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PREVALENCE OF SUBMICROSCOPIC MALARIA PARASITAEMIA AMONG ASYMPTOMATIC POPULATIONS IN NAVRONGO.

BY

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DECLARATION

I, ATELU GEOFFREY R. K. of the University of Ghana School of Public Health, do hereby declare that this research project was duly carried out by me, and the results obtained therein are a true reflection of work done under the supervision and direction of the following. The findings have not been submitted either in part or whole for the award of any degree elsewhere.

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(Supervisor)

Dr. Nancy Quashie …… Date…….

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Dr. Bismark Sarfo …… Date…….

(Supervisor)

DEDICATION

This thesis is dedicated to the memory of my late father, Mr. Humphrey Kwame Atelu and my mother, Mrs. Freda Atelu for the influence they have in my academic life.

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LIST OF ABBREVIATIONS

AFLP	Amplified fragment length polymorphism
A. gambiae	*Anopheles gambiae*
AP2-G	Apicomplexan-specific transcription factor
bp	Base pair
CDC	Centers for Disease Control and Prevention
cDNA	Complementary Deoxyribonucleic acid
Ct	Cycle threshold
CV	Coefficient of variation
DDT	Dichlorodiphenyltrichloroethane
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylene diamine tetraacetic acid
et al	and others
etc.	et cetera
FAM	6-carboxyfluorescein
Fig	Figure
g	gram
GDHS	Ghana Demographic and Health Survey
GHS	Ghana Health Service
GMAP	Global Malaria Action Plan
HbC	Hemoglobin C
HbS	Hemoglobin S
HRP-2	Histidine-rich protein 2
IPTi	Intermittent Preventive Treatment of malaria in Infants
IPTp	Intermittent Preventive Treatment of malaria in pregnancy
IRS	Indoor Residual Spraying
ITNs	Insecticide Treated Nets

KND	Kassena Nankana District
LAMP	Loop-mediated Isothermal Amplification
LLINs	Long Lasting Insecticide Treated Nets
malERA	Malaria Eradication Research Agenda
MOH	Ministry of Health
$MgCl_2$	Magnesium Chloride
ml	Milliliter
NDSS	Navrongo Demographic Surveillance System
NHRC	Navrongo Health Research Center
NMCP	National Malaria Control Program
NMIMR	Noguchi Memorial Institute for Medical Research
OPD	Out Patient Department
PCR	Polymerase Chain Reaction
P. falciparum	*Plasmodium falciparum*
Pfs25	*Plasmodium falciparum* Specific gene
RBM	Roll Back Malaria
pLDH	*Plasmodium* Lactate Dehydrogenase
PMI	President's Malaria Initiative
RAPD	Random Amplified Polymorphic DNA
RDT	Rapid Diagnostic Test
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SD	Standard Deviation
SP	Sulphodixine-Pyramethamine
Tm	Melting temperature
TAMRA	6-carboxytetramethyl-rhodamine
USA	United States of America

UV	Ultraviolet
WBC	White Blood Cell
WHA	World Health Assembly
WHO	World Health Organization
X^2	Chi squared
μl	Micro liter
^oC	Degree Celsius
>	Greater than
<	Less than

ABSTRACT

The recently observed decrease in cases of malaria achieved through the effective management and control of the disease has been a motivation to strive towards attaining the millennium goal of elimination and eradication of the disease. Asymptomatic infections also referred to as the parasite 'reservoir hosts' which could be microscopic or submicroscopic infections has been implicated in malaria transmission and therefore has become a targeted parasite population for malaria elimination efforts. In this regard, the relative contribution of the microscopic and submicroscopic infections to transmission, which is unknown, needs to be described. This study therefore determined the prevalence of submicroscopic *Plasmodium falciparum* parasite infections and gametocyte carriage in asymptomatic individuals in northern Ghana. A cross sectional study was conducted using the general population comprising of all age groups, during the dry season in Navrongo Township. Capillary blood samples collected from 209 study participants was used for microscopic identification of parasites and DNA extraction. Submicroscopic infections, for both asexual stage and gametocyte stage were detected by nested reverse transcriptase polymerase chain reaction (RT-PCR). The prevalence of asymptomatic *P. falciparum* carriage detected was 13.9% (29/209) by RT-PCR and 4.8% (10/209) using microscopy. All the parasites were identified as *P. falciparum* ring stage with mean parasitaemia of 732 parasites/μl ranging from 40 – 3,520 parasites/μl of blood. Overall submicroscopic prevalence was 9.1% (19/209). *P. falciparum* gametocytaemia was 3.8%. About 9.1% (19/209) of the asymptomatic infections were eclipsed by microcopy due to its low sensitivity (34.5%). However microscopy was found to be highly specific (100%). Gametocytemia was low, nonetheless, the ability of gametocytes to sustain transmission in an area of seasonal transmission; even at very low densities must be critically examined. Our study has shown that 9.1% (19/209) of the study population carry submicroscopic *P.*

falciparum parasites and also 3.8% carry gametocytes, which persist during the dry season. This study also revealed the importance of conducting active case surveillance to accurately determine submicroscopic malaria prevalence, and highlights the need for updating the malaria guidelines in tracking and treating of sub-microscopic malaria infections.

CHAPTER ONE

INTRODUCTION

1.1 Background

Malaria, which remains a major public health burden accounted for an estimated 198 million cases and 584, 000 deaths worldwide in 2013 (WHO, 2014). Of the estimated deaths, 90% occurred in sub-Saharan Africa and 78% were children under 5 years of age. However, the current integrated interventions against both the parasite and the vector have led to reduction in malaria mortality by 47% globally and 54% in WHO Africa Region from 2000 – 2013 (WHO, 2014). Within the same period, mortality rates among under-five age group have also declined by 53% globally and by 58% in Africa (WHO, 2014).

The causative agents of the disease are species of the genus *Plasmodium* from the family *Plasmodiidae,* namely, *P. falciparum, P. malariae, P. ovale, P. vivax* and more recently *P. knowlesi* which is zoonotic in parts of South-East Asia (WHO, 2014). Although *P. falciparum* and *P. vivax* are the most common parasites, the former is the most virulent of the five, widespread in Africa and responsible for about 95% of malaria cases reported in sub-Saharan Africa (Keba-Africa, 2012). The vectors for the transmission of the disease in sub-Saharan Africa are mainly females of the *Anopheles gambiae* species complex namely, *An. gambiae senso stricto, An. funestus, An. arabiensis, An. melas,* and *An. Colluzzii* (*Lee et al.,* 2013). The control of the disease involves the integrated interventions against both vector and parasite. Parasite elimination is mainly through chemotherapy and the vector is mainly controlled with insecticides. A number of malaria interventions are ongoing in malaria endemic regions of the world, which aim at reducing morbidity and mortality due to the disease. For Ghana, programs such as US President Malaria Initiative (PMI), Roll Back Malaria and Global Malaria Action Plan (GMAP) are being run which

involve the supply and distribution of insecticide treated bed nets (ITNs/LLINs), malaria prophylactic and therapeutic drugs.

The new malaria agenda is focused on the elimination at the country level and eradication of the disease globally due to the successes achieved in malaria treatment and vector control (Cotter *et al.*, 2013; Mendis *et al*., 2009). Among the planned strategies to achieve elimination is targeting the human reservoir of infection otherwise known as the 'asymptomatic reservoir' (Sturrock *et al*., 2013). The asymptomatic reservoir is the individual with asexual stage parasites without showing any symptoms of the disease. Most asymptomatic infections remain untreated and the possibility of transmission of the disease through this means cannot be ignored. As such, this targeted group of individuals in the population is justified for mass drug administration to kill the parasite and reduce disease transmission. However, lack of evidence-based data on the role of the asymptomatic reservoir in disease transmission is hampering efforts.

The identification and treatment of malaria infection in health facilities has been dependent on the use of microscopy, which is the gold standard for the parasite detection. Meanwhile, some asymptomatic infections are undetectable by microscopy and are referred to as submicroscopic or microparasitaemic infections. Such infections have very low parasitaemia beyond the limit of detection with microscopy and would therefore be missed during an intervention using only microscopy as the tool for diagnosis (Manjurano *et al*., 2011). In the advent of molecular epidemiology of diseases, the use of molecular tools assists tremendously in recognizing such latent infections. Thus, the Malaria Eradication Research Agenda which suggests the treatment of any level of parasitaemia because it represents a potential for transmission (malERA, 2011) will be enhanced.

It is certain that microscopic asymptomatic infections persist for long periods and develop into gametocytes, the parasitic stage picked up from humans and transmitted by mosquitoes (Bousema *et al*., 2014). Studies conducted in the Gambia and Kenya have shown that about 15-20% of untreated asymptomatic infections developed into transmissible gametocytes after four weeks (Bousema *et al*., 2004; Dunyo *et al*., 2006).

Most studies on submicroscopic malaria have been conducted in low transmission areas outside of Africa such as South-East Asia and the Amazons where most of the asymptomatic infections are submicroscopic (Harris *et al*., 2010; Steenkeste *et al*., 2010). In the Amazons, most of the research conducted on submicroscopic infections was conducted on *P. vivax* infections which normally exist as co-infections with *P. falciparum*. Unlike *P. falciparum*, *P. vivax* has a latent hypnozoite stage in the liver which can last for weeks or years and cause clinical relapse known as the hypnozoite reservoir (Lin *et al*., 2014). It is also evident that *P. vivax* gametocytes can be detected in early infections which are symptomatic and this is rarely seen in *P. falciparum* infections in Africa (Bousema & Drakeley, 2011). It was also observed in Thailand that about 2.4% of patients in a study had *P. falciparum* gametocytes on admission *(Price et al*., 1999). A study conducted in Ethiopia showed the prevalence of submicroscopic malaria carriage at 19.2% using PCR whilst microscopy-based prevalence was 3.2% and the prevalence was 6.9% using a rapid diagnostic test (RDT) alone (Golassa *et al*. 2013). From this study it could be seen that about 16% prevalence was eclipsed with microscopy.

The development of asexual stage malaria parasites into gametocytes occurs under unfavorable conditions such as stress or the presence of antimalarial drugs (Baker, 2010). Gametocytes can be detected in the bloodstream 7 to 15 days after asexual parasites are

seen (Day, Hayward, & Dyer, 1998). High asexual stage parasitaemia under unfavorable conditions may result in high level gametocytemia and subsequently more infectious to mosquitoes for transmission (Lin *et al*. 2014). Submicroscopic gametocytemia therefore will be expected to be non-infectious to the mosquito; however, studies conducted in Burkina Faso and Kenya showed that children with gametocyte blood film negative could infect comparatively fewer mosquitoes unlike those with microscopic gametocytemia (Ouedraogo *et al.*, 2009; Schneider *et al.*, 2007). The ability of a few mature gametocytes from submicroscopic infections to form gametes that will produce numerous oocytes which will develop into infectious sporozoites cannot be underestimated and therefore shows the contribution of such an infection to the overall transmission of malaria. The need to detect these very low level infections is therefore important for malaria elimination.

Microscopy is still the gold standard for diagnosing malaria infections and can detect from 5 to 50 parasite/μl (Amexo *et al* 2004). However, the average microscopist can only detect about 100 parasites/μl, which limits the use of microscopy for low parasitaemia detection (WHO, 1988). The use of molecular tools for malaria parasite detection comparatively has a higher sensitivity than microscopy. Molecular methods could detect the presence of parasite in microscopically undetectable limits with precision (Okell *et al.*, 2012). One of the methods known as the polymerase chain reaction (PCR) is able to detect specific nucleic-acid sequence in the parasite genome. PCR can detect five parasites or less per μl of blood (Guerin *et a*l., 2002) making it the appropriate method for the detection of submicroscopic asexual stage and gametocyte infection.

1.2 Problem Statement

Ghana is a malaria endemic country and the disease is the leading cause of morbidity and mortality accounting for 33% of hospital deaths in children under five years and about 38% of all outpatient illnesses as well as 36% of patients on admission (Ghana country Profile-PMI, 2014). In 2012, Ghana recorded an estimated 6.9 million cases of malaria out of which 17,000 deaths occurred (WHO, 2013). In this era where all efforts are geared towards malaria elimination, data on malaria transmission potential of asymptomatic malaria infections is most appropriate. The determination of the prevalence of submicroscopic infections and the relative contribution to the overall transmission of malaria in the country will provide significant information that will be useful towards the elimination efforts.

Blood smear microscopy and RDT are the methods being used routinely for diagnosis of malaria in Ghana. From the perspective of control agencies aiming to reduce transmission, the most important question is, to what extent do submicroscopic parasite carriers, who are missed during routine testing and surveys, contribute to the sustenance of transmission in Ghana. It is clear that smear-positive asymptomatic infections detectable by microscopy are an important reservoir because they often persist for months and develop into gametocytes.

The northern parts of Ghana with savanna/Sahel type of ecology experience seasonal malaria transmission, which occurs from June to November. This season is followed by the cessation of transmission during the dry season spanning six months. However upon the onset of the rains transmission occurs abruptly with an upsurge of malaria cases at the OPDs in health centers, clinics and hospitals, which is suggestive of the persistence of gametocytes throughout the dry season A recent modeling exercise based on findings with

molecular gametocyte detection tools concluded that gametocytes may persist for well beyond one month after clearance of asexual parasites and that drugs clearing immature and mature gametocytes can lead to substantial reduction of gametocyte carriage (Bousema *et al.*, 2010).

In most malaria endemic settings, asymptomatic infections are known to outnumber symptomatic infections and therefore the need to thoroughly describe the transmission potential of asymptomatic infections particularly at microscopically undetectable levels (Lindblade *et al*. 2013). It follows that the success of elimination strategies will rely on the ability to find and treat the asymptomatic reservoir.

1.2.1 Conceptual framework

Conceptual framework shows the relationships between relevant or salient factors that may influence project operation and the successful achievement of the study objectives.

Age and Immunity: A number of studies have linked increasing age to lower parasite density and prevalence (Bousema *et al.* 2014). More infections are found to be submicroscopic in older children and adults compared to younger children. This may be due to greater immunity in older individuals, likely due to cumulative exposure and a more developed immune system. Human behavior, such as the proportion of time spent outdoors (Liebman *et al*. 2014) may therefore influence the immunity of an individual. **Malaria Endemicity:** Due to the role of immunity in suppressing parasite densities, individuals in high transmission areas with greater exposure are expected to have higher prevalence of submicroscopic infections. **Genetic factor:** Studies have shown that HbC and HbS offer some protection to their carriers against clinical malaria leading to lower parasite density. (Verra *et al.*, 2007). **Anti-malaria treatment:** Treatment with

antimalarial drugs such as sulfadoxine pyrimethamine (SP) is found to result into a marked increase in the density of gametocytes within the individual (Bousema *et al*., 2004) and subsequently this enhances transmission of SP resistance strains. However, the use of artemisinin derivatives in combination with other drugs results in lower gametocyte carriage rates and density.

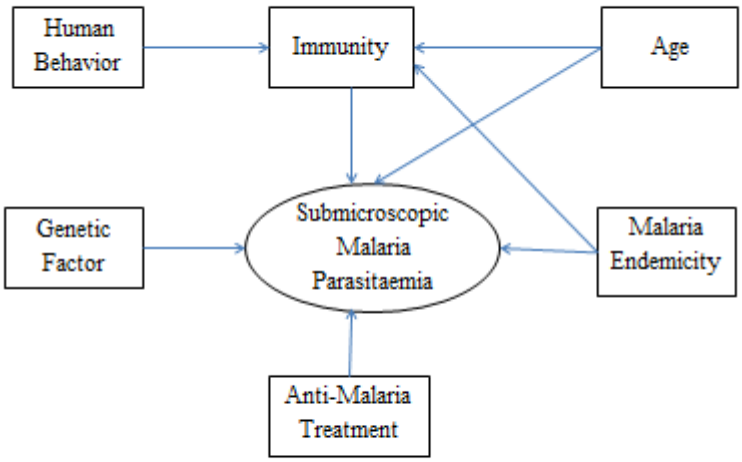

Fig 1.1 Conceptual framework

1.3: Justification

As malaria elimination programs pursue mass screening and treatment of asymptomatic individuals, further research is needed to define the degree to which submicroscopic malaria contributes to the transmission of parasites from the infectious reservoir and, in turn, what diagnostic detection threshold is needed to effectively interrupt transmission. To the best of our knowledge this study will be the first to describe such submicroscopic infections in the northern belt of Ghana where there is seasonal transmission of malaria.

