PREVALENCE OF *PLASMODIUM* INFECTION AND ANAEMIA IN PRIMARY SCHOOL CHILDREN FOLLOWING UNIVERSAL DISTRIBUTION OF INSECTICIDE TREATED BED NETS IN KASIPUL, HOMA-BAY COUNTY, KENYA

OMONDI ROBERT, BSc

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DECLARATION

This thesis is my original work and has not been presented for a degree or other awards in any other university.

Sign…….. Date……

Omondi Robert

Reg. No. I56/CE/24667/2012

Department of Zoological Sciences

Supervisors' Approval

This thesis has been submitted for examination with our approval as university supervisors.

Dr. Lucy M. Kamau Signature…….. Date…….

Department of Zoological Sciences

Kenyatta University

Dr. Eric K. Mwangi Signature…… Date……

Department of Zoological Sciences

Kenyatta University

DEDICATION

This thesis is dedicated to my loving wife, Rose Akinyi Omondi who steadfastly supported me during my studies, my lovely son Bradley Otieno for his continuous encouragement and inspiration during the period of my study. May this work spur you to achieve the best in your lives.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACRONYMS AND ABBREVIATIONS

DNA	Deoxyribinucleic Acid
IRIN	Integrated Regional Information Network
LLITN	Long-lasting Insecticide Treated Net
ITN	Insecticide Treated Nets
ITNs	Long-lasting Insecticide Nets
MIS	Malaria Indicator Survey
MOH	Ministry of Health
OR	Odds Ratio
QBC	Quantitative Buffy Coat
RBC	Red Blood Cell
RDSP	Rachuonyo District Strategic Plan
RDT	Rapid Diagnostic Tests
SPSS	Statistical Package for Social Sciences
WBC	White Blood Cells
WHO	World Health Organization

DEFINITION OF TERMS

Insecticide Treated Net: Referred to a Long-Lasting Insecticide Treated Net or any conventional net that had been re-treated with an insecticide.

Net coverage: The fraction of households that everybody uses ITN. Coverage can also be variably be referred to as net ownership or net possession.

School net coverage: Fraction of pupils owning ITN in a school.

Net use: The percentage of children who sleep under ITN.

ABSTRACT

Malaria is devastating diseases afflicting humans, in Kenya; the disease is endemic in areas around Lake Victoria and along the southern coast. Untreated malaria in school children, result in anaemia, reduced ability to concentrate and learn in school and if fallen sick may lead to school absenteeism. Insecticide treated nets (ITN) have been shown to provide significant protection against *Plasmodium* infection. Available data show that the overall prevalence of *Plasmodium* and anaemia among primary school aged children in Kasipul is 25.8 % and 14.1%, respectively. However, there is limited information on the *Plasmodium* and anaemia prevalence in Kasipul following mass distribution of ITN in 2014. The objective of this study was to assess the prevalence of *Plasmodium* and anaemia among school children living in Kasipul and their reported use of insecticide treated bed nets, one year after mass distribution of ITN in Kasipul, Homa-Bay County. A descriptive cross-sectional study of 398 primary school pupils was conducted in Kasipul. Pupil's fingers were pierced using a lancet to obtain blood sample for malaria parasite detection and haemoglobin level determination. Data on insecticide net use was collected using self-administered questionnaire. The overall prevalence of *Plasmodium* among children was 10.05% and anaemia was 2.3%. The association between net ownership and *Plasmodium* prevalence among pupils was significant ($\chi 2= 14.46$, df =1, p = 0.000). The difference in malaria prevalence in terms of sex was not statistically significant ($\chi 2= 0.814$, df= 1, p = 0.367). However, anaemia was slightly more prevalent in girls (3.6%) than boys (1.0%) were. Although the difference was not statistically significant ($\chi 2= 3.217$, df= 1, p = 0.073). The study established that only 51.0 % of the study population owned ITN, which is below the 80% target set by the government. A negative correlation of -0.3874 existed between the use of ITN and malaria prevalence. The study observed a significant decline in *Plasmodium* prevalence from 25.8% in 2011 to 10.05% in 2016, which is evidence that ITN, which was the major control strategy implemented in Kasipul reduced *Plasmodium* infection in the study population. Decline in *Plasmodium* infection could also have reduced the prevalence of anaemia in the study area from 14 % in 2010 to 2.3% in 2016. In conclusion, this inquiry revealed that the prevalence of *Plasmodium* and anaemia has significantly reduced following distribution of free ITN in Kasipul. *Plasmodium* prevalence was lower in schools, which recorded a large number of pupils using ITN. Prevalence of *Plasmodium* in Kasipul is still high compared to the national average of 5%; this study recommends that other control measures apart from insecticidal nets should also be introduced in Kasipul, by the Kenya government to eliminate *Plasmodium*. Ministry of health and other stakeholders should ensure that hang-up' campaigns to sensitize residence on the relationship between ITN and *Plasmodium* prevalence, forms an integral part of future treated nets distributions. Further studies using households as sampling units need to be conducted in Kasipul, since this study did not include pupils absent from school on the sampling days.

CHAPTER ONE

INTRODUCTION

1.1 Background

In 2013, approximately 198 million cases of malaria and 584,000 deaths occurred globally, and 90% of the deaths were in Africa. Sub-Saharan Africa bears the heaviest brunt, with 90% of all deaths, 77% among children under the age of five years (Sonko *et al.*, 2014). Eighty per cent of malaria cases in Africa occurred in thirteen countries, and over half were in Nigeria, Democratic Republic of Congo, Ethiopia, United Republic of Tanzania and Kenya (Aderibigbe *et al.*, 2014). Kenya has an estimated malaria mortality rate of 27.7 per 100,000 people, malaria accounting for almost 9 million outpatients cases annually (Odhiambo *et al.*, 2017). Kasipul experiences a 25.8 % *Plasmodium* prevalence among primary school children (Bousema *et al.*, 2013).

Infection with *Plasmodium falciparum* can give rise to neuro-cognitive impairments, affecting speech, language and memory in affected children (Kariuki *et al.*, 2014; Buffet *et al.*, 2011). Although, school children are included in standard malaria intervention strategies, often they have the lowest coverage of malaria preventive measures, such as bed net use net because of misuse such as ITNs being used for activities in agriculture and fishing (Kateera *et al.*, 2015). While reduction of infections is observed in children below five years that are targeted by interventions, parasite prevalence among school children may even increase, as observed after the national distribution of bed nets in Kenya (Idris *et al.*, 2016). In addition, as school children are rarely treated for asymptomatic infections, they may contribute significantly to the infectious reservoirs of *Plasmodium* (Kepha *et al.*, 2016).

Anaemia is prevalent among school children in the tropics. The aetiology of anaemia is usually multifactorial but it is likely that, in many communities, malaria plays a major role (Nankabirwa *et al.*, 2014). Asymptomatic *Plasmodium* infections, if untreated in children, persist and maintain malaria induced inflammation, that is commonly associated with iron deficiency anaemia. *Plasmodium* infection also impair intestinal iron absorption and iron release from hepatocytes and impairment of the recycling of iron derived from parasitized RBC phagocytosis (Doua *et al.*, 2013). Therefore, the prevalence of anaemia in a community, as measured in population-based surveys, is taken as the candidate indicator of the total malaria-related disease burden. If the response of anaemia to various malaria interventions is known, anaemia might serve as an indicator of the success of malaria control programmes (Korenromp *et al.*, 2004).

Insecticidal nets are dependable malaria control tools (Sedlmayr *et al.*, 2013). Since 2001, use of ITN has prevented over three million malaria-related deaths (Drakeley and Lines, 2014). When used by more than eighty per cent in a particular village, ITN protects everyone in the area, including households without nets since fewer people are bitten due to a reduced mosquito population and this provides some community protection (Kyama *et al.*, 2007). Few data exist on patterns of ITN use and effectiveness of nets in controlling malaria among primary school children (Gitonga *et al.*, 2010).

The ministry of health (MOH) in Kenya commonly uses mass net distribution campaigns done every three years as a strategy to control malaria in populations living in epidemic prone and endemic areas of the country. In 2011, ITN policy was

changed to cover the entire population at risk regardless of age and gender. In 2014, the third round of mass distribution was launched in Kenya to boost ITN coverage and replace old nets (Zhou *et al.*, 2016). In the last 10 years, ownership of ITN has increased from 6% to 68% in malaria endemic Lake Victoria basin of Kenya (Obala *et al.*, 2015). However, reported use of bed net among primary school children is considerably low (33%), mostly because these children do not sleep under beds and the increase in parasite prevalence among school children in the region likely reflects an increase in exposure to infective mosquito bites (Idris *et al.*, 2016).

There is a growing recognition of the importance of infection prevalence surveys in the design and evaluation of malaria control initiatives in Africa. However, national household sample surveys that include malaria infection measurements are expensive and labour intensive. In areas where school attendance is high and malaria transmission is stable, the use of school-based malaria surveys, offers a cheaper alternative to examine community-acquired infection prevalence and reported coverage of household vector control (Okoyo *et al.*, 2015). A study in Uganda found that reports by school children on household net ownership provide a rapid method to collect reliable ITN coverage data at the community level (Gitonga *et al.*, 2010).

1.2 Statement of the problem

Malaria control efforts in Africa have been intensified in young children; this has increased the risk of malaria transmission to school aged and older children (Brookers *et al.*, 2008). Reports indicate that school children are the age group most commonly infected with malaria parasites. Their infections are usually asymptomatic, go unnoticed and thus never get treated, resulting in anaemia, reduced ability to

concentrate and learn in school, and when sick may lead to school absenteeism (Nzobo *et al.*, 2015). Available data show that the overall prevalence of *Plasmodium* and anaemia among school children in Kasipul is 25.8 % and 14.1%, respectively following implementation of universal distribution of ITNs (Gitonga *et al.*, 2010; Bousema *et al.*, 2013). However, there is no current information on the *Plasmodium* and anaemia prevalence in Kasipul following mass distribution of ITNs in 2014. This study, determined the prevalence of *Plasmodium* and anaemia among primary school children living in Kasipul, which is an area of intense malaria transmission and their reported use of insecticide treated bed nets, one year after mass distribution of ITNs in September 2014.

1.3 Justification

Controlled trial of ITN usage in Asembo bay in Nyanza province has showed that ITNs reduced the incidence of new malaria parasite infections. Studies indicate that children protected by ITNs have a reduced risk of anaemia, compared to unprotected children (Snow *et al.*, 1997; Eisele *et al.*, 2011). In general school children normally aged 6–15 years, make up a large proportion of the population. Little is known about the effectiveness of ITNs at preventing *Plasmodium* infections among this group. In the absence of data from intervention studies, school-based cross-sectional surveys can provide insight into the potential efficacy of ITNs among school children (Gitonga *et al.*, 2012). Previous studies in other parts of Africa have found that even after universal net distribution, school children were significantly less likely to use ITNs compared to other age groups (Buchwald *et al.*, 2017). Given their contribution to overall *Plasmodium* prevalence in an area, there is a need for research into effectiveness of ITNs in controlling *Plasmodium* infection among primary school

children in particular malaria endemic areas such as Kasipul, which benefited from free distribution of ITNs in 2011 and 2014. The findings of this study shed light on the overall prevalence of *Plasmodium* and anaemia among primary school children following mass distribution of free ITNs as the main strategy in the control of *Plasmodium* infections in this part of Kenya. Such information provides a rationale for using schools and school children to assess effectiveness of ITNs in controlling *Plasmodium* infection.

1.4 Research questions

i. What is the current prevalence of *Plasmodium* infection among primary school children in Kasipul, following mass distribution of free insecticide treated mosquito bed nets?

ii. What is the prevalence of anaemia among primary school children following mass distribution of free insecticide treated mosquito bed nets in Kasipul?

iii. What is the relationship between insecticide treated mosquito bed net (ITNs) usage and *Plasmodium* prevalence among primary school children in Kasipul?

1.5 Null hypotheses

i. *Plasmodium* infections are still highly prevalent after mass distribution of insecticide treated mosquito bed nets among primary school children in Kasipul

ii. Anaemia is still highly prevalent after mass distribution of insecticide treated mosquito bed nets among primary school children in Kasipul

iii. There is no relationship between ITNs usage and the prevalence of *Plasmodium* infection among primary school children in Kasipul.

1.6 Objectives of the study

1.6.1 General objective

To assess the prevalence of *Plasmodium* infection and anaemia among primary school children after a universal bed net distribution campaign in Kasipul, Homa-Bay County, Kenya.

1.6.2 Specific objectives

i. To determine the current prevalence of *Plasmodium* infections among primary school children in Kasipul, Homa-Bay County.
ii. To determine the prevalence of anaemia among primary school children in Kasipul.
iii. To determine the relationship between ITNs use and *Plasmodium* infection prevalence among primary school children in Kasipul.

