PREVALENCE OF HAEMOPARASITES AMONG VOLUNTARY NON-RENUMERATED BLOOD DONORS OF NAKASERO BLOOD BANK

MALIK KAFI MAKI BAKU
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DECLARATION

I, **Malik Kafi Maki Baku,** hereby declare that the information presented in this dissertation is entirely out of my hard work and guidance from my supervisor, and that it has never been produced in any form for any academic award in any university or institution of learning. Where data or literature from related work has been used, this has been acknowledged.

Signature…….. Date……../……. /…….

MALIK KAFI MAKI BAKU

2014-BMLS-FT-010

APPROVAL

This is to certify that this dissertation has been developed under my supervision, has been submitted with my approval as the university supervisor.

Mr. TUSUUBIRA SHARIF KIRAGGA
Lecturer,
Institute of Allied Health Sciences,
International Health Sciences University.
P.O .BOX 7782, Namwongo, Kampala

Signature: …….. Date: ……

DEDICATION

I would like to dedicate this research to my mother, who has lived her life to see me do something like this, and to David who supported me throughout my education life. I am greatly indebted to my course mates whom I have spent four wonderful years with and who helped me greatly throughout the course.

To my sister Jennifer Telfer, who has been an inspiration to me and also supported me financially and always believed in me, thank you so much and this is for you.

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DEFINITION OF TERM

A Parasite: A disease causing organism that lives in or on a human or other animal and derives its nourishment from its host. Hemoparasites live within host blood stream.

Anemia: This is a condition characterized by weakness, fatigue, and paleness resulting from a deficiency of red blood cells.

Antibody: This is a protein that react with antigens on red blood cells and may destroy transfused red blood cells.

Antigen: This is a substance on the surface of red blood cells that may elicit an immune response
blood cells.

Blood: A fluid that circulates throughout the body carrying nourishment and oxygen to the cells and tissue, at the same time, removing waste matter and carbon dioxide.

Screening: This is a test conducted to detect a disease when there is little or no evidence of a suspected disease.

Transfusion: This refers to replacing blood or blood components a body has lost as a result of any medical interventions.

LIST OF ABBREVIATION AND ACRONYMS

TTM:	Transfusion Transmitted Malaria
TTPIs:	Transfusion Transmitted Parasitic Infections
UBTS:	Uganda Blood Transfusion Services
WHO:	World Health Organization

ABSTRACT

Background: The use of blood to patient management is potentially a lifesaving maneuver, which necessitates critical care. Consequently, demand for blood has greatly increased over the years. Despite several advances in the use of blood and its components in alleviating several ailments, challenges related to transfusion transmissible infections (TTIs) such as haemoparasites still stand, and transmission of haemoparasites through blood transfusion potentially negates the progress made in malaria control in malaria endemic areas. This study established the prevalence of haemoparasites in voluntary blood donors of Kampala donating blood to Nakasero National Blood Bank.

Methods: This was a laboratory based cross-sectional study with a quantitative approach carried out on all voluntary blood donors from 6th August to 28th September, 2018. Blood from the collected donor bag was used to make a thick and thin film for haemoparasites investigation. These were stained using Giemsa, and microscopically examined. Data was analyzed as proportions using 95% confidence interval.

Results: Of the 384, the prevalence of haemoparasites among donors was established as 2.86% (11/382). The distribution of haemoparasites was seen in 3 (27.3%) females, and 8 (72.7%) male donors. Their ABO distribution was group A (3, 27.3%), group B (1, 9.1%), and group O (7, 63.6%). The haemoparasites were all *Plasmodium species*, of which 90.9% (10 out of 11) were *Plasmodium falciparum*, and only one was *Plasmodium malariae*.

Conclusion: Based on this study, it was observed that haemoparasites occur in blood for transfusion, with *Plasmodium species* being the most prevalent. To this, the risk of transfusion transmissible malaria needs to be taken into account, and routinely screen all donors in order to enhance the safety of the blood supply chain from donors to recipients by means of appropriate diagnostic tools.

CHAPTER ONE: INTRODUCTION

1.0 This chapter discusses the background of the study, problem statement, significance of the study, objectives of the study, and the research questions, justification of the study and conceptual frame work of the study.

1.1 Background

Haemoparasites are parasite that can be found in the bloodstream of infected people; and can also be spread to other people through exposure to an infected person's blood by such procedures like blood transfusion with infected blood. They include malaria, African Trypanosomiasis, Babesiosis, Chagas disease, Leishmaniasis and Toxoplasmosis (Springer *et al*., 2015). Some of these parasites spend most of their life cycle in the bloodstream, like *Babesia* and *Plasmodium* species; while others are found in the blood early in an infection like the *Trypanosoma cruzi*. Their presence in blood stream depends on factors like, how much of the parasite's life cycle is spent in the blood; parasite density, how long the parasite stays in the body of the treated and untreated people; as well as how the parasite affects people (Posada-Guzmán *et al.,* 2015).

Blood transfusion (also termed hemotherapy) is an integral clinical care approach where blood or its products are administered to a recipient in whom it's indicated as a lifesaving intervention (Mattia and Andrade, 2016). Globally, there are approximately 108 million units of blood collected (WHO, 2015). The need is driven by an array of indications for transfusion like anaemia due to malnutrition, sickle cell disease, malaria and other parasitic infections, oncologic disorders, drug induced cytopenias (especially anti-retroviral and chemotherapeutic agents), surgical interventions, infection with human immunodeficiency virus (HIV), blood loss as a result of armed conflicts and road traffic accidents (American Red Cross, America's Blood Centers AABB, 2013).

In Africa, blood transfusions were first reported in the early 1920s, and have since gained an upper hand mainly sparked by remarkable bloodshed during conflicts (Schneider & Drunker, 2006). According to WHO (2016), 31.3 million units of blood are collected annually. As a limited resource set up, the risks of the less anticipated transfusion transmissible infections is balanced against the potency of lifesaving, however, as HIV and other transfusion transmissible infections (TTIs) befall

endemic in the region, this has set precedence to the donor community to support the strategies aimed at availability of sufficient and HIV free blood (Schneider, 2013). The current blood screening policy involves HIV, syphilis, hepatitis B and C. on the other hand, malaria haemoparasites remain unscreened for (Uganda Blood Transfusion Services report, 2016). In Uganda, there were 280, 145 units of blood that were collected in 2016 (UBTS report, 2016). This blood is collected from all the regional centers, screened for transfusion transmissible infections (TTIs) and distributed to the health care facilities.

