COLLEGE OF MEDECINE AND HEALTH SCIENCES

Prevalence of *Plasmodium* species and factors associated with non-*Plasmodium falciparum* among malaria cases attending health centers of Bugesera district, Eastern province, Rwanda, September – December, 2018

A thesis submitted to the University of Rwanda in partial fulfillment for the requirements for the degree of Master of Science in Field Epidemiology and Laboratory Management in the School of Public Health, College of Medicine and Health Sciences

By

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DECLARATION

I, Emmanuel SESHEMA, declare that the dissertation on "Prevalence of *Plasmodium* species and factors associated with non-*Plasmodium falciparum* among malaria cases attending Health centers of Bugesera district, Eastern province, Rwanda, September – December, 2018 ", submitted for the degree of Master of science in Field Epidemiology and Laboratory management at University of Rwanda /College of Medicine and Health Sciences/School of Public Health is my original work and has not before been submitted elsewhere. All the sources of information are listed in references.

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DEDICATION

To my lovely wife and children, parents and friends, academic facilitators,

To all who actively participated in this study,

I dedicated this work.

ACKNOWLEDGEMENT

My acknowledgments from the core of my heart are addressed to my supervisor Associate Professor Manassé NZAYIRAMBAHO for the support, comments and guidance. I thank also Dr Monique MURINDAHABI for the comments and inputs provided. My sincere appreciation goes to University of Rwanda - School of Public Health staff, AFENET and CDC-Rwanda for their support from the beginning until the end of my studies.

Thank you.

ABSTRACT

Background: Malaria disease caused by species of the genus plasmodia remains one of the leading causes of mortality and morbidity in Rwanda particularly in East Province localized Bugesera district where the prevalence of malaria in general population was 17% in 2017 according to Rwanda Malaria Indicators Survey (RMIS). The study aimed to determine the prevalence of *Plasmodium* species and determine the factors associated with non-*Plasmodium falciparum* among malaria cases.

Methods: A cross-sectional study was conducted from September-December 2018 in health centers of Bugesera catchment area. A systematic random sampling was used to select 238 malaria positive cases among confirmed malaria. Socio-demographic, environmental and clinical information of confirmed malaria patients was collected in patients file and laboratory logbook. Data was recorded and entered into computer Microsoft Excel and analyzed using STATA software. The logistic regression was performed to estimate the strength of association between independents variables and non –*Plasmodium falciparum*.

Results: Among 238 patients diagnosed malaria positive, 213(89.5%) was confirmed infected by *Plasmodium falciparum*, 13(5.5%) *Plasmodium malariae* and 12(5.0%) *Plasmodium ovale*. No *P.vivax* was found. All confirmed malaria received antimalarial coartem and quinine 98.7 and 1.3 % respectively. The multivariate analysis shows two factors associated with non-*Plasmodium falciparum* among people confirmed malaria. Public servant was 3 times more likely infected by non-*Plasmodium falciparum* [aOR= 3.39, CI (1.02 - 11.22)]; P. value=0.045. Patients diagnosed malaria positive more than one time within six months were 173 times more likely infected by non-*Plasmodium falciparum* [aOR= 173.74, CI (16.37-1842.92)]; P. value=0.001.

Conclusion: *Plasmodium falciparum* was found dominant among malaria cases .However, other non-*Plasmodium falciparum* constituted a substantial proportion of the malaria cases. Most of the non-falciparum malaria cases were in Nzangwa health center catchment area. The main factor associated with non-falciparum malaria was multiple consultations.

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LIST OF ACRONYMS AND ABBREVIATIONS

ACT: Artemisinin –based Combination Therapy

AIDS: Acquired Immune Deficiency Syndrome

CI: Confidence interval

CNS: Celebro-nerve system

G6PD: Glucose -6- Phosphate Dehydrogenase

HIV: Human Immune deficiency Virus

HMIS: Health management information system

ICAM-1: Intercellular Adhesion Molecule 1

ICCM: Integrated Community Case Management

IRB: Institution review board

IRS: Indoor Residual Spraying

ITN: Insecticide Treated Net

LLITNs: Long Lasting Insecticide Treated Nets

AOR: Adjusted odd ratio

P.: *Plasmodium*

RDHS: Rwanda Demographic Health Survey

RHIMS: Rwanda Health information management system

STATA: Statistical analysis software

TNF- α: Tumor necrosis factor

US $: United State of America dollar

WHO: World health organization

DEFINITIONS OF KEY TERMS

Artemisinin-based Combination Therapy (ACT): Artemisinin or its derivatives plus antimalarial of a different class(1).

Drug resistance: a parasite survives to a medicine after exposure to a dose which is not adequate or reduction of the drug susceptibility**.**(2).

Hyperparasitaemia: a high density of forms of parasites in the blood cells which may cause multiple sequestration of infected blood cells resulting to severe malaria. (2).

"Hypnozoites: dormant forms of parasites in the liver developed in malaria caused by P.ovale and P.vivax. They may take weeks to year before replication (2).

***Plasmodium*:** blood parasites causing malaria in humans. These include *Plasmodium falciparum, P. malariae, P. ovale* and *P. vivax*. *P. knowlesi* infected monkey has also reported (2).

Recrudescence: new illness from parasites not cleared in blood due to treatment failure. A small number of infected blood cells persist and develof to new infection. These differ to relapse whereby liver stage persist and mature in schizonts in hepatic cells then after weeks to year bust to liberate merozoites then the asexual life cycle start in red blood cells (2).

Recurrence: new inoculation by infected mosquito vector causing new infection by malaria parasites(2).

Severe falciparum malaria: Acute malaria caused by Plasmodium falciparum with vital organs dysfunctions(2).

CHAPTER ONE: INTRODUCTION

1.1. Background

Malaria is a febrile, acute, fatal disease spread by mosquitoes in human and caused by species of the genus plasmodia. The common *Plasmodium* species are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* (3). *P. falciparum*; is the most common species accounting for about 95% of infections, *P. malariae* accounting for about 2-3% of infections, *P. ovale*; accounting for 1-2% of infections and common in west Africa then *P. vivax* which is not common in Africa. The most common symptoms of malaria disease are: abdominal discomfort, headache, joint aches, muscle aches, abdominal discomfort, vomiting, lethargy and anorexia with fever in most cases(4–6).

In 2016 around 216 million malaria cases occurred worldwide compared to 237 million cases in 2010. Among these, 81% were reported in African Region, 13% in South East Asia, 5% in Eastern Mediterranean Region. World Health Organization (WHO) has estimated 212 million new cases of malaria in 2015 and 429,000 deaths worldwide. It was estimated that 445 000 deaths due to malaria had occurred globally. Among them, 407 000 deaths (approximately 91%) were in the WHO African Region and approximately 80% of all deaths in the same year occurred in 15 countries of African Region"(4,7,8).

About 90% of all malaria deaths in the world today occur in sub-saharian Africa. This is because the majority of infections in Africa are caused by Plasmodium falciparum, the most dangerous and most difficult to control of the six human malaria parasites 4 common plus *P.knolewsi* and *P.simuim* both zoonotic infections commonly used to affect monkeys but have evolved to cause malaria in human(9). Most of these deaths are children under 5 years old. Recent studies have shown that malaria causes at least 20% of all deaths in children under 5 years of age in Africa" (6,8).

Malaria develops seven days and longer(10). *P. falciparum* is most severe with variable clinical features that include fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhea and abdominal pain. Other symptoms related to organ failure may supervene, such as acute renal failure, pulmonary edema, generalized convulsions, circulatory collapse, followed by coma and death(4). It is important that the possibility of *falciparum* malaria is considered in all

cases of unexplained fever starting at any time between 7 days after the first possible exposure to malaria and 3 months (or, rarely, later) after the last possible exposure (10).

Falciparum malaria may be fatal if treatment is delayed beyond 24 hours after the onset of clinical symptoms. Young children, pregnant women, people who are immune-suppressed and elderly travelers are particularly at risk of severe disease. Malaria, particularly *P. falciparum*, in non-immune pregnant travelers increases the risk of maternal death, miscarriage, stillbirth and neonatal death. Human malaria caused by other *Plasmodium* species results in significant morbidity but is rarely life-threatening. Cases of severe *P. vivax* malaria have been reported among populations living in (sub) tropical countries with malaria risk. *P. vivax* and *P. ovale* can remain dormant in the liver; relapses caused by these persistent liver forms ("hypnozoites") may appear months – and, rarely, several years – after exposure. Relapses are not prevented by current chemo-prophylactic regimens, with the exception of primaquine. Latent blood infection with *P. malariae* may be present for many years, but it is very rarely life-threatening(10).

Three factors are associated with deaths due to malaria in Africa such as an overwhelming acute infection which leads to the cerebral malaria, repeated malaria infection which leads severe anemia and then after, low birth weight as consequence of malaria infection in pregnant women".

In African malaria-endemic countries, around 30% of all outpatient clinic visits are for malaria and between 20% and 50% of the hospitalized patients are malaria (see country profiles for details)(6).

People with low socio-economic status is more exposed and frequently to be at risk of being infected by malaria(6,8).

Malaria has been a major cause of morbidity and mortality in Rwanda for several years, with periodic epidemics in high-altitude areas. Malaria prevalence in individuals older than 6 months is 7 percentage points higher in rural areas (9%) than in urban areas (2%).Malaria prevalence in individuals older than 6 months is 17% in East province(11). These were infected with at least one form of malarial parasites(12)**.**

According to the statistical yearbook of 2016, malaria was the second cause of morbidity in Rwanda representing 7.4% of outpatient consultations and the sixth cause of mortality representing 6%.

1.2. Problem statement

Since 2012 to 2016, the government of Rwanda has implemented a contingency plan to fight malaria. The contingency plan was composed of reviewing malaria strategic plan 2013-2018, initiation of home based treatment of fever in adults, distribution of Long Lasting Insecticide Treated Nets(LLITNs), Indoor Residual Spraying(IRS) in targeted districts and Malaria prevention and control sensitization in the community(13).

Bugesera district is one of eleven districts with high malaria incidence in Rwanda. It is composed of fifteen sectors with a total population of 421,598. Its Population Average Annual Growth Rate is 3, 1%, with a population density of 282 people per km2 .Besides rivers Akagera and Akanyaru and 9 lakes(12). Climate is dry with temperature varying between 20 and 30°C(14).

Bugesera district has seventeen health facilities, one district hospital, Bugesera prison, and fifteen health centers in which this study was conducted (Nyamata,Mwogo,Gakurazo,Ntarama,Mayange,Rilima,Gashora,Nzangwa,Kamabuye,Ngeruka,Ruhuha,Nyarugenge,Gihinga,Mareba and Juru) (15).There are two rainy season: September-December and March-June. Additionally, the temperature average range is between 26^0C and 29°C. These environmental factors allow mosquito breeding and reproducing.

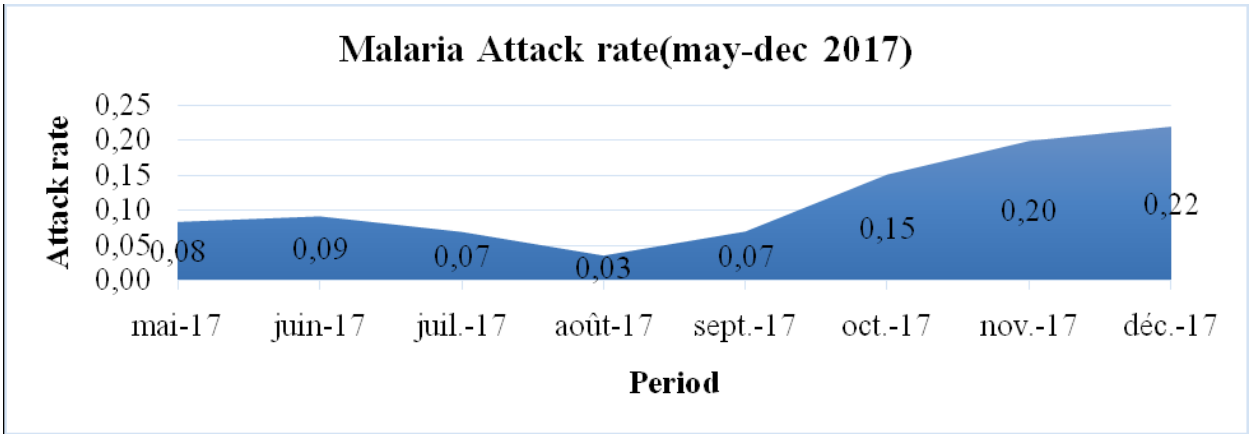
Bugesera district is classified as high-burden accounting for almost 10% of malaria morbidity according to MoH's Health Management Information System (HMIS, June 2018).