1.7 Significance of the study

The prevalence of *Plasmodium* infection in a country is an integral indicator for national malaria control programmes, which can be used to measure the success of intervention strategies and for appropriate resource allocation. Therefore, an accurate data on malaria parasite prevalence is crucial. With the health sector now devolved to county governments in Kenya, provision of quality health services will depend on a good understanding by county government of the disease distribution patterns and risk levels especially malaria. The finding of this study has revealed that the free distribution campaign of ITNs is effective in reducing the prevalence of *Plasmodium* infection among school children in Kasipul. The study indicates that INTs distribution should continue to sustain the reducing morbidity of malaria. The results also provide

detailed baseline data to inform the evaluation of ITN as a malaria control tool in Kenya.

1.8 Limitation of the study

This school-based surveys did not capture children found to be absent on the day of the survey, and some of them could have been absent due to illness, including malaria. Due to logistical constraints, no effort was made to follow-up absent children, thus introducing potential selection bias. The study did not include school children below 9 years (class 1-3 were not sampled); despite the fact that younger children could probably have more risk of infection. Use of a light microscope in *Plasmodium* detection during the study probably resulted in underestimating *Plasmodium* infection rates, especially in cases where pupils had low parasitaemia. A more sensitive method such as PCR-based parasite prevalence could be used in surveys in Kasipul.

CHAPTER TWO

LITERATURE REVIEW

2.1 General situation of human *Plasmodium* infection

Malaria has plagued humanity throughout history; it remains a challenge to global health and economic development. Over half a million residents of third world countries perished due to malaria in 2009 worldwide (Ngomane *et al.*, 2012). Apart from Africa, malaria remains, prevalent in Asia and South America (Malaria, 2014). Tanzanians record sixteen million episodes of malaria annually; while Kenya ranks second in eastern Africa with eight million cases of *Plasmodium* infections annually (Magesa *et al.*, 2005; Opiyo *et al.*, 2007).

2.2 Malaria transmission

Anopheles mosquito's body provides microenvironment needed for sexual phase of the *Plasmodium* life cycle, enabling it to be responsible for human malaria infections during feeding (Choices, 2014). When a female *Anopheles* mosquito sucks blood from humans, gametocytes, are ingested together with the blood meal. These undergo further development to form sporozoites before being introduced to the next human victim during feeding (Alan and Sarah, 2012). Other modes of *Plasmodium* transmission, such as blood transfusion and the sharing of needles are rare (Choices, 2014).

There is increasing evidence suggesting that school aged children (5–15 years) bear the highest burden of asymptomatic malaria irrespective of the transmission setting with a prevalence range between 14 and 64 %, and constitute nearly half of the population at risk of malaria worldwide. Moreover, due to a decline in transmission

and exposure in some areas, the peak age of clinical attacks of malaria is shifting from very young (under five) to older children (Kepha *et al.*, 2016).

2.2.1 Host parasite relationship factors influencing *Plasmodium* transmission dynamic

Certain substances produced by human beings exert an attraction to females *Anopheles* mosquitoes; a wide range of host, parasite and mosquito factors influences the likelihood that a mosquito will acquire the infection. These include hosts anaemia, immunity and drug treatment; parasite (gametocyte) density, maturity and clone diversity; and mosquito size, microbial gut flora, environment and immune response among others. Mosquito innate immunity, feeding behaviour, longevity, the duration of sporogonic development and the degree of contact between mosquitoes and humans will also influence the likelihood of successful infection (Churcher *et al.*, 2015).

The most important factor that influences the success of transmission is simply the abundance of vectors and the frequency with which they 'sample' infected blood. Contact between humans and mosquitoes differ between endemic settings and among individuals within settings. This is partly a consequence of differences in human behaviour, such as the proportion of time spent outdoors or indoors before going to bed, differences in attractiveness to mosquitoes due to odour profiles. Naturally, acquired immunity to *Plasmodium* sexual stage antigens may also reduce transmissibility (Bousema *et al.*, 2014).

Human behaviour, often dictated by social and economic reasons, can influence the risk of malaria for individuals and communities. For example, poor rural populations

in malaria endemic areas often cannot afford the housing and bed nets that would protect them from exposure to mosquitoes. Activities such irrigation and Rice cultivation can create breeding sites for malaria vector thus prompting *Plasmodium* infection. Raising domestic animals near the household may provide alternate sources of blood meals for *Anopheles* mosquitoes and thus decrease human exposure and indirectly protecting humans from *Plasmodium* infection. (CDC, 2012).

Studies in African indicate that males school children continue to be less affected with asymptomatic malaria than females because male school children tend to sleep earlier (reduced the exposure time to mosquito bites) at night than females who delay (increased exposure time to mosquito bites). In African cultures, female children had responsibilities to assist their mothers in several sociocultural activities once they came back from school until the late hours of a day, and hence the chances of being bitten by mosquitoes is higher than in males (Nzobo *et al.*, 2015).

2.3 Epidemiology of malaria

Malaria is endemic in 109 countries and territories in tropical and sub-tropical zones, spanning all continents of the world except Antarctica and Australia, with intensities of transmission that vary from very low to extremely high (WHO, 2008). *Plasmodium vivax* is geographically the most widely distributed (Mueller *et al.*, 2009). *Plasmodium falciparum* accounts for almost all the malaria mortality in Sub-Saharan Africa (Snow and Omumbo, 2006). The malaria vectorial system in Africa is dominated by four species of *Anopheles* mosquitoes, *A. gambiae*, *A. coluzzii*, *A. arabiensis* and *A. funestus* (Guelbeogo *et al.*, 2014). Biological, political, sociocultural, economic and behavioural factors influence the transmission of malaria

in Africa (Ngalame *et al.*, 2004). Environmental factors such as the presence of bushes and stagnant water around homes, rainfall, low altitude and high temperatures favour the breeding of malaria vectors, as well as parasite reproduction within them (Kimbi *et al.*, 2013).

Kenya is one of the malaria endemic countries in Sub-Saharan Africa with highly intense malaria transmission (Zhou *et al.*, 2014). Nearly 28 million Kenyans live in areas of malaria risk, a majority of them children under the age of 15 years (MOH, 2011). The epidemiology of malaria in Kenya has been changing with reported reductions in malaria associated hospital admissions and mortality in children under the age of five years, partly, attributed to the increase in coverage and access to malaria control interventions, such as ITNs, artemisinin-based combination therapy and indoor residual spraying (IRS) (Gitonga *et al.*, 2010).

Reductions in the densities of the major malaria vectors and a shift from human to animal feeding have contributed to the decreased burden of malaria along the Kenyan coast. *Anopheles arabiensis* has replaced *Anopheles gambiae* as the major malaria vector; this vector predominately rests and feeds on humans outdoors (Mwangangi *et al.*, 2013). In western Kenya, malaria is predominantly a rural disease, over the past four decades, deforestation and swamp cultivation have widely occurred in western Kenya, and these are now thought to be a major contributing factor to the abundance of breeding habits and the survival of malaria vectors (Imbahale *et al.*, 2012).

Malaria prevalence in the lake endemic zone remains concerning at 38%. In terms of *Plasmodium* species distribution the main etiology is caused by *P. falciparum*; *P.*

malariae, *P. ovale* and *P. vivax* infections are less common (Idris *et al.*, 2016). School surveys carried out in primary schools in Kasipul in 2011 indicated an average *Plasmodium* prevalence of 25.8% in 7 to 18 year old pupils. The main malaria vectors in the Kasipul are *Anopheles gambiae, An. arabiensis, and An. funestus.* Malaria transmission is seasonal, with two peaks in malaria cases reflecting the bimodal rainfall pattern; a peak corresponding to the heaviest rainfall typically occurs between March and June and there is a smaller peak between October and November each year (Bousema *et al.*, 2013). Despite a drop in malaria prevalence in other parts of the country, transmission has remained high in the lake endemic zone consequently, as part of the Division of Malaria Control's (DOMC) 2009-2017 National Malaria Strategy, prevention and control interventions are tailored to the current epidemiology of malaria, with a concentration efforts in the lake endemic zone (USAID, 2013).

2.4 Life cycle of *Plasmodium*

Plasmodium is a two-host parasite (Mandal *et al.*, 2011). Figure 2.1 illustrates a typical life cycle of *Plasmodium* (Morrow, 2007). The definitive host inject sporozoites into the intermediate host during feeding and hepatocytes are then infected via systemic blood circulation (Wijayalath *et al.*, 2014). Trophozoites then mature in the liver and begin, schizogony producing numerous daughter nuclei, which transform into schizonts, also known as cryptozoites. A clinically dormant stage referred to as hypnozoite is a hallmark of some species. Schizogony produced merozoites, which invade red blood cells (RBC) and develop to trophozoites and mature schizonts (Gerald and Larry, 2009). Gametocytes are taken up by a *Dipteran* host together with blood into the gut where gametogony occurs (Cowman *et al.*, 2012). Exflagellation of the microgametocyte produces microgametes. The

product of fertilization in malaria parasite is referred as ookinete. Sporozoites producing oocyst develop from ookinete and start producing thousands of sporozoites within a period of 2 to 3 weeks depending on environmental temperature (Collins and Geoffrey, 2007).

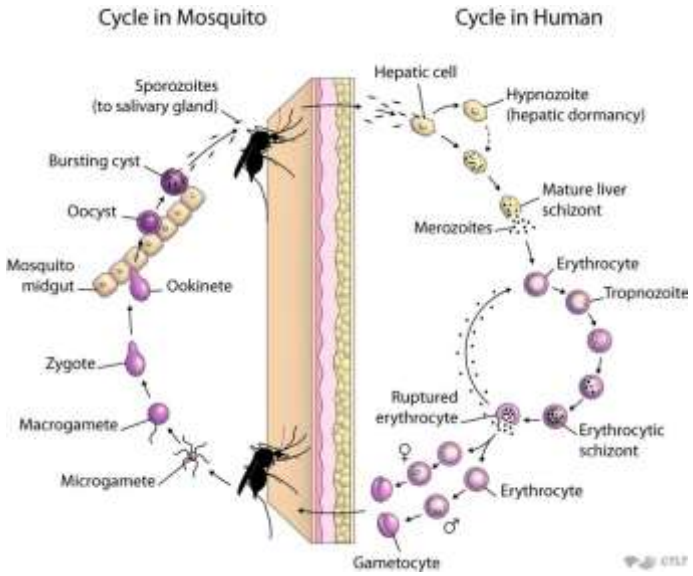

Figure 2.1 Malaria parasite life cycle (Marrow, 2007).

2.5 Types of Malarial parasites

Only five species of malaria parasites infect humans naturally and they exhibit a close phylogenetic relationship to non-human *Plasmodium* parasites (Waters, 1993; Spinello *et al.*, 2012).

2.5.1 *Plasmodium falciparum*

This is the most pathogenic of all the *Plasmodia* and hence, the name malignant tertian or pernicious malaria for its infection. The disease has a high rate of complications and unless treated, is often fatal. The species is responsible for almost all deaths caused by malaria (Paniker, 2013). Its distribution has become restricted in recent years, although it is still very common in tropical and sub-tropical regions such as Sub-Saharan Africa, South-East Asia, and parts of South America (Snow, 2005). It does not form hypnozoites but sudden relapses (recrudescence) can occur months or even a year or more after the last fever episode as a result of the parasite persisting in the blood at low sub-clinical levels. The merozoites are able to invade and develop in both young RBC (reticulocytes) and mature RBC. Consequently, *Plasmodium falciparum* malaria is often characterized by high levels of parasitaemia (Alan and Sarah, 2012). The erythrocytic schizogony takes about 48 hours or less, so that the periodicity of febrile paroxysms is 36–48 hours (Paniker, 2013).

2.5.2 *Plasmodium vivax*

Plasmodium vivax is the most common parasite in Asia and Latin America, but it is less common in Africa. Historically, this particular malaria parasite had the widest geographical distribution, occurring even in Europe but has since been eliminated (Spinello *et al.*, 2012). The species solely penetrate immature erythrocytes. Hypnozoite stage of the parasite is responsible for the sudden development of malaria symptoms in patients, after a period of sound health (Alan and Sarah, 2012). *Plasmodium vivax* account for 80% of all malaria infections causing benign tertian malaria with frequent relapses (Paniker, 2013).

2.5.3 *Plasmodium ovale*

It is the rarest of all *Plasmodia* infecting humans and is seen mostly in tropical Africa, particularly along the West Coast. Symptoms resemble *vivax* malaria (Paniker, 2013). The parasite is not exclusive to African since it has also been documented in health facilities in Asia and South America (Cheesbrough, 1998).

2.5.4 *Plasmodium malariae*

This parasite displays zoonotic characteristic and is common in blood samples from wild chimpanzees (Hayakawa, 2009). The parasite cannot be considered endemic to any zone. Low parasitaemia usually remains in the human body after initial infection and fulminant malarial paroxysm after a period of comparatively sound health is a product of loss of partial immunity among victims due to a reduction of malaria antigen specific memory cells (Collins and Geoffrey, 2007). It causes quartan malaria, in which febrile paroxysms occur every fourth day, with 72-hour interval between the bouts (Paniker, 2013).