Despite several advances in the use of blood and its components in alleviating numerous ailments, lack of quality assurance (QA) and suboptimal oversights could see some accidental risk of TTIs such as haemoparasites (Okocha *et al*., 2015; Verra *et al*., 2018). In sub-Saharan Africa 12.5% of patients who receive blood transfusions are at risk of post-transfusion haemoparasitic infections (Verra *et al*., 2018). In Egypt, a study to evaluate the risk of transfusion-transmitted haemoparasites reported 3.4% cases (Bakr *et al.*, 2017). Other reports seem to indicate re-emergency of malaria in most donors in sub Saharan Africa (Kenawy, 2015; El-Bahnasawy *et al.*, 2017) and attributed to the high endemicity of malaria due to climate change and anti-malaria resistance (WHO, 2016). The administration of blood to a patient is potentially a lifesaving procedure and the demand for blood has greatly increased over the years. The prevalence of parasitic infection especially haemoparasites remains a serious case which needs to be addressed, some diseases resulting from these parasites during transfusion including Trypanosomiasis, Filariasis, Bacteria and Viruses (Natukunda *et al*., 2014). Haemoparasites constitute a serious threat to the human race due to the fatality (Okocha *et al*., 2015), and as a result, there is a risk of transmission through donor blood since these haemoparasites are known to be endemic in Uganda (Torres *et al*., 2014).

Blood screening was revamped by the support from the European Commission which had hitherto been on the frontier of capacitating strategies for safe blood in low income countries to revitalize the Uganda Blood Transfusion Services (UBTS), which to present is mandated on coordinating the safety of blood. One such approach is by quality assured testing for TTIs. The screening algorithm sets out a sequence of steps in the blood screening process to be followed to determine the suitability of donated blood and its components for clinical use. It specifies the actual tests to be used and, based on each test result, directs the need for further testing. This ensures consistency in testing and decisions regarding the release of screened blood and blood components (UBTS report,

2014). In Uganda, donor blood screening for haemoparasites is under looked partly due to paucity of evidence to ascertain the extent of occult and residual infections that may go undetected, thus, there is need to explore blood safety through laboratory based screening tests. It is hoped that exploring the burden of haemoparasites. The study therefore seeks to determine the prevalence of haemoparasites among voluntary non remunerated blood donors at Nakasero National Blood Bank.

1.2 Problem statement

The practice of blood transfusion can lead to a risk of haemoparasitic transmission from the donor to the recipient (Singh and Sehgal, 2010; Maselli *et al.*, 2014; Abdullah and Karunamoorthi, 2016). Uganda Blood Transfusion Services (UBTS) implements methodical screening of all donor units for HIV, HBV, HCV and syphilis (Matte *et al*., 2013; UBTS, 2016). Even though the incidence of blood transfusion transmitted parasitic infections is lower compared to that of bacterial and viral infections (Verra *et al*., 2018), these organisms pose a considerable risk of illness especially in immune-compromised individuals (Fishman, 2017) including children, under ages less than five years and pregnant women (Liliane *et al*., 2016).

The most common parasitic infections implicated in transfusion transmitted parasitic infections are *Plasmodium* species, Toxoplasma, Trypanasomes, Babesia species and Filarial worms (Verra *et al*., 2018). These are common in most of Uganda's region, and they pose a potential risk of being carried and therefore transmissible through blood components (Roh *et al*., 2016). This study therefore seeks to establish the prevalence of haemoparasites among donor blood at Uganda Blood Transfusion Services, Nakasero in Uganda.

1.3 Objectives of the study

1.3.1 General objective

To determine the prevalence of haemoparasites in voluntary blood donors of Kampala donating blood to Nakasero blood bank.

1.3.2 Specific objectives

i) To establish the prevalence of haemoparasites in blood donors of Kampala donating blood to Nakasero blood bank.

ii) To determine the distribution of haemoparasites according to donor sociodemographic factors among blood donors of Kampala donating blood to Nakasero blood bank

iii) To establish the common blood parasites among donors of Kampala donating blood to Nakasero blood bank.

1.4 Research questions

i) What is the prevalence of haemoparasites in blood donors of Kampala donating blood to Nakasero blood bank?

ii) What is the distribution of haemoparasites in relation to donor sociodemographic factors among blood donors of Kampala donating blood to Nakasero blood bank?

iii) What are the common parasites among blood donors of Kampala donating blood to Nakasero blood bank?

1.5 Justification of the study

The study will be useful in providing information regarding the status of TTIs before this blood is released for hospital use to reduce the occurrence of fatal outcomes to blood recipients as the current screening methods do not involve laboratory confirmation. Blood serves as a vehicle for transmission of many haemoparasites and in many blood bank facilities in Uganda, the screening of donor blood for the same does not fulfill the standard protocols or is not practiced. Determination of the prevalence of haemoparasites in blood donors of Kampala will provide information that will call for need to or not to screen blood donors for haemoparasitic infections like malaria, African trypanosomiasis, Leishmaniasis and filariasis and will positively help in reviewing screening procedures and making health policy regulations. Also, this research shall still benefit future researchers by providing reference information to those interested in the similar study.

1.6 Conceptual framework

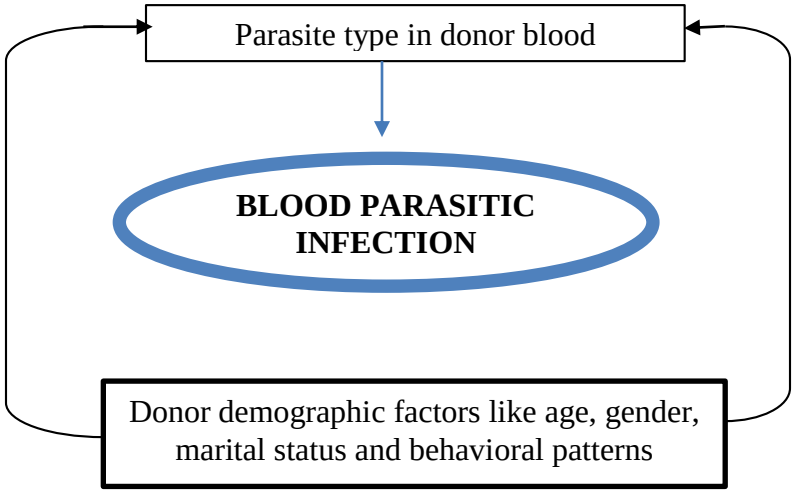


Figure 1: Conceptual framework

The conceptual frame work shows the interplay between dependent variable (blood parasitic infection) and the outcome variable of parasite infestation in the donor blood. The independent variable is also dependent on such factors like donor age, gender and marital status which may account for their behavioral patterns to the acquisition of infections. Also donor category that is voluntary, commercial or replacement donors coupled with the frequency of donation accounts for the risk of blood parasitic infection.

CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

This chapter presents the review of the related literature under the following titles of objectives; definition of haemoparasites, global prevalence of haemoparasites in blood units, status of haemoparasites in donated blood in Africa, Epidemiology of transfusion transmitted parasitic infections, most prevalent haemoparasites in blood donors, screening protocol for the different transfusion transmitted parasitic infections.

2.1 Global prevalence of haemoparasites in blood donors

Globally, blood safety remains an important public health concern. While safe blood is a universal requirement of hemotherapy, haemoparasites render blood unsafe for human use. Available data indicates that blood transfusion accounts for 5–10% of HIV infections and 4–12.5% post-transfusion hepatitis in sub-Saharan Africa (Yang *et al*., 2016). The epidemiological burden varies across the globe, for example in China, the prevalence of haemoparasites was 38% in donated blood (Li *et al*., 2012), and 5.8% in Sauda Arabia (Arshad *et al*., 2016). In India, haemoparasites accounts for 5–10% of TTIs (Abate and Wolde, 2016).