Malaria cases are managed from the community level to health facilities depending on the severity of the case and the complications associated may be different with *Plasmodium* species. Still now, malaria cases with different *Plasmodium* species identified are referred from the community level to health center and from health center to district hospital for further management. According to National Malaria Treatment in Rwanda 3^{th} version 2013, it's recommended that for every positive blood smear to mention the number of parasites per micro liter and species identification(1). However, antimalarial prescribed to treat malaria infection are prescribed without considering the species of *Plasmodium* identified *and* the degree of

parasitemia. Most of malaria cases receive coartem (Artemether/lumefantrine) which is normally indicated for malaria infections due to *Plasmodium falciparum*(16).

During this study specimen was collected on malaria suspected patients. The collected specimen was managed following malaria diagnosis standard operating procedures and then if confirmed positive the result was notified with the names of the species and the development stage of the parasite. Identification of malaria species among malaria cases help in malaria case management by prescribing adequate treatment related to specific *Plasmodium* species. This study was conducted within the rainy season started in September to December 2018 in which there is an increase of malaria. We determined the prevalence of *Plasmodium falciparum* and non-*Plasmodium falciparum* s and determine factors associated.

Figure 1: malaria attack rate in Bugesera district May-December 2017



Source: HMIS malaria data analysis Bugesera district May-December 2017

This figure above shows the increase of malaria from September to December 2017 in Bugesera district whereby the attack rate has increased from 0.07 in September to 0.22 in December respectively.

1.3. Objectives of the study

1.3.1. Main objective

To determine the prevalence of *Plasmodium* species and factors associated with non-*Plasmodium falciparum* among malaria cases attending Health Centers of Bugesera district from September to December 2018.

1.3.2. Specific objectives

a. To describe socio-demographic, environmental and clinical characteristics of patients with confirmed malaria
b. To determine the prevalence of different *Plasmodium* species
c. To determine geographical distribution of *Plasmodium* species
d. To determine factors associated with non- *Plasmodium falciparum*

1.4. Research question

The following are research questions:

a. What are socio-demographic, environmental and clinical characteristics of patients with confirmed malaria*?*
b. What is the prevalence of *Plasmodium* species among malaria cases in Bugesera district?
c. How *Plasmodium* species are distributed in Bugesera district?
d. What are factors associated with *non-Plasmodium falciparum* among malaria cases attending health center of Bugesera district?

1.5. Justification of the study

No study was conducted in Rwanda on prevalence of plasmodium species. It was conducted in Bugesera district with the aim of determining the prevalence of *Plasmodium* species and factors associated with non-*Plasmodium falciparum* among malaria cases attending health centers of Bugesera district. The identification of *Plasmodium* species will explore the magnitude of malaria species. The findings from this study will help to strengthen malaria case management by prescribing adequate antimalarial treatment. Also findings will help scientist and health professional to strengthen their knowledge in malaria infection and reinforce preventive measures within the population of Bugesera District and all Rwandan in general.

1.6. Organization of the study

This study was organized as follows:

a. Introduction: the background, problem statement, objectives, research questions, justification of the study and the organization of the study.
b. Literature review: what is known about this study; overview of malaria, geographical distribution of *Plasmodium* species, pathogenesis, life cycle of *Plasmodium*, laboratory diagnosis and treatment of malaria.
c. Methodology: The way the results were found.
d. Results: findings from data used and analyzed for the study
e. Discussions : compare findings from this study with other similar research conducted
f. Conclusion and recommendations
g. References

CHAPTER TWO: LITERATURE REVIEW

2.1. Overview

Malaria is a burden disease mainly affecting humans. It is one of the most important public health diseases worldwide, with economic impact and health implications in tropical and Sub-Saharan Africa(17).The disease remains a major cause of death and morbidity in over a 100 countries and is responsible for the annual death of approximately half a million people, mostly in Sub-Saharan Africa(17).

In Sub Saharan reported malaria cases were from 13.5 million in 2010 to 41.5millions in 2016.Malaria incidence were decreased from 49.9 million in 2010 to 46 million in 2016.Malaria deaths declined from 109000 to 89000 in 2016. Access to ITN was 61 % and the population sleeping under mosquito bed net was estimated to 54%. In this year, worldwide,445000 deaths were caused by malaria and 91% of them occurred in WHO African region(13,14).

Mainly, malaria risk factors are climate change, socio-economic status, socio-demographic practices, poor-quality housing, ,age group, lack of education, lack of malaria drug, increased rainfall, altitude, inappropriate bed net use, poor nutrition, inadequate vector control and living near stagnant water(19).

According to D.G.Ayele et al.,2006-2007, in the study conducted on 'Prevalence and risk factors of malaria in Ethiopia, they found that socio-economic factors are related to malaria risk: construction material of walls, roof and floor of house; main source of drinking water; time taken to collect water; toilet facilities and availability of electricity. They conclude that Individuals with poor socio-economic conditions are positively associated with malaria infection(20).

2.2. Geographical distribution of *Plasmodium* species

Plasmodium species causing malaria are distributed based on ecological characteristics and the mosquito host(17,18).

Plasmodium falciparum is found worldwide in endemic region and is the first cause of severe malaria accompanied with highest morbidity and mortality particularly in children under five years and pregnant women in Su- Saharan Africa(22).

Plasmodium vivax extends into temperate climate. Mortality from this parasite is lower and morbidity is high compared to *Plasmodium falciparum*(23). *P. vivax* accounts for 6% of all global malaria cases, and outside of Sub-Saharan Africa, it contributes to more than half of all malaria cases. Hypnozoites forms in liver of *P. vivax* are protected from host immune response and serve as reservoir of the parasite. The populations with Duffy antigen of red blood cells are more affected(24).

P. malariae is found in tropical region from Africa and South America. Because it causes low-grade chronic infections, is understudied. It can be found mixed with *Plasmodium falciparum* or monoparasite infection, therefore is frequently undiagnosed(25).

P. ovale is found in tropical Africa, New Guinea, the eastern parts of Indonesia and the Philippines. This species forms dormant stages in liver as *P. vivax* therefore it can invade host for weeks to years (26).

2.3. *Plasmodium* species life cycle

Malaria is transmitted to human from infected female Anopheles mosquito by injection in human of saliva containing sporozoites. Thousands of sporozoites are carried to the liver where they develop asexually to become schizonts (pre-erythrocytic schizonts) or the dormant hypnozoites produced by *P. vivax* and *P. ovale*. The development and maturation of merozoites cause infected hepatocytes to bust(27).

The released merozoites into the bloodstream are attached to erythrocytes surface receptors helping them to penetrate into red cells. In red cell, merozoites developed into trophozoite presenting ring forms which can be seen on light microscopy. Within a period of 24 to 36 hours, trophozoites enlarge and enter the stage of asexual division to form erythrocytic schizont for malaria caused by *P.ovale*, *P.malaria* and *P.vivax*. In *Plasmodium falciparum*, schizont form develops in deep capillaries. The process of asexual replication continues through repeated cycle of maturation, consequently a big number of infected red blood cells rupture responding in symptomatic infection(28).

The person infected by malaria disease present fever and chills as a consequence of parasite waste haemozoin released from the degradation of haemoglobin in the erythrocytes by the actively growing merozoites(2,6,27).

Sexual differentiation of merozoites develops into gametocytes which circulate in blood without causing infection. The ingestion of gametocytes male and female by female anopheles mosquito result in the form of zygotes in the mid gut of the mosquito. The maturation of this zygote in enlarged oocysts causes rupture and liberation of sporozoites which will be stored in the salivary gland of the mosquito. However, at the next biting, sporozoites will be transmitted in human and new infection appears(29).

In some species, hepatic stage do not divide immediately, they remain dormant form or hypnozoite in the liver. This happens for *P. vivax* and *P. ovale* infections. The dormancy period may take weeks to over one year several times from weeks to over one year before replication. This explains the tendency for relapse that is characteristic of *P. vivax* and *P. ovale* infections(29,30,31).

Multiple factors determine the epidemiology of malaria. These include environmental conditions (ambient temperature), mosquito vector density, travelers, geographical (altitude), parasitemia rates among endemic populations and the species of mosquito. In addition, misidentification in carriers of Plasmodium species represents a large unknown transmission factor for malaria(31)**.**

P. vivax and *P. ovale* generally infect reticulocytes, while *P. malariae* infect old cells with parasitemia level of 2%. *P. falciparum* infects both mature and immature red cells of all ages and can therefore manifest with parasitemia levels often >5%(32).

Relapse, recurrence and recrudescence are three important complications in untreated *Plasmodium* malaria. Relapse happen in malaria infection caused by P. *vivax* and *P. ovale* after taking non-responding schizonticide. Dormant hypnozoites replicate in the liver and develop into merozoites which reenter red blood cells. Thus, new malaria infection occurs. A small number of infected blood cell may persist to treatment. The malaria caused by the persistence of a small number of infected red blood cells is called recrudescence. In contrast, recurrence is caused by sporozoites inoculated from malaria vector(23).

2.4. Pathogenesis of *Plasmodium* species

The person infected by Malaria species present complications. In malaria caused by *P. falciparum*, malignant form is associated with the infection(33). The most serious important complication is cerebral malaria associated with delays in diagnosis and treatment(34,32).

As *Plasmodium falciparum* trophozoites infect and mature in the red blood cells, they form a small protein knobs appear on the surface of the red cell. The adherence of parasitized red cell by this protein to the endothelial cells leads to sequestration of these infected cells in deep tissues. ICAM-1 is important for sequestration in the brain. Infected red cells sequestration is specific for malaria caused by *P.falciparum* accompanied with cerebral malaria, coma and death. In malaria caused by *P. vivax, P. ovale,* or *P. malariae* no sequestrations**(35,36).**

In case of cerebral malaria, chemokines/cytokine is produced from the macrophages as a potent pro-inflammatory response to control the infection. This response may induce complications, such as anemia, hypoglycemia and cerebro-malaria. The maturation of parasite inside red cell leads to resetting, rigidity and spherical form of the cell. Due to these forms, infected cells become trapped in the capillaries. Consequently, increase sequestration of *P.falciparum* parasitized erythrocytes in the cerebral vasculature, stagnation of the cerebral blood flow, and secondary ischemia leading to tissue hypoxia, lactic acidosis, hypoglycemia, and prevention of delivery of nutrients to the tissues. High concentrations of TNF-α can precipitate cerebral malaria by increasing the sequestration of parasitized erythrocytes(37,38).

The process of malaria is cerebral Nerve System (CNS) result in delirium, impaired consciousness, convulsions, paralysis, coma, and ultimately, rapid death if not treated. Because there is no latent form of *P. falciparum* in the liver, as there is for *P. vivax* and *P. ovale,* cases of *P. falciparum* malaria should become clinically evident within a month after leaving an endemic area. *P. malariae* is known to cause long-term chronicity with low morbidity that can result in nephritic complications and splenomegaly (37). This species has also been associated with drug treatment failures in undiagnosed mixed-species infections by unknown mechanisms(38).

2.5. Laboratory diagnosis of *Plasmodium* species

Diagnosis of malaria is based on clinical signs and symptoms such as fever, headache, chills and abdominal pain are non-specific. They must be confirmed by the presence of *Plasmodium*

parasites in the blood with laboratory tests(18). As recommended by WHO, all suspected malaria cases are to be confirmed by parasitological diagnosis before treatment (referred to as "test, treat and track")(19,22,40).

Microscopy of Giemsa stain(40) of blood smear by a skilled laboratory technician, thin or thick smears, remains the gold standard for malaria diagnosis(11). They are the most common practices for identification of *Plasmodium* species. Microscopy helps to identify and differentiate *Plasmodium* species and also to determine by counting parasite density(41). Using light microscopic examination of blood smears techniques remain the most cost-effective methodology for diagnosis of malaria. They provide accurate results in a timely manner. Thick and thin films are prepared directly from finger prick blood samples, stained with 10% Giemsa solution and examined by trained laboratory technician(42).

In the study conducted in Thailand by R.coleman et al on "Comparison of field and expert laboratory microscopy for active surveillance for asymptomatic *Plasmodium falciparum* and *Plasmodium vivax* in western thailand", they found that in a total of 3,004 blood films were made from 585 individuals, of which 156 (5.2%) were positive for *P. falciparum*, 177 (5.9%) for *P. vivax*, 4 (0.1%) for both *P. falciparum* and *P. vivax*, and 2,667 (88.8%) were negative for both *P. vivax* and *P. falciparum* by expert microscopy(43).

2.6. Malaria treatment

The recommended treatment by WHO of malaria in endemic countries is the artemisinin combination therapies (ACTs).However, in pregnant women who are in their first trimester, quinine with clindamycin is recommended. The artemisinin react on asexual and sexual blood-stages of the *Plasmodium* parasites but has no effect on hepatocytes hypnozoite forms in malaria with *P. ovale* and *P. vivax*. Recommended ACTs combinations include artemether with lumefantrine, artesunate with either amodiaquine or mefloquine, dihydroartemisinin with piperaquine, and artesunate with sulfadoxine-pyrimethamine(6,13,19).