2.5.5 *Plasmodium Knowlesi*

This parasite is zoonotic and mainly infects monkeys in Asia. Incidences of *P. knowlesi*s infections in humans are common in Malaysia. The symptoms mimic *P. vivax* paroxysm, but unlike *vivax* malaria, the aftermaths can be fatal (Singh *et al.*, 2008). *Plasmodium knowlesi* and *P. vivax* show some phylogenetic resemblance; however, *P. knowlesi*s lack a dormant liver stage (Spinello *et al.*, 2012).

2.6 Pathogenesis of malaria

Malaria is characterized by overproduction of cytokines responsible for signalling and promoting systemic inflammation in the body. Severe malaria causes severe anaemia resulting from decreased erythropoiesis, and cerebral malaria, a product of occlusion of minute blood vessels in the central nervous system by erythrocytes (Gerald and Larry, 2009). Late stage schizonts of *P. falciparum* initiate changes on the surface of RBCs promoting aggregation of infected RBCs to adhere to other non-infected RBCs and capillary endothelial cells. The resultant effect is capillary plugging of cerebral microvasculature, which results in anorexia, ischaemia, and haemorrhage in the brain (Paniker, 2013). The presence of *P. falciparum* in the placenta induces a local inflammatory response, which interferes with exchanges between the mother and foetus, leading to low birth weight and premature labour and birth (Matangila *et al.*, 2014).

Malarial anaemia is caused by destruction of parasitized RBC, indirect destruction of non-parasitized RBC by immune mechanisms, and bone marrow suppression associated with imbalances in cytokine concentrations (Greenwood *et al.*, 2005). High levels of pro−inflammatory cytokines increase serum hepcidin and this limits the release of recycled red cell iron sequestered in macrophages and other reticuloendothelial cells (Glinz *et al.*, 2014).

2.7 Clinical features of Malaria

A typical malaria victim remains symptomless following the initial mosquito bite and the exoerythrocytic cycle of the malarial parasite. However, once the erythrocytic phase is initiated and large numbers of rupturing RBCs simultaneously occur, the

resulting merozoites and toxic waste by products in the systemic blood circulation trigger clinical indications. Malaria paroxysm is part of an allergic response of the body to the development of the schizonts and to the circulating parasitic antigenic determinants following the release of merozoites (Zeibig, 2013).

2.7.1 Benign malaria

Traditionally non-*P. falciparum* malaria is commonly referred to as benign. The designation tertian is derived from the ancient Roman custom of counting the days of an event in sequence; therefore, the first day of the fever peak is designated "day one," the intervening day is "day two," and the day of the next fever episode is "day three" although the time interval between peaks in *P. vivax* malaria is only forty eight hours, so "day three" then becomes "day one" when counting the next interval. In quartan malaria, merozoites rupture from the infected cell synchronously every seventy-two hours, with an accompanying fever paroxysm (Bruton *et al.,* 2013).

Typical benign, tertian or quartan malaria is triggered by the activation of the hypothalamus, accompanied by a rapid increase in body temperature to over 40°C; the victim goes through a feeling of extreme cold. Hot stage is ushered in an hour late, characterized by intense heat and headache. Sweating stage drench the patient in sweat and signals the end of the hot stage: the body temperature falls back to normal; the patient may fall into deep sleep and wake up with a normal feeling that lasts until the next paroxysm. Infection with *P. vivax* usually follows a chronic course with periodic relapses, whereas *P. ovale* malaria is mild; kidney failure is concomitant to *P. malaria*. Other features of benign malaria are anaemia, splenomegaly, and hepatomegaly (Paniker, 2013).

2.7.2 Malignant tertian malaria

The most life threatening type of malaria is the malignant tertian malaria caused by *P. falciparum* (Paniker, 2013). Patients may experience sudden fever, as the first sign of the infection. A severe headache is the usual presenting symptom, followed by drowsiness, confusion, and coma. The temperature falls rapidly, and the patient may become delirious. Symptoms of systemic circulation failure and shock develop quickly. *Plasmodium falciparum* infection involving sequestration of parasitized RBCs in cerebral micro vessels produces the most deadly form of malaria in untreated patients. Intestinal symptoms (nausea, vomiting, and diarrhoea) if developed, mimic those seen in malignant infections, hence, the name malignant tertian malaria (Voges and Markell, 2009).

2.8 Malaria chemotherapy

Despite Chloroquine having high efficacy and low perniciousness, uncontrolled use in most parts of the world has rendered it ineffective. Quinine is very effective and is one of the oldest anti-malarial drugs against malaria parasite (Goswami *et al.,* 2013). Of late, reduced susceptibility of *Plasmodium* to Chloroquine has prompted the adoption of Quinine as a standard treatment for Chloroquine-resistant malaria (Achan *et al.,* 2011). Currently, Chinese traditional medicinal herb *Qinghaosu* is the most efficacious anti-malarial drug. Artemisinin derivatives in combination with other anti-malarial compounds remain the most efficacious treatment option currently available to tackle this parasite (Mehta *et al.,* 2006; Parija *et al.,* 2011).

2.9 Diagnosis of malaria

2.9.1 Demonstration of parasite by microscopy

Routine diagnosis of *Plasmodium* infection anchor on microscopical demonstration of the parasites in stained thick and thin blood smears. Figure 2.2 shows unstained human blood smears used for malaria diagnosis (MIS, 2013). Thin smears offer the advantage of very little distortion of the parasite, but the disadvantage is that many fields must be examined to detect parasites when they are few in number. Although thick blood smear preparations often distort the parasite morphology, the chances of detecting parasites are enhanced. Thick smear technique concentrates blood; the same volume of blood can be screened about three times faster than that in a thin smear (Ruth and Russel, 2012).

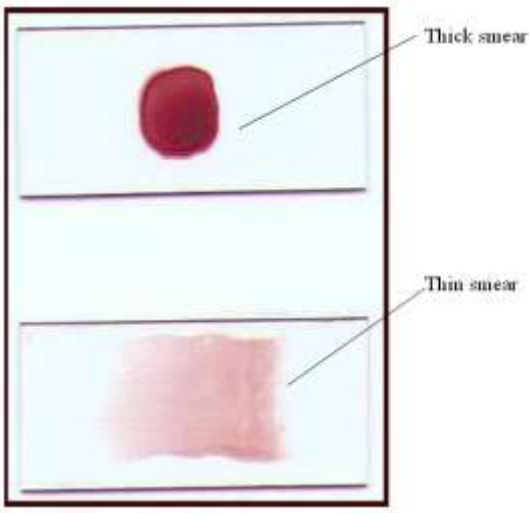

Figure 2.2 Blood smear for malaria parasite detection (MIS, 2013)

Morphological appearance of individual *Plasmodium* species is used in routine diagnosis of malaria. Only a few asexual forms of *P. falciparum* show up in blood smears. Figure 2.3 illustrates erythrocytic stages of all the four species of *Plasmodium*

(Srinivas, 2015). Blood smears from *P. falciparum* victims, show ring form alone or with gametocytes. Figure 2.4 shows trophozoite and gametocyte of *Plasmodium falciparum*, in a thin blood smear (Paniker, 2013). In *P. falciparum*, the infection does not alter the size of RBCs but they possess a large Maurer's dots. A careful search on the slide is recommended for mixed infections (Paniker, 2013).

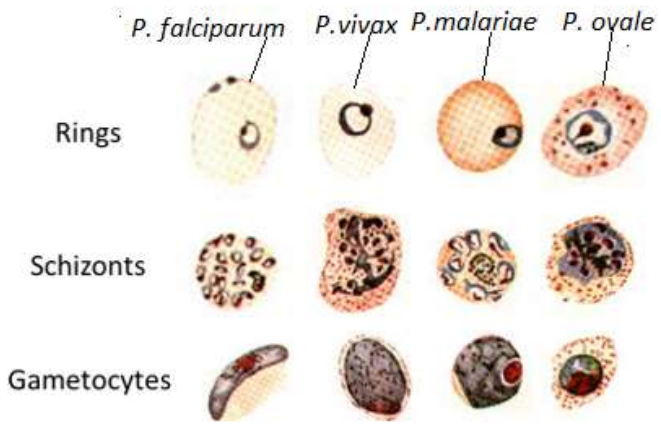

Figure 2.3 Malaria parasites in thin blood smear (Srinivas, 2015)

Thick film is mainly used as a screening procedure; unless the examiner has much experience, examination of the thick film should not be relied upon for specific diagnosis of *Plasmodium* infection (Voges and Markell, 2009). Thick blood smear technique destroys all RBC and only white blood cells and a distorted parasite can be seen, making it unsuitable for detecting morphological changes that occur in parasitized erythrocytes (Bruton, 2013).

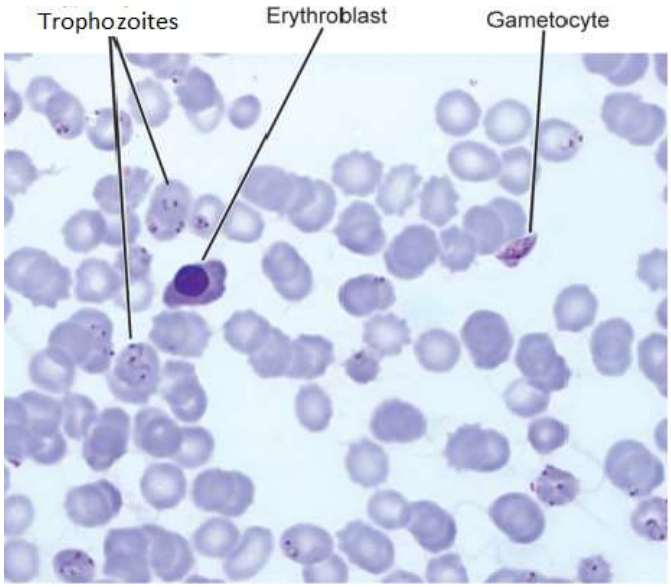

Figure 2.4 *Plasmodium* falciparum blood smear (Paniker, 2013)

2.9.2 Rapid antigen detection tests

Microscopic diagnosis of malaria requires considerable training and experience. Rapid diagnostic tests (RDTs) offer a simple and rapid complement to microscopic malaria diagnosis. Newer generation three-band tests display a control line and two test lines, one for detection of *P. falciparum*-specific antigen and another for detection of antigens common to the four species, such as pan-*Plasmodium*-specific parasite lactate dehydrogenase or aldolase (Van der Palen *et al.*, 2009). The Para Sight–F test is a dipstick antigen capture test specific for *P. falciparum.* The test is sensitive, specific and rapid. It easily differentiates between *Plasmodium falciparum* and other malaria species (Paniker, 2013).

2.9.3 Molecular diagnosis

Neither microscopy nor RDTs are able to detect low-density parasite infections and which are often asymptomatic. Nucleic acid amplification tests (molecular tests), such as PCR-based assays, have both, superior sensitivity and specificity compared to microscopy and RDTs. Studies indicate that PCR can detect as few as 1-5 parasites/µl of blood compared with around 50-100 parasites/µl of blood by microscopy (Tangpukdee *et al.*, 2009). However, some challenges such as the cost of assays exist that impede the implementation of molecular assays in large epidemiological surveillance studies (Lucchi *et al.*, 2014).

2.9.4 Quantitative Buffy coat, smear

The Quantitative buffy coat (QBC) test is a new simplified method for diagnosing malaria, where a small quantity of blood (50–110 µL) of blood is spun in QBC centrifuge at 12,000 revolutions per minutes for 5 minutes. Red blood (RBC) containing malaria parasites are less dense than normal RBCs and concentrate just below the buffy coat of leucocytes at the top of the erythrocytic column. Pre-coating of the tube with acridine orange induces fluorescence on the parasites, which can then be readily visualized under the oil immersion microscope because the parasite contains DNA, but the mature RBCs do not contain DNA and RNA. The nucleus of the parasite is detected by acridine orange stains and appears as fluorescing greenish yellow against red background (Paniker, 2013).

2.9.5 Serodiagnosis

Serodiagnosis is used mainly for seroepidemiological survey and to identify the infected donors in transfusion malaria. The tests used are indirect hemagglutination

(IHA), indirect fluroscent antibody test (IFA), and enzyme-linked immunosorbent assay (ELISA). However, these tests are not helpful in clinical diagnosis because they will not differentiate between an active and past infection (Paniker, 2013).

2.10 Control of *Plasmodium* infections

Malaria prophylaxis includes chemotherapy and, vector breeding control through environmental management and human protection from malaria vector (Ferede *et al.*, 2013). Habitat modification is a reliable tool in mosquito control, for example, altering mosquito harbourage to make it unsuitable for mosquitoes (Durden and Mullen, 2002). Sterile Insect Technique has gained attractiveness as a control strategy in malaria vector control. Introduction of mosquito predators such as the mosquito eating fish, *Gambusia afinis,* and judicious use of insecticides, appropriate drug treatment of persons with the disease as well as prophylactic drug treatment of newcomers to malaria endemic areas are integral parts of malaria control (Gerald and Larry, 2009).