2.2 Prevalence of haemoparasites among blood donors in Africa

In Burkina Faso, the prevalence of haemoparasites among donor blood is reported at 14.3% (Manzoor *et al*., 2009). In eastern Nigeria, haemoparasites were reported at 46.6% among donor blood (Okorafor *et al*., 2015); 28% in southern Nigeria (Agboola *et al*., 2010) and 6.2% in Ethiopia (Baye and Yohannes, 2014). Further, a study carried out in East Africa by World Health Organization (2012), reported the prevalence of haemoparasites at 8.6% among Kenyan blood donors, compared with 1.2% in Tanzania. In Uganda, there is limited data to report the prevalence of haemoparasites among blood donors.

2.3 Distribution of haemoparasites according to gender of blood donors

Of the 491 prospective blood donors (402 males, 89 females), the microfilaria ratio was 4.5:1 respectively. This was revealed in a study done by Mabayoje *et al*. (2006) in Nigeria.

In a study conducted on 80 asymptomatic blood donors to determine the prevalence of microfilaria, five (0.01%) were positive for microfilaria; 3 (60%) had *Mansonella perstans*, 1 (20%) had

Onchocerca volvulus while 1 (20%) had Loa loa. All the 5 donors were male aged 23, 25, 31, 50 and 55 years and were asymptomatic (Dover & Schultz, 2009). When four hundred and ninety-three blood donors in a tertiary care hospital in North India were screened for IgG and IgM anti-*Toxoplasma gondii* antibodies it was discovered that the prevalence was higher in females (males=51.6%, females=89.2%) (Elhence *et al.*, 2010).

In another study, the prevalence of malaria parasite was carried out among 200 voluntary blood donors at Lagos University Teaching Hospital Idi-Araba, Lagos and when the blood samples were examined for parasites, 56(28%) samples were positive for *Plasmodium falciparum*, with highest prevalence among the male donors which was 26.5% (Agboola *et al.*, 2010).

The study further recommended that there was a relatively high prevalence of malaria parasite among the blood donors and this called for the attention of the authority concerned that blood donors should also be screened for malaria parasite before such blood is transfused to avert the likely consequences on the recipients. The prevalence of malaria parasite among blood donors at the study site in Lagos Nigeria revealed that of the two hundred blood samples screened (192 males and 8 females), 56 (28%) blood were positive for *P. falciparum* (Agboola *et al.,* 2010).

2.4 The prevalence of haemoparasites according to the ABO blood group of the donor

A study carried out on 200 voluntary donors in Lagos-Nigeria revealed that among the 56 patients whose blood smears were positive for *Plasmodium falciparum*, 24(43.2%) were of ABO blood group O and further discussed that this was because this blood group was the predominant one sampled during the study accounting for 58% (116 donors) of the respondents (Agboola *et al.*, 2010). The blood group O Rhesus D positive was the commonest blood group type, accounting for two thirds of the donors. Also, most of the subjects with malaria parasite positive slides were of this blood group (White & Pvkrittaya, 2004).

2.5 Common parasites found in donor blood

There are numerous parasites that have been isolated from donated blood. In Nigeria, of the 520 units of donated blood, 121 (23.6%) were infected with *Plasmodium falciparum*; 11 (2.1%) with *Plasmodium malariae*; 7 (1.4%) with *Trypanosoma bruceigambiense*; 5 (1%) were infected with microfilariae, 0.8% had *Mansonell aperstans*, while 0.2% had Loa loa (Ike *et al*., 2017). Another study in Nigeria reported that 0.01% of blood donors had microfilaria; 1% had *Onchocerca*

volvulus while 0.71% had Loa loa (Mabayoje *et al*., 2014). In North India, the common parasites isolated were *Toxoplasma gondii* (2.2%) and Loa loa (0.44%) (Elhence *et al.,* 2010). In Lagos, another study reported the prevalence of malaria parasite at 28% and 0.91% had Leishmania (Agboola *et al.*, 2010). In Calabar, the following parasite were prevalent: *Loa loa* (1.3%), *Mansonella perstans* (15.6%), co-infection of *Loa loa* and *Mansonella perstans* (0.2%), *Plasmodium falciparum* (3.3%), *Plasmodium malariae* (1.0%) and a mixture of *P. falciparum* and *P. malariae* (0.2%) (Emeribe *et al*., 2015). A study conducted among 80 asymptomatic blood donors found five cases (0.01%) with microfilaria; 0.3% had *Mansonella perstans*, 1.4% had *Onchocerca volvulus* while 1.8% had *Loa loa* (Dover & Schultz, 2009). In Ethiopia, *Plasmodium falciparum* was the only parasite isolated (Garba *et al*., 2016).

2.5 Haemoparasites

There are numerous haemoparasites implicated in transfusion transmitted parasitic infections are *Plasmodium* species, *Trypanosoma cruzi*, *Toxoplasma gondii*, and *Leishmania* species. For parasites to be transmitted by blood transfusion, they must: circulate in the blood stream of donors, comprise of certain physical characteristics and resist processing steps leading to the preparation of labile blood products (packed red blood cells, therapeutic frozen plasma, or platelet concentrates), survive conservation; further, to generate infection in the blood receiver, such parasites must retain infectivity (Shulman, 2004). The blood borne protozoan parasite *Trypanosoma cruzi* (*T. cruzi*) is the causative agent of Chagas disease. An estimated 90 million people are at risk for this disease, mostly in Mexico, Central and South America. Among this population about 11 million persons carry the parasite chronically and present a potential source of infection through blood donation. *Plasmodium* species and filariasis are the most prevalent haemoparasites in developing countries (Tapko *et al*., 2006; Tagny *et al*., 2008).

2.5.1 Common haemoparasites

2.5.1.1 Malaria

Malaria has been reported to occur mainly from single-donor products. The major considerations are first, the malaria risk associated with any individual donor, and second, the ability of the systems to identify and manage the donor and any donation. It is here that there are fundamentally

different approaches taken by different blood transfusion services: differences in the overall approaches taken between endemic and non-endemic areas (Chiodini *et al*., 2014).

2.5.1.2 Microfilariae

Microfilariae are circulated in the recipient's blood but they do not develop into adult worms. Mortality associated with transfusion-associated filarial infection is not documented but it may give rise to morbidity in transfusion recipients in terms of allergic reaction (Choudhury, 2013).

2.5.1.3 Leishmania

This is the etiologic agent of visceral Leishmaniasis is transmitted by the bite of a sand-fly. The organism is an intracellular parasite that is present primarily in cells of the reticuloendothelial tissue and cells of the mononuclear phagocytic system (Choudhury, 2013).

2.5.1.4 Toxoplasma

Toxoplasmosis is a zoonosis caused by *Toxoplasma gondii*, a parasite that is hosted in cats and dogs and has three forms: trophozoites, cysts, and oocysts. *T. gondii* is transmitted through several routes: ingestion of *T. gondii* oocysts, eating undercooked contaminated pork or beef, direct contamination of open wounds, and vertical transmission from mother to infant. In addition, the agent has been reported to be transmitted through blood transfusion and organ transplantation. Nevertheless, the risk of *T. gondii* transmission through blood transfusion is extremely low, and serologic testing of antibodies to *T. gondii* in blood donors appears to be unnecessary. It has been suggested that people who are at increased risk of toxoplasmosis, such as immunosuppressed individuals and pregnant women, receive *T. gondii* antibody-negative blood components for transfusion (Ajayi, 2010).