According to WHO guidelines for the treatment of malaria ,primaquine is recommended to prevent relapses of P.ovale infections(2).

The National Malaria Treatment Guideline in Rwanda 3rd Version 2013 explains the diagnosis and treatment of uncomplicated and severe malaria caused by all types of malaria. It

recommends the use of artemisinin combination therapies (ACT) for the treatment of simple malaria cases. Artesunate in the treatment of severe malaria and recommends also compulsory laboratory diagnosis of all malaria cases including severe malaria in all age groups.

At community level cases of malaria in children under five years are managed using rapid diagnostic test and these case may be referred to health center for further management. After confirmation of positive malaria, prescription of the first line ACT is indicated.

At health facility level, also first line is prescribed after obtaining a positive malaria blood smear results using microscopy or rapid malaria diagnostic test in case of emergency malaria patients.

2.7. Theoretical framework

The theoretical framework of this study focused on socio-demographic, environmental and clinical factors which may be associated with *Plasmodium* species. It shows the whole study activities. The variables were identified based on the literature related to our study objectives.

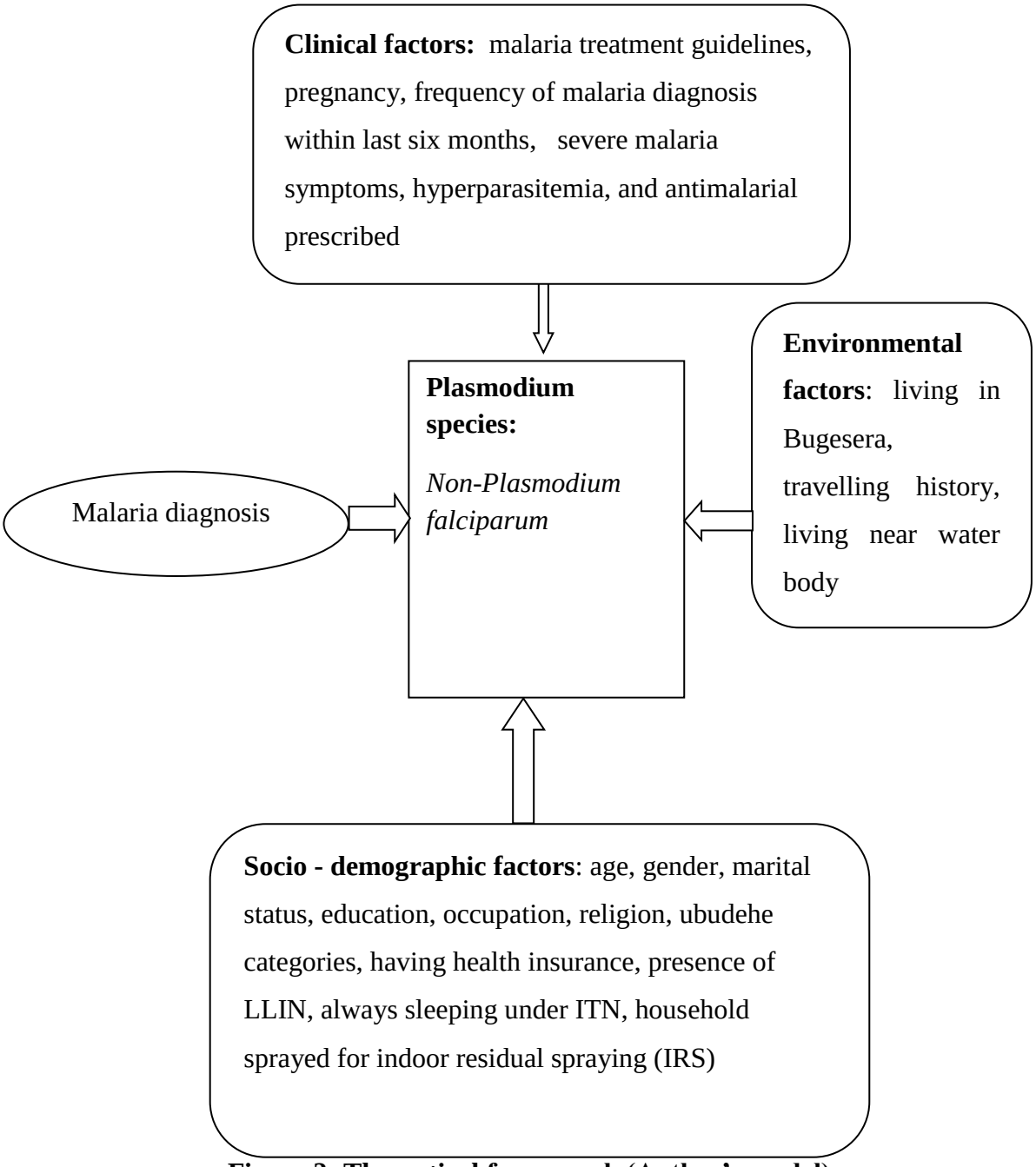


Figure 2: Theoretical framework (Author's model)

CHAPTER THREE: METHODOLOGY

3.1. Study design

This study was a cross-sectional study in which socio demographic and environmental information was collected from patients received at health facilities and confirmed to be malaria positive. We also collected clinical information regarding the patients affected by malaria parasites species from patient's file and the laboratory log book.

3.2. Study population

The target population for this study was patients confirmed to have malaria by laboratory blood smear. It targeted all new patients documented within the study period and confirmed to have malaria positive result using laboratory techniques (thin or thick smear) by the use of microscopy.

3.3. Sample size calculation

The sample size for this study was based on the recent malaria prevalence measured through the 2017 Malaria Indicator Survey which was17% in Eastern province, Rwanda (11). We used the following Kish Leslie's formula:

Formula: $n = \frac{(z_{1-\alpha/2})^2 \, p(1-p)}{d^2}$ Where:

n=Sample size

d= margin error =5%

P= estimate proportion equals to 17% representing the prevalence of malaria in Eastern province, Rwanda where Bugesera district is located(11).

$(z_{1-\alpha/2})^2$ = the level of confidence with which it is desired to be sure that the true population

(Positive cases with malaria species)

$$n \quad \frac{(1.96)^2 0.17(1-0.17)}{0.05^2} = 217$$

The sample size was 217

For this sample size we added 10 % representing possible non-responses. Hence, the total sample size was equal to 238. All selected patients accepted to be questioned.

The proportion of *Plasmodium* species in malaria was determined by computing the number of each type of *Plasmodium* divided by the total number of malaria cases.

3.4. Sampling method and strategy

We first used the proportionate sampling technique to determine the number of participants from each health facility. To found this, we based on the number of malaria confirmed cases for each health facility recorded in laboratory register from September to December 2017. After the proportionate technique, we used a systematic random sampling method to select the study participants from each health facility where data collectors selected the first malaria patients until the participant number from the health facility was reached independently on the number of spent days.

Here are a number of malaria cases from each health facility reported during the three months as highlighted above (September-December 2017).

This figure 3 below shows the number of expected participants by health center according to the number of tested malaria positive by microscope technique in fifteen health centers.

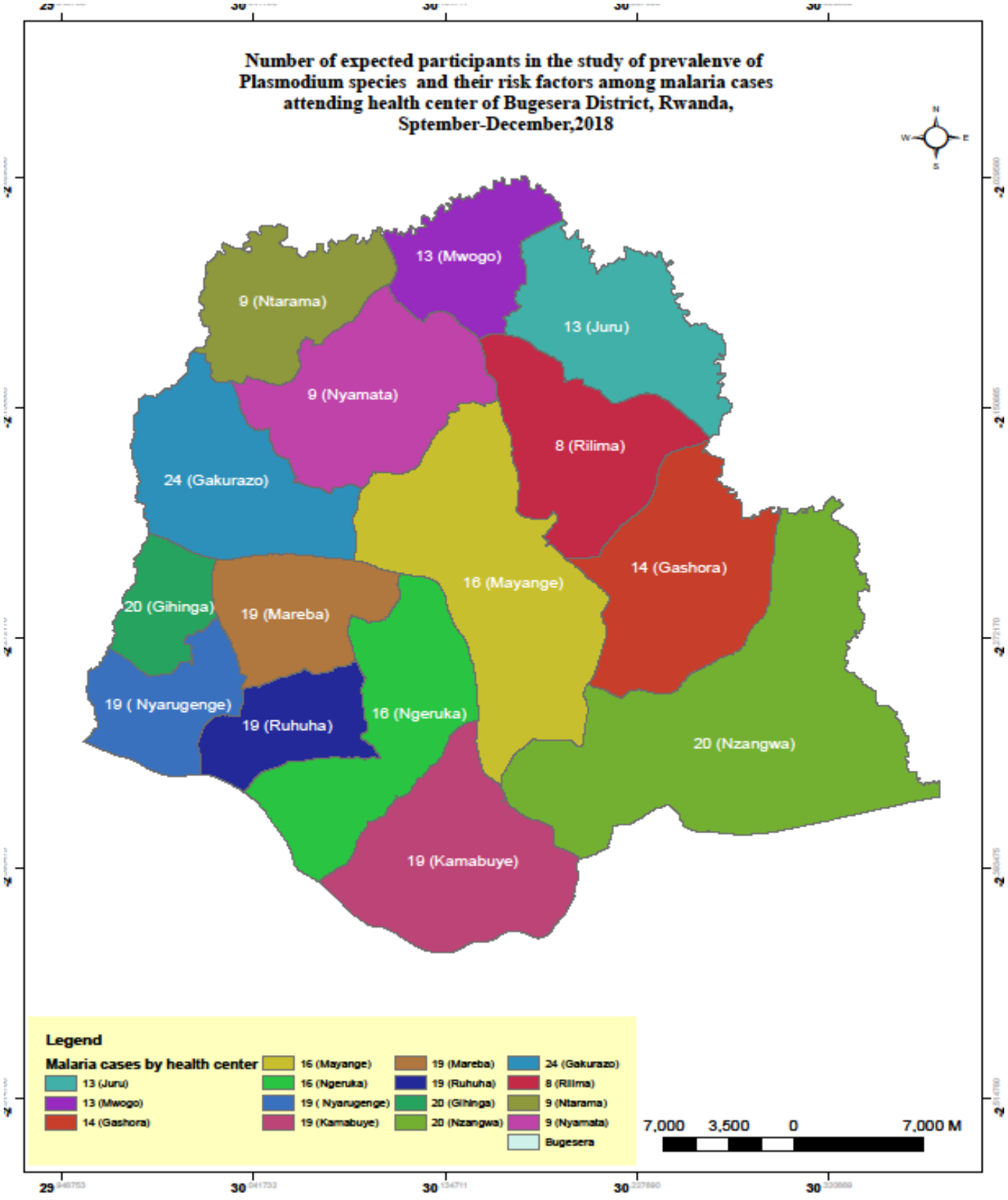


Figure 3: number of expected malaria cases by health center of Bugesera district

The expected study participants was obtained by computing the number of confirmed malaria positive by laboratory in each health center times the number of sample size divide by the total number of malaria positive in fifteen health centers of Bugesera district recorded in laboratory log book.

3.5. Inclusion and exclusion criteria

Inclusion Criteria

The participants for this study were malaria positive cases diagnosed and confirmed by laboratory in fifteen health facilities from September to December 2018 in Bugesera catchment area. All ages are included.

Exclusion criteria

The exclusion criteria for this study were:

- To be a confirmed malaria on treatment presented at health center for other investigations or after the research period
- To be in young aged while not accompanied by adult person to provide the information regarding your status.
- Any malaria positive returned for further management during the study period

3.6. Data collection, management and analysis

A questionnaire pre-established with independent variables (clinical, environmental, socio-demographic factors) and dependent variables plasmodium species (*P. falciparum* and non-*P. falciparum*) was used to collect data from the patient directly, the patient's file and the laboratory logbook. Data collected from the laboratory logbook was those which specify the *Plasmodium* species. The data entered into computer MS-Excel and analyzed using *STATA.* The descriptive analysis was computed to determine socio demographic, clinical and environmental characteristics of study participants. Cross-tabulations between independent (clinical, socio-demographic and environmental factors) and dependent variables were performed. The logistic regression was performed to determine the association at 95% CI, P-value <0.05.

