TRANSFUSION TRANSMISSIBLE PARASITIC INFECTION AMONG BLOOD DONORS IN IBADAN, SOUTH WEST NIGERIA

AMOO ABIMBOLA M. (MB, BS)

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DECLARATION

This thesis is my work and has not been presented for degree in any other University or any other award.

Abimbola Muideen AMOO

Signature .....

Date.....12/12/2018

SUPERVISORS

We confirm that the work reported in this dissertation was carried out by the student under our supervision.

DR. KARIUKI NJAANAKE — Signature..... Date.....

(PhD Tropical Infectious Diseases)

Lecturer, Medical Parasitology

Department of Medical Microbiology.

University of Nairobi.

DR. HANNAH O.DADA-ADEGBOLA — Signature..... Date..13/12/18

(MBBS(Ib), Msc., FMCPath)

Senior Lecturer, Consultant Medical Microbiologist

Department of Medical Microbiology

and Parasitology.

University of Ibadan.

DR GLORIA OMOSA-MANYONYI Signature…….
Date……

(MBBS. (UoN), Msc Infectious Diseases, University of London)

Lecturer, Medical Parasitology

Department of Medical Microbiology

University of Nairobi

DEDICATION

This thesis is dedicated to my lovely father Mr Amoo Inaolaji and my mother Bolatito, they are my strength in everything I do.

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TABLE CONTENTS

CHAPTER THREE: MATERIAL AND METHOD

CHAPTER FOUR: RESULTS

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
CNS	Central Nervous System
EIA	Enzyme Immunoassay
ERC	Ethical Research Committee
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus (HIV)
IFAT	Immunofluorescent Antibody Test
KNH	Kenyan National Hospital
mRDT	Malaria Rapid Diagnostic Test
NBTSC	National Blood Transfusion Service Centre
PCR	Polymerase chain reaction
TTT	Transfusion – Transmissible Infection
TTM	Transfusion-Transmitted Malaria
TTPI	Transfusion- Transmissible Parasitic Infection
UCH	University College Hospital
UI	University of Ibadan
UoN	University of Nairobi
WHO	World Health Organization

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

BACKGROUND

In Nigeria, the available reports on transfusion-transmissible infections among blood donors are mostly prevalent studies on bacterial and viral pathogens that can be acquired by blood recipients through blood transfusion. There is paucity of data on transfusion-transmissible parasitic infections that can cause post-transfusion illness, especially in immunocompromised and transfusion-dependent patients. This study was designed to bridge the gap by screening for *Plasmodium falciparum* and *Toxoplasma gondii* which can be transmitted by blood transfusion and determine the effect of storage duration at 4°C on parasitic infection.

OBJECTIVES

To determine the prevalence of *P. falciparum* and *T. gondii*, identify sociodemographic factors associated with toxoplasmosis, and employ serology and microscopy methods to screen blood donors for *P. falciparum* and *T. gondii*, at the Blood Bank Transfusion Service Centre, South-west Nigeria.

METHODOLOGY

Cross-sequential study in Oyo state National Blood Transfusion Service centre. Demographic data and clinical history were obtained using a pro-formal questionnaire. Donors blood samples were tested for *P. falciparum* using both malaria Rapid diagnostic test (mRDT) kit and Giemsa stained microscopy, on initial day, 3 days, 7 days and 21 days after storage at 4°C. The remaining samples serum were tested for *T. gondii* infection using IgG and IgM ELISA test kits.

RESULTS

A total of 248 donated blood samples were tested for *P. falciparum* on initial day also, on day 3, 7, and 21 after storage period. Overall prevalence of *P. falciparum* on initial day was 16.5% and 8.5% using Malaria *P. falciparum* Rapid Diagnostic Test and Giemsa microscopy respectively. While the storage duration of 3, 7, and 21 days was associated with significantly lower in *P. falciparum* prevalence of 8.1%, 7.3%, and 5.7%, respectively ($p< 0.001$). The seroprevalence of anti- *T. gondii* IgG and IgM was 19.8% and 42.7% respectively. There was a significant difference in anti-*T. gondii* seroprevalence in vegetarian and non-vegetarian (20% vs 47%; $p <0.002$).

CONCLUSION

This study showed that *P. falciparum* and *T. gondii* are prevalent among blood donors in the southwest, Nigeria. Storage of donor's blood can significantly reduce the risk of transfusion-transmitted *P. falciparum* malaria. The diet and age of blood donors are major risk factors for *T. gondii* infection. This can be attributed to the poor sanitary conditions in the study area under study.

CHAPTER ONE

1.1 Background information

A transfusion transmissible infection (TTI) is an infection transmitted from one person to another through blood transfusion (Momoh *et al.* 2007). Infectious pathogens that cause TTI include bacteria, viruses, and parasites. Some of these pathogens also can be transmitted by other means including intravenous drug use, vector, and sexual intercourse but as transfusion is an important mode of transmission of this pathogenic microorganism, there is a need to screen blood before transfusion (Eze & Eze, 2015, Momoh *et al.* 2007).

Current transfusion blood screening services in the sub-Saharan Africa have focused mainly on viruses, such as HIV, Hepatitis B and C, and bacteria such as *Treponema pallidum* (Momoh *et al.* 2007). However, there is a need to consider other important pathogens such as *Plasmodium* spp. and *T. gondii* which have been implicated in TTI (Murphy, Evan & Vermeulen 2013).

The epidemiologic characteristic of most of the developing tropical countries such as Nigeria are characterized by the occurrence of endemic parasitic infections and diseases, which are either absent or rare in developed countries. Another major difference is lack of or inadequate capacity, both in terms of manpower and infrastructure, to diagnose and manage the diseases (World Health Organization, 2017).

In the developed countries, especially in Europe and the United States, there is continuous improvement of screening procedures with the relatively recent development of advanced serological and nucleic acid amplification test (NAT). These have resulted in decreased residual

risk of TTI over the past three decades (Karimi, Mardani & Zadsar 2016). Since the incidence of blood transfusion- transmitted parasitic infections (TTP) is lower in non-endemic regions,

most blood banks do not screen donor blood samples for potentially pathogenic haemoparasites. However, in endemic regions if the same practice is copied, it poses a risk to those requiring blood transfusion. This risk is higher in multiple blood recipients, neonates, pregnant women and immunocompromised patients, who have less ability to mount an immune response against the parasites (Eze & Eze, 2015).

The WHO recommends quality-assured screening of all donated blood for transfusion-transmissible pathogens such as HIV, hepatitis B, hepatitis C, *Treponema pallidum* (syphilis), *Trypanosoma cruzi* and *Plasmodium* spp. in some countries (Pondei, Lawani, & Ndiok, 2012). In spite of this, blood screening for parasites is not routinely carried out in many endemic countries such as Nigeria (Oladeinde *et al.* 2014). The concern about HIV and transfusion-transmitted hepatitis infection has overshadowed the fact that other diseases, particularly parasites like *Plasmodium* spp. and *T. gondii* can be spread by transfusion and cause severe infection especially in immunocompromised patients (Attah, 2000).

In Nigeria, screening for malarial parasites, *T. gondii* and other endemic hemoparasites are not included in the current National Blood Transfusion Guidelines. This is because transmission of parasitic microorganisms through blood transfusion is generally not regarded as a serious problem in an adult whose level of immunity is thought to be sufficiently effective in combating transfusion-transmitted parasitic infection in an endemic region (Federal Ministry of Health, 1991). High level of occurrence of blood transfusion demanding health conditions increase the possibility of transmission of bloodborne parasitic diseases (Attah, 2000).

1.2 Problem Statement

Transfusion-transmissible infections are widely studied in blood transfusion medicine with a lot of attention on viruses and bacteria as agents of infection, however, there are paucity of data

on mode of prevention of transfusion-transmitted infection and formulation of policies on deferment for donors. Most centres where blood transfusion services take place usually screen for HIV, HBV, and HCV without consideration for parasitic infections.

The available reports on Transfusion-transmissible parasitic infections among blood donors are mostly prevalence studies on malaria parasite while no mention is made of other parasitic infections that can cause post-transfusion illness, especially in immunocompromised and transfusion-dependent patients.