This study will provide knowledge on the level of submicroscopic asexual stage and gametocyte carriage in northern Ghana. Subsequently, the study will aid in the development of strategies towards the control and elimination of malaria in the country.

1.4 General Objective

To determine the prevalence of submicroscopic *P. falciparum* parasitaemia among asymptomatic individuals in Navrongo, Ghana, during the dry season.

1.4.1 Specific objectives

1. To determine the prevalence of asymptomatic malaria infections in the population.
2. To determine the proportion of submicroscopic asymptomatic malaria infections in the population.
3. To detect submicroscopic *P. falciparum* asexual stage and gametocytes in the population.
4. To determine factors associated with *P. falciparum* infections in asymptomatic individuals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria: The Disease

Malaria is a parasitic infection caused by protozoa of the genus *Plasmodium* and is transmitted through the bites of infected female *Anopheles* mosquitoes. In the human body, the parasites multiply in the liver and then infect red blood cells leading to their rupture with subsequent development of anemia and other complications, depending on severity of the infection.

Hippocrates was the first to describe the symptoms of malaria and related them to the time of the year and where the patients lived (Freeman & Bradley, 1996) . Several names were given in describing the disease such as shakes intermittent fever, ague and chills. Then, there was this realization that the disease had an etiological relationship with swamps. This led the Romans to start drainage programs because of the bad air associated with fever-producing areas. The term *mala-aria*, written as *mal' aria* was then given to the disease and subsequently removing the apostrophe to get the present day term malaria (Cohen *et al.*, 2012).

The causative organism of malaria was discovered in 1880 by Alphonse Laveran and the transmission was later explored by Ronald Ross in 1898. The first known intervention against the disease was by American Indians in 1600, even before the discovery of the parasite and vector. They used the bitter back of cinchona tree for treatment (Freeman & Bradley, 1996). Vector control began in 1942 when DDT became an effective insecticide but lost its effectiveness as the vectors rapidly developed resistance to it. Chemotherapy

has been the main method for parasite control with the use of drugs like quinine, pyrimethamine-sulphadoxine (SP) and artesunate/amodiaquine combination.

Out of the five species of *Plasmodium* that affect humans, *P. falciparum* and *P vivax* are the most important (WHO, 2013). Infections due to *P. vivax* are more common than those due to *P. falciparum* outside the region of Africa, most especially the cooler regions. This is because *P. vivax* is able to thrive in the *Anopheles* mosquito vector at lower temperatures, and at higher altitudes and in cooler climates.

2.1.1 Symptoms of malaria

Malaria can be classified as uncomplicated or severe disease. It is classified as uncomplicated when the patient presents with a combination of symptoms such as fever, chills, headache, muscle and joint aches, tiredness, nausea, vomiting and diarrhea with the demonstration of parasites in the blood, usually by microscopy. The early symptoms of uncomplicated malaria are non-specific and therefore malaria is usually over-diagnosed on the basis of symptoms alone, especially in endemic areas (WHO, 2010). Accurate diagnosis, as in laboratory confirmation of the presence of the parasite, is essential for effective management of the disease in all settings. *P. falciparum* infections that are not promptly treated can quickly progress to severe malaria due to the ability to attain high levels of parasitaemia during its life cycle. The main symptoms usually include coma, severe breathing difficulties, low blood sugar, prostration and severe anemia. It is diagnosed on the basis of the presence of *P. falciparum* parasites and one of the above symptoms with no other obvious cause. Children, particularly those under five years are most affected since they have little or no immunity against the parasite. Severe malaria can lead to death if prompt treatment is not given. The results of a study conducted on children

with severe malaria in the remote communities of northern region of Ghana indicated that, severe anemia is indicative of severe malaria in majority of the patients, followed by prostration and respiratory distress (Mockenhaupt *et al.*, 2004). Furthermore, malaria is termed as cerebral when it is characterized by cerebral symptoms, such as coma. The pattern of malaria and the relative contribution of individual symptoms to mortality differ with endemicity, geographical location, access to health services, and age (Bejon *et al*., 2007).

2.1.2 Asymptomatic infections

Repeated exposure to malaria usually leads to the build-up of immunity in an individual. However, this varies widely depending on the intensity of transmission and geographical settings (Njama-Meya *et al.*, 2007) and does not necessarily inhibit infection but can reduce parasite density and symptoms (Tran *et al*., 2013). Community surveys have shown that over 75% of malaria infections are asymptomatic despite the fact that the disease occurs suddenly and quickly, and very severe to the point of lethality (Bousema *et al*., 2014). Some of these asymptomatic infections develop symptoms within days or weeks of initial detection (Nsobya *et al*., 2004), but most infections can persist for many months and remain asymptomatic at different parasite densities (Roucher *et al.*, 2012). The importance of asymptomatic malaria as a "reservoir host" depends on two main features; duration of infection and gametocyte density. Harboring infections for long periods of time could result in more mosquitoes taking blood meals with gametocytes which may enhance transmission. Moreover, infections with higher gametocyte densities are generally more infectious and will increase transmission. This targeted group of individuals in the population is therefore justified for mass drug administration to kill the parasite and reduce disease transmission. To be able to identify and treat the infection in asymptomatic

population, microscopy is normally the method of choice for the parasite detection. However, some asymptomatic infections are undetectable with microscopy and are referred to as submicroscopic or microparasitaemic infections.

2.2 Global Distribution of Malaria

Malaria which continues to be one of the devastating infectious diseases worldwide accounted for an estimated 198 million cases and 584, 000 deaths in 2013 (WHO, 2014). This represents a significant drop in incidence and mortality rates of 30% and 47% since 2000, respectively.

The burden is heaviest in the Tropical African Region, where an estimated 90% of all malaria deaths occur, and 78% are children under 5 years of age (WHO, 2014). An estimated 3.4 billion people are said to be at risk of malaria globally (WHO, 2014). The 2013 edition of World Malaria report indicates there are over 100 countries and territories in which malaria is considered endemic, and these have been divided into six WHO Malaria Regions (WHO, 2013). These include 43 in the African Region, 9 in the Eastern Mediterranean region, 8 in the European Region, 10 in the South-East Asian Region, 10 in the Western Pacific Region and 21 in the American Region (Fig. 2.1). These countries have reached different levels of progress towards achieving the World Health Assembly (WHA) and the Roll Back Malaria (RBM) partnership targets for 2015, which requires reduction of malaria cases by 75% from 2000 levels; and the Millennium Development Goal target of reversing the incidence of malaria. It is worth noting that countries such as Morocco, United Arab Emirates, Armenia and Turkmenistan have recently been certified as malaria free, while others are at various phases of elimination (WHO, 2013).

Malaria affects mostly persons with no or little immunity against the disease. In areas with high transmission, such as sub-Saharan Africa, the high risk groups are young children who have not yet developed partial immunity to malaria, pregnant women whose immunity is decreased by pregnancy, especially during the first and second pregnancies and travelers coming from areas with little or no malaria transmission, who lack immunity (Bousema *et al*. 2014). In areas such as Temotu Province where there is low transmission of the parasite, residents are less frequently infected (Harris *et al*. 2010). As such persons who may reach adult age without having built protective immunity are susceptible to the disease, including severe and fatal illness.

Out of the five species of *Plasmodium*, *P.vivax* has a wider distribution globally, due to its ability to develop in the vector at lower temperatures, and also to survive at higher altitudes and in regions of cooler climates. However, *P. falciparum* predominates in Africa while *P. vivax* survives mostly in temperate zone (World Malaria Report 2014*;* 2015)

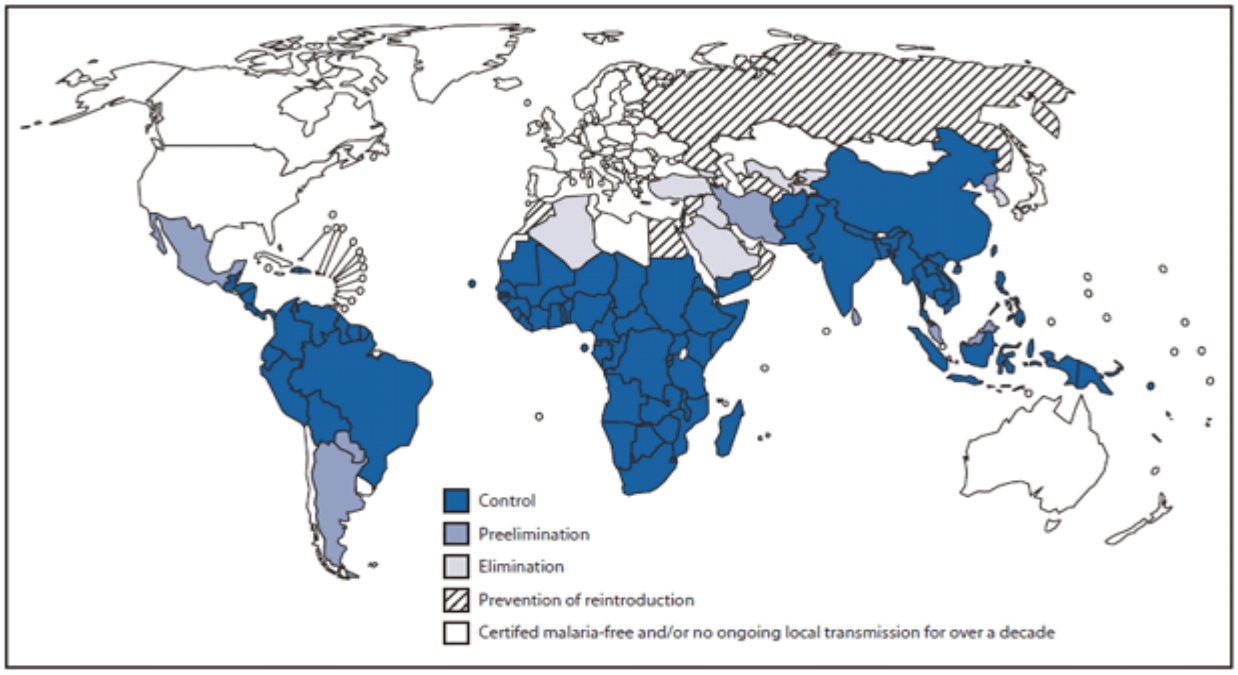

Figure 2. 1: Global Distribution of Malaria

Source: Roll Back Malaria, WHO

2.3 The Socio-Economic Impact of Malaria

Malaria has been a major obstacle to development in disease endemic regions, especially in Africa, which is the worst affected malaria region. Within endemic countries, the poorest and most marginalized communities are mostly affected, having the highest risks associated with malaria, but the least access to effective services for prevention, diagnosis and treatment.

The disease causes widespread premature death and suffering, imposing financial hardship on poor households, and holds back economic growth and improvements in living standards. Malaria impacts enormous costs to both individuals and governments. Costs to individuals and their families include purchase of drugs in treating malaria at home; expenses for travel to, and treatment at dispensaries and clinics; lost days of work; absence from school; expenses for preventive measures and expenses for burial in case of deaths. On the other hand, governments incur costs that include maintenance, supply and staffing of health facilities; purchase of drugs and supplies; public health interventions against malaria; lost days of work with subsequent loss of income and lost opportunities for joint economic ventures and tourism. While the direct costs (for example, illness, treatment, premature death) have been estimated to be at least US$ 12 billion per year, globally, the cost in lost economic growth is many times more than that (WHO Factsheet, 2014).

2.4 Epidemiology of Malaria

2.4.1 The life cycle and transmission of *Plasmodium* species.

The life cycle of the *Plasmodium* parasite begins in a vertebrate host and ends up in an insect vector (Fig 2.2). Human infection is initiated with a bite from an infected female *Anopheles* mosquito by injecting sporozoites into the blood stream while taking a blood

meal. Sporozoites travel to the liver and invade hepatocytes and multiply asexually to become schizonts that contain tens of thousands of rounded merozoites, per liver cell. This process known as pre-erythrocytic cycle or schizogony lasts for 6-8 days for *P. falciparum* (Bousema *et al*. 2014). The merozoites are then released into the bloodstream, which initiates the asexual parasite multiplication stage. Some malaria parasite species (*P. vivax*) remain dormant for extended periods in the liver, causing clinical relapses weeks or years later, known as the hypnozoite reservoir (Lin *et al*. 2014) . Within the erythrocytes, the parasites feed on hemoglobin and develop into ring-shaped trophozoites. Repeated cycles of asexual replication within red blood cells (48hrs for *P. falciparum*) result in thousands of parasite-infected red cells in the host bloodstream, leading to illness and complications of malaria that can last for months if not treated.

On the other hand, a fraction of the merozoites develop into sexual forms (micro and macrogametes), which are the only form of the parasite that can be transmitted from human to the vector. For *P. falciparum*, it takes 10-12 days for gametocytes to mature in the bone marrow (Farfour *et al.* 2012) before appearing in peripheral blood where they persist with an estimated mean circulation time of 3.4–6.5 days per gametocyte (Bousema *et al*. 2010). The matured gametocytes circulate in the blood stream until they are picked up by another vector as it takes a blood meal from the infected human. The asexual stage parasites may develop into gametocytes under unfavorable conditions such as stress or presence of antimalarial drugs (Baker, 2010). They can be detected in the bloodstream 7 to 15 days from when asexual parasites are spotted (Day *et al*., 1998). High asexual stage parasitaemia may result in high gametocytemia and subsequently more infectious to mosquitoes for transmission (Lin *et al*., 2014). The density of mature gametocytes in peripheral blood is usually less than 100 gametocytes per µl of blood (Bousema *et al*.,

2014) and vast majority of these occur at densities not detected by microscopy. Submicroscopic gametocytemia therefore will be expected to be non-infectious to the mosquito; however, studies conducted in Burkina Faso and Kenya showed that children with gametocyte blood film negative could infect comparatively fewer mosquitoes unlike those with microscopic gametocytemia (Ouédraogo *et al*. 2009). The ability of a few mature gametocytes from submicroscopic infections to form gametes that will produce numerous oocytes which will develop into infectious sporozoites cannot be underestimated and therefore shows the contribution of such an infection to the overall transmission of malaria. Each gametocyte ingested by mosquitoes, forms one female macrogamete or up to eight male microgametes. Within the mosquito mid-gut, male and female gametes fuse to produce a zygote that develops into a motile ookinete, which can penetrate the mid-gut wall and form oocysts. The oocysts undergo sporogony to produce many threadlike sporozoites, which are released when the oocysts busts. The sporozoites then migrate to the mosquito salivary gland, from where they can infect humans during the next blood meal. The presence of mature gametocytes in the peripheral blood is a pre-requisite for transmission and spread of the infection (Baker, 2010).