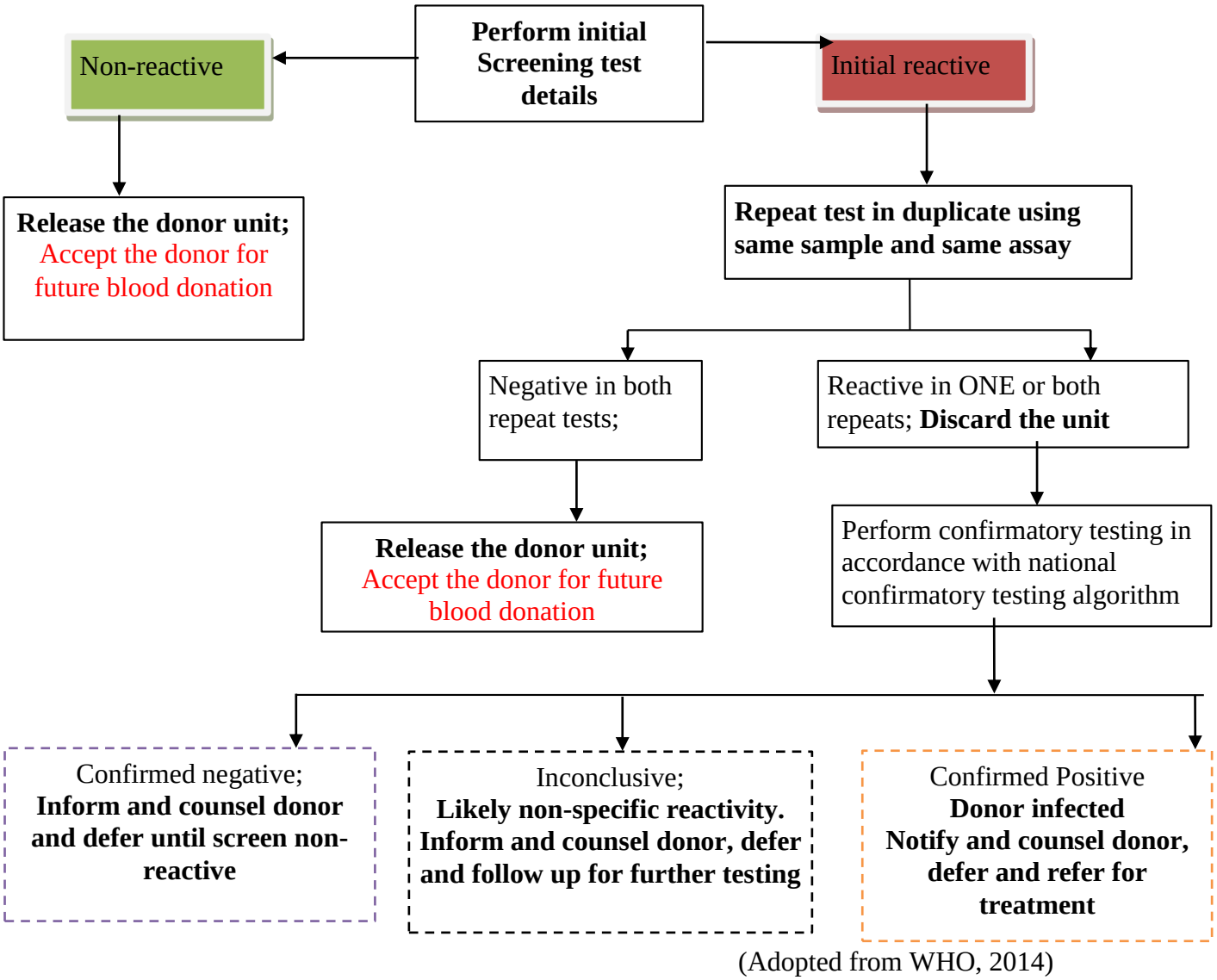
2.5.1.4 Babesiosis

Babesia species are intraerythocytic parasites and their examination requires staining with Giemsa. Unlike Plasmodium, Bebesia does not form pigments.

2.5.1.5 Trypanosomiasis

The diagnosis rests upon demonstrating trypanosomes by microscopic examination of chancre fluid, lymph node aspirates, blood, bone marrow, or, in the late stages of infection, cerebrospinal fluid. A wet preparation of blood is examined for motile trypanosomes in addition to a fixed smear stained with Giemsa (or Field's) stain.

2.6 The WHO model algorithm for blood donor screening and confirmatory testing



(Adopted from WHO, 2014)

Figure 2: The WHO model algorithm for blood donor screening and confirmatory testing

Over time, use of pathogen specific humoral immune response has been used for testing. This has however, ran short of detection accuracy due to long spells of window phase; consequently, more accurate diagnosis that incorporates nucleic acid testing (NAT) is desirable (Chatterjee *et al.*, 2012). It should be recognized, however, that all blood screening programs have limitations and that absolute safety, in terms of freedom from infection risk, cannot be guaranteed. These are used to

detect antibody, antigen or a combination of the two. Commonly used antigen detection assays are based on the use of immobilized antibody to capture pathogen-specific antigens present in the sample. The enzyme and chemiluminescent immunoassays are currently the most commonly used assays for screening donated blood for TTIs. They have high sensitivity and they can detect the target markers of infection required if used within a quality environment.

2.7 Screening protocol for haemoparasites

Some, but not all, parasitic infections can be detected by blood testing. Blood tests look for a specific parasite infection. There are two general kinds of blood tests.

2.7.1 Malaria microscopy

The accepted laboratory procedure for the diagnosis involves preparation and examination of Giemsa or Field's stained blood smears under the light microscope. In this, both thick and thin smears are examined and are considered the gold standard. Parasites are quantifies and reported per 200 white blood cells or microlitres of blood (WHO, 2014).

2.7.2 Antigen detection

These are capable of detecting fewer parasites and of producing a more rapid result with in 10-15 minutes. They are commercially available kits which includes all the necessary reagents and do not require extensive training or equipment to perform or to interpret their results. Two parasites antigens are in use, the rapid immunochromatographic test: Histidine rich protein-2 (HRP-2), a water soluble protein which is expressed by P.falciparum and parasite lactate dehydrogenase (pLDH) antigen present as separate isomers for all four Plasmodium species infecting humans. The latest antigen capture test is rapid and simple to perform and have detection limits comparable to those of high quality microscopy (WHO, 2014).

2.7.3 Use of Polymerase Chain Reaction (PCR) assays

These have been the latest advance for haemoparasites diagnosis. It's the main diagnostic test that uses successive invitro amplification of nucleic acid sequences using primers complementary to specific targets which yields million copies of the target genes, making it possible to detect minute

quantities of an organism in the blood. Its diagnostic preference is due to its high specificity, thus, the test offers accurate diagnosis in window stages of infection. The test is however limited by the high cost required to perform the test, making it less applicable in the low resource set up.