1.3 Justification

P. falciparum and *T. gondii* infections are common Transfusion transmissible parasitic infections with presentations ranging from asymptomatic carrier state to severe illness. They are common among immunocompromised individuals, pregnant women and those who require massive or frequent blood transfusion Sundar *et al.* (2007). There is a paucity of data on the prevalence and burden of Transfusion-transmissible parasitic infections, and how to reduce transmission risks among these high-risk populations in Nigeria, especially in the South-western part of the country.

Ibadan is a cosmopolitan city, in the rainforest belt of southwest Nigeria, with an open and stagnant drainage system, numerous substandard accommodation, and poor sanitary conditions. These create a favourable environment for the breeding of the *Anopheles* mosquito that serves as a vector of the *Plasmodium* spp. (Agbolade *et al*. 2013). Similarly, residents of Ibadan keep cat as pets and have easy contact with cats or cat faeces, which serve as host or aids the transmission of *T. gondii* to potential blood donors (Siransy *et al*. 2016).

CHAPTER TWO

LITERATURE REVIEW

2.1 Parasitic infections in blood transfusion medicine

P. falciparum and *T. gondii* are considered as threats to blood recipients' health especially immunocompromised and multiple unit's blood recipients (Sundar *et al.* 2007). The prevalence of *P. falciparum* infection ranges from 0.7 % to 55% in Nigeria (Owusu-Ofori *et al.* 2010). Seroprevalence of 20.8% was reported for *T. gondii* infection in a study by Uneke *et al* (2007) among healthy individuals in Nigeria, also in studies done by Ishaku *et al.* (2009), in Zaria, Northwest and Deji–Agboola *et al.* (2011), in Lagos, Southwest Nigeria revealed 29.9% and 40.8%, respectively.

Malaria infection caused by *Plasmodium* spp remain most common transmissible parasitic infection in the world (Cullen & Arguin 2014). The prevalence of malaria is significant in Nigeria because of heavy rainforest vegetation and poor drainage systems that enhance the breeding of the vector *Anopheles* mosquito.

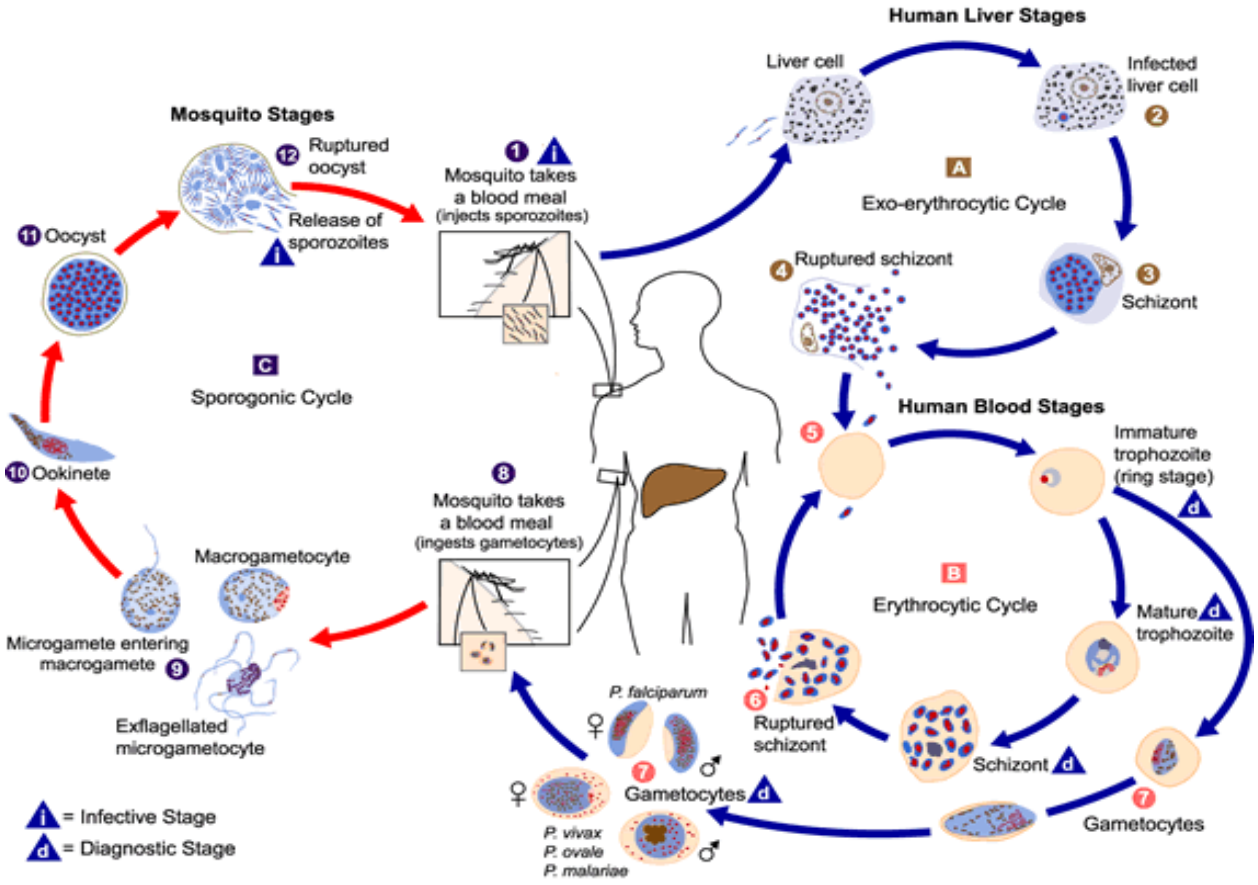
These two parasite species are transmissible via blood and blood product transfusions mainly due to: 1) the presence of asymptomatic stages in the course of parasitic infection; 2) the presence of an infective form of parasites within donor blood circulation, which can cause severe illness when transfused to immunosuppressed recipient in high concentration; 3) the ability of the parasite to survive in blood sample or blood product under storage conditions (Karimi, Mardani & Zadsar 2016).

The WHO recommends that donated blood should be screened for endemic parasites and other pathogenic organisms prior to transfusion (Pondei *et al.* 2012). Unfortunately, this is not done in most African countries (Ewunife *et al.* 2011). In Nigeria, the national blood transfusion service policy recommends that all donated blood be screened for Human immunodeficiency virus (HIV), Hepatitis B and C and, Syphilis (Federal Ministry of Health, 2005), thereby laying emphasis on screening for the viruses and syphilis and less or no attention is paid to the effect

of transfusion-transmitted *P. falciparum* and *T. gondii* despite their endemicity and public health importance.

2.2 Transfusion-Transmitted Malaria (TTM)

Human malaria is a mosquito-borne disease caused by intraerythrocytic protozoa of the genus *Plasmodium* namely; *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi. Plasmodium* spp. require two hosts to complete their life cycle. The sexual cycle occurs in the vector female *Anopheles* mosquito and the asexual cycle occurs in human in two phases involving the hepatocytes (pre-erythrocytic phase) and red blood cells (erythrocytic phase) (Figure 1). The incubation period of mosquito-transmitted malarial infection is about 1 - 2 weeks but that of transfusion-transmitted malarial infection takes about 2 – 4 days. This is partly because transfusion-transmitted parasites by-pass the extra-erythrocytic cycle (Karimi, Mardani & Zadsar 2016).



Source: Centre for Disease Control and Prevention

Figure 1: Life cycle of *Plasmodium falciparum*

The risk of acquiring transfusion-transmitted malaria is about 1 – 50 cases per million donor units in endemic regions including Nigeria. The factors influencing the prevalence of transfusion-transmitted malaria are seasonal variations with higher rates observed during the rainy season, criteria for blood donor selection and the blood donor screening methods (Pondei *et al.* 2012).

The majority of transfusion-transmitted malaria infections in Nigeria are caused by *P. falciparum.* According to Pondei *et al.* (2012) many blood donors in the endemic areas are asymptomatic carriers with parasite densities below the detection threshold of the currently available methods of diagnosis. These individuals have partially protective immune status and are generally the source of TTM (Pondei *et al.* 2012).

