (CQ) was discontinued and replaced with ACTs, due to antimalarial resistance leading to the introduction of specifically, artesunate-amodiaquine in 2005 and subsequently artemether-lumefantrine (AL) and dihydroartemisinin-piperaquine (DHAP) as first-line treatments for uncomplicated malaria in 2007 and 2009, (Abuaku et al., 2016). As a result of these interventions the frequency of CQR genotypes had significantly decreased (Salissou et al., 2014) This decrease was accompanied by an increase in the frequency of the CQ-susceptible *Pfcrt* CVMNK genotype and the emergence of a new genotype, *Pfcrt* CVINT, possibly selected under



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the pressure of the new treatments combining artesunate and AQ introduced into Ghana, Niger and across some African countries. Overall, these results suggest a gradual inversion of the pattern of chloroquine resistance (CQR) genotypes and phenotypes in Ghana following the withdrawal of CQ and the introduction of ACTs in this country. These results have important implications and must be further investigated to avoid any unpredictable drug resistance stains.

For *Pfdhps,* the key mutation associated with drug resistance is sulfadoxine, a single amino acid residue that changes from alanine (A) to glycine (G) at codon 437 of *Pfdhps* (Braun et al., 2015). The A437**G** selection by SP has been previously described during intermittent preventive treatment for infants (IPTi) (Flegg et al., 2022; Rupérez et al., 2016). As the most frequent mutation of *Pfdhps*, our findings illustrate the high prevalence of A437G. Furthermore, a higher proportion of A437G has been detected at 75.6% in Gabon (Bouyou-Akotet et al., 2015), 87.9% in Kenya (Lucchi et al., 2015), 97.6% (1416/1451) in Congo (Lucchi et al., 2015), and 96.4% (27/28) in Nigeria (Lucchi et al., 2015). As a result, these areas have a high frequency of SP-resistant parasites. Given the high incidence of *Pfdhfr* mutations, more focus should be paid to SP drug resistance surveillance.

In this study, parasites with a single mutant *Pfdhps* haplotype (SGKAA) and triple mutant *Pfdhfr,* (IRNI) were reported. Based on *Pfdhfr-Pfdhps* haplotypes, SP resistant parasites can be divided into three groups: "partially resistant", "fully resistant" and "super resistant" (Naidoo & Roper, 2013). Partial resistance is acquired by combining the triple *Pfdhfr* mutant N51I, C59R, S108N (haplotype IRNI) with *Pfdhps* A437G (haplotype SGKAA), and it is referred to as a quadruple mutation (IRN-GK). Full resistance is conferred by



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combining the double *Pfdhps* mutant of A437G, K540E (haplotype SGEAA) with the triple *Pfdhfr* mutant N51I, C59R, S108N (haplotype IRNI), which results in a quintuple mutation (IRN-GE. Also, the triple *Pfdhfr* mutation and triple *Pfdhps* mutations (A437G, K540E and A581G) give a sextuple mutation (IRN-SGEGA) which confers super resistance (Naidoo & Roper, 2013). These various haplotypes or mutational combinations may have an impact on the outcomes of Intermittent Preventive Treatment of pregnancy (IPTp) in infected pregnant women and children. Fortunately, our findings showed that partially SP-resistant (IRNI-SGKAA) parasites were only mildly prevalent but full resistance to SP-resistant (IRNI-SGEAA) haplotype could not be found. Neither the blood recipient nor the donor unit contained any parasites that were super SP-resistant. IPTp-SP is anticipated to encounter some partial resistance in Ghana.

*P. falciparum* multi drug resistance gene 1 (*Pfmdr1*) is one of the markers associated with decreased sensitivity to lumefantrine, particularly *Pfmdr1* N86 (Venkatesan et al., 2014). Parasites carrying *Pfmdr1* 86 N gene are killed by treatment with artemether-lumefantrine (AL) but their increased tolerance of residual drug levels allows them to re-infect a person more rapidly after his treatment, compared to parasites carrying 86Y form of the gene (Hastings and Ward, 2005). The *Pfmdr1* gene mutations have been associated with the development of multidrug resistance in *P. falciparum*. Globally, the most mutations implicated include N86Y, Y184F and N1246Y (Li et al., 2014). In Ghana, these alleles have also been identified to be associated with reduced sensitivity of ACTs and other anti-malaria mono-therapies (Abugri et al., 2018). The most prevalent SNPs in Africa, at codons 86, 184, and 1246, have been



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shown to influence both susceptibility and resistance to a variety of antimalarial drugs (Duraisingh et al., 1997; Reed et al., 2000). Thus, there is a substantial probability of artemether-lumefantrine treatment failure when the *Pfmdr1* N86 genotype is present. (Venkatesan et al., 2014).