2.7.4 Rapid diagnostic tests

These are provided in simple-to-use formats that generally require no additional reagents except those supplied in the test kit. They are read visually and give a simple qualitative result within minutes. Presently, RDTs are in common use as point of care tests (PoCT), but with varied diagnostic accuracy. The pitfall to RDT use is failure to differentiate passive from active infection.

CHAPTER THREE: MATERIALS AND METHODS

3.0 Introduction

This chapter discusses the study area, study design, scope of the study and content scope, study population, sample size, sampling techniques, selection criteria, study variables, sample analysis, statistical analyses, ethical consideration and dissemination plan.

3.1 Study area

The study was conducted from Nakasero blood bank located on Nakasero hill road 1km from Ministry of Public Service (see map in Appendix VI). Nakasero blood bank is the headquarters of Uganda Blood Transfusion Services (UBTS) mandated to collect adequate, safe and efficacious blood for use in hospitals. The main activities carried out at this blood bank include; blood donor recruitment, blood collection, blood testing for transfusion transmissible infections, blood component production, and blood distribution. The blood units are tested for HIV, HBV HCV and syphilis. The ABO and Rhesus factors for the blood units are also determined before distribution.

3.2 Study design

A laboratory based cross-sectional descriptive study was carried out.

3.3 Study population

The study used EDTA blood samples that were obtained from voluntary non-renumerated blood donors aged 17 years, and above.

3.4 Sample selection criteria

3.4.1 Inclusion criteria

The study included non-clotted blood samples collected from donor blood units, which was stored for less than 35 days.

3.4.2 Exclusion criteria

Units of blood samples with obvious signs of deterioration like clotting and hemolysis.

3.5 Sample size

This was determined using formula; $N = (Z/e)^2(p)/(1-p)$ (Fink and Kosecoff, 1965).

Where; N = sample size, Z= score on normal standard variance curve that corresponds to 95% (1.96) level of confidence; e = desired level of precision (proportion of sampling error 5% i.e. of 0.05); P =estimated prevalence of haemoparasites among donor blood (Approximately 50% for unknown prevalence).

Thus on substituting for: Z=1.96 (95% level of confidence)

P= 0.5 (prevalence of haemoparasites)

Then**;** $N= (1.96/0.05)^2 (0.5x0.5) = 385.16 \quad X\ 0.25 = 383.16$

N = 384

Therefore, a minimum of 384 donor units was considered for haemoparasites screening.

3.6 Study variables

The variables were haemoparasites among study samples.

3.7 Data collection methods

3.7.1 Laboratory methods

The study used rapid diagnostic test (CareStart™ Malaria pLDH Pf/PAN) and microscopic examination of blood samples for haemoparasites (As described in Appendix I), thick and thin blood films were stained using Giemsa technique (Appendix II) and were examined for the presence of haemoparasites (Appendix III) as already described (Cheesbrough, 2009). The microscopic examination result was considered as the gold standard test.

3.7.2 Data collection tools

We used a data capture to obtain donor socio-demographic information in regard to the risk of acquiring and preventive strategy of malaria infection as developed by WHO (2014) (Appendix IV).

3.8 Data processing and Analysis

Collected data was entered and analyzed using the SPSS (software version 20.0) program with the aid of a statistician. Microsoft office excel 2007 computer program was also used to present the results in form of tables, bar graphs and pie charts. To determine the prevalence and most prevalent haemoparasites, we were able to calculate the proportion using 95% confidence interval.

3.9 Quality control

Standard operating procedures for Giemsa stain were followed. The stain was quality controlled using known positive and negative control slides. Wet and stained thick and thin blood preparations were double checked by a second laboratory technician.

3.10 Ethical consideration

We obtained an introduction letter from the University, which was used to present to the management Uganda Blood Transfusion Services. Participation in the study was also voluntary after an informed consent. Laboratory numbers were used to identify the donors and the date. Results were handled with maximum privacy and confidentiality throughout the study.

3.11 Dissemination of findings

Copies of findings were also compiled into a report and submitted for marking to the Institute of Allied Health Sciences, Clarke International University (Formerly, International Health Sciences University) for the award of a Bachelor's of Medical Laboratory Science. Copies of the research report will be submitted to the management of UBTS and CIU.

CHAPTER FOUR: RESULTS

4.1 Baseline donor factors

Three hundred and eighty four non-remunerated blood donor samples were analyzed. Donor socio-demographic data indicated their mean age was 27.2 years (range, 17 to 61). There were 229 (59.6%) male donors, and had varied ABO and RhD blood group systems as given in table 1;

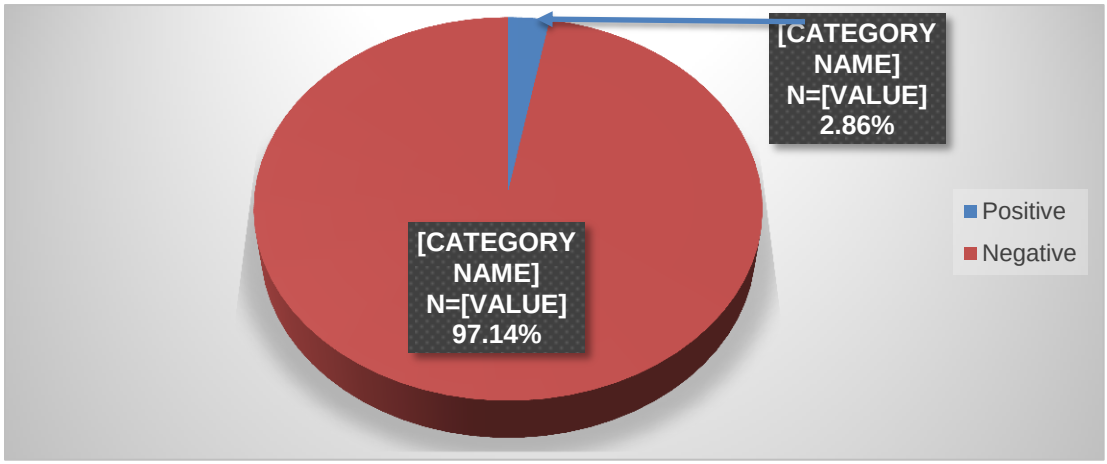
Table 1: showing the baseline donor factors

Variable	**Frequency (%)**	**Haemoparasitic status**	
		Positive (N=11)	**Negative (N=373)**
Age category (Years)			
17-19	63 (16.4)	1	23
20-24	74 (19.3)	4	175
25-29	56 (14.6)	5	129
30-34	25 (6.5)	1	27
≥35	166 (43.2)	0	19
Gender			
Male	229 (59.6)	8	221
Female	155 (40.4)	3	152
ABO blood group			
A	67 (17.4)	3	64
B	50 (13.1)	1	49
AB	14 (3.6)	0	14
O	253 (65.9)	7	246
RhD system			
D negative	17 (4.4)	1	16
D positive	367 (95.6)	10	357

4.2 Prevalence of haemoparasites in blood donors

Of the 384 blood samples tested for *Plasmodium species*, 14 screened positive with the malaria RDT. Of these, 11 were confirmed using the gold standard, giving a prevalence of 2.86% (95% Confidence Interval: 2.61-3.03). This is presented in the figure 1.