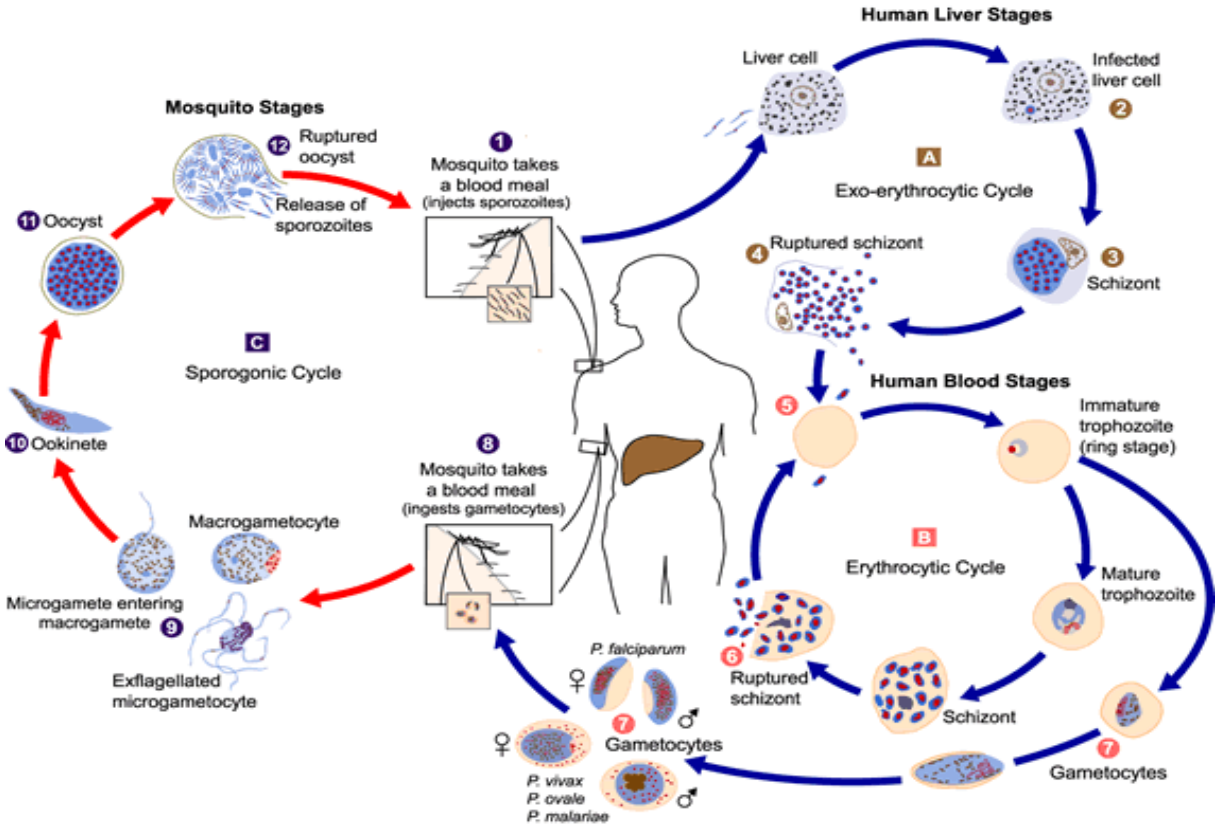

Figure 2. 2: The life cycle of malaria parasite.

(Source: http://www.cdc.gov/malaria/about/biology/)

2.4.2 Factors that influence parasite transmission.

The rate of transmission of malaria varies greatly and depends on several factors that are classified into parasite, mosquito and human factors.

Parasite factors: Commitment of malaria parasites to gametocytogenesis has recently been found to be controlled by a genetic factor AP2-G found in *Plasmodium* species (Kafsack *et al*., 2014). Treatment with antimalarial drugs such as sulfadoxine pyrimethamine (SP) is found to result into a marked increase in the density of gametocytes within the individual (Bousema *et al*., 2004) and subsequently this enhances transmission of SP resistance strains. This would therefore raise an argument against the use of SP for intermittent treatment. However, the use of artemisinin derivatives in combination with other drugs which results in lower gametocyte carriage rates and density and reduced

infectiousness in treated individuals should be enforced (Plowe, 2003). Additionally, parasite density appears to have an effect on gametocytogenesis as relatively higher concentration of gametocytes is seen in low-density infections compared with high-density infections (Bousema and Drakeley, 2011). Although studies have shown that success of transmission increases as gametocyte density increases, transmission still occurs at low gametocyte densities (Churcher *et al.*, 2013).

Mosquito factors: The ability of the sexual stage parasite to survive in the mosquito vector is crucial to its transmission. This is achieved by the role of parasite protein *Pfs47* which acts to block activation of the complement-like system of the host (Molina-Cruz *et al*., 2013). It is worth noting that, the most important factor that influences transmission success is simply the quantum of vectors and the frequency with which they bite.

Human factors: Contact between humans and mosquitoes are essential for parasite transmission. However, this differs between endemic settings and among individuals within the same area. As a result, human behavior, such as the proportion of time spent outdoors (Liebman *et al*. 2014), differences in attractiveness to mosquitoes, body size and odour profiles may influence parasite transmission. The suspicion is that major factors governing which individuals mosquitoes tend to bite quite often is the combine effect of individual characteristics and exposure time. For instance, adults who are larger in size may receive more bites than infants (Liebman *et al*. 2014). Studies have shown that HbC and HbS (b-globin genotype) offer some protection to their carriers against clinical malaria. However, the precise mechanism is yet to be unfolding (*Verra et al*., 2007). Environmental stresses, including host immunity, incomplete drug treatment and anemia also influence gametocyte development (Bousema and Drakeley, 2011).

2.5 The Control of Malaria

The Eighth World Health Assembly (WHA) in May 1955, adopted a Global Malaria Eradication Campaign based on the use of the insecticide Dichlorodiphenyltrichloroethane (DDT) for both indoor and outdoor spraying against adult mosquitoes as well as the use of antimalarial drugs to treat malaria and to eliminate the parasite in humans. The decision was taken on the grounds that, the transmission of malaria will be interrupted in the communities when a sufficient number of adult mosquitoes are eliminated before the parasite development in the vector is completed. This idea was the brain child of Professor George Macdonald (as cited by Trigg and Kondrachine, 1998). In fact, the campaign led to the eradication of malaria by 1967 from all developed countries where the disease was endemic and large areas of tropical Asia and Latin America were freed from the risk of infection. However, only three countries of tropical Africa launched this campaign since it was not considered feasible in the others. Although some achievements were realized it was certain that the objectives of global eradication could not be reached without a well-developed and public health-oriented infrastructure. Consequently, a new strategy was endorsed by a Ministerial Conference on Malaria Control in 1992 and confirmed by the WHA in 1993. This strategy differed considerably from the approach used in the eradication era. The new approach calls for flexible and decentralized programs based on disease rather than parasite control.

In 2006, WHO adopted a Global Malaria Control Strategy based on four basic technical elements to help reduce the devastating effect of malaria. These elements include the following: the provision of early diagnosis and prompt treatment of the disease; the planning and implementation of selective and sustainable preventive measures including vector control; early detection for the prevention or containment of epidemics; and the strengthening of local capacities in basic and applied research to permit and promote the

regular assessment of a country's malaria situation, in particular, the ecological, social, and economic determinants of the disease (WHO 2014).

In the past decades, malaria control efforts have been enhanced with increased international funding and greater political commitment. Consequently, the reported malaria burden has reduced globally, including some countries in tropical Africa. African countries such as Ethiopia, Gambia, Kenya, Mali, Niger as well as Togo are on their way to achieving high coverage with these effective malaria control interventions (WHO 2008). A further seven formerly endemic countries reported zero for locally acquired cases and these include Mauritius (1998), the United Arab Emirates (1998), Egypt (1998), Morocco (2005), Syrian Arab Republic (2005), Armenia (2006) and Turkmenistan (2006).

In line with these, a number of anti-malaria interventions are ongoing in Ghana, aiming at prevention and control of the disease. These interventions have reflected positively showing a downwards prevalence of malaria in the country, with a record of 34.9% in 2010 to 27.3% in 2014 (Ghana: DHS 2014-Key Indicators Report). These achievements have raised new hopes of eradicating malaria.

2.6 Methods of Detection of Malaria Parasites

2.6.1 Microscopy

Microscopic examination of Giemsa-stained thin and/or thick blood smears has been and still remains the gold standard for malaria diagnosis (Fig. 2.3). The method enables identification of various species of *Plasmodium* and quantification of parasites, both of which are important in assessing disease severity and prescribing adequate therapy. Thin

films (one layer of red cells) allow species identification because the parasite's appearance is best preserved in this preparation. Thick films (about five to six red blood cells thick) allow the microscopist to examine a relatively larger volume of blood and are >10 times more sensitive than the thin film (Warhurst and Williams, 1996). Hence detecting low levels of parasitaemia is easier on the thick film, but the appearance of the parasite is much more distorted and therefore distinguishing between the different species can be difficult. Considering the advantages and disadvantages of both thick and thin smears, it is better to use both smears while attempting to make a definitive diagnosis. The distinguishing features of the different *Plasmodium* species include the parasite nuclear and cytoplasmic material as well as the staining of cytoplasmic and membrane changes in infected red blood cells. These are better demonstrated by 10% Giemsa stain diluted with buffered water at pH 7.2. (WHO 2009).

Typically in *P. falciparum* infections, only ring stage parasites and gametocytes are found in peripheral blood. The rings have delicate cytoplasm, which stains blue and one or two chromatin dots that stains red (Fig. 2.4). Microscopic detection of *Plasmodium* species is an inexpensive, rapid and relatively sensitive procedure that allows detection of 50 to 500 parasites/µl (Rougemont *et al*. 2004). Assessment of this technique in European reference laboratories reliably detected parasite densities >10 parasites per µl of blood (Bousema *et al*. 2014). Outside research settings and reference laboratories, the sensitivity of microscopy is considerably reduced to100 parasites per µl of blood in approximately 5µl of whole blood (Bousema *et al.* 2014). The limited sensitivity of microcopy in detecting malaria infections has led to misdiagnosis of *Plasmodium* species and consequently wrong treatment. Furthermore, the lack of skilled microscopist has significantly hampered the efficacy of this traditional technique.

2.6.2 Rapid diagnostic test

The shortfalls associated with microcopy have led to the development of rapid diagnostic tests (RDT). This method of malaria diagnosis has been developed to improve the timeliness, sensitivity, and objectivity of malaria diagnosis. The test is based on the detection of malarial antigen in an infected blood by the use of monoclonal antibodies to target the conserved element of *Plasmodium* lactate dehydrogenase (pLDH) for specific regions which are unique to *P. falciparum* or *P. vivax (*Murray *et al 2003)* . Currently the technology is centered on detection of Histidine-rich protein 2 (HRP-2) from *P. falciparum* and parasite-specific lactate dehydrogenase or *Plasmodium* aldolase from the parasite glycolytic pathway, found abundantly in all asexual and sexual stages of the parasite. Although this technique is rapid and can produce instant results without employing expert hands, it is limited in sensitivity in cases of low parasitaemia and also in diagnosing mix infections. Another limitation to this method is the possibility of producing false positive results. This can be attributed to the persistence of *Plasmodium* antigens weeks beyond the actual infection (Mangold *et al*., 2005).

Thick film Thin film

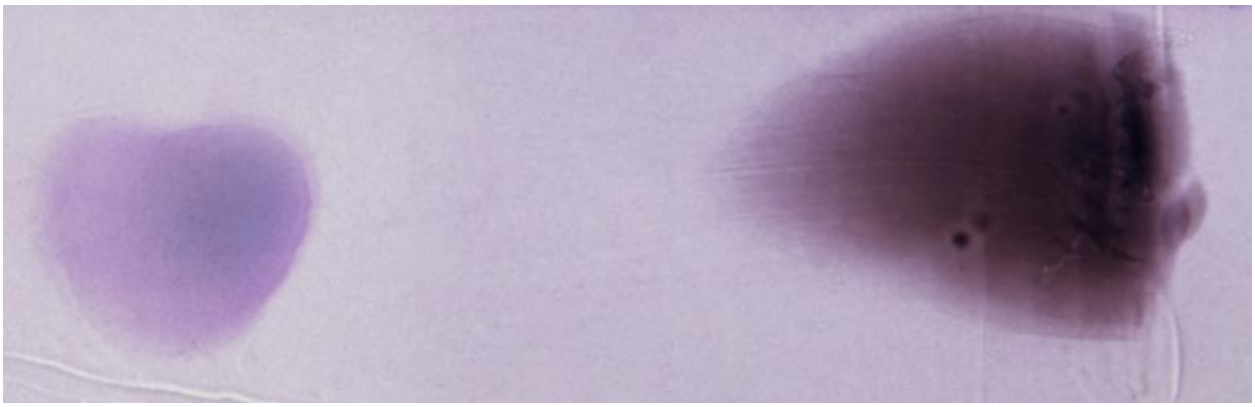

Figure 2. 3: Thick and thin blood smears prepared on the same slide and stained with Giemsa for microscopic analysis.

(Obtained from the CDC [http://phil.cdc.gov/phil/home.asp Public Health Image Library).

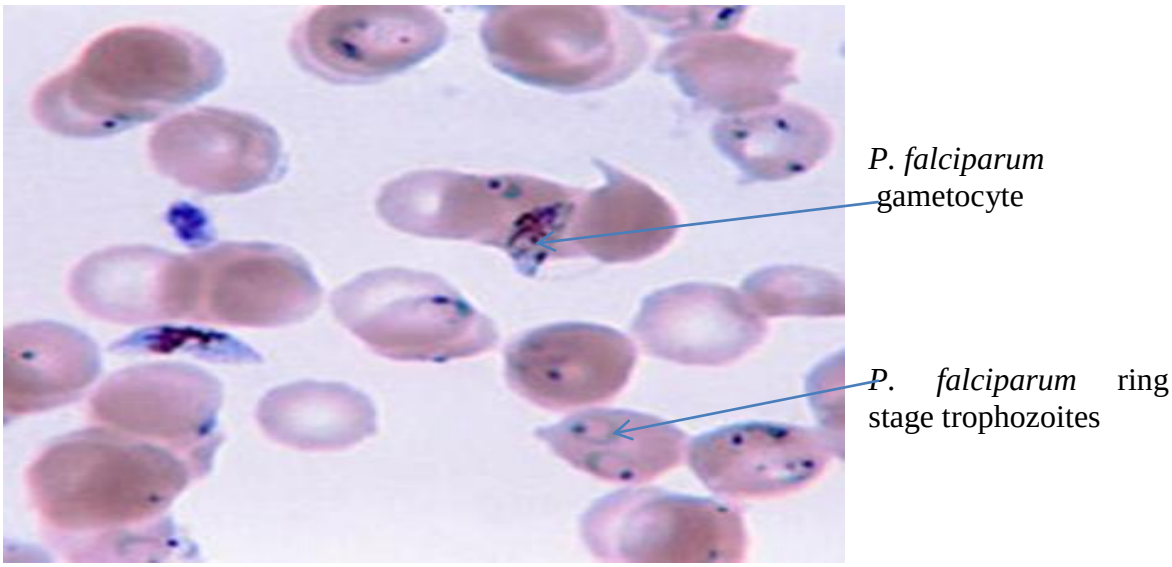

Fig 2.4: Geimsa stained thin blood film showing ring stage and gametocytes of *P. falciparum* parasites.

2.6.3 Molecular methods

Molecular technology allows the identification of parasites based on their antigenic components or DNA segments. Current methods for the identification of parasites include: polymerase chain reaction (PCR), random amplified polymorphic DNA (RAPD),

amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), loop-mediated isothermal amplification (LAMP), and the recently added reverse transcriptase as well as real-time PCR (Singh, 1997).

PCR was first applied to malaria diagnostics in the early 1990s (Anthony, Mahmud *et al*. 2013) and has since been used extensively. This technique for amplifying DNA has become the cornerstone of modern molecular biology. Indeed, these molecular methods based on selective DNA amplification from complex genomes, have brought about much improvement in the diagnosis of malaria. The amplification process is required to make several copies of the DNA to give a detectable signal for quantification. This technique therefore requires small pieces of DNA complimentary to your gene sequences of interest (primers) as the starting point for thermo-stable polymerase enzyme to begin making several copies of the target gene. After amplifying your gene it is possible to run the amplified DNA out on an agarose gel and stain it with a fluorescent dye which makes it visible. The brighter the visible band, the more copies of the target that have been made. This conventional PCR assay described above is technically demanding, time-consuming and is subject to the carrying over of contamination during the manipulation of final DNA products. Nonetheless conventional PCR has advanced from detection at the end-point of the reaction to detection while the reaction is occurring, thus referred to as Quantitative Real-time-PCR (qPCR).

A qPCR is a laboratory technique of molecular biology based on the PCR method and it is used to amplify and simultaneously detect or quantify a targeted DNA molecule. With this technique, amplification of DNA is linked to the generation of fluorescence which can simply be detected with a camera during each PCR cycle. Hence, as the number of gene copies increases during the reaction, so the fluorescence increases. In the case of RNA

quantitation, the template is complementary DNA (cDNA), which is obtained by reverse transcription of RNA followed by RT-PCR (Bousema *et al*. 2014)

CHAPTER THREE

METHODS

3.1 Study Site

The study was carried out in Navrongo, the administrative capital of Kassena- Nankana District (KND) in the Upper East Region of Ghana (Fig. 3.1 and 3.2). Navrongo has an estimated population of 25,470 and lies in guinea savannah zone with distinct wet and dry seasons. The wet season extends from June to November, with the heaviest rainfall occurring between June and October. The dry season starts from late November and ends in the latter part of March. The population consists of two distinct ethnic groups, the Kassena forming 49% of the district's population and the Nankani about 46%. The Builsa and migrants make up the remaining 5%. The inhabitants live within traditional compounds of extended family groups. The site is served by the Navrongo Health Research Centre (NHRC), which uses the Navrongo Health and Demographic Surveillance System (NHDSS), a computerized database, to track births, deaths, migration and other population features. Fieldworkers visit compounds every three months to collect data on these health and socio-demographic information (Oduro *et al*., 2012).