Asymptomatic malaria, as the case is, in blood donors will always put blood recipients at risk of transfusion-transmitted malaria. It is, therefore, essential to screen blood donors for malaria. In furtherance to this, PCR technology has been used to classify the geographical association of asymptomatic *Plasmodium* infections into three categories: low, moderate and high transmission areas. Low transmission areas (Cambodia, Thailand, Haiti, Solomon Islands and Sri Lanka) are geographical areas with asymptomatic infections in the range 0-2.9% (Atkinson et al., 2012; Congpuong et al., 2014; Hoyer et al., 2012), moderate transmission areas (Uganda, Zambia, Brazil, Senegal and Congo) are geographical areas with asymptomatic infections in the range 3.0-20% (Hsiang et al., 2010; Koukouikila-Koussounda et al., 2012; Ladeia-Andrade et al., 2009; Stresman et al., 2010; Vafa et al., 2008) and high transmission areas (Tanzania, Gabon, Ghana, São Tome and Kenya) with asymptomatic infections above 20% (Bereczky et al., 2004; Dal-Bianco et al., 2007; Gahutu et al., 2011; Lindblade et al., 2013; Pinto et al., 2000).

The situation is quite worrying when there is a reported huge prevalence of asymptomatic malaria in Ghana. The forest zone of Ghana has been identified as an area with a high prevalence of asymptomatic malaria during the rainy season, as evidenced by a PCR prevalence of 73% among infected adults with the *P. falciparum* parasite.(Heinemann et al., 2020). Asymptomatic adults represent a relevant reservoir for malaria parasites. Any attempt at malaria



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eradication therefore must target a wider population and should not only focus on children or individuals with a positive malaria RDT. In endemic regions, surveys should be conducted on a regular basis. A study conducted in 1998 observed a prevalence detected by microscopy of 51% in the forest area of the Ashanti region with a peak among 8-year-old children and a plateau at about 20% in adults (Browne et al., 2000). In a study including 160 asymptomatic adults and children from the Greater Accra Region in Southern Ghana, the prevalence of asymptomatic parasite carriers based on microscopy was 33.75% (27/80) in Obom, a high malaria transmission area and 3.75% (3/80) in Asutsuare, a low malaria transmission area respectively (Abukari et al., 2019). In the Upper East region of Ghana, which is considered a part of the Guinea Savannah Zone, asymptomatic *P. falciparum* carriage rates detected by PCR among all age-groups were 14% during the dry season (Atelu et al., 2016). In the same region, the molecular prevalence of *P. falciparum* infection was 72% in asymptomatic adults above 19 years recruited in the rainy season in 2000 (Owusu-Agyei et al., 2002). Among adult residents (> 20 years) of the Guinea Savannah Zone recruited in 2012–2013, the prevalence of asymptomatic *P. falciparum* infection assessed by combined microscopy and PCR was 64% and 27% in the wet and dry seasons, respectively (Tiedje et al., 2017). In a cross-sectional study performed in the Eastern region of Ghana in 2017, the positivity rate among adults 20 years old and above was 14% by RDT and about 55% by PCR (Amoah et al., 2019).



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# CHAPTER FIVE

# SUMMARY, CONCLUSIONS, NOVELTY FINDINGS AND RECOMMENDATIONS

## Summary

Transfusion-transmissible malaria (TTM) has become a concern in low endemic nations over the past ten years and it is expected to worsen in sub-Saharan Africa where *Plasmodium* parasite asymptomatic carriage is prevalent. In Ghana, asymptomatic malaria infections have been found to be common, with a 10% prevalence of *P. falciparum* parasitaemia in blood donors. It is possible that the parasitemia found in a transfusion recipient was not from the transfused blood but from a mosquito bite, making genotyping necessary to prove TTM by showing genetic similarity between the parasites in the recipient and the transfused blood.