A parasite identified in a thick blood film is equivalent to about 10,000 parasites in a 450-mL unit of blood (Owusu-Ofori *et al.* 2013). This size of inoculum from transfused blood may pose a greater threat when compared to a bite from an infected mosquito, which has as few as 15 parasites (Owusu-Ofori *et al*. 2013).

In Nigeria, screening for malarial parasite is not stipulated in the current National Blood Transfusion Guidelines (Faruk, Ogunrinde & Mamman 2017). This is because transmission of malaria through blood transfusion is generally not regarded as a serious problem in an adult whose levels of immunity is thought to be effective in combating transfusion-transmitted malaria in an endemic region (Federal Ministry of Health, 2005). However, the high number of blood transfusion-demanding health conditions such as victims of road traffic accidents, pregnancy-related haemorrhage, and severe immunosuppression enhance the possibility of the transmission of blood-borne diseases (Ewunife *et al*. 2011).

The sensitivity of giemsa stained microscopy examination of malaria parasite decreases with decrease in parasitaemia, it is therefore, not sufficiently sensitive to be use as a screening tool

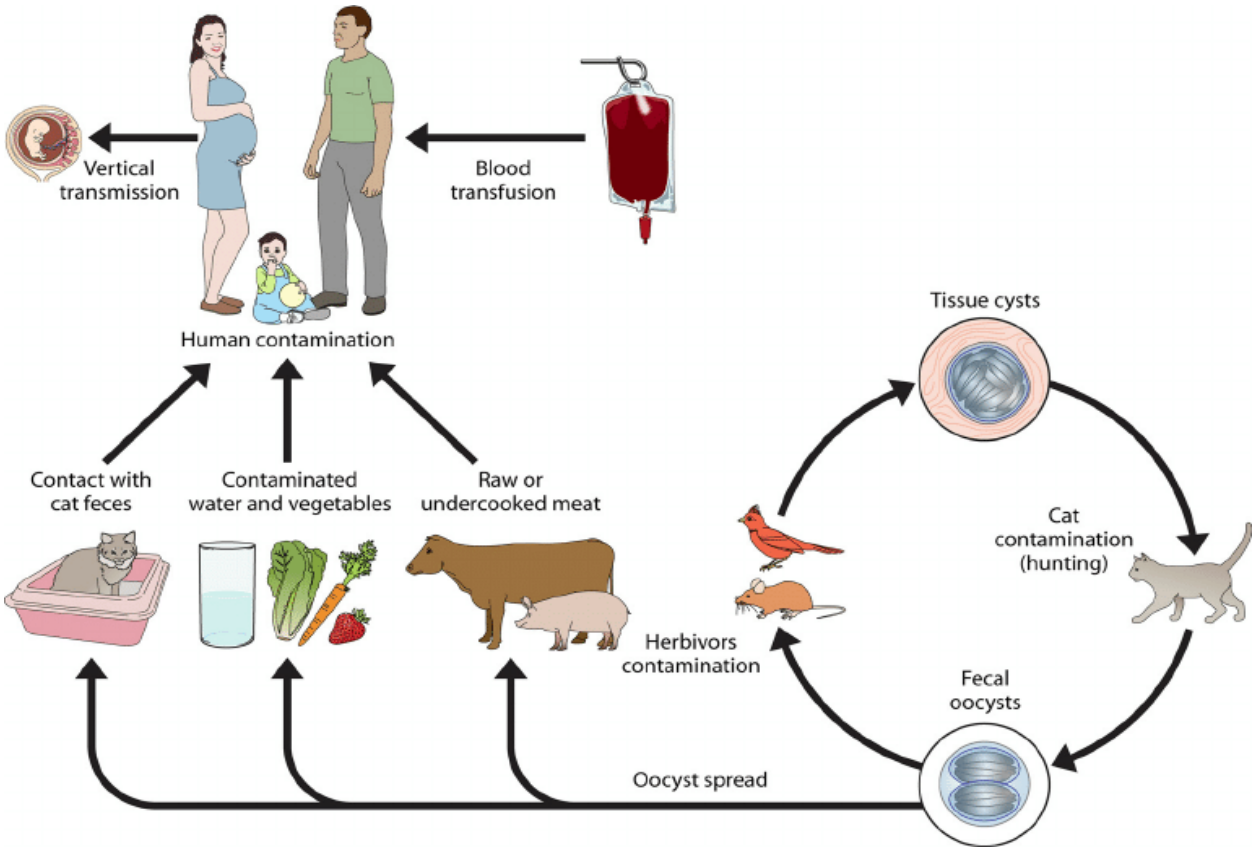
among asymptomatic *P. falciparum* infected carriers (Karimi, Mardani & Zadsar 2016). Other methods of diagnosis judged to be more sensitive include immunoassays such as indirect hemagglutination, Immunofluorescent assay, Radioimmunoassay, Enzyme-linked immunosorbent assay, and antigen-based rapid diagnostic test, as well as molecular amplification methods such as using agar-gel diffusion and immunoassay, for the detection of *P. falciparum* antigen and DNA (Abdel-wahab *et al*. 2012).

2.3 Transfusion-Transmitted Toxoplasmosis (TTT)

Toxoplasmosis is an opportunistic infection caused by the obligate intracellular blood protozoan called *T. gondii*, first discovered by Nicolle and Manceaux in 1908 in a small North African rodent- called *Ctenodactylus gundi*. It is an important zoonotic infection in which feline are the definitive hosts. The parasite infects about one-third of the world's population. (Babatoundé et al. 2017). Two forms of toxoplasmosis are found in humans, the actively proliferating *tachyzoites*, usually seen in the initial, more acute phase of the infection and the slowly dividing *bradyzoites*, which form cysts in brain and skeletal muscle, as a result of the immune response from the host (Babatoundé *et al.* 2017).

Routes of *T. gondii* infection in humans include 1) ingesting food or water that is contaminated with oocysts shed by cats, in the soil or in garbage; 2) eating undercooked or raw meat containing tissue cysts; 3) Contamination of open wounds, arthropod bites, transplantation and blood transfusion; 4) Congenitally, from infected mothers to their babies (Agbolade *et al.* 2013) and; 5) sexual transmission (Flegr *et al*, 2014) (Figure 2).

Following infection, the parasite invades human tissue and cells (Chiang *et al.* 2012), multiply in all nucleated cells by internal budding (*endodyogeny*). They proliferate within the host cell during the acute stage toxoplasma infection and form *pseudocyst*. These asexual stages of *T. gondii* give rise to merozoites, which enter the blood circulation and form cysts in tissue (Ogoina *et al*. 2013).



Source: [https://www.researchgate.net/figure/Toxoplasma gondii life cycle Domesticated and wild cats are the definitive](https://www.researchgate.net/figure/Toxoplasma-gondii-life-cycle-Domesticated-and-wild-cats-are-the-definitive) **hosts**

Figure 2: Life cycle of *Toxoplasma gondii*

The cysts remain viable in human tissues for many years and serve as the sources of transmission of infection via blood transfusion. The parasite disseminates through the bloodstream to different tissues and organs of the human body where it invades cells and multiplies (Ogoina *et al*. 2013). *T. gondii* infection acquired in pregnancy can result in congenital infection, that can manifest as mental retardation, epilepsy, blindness, chorioretinitis, stillbirth or abortion (Oyibo *et al*. 2009)

Toxoplasmosis acquired via blood or blood product transfusion is the main cause of ocular toxoplasma infection, which occur as result of a chronic proliferation of tachyzoites in the retina or as result of hypersensitivity response to ruptured tissue cyst (Frenkel, 1973). This may present as uveitis, chorioretinitis, or choroiditis, especially in immunocompromised patients (Frenkel, 1973). Other severe consequences of toxoplasmosis include myocarditis and encephalitis as a result of *T. gondii* infection transmitted via blood transfusion and may survive in citrated donor blood, at 4^{o}C, up to 50 days (Sundar *et al*. 2007).

It is possible to acquire toxoplasmosis through leukocytes or whole blood transfusions, especially if the parasitized cells are transfused in multiple units or in high concentration. Multiple transfusions are regularly administered to patients with sickle cell anaemia, aplastic anaemia and thalassemia, who need frequent and regular blood transfusion sourced from different donors for survival (Modrek, Mousavi & Saravani 2014). As a result, infected asymptomatic blood donors may transmit *T. gondii* (Siransy *et al*. 2016).