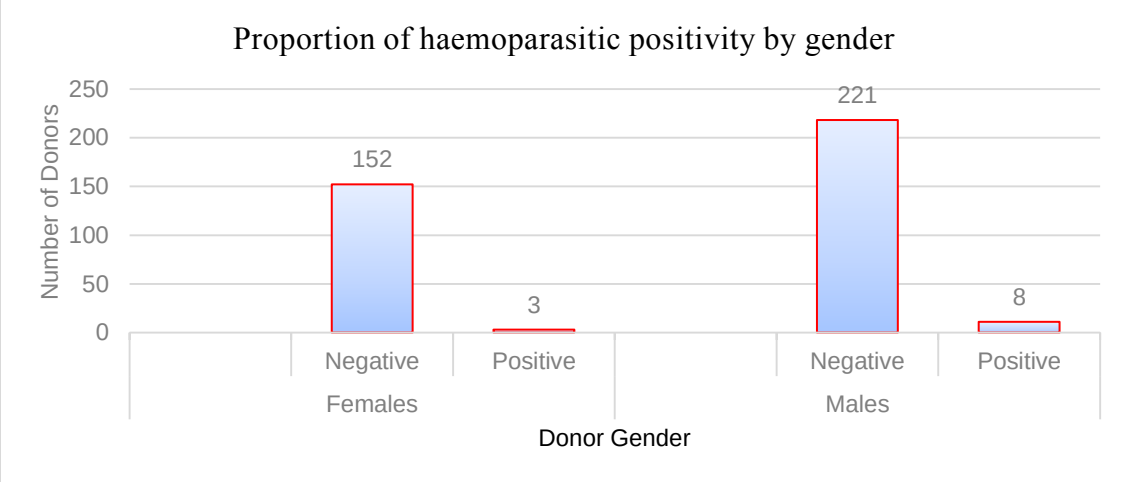
Figure 3: A pie-chart showing the prevalence of *Plasmodium species*



4.3 Distribution of haemoparasites according to donor sex

Among the 11 donors who tested positive for the haemoparasites, 3 (27.3%) were females, while 8 (72.7%) were males, as indicated in the bar graph below;

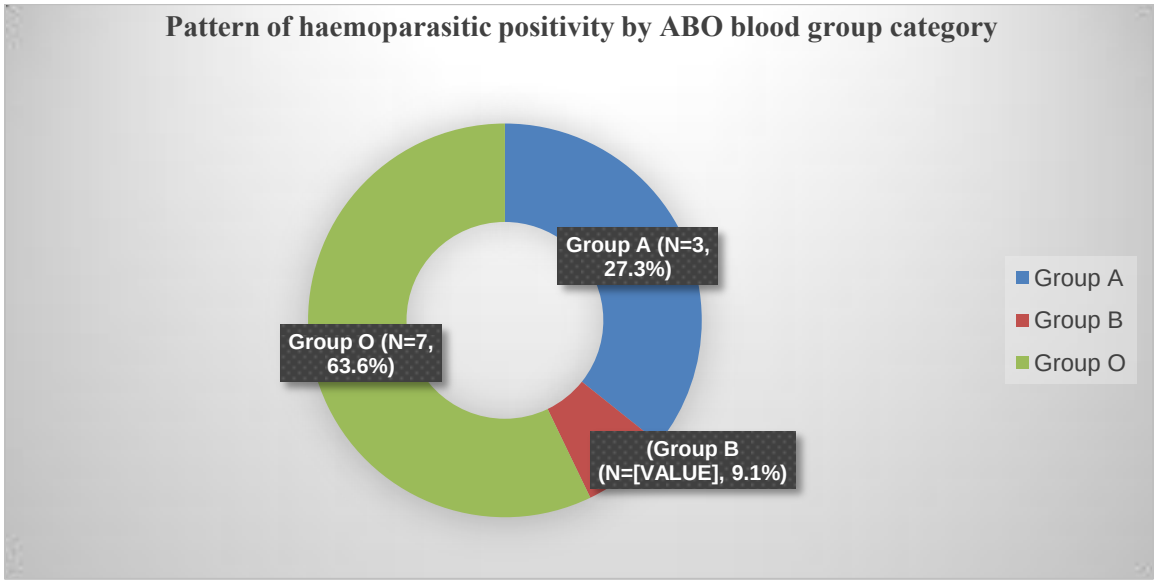
Figure 4: A bar graph showing the proportion of haemoparasitic positivity by gender



4.4 Pattern of haemoparasitic positivity by ABO blood group category

Out of the 11 donors who tested positive for the haemoparasites, their ABO blood group category was distributed among three groups. Accordingly, 3 donors (0.78%) were group A, 7 (1.82%) were group O, and 1 (0.26% was of blood group B). The pattern is summarized in the pie-chart below;

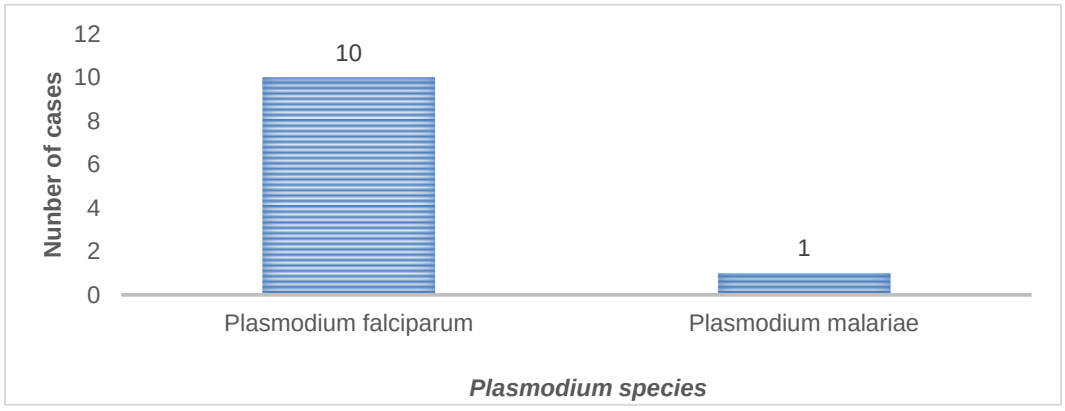
Figure 5; A pie chart showing the pattern of haemoparasitic positivity by ABO blood group category



4.5 Common parasites found in donor blood

Among the 11samples that tested positive for *Plasmodium species*, 10 (90.9%) were of *Plasmodium falciparum*, while one participant had *Plasmodium malariae*. The distribution of *Plasmodium species* is summarized in figure 4.