Malaria is endemic in the area and is the most important cause of morbidity and mortality in KND (Kasasa *et al*., 2013) . *P. falciparum* is the predominant parasite species, being transmitted in the area by both *An. gambiae* s.s and *An. funestus*. KND is a high malaria transmission area and several interventions have been done to reduce the devastating effect of malaria. These include; indoor residual spraying (IRS), sleeping under insecticide treated bed nets (ITNs), Intermittent preventive treatment of malaria in pregnancy (IPTp) and in infants (IPTi) as well as the introduction and distribution of artesunate-amodiaquine tablets to all communities at subsidized rates.

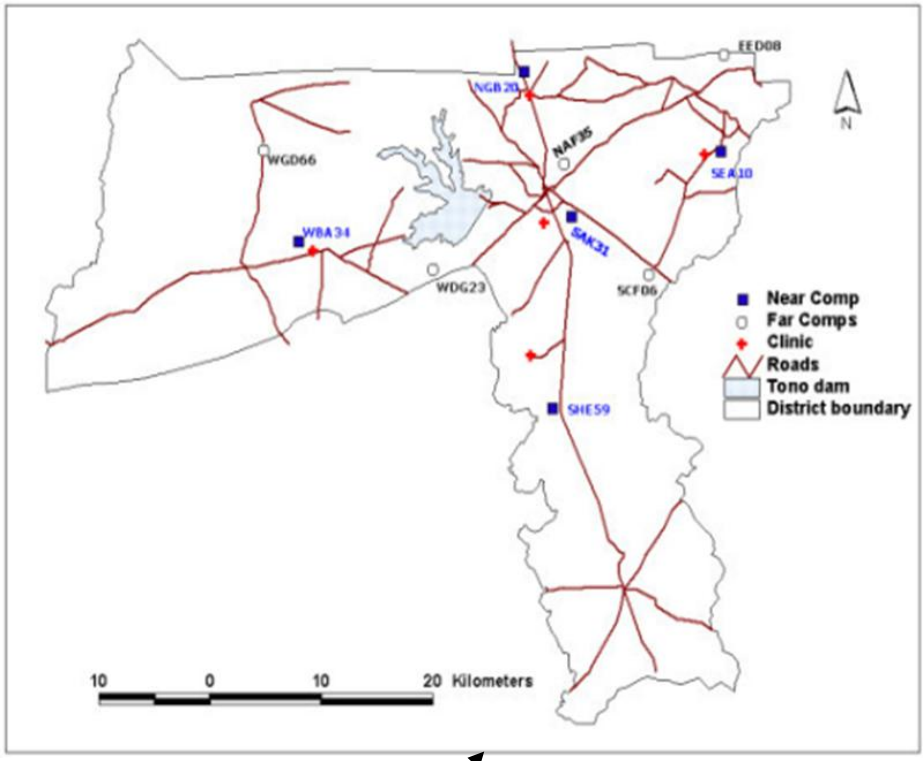

Figure 3. 1: Map of KND showing location of health facilities and index compound

Figure 3. 2: Map of Ghana showing the location of the KND.

3.2 Study Design

A cross sectional study was conducted using the general population comprising of all age groups during the months of February and March 2015, a period of low malaria transmission in the northern part of Ghana.

3.2.1 Study population

Inclusion criteria

Individuals of all ages living in the communities of Navrongo town who showed no signs and symptoms of malaria were recruited for the study.

Exclusion criteria

All neonates were excluded.

Variables

Dependent variable is submicroscopic malaria parasitaemia.

Independent variables include:

- Age
- Sex
- Occupation
- Educational level
- ITN use
- Reported fever and malaria treatment in previous 2weeks
- Marital status

Sample size determination

The sample size determination was based on achieving a 90% power of detecting submicroscopic malaria parasite carriers, with 95% confidence that the sample proportions obtained will differ from the population proportions by not more than 5%. Using submicroscopic malaria prevalence of 15%, a mean value from previous studies (Diallo *et al*., 2012) sample size (n) of 196 participants was arrived at using the formula:

$n = Z^2 \times p (1- p)/E^2$ where,

n = required sample size

Z = Confidence level at 95% (standard value of 1.96)

P = Estimated prevalence of sub microscopic malaria carriage.

E = Margin of error at 5% (standard value of 0.05)

Hence, $n = 1.96^2 \times 0.15 (1-0.15)/ 0.05^2$

n = 195.92.

For participant's non-response, we adjusted the sample size by 35%.

Thus, adjusted sample size (N) = 196 x 135/100 = 264.6 or 265

3.2.2 Sampling

Stratified random sampling method was employed in selecting the study participants. The total population of Navrongo central was categorized into six age groups, <5, 5-14, 15-24, 25-34, 35-44 and >44 using the database of the NHDSS. Having in mind the adjusted sample size of 265 and six age groups, forty-four individuals were then selected randomly among the age groups by a computerized system. This number was selected to make room for absenteeism and non-consenting persons as well as those who may not meet the inclusion criteria. A list of the selected individuals with household and compound identification numbers was generated to guide us through the fieldwork in selecting the participants. We move from compound to compound with the assistance of a trained fieldworker of NHDSS to seek the consent of the selected persons. Those who consented and met the inclusion criteria were recruited and sampled. Individual informed consent was obtained after the consent form was read and interpreted to them in the local language. We finally recruited 209 participants due to the overwhelming desire of the population to participate.

Thick and thin blood films were prepared on the same slide with blood from a finger prick and labeled with participant's identification number. A further 250µl of blood taken into

EDTA tube was added to 750µl of Trizol reagent (Life Technologies, USA) placed on ice and later stored at -80^{o}C in the NHRC laboratory, until samples were transported on ice to Noguchi Memorial Institute of Medical Research (NMIMR), Legon, Ghana.

3.3 Data Collection

3.3.1 Questionnaire administration

Information on demographic characteristics of each participant was collected by means of a structured questionnaire (Appendix II).

3.3.2 Detection of malaria parasites by microscopy

Laboratory analyses were carried out at the Epidemiology Department of NMIMR under the supervision of highly trained laboratory professionals. The blood smears prepared from the field were air dried, the thin films fixed with absolute methanol and both stained with Giemsa stain. To obtain the best staining reaction, a 10% Giemsa working solution was prepared with buffered deionized water at pH 7.2 and stained for 10 minutes. The stained slides were examined at two different laboratories by me and another experienced microscopist. A slide was considered negative only after 200 oil emersion fields have been examined without seeing a parasite. Any discordant result was again assessed by a third microscopist before the final result recorded. Parasitaemia was determined by the number of parasites in relation to standard number of white blood cells (WBC) /µl (8000). Parasites in thick film were counted until 200 leucocytes have been seen.

3.3.3 Molecular analysis for the detection of parasites

Genomic RNA was extracted from whole blood preserved in Trizol at -80^{o}C, using the Trizol reagent (Life Technologies, USA) protocol for isolation of high quality RNA. The manufacturer's protocol was optimized using *P. falciparum* 3D7 in vitro culture strains to obtain RNA with high purity and integrity.

The homogenized blood samples stored at -80^{o}C were allowed to thaw at room temperature after which 200µl of chloroform was added to each tube. The tubes were then shaken vigorously by hand for 15 seconds. After incubating for 3 minutes at room temperature, the samples were centrifuged at 12,000 × g for 15 minutes at 4°C. The aqueous phase of the sample containing RNA was separated into another tube by pipetting the solution and leaving the interphase and the organic phenol-chloroform phase. Five hundred micro liters of 100% isopropanol was then added to the aqueous phase, per 1 ml of Trizol reagent used for homogenization. The samples were then incubated at room temperature for 10 minutes and centrifuged at 12,000 × g for 10 minutes at 4°C. After the centrifugation, a gel-like pellet of RNA was visible on the side and bottom of the tubes. The supernatant was removed leaving the RNA pellets which were washed with 1 ml of 75% ethanol and vortex briefly. It was then centrifuged at 7500 × g for 5 minutes at 4°C. The wash was discarded while the pellets air dried for 10 mins. RNA pellets were re-suspended in 30µl of RNase-free water by passing the solution up and down several times through a pipette tip. Extracted RNA achieved was then stored at -70°C until ready to be used. In order to determine the efficiency of the Trizol extraction protocol, *P. falciparum* 3D7 in vitro culture strains were initially taken through the protocol. The yield and purity of extracted RNA was determined by measuring the absorption of ultraviolet (UV) light, using Nano Drop spectrophotometer (Thermo Scientific, USA). According to the Trizol

extraction protocol, the ratio of the absorbance of pure RNA at 260nm to that at 280nm is expected to be 1.6 and above. These measures were also applied to two samples from each of the five badges of samples worked on, for quality control purposes.

Certain precautions were taken: (1) During homogenization, the appropriate amount of Trizol Reagent (0.75 mL of Trizol per 0.25 mL of sample) was used because an insufficient volume could result in DNA contamination of isolated RNA; (2) To minimize handling time during the RNA extraction procedure, samples were processed in relatively small batches of 24 to minimize RNA degradation; and (3) When removing the aqueous phase that contained RNA, caution was taken to avoid drawing any of the interphase or organic layer into the pipette, to prevent DNA contamination of RNA extract.

Reverse transcription polymerase chain reaction (RT-PCR) for *Plasmodium* genus detection.

The PCR assay was performed using RNA samples isolated for the detection of *Plasmodium* genus. All the 200 samples were analyzed for positivity for *Plasmodium* parasites. A published protocol on a genus-specific RT-PCR for the detection of *Plasmodium* was followed with minor changes (Kamau *et al.*, 2011).

The assay procedure involves three steps:

First step: This step involves a reaction that essentially cleans RNA template by removing contaminants, typically genomic DNA. Following the manufacturers protocol (Quantitect Reverse Transcription Kit, Qiagen, USA), genomic DNA was eliminated by a reaction with 2µl of gDNA Wipeout buffer and 11µl of RNase-free water added to 1µl of template RNA per test. This was mixed, incubated for 2min at 42^{o}C and placed immediately on ice for the next reaction.

Second step: This step involves the reverse-transcription (RT) reaction which was set-up by adding 1µl of Quantiscript reverse transcriptase, 4µl of Quantiscript RT buffer and 1µl RT primer mix to the entire reaction from step one. The components were mixed and incubated for 15minutes at 42^{o}C, followed by a further 3 minutes at 95^{o}C. The cDNA products were then stored on ice to proceed directly with RT-PCR or stored at -20^{o}C until ready to use.

Third step: R-T PCR for detection of *Plasmodium* species

The primers and probe were designed from 18S rRNA sequences to amplify all the units of rRNA in the chromosomes 1, 5, 7, 11, and 13. The regions of the sequences are highly conserved and found only in the genus *Plasmodium*. The sequence of the forward primer is 5'-GCTCTTTCTTGATTTCTTGGATG-3'and that of the reverse primer is 5'-AGCAGGTTAAGATCTCGTTCG-3'. The probe sequence is 5'-ATGGCCGTTTTTAGTTCGTG-3' labeled with 5'FAM (6-carboxyfluorescein) and 3'TAMRA (6-carboxytetramethyl-rhodamine) as the reporter and quencher, respectively. Amplification and real-time measurements were performed in the Applied Biosystems 7300 analytical PCR system with the following thermal profile; 50^{o}C for 30 minutes for reverse transcription process followed by 10 min at 95°C, 40 cycles of 15 seconds at 95°C, and 1 minute at 60°C. 1µl of template is added to 9µl of reaction master mix containing primers, probes, QuantiTect Probe RT-PCR Master Mix (Qiagen), 0.4µM each primer, 0.2µM probe, and 4 mM MgCl2. QuantiTect RT Mix (a blend of Omniscript and Sensiscript Reverse enzymes) was added to the reaction master mix as recommended by the manufacturer at a rate of 1µl per 100 µl of the reaction master mix.

Nested RT-PCR method for *Plasmodium falciparum* Gametocyte Detection

The gametocyte *Pfs25* transcript was detected using nested RT-PCR following published protocols (Jones *et al*., 2012; Wampfler *et al*., 2013). The cDNA generated from the RT reaction described above was used for the nested PCR; making use of two sets of primers. The primary PCR was conducted by adding two microliters of cDNA from the RT step to 23 microliters of master mixture containing: 100µM of each dNTP, 1X PCR buffer(50Mm KCL, 20mM Tris-HCL, pH 8.3, 1.5 mM $MgCl_2$, 1.25 U of enzyme *Taq* polymerase and 0.4µM of a set of primers (Forward:5'-TAATGCGAAAGTTACCGTGG-3';Reverse: 5'-TCCATCAACAGCTTTACAGG-3'). The final volume of all the reactions was 25µl. The PCR cycling conditions were an initial denaturation at 94^{o}C for 2 minutes, 45 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 52^{o}C for 60 seconds and extension at 68^{o}C for 2.5 mins. Two microliters of the product of the first PCR was used as template for a nested PCR using a set of internal primers: sense (5' - TAATGCGAAAGTTACCGTGG-3') and anti-sense (5'-TCCATCAACAGCTTTACA GG-3'). Cycling conditions were the same as the primary PCR but 30 cycles were used instead.

Analysis of PCR products

Following the PCR, 10µl of the products were electrophoresed on 2% Agarose gel stained with 5µg/ml ethidium bromide. The electrophoresis was ran in 1X Tris-Acetate-EDTA (TAE) buffer at 80volts for 1hour and visualized with ultra violet illumination. Photograph of the gel was taken using a Polaroid camera. The sizes of the PCR products were estimated by comparison with the mobility of a standard of known band sizes. The diagnostic size expected of the PCR amplified fragments is 124bp for *P. falciparum* gametocytes.

Nested RT-PCR Assay for detection of *Plasmodium* species

The entire samples positive by genus assay were further analyzed by species specific RT-PCR method, using the cDNA generated during the genus specific assay conducted in the earlier analysis. The initial amplification reaction involved the use of genus specific oligonucleotide primer pair, r PLU5 and r PLU6 to amplify DNA targets from the four malaria species (Table 3.2). The PCR products obtained was used as template for the nested PCR using species-specific oligonucleotide primer pairs for *Plasmodium* species. The primer pairs were rFAL1 and rFAL2 for *P. falciparum,* rMAL1and rMAL2 for *P. malariae,* rOVA1 and rOVA2 for *P. ovale and* rVIV1 and rVIV2 for P. vivax (See table 3.2). All reactions were carried out in final volume of 25µl, which contained 1X PCR buffer, 4mM $Mgcl_2$, 200µM DNTP, 0.25µM of each primer, 1U of *Taq* polymerase and 2µl of cDNA for primary amplification. This was thoroughly mixed and covered with adhesive foil to prevent evaporation of the reaction mixture. The nested reaction was carried out with similar reaction mix except that 1µl of primary products was used as template.

Both reactions were performed in the Applied Biosystems 7500 analytical PCR system with the following thermal profile; Initial denaturation at 94^{o}C for 1 minute, 35 cycles of denaturation at 94^{o}C for 1 minute, annealing at 58^{o}C for 2 minutes, extension at 72^{o}C for 5mins with a final extension at 72^{o}C for 5 minutes. However, the nested reaction was carried out with 30 cycles. Finally the PCR products were resolved by electrophoresis in 2% agarose gel as described above. The expected sizes of the PCR amplified fragments are 205bp for *P. falciparum,* 144bp for *P. malariae,* 800bp for *P. ovale* and 120bp for *P. vivax.*

Table 3. 1: **DNA Sequences of the synthetic oligonucleotide primers for PCR identification of *Plasmodium* species**

Name of Primer	DNA Sequence (5' – 3')	Melting temperature ($Tm^{o}C$)
r PLU5 (f)	CCT GTT GTT GCC TTA AAC TTC	58
r PLU6 (r)	TTA AAA TTG TTG CAG TTA AAA CG	58
rFAL1 (f)	TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT	76
rFAL2 (r)	ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC	86
rMAL1 (f)	ATA ACA TAG TTG TAC GTT AAG AATAAC CGC	82
rMAL2 (r)	AAA ATT CCC ATG CAT AAA AAA TTATAC AAA	72
rOVA1 (f)	ATA TCT TTT GCT ATT TTT TAG TAT TGG AGA	76
rOVA2 (r)	GGA AAA GGA CAC ATT AAT GTG ATC CTA GTG	74
rVIV1 (f)	CGC TTC TAG CTT AAT CCACAT AAC TGA TAC	82
rVIV2 (r)	ACT TCC AAG CCG AAG CAA AGA AAG TCC TTA	86

Where f is forward and r is reverse

Optimization and standardization of reagents and determination of critical control parameters

Sample collection, preparation, transport, and nucleic acid extraction methods are all critical parameters in test performance and so were optimized for *P. falciparum* detection. All equipment used during the process were calibrated according to the laboratory's quality assurance protocol. Each badge of samples run included an internal positive control (*P. falciparum* 3D7 cultured clones) and a negative control. The precision of the methods was assessed by calculating the percentage coefficient (CV %) of variation between badges of samples that were ran by using Ct values of *P. falciparum* 3D7 cultured clones.