Studies have shown a high prevalence of anti-*T. gondii* antibodies among blood donors in sub-Sahara Africa but still screening of blood for T. *gondii* is not routinely done (Siransy *et al.* 2016). Similarly, studies on the epidemiology of *T. gondii* infection among groups of patients and healthy individuals have been carried out but only a few studies have been conducted among blood donors in Nigeria (Ogoina *et al*. 2011).

The rate of *T. gondii* infection among blood donors varies in different part of the world and depends on sociodemographic variables such as age, cultural lifestyle, food habits such as eating rats or cats, keeping cats as pet in the community (Mansouri *et al.* 2017). In a study among healthy individuals in northern part of Nigeria Ogoina and colleagues reported 32% seroprevalence of anti-Toxoplasma IgM and IgG (Ogoina *et al.* 2011).

The prevalence of asymptomatic *T. gondii* in blood donors as reflected by anti-*T. gondii* IgG/IgM seropositivity ranges from as low as 4.1% in Thailand to 75% in Brazil, and there is a general trend towards an increasing prevalence with age, and is higher in female than male donors (Gharib and Mayam 2016). *T. gondii* transmitted from healthy blood donors to recipients has become a major concern in transfusion medicine especially among groups of recipients with an impaired immune system (Chiang *et al*. 2012).

Screening for *T. gondii* infection among blood donors is mainly by serological tests, which includes Sabin-Feldman test, Indirect haemagglutination test, Direct haemagglutination, Indirect immunofluorescence test and Enzyme linked immunosorbent assay detection of anti-Toxoplasma IgG/ IgM antibodies in serum. Molecular screening, immunoblotting, and tissue biopsy have also been used for the detection of active *T. gondii* infections (Sarkari *et al.* 2014).

2.4 Research Questions

1. What is the prevalence of *P. falciparum* and *T. gondii* infections among blood donors in Ibadan, Southwest Nigeria?

2. What is the effect of donor blood storage and *P. falciparum*?

2.5 Hypothesis

Null: There is no transfusion transmissible *P. falciparum* and *T. gondii* in the blood of potential donors in Ibadan.

Alternative: There are transfusion transmissible *P. falciparum* and *T. gondii* in the blood of potential donors in Ibadan.

2.6 Objectives

2.6.1 Broad objective

To determine the prevalence of *P. falciparum* and *T. gondii* infections and related sociodemographic factors among blood donors at the Blood Bank Transfusion Service Centre, in Ibadan, South-west Nigeria.

2.6.2 Specific objectives

1. To compare malaria rapid diagnostic tests (mRDT) and Giemsa stained microscopy of thick and thin blood for method to screen freshly donated blood for *P. falciparum* infection at the Blood Bank Transfusion Service Centre, in Ibadan.
2. To determine the prevalence of *P. falciparum* in donated blood after refrigeration for 3, 7, and 21 days.
3. To determine the seroprevalence of *T. gondii* in blood donated for transfusion in Ibadan.
4. To identify sociodemographic factors associated with *T. gondii* infection seropositivity among donors.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study setting

Ibadan city is an important trade and educational Centre. It houses one of the largest and foremost teaching and general hospitals in Africa, with population of 1.3 million people. The city is located at an altitude range from 152 - 213m, latitude 7º 23' north of equator and longitude 3º 5' east, a distance of about 145km northeast of Lagos. The city being the largest in Africa south of Sahara, is characterized by a low level of sanitation, poor housing, and improper management of wastes. Majority of Ibadan residents have easy contact with a cat or own a cat, especially in the indigenous core areas, characterized by high population density and low-income (Afolabi *et al.* 2013). The present study was conducted at the University College Hospital, Ibadan Blood bank Service Centre.

3.2 Study population

The study population consisted of blood donors, at the Oyo state National blood transfusion service centre, Ibadan, majority of whom were members of voluntary nonremunerated blood donors.

3.3 Study design

The study was a cross-sequential study among blood donors in Oyo state National blood transfusion service centre conducted between May 2018 and July 2018. Only blood donors that were fit for blood donation and gave their consent were recruited into the study. Their venous blood samples were taken from the cord of the blood bags and examined for *P. falciparum* and *T. gondii* infection. Examination for *P. falciparum* was done on 0, 3, 7 and 21 days post collection.

3.4 Sampling methods

A random sampling technique was used to select all eligible and fit participants for the study. From the blood bank, 248 consenting donors aged between 18 and 59 years were randomly recruited following their informed consent. Those that have been treated for malaria parasite or *T. gondii* in last one month or on treatment for malaria were excluded.

3.5 Sample size determination

The sample size required to detect the prevalence of *P. falciparum* and *T. gondii* among donor blood in Southwest region of Nigeria was determined using the standard for cross-sequential study design as follows:

$n=Z^2*p*1-p/d^2$ (Israel, 1992).

Where,

n = required sample size.

Z = critical value at 5% level of significance=1.96

p=prevalence of malaria parasitemia in the fresh blood sample from previous study =17.5% (Okonkwo *et al.* 2012).

d=margin of error, set at 5% (0.05)

Therefore, substituting the respective values into the formula.

n=221 blood samples

Doing the same for the prevalence of parasitemia in stored blood, p=10%

$n=1.96^2x0.1x0.9/0.05^2$

n=138

Using the prevalence that gives the larger sample size between fresh and stored blood, i.e. nf = 221 blood samples.

Factoring nonresponse and attrition rate of 10%, i.e. 1- r.

The final sample size, n = nf/1- r.

n =221/1-0.1

=221/0.9

n=248

3.6.1 Inclusion Criteria

1. All consenting healthy donors in the Oyo state National Blood Transfusion Service Centre, Ibadan.
2. Blood donors who have been residing in Ibadan for the past three years.

3.6.2 Exclusion Criteria

Participants who had been on treatment for malaria.

3.7 Data and specimen collection of *P. falciparum* and *T. gondii* infection

3.7.1 Data and specimen collection

Structured closed- ended self - administrated questionnaire was used (Appendix 2). Section A covered the socio-demographic characteristics of blood donors such as age, race, marital status, and determinants socio-economic status such as level of education and occupation. Section B explored the risk factors determining the susceptibility of donors to *P. falciparum* and *T. gondii*

infection such as open and stagnant drainage system, raring cats, and drinking or eating boiled blood.

Ten millilitres (ml) of whole blood sample was obtained from each study participant's freshly donated blood through the tap attached to the blood bags using a sterile 10 mls syringe and needle. Two and a half (2.5) mls of each sample was aliquoted into four different universal bottles labelled f0, f3, f7, and f21 respectively. The blood in bottles labelled f0 was used to screen for *P. falciparum* and *T. gondii* immediately after collection. The remaining samples (f3, f7, and f21)

were stored at 4°C in the refrigerators at the Department of Medical Microbiology, University College Hospital, Ibadan, until use in microscopy for *P. falciparum* on days 3, 7 and day 21, respectively.

P. falciparum antigen was detected using Care start Pf Rapid Diagnostic Test kits, which detects *P. falciparum* Histidine-rich protein II. Presence of *P. falciparum* trophozoites stages were confirmed using Microscopy of Giemsa stained thick blood smear (Murray *et al* 2009). *P. falciparum* trophozoites, as well as parasite density count, were done by experienced microscopist who was blinded for RDT results. Anti-*T. gondii* IgG and IgM blood sample were detected by using qualitative anti-*T gondii* ELISA kits (Gharib & Maryam, 2016).

3.7.2 Examination of blood samples

Five microlitres (5 µl) blood was used to prepare thin and thick blood smear, which was stained with Giemsa and examined for malaria parasites under a light microscope. Another 5 µl of the blood sample was examined for the presence of *P. falciparum* Histidine-rich protein II antigen using Care Start kit, according to the manufacturer's instructions.