Figure 4: A bar graph showing the distribution of *Plasmodium species*



CHAPTER FIVE:

5.1 DISCUSSION

Prevalence of haemoparasites in blood donors was 2.86%. This value is comparable to 2.5% prevalence reported among donors in Mbarara Regional Blood Bank (Akankwasa, 2016 – unpublished), however, slightly higher than the reported value in Kenya and Tanzania of 0.6% and 1.2%, respectively (WHO, 2014). Further, the prevalence of Plasmodium species is lower than 26.5% that reported among blood donors at the study site in Lagos Nigeria (Agboola *et al.,* 2016), in Burkina Faso where the prevalence was 14.3% (Manzoor *et al*., 2009), and 6.2% in Ethiopia (Baye and Yohannes, 2014). The lower prevalence of haemoparasites in this study is ascribed to the fact that blood donors are usually adults, and in regions with high rates of malaria, adults often develop some immunity against the parasite, meaning they could have the parasites in their blood but not feel sick (WHO, 2016).

Among the 11 donors who tested positive for the haemoparasites, 3 (27.3%) were females, while 8 (72.7%) were males. This trend is consistent with the findings of a study conducted on 80 asymptomatic blood donors to determine the prevalence of haemoparasites in which all the 5 donors who tested positive were male (Dover & Schultz, 2015). Similarly, another study carried out among 200 voluntary blood donors at Lagos University Teaching Hospital Idi-Araba, Lagos indicated that 56(28%) samples were positive for *Plasmodium falciparum*, with highest prevalence among the male donors which was 26.5% (Agboola *et al.,* 2016). Although this study did not establish the likely causative effect of gender and the risk of haemoparasites among blood donors, it is thought that male gender is more at risk owing to lifestyle patterns, as they work outdoors till late hours such as in fields or forests at peak biting times, or migrate to areas of high endemicity for work (Tin-Oo *et al*., 2015; Vlassoff and Manderson, 2015). On the other hand, the cases detected among women are probably due to the fact that they get up before dawn to perform household chores, and may also be exposed to mosquitoes and consequently to malaria infection (Reuben, 2014).

The haemoparasitic positivity by ABO blood group category found donors of group A, B and O to be positive. This is in agreement with previous research such as a study carried out on 200 voluntary donors in Lagos-Nigeria revealed that among the 56 patients whose blood smears were positive for *Plasmodium falciparum,* 24(43.2%) were of group O (Agboola *et al.,* 2016), and a

similar finding was reported in Turkey (White & Pvkrittaya, 2014). This is explained by the fact that these three groups were the dominant and therefore correlated well with the malaria positivity.

Among the 14 samples that were positive with the RDT, 11of them tested positive using microscopy for *Plasmodium species.* Of these, 10 (90.9%) were of *Plasmodium falciparum,* while one participant had *Plasmodium malaria.* The difference in the malaria RDT and smear microscopy result is explained by false positivity of the RDT due to passive donor antibodies as malaria is endemic in our setting (WHO, 2016; Garba *et al*., 2016; Bakr *et al*., 2017). The other likely cause of positive RDT but microscopy negative is attributed to autolysis of *Plasmodium species* as some of those parasites died in the process of storage (2-8^{0}C storage conditions), however, the anti-bodies remained (Garba *et al*., 2016). Also, the gametocyte stage of *Plasmodium* species as were observed in the case of *P. falciparum.* In asymptomatic stage, gametocytes are seen and this explains the finding of this study in whom, the was a big risk of asymptomatic donor carriage that increases receipt transmission (WHO, 2016). The obtained prevalence of *Plasmodium* infestation agrees with White and Pvkritaya's study (2014) carried out on 200 voluntary donors in Nigeria which revealed that 56 patients whose blood smears were positive for *Plasmodium falciparum.* Also, in Nigeria, of the 520 units of donated blood, 121 (23.6%) were infected with *Plasmodium falciparum*; and 11 (2.1%) with *Plasmodium malariae* (Ike*et al*., 2017). In Lagos, another study reported the prevalence of *Plasmodium species* at 28% (Agboola *et al.*, 2016). In Calabar, the following parasite were prevalent: *Plasmodium falciparum* (3.3%), and *Plasmodium malariae* (1.0%) (Emeribe *et al*., 2015). In Ethiopia, *Plasmodium falciparum* was the only parasite isolated (Garba *et al*., 2016). This is explained by the trend of *Plasmodium specie* intensity in the region. The fact that we found parasites in those units even if they were a week or older when typically parasites outside the hosts for that long as supposed to be dead, shows the threat posed and the urgent need to consider malaria donor screening. This coupled with the fact that trophozoites are found shows the immediate threat to the receipts, who are normally immune-compromised.

5.2 CONCLUSIONS

The prevalence of haemoparasites among asymptomatic blood donors of Kampala is moderate, with *Plasmodium species* being the most prevalent. In this regard, a significant number of blood donors harbor malaria, which is a cause for concern. As the pregnant women and children receive the majority of transfusions in this setting, the need to assess the risk and put interventions in place to mitigate the risk are timely.

5.3 RECOMMENDATIONS

Based on our study findings, it is imperative that new approaches are instituted for better use of blood. First, the Uganda Blood Transfusion Services ought to review its screening procedures, and initiate screening out all blood donors from highly endemic parts Uganda for haemoparasites. Then Ministry of Health (Uganda) ought to put in place sensitization programs about the causes, modes of transmission and preventive measures of haemoparasites in order to increase the level of awareness among the general population.

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APPENDICES

APPENDIX I: PROCEDURE FOR VENOUS BLOOD COLLECTION

Arrange all the required materials.

Allow the patient to sit comfortably with one arm resting on the bench or table.

Fit the needle onto the syringe.

Apply the tourniquet to the arm. Ask the subject to open and close the fist several times.

Disinfect the skin over the vein using an alcohol swab. Allow the skin to dry.

With the bevel of the needle facing up, carefully insert the needle into the vein.

Release the tourniquet

Slowly draw out the blood until the required amount of blood fills the syringe or blood bag.

Withdraw the needle from the vein and immediately apply pressure on the puncture site with a plug of dry cotton wool.

Detach the needle from the syringe and transfer the blood sample into the EDTA anti coagulated vacutainer.

Replace the cap firmly onto the specimen bottle and mix the blood gently with the anti coagulant by rotating the container in the palms of the hands.

Label each tube with the subject's laboratory number using a grease pencil.

Discard the needles in the container labeled "SHARPS" and syringes in the container labeled "SYRINGES".

Place the used cotton wool in the container marked "INCINERATION"

APPENDIX II: STAINING OF THICK AND THIN BLOOD FILMS USING GIEMSA STAIN

Mix 1 part of Giemsa stain with 9 parts of buffered water (PH 6.8-7.2) to make a 1 in 10 dilution (10% Giemsa stain)

Cover the thick film with 10% Giemsa stain for 10 minutes

Wash off the stain buffered water pH (6.8-7.2)

Wipe the back of the slide clean

Stand the slide on the drying rack to dry

APPENDIX III: EXAMINATION OF STAINED BLOOD FILMS

Place immersion oil on the thick blood film

Swivel the x100 oil immersion objective over the selected portion of the blood smear that is well stained.

Lower the objective so that it touches the immersion oil.