Screening for *Plasmodium* parasites and other haemo-parasites in blood for transfusions is not routinely performed in most malaria-endemic countries in sub-Saharan Africa despite potential asymptomatic carriers. The lack of data on the clinical effects of malaria transmitted through transfusions and absence of a reliable TTM screening procedure makes it difficult to make informed decisions. The way to confirm TTM is through genotyping which maps the genetic similarity between the parasites in the recipient and the transfused blood. In this study the clinical effects of exposure to *Babesia* spp., *Leishmania* spp., *Toxoplasma gondii*, and *P. falciparum* in blood donors has been assessed. Selective whole genome sequencing was used to confirm TTM, as well as profiling *P. falciparum* drug-resistant genes in donor blood infected with *P. falciparum.* The study was conducted at Nsawam Government Hospital in the



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Eastern Region of Ghana. It was a cross-sectional study, which recruited in-patients due to blood transfusions. Most of the blood donors were family replacement donors and the majority of the transfusions were in the form of whole blood. All donor blood used in the study was collected from August 2020 to May 2021.

Ethical approval was obtained from, and consent was taken from all participants or their guardians. Patients were monitored within 24 hours for possible acute transfusion reaction. PfHRP2 antigens RDT and microscopy was performed on the recipient's blood prior to transfusion. ELISA was used in the detection of *Babesia* spp., *Leishmania* spp., and *Toxoplasma. gondii.* For the patient who received the infected blood, the remnant of the donor blood was screened for malaria, for a positive result with periodic follow-up visits up to 35days.

A total of 571 donor blood units were transfused to 440 recipients, most of whom were of blood group O and received whole blood. Results showed that 106 (18.6%) of the donor blood samples were infected with *P. falciparum, Babesia* spp.*, Leishmania* spp.*, or Toxoplasma. gondii*. Out of the 440 blood recipients, 31 (7%) experienced transfusion reactions, with fever and sweating associated with recipients of *P. falciparum*-exposed blood and sweating alone associated with *Leishmania spp.* exposed blood. None of the recipients experienced respiratory distress or haematuria, but 3 recipients of unexposed blood developed transient haematuria.



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## Conclusion

**Exposure to *P. falciparum, Babesia* spp., *Leishmania* spp. and *Toxoplasma. gondii* was prevalent in approximately one in five recipients.**

This study demonstrates various levels of exposure rates of parasitic infections in blood donors. The prevalence of exposure to *P. falciparum, Babesia* spp., *Leishmania* spp., and *Toxoplasma. gondii* was 106 (18.6%). 69 (12%) of the donor blood samples contained *P. falciparum* histidine-rich protein 2 (PfHRP2) antigens, while exposure rates of *Babesia* spp. *Leishmania* spp. and *T. gondii* were 6 (1.1%), 16 (2.8%) and 22 (3.9%) by ELISA. These findings underscore the fact that *P. falciparum*, *Babesia* spp., *T. gondii,* and *Leishmania* spp. are endemic in Ghana. Therefore, concerted efforts must be made to determine the actual burden and its associated risk factors in Ghana, with the ultimate goal to reduce the exposure and incidence in adults of blood donating age.

***Babesia* spp. was found to have a co infection with malaria.**

Babesiosis may at times be misdiagnosed as malaria because of the similarity between the two diseases. It's crucial to apply more sensitive and specific diagnosis methods to set up rapid response mechanisms for the important infectious diseases in babesiosis and malaria in these areas, particularly in places which are weak in surveillance and response systems.

**MRDT identifies more cases of malaria in blood donors compared to microscopy**.

The prevalence of *P. falciparum* infection in donor blood unit was 12.1% by mRDT whereas parasitemia was 8.4% by microscopy. Unlike other studies which found associations of blood groups and malaria infection, in this



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study, no blood group was associated with malaria infection. Malaria transmission via blood transfusion remains an issue of public health concern, as indicated in the results of this current study.