3.7.3 Rapid Diagnostic Test for P. falciparum

Care Start (Pf) cat No. G0140 RDT kits were used in the entire study. The test kit employs lateral-flow immunochromatographic technology (ICT), to detect *P. falciparum* antigens. The kits were used to test for the presence of Histidine-rich protein II antigen in each fresh blood sample on day 0 (f0) only, according to the manufacturer instructions. About 5µl of blood picked and transferred into the sample well of the cassette using capillary tube, then two drops of the assay diluent were added into the square assay diluent well of each cassette. The blood sample migrated as a liquid across the surface of the nitrocellulose membrane by means of capillary action and was captured by monoclonal antibodies bound to the membrane in an immobile phase. The antibodies were bound to *P. falciparum* Histidine-rich protein II antigen from the migrating blood sample. The second set of antibodies are conjugated to an indicator, typically gold particles, in a mobile phase.

The antibody-indicator complexes are bound to the parasite antigen, if present, that was captured by the immobile antibody on the membrane, producing a visible line thus indicating the present parasite

Histidine-rich protein II in the blood sample. The test was read after initial 15 minutes, the presence of two colour bands (Pf test line and C control line) within the P.f test window was considered *a P. falciparum* positive result.

3.7.4 Laboratory investigation for *P. falciparum* by Microscopy

Thin and Thick blood smears were prepared from each fresh donor blood sample and air dried, then stained with freshly prepared 10% Giemsa's stain solution for ten minutes. The stain was washed off gently and slides air-dried before they were examined under a microscope at X 100 oil immersion magnification. The slide examination was done by two microscopists who were blinded for the mRDT results.

Where there was disagreement on the reading between the two microscopists, a third microscopist was involved as a tiebreaker. For slide positive malaria parasite, the number of parasites was counted against 200 leucocytes and quantification of parasite density estimated by assuming 8,000 leucocytes/µl of blood (Junior *et al.* 2014), this was repeated on day 3 (f0), day 7 (f7) and day 21 (f21). Slides were reported negative when no *P. falciparum* was detected in 100 fields of each thick smear. A sample with known high number of *P. falciparum* trophozoite was used as positive control.

3.7.4 *T. gondii* Antibody Detection using IgG and IgM

Anti-T. gondii IgM and IgG were detected using commercially available anti-toxoplasma antibody Enzyme-linked immunosorbent assay (ELISA) kits, according to the manufacturer's instructions.

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

A chromogenic substrate was added to produce yellow colour whose intensity depends on the amount of antibody in the complex. The absorbance of the microwell contents was read by a spectrophotometer with a filter of 450 nm. The calibrators and controls are utilized in internal quality control and in the qualitative interpretation of the assay. A sample that turned positive for anti-*T. gondii* IgG and negative for IgM was interpreted to mean a chronic *T. gondii* infection. A sample with both anti-*T.* gondii IgG and IgM *T. gondii* was considered to be a presence of acute on chronic *T. gondii* infection. A positive IgM anti-*Toxoplasma gondii* only sample was considered to be the presence of acute and recent *T. gondii* infection.

3.7.6 Quality Assurance

All the requirements to obtain quality, relevant and reliable test results were fulfilled during the pre-analytical, analytical and post-analytical stage of the project. At the pre-analytical

stage, there was proper documentation and correct labeling of collected blood samples per donor from the blood bags and samples were transported to the University College Hospital, Medical Microbiology laboratory for processing. At the analytical stage, all equipment, instruments, and materials used for microscopy such as Giemsa staining reagent, pipette and light microscope were calibrated for proper working conditions by checking whether appropriate maintenance was done.

Serological analysis was done by an experienced technologist in the most aseptic methods under biosafety level II cabinets with personnel wearing protective clothing such as lab coats and gloves to avoid contamination. All reagents and diagnostic kits used were within their valid date. Positive controls and negative control were analysed for each IgG antibody as well as IgM antibody, according to the manufacturer's instructions. At the analytical stage, correct and legible documentation was done for results which were stored in both soft and hard copies. All contaminated material was autoclaved before disposal to avoid environmental contamination.

3.8 Data analysis

Data were entered into Microsoft Excel spreadsheet and double-checked for accuracy. Data on prevalence of *P. falciparum* and *T. gondii* infection, and sociodemographic risk factors were analyzed using STATA version 12 (Stata Corporation, College Station, Texas, USA). Categorical variables such as gender and occupation were presented in frequencies and percentages. Comparative statistics were carried out using Student's *t*-test for continuous variables and Chi-square test for categorical variables. Correlation between parasite positive samples with socio-demographic characteristics was tested using the Pearson's correlation coefficient. The effect of storage duration on *P. falciparum* parasite density was compared using Kendall's Tau test. For all tests of significance, confidence level was set at 95% and p-value < 0.05 were regarded as statistically significant.

3.9 Ethical Considerations

Ethical review and approval for the study was obtained from Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH-ERC) prior to the inception of the study (Appendix I). Permission to conduct the study was also obtained from Oyo State ethical review committee in Oyo state, Ibadan, Nigeria (Appendix II). Written informed consent was obtained from each study participant before enrolment into the study (Appendix III). The participation was totally voluntary. Confidentiality was maintained at all times during and after the data collection. Specimen and data were stripped of all personal identifiers.

CHAPTER FOUR

RESULTS

4.0 Study Population

A total of 248 asymptomatic blood donors aged between 18 and 60 years were enrolled into the study. Eighteen (7.2%) of the participants were females and 230 (93%) were males, with 246 (99.2%) voluntary nonremunerated blood donors while 2 (0.8%) were commercial blood donors. Two hundred and thirty-two (94%) participants were residents of Ibadan and 194 (78.2%) were natives of Southwest Nigeria. About 42.7% were between aged 29 and 39 years, and 58% of the participants were higher institution graduates. One hundred and twenty-two (49.1%) of the donors reported sleeping under mosquito bed nets regularly and 1.6% were on malaria prophylaxis during the sample collection.

4.1 Seroprevalence of *Plasmodium falciparum* infection using mRDT

Determination of *P. falciparum* seroprevalence was based on mRDT results. A total of 248 blood samples were tested, and each serum was tested for *P. falciparum* antigen at initial day 0, and 41 samples (16.5%) were positive for *P. falciparum* antigen.

4.2 Proportions of *P. falciparum* parasitaemia by microscopy

Blood film microscopic examination was conducted on each donor blood sample at day 0 (f0) then on day 3, day 7 and day 21. A total of 248 thick and thin films were observed at the initial day 0, and trophozoite of *P. falciparum* were seen in 21 (8.5%) donor samples. Ten (4%) donors' samples *P. falciparum* positive by mRDT were confirmed *P. falciparum* positive using Microscopy, while 11 donor (4.4 %) sample were mRDT negative and microscopy positive on day 0, as shown in Table 1.

Table 1: Comparison between mRDT and Microscopy seropositivity on day 0

Malaria RDT day 0	Microscopy day 0		Total
	Negative	Positive	
Negative	196	11	207
Positive	31	**10**	**41**
Total	227	**21**	**248**

($p = 0.000$)

4.3 Proportion of *P. falciparum* parasitaemia in follow-up stored blood samples by Microscopy

The first follow-up microscopy was carried out on each blood sample on day 3 (f3), following storage at 4°C. The prevalence of *P. falciparum* infection on day 3 was (8.1%) when compared with microscopy result (8.5%) on day 0, which was a statistically significant difference (p <0.001). The second follow-up was conducted after 7 days (f7) of storage at 4°C. Each blood sample was examined for *P. falciparum* using microscopy. The prevalence of *P. falciparum* infection, also slightly reduced on day 7, to 7.3% (p<0.001).

Third follow-up was carried out on day 21. The prevalence of *P. falciparum* infection significantly dropped on day 21, to 5.7% compared to 7.3% prevalence on day 7, the result was statistically significant (p<0.001). In general, the prevalence of *P. falciparum* infection decreased with increase in duration of storage at 4°C (Figure 3).