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3.7. Variables

Independent variables:

Clinical factors: malaria treatment guidelines, pregnancy, frequency of malaria diagnosis within last six months, severe malaria symptoms, hyperparasitemia, and antimalarial prescribed

Socio-demographic factors: age, gender, marital status, education, occupation, religion, ubudehe categories having health insurance, presence of LLIN, always sleeping under ITN, household sprayed for indoor residual spraying (IRS)

Environmental factors: living in Bugesera, travelling history, living near water body

Outcome: non-*Plasmodium falciparum*

3.8. Quality assurance and quality control

To ensure that laboratory results provided to the patients during this study are valid and according to WHO recommendations, microscopy diagnostic test should be supported by a quality insurance programme(2). Hence, more than ten per cent of the total examined slides chosen randomly were reread by laboratory scientists of National Reference Laboratories microbiology section specialized in malaria slides examination. Result from quality control was concordant with those found at health center selected for the study. *Plasmodium* species was identified and quantified by semi-quantitative methods.

3.9. Ethical consideration

The University of Rwanda, College of Medicine and Health Sciences through its Institutional Review Board (IRB) provided the permission to conduct this research. Also the Director General of Nyamata Hospital through its ethic committee approved the permission to carry out this research in health centers. Before data collection, a meeting with health center management was held to explain the purpose and the benefits of the study. All rules regarding confidentiality was applied. Code representing participant's information was used. No names of participants were used for identification.

The risks of participating in this study were minimal. However, there was a little pain from finger prick but it was only rest for short time. There was no risk of transmitting infection because safety precautions were applied according to existing bio-safety and bio-security policies and procedures. All positive cases were managed following the National Guidelines

of malaria diagnosis. Blood smear slides was kept in a closed area. Indeed there was no remuneration for participants. Finally, the participants had right to withdraw from this study at any time without risks.

3.10. Data management

Data collected from questionnaire was entered in computer. This computer was protected by a strong password. To ensure the confidentiality, questionnaire was kept in a locked cupboard .They was no access on the completed questionnaire and computer without the permission of the principle investigator.

3.11. Study limitation

Thick smear is used as a gold standard for malaria diagnosis but this method is sensitive but not able to distinguish *P.ovale* spp. (*P. ovale curtisi* and *P.ovale wallikeri).*

CHAPTER FOUR: RESULTS PRESENTATION

3.4. Characteristics of respondents

3.4.1. Distribution of socio-demographic characteristics

The following table 2 shows us the frequency of different variables within the socio-demographic and economic, environmental and clinical characteristics of respondents. Based on this table the most frequent age group is between 5-30years old 106(44.5%). Females are more frequent than males 130(56.6%) and 108(45.4%) respectively. According to marital status, single, those who live with parents and married people are more affected 81(34.0%), 78(32.8%) and 72(30.3%) respectively. Considering the education, the most frequent are those with primary level 149(62.6%) followed by illiterate 54(22.7%) and with secondary level 35(14.7%). Public servant and people doing other activities are also more affected 108(45.4%) and 102(42.9%) respectively. According to religion, Christians are 225(94.5%). About 237(99.6) had health insurance. Those categorized in Ubudehe two and three was equally distributed at 39%. The LLITNs are available in household as declared by 164(69.9%) of respondents and 151(63.5%) declared to sleep under bed net, 87(36.5%) do not sleeping under Insecticide Treated Net (ITN). For reason of not sleeping under ITN, 159(66.8%) of respondents declared causing other disease. Indoor residual spraying was available in 216(90.8%) and 22(9.2%) was not spayed" (Table 1).

3.4.2. Distribution of environmental characteristics

About 222(93.8%) lived in Bugesera district, 16(6.7%) where from out of Bugesera. The distribution of patients travelled out of Rwanda was 224 (94.1%). Approximately, 116(48.7%) of respondents lived near water body' (Table 1).

3.4.3. Distribution of clinical characteristics

The patients diagnosed malaria positive once within the last six month was 217(91.2%). Patients presenting and those not presenting severe malaria symptoms was 23(9.7%) and 215(90.3%) respectively. High parasitemia was examined in 23(9.7%). *Plasmodium* species identified among malaria positive cases participants in this study was *Plasmodium falciparum*, *Plasmodium malariae* and *Plasmodium ovale* 213(89.5%), 13(5.5%) and 12(5.0%) respectively. No *Plasmodium vivax* was found. The malaria patients treated by coartem and quinine 235(98.7%) and 3(1.3%) respectively (Table 1).

Table 1: Distribution of socio-demographic, environmental and clinical characteristics

Variables	Frequency	Percentage
1. Socio-demographic and economic characteristics		
Gender		
Male	108	45.4
Female	130	54.6
Age group		
0- 4 years	30	12.6
5- 30 years	106	44.5
31-55 years	57	23.9
56- 85 years	20	8.4
Marital status		
Single	81	34.0
Married	72	30.3
Separated	4	1.7
Widower	3	1.3
Live with partner	78	32.8
Education level		
Primary	149	62.6
Secondary	35	14.7
University	0	0.0
Illiterate	54	22.7
Occupation		
Farmer	9	3.8
Public servant	108	45.4
Private sector	5	2.1
Fisher	8	3.4
Rice farmer	2	0.8
Wetland farmer	4	1.7
Other	102	42.9
Religion		
Christian	225	94.5
Muslims	5	2.1
None	8	3.4
Other	0	0.0
Ubudehe category		
Category 1	50	21.0
Category 2	93	39.1
Category 3	95	39.9
Category 4	0	0.0
Having health insurance		
Yes	237	99.6
No	1	0.4

Presence of Long Lasting Insecticide Treated Nets		
Yes	205	86.1
No	33	13.9
Sleeping under Long Lasting Insecticide Nets		
Yes	151	73.2
No	54	26.1
Reason of not sleeping under Insecticide Treated Net		
Unknown	0	
Cause other diseases	36	66.8
Used for other purpose	0	0.0
Too hot	0	0.0
Availability of bed bugs	0	0.0
Too old	18	33.2
Other	0	0.0
Indoor residual spraying (IRS) in household		
Yes	216	90.8
No	22	9.2
2. Environmental characteristics		
Travelling history out of Rwanda		
Yes	14	5.5
No	224	94.1
Living in Bugesera		
Yes	222	93.3
No	16	6.7
Living near water body		
Yes	116	48.7
No	122	51.3
3. Clinical characteristics		
Availability of malaria treatment guidelines		
Yes	137	99.6
No	1	0.4
Pregnancy history		
Yes	10	4.2
No	118	49.6
Not applicable	110	46.2
Frequency of malaria diagnosis within last six month		
Once	217	91.2
Twice	11	4.6
More than twice	10	4.2
Malaria patients present severe malaria symptoms		
Yes	23	9.7
No	215	90.3
Malaria patients confirmed to have hyperparasitemia		
Yes	23	9.7
No	215	90.3

***Plasmodium* species**		
P. falciparum	213	89.5
P. malariae	13	5.5
P. ovale	12	5.0
P.vivax	0	0.0
Antimalarial taken		
Qoartem	235	98.7
Quinine	3	1.3

3.5. Prevalence of *Plasmodium* species among malaria patients

Out of 238 patients diagnosed malaria positive, 213(89.5%) was confirmed infected by *P. falciparum*, 13(5.5%) *P. malariae*, 12(5.0%) *P.ovale*. No *P.vivax* found(Figure 4).

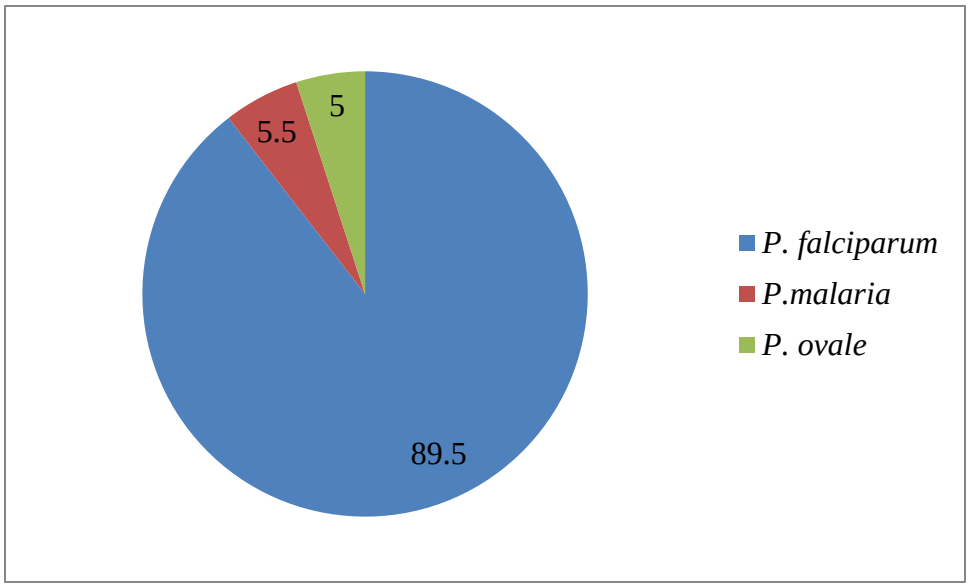

Figure 4: Prevalence of *Plasmodium* species among malaria patients

P. =Plasmodium

3.6. Geographical distribution of *Plasmodium* species by health center of Bugesera district

This figure 4 below shows the distribution of *Plasmodium* species by health center of Bugesera district. *Plasmodium falciparum* was identified among malaria cases in all health centers; *Plasmodium* malaria was found in seven health center; Nzangwa, Gashora, Mayange, Ngeruka, Mareba, Nyamata and Mwogo. *Plasmodium ovale* was identified in four health centers in Nzangwa, Ruhuha, Gakurazo and Mwogo(Figure5).

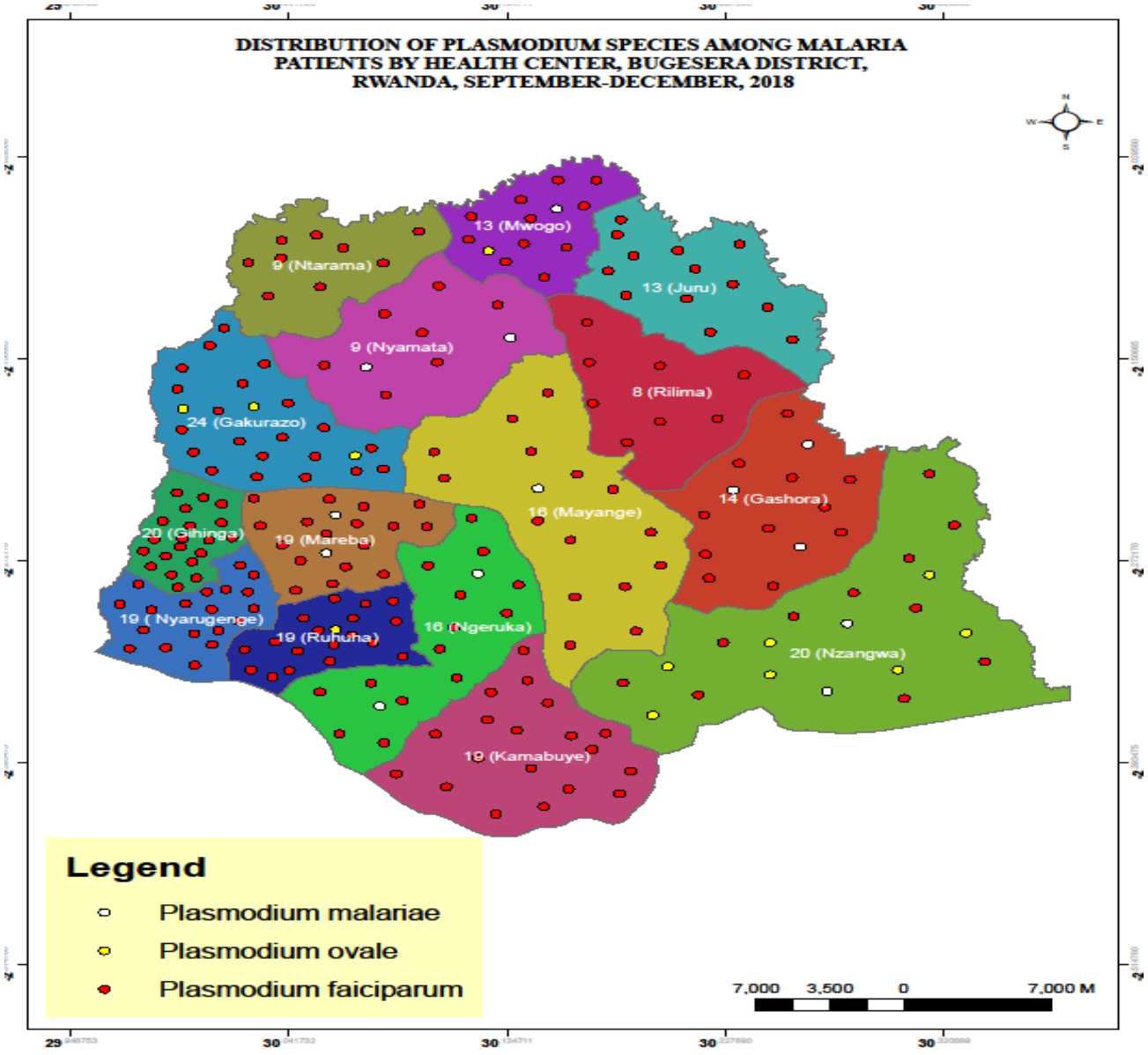

Figure 5: Geographical distribution of *Plasmodium* species among malaria cases by health center of Bugesera district.