2.10.1 Insecticide treated nets

Bed nets have offered protection against biting insects long before the discovery of female *Anopheles* mosquito as the vector responsible for human *Plasmodium* transmission (Beer *et al.*, 2010). Mosquito nets are tent-like fabrics used for covering the bed to protect the occupant against insect vectors. Deltamethrin and Permethrin have been adopted recently as routine insecticides used in the treatment of ITN (Mosquito net, 2014). There are two types of ITN available in the market: Olyset® net made of permethrin incorporated netting material and Permanet® nets made of Deltamethrin coated net fabrics (Dabiré *et al.*, 2006). This nets repel and kill malaria

vectors, and they prevent sporozoite-bearing *anopheline* mosquitoes from biting the occupants lying under or near the bed net (Smithuis *et al.*, 2013).

High coverage and utilization rates are required for ITN to make a substantial impact on the prevention of malaria transmission (Sichande *et al.*, 2014). If used by almost all members of a community, insecticidal nets kill large numbers of the local malaria vectors, reducing the mean survival, sporozoite rate and population density of the vector population and hence substantially reducing its entomological inoculation rate. This "bonus" effect of community-wide coverage of ITN can equal or exceed the personal protection effect (Maxwell *et al.*, 2006). ITN is considered as the most efficacious of all the currently feasible interventions for malaria control in Africa. It is cost-effective in preventing malaria-related morbidity (Ordinioha, 2012).

Currently, most African countries are increasing overall ITN household ownership to over sixty per cent from an initial figure of almost zero (Lim *et al.*, 2011). The WHO and Roll Back Malaria partnership now recommend that distribution of ITN be free or heavily subsidized to achieve greater equity of coverage, and that a variety of distribution systems be used to achieve universal access, including targeted campaigns to deliver nets to most-at risk populations, which include pregnant women and children. The investment into this simple yet effective intervention has been substantial. In 2010, there were enough ITN, procured on the African continent to cover 73% of the at risk population, yet achieving equitable distribution and providing on-going supply remain a challenge (Singh *et al.*, 2013).

Kenya adopted WHO universal bed net program in 2011 to increase ownership and usage of ITN to eighty per cent by giving out two persons in each family a net, over 7.6 million ITN were distributed to priority areas in Nyanza, Western and Rift valley provinces during the free ITN mass distribution campaign. The government distributed additional 500,000 ITNs to all malaria endemic zones in Kenya during the 2014 mass distribution campaign (USAID, 2014; Ototo *et al.,* 2015). Despite periodic distribution of ITNs in Kenya, studies indicate that ITN coverage show highest coverage among children aged less than five years, dropping to lowest levels of coverage among children and adolescents aged 5-19 years. As expected, this group represents the largest threat to the success of scaled, universal coverage of ITN likely to impact upon reduced community-levels of transmission, mainly because this age group have not developed a parasitic immunity that regulates the risk of blood stage infection (Noor *et al.*, 2009).

2.10.2 Factors influencing the efficiency of ITN in lowering risk of *Plasmodium* infection

The main entomological justification for the use of ITN was that most biting by the anthropophilic, endophagic and endophilic vector mosquitoes occurred at hours of the night when most people were in bed and under nets if they had them. Peak-biting activity of *Plasmodium*-transmitting mosquitoes is known to be between 22.00 and 05.00 hours, and the majority of mosquitoes bite humans indoors and rest indoors. Behavioural change in the biting pattern of a greater proportion of the malaria vectors to biting earlier or later in the night and biting outdoors when many people are not in bed, is rendering bed nets less effective, hence causing an increase in the malaria infection rates (Kabbale *et al.*, 2013).

In Benin, three years after universal coverage with ITN, *Anopheles* mosquitoes have showed substantial diurnal and early biting activity and more frequent outdoor biting. This change in mosquito biting habits could jeopardize the success of control operations and may affect the epidemiology of malaria transmission globally (Sougoufara *et al.*, 2014). Another recent study in western Kenya showed an overlap of early biting habit of the malaria vectors and human activity. In Tanzania, a studies indicate that prolonged use of insecticide treated bed nets has triggered a behavioural adaptation in mosquitoes to avoid contact with ITN by biting in the early hours of the night (Kabbale *et al.*, 2013).

Across African countries, women of reproductive age are most likely to use the net; least likely are children of age 5-14 and adult male (Baume and Marin, 2007). A key determinant of ITN impact is bed net use, with previous studies showing disparities between bed net ownership and use. One such determinant of bed net use is seasonality. While higher net use has been reported more in the rainy season due to the associated high mosquito density, lower net use has been associated with hot dry months due to heat-related discomfort. Other previously reported determinants of net use include number of nets owned per household, disruptive sleeping arrangements, and net misuse such as bed nets being used for activities in agriculture and fishing (Kateera *et al.*, 2015).

2.11 Anaemia prevalence among school aged children

Anaemia is defined as a condition when haemoglobin level is less than 11.5 g dl (Assefa *et al.*, 2014). Overall prevalence of anaemia among school children is about 14.1% in areas bordering Lake Victoria in Kenya (Gitonga *et al*., 2010). Chronic or

repeated episodes of malarial anaemia due to any *Plasmodium* species have been associated with adverse developmental effects as well as school attendance (Sumbele *et al.*, 2015). Although nutritional deficiencies, hookworm infection, HIV and haemoglobinopathies all predispose to the development of anaemia in children, evidence suggests that, in endemic countries, malaria is one of the most important factors (Otupiri *et al.*, 2012). Studies indicate that 23 % of primary school children in Nyanza in are anaemic (Leenstra *et al.*, 2004).

2.12 Use of school-based cross-sectional study in malaria monitoring and evaluation of interventions

To date, malaria monitoring and evaluation of interventions in malaria endemic countries in Africa has been mainly based on periodic national household surveys. The principal advantages of such household surveys are that they adequately capture the underlying variation in the sampled population and the flexibility of data collection instruments, which can accommodate a number of questions on a variety of topics (Ototo *et al.*, 2015). However, household surveys are expensive, time consuming and labour intensive, and generally only undertaken every 3-5 years and therefore not ideal for routine monitoring at local levels (Okoyo *et al.*, 2015). A cheaper and rapid complementary approach to household surveys would be to use the existing school system for school-based malariometric surveys. Routine school survey stopped in the 1980 s due to financial constraints and deteriorating school enrolment rates. The renewed potential for school malaria surveys builds on the increased funding for malaria surveillance but also recent improvements in primary school enrolment in Kenya and it currently form an integral part of malaria surveys in Kenya (Gitonga *et al.*, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.0 Study area

The study was conducted in Kasipul located within Homa-Bay County (34.75 to 34.95°E, 0.41 to 0.52°S). Kasipul lies within the Lake Victoria basin, which has high malaria prevalence of 40%, among the residents. This region benefited from free distribution of ITN in 2014 (Minakawa *et al.*, 2012; Zhou *et al.*, 2016). It has an area of 365.5 sq. Kms and a population of 129,854. Eighteen thousand children are enrolled in sixty-seven primary schools within Kasipul (RDSP, 2005). It has an altitude of between 1400 m to 1600 m. Figure 3.1 shows a map of Kasipul (Softkenya, 2015). Malaria transmission is seasonal, with two peaks in cases coinciding with the bimodal rainfall pattern; a peak corresponding to the heaviest rainfall typically occurs between March and June and there is a smaller peak between October and November each year. Most malaria is caused by *P. falciparum* (Bousema *et al.*, 2013).

Figure 3.1 Map of Kasipul: Insert map of Kenya showing the location of Kasipul (Softkenya, 2015).

3.1 Study design

School-based cross-sectional study was undertaken to establish the prevalence of *Plasmodium* infections, and anaemia among primary school children in Kasipul, Homa-Bay County. This type of study provides data on disease prevalence in a delimited population at a specific time (Levin, 2006). Studies indicate that in areas where school attendance is high and malaria transmission is stable, the use of school-based malaria surveys offers a cheaper alternative to examine community-acquired infection prevalence and reported coverage of household vector control such as ITNs (Okoyo *et al.*, 2015). In contrast, household surveys are expensive, time consuming and labour intensive, and generally only undertaken every 3-5 years and therefore not ideal for routine parasite monitoring at local levels (Gitonga *et al.*, 2010).

3.2 Study period

The period from survey preparation to conclusion was from December, 2015 to June, 2017. Preparation of study materials including recruitment and training of research assistant was conducted in February 2016. While actual data collection was conducted between March to May 2016. To coincide with high peak malaria transmission season in Kasipul.

3.3 Study population.

The study population for this survey comprised of all primary school children registered in primary schools within Kasipul, Homa-Bay County.

3.4 Target population

The survey targeted primary school children from nine randomly selected schools within Kasipul, Homa-Bay County. At least a school was selected from each of the five administrative divisions of Kasipul.

3.5 Inclusion criteria

All primary school children between 9 to 18 years who agreed to participate and their parents or guardians signed informed consent form and were physically present in school on the days when data collection was done were enrolled in this study. This particular age group are in class four to eight base on Kenyan education system and were sampled because they are able to fill in self-administered questionnaire, which was used in this study to collect data on net ownership and usage.

3.6 Exclusion criteria

Pupils who did not attend school on data collection days, children less than nine years of age and those whose parents or guardians did not sign informed consent form, were excluded from this survey. Children who had stayed in Kasipul for fewer than 16 days did not participate in the study because blood smear technique used for malaria diagnosis in this survey only detect *Plasmodium* in blood (Paniker, 2013). Pre-existing illness and treatment history were not considered as part of exclusion criteria.

3.7 Sample size estimation

In estimating the sample size, the researcher used 40% prevalence of *Plasmodium* for this region as reported in previous studies (Minakawa *et al.*, 2012). A formula

provided by Charan and Biswas (2013) was then used to calculate sample size as outline below:

$\mathbf{n} = \frac{\mathbf{t^2 \times p(1-p)}}{\mathbf{m^2}}$ (Charan and Biswas, 2013).

n = required sample size, **t** = confidence level at 95% (standard value of 1.96), **p** = estimated prevalence of malaria in the study area**, m** = margin of error of 5% (standard value of 0.05). Sample size was calculated as;

$n = \frac{1.96^2 X\ 0.4\ (1-0.4)}{0.05^2}$ = 368.79 = 369 children.

Cochran's (1977) correction formula was then used to calculate the final minimum sample size $\mathbf{n_1}$.

$\mathbf{n_1} = \frac{n}{1+\left(\frac{n-1}{N}\right)}$ (Cochran, 1977).

Where **n** is the sample size and **N** is the total population size of the sampled schools;

$n_1 = \frac{369}{1+\left(\frac{369-1}{3420}\right)}$ = 336 + 10% = 370 children

The sample size was increased to 450 children to account for eventualities such as failure to sign informed consent form. Number of observations per school was calculated by dividing the sample size by the number of participating schools;

$= \frac{\text{Sample size}}{\text{Number Schools}}$ = school sample size = $\frac{450}{9}$ = 50 children

Sample size per class = $\frac{50}{5}$ = 10 pupils per class

3.8 Sampling technique

A random cluster sampling technique was used to select school and proportional sampling technique used to select participants. From the list of all primary schools in Kasipul, a random cluster sampling technique was employed to select nine schools out of the total number of schools in the study area, and then within each selected school proportional sampling was used to select sample subjects from the list of all children who attend the school during the sampling day. At least ten pupils were selected from each class. Sampled pupils were provided with written informed consent form (Appendix I), to take to their parents for signing as a prerequisite to participate in the study. Before data collection, a meeting was held between the researcher and parents/guardians to explain to them the purpose of the study. The researcher also read and interpreted the requirement of the study to all parents in Kiswahili and local language.

3.9 Recruitment of research assistants and piloting of research tools

Two medical laboratory technologists with phlebotomists training from Rachuonyo district hospital and Kokwanyo Health Centre were recruited and given an orientation to the study. Prior to commencing the study, questionnaires were pretested in Kasimba primary school in Kasipul to check the validity of the questions.

3.10 Data collection procedures

The researcher and two medical laboratory technologists trained as research assistants to collect data on *Plasmodium* infection did a laboratory investigation of peripheral blood for malaria parasite and haemoglobin level determination for anaemia between March to May 2016. Self-administered questionnaires were used to collect data on

insecticidal net ownership and characteristics of participants in terms of school, gender age and level of education (Appendix II). Other variables analysed were time of retiring to bed and knowledge of ITN use. All participating children were given a self-administered questionnaire to fill individually before blood collection for malaria parasite and anaemia testing.

3.11 Collection of blood samples

WHO (2010) guideline on malaria microscopy was used for blood collection from selected participants. After recording the child's demographic data on the laboratory form and putting on a new set of protective rubber gloves, the third finger from the thumb was sterilised using seventy per cent alcohol and dried using sterilised cotton gauze. Spring-loaded lancet was then used to puncture the sterilised finger. Slight pressure was applied to the punctured finger and about 5 µl of blood collected in the middle of the slide to prepare thin blood film. Another 5 µl of blood was placed on a second clean microscope slide and used for thick smear preparation. Lastly, the tip of the HemoCue microcuvette was placed in the middle of the blood drop on the punctured finger and allowed to fill itself automatically by capillary action.