Precautions taken to avoid false-positive and false-negative results

False-positive results may arise from either laboratory-related issues or assay-related factors, such as inefficient optimization. Product carry-over from positive samples or, more commonly, from cross-contamination by PCR products from earlier experiments is a possible source of error. However, various practices and tools have been introduced to prevent false-positive PCR results. These include the following: (1) Samples and reagents were handled in separate laminar air-flow hoods which are regularly decontaminated using UV light and bleach. (2) As part of good laboratory practices, the basic steps of the procedure (RNA extraction, master mix preparation, sample preparation, etc.) were performed in separate laboratory areas. (3) Different sets of pipettes were used for each of the steps (4) The use of positive displacement and filtered pipette tips was applied (5) Positive internal controls and negative controls were analyzed alongside each plate of samples.

3.4 Data Processing and Analysis

Parasitaemia was determined by multiplying the parasite count per 200 leucocytes, by 40 to give parasites per µl of blood. Thus, Parasites/ µl of blood = Number of parasites counted x 8000/No. of WBCs counted.

The study results were entered into Epi-data 3.0 and exported to stataMP 11 software for analysis. Univariate analysis was done to express background characteristics as frequencies and proportions. Baseline characteristics were cross tabulated to determine the prevalence of microscopic and submicroscopic parasitaemia. Bivariate and multivariate logistic regression models were used to assess the associations between the characteristics of the participants and parasitaemia.

3.5 Ethical Issues

Ethical clearance was sought from the ethical review committees of GHS Research and Development Division, Accra and NMIMR as well as the NHRC. All participants were given detailed information about the study aim and procedures, both in English and interpreted in local language, before they signed or thumb printed individual consent forms. Parents and guardians signed or thumb printed on behalf of their children below 12 years while assent forms were signed or thumb printed by children between 12 and 17 years. Participants were assured of their privacy and confidentiality by allocating codes/numbers to samples instead of their names.

CHAPTER FOUR

RESULTS

4.1 Study Population

A total of 209 individuals from Navrongo township were randomly selected for this study. The study population comprised of 98 (46.9%) males and 111 (53.1%) females. Their midian age was 24 years which ranged from 1-82 years with a standard deviation (SD) of ±21.02. Out of the 209 participants, 162 (77.5%) belong to the Kassena tribe, 18 (8.6%) were Nankams, 3 (1.4%) were of the Builsa ethnic group and migrants resident in Navrongo who participated were 26 (12.4%). The details of the demographic information, parasitaemia levels and PCR data for each participant are given in Appendix I. The study population was almost evenly distributed among the six age groups as shown in Table 4.1.

4.2: Demographic Information on the Study Population

Information on demographic characteristics of each participant was collected by means of a structured questionnaire. A large proportion (97.6%) of the participants investigated had not taken any anti-malaria drugs in the two weeks before sampling. Out of the five participants (2.4%) who took some anti-malaria drug, three of them took either Fansidar, SP or Malafan; while the other two took Artesunate/Amodiaquine combination and Artemether/Lumifantrim combination respectively. Investigation on ITN use reveals a very poor patronage in the utilization of ITNs in Navrongo town. Only 26 (12.4%) said they use ITN consistently. Nonetheless, there was no association between prevalence of malaria infection and ITN use ($X^2 = 0.0566$; $P = 0.812$). However, there was a significant association between marital status and malaria prevalence ($X^2 = 14.8554$; $P = 0.001$). The prevalence of *P. falciparum* parasite carriage among various characteristic groups is shown in Table 1.

4.3: Prevalence of parasitaemia detected by microscopy in study population

The microcopy-based prevalence of asexual *P. falciparum* parasites was 2.7% (3/111) among women and 7.1% (7/98) in males, while the overall point prevalence was 4.8% (10/209) among the study population (Table 4.1). All the parasites were identified as *P. falciparum* ring stage. The parasitaemia ranged between 40 and 3,520 parasites/ul of blood with the mean value of 732 parasites/ul of blood. The highest prevalence (10.3%) was among age group <5 years, with a gradual decrease as the age increases (Fig 4.1). Among the study population, only two individuals (1%) were found to carry gametocytes at a density of 240 and 720 parasites/ul of blood.

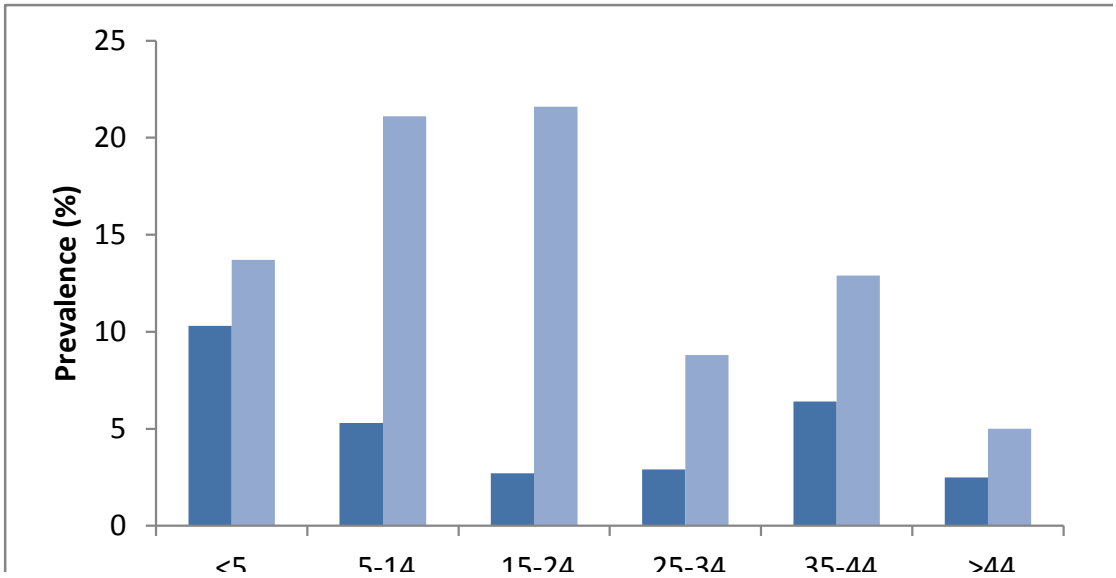

Figure 4. 1: Prevalence of *P. falciparum* asexual parasites by age group, Navrongo, 2015.

Table 4. 1: **Characteristics of the Study Population and Parasite Prevalence, Navrongo, 2015**

Characteristics	Number Examined N (%)	Prevalence by Microcopy N (%)	Prevalence by PCR N (%)
Age groups(years)			
(N= 209)			
<5	29 (13.9)	3 (10.3)	4 (13.7)
5 – 14	38 (18.2)	2 (5.3)	8 (21.1)
15 – 24	37 (17.7)	1 (2.7)	8 (21.6)
25 – 34	34 (16.3)	1 (2.9)	3 (8.8)
35 – 44	31 (14.8)	2 (6.4)	4 (12.9)
>44	40 (19.1)	1 (2.5)	2 (5.0)
Malaria treatment in the past 2 weeks (n=209)			
Treated	5 (2.4)	0 (0)	0 (0)
Not treated	204 (97.6)	10(4.9)	29 (14.2)
ITN use (n=209)			
Use net	26 (12.4)	2 (7.7)	4 (15.4)
Does not use net	183 (87.6)	8 (4.4)	25 (13.7
Occupation (n=209)			
Farmer	18 (8.6)	2 (11.1)	2 (11.1)
Public Servant	8 (8)	0 (0)	0 (0)
Trader	64 (30.6)	2 (3.1)	6 (9.4)
Unemployed	119 (56.9)	6 (5.0)	21 17.6)
Level of Education (n=209)			
No formal Education	53 (25.4)	5 (9.5)	19 (18.1)
Basic Education	105 (50.2)	4 (3.8)	2 (5.9)
Senior High	34 (16.3)	1 (2.9)	1 (5.9)
Tertiary	17 (8.1)	0 (0)	7 (13.2)
Marital Status (n=209)			
Married	98 (46.9)	2 (2.0)	4 (4.1)
Single	103 (49.3)	8 (7.8)	23 (22.3)
Divorced	8 (3.8)	0 (0)	2 (25.0)

4.4: Prevalence of *Plasmodium* parasites as detected by RT-PCR

Following microscopy examination of blood smears, all the 209 samples collected from the field were also tested for *P. falciparum* parasites by PCR techniques. Out of the total samples tested, 29 were positive, bringing the overall PCR-based prevalence to 13.9%; approximately three folds that of microscopy. Thus, the prevalence of asymptomatic infections in this study was 13.9%. The proportion of males (51.7%) detected as carriers of the parasites was more than that of the females (48.3%), though not statistically significant (x^2 = 2.25; P>0.05). The highest prevalence of *P. falciparum* parasites was observed among the 5 – 24 year age group while the lowest was among age >44 (Fig 4.1).

The nested RT-PCR conducted on all samples identified eight individuals as having *P. falciparum* gametocytes using *Pfs*25 rRNA transcript detection. This gives gametocyte prevalence of 3.8%. Three of these did not have detectable asexual parasitaemia. Interestingly, only one female was among gametocyte carriers. Gametocyte prevalence was highest (6.9%) among children under 5years (Fig 4.2).

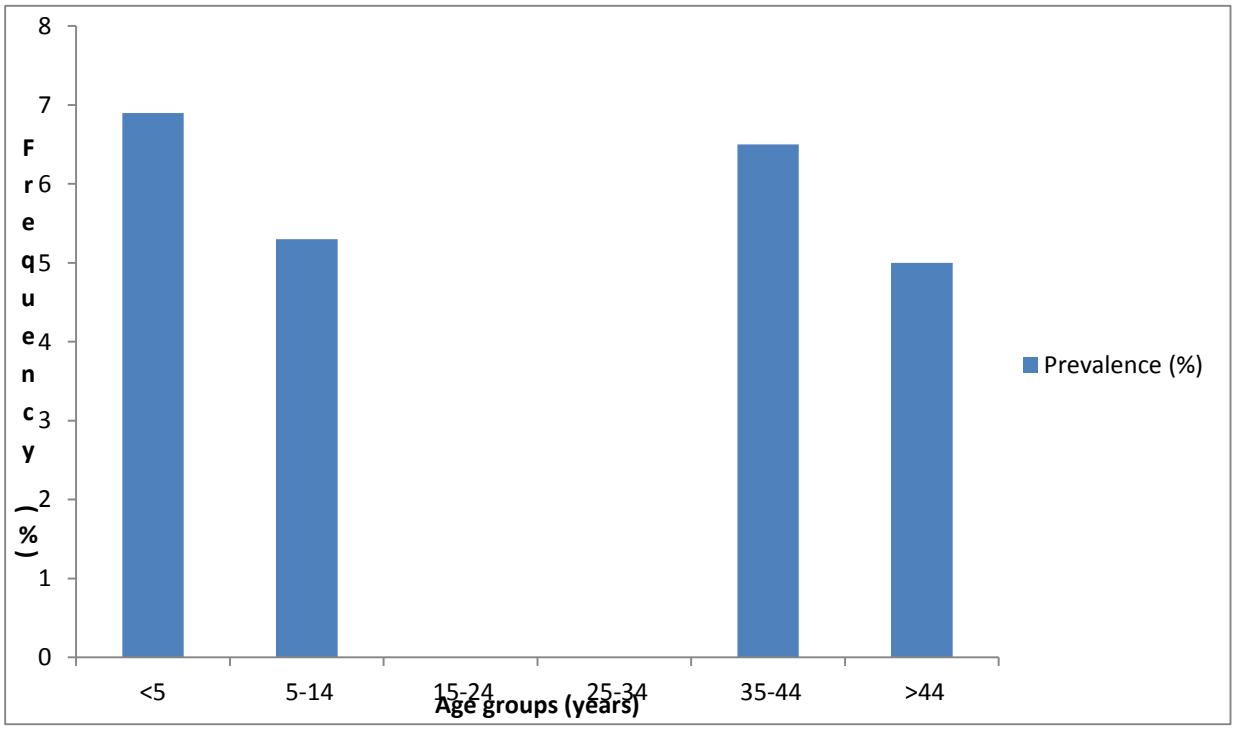

Figure 4.2: Prevalence of *P. falciparum* gametocytes among age groups, Navrongo, 2015.

4.5: Comparison of microscopy and PCR results for detection of *Plasmodium falciparum* infection

Out of the 209 samples analyzed, 4.8% (10/209) were positive for both microscopy and PCR while 86% (180/209) were negative for both methods. However, PCR identified more parasite-positive individuals than microscopy, 13.9% (29/209) against 4.8%. All the individuals positive for *Plasmodium* parasites by microcopy were also confirmed by PCR. Thus, using PCR as the standard method, sensitivity and specificity of microscopy were 34.5% and 100% respectively. Similarly, the positive predictive value and negative predictive value were 100% and 90.5% respectively.

4.6: Prevalence of submicroscopic infections

The RT-PCR identified 19 more individuals as having 18s rRNA transcript than microscopy did. Overall submicroscopic prevalence was 9.1% and that among males and

females was 8.4% and 9.9% respectively. Submicroscopic infections were low among children below 5 years but highest among the middle age group (15-24). The trend is shown in Fig 4.3.

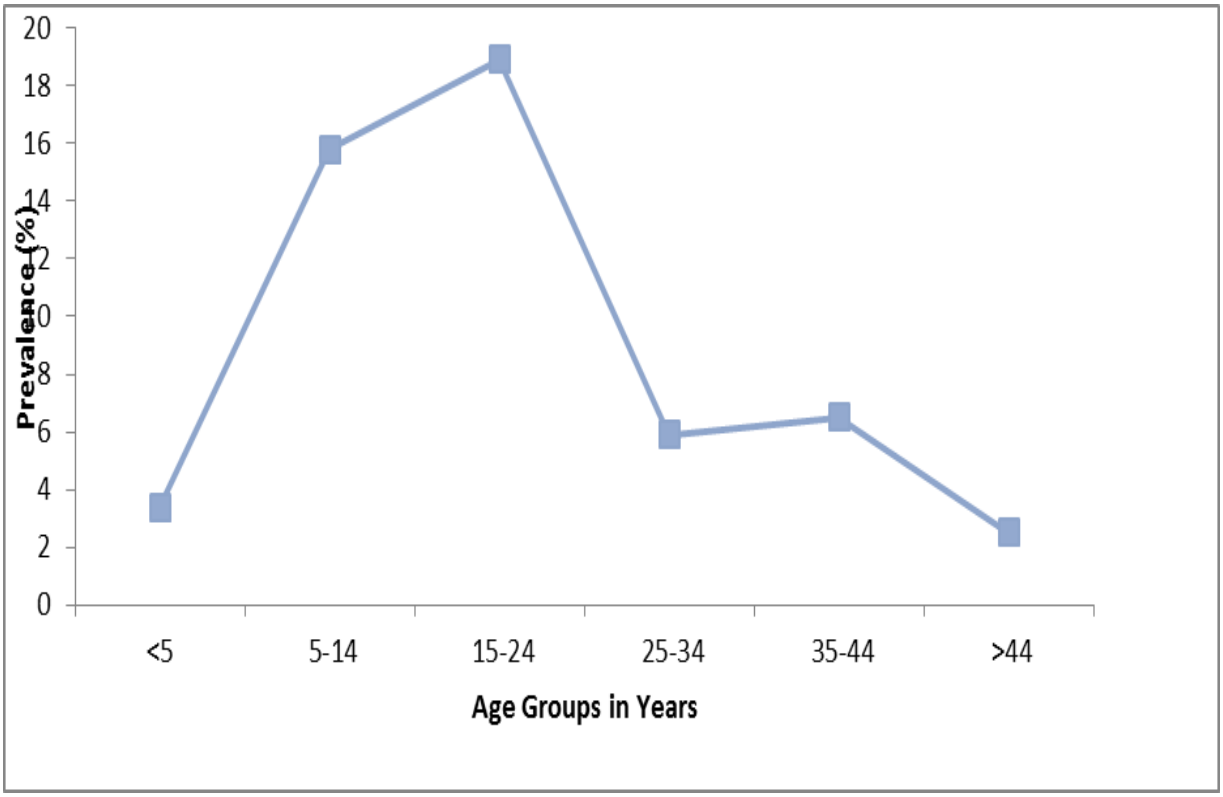

Figure 4.3: The trend of submicroscopic *P. falciparum* infections in the study population, Navrongo, 2015.