**The study concluded that transfusion-transmitted malaria was 7%.**

The confirmation of TTM albeit at a relatively low percentage, indicates that blood transfusers are still at-risk of malaria infections. These infections can be fatal leading to possible death in the immunocompromised. Semi-immune people present the biggest challenge for TTM screening because they may become asymptomatic carriers with a very low parasite density, which is difficult to detect. The best strategy to reduce the risk of transfusion-transmitted malaria without needlessly barring blood donations is still up for debate.

**The prevalence of *Plasmodium falciparum* anti-malaria drug resistant markers in blood donors was found to be approximately 20%**

The proportions of mutations in the *P. falciparum* found in the donor and recipients' samples were for *Pfcrt* 31 (5.7%), *Pfdhfr* 28(12.9%), *Pfdhps* 23 (14.4%) and *Pfmdr1* 29 (14.4%) and *Kelch*13 28 (9.4%). These confer resistance to antimalaria drugs.

**New putative malaria drug resistance genes were identified.**

The study identified an increase in the frequency of the CQ-susceptible *Pfcrt* CVMNK genotype and emergence of the new *Pfcrt* CVINT genotype in Ghana. This new genotype was possibly due to the introduction of new treatments. A high prevalence of *Pfdhps* A437G was observed indicating that a high prevalence of SP-resistant parasites is present in the region. *Pfmdr1* new mutants YYD, NYN and YYY which has been associated with reduced susceptibility to a number of antimalarial drugs were identified. *Kelch 13*



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G449A mutation which has been shown to reduce the susceptibility of the malaria parasite to artemisinin-based drugs was also observed. A prophylactic dose of antimalarials for transfused patients has been suggested by some authors. However, this may lead to drugs resistance.

**Novelty findings**

The following novelty findings were made to improve the safety of blood.

1. The prevalence of exposure rate of *Babesia* spp. (1.1%), *Leishmania* spp. (2.8%) and *Toxoplasma. gondii* (3.9%) have been determined for the first time among blood donor units in Ghana.
2. Association between *Babesia* spp., *Leishmania* spp., and *Toxoplasma gondii,* exposed donor blood with acute transfusion reaction in recipients has been established.
3. *Babesia* was found to be a co-infection with malaria.
4. Novel antimalaria drug resistance genes were identified for the first time in Ghana.
    - *Pfcrt* mutant gene: CVINT
    - *Pfmdr1* mutant gene: YYD which have been implicated in Chloroquine Amodiaquine and mefloquine in s. NYN is linked to chloroquine and Amodiaquine resistance and YYY which has been associated with reduced susceptibility to a number of antimalarial drugs, including mefloquine and lumefantrine.
    - *Kelch 13* G449A mutation has been identified in this study in Ghana for the first time. This mutation has been shown to reduce the efficacy of the artemisinin-based drugs for the malaria treatment.



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## Recommendation

Below are the recommendations from this study:

1. Considering the prevalence of the parasite in Ghana and the subregion, the screening of these parasites should ideally be part of the donor screening algorithm within the Ghana Health Services. All blood donors or donor blood units should be screened for malaria and other haemoparasites as recommended by WHO. These parasites have been found to trigger adverse post transfusion reactions.
2. Further studies should be carried out on malaria prevention, pre-transfusion, post-transfusion and pathogen reduction technology.
3. Further research is needed to quantify the risk and associated burden of Transfusion-Transmitted Malaria (TTM) in malaria endemic regions.

## Limitations of the study

1. Pre-transfusion samples were only screened with microscopy and rapid tests but not molecular techniques.
2. 18% of the recipients lost to follow up, therefore, this study could not determine TTM in them.
3. Not screening for bacteria that can cause septic transfusion reactions is a limitation it introduces a potential confounder the presence of these bacteria is associated with the occurrence of transfusion reactions.