3.4. Risk factors

3.4.1. Bivariate analysis of factors associated with non-*Plasmodium falciparum*

The following table 3 shows the bivariate analysis includes all variables and the aim was to identify the factors associated with non-*Plasmodium falciparum* among malaria patients. The results show that being public servant was associated with having *non-Plasmodium falciparum* (P. value = 0.048). Several consultation at health center for malaria diagnosis, travelling out of Rwanda , lack of health insurance and treatment taken by malaria patients are also associated with having *Plasmodium* species (P.value =0.0001.) Also treatment taken was likely assocaiated with p-value=0.019(Table 2).

Table 2: Bivariate analysis

	***Plasmodium* species**					
Variables	***P. falciparum***		**Non *P. falciparum***		**Statistical tests**	
	N	**%**	**N**	**%**	**cOR (95% CI)**	**P. value**
Age group						
31-55 years	44	91.67	4	8.33	**1**	
0-4 years	29	90.63	3	9.38	0.87 (0.18 - 4.21)	0.872
5-30 years	128	87.67	18	12.33	0.64 (0.20 - 2.01)	0.452
56-74 years	12	100.00	0	0.00	1 (empty)	-
Gender						
Female	94	87.04	14	12.96	1	
Male	119	91.54	11	8.46	1.61 (0.69 - 3.71)	0.263
Residence						
Out of Bugesera	13	81.25	3	18.75	**1**	
Bugesera	200	90.09	22	9.91	2.09 (0.55 - 7.93)	0.275
Marital status						
Married	71	89.87	8	10.13	**1**	
Single	142	89.31	17	10.69	0 .94 (0 .38 - 2.28)	0.893
Education level						
Literate	164	89.13	20	10.87	**1**	
Illiterate	49	90.74	5	9.26	1.06 (0.75 - 1.49)	0.735
Occupation						
Nonpublic servant	92	85.19	16	14.81	**1**	
Public servant	121	93.08	9	6.92	2.33 (1.08 - 5.52)	0.048*
Religion						
Others	11	84.62	2	15.38	**1**	

Christians	202	89.78	23	10.22	1.59 (0 .33 - 7.65	0.558
Ubudehe category						
Category 1	46	92.00	4	8.00	**1**	
Category 2	86	92.47	7	7.53	1.06 (0.29 - 3.84)	0.919
Category 3	81	85.26	14	14.74	0.50 (0.15 - 1.61)	0.249
How often did diagnosed for malaria parasites within six Months						
Once	207	95.39	10	4.61	**1**	
More one	6	28.57	15	71.43	51.75 (16.55 - 161.77)	**< 0.001***
Family have health insurance						
Yes	213	89.87	24	10.13	**1**	
No	0	0.00	1	100.00	**1 (omitted)**	**-**
Travel history out of Rwanda						
No	208	92.86	16	7.14	**1**	
Yes	5	35.71	9	64.29	**0.04 (0.01 - 0.14)**	**< 0.001***
Pregnancy						
No	9	90.00	12	10.17	1	
Yes	106	89.83	1	10.00	1.01 (0.11 - 8.75)	0.986
Presence of Long Lasting Insecticide Nets						
Yes	148	90.24	16	9.76		
No	65	87.84	9	12.16	0.78 (0.32 - 1.85)	0.576
Sleeping under Long Lasting Insecticide Nets						
Yes	136	90.07	15	9.93	1	
No	77	88.51	10	11.49	0.84 (0.36 - 1.98)	0.706
Reason of not sleeping under Insecticide Treated Nets						
Unknown	143	89.94	16	10.06	1	
Cause other diseases	42	91.30	4	8.70	1.17 (0 .37 - 3.70)	0.783
Others	28	84.85	5	15.15	0 .62 (0 .21 - 1.85)	0.397
Living near water body						
No	112	91.80	10	8.20	1	
Yes	101	87.07	15	12.93	0 .60 (0 .25 - 1.39)	0.237
IRS household						
Yes	194	89.81	22	10.19	1	
No	19	86.36	3	13.64	0 .71 (0 .19 - 2.62)	0.616
Malaria treatment guideline available at Health Center						
Yes	212	89.45	25	10.55	1	
No	1	100.00	0	0.00	1 (omitted)	-
The patient present a symptoms of severe malaria						
Yes	1	100.00	0	0.00	1	
No	212	89.45	25	11.63	1 (omitted)	-
Hyperparasitaemia						
Yes	23	100.00	0	0.00	1	
No	190	88.37	25	11.63	1 (omitted)	-
Treatment taken						
Coartem	212	90.21	23	9.79		

Quinine	1	33.33	2	66.67	0.05 (0.004 - .62)	0.019*

* Statistically significant with P.value <0.05

3.4.2. Multivariate analysis of factors associated with non-*Plasmodium falciparum*

The multivariate analysis shows two factors associated with non-*Plasmodium falciparum* among people confirmed malaria. Public servant was 3times more likely infected by non-*Plasmodium falciparum* [aOR= 3.39, CI (1.02 - 11.22)]; P. value=0.045. Patients diagnosed malaria positive more than one time within 6 months were 173times more likely infected by non-*Plasmodium falciparum* [aOR= 173.74, CI (16.37-1842.92)]; P. value=0.001.

Table 3: Multivariate analysis

Risk Factor	Adjusted Odds Ratio (AOR) 95% confidence interval (CI)	P. value
Occupation		
Nonpublic servant	1	
Public servant	3.39 (1.02 - 11.22)	**0.045**
How often did diagnosed for malaria parasites within six Months		
Once	1	
More than once	173.74 (16.37 - 1842.92)	**0.001**
Family have health insurance		
Yes	1	
No	1 (omitted)	**-**
Travel history out of Rwanda		
Yes	1	
No	5.52 (0 .44 - 68.02)	0.182
Treatment taken		
Coartem	1	
Quinine	0 .33 (0 .01 - 10.94)	0.537

CHAPTER FIVE: DISCUSSION

This study aimed to determine the prevalence of *Plasmodium* species and factors associated with non-*Plasmodium falciparum* among malaria cases attending health centers of Bugesera district, eastern province, Rwanda, September-December 2018.

The findings from this study show that the prevalence of *P. falciparum* which is known to be predominant among other *Plasmodium* species causing malaria and responsible of severe malaria was 89.5% in the study area. *P.malariae* and *P.ovale* were 5.5 and 5.0% respectively. Other previous study conducted on prevalence of malaria parasite infection and vector species abundance in Huye district, southern Rwanda (2014) found only *P. falciparum* but did not found others *Plasmodium* species(44). In the same area in the study conducted by Gahutu et al. found the presence of *P.falciparum, P.ovale* and *P.malariae*(45). Doctor et al. worked on Low prevalence of *P.malariae* and *P.ovale* mono-infections among children in the Democratic Republic of the Congo: a population-based, cross-sectional study and they found that *P.falciparum, P.malariae* and *P.ovale* were 46,6, 12.9 and 8.3%, respectively(46).

Similar findings were reported in Uganda malaria indicator survey 2014-2015 in which 96.0% of children with malaria had *P.falciparum*, 6 % with *P. malariae*, 1% *P. ovale*, and less than 1% *P. vivax*. Among thsese 4% were mixed infections where two or more *Plasmodium* species were identified(47).

All diagnosed malaria cases in this study received antimalarial drug treatment. A number of 98.73% received artemisinin- based combination therapy(artimether/lumefantrine) as the recommended first-line antimalarial drug for the treatment of uncomplicated malaria in Rwanda(1) and also recommended by WHO(Worl Health Organization)(9). A few number 1.27% received quinine. However, in the clinical studies conducted on malaria cases, the efficacy of coartem(artimether and lumefantrine) was evaluated for the treatment of acute, uncomplicated malaria caused by *Plasmodium falciparum*(16). According to current WHO guidelines, primaquine is recommended to prevent relapses of *P.ovale* spp. infections(48). However, primaquine was not prescribed for the patients found to be affected by *Plasmodium* ovale spp. Consequentry , the absence of treatment of may be the cause of *P.ovale* transmission(49). Using our data, we cannot determine whether this is a reason of the presence of these parasites among our study participants.

As for *Plasmodium falciparum*, Altimeter Combination Therapy is efficacious against asexual *Plasmodium malariae* and *Plasmodium ovale* spp.

Our findings also show that the patients who frequent more than one time for malaria diagnosis and confirmed malaria positive are infected with non-*Plasmodium falciparum*. As mentioned, this study shows the presence of *P. malaria* and *P.ovale* among malaria cases. These *Plasmodium* species in hepatic stage do not divide immediately; they remain dormant in hepatocytes cell and form hypnozoite in the liver. The dormancy period may take several times weeks to over one year before replication. This explains the tendency for relapse that is characteristic *P. ovale* infections((29, 30,31).

According to the study conducted by Sindair *et al*. on ACT for treating uncomplicated malaria, the additional of other treatment to cure completery malaria patients was recommanded(50).

The treatment with artemisinin react on sexual(gametocytes) and asexual red blood-stages of the *Plasmodium* parasites but on hypnozoite in hepatocytes cells no effect in malaria with *P. ovale* and *P. vivax*(51) **.**

The findings from this study highlight the presence of of *P.ovale* spp. And *P.malariae*. No malaria patient was infected by *P. vivax*. These findings emphasize the need to carefully identify *Plasmodium* species by health workers in order to improve their understanding in epidemiology of malaria parasites. And also to establish specific interventions to achieve malaria control and elimination(51,52). *Plasmodium* species appear to share the same primary malaria vectors(53) These vectors are antropophilic predominantly indoor biting at night. Efficient use of available preventive measures may reduce malaria infection and transmission(54).

In our study, *Plasmodium* species was found as mono infection among malaria patients. Furthermore, our results indicate that patients with *P.ovale* and *P. malariae* was presented at health center more than one time for malaria diagnosis and treatment. The similar findings was founds in the study conducted by denko et al.(55). Inefficient malaria treatment cause recrudescence malaria infections, more anemia following repeated infections, increased gametocyte carriage and transmission as well as increase incidence of malaria"(56).

"The limitations from this study were that to identify *Plasmodium* species thick smear is used as a gold standard for malaria diagnosis. This method is sensitive but is not able to distinguish *P.ovale* spp. (*P. ovale curtisi* and *P.ovale wallikeri*).

In summary, *P.falciparum* is predominant among malaria cases in Bugesera district. The presence of non-*Plasmodium falciparum* could not be neglected while they form dormant forms and relapse if not treated efficiently. In addition, strategies designed for reducing malaria transmission may be less effective. To achieve the goals of malaria elimination, identification and adequate treatment as well as specific techniques for malaria diagnosis are required.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

This study has confirmed the predominance of *P.falciparum* among malaria patients in Bugesera district. However, other non-falciparum constituted a substantial proportion of the malaria cases Most of the non-falciparum malaria cases were in Nzangwa HC catchment area. The main factor associated with non-falciparum malaria was multiple consultations.

6.2. Recommendations

To Ministry of Health/Rwanda Biomedical Center:

- To review guidelines of malaria diagnosis and treatment
- To train all health workers on available malaria treatment guideline.

To Health facilities:

- Implementation of malaria treatment guidelines

To scientists/researchers:

- Conduct a research on efficacy of coartem in patients affected by malaria infection
- Conduct a research on plasmodium species countrywide

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APPENDICES

Appendix 1: Budget

This study required the helping staff, equipment as well as financial resources. It is the reason why, the budget below helped in carrying out the whole study.

The table below shows in detail, the budget used in the study on prevalence of *Plasmodium* species and their risk factors among malaria cases attending health centers of Bugerera District, Eastern province, Rwanda, from September to December 2018.