4.7: Inter-batch precision of Real Time RT-PCR technique

The precision of the method was expressed as percentage coefficient of variation (CV %), which has been determined using Ct values obtained from synchronized 3D7 cultured clones used as controls. Inter-batch variation of the method was calculated from the Ct values of controls analyzed along each batch of samples. The coefficient of variation for RT-PCR was 2.7%.

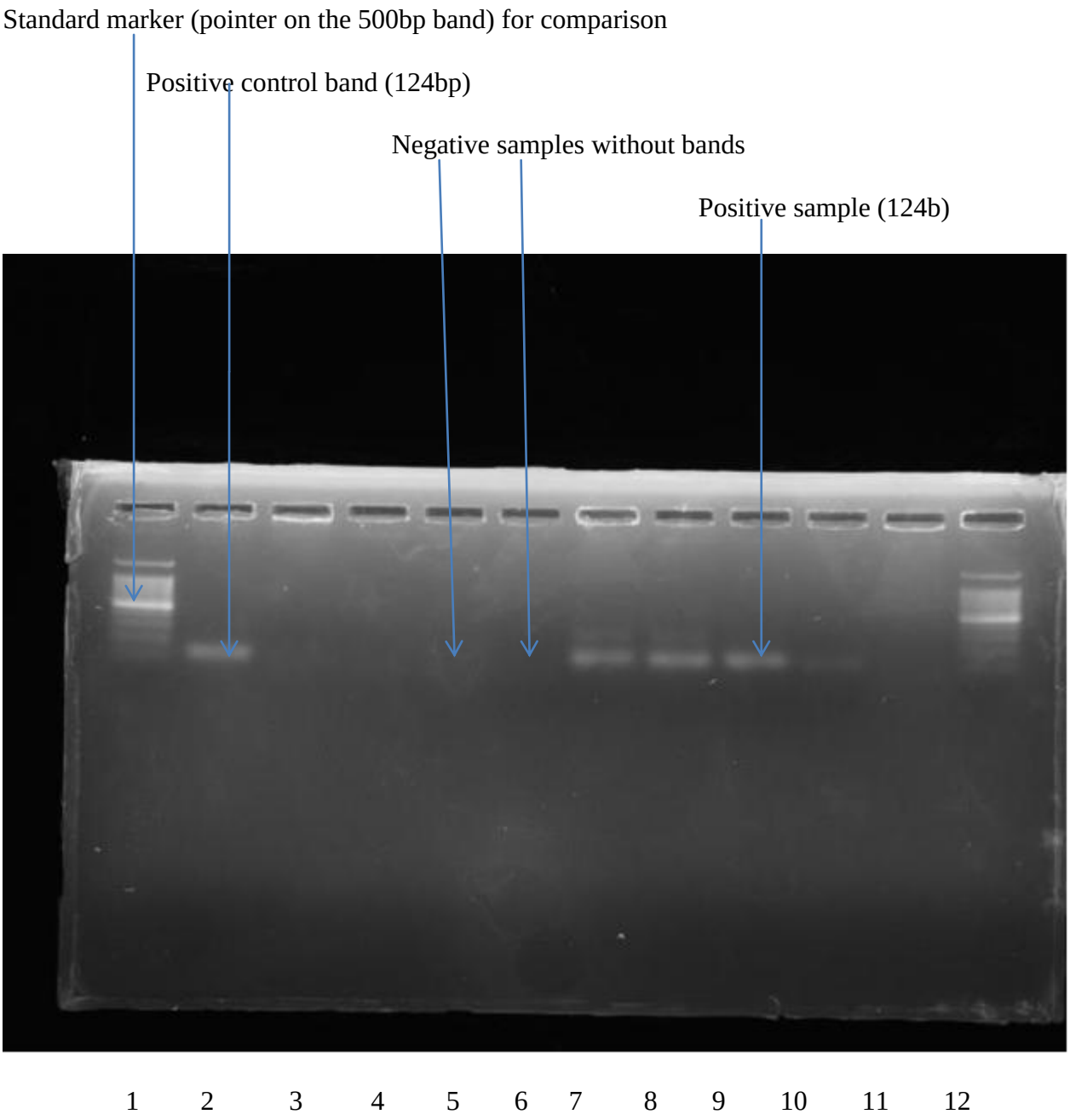

Figure 4. 4: Photograph of agarose gel showing DNA bands from the nested PCR products for identification of *Pfs25* transcript.

A positive control and four other samples were run along with a marker on both outer lanes for comparison. Besides the positive control (lane 2), three samples in Lanes 7th, 8th and 9th have positive bands (124bp).

CHAPTER FIVE

DISCUSSION

Our study showed that 13.9% of people living in Navrongo are asymptomatic *P. falciparum* carriers and therefore may go about their duties normally without seeking treatment, subsequently becoming reservoirs of parasites for onward transmission. A further 9.1% who carried malaria parasites at levels undetected by the routine method of diagnosis, microcopy, were also identified. Studies conducted in the Gambia and Kenya have shown that about 15-20% of untreated asymptomatic infections developed into transmissible gametocytes after 4 weeks (Dunyo *et al*., 2006). Microscopic asymptomatic infections are a great threat to malaria control and elimination efforts because they harbor parasites for long periods undetected, which may develop into gametocytes and are transmitted to others during the transmission season in the community (Bousema *et al.*, 2014).

The highest PCR-based prevalence of *P. falciparum* parasites was observed in the age group 5-24 years, which could be due to the fact that the protective immunity acquired over the years with numerous encounters with parasite infections have kept the parasite population at very low thresholds . On the other hand, prevalence by microscopy was highest among children <5 years (10.3%); an indication of higher parasite density among the children who are comparatively (with the older age group described above), still in the process of building up protective immunity against disease. Parasite density is controlled by acquired immunity in the infected host (Bousema *et al*., 2014) as such, adults are more likely than children to carry submicroscopic infections (Mosha *et al*., 2013; Okell *et al*., 2012). Our study showed a higher prevalence of submicroscopic infections among older children and adults (>5years) than among children <5years (10.3% versus 3.4%). Similar findings have been reported in a study conducted in Misungwi district located in the north-

west Tanzania, where malaria transmission is described as mesoendemic (Mosha *et al.*, 2013). A meta-analysis conducted by Okell *et al*. (2014) revealed that submicroscopic infections could contribute to 20-50% of transmission in areas where slide prevalence is 4% and below, but contribute considerably less in areas of high transmission intensity (Okell *et al*., 2012). The slide prevalence of malaria parasites in this study was 4.8% and so submicroscopic infections detected may contribute similar magnitude of transmission in Navrongo.

The presence of infectious *P. falciparum* gametocytes in the human peripheral blood is a pre-requisite for parasite transmission (Ouédraogo *et al*. 2009). Henceforth, prevalence of gametocytes is commonly used as a measure for malaria transmission. Some studies have shown that success of transmission increases as gametocyte density increases, however, transmission still occurs at low gametocyte densities (Churcher *et al*., 2013). The low prevalence of *P. falciparum* gametocytes, 1% and 3.8% as detected by microscopy and PCR respectively, cannot be overlooked due to the transmission potential of gametocytes. A few gametocytes when successfully picked up by a mosquito can produce numerous oocytes which will develop into infectious sporozoites, which can contribute immensely to the overall transmission of malaria. It has been shown that, in areas of high malaria transmission intensity, gametocyte carriage is most prevalent in the younger age groups, who also have the highest prevalence and densities of asexual malaria parasites (Bousema & Drakeley, 2011), however the gametocytemia observed in this study was common among children below 5 years (6.9%). This finding compares favorably with a study conducted in Madang Province, Papua New Guinea where 9.8% of asymptomatic individuals carried *P. falciparum* gametocytes; with highest prevalence (10.9%) among children 0–3 years (Koepfli *et al*., 2015). The two individuals in this study with

microscopic gametocytes also had the highest asexual parasitaemia which is consistent with the fact that high asexual parasitaemia results in high gametocytemia (Bousema *et al.*, 2014).

As in many other studies, our study also identified PCR as a more sensitive method for detection of *Plasmodium* parasites, particularly infections with very low parasitaemia. About 9.1% of the asymptomatic infections were eclipsed by microcopy due to its low sensitivity, which was estimated to be 34.5% in this study using PCR as the standard method. However microscopy was found to be very specific (100% specificity) meaning the method in this study adequately identified individuals without the disease but produced a great number of false negatives. One can therefore imagine the implication of this during this era of malaria elimination where parasite transmission is declining, and the need to detect sub-microscopic infections is becoming important. Low-density infections are likely to increase, which may limit the use of microscopy in diagnosis of malaria. Studies have shown that microscopic identification of *P. falciparum* parasites misses on average 50% of the infections in endemic areas compared to PCR (da Silva-Nunes *et al.*, 2012). It is then certain that, in line with the assessment of progress in reducing malaria transmission, PCR proves to be the best tool for estimation of parasite prevalence among the general population.

Our study revealed a significant number of *P. falciparum*-infected-individuals who were asymptomatic and carry sub-microscopic parasite densities below the level of detection of microscopy. Even though one was expecting high level of submicroscopic infections from Navrongo, an intense *P. falciparum* transmission area, 9.1% prevalence cannot be overlooked. Gametocytemia was low in the study area. Nonetheless, the ability of

gametocytes to sustain transmission in an area of seasonal transmission; even at very low densities must be critically examined. It is very important to step up detection of symptomatic and submicroscopic asymptomatic individuals using enhanced diagnostic methods. RT-PCR is more sensitive in detecting submicroscopic parasitaemia and prevalence determination in a given population. The Ghana Demographic and Health Survey (GDHS) indicated that the nation is still at the control stage and would need greater efforts to reach the pre-elimination stage of the disease (GDHS, 2014). Thus in the phase of malaria elimination it is most paramount to adopt very sensitive diagnostic tools such as RT-PCR in surveys aim at establishing prevalence of malaria to inform choice of elimination strategies.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion this study determined the prevalence of asymptomatic infections to be 4.8% and 13.9% by microscopy and PCR respectively in the study population, thus revealing the limitations of microscopy. Also, the proportion of submicroscopic asymptomatic infections in the population was 9.1%, while the gametocyte prevalence was 3.8%, which persists during the dry season. Marital status was also found to be associated with the prevalence of *P. falciparum* infections. This study therefore revealed that, submicroscopic asymptomatic infections persist during the dry season and can contribute to malaria transmission in the northern part of Ghana.

6.2 Recommendations

Ghana is still endemic with malaria despite remarkable efforts being made in the control and prevention of the disease over the years. It is therefore recommended that,

(1) National Malaria Control Program should design studies to identify the contribution of sub-microscopic asymptomatic infections to transmission; which is necessary for monitoring and evaluation of future control and elimination efforts in Ghana. (2) NMCP should adopt new and improved screening tools and strategies for detection and management of very low density parasitaemia.

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APPENDIX I: Data from the field and laboratory (section 1)

a2	a3	a4	a5	a6	a7	b1	b2	b3	b4	b5	b6	b7	c1
1	FEMALE	CAA019037	24/06/2013	2	KASSENA	2	2	2	2		2	2	2
2	MALE	CAA024001	15/06/1959	56	KASSENA	2	2	2	2		2	2	2
3	FEMALE	WEE029252	5/5/1988	27	KASSENA	2	2	2	2		2	2	2
4	FEMALE	CABO41010	3/4/1975	40	KASSENA	2	2	2	2		2	2	2
5	FEMALE	CAA045002	7/4/1969	46	KASSENA	2	2	2	2		2	2	2
6	FEMALE	CAA440701	21/05/2000	15	KASSENA	2	2	2	2		2	2	2
7	FEMALE	CAAO44007	14/01/1965	50	KASSENA	2	2	2	2		2	2	2
8	FEMALE	CAA07	12/8/1936	79	KASSENA	2	2	2	2		2	2	2
9	FEMALE	CAA07	15/06/1978	37	KASSENA	2	2	2	2		2	2	2
10	FEMALE	CAA07	4/4/2001	14	KASSENA	2	2	2	2		2	2	2
11	FEMALE	CAA07	22/08/2009	6	KASSENA	2	2	2	2		2	2	2
12	FEMALE	CAA07	15/06/2005	10	KASSENA	2	2	2	2		2	2	2
13	MALE	CAA007	9/1/2012	3	KASSENA	2	2	2	2		2	2	2
14	MALE	CAA007007	15/06/1976	39	KASSENA	2	2	2	2		2	2	2
15	MALE	CAA023	20/09/1961	53	KASSENA	2	2	2	2		1	1	1
16	FEMALE	CAA023	10/12/1981	33	KASSENA	2	2	2	2		2	2	2
17	FEMALE	CAA0023	15/08/1936	79	KASSENA	2	2	2	2		2	2	2
18	MALE	CAA028	19/08/1999	16	KASSENA	2	2	2	2		2	2	2
19	MALE	CAA013	11/12/1997	17	KASSENA	2	2	2	2		2	2	2
20	FEMALE	CAA013083	20/10/2011	3	KASSENA	2	2	2	2		2	2	2
21	MALE	CAA034002	15/06/1939	76	KASSENA	2	2	2	2		2	2	2
22	MALE	CAC048002	16/06/1940	75	OTHER	2	2	2	2		2	2	2
23	FEMALE	SGSO49071	15/04/1978	37	NANKANA	2	2	2	2		2	2	2
24	FEMALE	CAA073	1/7/1964	50	NANKANA	2	2	2	2		2	2	2
25	MALE	CAA073	3/4/2012	3	NANKANA	2	2	2	2		2	2	2
26	FEMALE	CAA070049	7/8/1964	50	KASSENA	2	2	2	2		2	2	2
27	MALE	CAA029010	22/03/1991	24	NANKANA	2	2	2	2		2	2	2
28	MALE	CAA028004	22/06/1998	17	KASSENA	2	2	2	2		2	2	2
29	FEMALE	CAA028007	28/09/2007	8	KASSENA	2	2	2	2		2	2	2
30	MALE	CAA028005	13/10/2010	4	KASSENA	2	2	2	2		2	2	2
31	FEMALE	CAA056001	13/10/1956	58	KASSENA	2	2	2	2		2	2	2
32	FEMALE	CAA056002	1/7/1989	26	BUILSA	2	2	2	2		2	2	2
33	MALE	CAM02	10/1/1995	20	KASSENA	2	2	2	2		2	2	2
34	MALE	CAM02	4/12/1992	22	KASSENA	2	2	2	2		2	2	2
35	FEMALE	CAMOO2	15/02/1991	24	KASSENA	2	2	2	2		2	2	2
36	FEMALE	CAM002	23/03/1997	18	NANKANA	2	2	2	2		2	2	2
37	FEMALE	CAM002	11/10/1940	74	KASSENA	2	2	2	2		2	2	2
38	FEMALE	CAM091	21/02/1982	33	OTHER	2	2	2	2		2	2	2
39	MALE	CAM091044	15/06/1973	42	KASSENA	2	2	2	2		2	2	2
40	FEMALE	CAM091004	20/04/1935	80	KASSENA	2	2	2	2		2	2	2
41	FEMALE	CAM062	13/07/1998	17	OTHER	2	2	2	2		2	2	2
42	FEMALE	CAM062	1/1/1962	53	OTHER	2	2	2	2		2	2	2
43	MALE	CAM062	10/3/1985	30	OTHER	2	2	2	2		2	2	2
44	MALE	CAM087	21/03/2012	2	OTHER	2	2	2	2		2	2	2
45	MALE	CAM076	9/7/1993	22	KASSENA	2	2	2	2		2	2	2
46	MALE	CAM076	14/12/1978	36	KASSENA	2	2	2	2		2	2	2
47	FEMALE	CAM076	15/06/1987	28	OTHER	2	2	2	2		2	2	2
48	MALE	CAM076	12/3/2014	1	NANKANA	2	2	2	2		2	2	2
49	MALE	CAM076	15/07/1989	26	KASSENA	2	2	2	2		2	2	2
50	MALE	CAM076	2/9/1994	20	KASSENA	2	2	2	2		2	2	2
51	MALE	CAM076	6/3/1996	19	KASSENA	2	2	2	2		2	2	2