3.12 Preparation of thick and thin blood smears

To prepare a thin smear a second clean microscope slide was held at 45^{o} and used to spread the blood drop on the first slide by pushing it along the length of the slide while maintaining the angle of inclination (Appendix III). To prepare thick film the corner of a microscope slide was brought into contact with the blood drop meant for thick smear preparation and using a circular motion to spread and make an even thick

film. Blood slides were labelled, air dried and then fixed with methanol in the laboratory (Lynne, 2007).

3.13 Malaria parasite diagnosis

The slides were placed in a staining dish and three per cent Giemsa stain poured into the dish. The slides were allowed to bath in the staining solution for 45 minutes before floating off the iridescent scum using distilled water (Appendix IV). Two technicians 'blind' read each pupils blood smear and classified either it as negative or positive. A third microscopist and the researcher performed another reading until a consensus of negative or positive was reached. Test results were recorded in the pupil's laboratory form. Morphological features were used in identification of malaria parasite species in a thin blood smear (Appendix V and VI).

3.14 Haemoglobin determination

Haemoglobin level was determined using handheld HemoCue machine. Blood filled cuvette was inserted into a HemoCue Hb 201+ Analyser and haemoglobin level (Hb) read and recorded in g/dl. The readings were subsequently classified as normal, mild, moderate or severe anaemia based on the WHO (2011), recommended cut off points.

3.15 Data analysis

The data collected from both laboratory and closed ended questionnaires were recorded and analysed using Statistical Package for Social Sciences (SPSS) version 22.0 computer software. The association between variables was tested using Chi-Square. Odds ratio (OR) was used to assess the strength of association between various risk factors and *Plasmodium* infection. A difference giving a p-value <0.05

was considered statistically significant. Correlation coefficient (r) was the main statistical tool used to test the relationship between use of insecticidal nets and malaria prevalence.

3.16 Variables

Dependent variables in this study were *Plasmodium* infection among school children and blood haemoglobin level. Use of ITNs, school, age and sex constituted independent variables.

3.17 Ethical consideration and clearance

Kenyatta University based Ethical Review Committee issued a clearance certificate for the study after receiving a research authorization letter from the Graduate School (Appendix VIII and IX). The researcher also obtained permission from Homa-Bay County director of education (Appendix X) and National Commission for Science, Technology and Innovation (Appendix XI) respectively. Authorization from Head teachers of the schools concerned and consent from the parents/guardians of participating children was obtained. On the survey day, the researcher informed all children in the school about the sampling and survey procedures, making it clear to them that their participation was voluntary and that they may opt out of the testing at any time if they choose to. Individual assent was also obtained from each child before participation in the study.

CHAPTER FOUR

RESULTS

4.1 Socio-demographic characteristics of study participants

This survey included school children from Kalando, Kokwanyo, Agawo, Umai, Andingo, Nyawango, Omiro, Got and Manga primary schools in Kasipul. Fifty-two parents/guardians out of four hundred and fifty refused to allow their children to participate in this study by refusing to sign informed consent form. Of the total 398 pupils studied, male were 206 (51.76%) and female were 192 (48.24%). The minimum age was 9 years and maximum age was 18 years (Table 4.1). Each of the three hundred and ninety-eight pupils sampled filled a self-administered questionnaire before malaria and anaemia testing.

Table 4.1 Demographic characteristics of study participants

Variable	Attribute	Number	Percentage (%)
Sex	Male	206	51.76
	Female	192	48.24
Age group (years)	9-11	94	23.62
	12-14	238	59.8
	15-17	64	16.08
	Above 17	2	0.5
School	Kalando	42	10.55
	Kokwanyo	45	11.31
	Agawo	44	11.6
	Umai	49	12.31
	Andingo	39	9.8
	Nyawango	49	12.31
	Omiro	44	11.08
	Got	43	10.8
	Manga	43	10.8

4.2 Prevalence of *Plasmodium* by age and sex of participants

Analysis of blood smears from study participants revealed an overall *Plasmodium* prevalence of 10.05 %, (40 out of 398 children positive for malaria), while 358 (89.9%) were negative, for blood parasite, as they had no malaria parasite in their blood samples. Eighteen (8.74%) out of the 206 male children had malaria parasites

against 22 (11.46%) of the 192 females (Table 4.2). Pupils' gender had no significant effect on malaria prevalence ($\chi 2$= 0.814, df = 1, p = 0.367).

The highest infection rate of 11.4% was recorded in respondents in 12-14 age set, compared to 15-17 age groups who recorded 10.8% pupils testing positive for malaria parasites. Age group 9-11years recorded only 6.38% infection (Table 4.3). Although the study established that age set had no effect on malaria prevalence in Kasipul ($\chi 2$ =2. 131, df =3, p =0. 546), children aged 12 -14 were 1.10 times (95% CI: 0.46, 2.66) more likely to be infected with malaria parasite compared to children aged above 14 years of age.

Table 4.2 Prevalence of malaria parasite by age and sex of the children

Variable	**N**	**Malaria parasite infection**		**$\chi 2$ (df)**	***p*-value**	**OR**	**95%**
		Positive n (%)	**Negative n (%)**				CI
Sex							
Males	206	18 (8.7)	188 (91.3)	0.392	0.531	1.35	0.70-2.60
Female	192	22 (11.46)	170 (88.5)	0.421	0.516	1	
Totals	**398**	**40 (10.05)**	**358 (89.9)**	**0.814 (1)**	**0.367**		
Age (Years)							
9-11	94	6 (6.4)	88 (93.6)	1.398	0.237	0.59	0.19-1.83
12-14	238	27 (11.4)	210 (88.6)	0.473	0.492	1.10	0.46-2.66
15-17	66	7 (10.8)	60 (89.2)	0.2601	0.847	1	
Totals	**398**	**40 (10.05)**	**358 (89.9)**	**2.131 (3)**	**0.546**		

Overall *Plasmodium* prevalence was 10.05% and was slightly higher in females than males ($\chi 2$= 0.814, df = 1, p = 0.367).

4.3 Parasitological characteristics of the study site

Majority (92.5%) of the blood sample were found to have *P. falciparum*, while the remainder had *P. ovale* (7.5 %) as illustrated in table 4.3. The study established that *Plasmodium falciparum* was the most common malaria parasite species among children in Kasipul.

Table 4.3 Parasitological characteristics of the study site

Site	**Infection**	***Plasmodium* species**	
	N (%)	***P. falciparum***	***P. ovale***
Kalando	8 (19.5)	6	2
Kokwanyo	4 (8.9)	4	0
Agawo	1 (0.0)	0	0
Umai	10 (20.4)	9	1
Andingo	3 (7.7)	3	0
Nyawango	4 (8.2)	4	0
Omiro	4 (9.1)	4	0
Got	5 (14.0)	6	0
Manga	1(2.3)	1	0
TOTAL	**40 (10.05)**	**37**	**3**

Plasmodium falciparum was the most common malaria parasite in Kasipul.

4.4 ITN ownership and *Plasmodium* prevalence per school

Table 4.4 shows that children from Kalando primary school which registered the lowest ITN ownership (26.2%) were 9.9 times (95% CI: 1.18, 82.95) more likely to be infected with *Plasmodium* compared to children from Manga primary school.

Majority of schools, which recorded high ITN ownership, registered low *Plasmodium* prevalence among children.

Table 4.4 ITN ownership and *Plasmodium* prevalence per school

School	n	ITN ownership	Malaria	OR	95% CI		P-value
		n (%)	Positive No. (%)		Lower	Upper	
Kalando	42	11 (26.2)	8 (19.5)	9.88	1.177	82.95	**0.04**
Kokwanyo	45	33 (73.3)	4 (8.9)	4.10	0.439	38.23	0.22
Agawo	44	26 (59.1)	1 (2.27)	0.98	0.059	16.13	0.99
Umai	49	17 (34.7)	10 (20.4)	10.77	1.317	88.06	**0.03**
Andin'go	39	23 (59.0)	3 (7.7)	3.50	0.349	35.14	0.29
Nyawango	49	28 (57.1)	4 (8.2)	2.74	0.274	27.36	0.39
Omiro	44	15 (34.1)	4 (9.1)	4.20	0.450	39.20	0.21
Got	43	21 (48.8)	5 (14)	6.81	0.783	59.21	0.08
Manga	43	29(67.4)	1 (2.3)	1			
TOTAL	**398**	**203 (51.0)**	**40(10.05)**				

Children from Umai were 10.8 times (95% CI: 1.32, 88.1) more likely to be infected with *Plasmodium* compared to children from Manga.

4.5 Effects of bedtime on *Plasmodium* prevalence

Analysis of time for retiring to bed revealed that 11.9% of pupils who slept before 9.00 pm were positive for malaria parasite compared to 9.6% who retired to bed after 9.00pm (Table 4.5). Bedtime effects on *Plasmodium* prevalence was not statistically significant ($\chi 2 = 0.4051$, df =1**,** P =0.5245).

Table 4.5 Effects of bedtime on *Plasmodium* prevalence

Time (pm)	Number (%)	Malaria parasite		$\chi 2$ (df)	p-value
		Positive n (%)	Negative n (%)		
Before 9.00	84 (21.1%)	10 (25%)	74 (20.7%)	0.31957	**0.5245**
After 9.00	314 (78.9%)	30 (75%)	284(79.3%)	0.08549	
Total	**398 (100%)**	**40 (100%)**	**3589(100%)**	**0.40506 (1)**	

Going to bed before or after 9.00 pm had no effect on malaria prevalence among pupils ($\chi 2$= 0.4051, df =1, p =0.5245).

4.6 Pupils knowledge on proper usage of ITN

When respondents were asked about whether they have knowledge on proper usage of ITN, 324 (81.4%) responded yes, while 74 (18.6%) said they had not heard about ITN (Table 4.6).

Table 4.6 Pupils' knowledge on proper usage of ITN

Knowledge of ITN proper use	Number	Percentage
Yes	324	81.4
No	74	18.6

Majority of pupils (81.4%) who participated in this study had heard of ITN.

4.7 Proportion of pupils owning ITN in terms of sex

Table 4.7 indicates that 203 pupils representing 51.0 % of the study population owned ITN. However, with respect to sex 104 (54.2 %) females possessed ITN compared to

99 (48.1%) of males. Pupils gender did not affect ITN ownership (χ^2=1.484, df =1, p =0. 223).

Table 4.7 ITN ownership among pupils in terms of sex

Sex	N	ITN ownership		χ^2(df)	p-value
		Yes	No		
Male	206	99(48.1%)	107(51.9%)	0.7158	**0.223**
Female	192	104(54.2%)	88 (45.8%)	0.769	
Total	**398**	**203(51.0%)**	**195(49.0%)**	**1.484(1)**	

Slightly more female pupils owned nets compared to males, although the difference was not significant (χ^2=1.484, df =1, p = 0. 223).

4.8 Ownership, type and source of nets used by pupils in Kasipul

Analysis of data on overall net possession with a focus on type of net and source revealed that out of 250 respondents who had mosquito bed nets, 203 (81.2 %) had ITNs while 47 (18.8%) owned other types of bed nets. Among 203 pupils who reported to have ITN at home, 189 (93.1%) obtained them free from the government during the last mass distribution campaign, while 14 (6.9%) bought the nets as illustrated in the (Table 4.8). There was evidence of a significant association between type and source of net used by pupils (χ^2= 4.875, df = 1, p = 0.027).

Table 4.8 Ownership source and type of nets used by pupils

Type of net	Net ownership	Source		χ^2(df)	p-value
	n	Free	Bought		
ITN	203	189(93.1%)	14(6.9%)	0.9164	**0.027**
Others	47	39(83.0%)	8(17.0%)	3.9582	
Total	**250**	**228(91.2%)**	**22(8.8%)**	**4.875(1)**	

Majority of the pupils used ITNs given free by the government, only a few bought ITNs. Difference in source and type of net used was statistically significant (χ^2= 4.875, df = 1, p = 0.027).

4.9 Association between type of net used and *Plasmodium* infection

The study revealed that 3.9 % of primary school children who reported having ITN in their bedrooms were positive for malaria parasite compared to 2.3% reported for other types of nets (Table.4.9). Malaria parasite infection was not affected by type of net used by children (χ^2= 0.362, df = 1, p = 0.548).

Table 4.9 Association between type of net used and *Plasmodium* infection

Type of net	Number	*Plasmodium* infection		χ^2(df)	p-value
		POSITIVE	NEGATIVE		
ITN	203	8 (3.94%)	195 (96.01%)	0.362 (1)	0.548
Others	47	1 (2.13%)	46 (97.87%)		
Total	**250**	**9 (3.6%)**	**241(96.4 %)**		

Type of net used by children did not influence *Plasmodium* infection (χ^2= 0.362, df = 1, p = 0.548).