No	Designation	Qty	Frequency	Unit price	Total/RWF
1	Data collectors restaurant fees	15	4	6000 RWF	360000
2	Facilitator in the training data c	1	4	50000	200000
2	Health center visit for supervision of collectors	15	1	6000RWF	96000
4	Quality control of blood smear slides	40	1	5000	200000
	Moto transport fees	15	1	10000RWF	150000
4	Communication fees	15	1	5000 RWF	75000
6	Paper	1	1	4000 RWF	4000
7	Pens	15	1	150RWF	2250
8	Orientation/training of data collectors on the study tools	15	1	10000RWF	150000
9	Flipchart	1	1	5000RWF	5000
10	Markers	2	1	1500	3000
Total					**1,245,250**

Appendix 2: Timeframe

Activities	**September 2018**	**October 2018**	**November 2018**	**December 2018 to September 2019**
Research proposal preparation				
Research proposal submission				
Proposal submission in IRB				
Ethical clearance and permission to do the work				
Meeting with health center management and staff				
Data collectors training				
Data collection				
Data entry into computer				
Data cleaning				
Data analysis				
Report writing draft				
Presentation of final report				
Final report				

Appendix 3: Consent form (English version)

Participant's code: ……..

Date: …../……/…….

Study title: Prevalence of *Plasmodium* species and their risk factors among malaria cases attending health centers of Bugesera district, Eastern province, Rwanda, September to December 2018

Investigator: Mr. Emmanuel SESHEMA, FELTP resident 4th cohort
Supervisor: Associate Professor Manassé NZAYIRAMBAH,PhD, UR-CMHS-SPH

Co-supervisor: Mrs. Stella MATUTINA UMUHOZA *Phone: +250788696732*

Investigator's statement:

I am requesting you to kindly participate in this study. The purpose of this consent form is to provide you with the information you will need to help you decide whether or not to participate in the study.

Introduction

Malaria remains one of the leading cause of high mortality globally especially in Africa. Malaria is a febrile, acute, fatal disease spread by mosquitoes in human and caused by species of the genus plasmodia. The common *Plasmodium* species are *Plasmodium falciparum, Plasmodium vivax, Plasmodium malaria and Plasmodium ovale.* P. falciparum; is the most common specie accounting for about 95% of infections, *P. malariae* accounting for about 2-3% of infections,*P. ovale*; accounting for 1-2% of infections and common in west Africa then P. vivax which is not common in Africa. The identification of plasmodium species is requiring in order managing efficiently and effectively malaria cases. Thus this study seeks to determine malaria species prevalence among malaria cases attending fifteen health centre of Bugesera District. The participant will be asked to answer to a number of questions that will take an average about 15 minutes of your time.

The sample collected from patients will be analyzed and then if positive you will answer to some questions regarding your status and knowledge on malaria treatment and prevention. Your daily duties will not be interrupted by your agreement to participate in the study. And all information will be kept with confidentiality.

Benefits: The results of this study will be used by stakeholders to improve and update the existing guidelines of malaria treatment. Participant tested positive during this study will be treated following malaria protocol.

Risks: The risks of participating in this study are minimal, there will be a little pain from finger prick but it will only rest for short time. There is no risk of transmitting infection because the sterile needle will be used. No change on the existing policy and procedure of malaria diagnosis and treatment. The interview will take short time almost 15 minutes.

Voluntariness: Participation in the study will be fully voluntary. You are free to refuse to participate or withdraw from the study at any time. There will be no financial reward to you for participating in the study.

Confidentiality: The information obtained about you and the result of blood test will be treated with most confidence and identification will not be released to any person or forum without your permission. All data collected in hard copy will be kept in a lockable cabinet where the researcher only will access to maintain confidentiality. Information stored in soft copies will be protected from access from unauthorized persons by password which will be changed periodically. All records will be identified by study identification number.

Questions: For any questions, contact the University of Rwanda, College of Medicine and Health sciences the Chairperson of the CMHS IRB (**0788 490 522**) and of the Deputy Chairperson (**0783 340 040**),the supervisor of this research on **0785255388** or use my cell phone **0788841781**.

Participant's statement:

I, , after having received adequate information regarding the study research, risks, and benefits hereby AGREE to participate in the study with malaria species prevalence. I understand that the participation is fully voluntary and that I am free to withdraw at any time. I agree that the researcher can collect sample blood for malaria testing and ask questions. I have been given adequate opportunity to ask questions and seek clarification on the study and these have been addressed agreeably.

Participant's Signature Date …. /…./…..

Investigator's declaration

I, , declare that I have sufficiently explained to the participant the study procedure, risks, and benefits and given him /her time to ask questions and seek clarification regarding the study. I have answered all the questions raised to the best of my ability.

Interviewer's Signature Date ……/……. /……..

Appendix 4: CONSENT FORM (IKINYARWANDA VERSION)

Kode y'usubiza: ……Tariki: ……/……. /…….

Umutwe w' ubushakashatsi: Kwerekana impuzandengo y'udukoko dutera malaria mu barwayi ba malaria bivuriza ku bigo nderabuzima byo mu karere ka Bugesera, mu ntara y' iburasirazuba, Rwanda

Umushakashatsi: Bwana Emmanuel SESHEMA. Nomero y'itumanaho: 0788841781

Umuyobozi w' ubushakashatsi: Prof Manassé NZAYIRAMBAHO *phone: +250785255388*

Umuyobozi wungirije: Mrs Stella MATUTINA UMUHOZA *Phone: +250788696732*

Ijambo ry'ukora ubushakashatsi:

Murahoneza! nitwa Emmanuel SESHEMA, ndi umunyeshuli mu kiciro cya gatatu muri kaminuza y' u Rwanda nkaba niga ibijyanye n'ikwirakwizwa no gukumira indwara. Nabasabaga ko mwakwitabira ubu bushakashatsi. Iyi nyandiko igamije kugirango usobanukirwe neza ibijyanye nubu bushakashatsi hanyuma utwemerere cg se wange kubwitabira byose nuburenganzira bwawe.

Iriburiro: Malariya iracyari imwe mu ndwara zitera imfu nyinshi kwisi hose byumwihariko muri Afurikaari naho u Rwanda ruherereye. Malariya yibasira abantu bose ikaba iterwa n' udukoko twitwa plasmodium Kumenya impuzandengo y' utu dukoko n' uko dukwirakwizwa bizatuma turushaho gushimangira ingambazo kwivuza hakiri kare ndetse no kudukumira.

Uri bubazwe ibibazo bike bizagufata iminota nka cumi n'itanu yonyine, turafata amaraso yo mugatoki tuyapime nk' uko dusanzwe tubikora.Uwitabiye ubushakashatsi wese dusanga arwaye turamubaza ibibazo bijyanye n' uburwayi kugirango tubihuze n' ubwokobw' agakoko turabatwabonye. Arahabwa kandi imiti nk' uko bisanzwe.Ibisubizo by' ibibazo mbabaza byose n'ibanga.

Inyungu: Ibizava muri ubu bushakashatsi bizafasha abakozi b'urwego rw'ubuzima kongera ubumenyi kudukoko dutera malariya, gushimangira ingamba ziriho zijyanye no kuvura malariya no kuyirinda. Uwo bazasangana malariya azavurwa nk' uko bisanzwe.

Ingaruka: Ingaruka zo kwitabira ubu bushakashatsi ni nkeya cyane, hashobora kubaho ububabare buke butewe n'amaraso dufata mu gatoki ariko bimara akanya gato cyane, nta ngaruka zo kwandura indwara zandurira mu byuma bikomeretsa kuko buri wese akoreshwaho

urushinge rushya. Ikiganiro tugirana kiramara nk'iminota cumi n'itanu.
Ubushake: Kwitabira ubu bushakashatsi ni ubushake. Ni uburenganzira bwawe kwitabira cg kubyanga igihe ushakiye nta ngaruka byakugiraho.Kandi nta mafaranga ateganijwe kugirango witabire.

Ibanga: Nkwijeje kugira ibanga ku makuru uri bumpe kandi amakuru yatuma umuntu agusobanukirwa ntabwo azerekwa undi muntu uwo ariwe wese keretse abonye uruhushya rwawe. Amakuruyandikwa ku rupapuro azabikwa mu gasanduku gafungwa. Naho ayo murimu dasobwa azafungwa n' umubarew'ibanga uzwi n'umushakashatsi gusa kandi uzajya uhindagurwa kugirango hatazagira uwumenya.

Ibibazo:

Muramutse mugize ikibazo kubijyanye n'ubu bushakashatsi wahamagara ni mero zikurikira: Uhagarariye ubushakashatsi muri Kaminuza y'Urwanda (**0788 490 522**) umwungirije (**0783 340 040**). ukurikirana ubu bushakashatsi (**0785255388),** uri gukora ubushakashatsi **(0788841781).**

Nimwemera ko dufatanya muri ubu bushakashatsi, murasinya ku rupapuro ahabugenewe

Amagamboy'uwitabira:

Njyewe, , maze kumva neza ibijyanye n'ubu bushakashatsi, ingaruka,inyungu nemeye kwitabira ubu bushakashatsi, nemereye umushakashatsi gufata ikizamini cy'amarasoyo mu gatoki kugirango ayapimemo malariya n' ubwokobw' udukoko tuyitera. Nasobanuriwe neza ko kubwitabira cg kutabwitabira ari uburenganzira bwanjye ndetse n'igihe cyose nashakira navamo.Nahawe n'uburyo busesuye bwo kuba nabaza ibibazo byo kugirango mbashe gusobanukirwa neza n' ubu bushakashatsi.

Umukono w'usubiza:

Tariki……/……/….

Amagambo y' ubaza:

Njyewe,……..……, ndemeza ko nasobanuriye bihagije uvugwa haruguru witabiriye ubu bushakashatsi, ibyerekeye ububushakashatsi, ingaruka, n'inyungu ndetse muha n'igihe ngo abaze ibibazo bishoboka byose kugirango asobanukirwe cyane n'ububushakashatsi. Nasubije neza uko nshoboye kose kugirango asobanukirwe.

Umukono w'ubaza

Tariki……/……/….

Appendix 5: QUESTIONNAIRE ENGLISH VERSION

Topic: Prevalence of *Plasmodium* species and their risk factors among malaria cases attending health centers of Bugesera district, Eastern province, Rwanda, September-December 2018

Name of health facility----------

Date:……./……./……

I. Patient/Clinical information

1. Number of participant: ……

2. Residence: Bugesera=1 ☐ rural=2 ☐ urban=3 ☐ out of Bugesera=4 ☐

3. Sex: male=1 ☐ Female=2 ☐

4. Age ☐

5. Martial status?

Single=1 ☐ Married=2 ☐ separated=3 ☐

widower=4 ☐ live with parent=5 ☐

6. Education:

primary=1 ☐ Secondary=2 ☐ University=3 ☐ not educated=4 ☐

7. Occupation

Farmer=1 ☐ Public servant=2 ☐ private servant=4 ☐ fisher=5 ☐

Rice farmer=5 ☐ wetland farmer=6 ☐ Other occupation=7:…….

8. Religion

Christian=1 ☐ Muslims=2 ☐ No religion=3 ☐ other(specify)=4: ☐

9. Ubudehe category

Ubudehe= 1 ☐ Ubudehe =2 ☐ Ubudehe= 3 ☐ Ubudehe= 4 none=5 ☐

10. **Do you have health insurance**? Yes=1 ☐ no=2 ☐

11. What kind of insurance do you have: mituelle=1 ☐ RSSB=2 ☐ MMI=3 ☐ Others=4 ☐ specify…….

12. Did you travel out of Rwanda within last month?

yes=1 ☐ no=2 ☐

13. Which country did you visit?

DRC=1 ☐ Uganda =2 ☐ Burundi=3 ☐ Kenya=4 ☐ Tanzania=5 Other visited countries=6 ☐ (specify)……

II. Malaria prevention

14. Are you pregnant?

Yes=0 No=1

15. If yes how many months are you pregnant? .....

16. Have you Insecticide-treated bed Net in your household? Yes=0 No=1

Yes=0 ☐ No=1 ☐

17. If no, which reason do you not have?

Not provided by ☐ government=1 ☐ exper ☐ e=2 ☐ 3= ☐ and replaced=3 cause other diseases=4 Used for other purpose=5 Others =6 specify……..

18. Do you always sleep under Insecticide Treated Net (ITN)?

Yes=0 ☐ No=1 ☐

19. If you do not sleep under ITN, which reason?

1= Causes other diseases=1☐ used for other purpose=2 ☐ too hot=3☐ availability of bed bugs=4 old=5 ☐ her spec..y=6 ☐

20. Is the household close the water body such as rice cultivation area, wetland, water dam?

Yes=0 ☐ no=1 ☐ ?

21. If yes how long is the distance between the water body and the household?

22. Is your house splayed for indoor Residual Splaying (IRS) during the last 12 months??

yes=0 ☐ no=1 ☐

III. Malaria clinical information

23. Is Malaria treatment guideline available at health facility?

Yes=0 ☐ no=1☐

24. Are health workers trained on malaria treatment guidelines?

yes=0☐ no=1 ☐

25. Date of onset:

26. **Date of consultation? .....**

27. How often did you do consultation within this month?

Once=1 ☐ twice=2 ☐ more than twice=3 ☐ other =4 ☐

28. Which symptoms did you present?

headache=1 ☐ fever=2 ☐ vomiting=3☐ muscle pain=4☐

joint pain=5 ☐ chills=6 ☐ 7=stiffness=7 ☐ 8=diarrhea=8☐ weakness=9 ☐

poor appetite=10 ☐ nausea=11☐ abdominal pain=12☐, others ☐ (specify)=13….