52	FEMALE	CAM065	5/7/1964	51	KASSENA	2	2	2	2	2	2	2
53	MALE	CAM 065	26/03/1973	42	KASSENA	2	2	2	2	2	2	2
54	MALE	CAM065	1/7/1944	71	KASSENA	2	2	2	2	2	2	2
55	MALE	CAM011	15/06/1959	56	KASSENA	2	2	2	2	2	2	2
56	FEMALE	CAM011	15/05/1963	52	KASSENA	2	2	2	2	2	2	2
57	MALE	CAM099	25/05/1977	38	KASSENA	2	2	2	2	2	2	2
58	MALE	CAM099	17/06/1977	38	KASSENA	2	2	2	2	2	2	2
59	FEMALE	CAM099	14/05/1995	20	KASSENA	2	2	2	2	2	2	2
60	FEMALE	CAM087	24/09/2000	14	OTHER	2	2	2	2	2	2	2
61	MALE	CAM093	15/06/2010	4	KASSENA	2	2	2	2	2	2	2
62	MALE	CAA032	3/3/2006	9	NANKANA	2	2	2	2	2	2	2
63	MALE	CAA032	18/03/2009	6	NANKANA	2	2	2	2	2	2	2
64	MALE	CAN044	3/3/1987	28	KASSENA	2	2	2	2	2	2	2
65	FEMALE	CAN044	1/2/1933	82	KASSENA	2	2	2	2	2	2	2
66	MALE	CAN044	11/6/1980	35	KASSENA	2	2	2	2	2	2	2
67	FEMALE	CAN044	6/12/1933	81	KASSENA	2	2	2	2	2	2	2
68	MALE	CAN044	2/3/1972	43	KASSENA	2	2	2	2	2	2	2
69	FEMALE	CAN044	21/07/2000	15	KASSENA	2	2	2	2	2	2	2
70	MALE	CAN038	25/02/1960	55	KASSENA	2	2	2	2	2	2	2
71	FEMALE	CAN038	30/07/2004	11	KASSENA	2	2	2	2	2	2	2
72	MALE	CAN044	1/7/1968	47	KASSENA	2	2	2	2	2	2	2
73	MALE	CAN042	28/04/1992	23	KASSENA	2	2	2	2	2	2	2
74	MALE	CAN037	18/08/1990	24	KASSENA	2	2	2	2	2	2	2
75	MALE	CAB001	9/1/1989	25	KASSENA	2	2	2	2	2	2	2
76	MALE	CAN037002	16/09/1990	24	KASSENA	2	2	2	2	2	2	2
77	MALE	CAN049	2/9/1988	26	KASSENA	2	2	2	2	2	2	2
78	MALE	CAN06	2/4/1992	25	KASSENA	2	2	2	2	2	2	2
79	MALE	WEF020	12/7/2004	11	OTHER	2	2	2	2	2	2	2
80	FEMALE	WEF020	3/3/2002	13	OTHER	2	2	2	2	2	2	2
81	MALE	WEF020	5/4/2010	5	OTHER	2	2	2	2	2	2	2
82	MALE	WEF020	12/1/2007	8	OTHER	2	2	2	2	2	2	2
83	FEMALE	WEF020	7/4/1998	17	OTHER	2	2	2	2	2	2	2
84	MALE	WEF020	24/07/2012	3	OTHER	2	2	2	2	2	2	2
85	MALE	CAN024	17/05/2005	10	KASSENA	2	2	2	2	2	2	2
86	MALE	CAN024	25/10/2010	4	KASSENA	2	2	2	2	2	2	2
87	FEMALE	CAN024	15/06/2004	11	KASSENA	2	2	2	2	2	2	2
88	FEMALE	CAN024	29/04/2009	6	KASSENA	2	2	2	2	2	2	2
89	MALE	CAN024	15/06/2004	11	KASSENA	2	2	2	2	2	2	2
90	MALE	CAN024	19/09/1971	43	KASSENA	2	2	2	2	2	2	2
91	MALE	CAA076	2/5/1988	27	OTHER	2	2	2	2	2	2	2
92	MALE	CAL025	28/07/1989	26	KASSENA	2	2	2	2	2	2	2
93	MALE	CAL025	13/04/1993	22	KASSENA	2	2	2	2	2	2	2
94	MALE	CAL025	19/10/1986	28	KASSENA	2	2	2	2	2	2	2
95	FEMALE	CAL025	15/05/1968	47	KASSENA	2	2	2	2	2	2	2
96	FEMALE	CAL025	4/4/1991	24	KASSENA	2	2	2	2	2	2	2
97	MALE	CAL025	21/09/2013	2	KASSENA	2	2	2	2	2	2	2
98	MALE	CAL039	17/01/2001	14	KASSENA	2	2	2	2	2	2	2
99	FEMALE	CAL039	10/6/2956	59	KASSENA	2	2	2	2	2	2	2
100	MALE	CAL103	27/08/1993	22	KASSENA	2	2	2	2	2	2	2
101	FEMALE	CAL004	25/09/1983	32	KASSENA	2	2	2	2	2	2	2
102	FEMALE	CAL004	9/9/1977	38	KASSENA	2	2	2	2	2	2	2
103	MALE	CAL068001	15/06/1940	75	KASSENA	2	2	2	2	2	2	2
104	MALE	CAL015	19/07/1978	37	OTHER	2	2	2	2	2	2	2
105	MALE	CAL0151	15/12/1975	39	NANKANA	2	2	2	2	2	2	2

106	FEMALE	CAL004	21/05/2006	9	KASSENA	2	2	2	2	2	2	2
107	FEMALE	CAL004	29/01/2011	4	KASSENA	2	2	2	2	2	2	2
108	MALE	CAL004	1/5/2007	8	KASSENA	2	2	2	2	2	2	2
109	MALE	CAL004	16/07/2010	5	KASSENA	2	2	2	2	2	2	2
110	MALE	CAL004	5/7/2003	11	KASSENA	2	2	2	2	2	2	2
111	FEMALE	CAL004	17/04/1974	41	KASSENA	2	2	2	2	2	2	2
112	MALE	CAL004	22/06/2011	4	KASSENA	2	2	2	2	2	2	2
113	MALE	CAL068	2/10/1990	24	NANKANA	2	2	2	2	2	2	2
114	MALE	CAL068	31/03/1998	17	KASSENA	2	2	2	2	2	2	2
115	FEMALE	CAL068	15/12/1960	54	KASSENA	2	2	2	2	2	2	2
116	MALE	CAL131	18/09/2008	7	KASSENA	2	2	2	2	2	2	2
117	FEMALE	CAL131	5/2/1998	17	KASSENA	2	2	2	2	2	2	2
118	FEMALE	CAL131	18/09/2008	7	KASSENA	2	2	2	2	2	2	2
119	FEMALE	CAL131	10/5/2012	3	KASSENA	2	2	2	2	2	2	2
120	FEMALE	CAL131	30/08/1983	32	KASSENA	2	2	2	2	2	2	1
121	FEMALE	CAL131	23/03/1989	26	KASSENA	2	2	2	2	2	2	1
122	MALE	CAL090	25/08/1994	21	KASSENA	2	2	2	2	2	2	2
123	FEMALE	CAL090	8/5/2004	11	KASSENA	2	2	2	2	2	2	2
124	MALE	CAL090	21/08/1998	17	KASSENA	2	2	2	2	2	2	2
125	FEMALE	CAL090	8/9/1957	58	KASSENA	2	2	2	2	2	2	2
126	MALE	CAL090	20/07/2001	4	KASSENA	2	2	2	2	2	2	2
127	FEMALE	CAL090	17/11/1971	43	KASSENA	2	2	2	2	2	2	2
128	FEMALE	CAL090	12/5/1999	16	KASSENA	2	2	2	2	2	2	2
129	FEMALE	CAL090	10/10/2014	1	KASSENA	2	2	2	2	2	2	2
130	FEMALE	CAL144	1/11/2000	14	KASSENA	2	2	2	2	2	2	2
131	FEMALE	CAL144	15/06/2005	10	KASSENA	2	2	2	2	2	2	2
132	FEMALE	CAL144	15/02/2008	8	KASSENA	2	2	2	2	2	2	2
133	MALE	CAL056	7/5/2009	6	OTHER	2	2	2	2	2	2	2
134	MALE	CAL056	13/02/2013	2	OTHER	2	2	2	2	2	2	2
135	FEMALE	CAL056	1/1/2000	15	OTHER	2	2	2	2	2	2	2
136	FEMALE	CAL056	27/07/2013	2	OTHER	2	2	2	2	2	2	2
137	MALE	CAD58	12/12/1981	33	KASSENA	2	2	2	2	2	2	2
138	FEMALE	CAD58	14/05/1986	29	KASSENA	2	2	2	2	2	2	2
139	FEMALE	CAD058	15/11/2012	2	KASSENA	2	2	2	2	2	2	2
140	MALE	CAD058	23/04/2012	3	KASSENA	2	2	2	2	2	2	2
141	FEMALE	CAD058	21/10/1982	32	KASSENA	2	2	2	2	2	2	2
142	FEMALE	CAD031	5/10/1962	52	KASSENA	2	2	2	2	2	2	2
143	FEMALE	CAD31	22/07/2015	10	KASSENA	2	2	2	2	2	2	2
144	FEMALE	CAD310	30/05/2010	5	KASSENA	2	2	2	2	2	2	2
145	FEMALE	CAF022	13/08/1980	35	KASSENA	2	2	2	2	2	2	2
146	FEMALE	CAF022	8/12/1982	32	KASSENA	2	2	2	2	2	2	2
147	FEMALE	CAF022	30/09/1987	28	NANKANA	2	2	2	2	2	2	2
148	MALE	CAF022	6/11/2014	1	KASSENA	2	2	2	2	2	2	2
149	FEMALE	CAQ044	12/2/2014	1	KASSENA	2	2	2	2	2	2	2
150	FEMALE	CAQ044	28/11/1983	31	NANKANA	2	2	2	2	2	2	2
151	MALE	CAQ001	15/06/1979	36	KASSENA	2	2	1	2	2	2	1
152	MALE	CAQ001	1/1/1962	53	KASSENA	2	2	2	2	2	2	2
153	FEMALE	CAQ001	3/4/2009	6	KASSENA	2	2	2	2	2	2	2
154	FEMALE	CAQ001	29/03/2012	3	KASSENA	2	2	2	2	2	2	2
155	FEMALE	CAQ001	12/8/1985	30	OTHER	2	2	2	2	2	2	2
156	FEMALE	CAQ023	26/01/2001	14	KASSENA	2	2	2	2	2	2	2
157	FEMALE	CAQ023	19/06/2007	8	KASSENA	2	2	2	2	2	2	2
158	MALE	CAQ023	15/01/2002	13	KASSENA	2	2	2	2	2	2	2
159	MALE	CAQ023	9/5/2011	4	KASSENA	2	2	2	2	2	2	2

160	MALE	CAQ023	12/1/1968	46	KASSENA	2	2	2	2	2	2	2
161	MALE	CAQ023	3/1/2009	6	KASSENA	2	2	2	2	2	2	2
162	FEMALE	CAQ023	28/07/1978	37	KASSENA	2	2	2	2	2	2	2
163	MALE	CAQ023	25/06/2006	9	KASSENA	2	2	2	2	2	2	2
164	FEMALE	CAF035	10/10/1975	39	OTHER	2	2	2	2	2	2	2
165	MALE	CAF035	10/8/2000	15	KASSENA	2	2	2	2	2	2	2
166	MALE	CAF090	4/4/1981	34	OTHER	2	2	2	2	2	2	2
167	FEMALE	CAF090	15/06/1982	33	KASSENA	2	2	2	2	2	2	2
90	FEMALE	CAF090	22/03/1994	21	KASSENA	2	2	2	2	2	2	2
169	FEMALE	CAF090	14/10/2013	1	KASSENA	2	2	2	2	2	2	2
170	MALE	CAF010	17/07/1948	67	KASSENA	2	2	2	2	2	2	2
171	MALE	CAF010	19/11/2012	2	KASSENA	2	2	2	2	2	2	2
172	FEMALE	CAF010	27/09/1938	77	OTHER	2	2	2	2	2	2	2
173	FEMALE	CAF103	6/6/1984	31	KASSENA	2	2	2	2	2	2	2
174	FEMALE	CAF103	15/06/1945	70	KASSENA	2	2	2	2	2	2	2
175	FEMALE	CAF103	23/03/1987	28	NANKANA	2	2	2	2	2	2	2
176	FEMALE	CAK088	20/09/1962	53	KASSENA	2	2	2	2	2	2	2
177	MALE	CAK004	25/02/1971	44	KASSENA	2	2	2	2	2	2	2
178	FEMALE	CAK004	6/6/1980	35	BUILSA	2	2	2	2	2	2	2
179	FEMALE	CAK004	18/12/2012	2	KASSENA	2	2	2	2	2	2	2
180	FEMALE	CAK004	1/6/1985	30	KASSENA	2	2	2	2	2	2	2
181	MALE	CAK004	1/9/2014	1	KASSENA	2	2	2	2	2	2	2
182	FEMALE	CAK004	25/07/1974	41	KASSENA	2	2	2	2	2	2	2
183	MALE	CAK004	19/10/2009	5	KASSENA	2	2	2	2	2	2	2
184	MALE	CAK004	11/9/1972	43	KASSENA	2	2	2	2	2	2	2
185	MALE	CAC017	25/05/1954	61	NANKANA	1	1	2	2	2	2	1
186	FEMALE	CAC017	2/4/1934	81	NANKANA	2	2	2	2	2	2	2
187	FEMALE	CAC017	16/05/1959	56	NANKANA	2	2	2	2	2	2	2
188	FEMALE	CAC017	1/1/1934	81	KASSENA	2	2	2	2	2	2	2
189	FEMALE	CAC075	15/05/1995	20	KASSENA	2	2	2	2	2	2	2
190	FEMALE	CAC075	15/06/1971	44	KASSENA	2	2	2	2	2	2	2
191	FEMALE	CAC075	23/12/1981	33	KASSENA	2	2	2	2	2	2	2
192	FEMALE	CAC075	3/11/1994	20	KASSENA	2	2	2	2	2	2	2
193	MALE	CAC075	24/09/1953	62	KASSENA	2	2	2	2	2	2	2
194	FEMALE	CAC059	25/11/1993	21	OTHER	2	2	2	2	2	2	2
195	MALE	CAC059		33	KASSENA	2	2	2	2	2	2	2
196	MALE	CAE03	15/06/1966	49	KASSENA	2	2	2	2	2	2	2
197	FEMALE	CAE03	6/10/2012	2	KASSENA	2	2	2	2	2	2	2
198	FEMALE	CAE03	26/06/1977	38	KASSENA	2	2	2	2	2	2	2
199	FEMALE	CAE017	25/07/1977	38	OTHER	2	2	2	2	2	2	2
200	FEMALE	CAE017	22/09/1993	22	BUILSA	2	2	2	2	2	2	2
201	FEMALE	CAI02	12/11/1982	32	KASSENA	2	2	2	2	2	2	2
202	MALE	CAI002	23/11/1993	21	KASSENA	2	2	2	2	2	2	2
203	MALE	CAH059	10/10/1986	28	KASSENA	2	2	2	2	2	2	2
204	FEMALE	CAH059	13/06/1976	39	KASSENA	2	2	2	2	2	2	2
205	FEMALE	CAH059	15/10/2006	8	KASSENA	2	2	2	2	2	2	2
206	MALE	CAH059	14/07/1974	41	KASSENA	2	2	2	2	2	2	2
207	MALE	CAH044	25/06/1980	30	NANKANA	2	2	2	2	2	2	2
208	FEMALE	CAH044	18/04/1992	23	NANKANA	2	2	2	2	2	2	2
209	FEMALE	CAH044	2/6/1999	16	KASSENA	2	2	2	2	2	2	2