4.10 Proportion of ITNs use the night before the interview among children owning ITNs

To minimize recall bias on the usage of ITN at home, children were asked about the use of an ITN the night before the interview: one hundred and sixty-one (161) representing 79.3% of pupils with ITN used the nets on the eve of the survey, while the remaining forty-two representing 20.7 % failed to deploy the insecticidal nets (Table 4.10). The survey reported more females using insecticidal nets on the eve of the study compared to males; their proportions were 83.7% and 74.7% respectively. However, gender did not influence the use of insecticidal nets in this study (χ^2 = 2.452, df =1, p-value = 0.117).

Table 4.10 Proportion of ITNs used on the eve of the study among children who reported to own ITN.

ITN USE THE NIGHT BEFORE THE INTERVIEW					
Demographic characteristic	**n**	**Used n (%)**	**Not used n (%)**	**χ^2 (df)**	**p-value**
Sex					
Males	99	74 (74.7%)	25 (25.3%)	1.2561	0.2624
Female	104	87 (83.7)	17 (16.3)	1.1957	0.2742
Total	**203**	**161(79.3%)**	**42 (20.7%)**	**2.452(1)**	**0.117**
Age (years)					
9-11	52	43 (82.7%)	9 (17.3%)	0.36246	0.5471
12-14	126	97 (77.0%)	29 (23.0%)	0.41552	0.5192
15-18	25	21 (84.0 %)	4 (16 %)	0.33508	0.5627
Total	**203**	**161(79.3%)**	**42 (20.7%)**	**1.113 (2)**	**0.573**

Majority of the schoolchildren of all age sets and sex had used ITN the night before the interview ($\chi^2 = 2.452$, df =1, p-value = 0.117).

4.11 Ownership and usage of ITN among pupils

Out of those pupils who indicated that they deployed insecticidal nets on the eve of the survey, 152 (74.9%) hanged their nets daily on the bed while 51 (25.1%) did not (Table 4.11). Females were observed to use ITN slightly more than males; their proportions were 70.7% and 78.8 %, respectively ($\chi 2$ =1.786, df =1, p = 0. 181). Moreover, school children in age group 9-11 years were observed to use ITNs more (84.6 %) compared to the age group 12-14 years ($\chi 2$ =3.844, df =2, p =0. 146). There was no statistical significance in the observed difference for the use of ITN by age groups and sex.

Table 4.11 Ownership and usage of ITNs

Demographic Characteristic	Net ownership n	Daily use of ITN YES	NO	χ^2 (df)	p-value
Sex					
Males	99	70 (70.7%)	29 (29.3%)	0.9151	0.338
Females	104	82 (78.8%)	22 (21.2%)	0.871	0.3507
Total	**203**	**152 (74.9%)**	**51(25.1%)**	**1.786(1)**	**0.181**
Age					
9-11	52	44 (84.6%)	8 (15.4%)	2.6216	0.1054
12-14	126	89 (70.6%)	37 (29.4%)	1.2052	0.2723
15-17	25	19 (76.0%)	6 (24.0%)	0.0168	0.8969
Total	**203**	**152 (74.9%)**	**51(25.1%)**	**3.844(2)**	**0.146**

Both males and female school children of all the three age sets used the nets similarly. Use of bed net with respect to age sets was not statistically significant (χ^2= 3.844, df = 2**,** p = 0.146).

4.12 Association between daily ITN usage and *Plasmodium* prevalence among pupils

Majority of respondents (74%) reported using ITN daily. Only 3.3% of pupils using ITN daily were positive for malaria parasite. However, 7.8% of those who slept under insecticidal nets on the eve of the interview, but did not normally use the nets on a daily basis were positive for malaria parasites (Table 4.12). The findings of this study indicated that sleeping under ITN daily did not lower the risk of a child contracting malaria infection (χ^2= 1.869, df =1, p = 0.172).

Table 4.12 Association between daily ITN usage and *Plasmodium* prevalence among pupils

Daily ITN use	n	Malaria		χ^2 (df)	p-value
		Positive	Negative		
Yes	152	5(3.3%)	147(96.7%)	0.4695	**0.172**
No	51	4(7.8%)	47(92.2%)	1.3993	
Total	**203**	**9(4.4%)**	**194(95.6%)**	**1.869(1)**	

Daily use of ITN was not associated with reduced risk of *Plasmodium* infection (χ^2= 1.869, df =1, p = 0.172).

4.13 Duration of ITN use by pupils

Further assessments of net usage among pupils in Kasipul, revealed that 67.7% of the male pupils and 64.4 % of female pupils had been using ITN for more than four months. Table 4.13 illustrates that 12-14 age set had the highest proportion (72.0%) of pupils who had been using ITN for more than four months.

Table 4.13 Duration of ITN use by pupils

Demographic Characteristic	1 week to 1 months n (%)	2 to 3 months n (%)	More than 4 months n (%)	χ^2 (df)	P- value
Overall	42(20.7%)	27(13.3%)	134 (66.0%)		
Sex					
Males	22 (22.2%)	10 (10.1%)	67 (67.7%)	1.788(2)	0.409
Females	20 (19.2%)	17 (16.3%)	67 (64.4%)		
Age					
9-11	12 (22.6%)	9 (17.0%)	32 (60.4 %)	7.098(4)	0.131
12-14	21 (16.8%)	14 (11.2 %)	90 (72.0 %)		
15-18	9 (36.0 %)	4 (16.0 %)	12 (48.0 %)		

Most of the pupils (72.0%) who had been using ITN for more than four months were in 12-14 years age set. Difference in duration of ITN use in terms of age set was not statistically significant (χ^2= 7.098, df =4, p = 0.131).

4.14 Reasons why pupils avoided insecticidal nets

Among those pupils who possessed ITN but reported not using them the night before the survey, the main reasons given included: too hot (28.6%) and forgetting to hang the net (45.2%). Other reasons given were no mosquitoes (7.1%), and that the nets were dirty (9.5%) (Table 4.14).

Table 4.14 Reasons for not using a Bed net the night before the interview

Reason	Number	Per cent
Too hot	12	28.6
Forgot to hang the net	19	45.2
Difficult to hang the net daily	1	2.4
Dirty	4	9.5
Used mosquito coils	3	7.2
There were no mosquitoes	3	7.1
Total	**42**	**100**

Most pupils (45.2%) did not use ITN the because they forgot to hang them.

4.15 Overall usage of ITN by households in Kasipul

In 51.0 % of the households, all family members possessed and protected themselves using ITN, while in 29.4 % children lacked nets and only parents were protected from mosquito bites. None of the household members slept under ITN in 18.1 % of the households (Table 4.15).

Table 4.15 Use of Bed net by households

Net use in household	Number	Percentage
All household members using ITN	203	51.0
Children only	6	1.5
Parents only	117	29.4
None of the household members using ITN	72	18.1

None of the household members slept under a net in 18.1 % of the households.

4.16 Association between ITN ownership and malaria parasite prevalence

Only 4.4 % of blood sample from pupils owning ITN tested positive for *Plasmodium*, compared to 15.9% recorded from pupils not owning ITN. In Kasipul, owning ITN was significantly associated with a lower risk of malaria parasitaemia among pupils ($\chi 2$= 14.46, df =1, p = 0.00) (Table 4.16).

Table 4.16 Effects of ITN ownership on *Plasmodium* prevalence

ITN Ownership	**n**	**Malaria parasite infection**		**$\chi 2$ (df)**	***p*-value**
		Positive n (%)	**Negative n (%)**		
Yes	203	9(4.4%)	194(95.6%)	7.1714	**0.000**
Not	195	31(15.9%)	165(84.1%)	7.2878	
Totals	**398**	**40(10.05%)**	**358(89.9%)**	**14.46(1)**	

Increased ownership of ITN significantly reduced the risk of malaria among participants ($\chi 2$= 14.46, df =1, p = 0.00).

4.17 Correlation between daily use of ITN and *Plasmodium* prevalence

Analysis of data on bed net usage per school in relation to *Plasmodium* prevalence revealed that bed net usage and *Plasmodium* infection rate had a negative correlation (r = -0.592, p= 0.093). Although not significant, malaria prevalence was higher among pupils not using ITN daily (Table 4.17 and Figure 4.1).

Table 4.17 Correlation between daily use of ITN and *Plasmodium* prevalence per school

Locality		School ITN possession	ITN in Daily use	Malaria prevalence		
	n	No.	No.	(%)	Correlation (r)	P-value
Kalando	42	11	8	19.05	-0.592	0.093
Kokwanyo	45	33	24	8.89		
Agawo	44	26	20	0.00		
Umai	49	17	11	20.41		
Andingo	39	23	19	7.69		
Nyawango	49	28	23	8.16		
Omiro	44	15	4	9.09		
Got	43	21	18	13.95		
Manga	43	29	25	2.33		

Increased use of ITN reduced prevalence of *Plasmodium* among pupils ((r = -0.592), though not significant

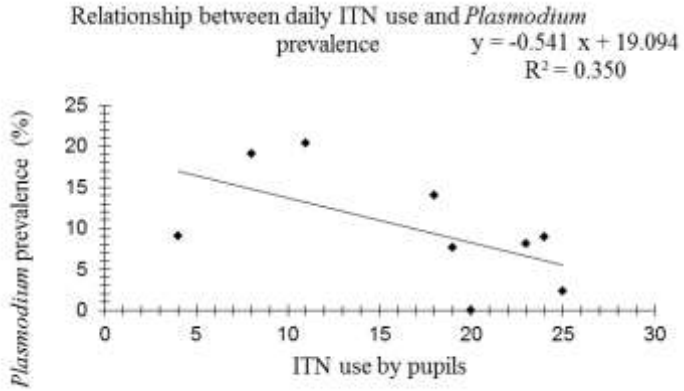

Figure 4.1 Correlation between ITN daily use and *Plasmodium* infection

4.18 Correlation between overall ITN use and *Plasmodium* prevalence

Data on overall ITN use and *Plasmodium* prevalence per school revealed a negative correlation which was not significant (r = -0.3874; p = -0.3029). Increased use of ITN resulted in a reduction in malaria prevalence among pupils (Table 4.18). However the slope of the regression line (- 0.612) was not significantly different from zero (Figure 4.2).

Table 4.18 Correlation between overall ITN use and *Plasmodium* prevalence per school

School	n	ITN usage n.	Malaria prevalence (%)	Slope	r	p-value
Kalando	42	11	19.05	-0.612	-0.3874	0.3029
Kokwanyo	45	33	8.89			
Agawo	44	26	2.28			
Umai	49	17	20.41			
Andingo	39	23	37.69			
Nyawango	49	28	8.16			
Omiro	44	15	9.09			
Got	43	21	13.95			
Manga	43	29	2.33			

Overall ITN use and malaria prevalence per school had a negative correlation (r = -0.3874). In most schools, increased use of ITN reduced prevalence of malaria.

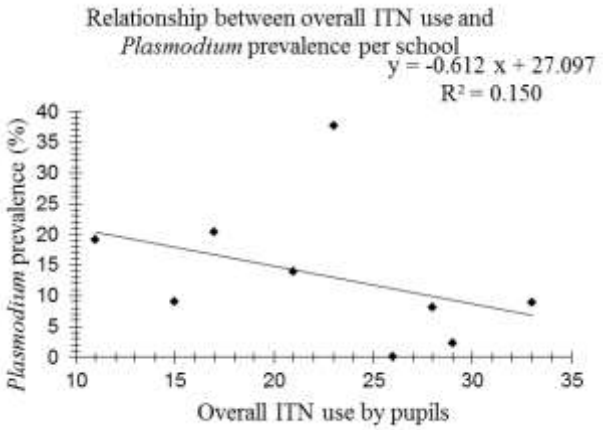

Figure 4.2 Correlation between overall ITN use and *Plasmodium* prevalence per school

4.19 Anaemia prevalence among school pupils by age set and sex

The overall prevalence of anaemia (haemoglobin level <11 g/dl) was 2.3 %. The mean haemoglobin level was 12.78 g/dl. Analysis of haemoglobin levels by age groups revealed that 15-17 years age set had the highest prevalence of anaemia at 4.6%, followed by 9-11 years age set (3.2 %) (Table 4.19). The study established that age group was not a significant factor influencing haemoglobin level among participants ($\chi 2$= 3.107, df = 3, P = 0.375). Further analysis of haemoglobin based on gender revealed that, girls had a 3.6% prevalence of anaemia, which was slightly higher than 1.0% for boys, although the difference was not statistically significant ($\chi 2$= 3.217, df =1, *P*= 0.073).

Table 4.19 Anaemia incidence by age and sex

Gender	Anaemia		Totals	χ2 (df)	*p*-value
	Positive n (%)	Negative n (%)	N		
Male	2 (1.0%)	204 (99.0%)	206	1.5521	0.2128
Female	7 (3.6%)	185 (96.4%)	192	1.6653	0.1969
Total	**9 (2.3%)**	**389 (97.7%)**	**398**	**3.217(1)**	**0.073**
Age set (Years)					
9-11	3 (3.2%)	91(96.8%)	94	0.36799	0.5441
12-14	3 (1.3%)	234(98.7)	237	1.06265	0.3026
15-17	3 (4.6%)	62 (95.4%)	65	1.62978	0.2017
Above 17	0 (0.0%)	2 (100%)	2	0.04628	0.8297
Total	**9 (2.3%)**	**389 (97.7%)**	**398**	**3.107 (3)**	**0.375**

Anaemia was slightly more prevalent in females (3.6%) than male pupils (1.0%). All age sets were equally predisposed to anaemia ($\chi 2 = 3.217$, df=1, $P = 0.073$).