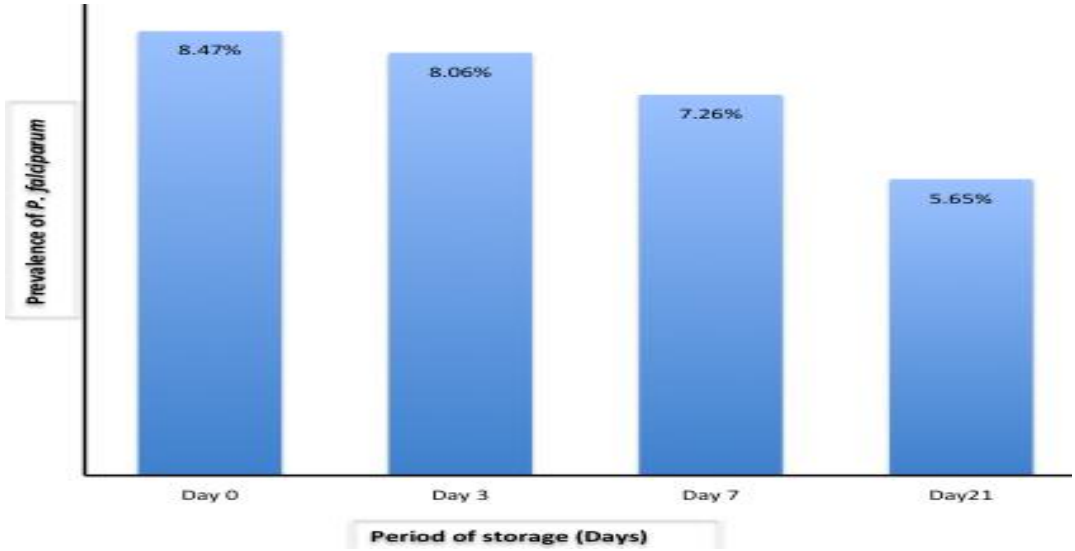


Figure 3. *P. falciparum* by microscopy in relation to days of blood refrigeration

4.4 Effect of blood storage duration on *P. falciparum* density

During the baseline survey on day 0 and the three subsequent follow-ups at day 3, 7, and 21, the parasite density for the initially positive samples by microscopy ranged between 75 and 8679 parasite/µl of blood. In the first follow-up, parasite density was between 37 and 7885 parasite/µl of blood, the 3 days period of storage was associated with significantly lower parasite densities (Kendall's $\tau b = 0.9738$, $p < 0.001$).

In the second follow-up, after 7 days parasite density was between 36 and 3680 parasite/µl of blood, the period of storage was associated with significantly lower densities of *P. falciparum* (Kendall's $\tau b = 0.9196$, $p < 0.001$). The third follow-up *P. falciparum* parasite density was between 15 and 379parasite/µl of blood. The 21 days period of storage was associated with significantly lower densities of *P. falciparum* parasite (Kendall's $\tau b = 0.8042$, $p < 0.001$).

4.5 Prevalence of *Plasmodium falciparum* infection among donors

P. falciparum infection prevalence was determined using mRDT on day 0. The prevalence was 16.5% whereas the proportion of participants positive for *P. falciparum* by microscopy on day 0 were 8.5%, 8.1%, 7.3% and 5.4%, during the 0, 3, 7, and 21, respectively.

4.6 Association of *P. falciparum* infection prevalence using mRDT and socio-demographic characteristics

Chi-square (χ^2) test was used to determine the association between mRDT *P. falciparum* results and gender, age group, education level, occupation, antimalaria prophylactic use, regions of origin, ABO blood group, and use of mosquito bed nets. There was no significant difference in prevalence *P. falciparum* and occupation ($p = 0.102$). The highest *P. falciparum* prevalence (58%) was found in the 29 - 39 age group, whereas no prevalence was reported in the age group less than 19 years. There was no significant difference in prevalence of *P. falciparum* and higher age groups (χ^2 test; $p = 0.475$).

However, there was significantly higher seropositivity among group of students that are yet to attain graduate from higher institution compared to graduates and postgraduate level of education. The difference between education level and presence of *P. falciparum* antigen was statistically significant ($p = 0.002$). No significant differences in *P. falciparum* infections were found with regard to region ($p = 0.676$), use of mosquito bed nets ($p = 0.531$), ABO blood group ($p = 0.141$) or use of prophylaxis ($p = 0.069$), as shown in Table 2.

Table 2: Association of *Plasmodium falciparum* infection seroprevalence using mRDT seroprevalence socio-demographic characteristics

Characteristics	mRDT Positive	mRDT Negative	χ^2 test	P value
Malaria prophylaxis			3.3001	0.069
Prophylaxis	2	2		
Not prophylaxis	39	205		
Mosquito bed nets			0.3918	0.532
Used	22	100		
Not used	19	107		
Region of Nigeria			4.2049	0.240
Southwest	30	164		
North-centre	9	23		
South-south	1	8		
Other	1	12		
Level of Education			12.2342	0.002*
Non-educated	7	15		
Educated	20	62		
Graduated	14	130		

*Statistically significant level of

4.7 Prevalence of T. gondii IgG among blood donors

Anti-*T. gondii* IgG ELISA test was conducted on donor serum samples stored at 4°C. Of the 249 blood donors, 49 (19.8%) tested positive for *T. gondii*. With regard to age, the highest prevalence of anti- *T. gondii* (58%) was found in the age group 29-39. The difference between age group and presence of anti-*Toxoplasma gondii* IgG antibodies was not statistically significant (p = 0.148). However, there was statistically significant higher seropositivity among vegetarians compared to non-vegetarians (χ^2 test; $p = 0.033$).

4.8 Prevalence of anti-*T. gondii* IgM among blood donors

Toxoplasma gondii IgM antibody ELISA test was conducted after 45 days of samples storage at 4°C. ELISA qualitative test was done for each sample following the manufacturers' instructions, independent of the ELISA anti-*T. gondii* IgG antibody results. Out of 248 stored blood samples tested, *T. gondii* IgM antibody were detected in 106 samples, corresponding to 42.7% IgM seropositive sera.

4.9 Association of anti-*T. gondii* infection seropositive and socio-demographic characteristic

χ^2 was used to determine the association between *T. gondii* infection and the socio-demographic characteristics as shown in Table 3. The highest prevalence (30%) and (43.3%) of anti-*T.*- *gondii* IgM positivity was reported in the 19 - 29 and 29 – 39 years age group respectively, and the lowest (0.01%) was found in the less than 19 years age group. The difference between age groups in prevalence of anti-*Toxoplasma gondii* IgM antibodies was not statistically significant ($p = 0.917$).

There was significant association between seroprevalence of *T. gondii* IgM antibodies in vegetarians (20 %) and (47%) non-vegetarians (χ^2 test; $p<0.01$). There was no significant association between anti-*T. gondii* IgM antibodies in drinking unpasteurized milk ($p = 0.051$). There was no significant association between seroprevalence of anti-*T. gondii* IgM in contact with a cat ($p = 0.470$), eating a game animal ($p =0.659$), education ($p = 0.705$), previous blood transfusion ($p = 0.470$) or eating undercooked meat ($p = 0.498$), as shown in Table 3.

Table 3. Prevalence of anti- *T. gondii* IgM in relation to socio-demographic characteristics among blood donors.

Sociodemographic Characteristics	Anti-Toxoplasma IgM		χ^2 test	P value
	Positive	Negative		
Vegetarian			10.0789	0.001*
Yes	8	32		
No	98	110		
Eating undercooked meat			0.4589	0.498
Yes	65	81		
No	41	61		
Contact with cat			0.5229	0.470
Yes	3	1		
No	103	141		

*Statistically significant at level of

CHAPTER FIVE

DISCUSSION

5.0 Parasitic infection among blood donor

Screening of blood donors is important for prevention and control of transfusion-transmissible pathogens to the blood recipients. Although microscopy is the diagnostic method commonly used for the detection of parasitic infection, serological screening tools such as RDT and ELISA are essential for detection of parasite antigen and antibodies, respectively.