Data from the field and Laboratory (section 2)

A2	microscopy	asexualm	countm	Pcr	asexualp	itn	occupation	education	marital
1	2	2	0	2	2	2	4	1	1
2	2	2	0	2	2	2	5	4	2
3	2	2	0	2	2	2	4	1	3
4	2	2	0	2	2	2	2	3	3
5	2	2	0	2	2	2	4	1	2
6	2	2	0	2	2	2	4	1	1
7	2	2	0	2	2	2	5	1	1
8	2	2	0	2	2	2	4	2	1
9	2	2	0	2	2	2	5	1	2
10	2	2	0	1	1	2	5	1	3
11	2	2	0	2	2	2	5	1	2
12	2	2	0	1	1	2	5	1	2
13	2	2	0	2	2	2	1	4	1
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15	2	2	0	2	2	2	5	1	2
16	2	2	0	2	2	2	5	4	1
17	2	2	0	2	2	2	5	1	2
18	2	2	0	1	1	2	5	1	2
19	2	2	0	2	2	2	1	4	1
20	1	1	40	1	1	2	4	4	2
21	2	2	0	2	2	2	5	1	3
22	2	2	0	2	2	2	5	1	1
23	2	2	0	1	1	2	5	1	2
24	2	2	0	1	1	2	4	1	3
25	2	2	0	2	2	2	5	2	2
26	2	2	0	2	2	2	5	1	2
27	2	2	0	2	2	2	5	1	2
28	2	2	0	1	1	2	5	1	2
29	2	2	0	2	2	2	4	1	1
30	2	2	0	2	2	2	5	4	1
31	2	2	0	2	2	2	5	2	2
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36	2	2	0	2	2	2	5	4	1
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38	2	2	0	2	2	2	4	4	1
39	2	2	0	2	2	2	5	2	2
40	2	2	0	2	2	2	4	1	1
41	2	2	0	2	2	2	4	2	1
42	2	2	0	2	2	2	5	4	2
43	2	2	0	2	2	2	4	2	2

44	2	2	0	2	2	2	4	1	1
45	2	2	0	2	2	2	4	1	1
46	2	2	0	2	2	2	4	4	2
47	2	2	0	2	2	2	5	3	2
48	2	2	0	2	2	2	5	2	2
49	1	1	200	1	1	2	4	2	2
50	2	2	0	2	2	2	5	4	1
51	2	2	0	2	2	2	5	3	2
52	2	2	0	2	2	2	5	3	1
53	2	2	0	2	2	2	4	2	1
54	2	2	0	2	2	2	5	3	1
55	2	2	0	2	2	2	4	2	1
56	2	2	0	2	2	2	4	4	1
57	2	2	0	2	2	2	1	4	1
58	1	1	1440	1	1	2	5	1	2
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63	2	2	0	2	2	2	5	1	1
64	2	2	0	2	2	2	4	1	1
65	2	2	0	2	2	2	5	3	1
66	2	2	0	2	2	2	4	2	1
67	2	2	0	2	2	2	5	4	1
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72	2	2	0	2	2	2	1	4	1
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74	2	2	0	2	2	2	5	3	2
75	2	2	0	1	1	2	5	1	2
76	2	2	0	1	1	2	4	1	2
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79	2	2	0	2	2	2	5	4	2
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84	1	1	3520	1	1	2	5	4	2
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88	2	2	0	2	2	2	5	1	2
89	2	2	0	2	2	2	5	1	2

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92	2	2	0	2	2	2	4	1	1
93	2	2	0	2	2	2	5	3	2
110	2	2	0	2	2	2	5	2	2
95	2	2	0	2	2	2	1	1	1
96	2	2	0	2	2	2	4	1	1
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98	2	2	0	2	2	2	5	1	2
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100	2	2	0	2	2	2	1	1	2
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102	2	2	0	2	2	2	5	1	1
103	1	1	240	1	1	1	1	4	1
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106	2	2	0	1	1	1	5	1	2
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108	2	0		2	2	1	5	1	2
109	2	2	0	2	2	1	5	1	2
110	2	2	0	2	2	2	5	1	2
111	2	2	0	2	2	2	4	1	1
112	2	2	0	2	2	2	4	2	1
113	2	2	0	1	1	2	5	2	2
114	2	2	0	2	2	2	5	2	2
115	2	2	0	2	2	2	5	4	1
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117	1	1	40	1	1	2	5	1	2
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121	2	2	0	2	2	2	4	1	1
122	2	2	0	2	2	2	5	2	2
123	2	2	0	2	2	2	5	1	2
124	2	2	0	2	2	2	5	1	2
125	2	2	0	2	2	2	4	2	3
126	2	2	0	2	2	1	5	1	2
127	2	2	0	2	2	2	4	4	1
128	2	2	0	1	1	2	5	1	2
129	2	2	0	2	2	2	5	4	2
130	2	2	0	2	2	2	5	1	2
131	2	2	0	2	2	2	5	1	2
132	2	2	0	2	2	2	5	1	2
133	2	2	0	2	2	2	5	1	2
134	2	2	0	2	2	2	4	4	1
135	2	2	0	2	2	2	5	4	1

136	2	2	0	2	2	2	4	4	1
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138	2	2	0	2	2	2	4	1	1
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140	2	2	0	2	2	2	5	4	2
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145	2	2	0	2	2	2	4	2	2
146	2	2	0	2	2	2	4	1	1
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148	1	1	80	1	1	2	5	4	2
149	2	2	0	2	2	1	5	4	2
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152	2	2	0	2	2	1	1	1	1
153	2	2	0	2	2	2	5	1	2
154	2	2	0	2	2	2	2	3	1
155	2	2	0	2	2	2	4	4	1
156	2	2	0	2	2	2	5	1	2
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163	2	2	0	2	2	1	5	1	2
164	2	2	0	2	2	1	4	1	1
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173	2	2	0	2	2	2	2	2	1
174	2	2	0	2	2	2	5	4	1
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178	2	2	0	1	1	2	4	1	1
179	2	2	0	2	2	2	5	4	2
180	2	2	0	2	2	2	4	1	1
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182	2	2	0	2	2	2	4	4	1
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184	2	2	0	2	2	2	1	4	1
185	2	2	0	2	2	2	5	3	1
186	2	2	0	2	2	2	5	4	1
187	2	2	0	2	2	2	5	4	1
188	2	2	0	2	2	2	5	4	1
189	2	2	0	2	2	2	4	4	1
190	2	2	0	2	2	2	4	4	1
191	2	2	0	2	2	2	4	1	3
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194	2	2	0	2	2	2	5	2	2
195	2	2	0	2	2	2	4	1	2
196	2	2	0	2	0	2	1	2	1
197	2	2	0	2	2	2	5	1	2
198	2	2	0	2	2	2	4	2	1
199	2	2	0	2	2	2	4	2	1
200	2	2	0	2	2	2	4	1	1
201	2	2	0	2	2	2	4	4	1
202	2	2	0	1	1	1	5	1	2
203	2	2	0	2	2	1	5	2	2
204	2	2	0	2	2	1	4	4	1
205	2	2	0	2	2	1	5	1	1
206	2	2	0	2	2	1	4	2	1
207	2	2	0	2	2	1	2	3	1
208	2	2	0	2	2	1	4	1	1
209	2	2	0	2	2	2	5	1	2

Note: Definition of the codes is available in the participant's questionnaire

APPENDIX II: Participants Questionnaire

THE PREVALENCE OF SUBMICROSCOPIC MALARIA INFECTIONS AMONG ASYMPTOMATIC POPULATIONS IN NAVRONGO TOWN.

PARTICIPANT QUESTIONNAIRE.

A. INDIVIDUAL BACKGROUND CHARACTERISTICS

A1. Name: ---------- **A2. Serial Number** ☐☐☐

A3. Gender: Male (1) ☐ **Female (2)** ☐ **A4. Individual ID:** ☐☐☐☐☐

☐☐☐☐☐☐☐☐

☐☐

A5. Date of Birth: DD/MM/YYYY **A6. Age in completed years**

A7. Tribe:

1	**Kassena**
2	**Nankana**
3	**Builsas**
4	**Other (specify)**

A8. Occupation:

1	**Famer**
2	**Public Servant**
3	**Watchman**
4	**Trader**
5	**unemployed**

A9. Education:

1	**Junior High**
2	**Senior High**
3	**Tertiary**
4	**No Education**

A10. Do you always sleep under Insecticide Treated Net (ITN)? Yes (1) ☐ **No (2)** ☐

A11. Marital Status:

1	**Married**
2	**Single**
3	**Divorced**
4	**Co-habitation**

HISTORY OF MALARIA DURING THE PAST TWO WEEKS

B1. Symptoms	**Yes (1)**	**No (2)**	**Duration in days (if yes)**
B2. Fever			

B3. Headache			
B4. Vomiting			
B5. Chills and Rigors			
B6. Convulsions			
B7. Diarrhoea			
B8. Others (specify)			

TREATMENT FOR MALARIA

C1. ANY TREATMENT GIVEN?	**Yes (1)** ☐	**No (2)** ☐
C2. If yes, what anti-malaria was given?	**Yes (1)**	**No (2)**
SP/ Fancider/ malafan		
Chloroquine		
Camoquine		
Quinine		

Artesunate/Artemisinin		
Artesunate/Amodiaquine combination		
Artemather/Lumifantrim conbination		
Others (specify)		

Investigator's Observations

COMMENTS ABOUT RESPONDENT: ----------

Name.....

Signature.....

Date.....

APPENDIX III: Informed Consent Form

INFORMED CONSENT FORM.

Title: The prevalence of submicroscopic malaria infections among asymptomatic populations in Navrongo Town.

Principal Investigator: Atelu Geoffrey Robert

Address: Department of Epidemiology and disease Control, School of Public Health, College of Health Sciences, University of Ghana. P.O. Box LG13, Legon-Accra, Ghana.

Telephone: +233-21-517505, Fax number: +233-21-517501

General Information about Research: Malaria continues to be a major public health problem in Ghana and the world at large, claiming several lives annually. Recent studies conducted indicate that many people moving about without signs and symptoms of malaria may harbour malaria parasites at very low levels undetected by microscopy. These infections often persist for months and harbour gametocytes, which results in malaria transmission.

You are invited to participate in the above mentioned research study. The purpose of the study is to determine the proportion of the population that has sub microscopic malaria infections. This will aid the development of strategies towards the control and elimination of malaria in the country. As a participant, you will be asked to answer a few questions and your finger will be pricked to collect about 5 drops of blood. The blood will be used to test for malaria by microscopic examination and also PCR for the parasite DNA. Your finger will be cleaned with diluted alcohol as disinfectant and the blood will be taken under aseptic conditions. Participation will take approximately 30munites.

Possible Risks and Discomforts: You may only feel a sharp pain in your finger when blood is been taken from you.

Possible Benefits: This study may help you and other participants who are at risk of developing malaria, as well as the nation.

Confidentiality: All the information gathered from this study will be kept confidential, and you will not be identified when the report is published.

Compensation: There will be refreshment in the form of beverages for all participants at the end of participation.

Voluntary Participation and Right to Leave the Research: Your participation in this research is voluntary and you may choose to answer as many questions as you wish, or stop at any time. There will be no negative consequences if you choose not to complete the study.

Contacts for Additional Information: In case you need further clarification or you encounter any discomfort after participation, you may call Geoffrey Atelu on mobile number, 0277489683. You may as well contact him via this E-mail address: atiloosjeff@yahoo.co.uk

Your rights as a Participant

This research has been reviewed and approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR-IRB). If you have any questions about your rights as a research participant you can contact the IRB Office between the hours of 8am-5pm through the landline 0302916438 or email addresses: nirb@noguchi.ug.edu.gh or hbaidoo@noguchi.ug.edu.gh. 3

Volunteer agreement

The above document describing the benefits, risks and procedures for the research titled above has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

_____ _____

Date Name and signature or mark of volunteer

If volunteers cannot read the form themselves, a witness must sign here: I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

_____ _____

Date Name and signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____ _____

Date Name Signature of Person Who Obtained Consent

Appendix iv: Child assent form

CHILD ASSENT FORM.

Title: The prevalence of submicroscopic malaria infections among asymptomatic populations in Navrongo Town.

Principal Investigator: Atelu Geoffrey Robert

Address: Department of Epidemiology and disease Control, School of Public Health, College of Health Sciences, University of Ghana. P.O. Box LG13, Legon-Accra, Ghana.

Telephone: +233-21-517505, Fax number: +233-21-517501

General Information about Research: We want to tell you about a research study we are doing. A research study is a way to learn information about something. We would like to find out more about how malaria is transmitted from one person to another. Malaria continues to be a major public health problem in Ghana and the world at large, killing several people annually. Recent studies conducted indicate that many people moving about without signs and symptoms of malaria may harbour malaria parasites at very low levels undetected by microscopy. These infections often persist for months and develop into gametocytes, which results in malaria transmission. If you agree to join this study, you will be asked to answer a few questions and some little blood (about 5 drops) will be taken from your finger. Participation will take approximately 30munites.

Possible Risks and Discomforts You may only feel a sharp pain in your finger when blood is been taken from you.

Possible Benefits: This study may help you and other participants who are at risk of developing malaria, as well as the nation as a whole.

Confidentiality: Anything we learn about you from this study will be kept as secret as possible. The samples would be coded and so your real names would not be used or disclosed.

Compensation: There will be refreshment in the form of beverages for all participants at the end of participation.

Voluntary Participation and Right to Leave the Research: Your participation in this research is voluntary and you may choose to answer as many questions as you wish, or stop at any time. No one will be mad with you if you choose not to complete the study.

Contacts for Additional Information: In case you need further clarification or you encounter any discomfort after participation, you may call Geoffrey Atelu on mobile number, 0277489683. You may as well contact him via this E-mail address: atiloosjeff@yahoo.co.uk

Your rights as a Participant

This research has been reviewed and approved by the Institutional Review Board of Noguchi Memorial Institute for Medical Research (NMIMR-IRB). If you have any questions about your rights as a research participant you can contact the IRB Office between the hours of 8am-5pm through the landline 0302916438 or email addresses: nirb@noguchi.mimcom.org or HBaidoo@noguchi.mimcom.org .

Volunteer agreement

The above document describing the benefits, risks and procedures for the research title has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

_____ _____

Date Name and signature or mark of volunteer

If volunteers cannot read the form themselves, a witness must sign here: I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

_____ _____

Date Name and signature of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____ _____

Date Name Signature of Person Who Obtained Consent

Appendix v: Chemicals Reagents and Equipment

RNA extraction

Trizol Reagent (Cat. no. 15596-018) by Life Technologies

Chloroform (99+ %, C- 2432: SIGMA)

Isopropyl alcohol (≥ 99% : SIBMA)

75% ethanol (in DEPC-treated water)

RNase-free water

Centrifuge and rotor capable of reaching up to 12,000 × g

Polypropylene micro centrifuge tubes

Water bath (55–60°C)

Preparation of 2% Agarose gel for the separation of DNA fragments

2g of Agarose (type 1, Fisher) was weighed into a 250ml conical flask. 100mls of TAE buffer (1X) was added to the Agarose and swirled to mix. The mixture was heated in a microwave at maximum power to melt completely. The Agarose was allowed to cool to about 60^{o}C before ethidium bromide (SIGMA) was added at a rate of 0.5ug/ml. The gel mold was prepared by placing the gel tray into the casting apparatus which is laid on a level surface. An appropriate comb was placed in the gel mold to create the wells. The molten gel was then poured into the gel mold and allowed to set at room temperature. Once the Agarose is set the comb was removed and the gel ready for use.

Preparation of 50X TAE electrophoresis buffer (1000ml, pH 8.2)

Tris (hydroxylmethyl) aminomethane (SIGMA)	242 g
0.5M EDTA	100ml
Glacial acetic acid	57.1ml
Deionized water	adjust to 1000mls

Add 20mls of 50X TAE buffer stock to 980ml deionized water to obtain 1X TAE for use.

Other reagents.

Ethidium bromide solution (0.5ug/ml; SIGMA)

100bp molecular weight marker (Gibco BRL).