4.20 Effects of ITN ownership on anaemia prevalence

It was found that 3.1% of the pupils suffering anaemia did not own ITN, compared to 1.5% who reported to possess, bed nets, the observed difference was not influenced by ITN ownership ($\chi 2 = 1.151$, df = 1, p 8= 0.283) (Table 4.20).

Table 4.20 Effects of ITN ownership on anaemia prevalence

ITN ownership	Anaemia		Totals	χ2 (df)	*p*-value
	Positive n (%)	Negative n (%)	N		
Yes	3(1.5%)	200(98.5%)	203	0.56379	**0.283**
No	6(3.1%)	189(96.9%)	195	0.58692	
Totals	**9(2.3%)**	**389(97.7%)**	**398**	**1.151(1)**	

ITN ownership did not significantly affect anaemia prevalence ($\chi 2 = 1.151$, df = 1, p-value 0.283).

CHAPTER FIVE
DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

5.1.1 Prevalence of malaria among primary school children in Kasipul

This survey of 398 primary school children in Kasipul reported a 10.05% prevalence of *Plasmodium*, which was high compared to other parts of the country. Similar primary school malaria surveys in Kasipul, reported a prevalence of 25.8% in 2011 (Bousema *et al.*, 2013). The decrease in prevalence in the present study may be conceivably explained to be due to the effects of mass distribution of free ITNs. Kyama *et al.* (2007) noted that ITNs nets kill and repel mosquitoes within an environment. The study revealed that enhanced insecticidal net ownership, reduced *Plasmodium* prevalence, thus, the null hypothesis that *Plasmodium* infections are still highly prevalent after mass distribution of insecticide treated mosquito bed nets among primary school children in Kasipul was rejected.

Children aged 12-14 (11.4%) years were slightly predisposed to malaria, than children older than 15 years (10.8%) and malaria was slightly higher among female children (11.5%) compared to males (8.7%). Similar studies in Bumula, western Kenya, also revealed that malaria was more prevalent in primary school girls compared to boys (Kepha *et al.*, 2016). Girls assist their mothers in domestic activities at home until late hours of the night exposing themselves to more mosquito bites than boys (Nzobo *et al.*, 2015). Studies in Nigeria recorded low prevalence in 15-16 years age group. *Plasmodium* prevalence in both sexes followed a similar pattern; and infection rate in terms of sex was found not to be significant and therefore not sex dependent (Ani, 2004).

The predominant malaria parasite in Kasipul was *P. falciparum* (92.5%) majority of the blood samples, which were positive for malaria parasite were positive for *P. Plasmodium*. Studies in Umuchieze region of Nigeria also revealed that *P. falciparum* is the most common malaria species with an infection rate of 72.09% (Kalu *et al.*, 2012). In Ghana, studies indicate that, *P. falciparum* account for 95.9% all cases of parasitaemia, while *P. ovale* account for only 0.2% (Sarpong *et al.*, 2015). In Kenya, *Plasmodium falciparum* is the most prevalent malaria parasite species at 96 per cent, of which 16 per cent comprises mixed infections with *P. ovale*, *P. malariae* or both (MOH, 2011).

5.1.2 Relationship between ITNs use and malaria parasite infection in Kasipul

Despite about 80% of the pupils using nets daily, overall household utilization of the ITNs was 51%, which is far below the universal bed net programme target, which was expected to increase household ITNs use by 80% in Kenya (Ototo *et al.*, 2015). The findings are comparable to those presented by Atieli *et al.* (2011) who reported that only 53% of households protect themselves using free insecticidal nets in Emuhaya district. The current study revealed that, in about 30% of the households; only parents used ITNs; while in fewer cases the children were given preference of using nets. Among the nine schools sampled, Kokwanyo Primary school had the highest overall pupil net possession of 73.3% compared to the least in Kalando primary. From the study results, both boys and girls owned nets similarly, Similar to previous reports in Nigeria (Garley, 2013).

The present study recorded only 3.3% of blood samples from primary school children who protect themselves with ITNs nets and were positive for malaria parasite as

opposed to 7.8% who did not use and were positive for malaria. Studies indicate that *Anopheles arabiensis* is a common malaria vector in Kasipul (Bousema *et al.*, 2013). Insecticide treated nets are indoor malaria control tools and mainly target indoor biting and resting species of *Anopheles* mosquitoes. They are ineffective in controlling vectors that rest and bite outdoor (Sangoro *et al.*, 2014). The recorded 3.3% infection in this study may be due to *Plasmodium* parasite inoculations outside the house by *Anopheles arabiensis*. Unlike *Anopheles gambiae*, *Anopheles arabiensis* readily feeds and rests outside as well as indoors (Mayagaya *et al.*, 2015). In Kilombero Valley in Tanzania, despite high coverage and usage of ITNs, *Anopheles arabiensis* has continued to maintain intense transmission of malaria over several years (Killeen *et al.*, 2016).

The study recorded non-significant negative correlation between malaria prevalence and ITNs use among pupils indicating that enhanced use of nets slightly reduced malaria prevalence among pupils. The null hypothesis that there was no relationship between ITNs use and malaria prevalence was subsequently accepted. However, the slope of the regression line indicated that pupil use of ITNs alone was probably not the only factor responsible for the observed reduction in *Plasmodium* prevalence among the participants. The study found household ITNs possession of about 50% and the observed decline in malaria prevalence in the area can be partly attributed to community-wide effects of ITNs. Reports from other African countries indicate that community-wide effect of ITNs enables them to offer protection even to those individuals not owning or using these nets (Maxwell *et al.*, 2006).

5.1.3 Prevalence of anaemia among primary school children in Kasipul

The overall prevalence of anaemia in primary school children was 2.3%, with an average haemoglobin concentration of 12.78 g/dl. This was a significantly lower prevalence compared to a WHO estimate of 27% anaemia prevalence in developing countries (Tatala *et al.*, 1998). Although not significant, slightly more females (3.6%) were positive for anaemia compared to males (1.0%). These findings are consistent with those from Abudayya (2007) who also found out that the prevalence of anaemia was higher in girls in Gaza than boys. However, net ownership effect on anaemia prevalence was insignificant, probably because anaemia results from complicated malaria cases which were reduced by ITNs interventions thus, the null hypothesis that anaemia is still highly prevalent after mass distribution of insecticide treated mosquito bed nets among primary school children in Kasipul was accepted.

In terms of age, the study revealed that anaemia prevalence was higher (4.6%) in the 15-17 age groups compared to 9-11 age set. This supports the findings of another study in African countries which reported higher anaemia prevalence among adolescents living in Sub-Saharan Africa (Elder and Ranson, 2003). There was no significant association of anaemia with possible malaria risk factors such as status of using ITNs, age and sex.

5.2 Conclusions

Based on the data obtained from this survey, the following conclusions were drawn:

i. This study established that the overall prevalence of malaria among primary school children was 10.05%. Malaria infection was found to be most prevalent among 12-14 years old, while age group 9-11 had the least infection. Both

male and female children were similarly predisposed. *Plasmodium falciparum* was found to be the most predominant, species of malaria parasite in the study population.

ii. Only 2.3% of Kasipul school children suffered from anaemia and the disease was slightly more prevalent in girls than boys. This low prevalence of anaemia compared to other developing countries could be attributed to decrease in severe malaria of which there were no such cases detected in this study.

iii. The results of this study have shown an average (51%) coverage of ITNs and moderate use (74.9%). Only 18.8% of net in use were not ITNs. Bed net ownership was not affected by gender. Increased ITN ownership among participants significantly reduced *Plasmodium* prevalence. Schools, such as Kokwanyo and Manga, which had a large number of pupils owning ITNs, recorded lower prevalence of malaria infections compared to schools with low bed net possession such as Umai and Kalondo.

The significant decline in malaria prevalence observed in this study provides evidence supporting the health benefits of ITNs use in Kenya. According to the findings from this study, malaria infection has dropped from 25 % in 2011 to 10.05% in 2016, following free distribution of ITNs in 2014 by Kenya government agencies, to replace ITNs distributed in 2011. Finally, the study has revealed that outdoor inoculation of malaria parasite may be going on despite high ITNs coverage as indicated by the presence of *Plasmodium* in 3.3 % of blood samples of pupils using ITNs daily.

5.3 Recommendations

5.3.1 Recommendations from the study

i. The study recorded moderate decrease in malaria prevalence following mass distribution of ITNs from 25.8% in 2011 to 10.05% recorded in this survey. However, prevalence of 10.05 per cent is still high compared to 5 per cent in the coastal endemic zone, 3 per cent in the highland epidemic zone, and less than 1 per cent in semi-arid areas reported in MOH (2015) report. Both national and county government should also promote other control strategies apart from ITNs.

ii. The ITNs interventions have significantly reduced anaemia prevalence, in the study area from 14 % in 2010 to 2.3% in 2016. This can be attributed to the reduction of severe malaria infections among the school children in Kasipul. The ministry of health and other stakeholders should ensure that other causes of anaemia should be checked and pupils treated to eliminate the anaemia cases.

iii. Data on bed net usage and malaria infection indicated that only 3.3 % of pupils using ITN daily were infected with *Plasmodium*. There is a need to intensify health education on the benefits of ITNs to create an understanding of the connection between malaria and mosquitoes. In addition, campaigns on the importance of sleeping under the net daily should be carried out to increase use compliance and the benefits of malaria reduction.

5.3.2 Recommendations for further studies

More studies on the prevalence of malaria in children, using households as sampling units need to be conducted in Kasipul; practically, school survey can report lower malaria prevalence because it does not capture pupils absent from school due to illness, which may include malaria. Use of a light microscope in *Plasmodium* detection during the study probably resulted in underestimating *Plasmodium* infection rates, especially in cases where pupils had low parasitaemia. Future *Plasmodium* prevalence survey in the study area should use a method that is more sensitive such as PCR-based techniques to provided true malaria parasite prevalence in Kasipul.

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APPENDICES

APPENDIX I. CONSENT FORM

This informed consent form is for parents of pupils participating in the research titled: **EFFECTS OF LONG-LASTING INSECTICIDE TREATED BED NETS ON MALARIA PREVALENCE IN SCHOOL CHILDREN IN KASIPUL, HOMA-BAY COUNTY, KENYA**

Name of Principal Investigator: **OMONDI ROBERT.**

Name of University: **KENYATTA UNIVERSITY.**

This informed consent form has two parts:

- Information Sheet (to share information about the study with you)
- Certificate of Consent (for signatures if you agree that your child may participate)

You will be given a copy of the full Informed Consent Form

Part I: Information Sheet

Introduction

Greetings! My name is **Omondi Robert** from Kenyatta University, School of Pure and Applied Sciences, MSc student in Applied medical Parasitology. I am conducting a research project with the objective of investigating "**Effects of long-lasting insecticide treated bed nets on malaria prevalence in school children in Kasipul, Homa-Bay County, Kenya."**

Study Purpose.

The study is intended to collect information about proportional of malaria parasitemia, anaemia and use of ITN among school children in the constituency. Findings from the

study will help to map the current situation of the problem and strengthen the malaria control measures in this county.

Selection of Participants

Your child has been chosen to participate in this study because she/he has been living in Kasipul Constituency, Homa-Bay County, which has very high prevalence of malaria (40%) in Kenya.

Voluntary Participation

Please note that your participation in this study is entirely voluntary and you have a right to refuse to participate. If you agree to take part, you have the right to withdraw from study at any time if you wish to do so, without giving a reason. Your decision to withdraw will not affect anything on the relationship between you and the researcher or any other person who is involved in this study.

Benefits

If you agree to participate in this study there may be direct and indirect benefit to you. The direct benefit is that once your child is found either infected with malaria parasite or having low haemoglobin levels (anaemia) that requires medical attention, he/she will be referred to the nearby health facility for appropriate treatment. You will also be notified if your child does not require medical attention.

Procedure

1. Your daughter/son will fill out a questionnaire which will be provided by the researcher and collected after the investigation.
2. Provide a finger prick blood sample for malaria and anaemia investigation at the District Hospital laboratory.

Risks and Discomforts

I assure you that no harm will be expected to happen to your child because of participation in this study however during finger prick one may feel some slight discomfort.

Reimbursements

Your daughter/son will not be provided with any payment to take part in the study.

Confidentiality:

All information collected from this study will be kept confidential and no one will be told on what you have said, your identity and laboratory findings of the sample taken from your child. Only people working in this study will have access to the information and laboratory findings.