5.1 *Plasmodium falciparum* infection among blood donors

The proportion of *P. falciparum* infection among blood donors detected by mRDT of 16.5% at day 0 is similar to previous report in Ghana, in a study by Owusu-Ofori *et al* (2013). Similarly, the finding of 8.5% prevalence of *P. falciparum* by microscopy agreed with findings of 8.2% reported amongst blood donors in Ibadan, Nigeria (Okonkwo *et, al.* 2012). The mRDT results showed a higher prevalence of *P. falciparum* when compared to microscopy results during the entire study period. Although mRDT has an accuracy of 86-99% compared to microscopy and has a high specificity for *Plasmodium* spp. (El-Sayed *et al.,* 2015), it may still be positive after treatment and clearance of parasites from blood circulation.

Since erythrocytes in blood remain alive during storage, it was assumed that the parasites in the erythrocytes were also alive and transmissible. However, there was a significant decline in parasiteamia of *P. falciparum* with increased period of blood storage from day 0, to day 21. Storage in low temperatures has been reported to have significant effect on *P. falciparum*. A similar study done in United State, there was a reduction in viability of malaria parasite, as no asexual multiplication was observed, after storing for more than 14 days at 4°C (Chattopadhyay *et al.* 2015). The results from the present study suggest that stored blood is less likely to contain *P. falciparum* with increased duration of storage and may be safer for malaria non-immune

individuals who require blood transfusion. Although, the prevalence of *P. falciparum* by microscopy in the study is low, this could be because the study was carried out during low transmission season (May to July) (Chaponda *et al.*, 2015).

5.2 *Toxoplasma gondii* among blood donors

The 19.8% prevalence of anti-*T. gondii* IgG found in this study is low compared to 32% seroprevalence reported among healthy individuals in northern part of Nigeria (Ogoina *et al*. 2011), but similar to findings of the studies among voluntary blood donors in Karnataka Indian (Sundar et al., 2007). However, the findings were discordant to seroprevalence among blood donors in Iran (Sarkari *et al.* 2014) and Thailand (Somchai *et al.* 2000), with lower results of 12.3% and 4.1%, respectively. This result suggests that there was less chronic *T. gondii* infection among blood donors in Ibadan, Southwest Nigeria.

The seroprevalence of anti- *T. gondii* IgM in this study was 43%, this was high compared to prevalence of 11.3%, 5.5% and 0.3% among blood donors in Abidjan, Côte d'Ivoire (Siransy *et al.* 2016), Southern Iran (Sarkari *et al.* 2014) and Taiwan (Chiang *et al.* 2012), respectively. This implies that many of the blood donors will seroconvert from acute infection to chronic toxoplasmosis, which may lead to retinochoroiditis, encephalitis or epilepsy later in life (Lappalainen & Hedman, 2004).

These variations in the seroprevalence of anti- *T. gondii* antibodies among blood donors might be due to difference in sex, ages, food habits, residences, level of education, environmental hygiene, contact with cat and socioeconomic status (Mansouri *et al.* 2017) in the study area. Moreover, the types of antibodies for detection, the types of test kit as well as sensitivity and specificity of the test kit also varies (Chiang *et al*. 2012).

In addition, the high seroprevalence rate of 12.9% and 18.5 % were found in age group 19 – 29 years and 29 – 39 years, respectively. This has been reported from another study among blood donors in Northeast Thailand (Somchai *et al*. 2000), although contrary to seroprevalence

of anti-*T. gondii* IgM reported among similar age groups (5.4%) in the southern Iran (Moderek *et al.* 2014). This indicates an increase in prevalence with increase in age, however, acute or recent infection was observed in younger age group blood donors. The reason might be due to increased risk to exposure to infection source with age.

Seropositivity of *T. gondii* IgM antibodies was higher in non-vegetarian (47%) than in vegetarian (20%) as similarly reported in 98% to 2% in Karnataka India (Sundar *et al*. 2007), and 96% to 4 % in Southwest Iran (Velayutharaj A. *et al.* 2017) among blood donors as well as in nationwide study in Germany (Hussain, Stitt, Szabo, & Nelan, 2017). The reason may be due to frequent consumption meat infected by *T. gondii* that is not well cooked.

Conclusions

1. The study showed that mRDT kits are effective in the screening of *P. falciparum* compared to microscopy, especially in routine blood bank screening where microscopy may be a challenge.
2. Malaria parasites reduced with duration of storage, and therefore, the risk of transmission through blood transfusion can be reduce, if the blood can be stored for a longer duration before transfusion.
3. Level of education was negatively associated with risk of *P. falciparum* carriage, probably due to level of awareness.
4. The seroprevalence of *T. gondii* was high among blood donors from Southwest region of Nigeria.
5. The anti- *T. gondii* IgM prevalence was relatively higher compared anti-*T. gondii* IgG, implying the majority of seropositive donors had an acute or recent infection, might seroconvert to chronic infection.
6. There was a lower seropositivity of *T. gondii* among vegetarian, suggesting that eating meat may pose a major risk of exposure to *T. gondii* infection in the region.

7. Age was negatively correlated with risk of T. gondii infections among the donors.

Recommendation

i. Mass screening of blood donors should be considered to identify asymptomatic voluntary non-remunerated blood donors, that are at risk of transmitting *P. falciparum* and *T. gondii* via blood transfusion.

ii. The prevalence of the malaria and *T. gondii* infection in the larger population in every region should be determined.

iii. Parasites control efforts should be enhanced to reduce the parasite reservoir in the larger population.

Further Research

i. Further study on molecular diagnosis is needed to determine other parasitic infection that can be transmitted via blood, and to reduce false positive diagnosis.

ii. There is a need to determine the incidence of transfusion-transmitted parasitic infection in pre and post-transfusion among immunocompromised blood recipients.

iii. It is necessary to conduct a comparative study of effective modes of diagnosis malaria among blood donors in malaria hyperendemic regions in Nigeria, to compare with the current study findings.

iv. Also, there is need to conduct a study on Toxoplasmosis among HIV/AIDS patients to compare it with high acute *T. gondii* infections in current study findings.

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APPENDICES

Appendix I: Institution ethical clearance form

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 09 MAY 2018 KNH/UON ERC P.O Box 20723-00202 NAIROBI

Ref: KNH-ERC/A/169

May 9, 2018

Dr. Amoo Abimbola M.
Reg. No.H56/5200/2017
Dept. of Medical Microbiology
School of Medicine
College of Health Sciences
University of Nairobi

Dear Dr. Ambimbola

RESEARCH PROPOSAL – TRANSFUSION TRANSMISSIBLE PARASITIC INFECTIONS AMONG BLOOD DONORS IN IBADAN, SOUTH WEST NIGERIA (P659/11/2017)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is from 9th May 2018 – 8th May 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

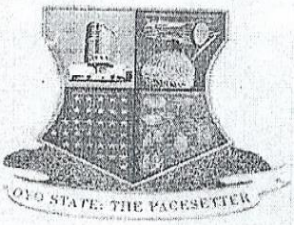
For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Chairperson, KNH-UON ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine, UoN
The Chair, Dept. of Medical Microbiology, UoN
Supervisors: Dr. Gloria Omosa-Manyonyi, Dr.Kariuki Njaanake

TELEGRAMS..... TELEPHONE.....



MINISTRY OF HEALTH

DEPARTMENT OF PLANNING, RESEARCH & STATISTICS DIVISION

PRIVATE MAIL BAG NO. 5027, OYO STATE OF NIGERIA

Your Ref. No. .....

All communications should be addressed to
the Honorable Commissioner quoting
Our Ref. No. AD 13/ 479/ 202

28th September, 2016

The Principal Investigator,
Department of Medical Microbiology and Parasitology,
University College Hospital,
Ibadan,
Oyo State.

Attention: Abimbola Amoo

ETHICAL APPROVAL FOR THE IMPLEMENTATION
OF YOUR RESEARCH PROPOSAL IN OYO STATE

This is to acknowledge that your Research Proposal titled: "Transfusion Transmissible Parasitic Infections among Blood Donors in Ibadan" has been reviewed by the Oyo State Review Ethical Committees.

2. The committee has noted your compliance. In the light of this, I am pleased to convey to you the full approval by the committee for the implementation of the Research Proposal in Oyo State, Nigeria.

3. Please note that the National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations, in line with this, the Committee will monitor closely and follow up the implementation of the research study. However, the Ministry of Health would like to have a copy of the results and conclusions of findings as this will help in policy making in the health sector.