Examine the slide for 100 oil immersion fields following a systematic pattern

Identify the species by noting the arrangement of the nuclei towards the end of the tail and the presence of the sheath. Record the findings

APPENDIX IV

1. Donor Unit Number: ……..
2. Age (Years): ……..

3. Gender: Male ☐ Female ☐

4. What level of education have you attained? None ☐ Primary ☐
Secondary ☐ Tertiary ☐

5. What is your employment status/ source of income? Government employee ☐
NGO employee ☐ Self employee ☐ Business employee ☐
Others (specify) ……

6. Type of donation: Voluntary non remunerated ☐ Replacement ☐ Others ☐

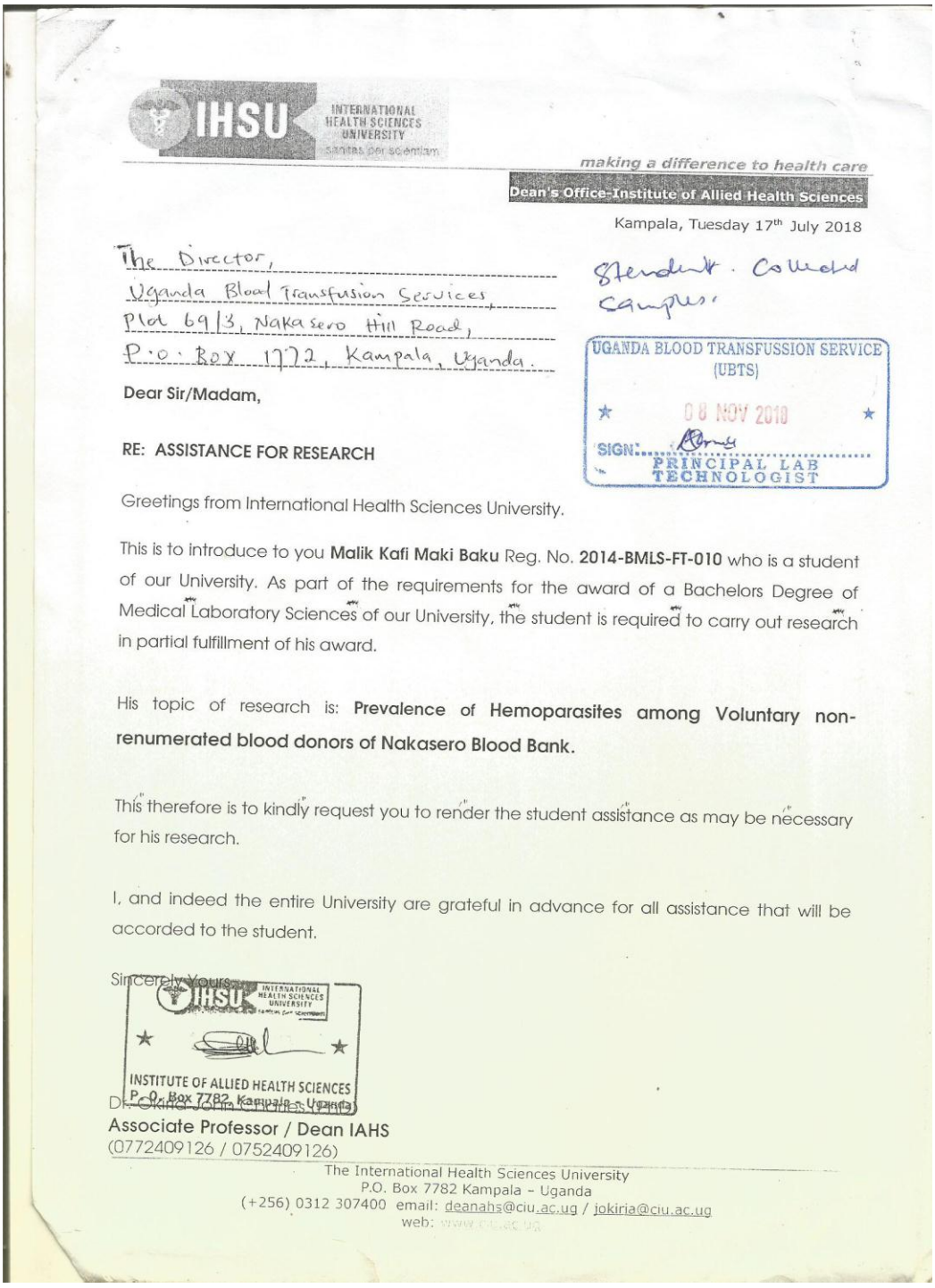
7. Donor assessment on the risk of malaria and treatment history

	Yes	No	
Are you Currently taking any medication	☐	☐	
Please read the Medication Deferral List.			
Are you now taking or have you ever taken any medications on the Medication Deferral List?	☐	☐	
Have you taken aspirin or anything that has aspirin in it?	☐	X	
In the past **6 weeks**			
Female donors: Have you been pregnant or are you pregnant now? (Males: check "I am male.")	☐	☐	☐ I am male
Have you traveled to a malarial endemic place for a 2 week vacation	☐	☐	
Have you had any of the following signs and symptoms: Fever	☐	☐	
Sweating	☐	☐	
Headache	☐	☐	
Loss of appetite	☐	☐	
Vomiting	☐	☐	
Dizziness	☐	☐	

8. Which ways do you use to prevent and control Malaria? Sleeping in bed nets ☐ wearing long sleeved clothes ☐ Making fire and smoke ☐ clearing bushes around the home ☐ Using insecticides to spray the house ☐

Thank you for your co-operation

APPENDIX V: AUTHORIZATION LETTER FROM UBTS



IHSU INTERNATIONAL HEALTH SCIENCES UNIVERSITY
Sanitas per scientiam

making a difference to health care

Dean's Office-Institute of Allied Health Sciences

Kampala, Tuesday 17th July 2018

The Director,
Uganda Blood Transfusion Services
Plot 6913, Nakasero Hill Road,
P.O. Box 1772, Kampala, Uganda.

Student. College campus.

UGANDA BLOOD TRANSFUSSION SERVICE (UBTS)
08 NOV 2018
SIGN:
PRINCIPAL LAB TECHNOLOGIST

Dear Sir/Madam,

RE: ASSISTANCE FOR RESEARCH

Greetings from International Health Sciences University.

This is to introduce to you **Malik Kafi Maki Baku** Reg. No. **2014-BMLS-FT-010** who is a student of our University. As part of the requirements for the award of a Bachelors Degree of Medical Laboratory Sciences of our University, the student is required to carry out research in partial fulfillment of his award.

His topic of research is: **Prevalence of Hemoparasites among Voluntary non-renumerated blood donors of Nakasero Blood Bank.**

This therefore is to kindly request you to render the student assistance as may be necessary for his research.

I, and indeed the entire University are grateful in advance for all assistance that will be accorded to the student.

Sincerely Yours,

IHSU INTERNATIONAL HEALTH SCIENCES UNIVERSITY
INSTITUTE OF ALLIED HEALTH SCIENCES
P.O. Box 7782, Kampala - Uganda

Dr. Okiria John Charles (PhD)
Associate Professor / Dean IAHS
(0772409126 / 0752409126)

The International Health Sciences University
P.O. Box 7782 Kampala - Uganda
(+256) 0312 307400 email: deanahs@ciu.ac.ug / jokiria@ciu.ac.ug
web: www.ciu.ac.ug