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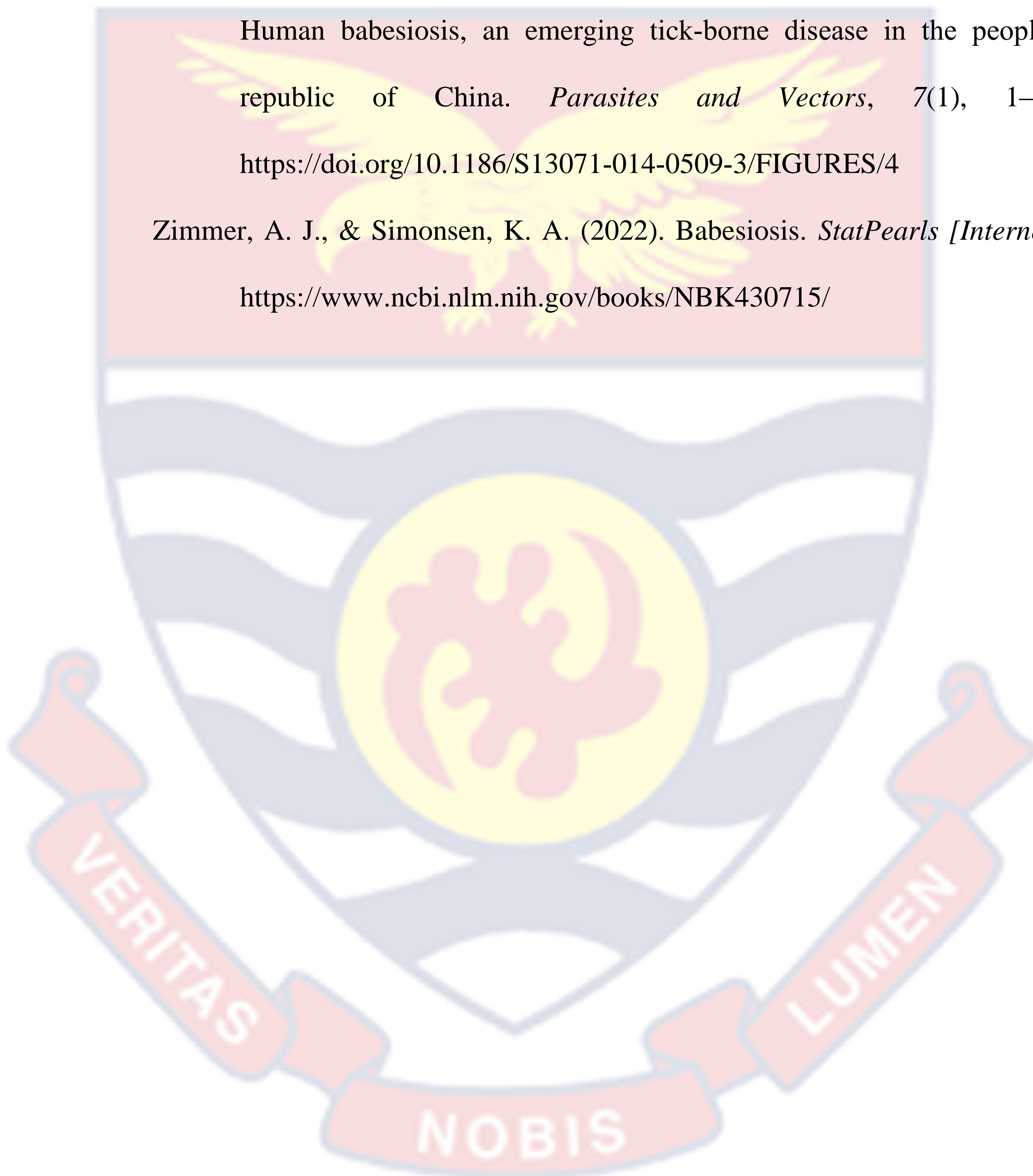