29. Blood specimen taken

Yes=0☐ no=1☐

30.Laboratory result

Plasmodium falciparum=1 ☐Plasmodium malaria=2☐

Plasmodium ovale=3 ☐ Plasmodium vivax=4 ☐ other plasmodium=5 (specify):…….

31. Laboratory results shows high parasitemia: yes ☐ no ☐

32. Drug prescribed, received?

yes=0☐ no=1 ☐

33. Names of drug taken

Quartem=1 quinine=2 ☐ Altesinate =4 Other drugs ☐ (specify) :……

Names and signature of investigator **Names and signature of participants**

Done on……/……../…….

Appendix 6: IBIBAZO MU KINYARWANDA

IBIBAZO BIBAZWA UWITABIRIYE UBUSHAKASHATSI

IZINA RY'UBUSHAKASHATSI:

KWEREKANA IMPUZANDENGO Y'UDUKOKO DUTERA MALARIA MU BARWAYI BA MALARIA BIVURIZA KU BIGO NDERABUZIMA BYO MU KARERE KA BUGESERA KUVA NZERI KUGERA UKUBOZA 2018

Ikigo nderabuzima cya: …….

Itariki: ……/……./…….

Amakuru rusange areba umurwayi

1. Umubare uranga uwitabiriye unushakashatsi:……

2. Aho mutuye mutuyemo:

Bugesera=1 ☐ Ahandi hatari Bugesera=2☐

Mu mujyi=3 ☐ mu cyaro=4☐

3. Igitsina: Gabo=1 ☐ Gore=2 ☐

4. Mufite imyaka ingahe?

5. Irangamimerere yanyu ni iyihe?

Ndibana=1 ☐ ndubatse=2 ☐ Twaratandukanye=3 ☐

Umupfakazi/re=4 ☐ Mbana n'ababyeyi=5 ☐

6. Amashuri mwize: ☐

Abanza=1 ☐ Ayisumbuye=2 ☐ Kaminuza=3 ntayo=4 ☐

7. Akazi mukora

Umuhinzi=1☐ Umukozi wa Leta=2 ☐ Mukorera abigenga=3 ☐ Umurobyi=4 ☐

Umuhinzi w' umuceri =5☐ Utunganya ibishanga=6 Ikindi mwaba mukora=7:……..

8. Idini

Umukiristu=1☐ Umusiramu=2☐ nta dini ngira=3 ☐ Ahandi mwaba musengera=4

9. Uri mucyiciro cya kangahe cy' ubudehe

Ubudehe =1 ☐ Ubudehe= 2 ☐ Ubudehe= 3 ☐ Ubudehe= 4 ☐ Ntacyo mfite=5 ☐

10.Do you have health insurance? Yes=0☐ No=☐

11. Ni ubuhe bwishingizi mukoresha? Mituweli=1☐ RSSB=2 ☐ MMI=3 ☐ Ubundi bwishingizi mwabuvuga=4 ☐

12. Mwaba mwaragiye mu bihugu duturanye mu kwezi gushize?

Yego=0 ☐ Oya=1 ☐

13. Ni ikihe gihugu mwagiyemo?

DRC=1 ☐ Uganda=2 ☐ Burundi=3☐ Kenya=4☐ Tanzania=5 ☐

6=Ahandi mwahatubwira=6:

I. Uburyo bwo kwirinda malariya

14.Mwaba mutwite? Yego=0 Oya=1

15. Niba igisubizo ari yego, hashize igihe kingana iki mutwite?

16. Mwaba mufite inzitiramibu mu rugo rwanyu?

Yego=0 ☐ oya=1☐

17. Niba igisubizo ari oya, yaba ari iyihe mpamvu mutayifite?

ntazo Leta yaduhaye=1☐ zirahenda=2 ☐ zarashaje ntizasimburwa=3☐

zitera ubundi burwayi=4☐ ibindi mwabitubwira=5

18. Mwaba muryama buri gihe mu nzitiramibu iteye umuti ?

Yego=1☐ Oya=1 ☐

19. Niba mutaryama mu nzitiramibu iteye umuti impamvu yaba ari iyihe?

zitera ubundi burwayi(kwishima, ibiheri)=1☐ Tuzikoresha mu bindi bikorwa =2 zirashyuha=3

Turazibitse=4 ☐ zarashaje=5 ☐

20. Aho mutuye mwegereye amazi y' ibiyaga, ibishanga, ibidendezi cyangwa se aho bahinga umuceri?

Yego=1☐ Oya=2 ☐

21.Niba igisubizo ari yego, hari intera ingana iki hagati yahoo mutuye n' ahari ibiyaga, ibishanga, ibidendezi cyangwa se aho bahinga umuceri?.....

22. Inzu yanyu yatewemo umuti wica umubu muri aya mezi cumi n' abiri ashize ?

0=yego 1=Oya

II. Ibijyanya no kwivuza k'umurwayi wa malariya

23. Hari amabwiriza yo kuvura malariya ku kigo nderabuzima?

Yego=0 0ya=1

24. Abavura bahuguwe ku bijyanye no kuvura malariya? Yego=0 Oya=1 ☐

25. Hashize igihe kinga iki ugaragaje ibimeneyetso bya malariya?.....

26**. Mwaje kwisuzumisha ryari? Itariki/ukwezi ……./……..**

27. Ni ku nshuro ya kangahe muri uku kwezi muje kwivuza malariya?

1=Ni ubwa mbere ☐ 2=Ni ubwa kabiri ☐ 3=Ni ubwa gatatu ☐ 4=Ni kenshi ☐

28. Ni ibihe bimenyetso bya malariya mwagaragaje

1=Kuribwa umutwe ☐ 2=Kugira umuriro ☐ 3=Kuruka ☐ 4=Kubabara umubiri wose ☐

5=Kubabara mu ngingo ☐ 6=Gutitira ☐ 7=Kugagara ☐ 8=Gucibwamo ☐ 9=Kugira intege nke ☐

10=Amaraso make ☐ 11=Kudashaka kurya ☐ 12=Kugira iseseme ☐ 13=kuribwa mu nda ☐ ibindi bimenyetso…….

29. Bagufatiye amaraso 0=Yego ☐ 1=0ya ☐

30. Ibisubizo bya laboratwari

1=Plasmodium falciparum ☐ 2=Plasmodium malaria ☐ 2=Plasmodium ovale ☐

4=Plasmodium vivax ☐ 5= Izindi:…..

31 .ibisubizo birerekana ko afite udukoko twinshi mu maraso ? Yego=0, Oya=1

31. Mwaba mwabonye imiti babandikiye 0=oya ☐ 1=yego ☐

32. Ni ubuhe bwoko bw' imiti babahaye

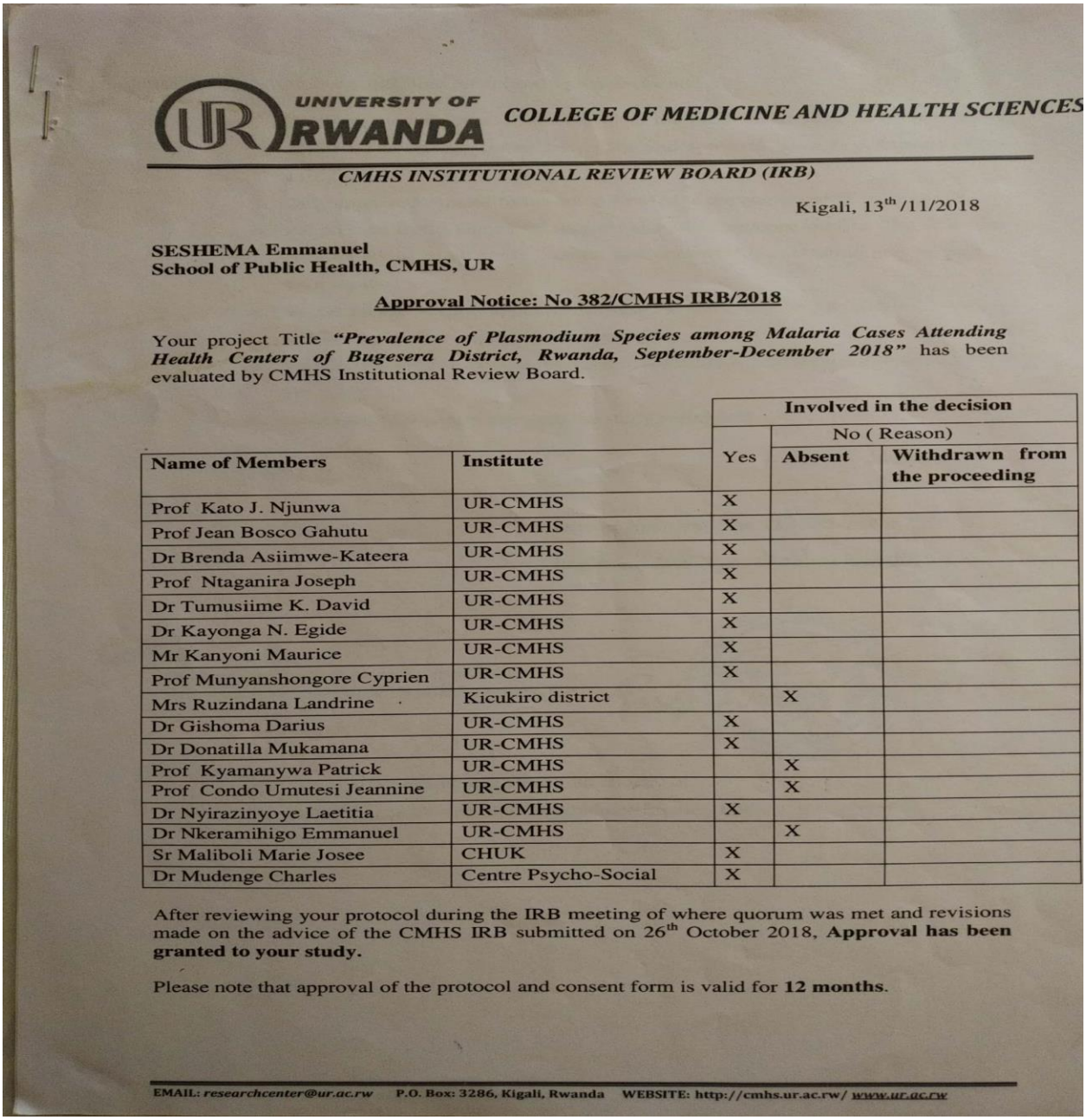
1=paracetamol ☐ 2=quartem ☐ 3=quinine ☐ 4=Altesinate ☐ Undi muti :……

Amazina n' umukono by' ubaza **Amazina n' umukono by' ubazwa**

Kuwa……/……../…….

MURAKOZE!