4. Wishing you all the best.

Signature & Date

Dr. Abbas Gbolahan
Director, Planning, Research & Statistics
Secretary, Oyo State, Research Ethical Review Committee

INFORMATION SHEET AND CONSENT FORM

TRANSFUSION TRANSMISSIBLE PARASITIC INFECTIONS AMONG BLOOD DONORS IN IBADAN, SOUTHWEST NIGERIA.

INFORMATION SHEET

My name is Amoo Abimbola M. of the Department of Medical Microbiology and Parasitology, University of Nairobi, Kenya. We are interviewing blood donors in Ibadan in order to design a protocol for screening donate blood with a view to prevent transfusion transmissible infections (including parasitic infection) in the blood recipients.

Procedure of the Research: A total of 247 participants will be recruited into this study, they are blood donors in National blood transfusion service centre, Ibadan and Blood bank, University College Hospital, Ibadan. The informed consent form as well as questionnaire to assess the risk factors will be given to each participant to fill,after the donor consent to the study,10ml of donated blood will be obtained from their blood bags and divided into four. The first will be screened immediately for the parasitic agents while the remaining three will be stored at 4°C for 3, 7 and 21 days respectively after which it will be screened for the same set of parasitic agents.

Expected duration of research: This research is expected to last about three months, your active involvement will be at the point of blood donation which may take about 2 hours, after which your physical presence may no longer be necessary.

Risks: Your involvement in this research puts you at no risk.

Costs to the participant: This research will not cost you anything.

Benefit: This study will contribute to the body of scientific knowledge in establishing policy on effective blood donor screening, according to WHO guidelines. The knowledge about the viability of transfusion transmissible haemoparasites in the stored donor bloods will help in reducing post transfusion parasitic infections.

Confidentiality: All information collected in this study will be given code numbers and no name will be recorded. This cannot be linked to participants in any way and their name or any identifier will not be used in any publication or reports from this study.

Voluntariness: Your participation in this is entirely voluntary.

Alternatives to participation: If you choose not to participate in this study, there will be no threat to you.

Due inducement: You will not be paid any fee for participating in this research but your results will be made available to you.

Consequences of participant's decision to withdraw from research and procedure for orderly termination of participation: You can also choose to withdraw from research at any time. Please note that some of the information that has been obtained about you before you choose to withdraw may have been modified or used in reports and publications. These cannot be removed anymore. However, the researcher promises to make effort to comply with your wishes as much as it is practicable.

Modality of providing treatments and actions to be taken in case of injury or adverse events: If you suffer any injury as a result of donating blood in this research. You will be treated at the University College Hospital, Ibadan, and the researcher will bear the cost of this treatment.

What happens to research participants and communities when the research is over?

I will inform you of the outcome of the research through text messages. During the course of this research, you will be informed about any information that may affect your continued participation or your health.

Statement about sharing of benefits among researchers and whether this includes or exclude research participants: If this research produces any benefits, it shall be owned by me. There is no plan to contact any participant now or in future, about such benefits.

Any apparent or potential conflict of interest: Neither the investigator nor the supervisor has shares in any company that produces/sell assay kits.

Statement of person obtaining informed consent: You are free to refuse to take part in this project.. You have a right to withdraw at any giving time if you choose to. We will greatly appreciate your help in responding to the survey or questionaire and taking part in the study.

Statement of person giving consent: Now that the study has been explained to me and I fully understand the content of the process, I will be willing to take part in the programme.

Signature/Thumbprint participant: ..... Interviewer date.....

Witness' signature/ Thumbprint of witness ..... Date.....

Contact Details

This research has been approved by the Kenyatta National Hospital/ University of Nairobi / Ethics & Research Committee and the chairperson of this committee can be contacted at; College of Health Sciences P.O. Box 19676 code 00202 Nairobi. Tel. (254-020) 2726300-9 Ext 44355.

Email: uonknh_erc@uonbi.ac.ke. In addition, if you have any question about participation in this research you can contact me: Amoo Abimbola, Department of medical microbiology. University of Nairobi, Kenya.

Tel: +2348060365704, +254796088165.

PLEASE KEEP A COPY OF THE SIGNED INFORMED CONSENT

Appendix IV

A SURVEY OF RISK FACTORS TO TRANSFUSION TRANSMISSIBLE PARASITIC INFECTIONS AMONG BLOOD DONORS IN IBADAN, SOUTHWEST NIGERIA.

Good day Sir/ Ma, this questionnaire is being administered to know whether a donor carries transfusion transmissible haemoparasite which can be transmitted to the potential recipients of your blood. It will take about 5 minutes to fill, please respond honestly to the questions below. The confidentiality of your response is guaranteed. Thank you.

SECTION A (DEMOGRAPHIC DATA)

Date ……... Serial Number ……

Age (years)…… Residential area …….

Sex…….…… City/Town……..

Nationality: …….. State ……..

Educational Status: No formal Education [ ] Primary Six Certificate [ ] Junior School Certificate [ ] Senior School Certificate [ ] Graduate [ ] Postgraduate [ ]

Profession/ Occupation: ……

SECTION B (MEASUREMENTS)

Body weight (kg)_____

SECTION C (MEDICAL HISTORY) PART I

Please tick the appropriate answer

1. Do you surfer from any other disease condition [YES] [NO]

2. Are you on any drugs [YES] [NO]
3. If yes please specify?
4. When last did you travel out of country? Please specify……
5. Where did you travel to? Please specify…….
6. Any history of yellowness of eye? [YES] [NO]
7. Any history of weight loss? [YES] [NO]
8. Do you live in riverine area [YES] [NO]
9. Any history of night sweat? [YES] [NO]
10. Any history transfusion/ organ transplant? [YES] [NO]
11. Do you reside in the city? YES [ ] NO[ ]
12. If yes, how long have you being residing in the city?
13. Did you sleep under mosquito bed nets (ITN)? [YES] [ ] [NO] [ ]
14. Are you on any antimalarial prophylaxis drugs? [YES] [ ] [NO] [ ]
15. If yes, please specify the drugs……..

SECTION C (MEDICAL HISTORY) PART II

Please tick the appropriate answer

1. Any Febrile illness [YES] [ ] [NO] [ ]
2. Do you surfer from any other disease condition? [YES] [ ] [NO] [ ]
3. If yes, please specify ……
4. When last did you travel out of country? [YES] [ ] [NO] [ ]
5. If yes, please specify……
6. Do you rear animals [YES] [NO]
7. If yes, what animal do you rear? ……..
8. Did you have cat at home or have been in contact with cat/eat cat before? [YES] [NO]
9. If yes, please specify……..

10. Do you eat cat? [YES] [NO]

11. Do you eat game/bush animal? [YES] [NO]

12. Do you reside in the city? YES[ ] NO[ ]

13. If yes, how long have you being residing in the city?

14. Do you eat undercooked meat? [YES] [ ] [NO] [ ]

15. Do you eat raw meat? [YES] [ ] [NO] [ ]

16. Do you eat raw vegetable outside the home? [YES] [ ] [NO] [ ]

17. Do you like eating meat free diet or you are vegetarian? [YES] [ ] [NO] [ ]

18. Do you live in hilly area? [YES] [ ] [NO] [ ]

19. What type of water do you drink? Boiled water [ ] Tap water [ ] Well [ ]

20. Do you consume unpasteurized milk? [YES] [ ] [NO] [ ]

21. Do you consume raw fruit/ unwashed fruit? [YES] [ ] [NO] [ ]

22. Are you on any chemoprophylaxis drugs? [YES] [ ] [NO] [ ]

23. If yes, please specify the drugs …….

SECTION D (DONATION INFORMATION)

1. Type of donor: Voluntary [ ] Commercial [ ]
2. If commercial donor, what is the incentive? Please specify
3. How many times do you donate blood in a year [Once] [Twice] [Three times] [More than three times}

PARASITIC PARAMETER

1. ABO Blood.....
2. Rh Blood .....
3. HIV 1 and 2 Screening.....
4. *Hepatitis B*sag Screening.....
5. *Hepatitis C* Screening.....
6. *Plasmodium falciparum*.....
7. *Toxoplasma gondii*.....