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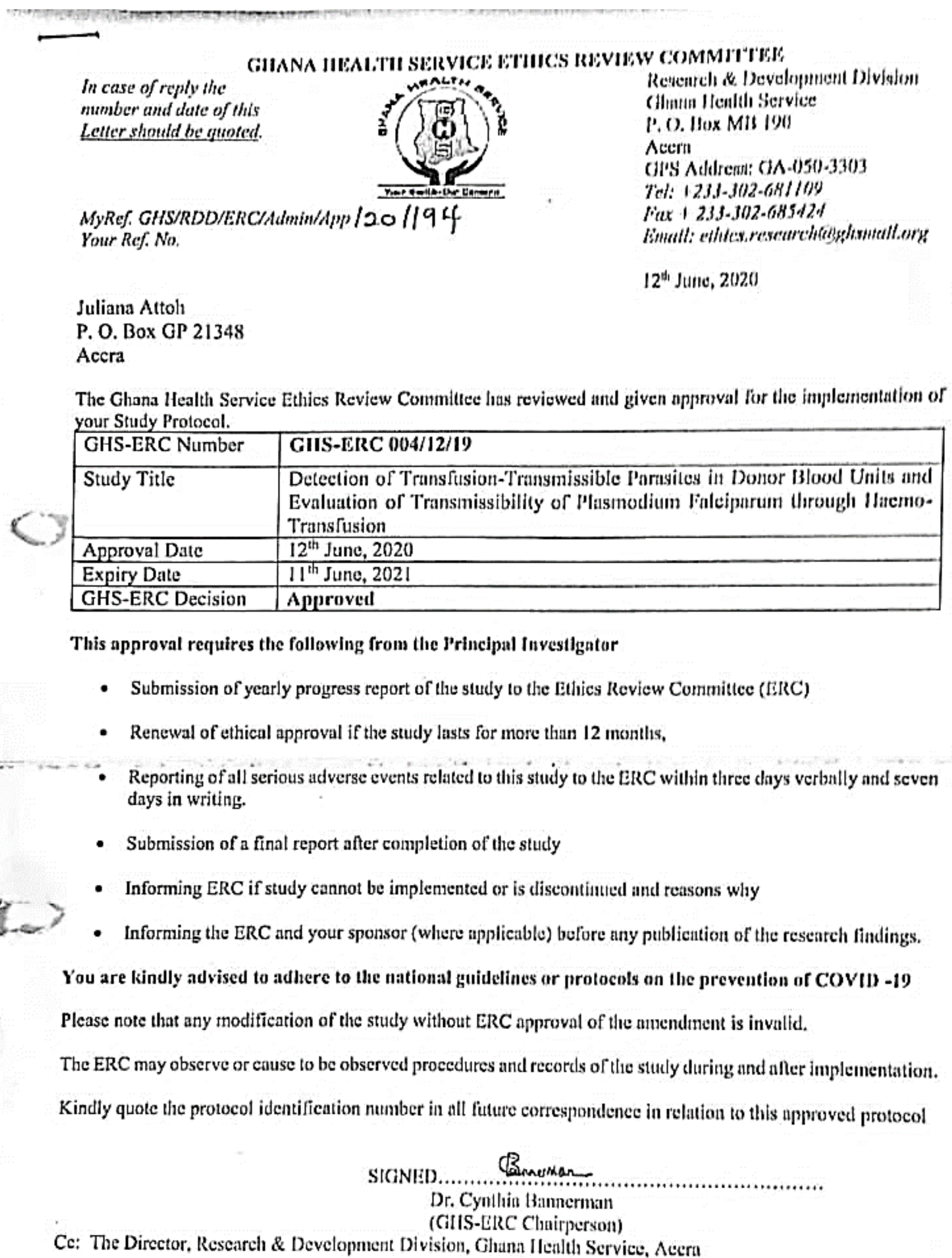
# APPENDICES

## APPENDIX 1: ETHICAL CLEARANCE APPROVAL LETTER



GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

*In case of reply the number and date of this Letter should be quoted.*

Research & Development Division
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P. O. Box MB 190
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*MyRef. GHS/RDD/ERC/Admin/App* /20/194
*Your Ref. No.*

12th June, 2020

Juliana Attoh
P. O. Box GP 21348
Accra

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

| GHS-ERC Number | GHS-ERC 004/12/19 |
|---|---|
| Study Title | Detection of Transfusion-Transmissible Parasites in Donor Blood Units and Evaluation of Transmissibility of Plasmodium Falciparum through Haemo-Transfusion |
| Approval Date | 12th June, 2020 |
| Expiry Date | 11th June, 2021 |
| GHS-ERC Decision | Approved |

**This approval requires the following from the Principal Investigator**

- Submission of yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

**You are kindly advised to adhere to the national guidelines or protocols on the prevention of COVID -19**

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED.............................................................
Dr. Cynthia Bannerman
(GHS-ERC Chairperson)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra



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# APPENDIX 2: PUBLICATION FROM THIS STUDY

Parasitology Research
https://doi.org/10.1007/s00436-022-07476-w

PROTOZOOLOGY - ORIGINAL PAPER

## Immunochromatographic and microscopic detection of *Plasmodium falciparum* in recipients of *P. falciparum*-infected donor blood

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**Abstract**
Blood transfusion practice is an essential medical intervention; however, it poses problems of transmissibility of infectious diseases including malaria. This study was designed to determine the potential of transfusion-transmitted malaria (TTM) by detecting malaria antigens and parasites in recipients of infected donor blood. After successful blood transfusion, remnants of transfused blood were screened for *Plasmodium falciparum* HRP2 antigen and parasitemia using CareStart malaria RDT and 10% Giemsa stain microscopy respectively according to established protocols. Recipients of microscopy detectable *P. falciparum* in infected blood who tested negative for malaria by both microscopy and mRDT prior to receiving infected donor blood were followed up weekly for 35 days. Donor *P. falciparum* antigenemia and parasitemia were 12.1% and 8.4%, respectively, while the prevalence of blood recipient parasitemia was 3.2%. Blood stored for 2–5 days recorded mean parasitemia higher than those stored for a day and after 5 days. Additionally, parasitemia was observed in all follow-up days with marginally high frequencies in days 7, 14, and 35. There was no association between the attributes (storage days, blood group, and parasite count range) of the infected donor blood units and the characteristics of blood recipients with post-transfusion parasitemia. This study provides baseline data on TTM in Ghana. However, further studies should establish the genetic relatedness of the implicated parasites since new infections and/or recrudescence of previous infections could account for this observation.

**Keywords** Transfusion-transmitted malaria · *Plasmodium falciparum* · Blood recipients · Malaria · Post-transfusion parasitemia

## Background

Malaria, a tropical disease caused by the protozoan intracellular *Plasmodium* parasite, is a major cause of morbidity and mortality especially in malaria endemic countries in sub-Saharan Africa and South East Asia (Dhiman 2019). Human malaria is caused by five different species of the *Plasmodium* parasite, namely, *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi* (WHO 2018). *P. falciparum* is mainly transmitted from an infected human to another through the bite of the female *Anopheles* mosquito (Cox 2010); however, transmission through infected donor blood has not been discounted.

Blood transfusion practice is a very essential medi-
cal intervention meant to save life through replacing vital

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# APPENDIX 3: PUBLICATION FROM THIS STUDY

## BLOOD TRANSFUSION MONITORING FORM

Health Facility

Surname: ____________ First Name: ____________

DOB/Age ____________ Sex: ______ Ward: ____________

Hospital Number: ____________ NHIS ____________

*Blood component transfused:*

☐ Whole blood ☐ CRC ☐ FFP ☐ Cryoprecipitate ☐ Platelet Concentrate

Time STARTED ____________ Time COMPLETED ____________

| | START | FINISH |
|---|---|---|
| BP: | | |
| TEMP: | | |
| PULSE: | | |
| RESP: | | |

| | 15 MIN | 30 MIN | 1 HR | 2 HR |
|---|---|---|---|---|
| PULSE: | | | | |
| RESP: | | | | |

Was the whole unit transfused uneventfully? ☐ Yes ☐ No

If No, tick reasons for discontinuing transfusion:

☐ Challenges with venous access ☐ Patient reacted to transfusion

☐ Other reason (Please state) ____________

*In the event of a transfusion reaction, please complete a transfusion reaction form and return all blood units, required specimens and the completed form to the Blood Bank for investigation.*



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# APPENDIX 4: RESEARCH QUESTION