Appendix 7: IRB approval (a)



UNIVERSITY OF RWANDA — COLLEGE OF MEDICINE AND HEALTH SCIENCES

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, 13th /11/2018

SESHEMA Emmanuel
School of Public Health, CMHS, UR

Approval Notice: No 382/CMHS IRB/2018

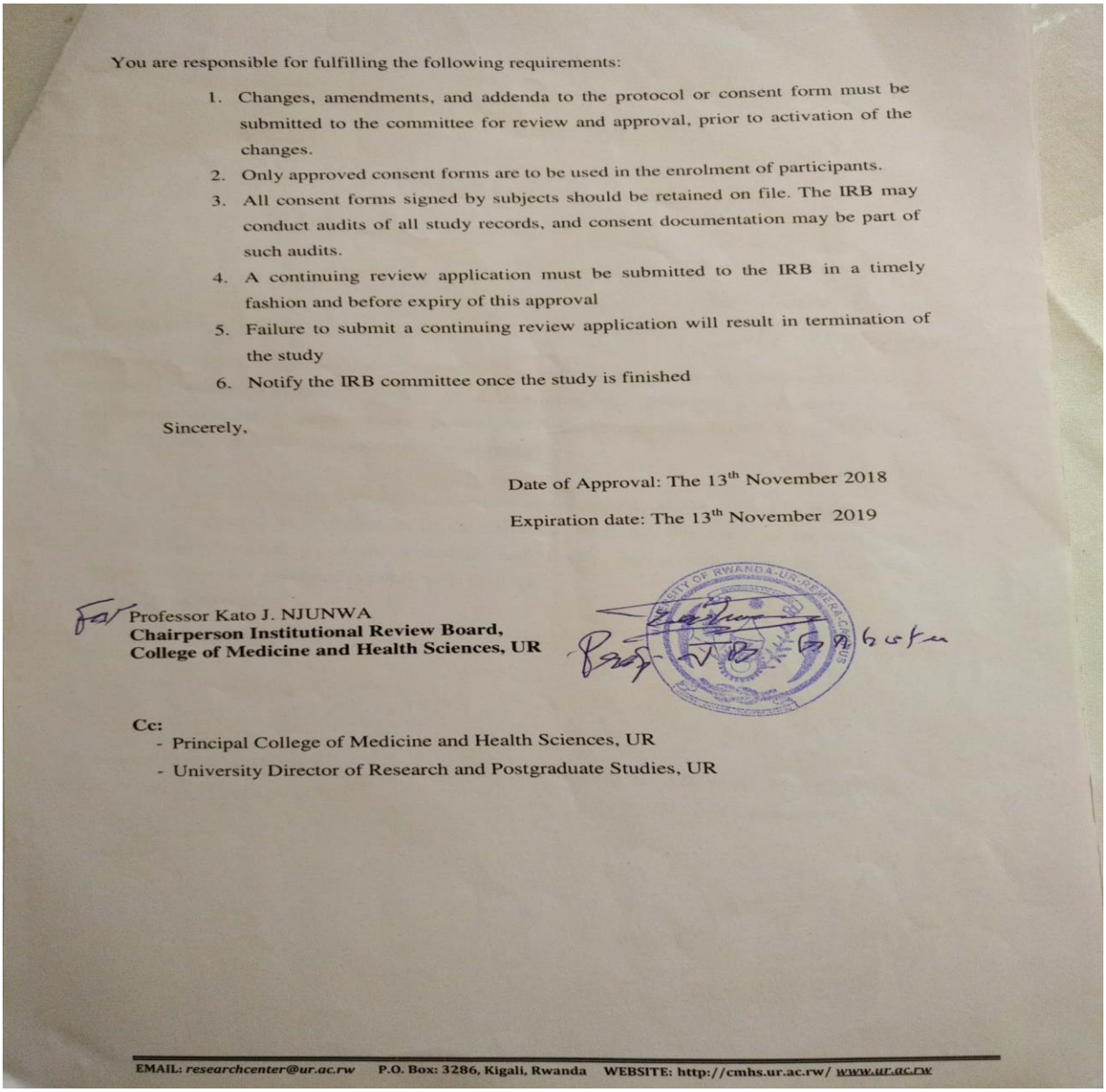
Your project Title ***"Prevalence of Plasmodium Species among Malaria Cases Attending Health Centers of Bugesera District, Rwanda, September-December 2018"*** has been evaluated by CMHS Institutional Review Board.

Name of Members	Institute	Involved in the decision		
		Yes	No (Reason)	
			Absent	Withdrawn from the proceeding
Prof Kato J. Njunwa	UR-CMHS	X		
Prof Jean Bosco Gahutu	UR-CMHS	X		
Dr Brenda Asiimwe-Kateera	UR-CMHS	X		
Prof Ntaganira Joseph	UR-CMHS	X		
Dr Tumusiime K. David	UR-CMHS	X		
Dr Kayonga N. Egide	UR-CMHS	X		
Mr Kanyoni Maurice	UR-CMHS	X		
Prof Munyanshongore Cyprien	UR-CMHS	X		
Mrs Ruzindana Landrine	Kicukiro district		X	
Dr Gishoma Darius	UR-CMHS	X		
Dr Donatilla Mukamana	UR-CMHS	X		
Prof Kyamanywa Patrick	UR-CMHS		X	
Prof Condo Umutesi Jeannine	UR-CMHS		X	
Dr Nyirazinyoye Laetitia	UR-CMHS	X		
Dr Nkeramihigo Emmanuel	UR-CMHS		X	
Sr Maliboli Marie Josee	CHUK	X		
Dr Mudenge Charles	Centre Psycho-Social	X		

After reviewing your protocol during the IRB meeting of where quorum was met and revisions made on the advice of the CMHS IRB submitted on 26th October 2018, **Approval has been granted to your study.**

Please note that approval of the protocol and consent form is valid for **12 months**.

EMAIL: researchcenter@ur.ac.rw P.O. Box: 3286, Kigali, Rwanda WEBSITE: http://cmhs.ur.ac.rw/ www.ur.ac.rw



You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrolment of participants.
3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval
5. Failure to submit a continuing review application will result in termination of the study
6. Notify the IRB committee once the study is finished

Sincerely,

Date of Approval: The 13th November 2018

Expiration date: The 13th November 2019

For Professor Kato J. NJUNWA
Chairperson Institutional Review Board,
College of Medicine and Health Sciences, UR

Cc:
- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Postgraduate Studies, UR

EMAIL: researchcenter@ur.ac.rw P.O. Box: 3286, Kigali, Rwanda WEBSITE: http://cmhs.ur.ac.rw/ www.ur.ac.rw

Appendix 8: IRB approval (b)

REPUBLIC OF RWANDA

BUGESERA DISTRICT

ADEPR

NYAMATA HOSPITAL

B.O.Box : 7112 Kigali

Tel : 0788130100

E-mail: nyamata.hospital@moh.gov.rw

23rd February, 2018

For receipt
SESHEMA Emmanuel
17/3/2018

Dear Emmanuel SESHEMA,

RE: Approval of conducting research

After review of your research protocol entitled **"plasmodium species among patients attending Bugesera Health Facilities"** aiming at **determining the prevalence of plasmodium falciparum and other plasmodium species**, the Nyamata Hospital Ethics Committee has decided to give you permission to conduct your research.

The Ethics Committee finally recommends you to ensure confidentiality of health information from patients and health professionals of the health facilities; to submit a final copy of your findings after completion of the research; and finally, to consult Nyamata Hospital administration in case you need to publish the findings.

Wishing you all the best!

Sincerely,

Dr Samuel NDAYISHIMIYE

Chair Person of Ethics Committee

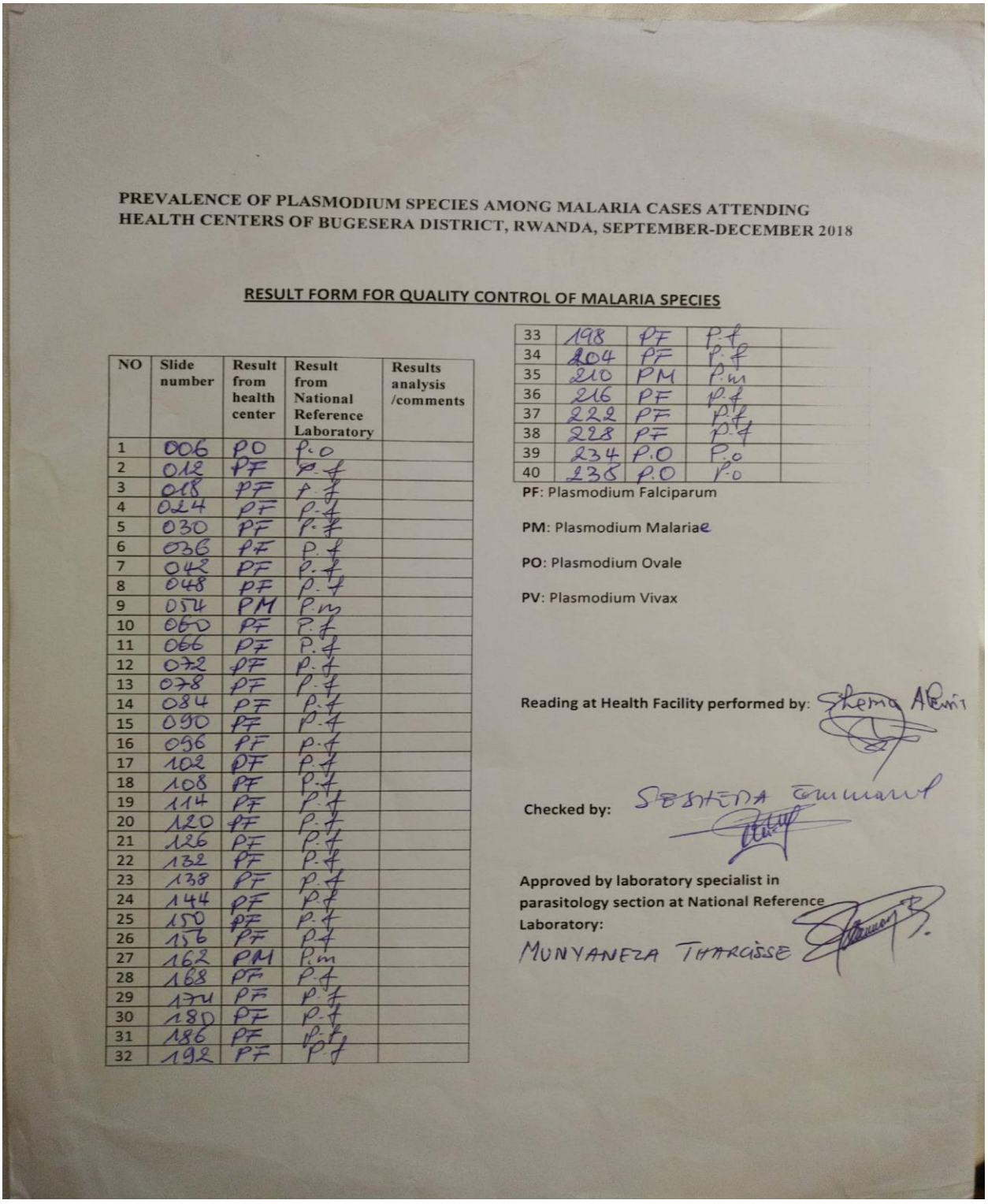
Dr Eldo BALIKWISHA
Medecin Generaliste
Hopital Nyamata
OMS 3756

Approved by **Dr Alfred RUTAGENGWA**

Director General

EGLISE DE PENTECOTE EN RWANDA ADEPR
Hopital Nyamata
BP 7112 Kigali
District de Bugesera

Appendix 9: Nyamata District Hospital approval



PREVALENCE OF PLASMODIUM SPECIES AMONG MALARIA CASES ATTENDING HEALTH CENTERS OF BUGESERA DISTRICT, RWANDA, SEPTEMBER-DECEMBER 2018

RESULT FORM FOR QUALITY CONTROL OF MALARIA SPECIES

NO	Slide number	Result from health center	Result from National Reference Laboratory	Results analysis /comments
1	006	PO	P.O	
2	012	PF	P.f	
3	018	PF	P.f	
4	024	PF	P.f	
5	030	PF	P.f	
6	036	PF	P.f	
7	042	PF	P.f	
8	048	PF	P.f	
9	054	PM	P.m	
10	060	PF	P.f	
11	066	PF	P.f	
12	072	PF	P.f	
13	078	PF	P.f	
14	084	PF	P.f	
15	090	PF	P.f	
16	096	PF	P.f	
17	102	PF	P.f	
18	108	PF	P.f	
19	114	PF	P.f	
20	120	PF	P.f	
21	126	PF	P.f	
22	132	PF	P.f	
23	138	PF	P.f	
24	144	PF	P.f	
25	150	PF	P.f	
26	156	PF	P.f	
27	162	PM	P.m	
28	168	PF	P.f	
29	174	PF	P.f	
30	180	PF	P.f	
31	186	PF	P.f	
32	192	PF	P.f	
33	198	PF	P.f	
34	204	PF	P.f	
35	210	PM	P.m	
36	216	PF	P.f	
37	222	PF	P.f	
38	228	PF	P.f	
39	234	P.O	P.o	
40	238	P.O	P.O	

PF: Plasmodium Falciparum

PM: Plasmodium Malariae

PO: Plasmodium Ovale

PV: Plasmodium Vivax

Reading at Health Facility performed by:

Checked by: SEBATEZA Emmanuel

Approved by laboratory specialist in parasitology section at National Reference Laboratory:

MUNYANEZA THARCISSE

Appendix 10: Blood slides quality assurance quality control result

World Health Organization

amref health africa

CERTIFICATE OF COMPETENCE

Tharcisse Munyaneza

IS CERTIFIED

Level 1 Malaria Microscopist*

According to the WHO Standard**
in the

External Competence Assessment of Malaria Microscopy
from 30 April to 4 May 2018

AF00565

* This certificate is valid for three years from date of issuance
** WHO. *Malaria Microscopy Quality Assurance Manual, Version* 2 (2016)

Dr Jackson Sillah
Ag. Malaria Team Leader
WHO Regional Office for Africa

Mr David Isaboke
Assessment Facilitator
WHO-Certified Level 1
Microscopist

Dr Jane Carter
Technical Director
Clinical and Diagnostics
Amref Health Africa

Appendix 11: Certificate of level 1 malaria microscopist national reference laboratory who performed blood slides quality control