## CLINICAL SEQUELAE OF TRANSFUSION OF DONOR BLOOD EXPOSED TO SELECTED TRANSFUSION-TRANSMISSIBLE PARASITES AND MOLECULAR CONFIRMATION OF TRANSFUSION-TRANSMISSIBLE MALARIA

| S/No | Date |
|---|---|
| 1 | Study ID Number |
| 2 | Age of patient (yrs) |
| 3 | Department: Ward: |
| 4 | Occupation |
| 5 | Marital status: [ ]single [ ] married [ ] divorced |
| 6 | Gender[ ] Male [ ] Female |
| 7 | Temperature( °C) |
| 8 | Date donor blood collected : / / |
| 9 | Blood type of donor: |
| 10 | Date blood transfused: / / |
| 11 | Blood type of recipient: |
| 12 | Number of storage days |
| 13 | Is this your first time of receiving blood?[ ] Yes [ ] No |
| 14 | Current Medical Condition |
| 15 | Have you received blood within [illegible] [ ] Yes [ ] No |
| 16 | Have you taken any anti-malar[illegible] the past five days? [ ] Yes [ ] No |
| 17 | Number of blood units received[illegible] [ ] 4 [ ] 5 |
| 18 | Number of blood units received |
| 19 | Number of admission days |
| | |
| | **Home verification / follow up** |
| 20 | House Description: |
| 21 | How do you set home from hospital? [ ]Home [ ] Bike [ ] Car [ ]By foot |
| 22 | Landmarks that surrounds the direction to your home:<br>……………………………………………………………………<br>……………………………………………………………………<br>……………………………………………………………………<br>……………………………………………………………………<br>……………………………………………………………………<br>…………………………………………………………………… |
| 23 | Popular names: |
| 24 | GPS/ Ghana Post Address |
| 24 | Phone number:…(1) (2) |
| 26 | If number is not reachable who should contact: |
| 27 | Name: |
| 28 | Phone number:…(1) (2) |
| 29 | GPS/ Ghana Post Address |

This is to Certify that this Study's Inform Consent Form Has Been Approved by GHS-ERC for the Period 13/06/2020 to 11/06/2021
for Sign.... Date 03/07/2020
Name Celma Allotey
GHC-ERC Administrator

1



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**CLINICAL SEQUELAE OF TRANSFUSION OF DONOR BLOOD EXPOSED TO SELECTED TRANSFUSION-TRANSMISSIBLE PARASITES AND MOLECULAR CONFIRMATION OF TRANSFUSION-TRANSMISSIBLE MALARIA**

| S/No. | Indicator | Response |
|---|---|---|
| | ID Number(indicate F1, F2, F3 etc ): | |
| | Date: | |
| | Was recipient present on first visit? | [ ] Yes [ ] No |
| | Temperature | |
| | Was sample collected? | |
| | Next visit due on | |
| | | |
| | | |
| | | |
| | | |

This is to Certify that this Study's Inform Consent Form Has Been Approved by GHS-ERC for the Period 15/06/2020 to 11/06/2021
for Sign.......... Date 03/07/2020
Name
GHC-ERC Administrator

1

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# APPENDIX 5: CONSENT FORM

CLINICAL SEQUELAE OF TRANSFUSION OF DONOR BLOOD EXPOSED TO SELECTED TRANSFUSION-TRANSMISSIBLE PARASITES AND MOLECULAR CONFIRMATION OF TRANSFUSION-TRANSMISSIBLE MALARIA

## PARTICIPANTS' STATEMENT

On behalf of my child/ward, I acknowledge that I have read or have had the purpose and contents of the Participants' Information Sheet read and all questions satisfactorily explained to me in a language I understand (Twi, Ga, English, other: ................). I fully understand the contents and any potential implications as well as my right to change my mind (i.e. withdraw my child/ward from the research) even after I have signed or thumb-printed this form.

I voluntarily agree that my child/ward should be part of this research.

Name of Parent /Guardian ................................

Parent /Guardians' Signature ........................... OR Thumb Print.....................

Date:................................

## INTERPRETERS' STATEMENT

I interpreted the purpose and contents of the Participants' Information Sheet to the afore named participant to the best of my ability in the (Twi, Ga, English, other: ................).language to his /her proper understanding.

All questions, appropriate clarifications sort by the participants' Parent /Guardian and answers were also duly interpreted to his/her satisfaction.

Name of Interpreter................................

Signature of Interpreter............................ OR Thumb Print ....................................

Contact .................................. Date:...........................

This is to Certify that this Study's Inform Consent Form Has Been Approved by GHS-ERC for the Period 15/06/2020 to 17/06/2021
Sign...
Name... Zelma Blay (for)
GHS-ERC Administrator

1



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