Who to Contact: If you ever have questions about this study, you may contact the study Principal Investigator: **Omondi Robert, Kenyatta University School of Pure and Applied Sciences. P. O. BOX 43844 Nairobi. Tel. 0722698303**

PART II: **Certificate of Consent**

I have been asked to give consent for my daughter/son to participate in this research study which will involve her completing one questionnaire and finger prick. I have read the foregoing information, or it has been read to me. I consent voluntarily for my child to participate in the study.

Print Name of Parent or Guardian …….

Signature of Parent or Guardian…….

Date…….

Day/Month/Year

If a participant cannot read:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to allow his/her child to take part in the research.

Signature of Witness……Name……. Relationship……

Signature of researcher or his assistant……

Date of signed consent…….

APPENDIX II. QUESTIONNAIRE

Serial No. ☐

Date _____/____/ 2015

PART A. Student's Particulars

1.0. School's name ……..

2.0. Name of the child: ……....

3.0. Sex: Male ☐ Female ☐ Age ☐ Class ☐

4.0. Place of residence……Duration of stay……

PART B: Answer by indicating YES or NO in the space provided

1. What time of the night do you normally go to sleep

…….

2. Have you ever heard of ITN? ……

3. How are they used?.....

4. Do you have mosquito bed net at home?

……...

4. What type of mosquito bed net do you have at home?

1. Insecticide treated net ☐ 2. Other type of net ☐

5. Where did your parent get the bed net you are currently sleeping under?

1. They were given free 2. ☐ It was bought from a shop/supper market ☐

6. What is the colour of your bed net?

1. Green. ☐ 2. White. ☐ 3. Blue. ☐

7. What is the shape of mosquito bed net you are using currently?

1. Round at the top ☐ 2. Square at the top. ☐

8. Did you cover yourself with net last night while sleeping?

1. Yes ☐ 2. No ☐

9. If **no,** give reasons why?

1. It very hot to use it ☐

2. I do not have a mosquito net ☐

5. I forgot to hang my net ☐

6. It is difficult to hang the net daily ☐

7. It was dirty ☐

8. I used a mosquito coil ☐

9. There were no mosquitoes ☐

10. If **yes,** do you always sleep under a mosquito Bed net?

1. Yes 2. No ☐ ☐

11. How long have you been using the net?

……

12. Do you have brothers and sisters have nets at home?

1. Yes 2. No ☐ ☐

13. If yes, did they also sleep under mosquito bed nets at night?

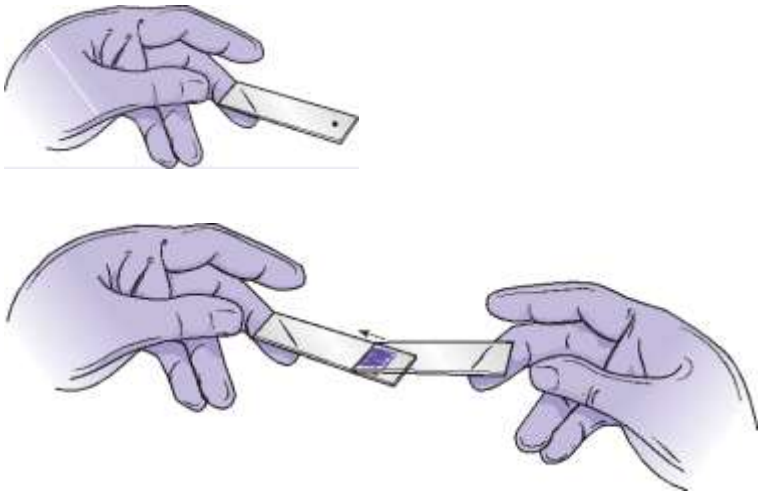
1. Yes 2. No ☐ ☐

14. Do your parents also sleep under a mosquito bed net at home?

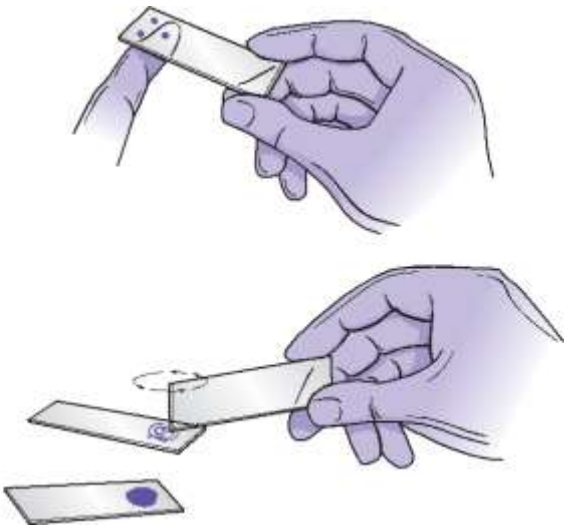
1. Yes 2. No ☐ ☐

APPENDIX III. PROCEDURE FOR MAKING BLOOD SMEAR

A) Preparation of thin blood smear



B) Preparation of thick blood smear



SOURCE : Ruth and Russel, 2012

APPENDIX IV. PROCEDURE FOR STAINING BLOOD FILMS

1. Fix the dried slide in methanol for 2 seconds
2. Place the slide in staining dish full of stain
3. Stain for forty min
4. Use water flotation to Remove the ‘scum’ and rinse the slide in water
5. Remove slides air and air dry
6. Observe under high power

APPENDIX V. *PLASMODIUM* SPECIES IDENTIFICATION GUIDE

Finding	***P. falciparum***	***P. vivax***	***P. malariae***	***P. ovale***
RBC size	Not enlarged	Enlarged	Not enlarged	Enlarged
RBC Shape	Round, sometimes crenated	Round or oval, frequently bizarre	Round	Round or oval, often fimbriated
Stipling	Maurer's spots, appear as large red spots, loops and clefts; up to 20 or fewer.	Schuffner's dots, appear as small red dots, onumerous.	Ziemann's dots, few tiny dots, rarely detected.	Schuffner's dots (James's dots). Numerous small red dots.
Pigment	Black or dark brown; in asexual forms as one or two masses; in gametocytes as about 12 rods	Seen as a haze of fine golden brown granules scattered through the cytoplasm	Black or brown coarse granules; scattered	Intermediate between *P. vivax* and *P. malariae*
Early trophozoite (ring)	Smallest, delicate; sometimes two chromatin dots; multiple rings commonly found	Relatively large; one chromatin dot, sometimes two; often two rings in one cell	Compact; one chromatin dot; single	Compact; one chromatin dot; single
mSchizont	Medium size; compact; numerous chromatin masses; coarse pigments; rarely seen in peripheral blood	Large; amoeboid; numerous chromatin masses; fine pigments	Small; compact; few chromatin masses; coarse pigments	Medium size; compact; few chromatin masses; coarse pigments
Gametocyte	Cresent shaped, larger and slender; central chromatin	Spherical; compact	Similar to *P. vivax*, but smaller and less numerous	Like *P. vivax*, but smaller

(Source**:** Microscopic, 2013)

APPENDIX VI. LABORATORY FORM

Form serial No…… (Should be the same as a questionnaire)

Name of pupil…… Age……

Class……..

Blood Sample Results

1. Haemoglobin level (g/dl) ☐

2. Blood results for malaria parasites

1. Positive ☐

2. Negative ☐

5. Parasites species detected

……..

(To be completed by the researcher and medical laboratory technologist only)

APPENDIX VII. HEMOCUE HB PROCEDURE FOR HB DETERMINATION

1. Put on gloves. Observe Standard Precautions.
2. Turn on the HemoCue Hb machine.
3. Enter Operator ID.
4. Run LQC if it has not been performed.
5. To run a patient, press the microcuvette symbol.
6. The Analyser will display “Please Fill and Insert a cuvette”.
7. Remove a microcuvette by sliding it out onto a clean surface.
8. Sample obtained by fingers prick:
    a. Wipe away the first drop of blood.
    b. Apply light pressure to the finger until a drop of blood forms. Do not squeeze the finger.
    c. Hold the microcuvette with the pointed end away from you.
    d. Touch the pointed tip of the microcuvette to the drop of blood.
    e. Allow the microcuvette to fill by capillary action.
9. Gently wipe the outside of the microcuvette with a Kimwipe or gauze using a sideways motion.
10. Examine the microcuvette for bubbles. If bubbles are present, use a new microcuvette and repeat sampling.
11. Place the filled microcuvette in the black cuvette holder and push the.

cuvette holder to the “MEASURING” position.

12. Upon completion, the Analyser will beep and the patient test results will

be displayed in g/dL. If the results are acceptable, press OK

APPENDIX VIII. ETHICAL APPROVAL LETTER

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE

Email: chairman.kuerc@ku.ac.ke
secretary.kuerc@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844 - 00100 Nairobi
Tel: 87105071/15
Fax: 8711243/8711575

Our Ref: KU/R/COMM/51/710

Date: 25th May, 2016

Robert Omondi
Kenyatta University,
P.O Box 43844,
Nairobi

Dear Omondi

RE: APPLICATION PKU/419/1388- "EFFECTS OF LONG LASTING INSECTICIDE TREATED BED NETS ON MALARIA PREVALENCE IN SCHOOL CHILDREN IN KASIPUL, HOMA-BAY COUNTY, KENYA."- VERSION 2

1. IDENTIFICATION OF PROTOCOL
The application before the committee is with a research topic "Effects of long lasting insecticide treated bed nets on malaria prevalence in school children in Kasipul, Homa-Bay County, Kenya." - Version 2
2. APPLICANT
Robert Omondi
3. STUDY SITE
Kasipul, Homabay County, Kenya
4. DECISION
The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines AND APPROVED that the research may proceed for a period of ONE year from 23rd May, 2016.

5. ADVICE/CONDITIONS
i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this board immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the KU/ERC/ number above.
If you accept the decision, proceed and advice and conditions given please sign in the space provided below and return to KUERC a copy of the letter

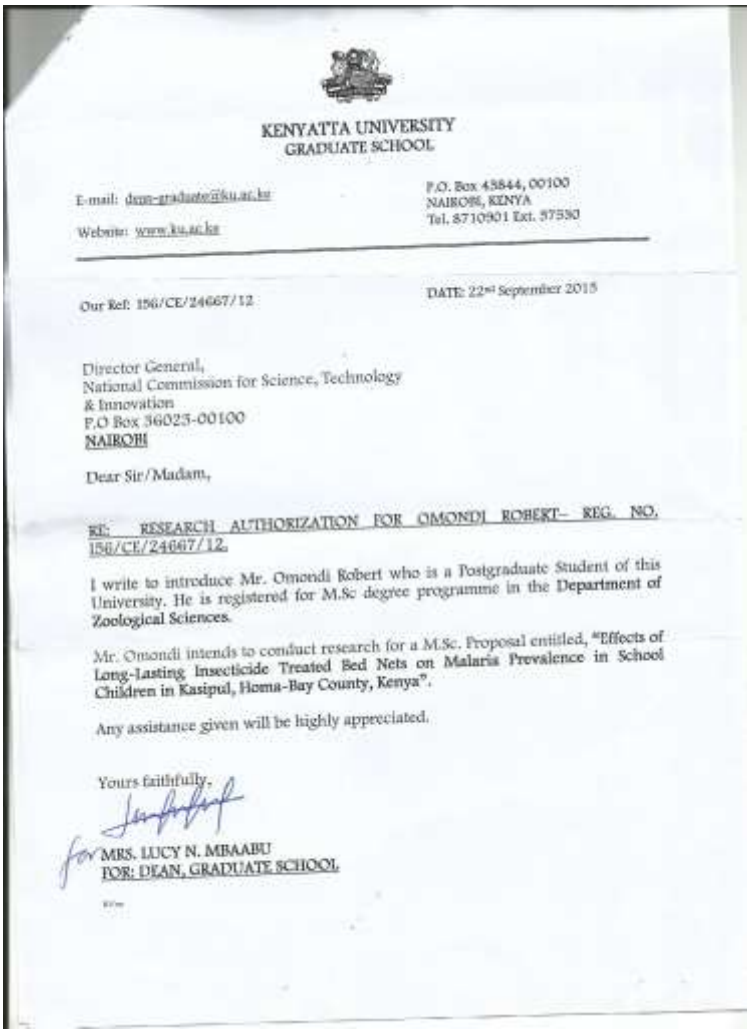
DR. TITUS KAHIGA
CHAIRMAN ETHICS REVIEW COMMITTEE

I [illegible] accept the advice given and will fulfill the conditions therein.

Signature ..... Dated this day of 25/5/ ..... 2016.

cc. Vice-Chancellor
DVC-Research Innovation and outreach

APPENDIX IX. RESEARCH AUTHORIZATION LETTER



KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: 156/CE/24667/12

DATE: 22nd September 2015

Director General,
National Commission for Science, Technology
& Innovation
P.O Box 36023-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR OMONDI ROBERT– REG. NO. 156/CE/24667/12.

I write to introduce Mr. Omondi Robert who is a Postgraduate Student of this University. He is registered for M.Sc degree programme in the Department of Zoological Sciences.

Mr. Omondi intends to conduct research for a M.Sc. Proposal entitled, "Effects of Long-Lasting Insecticide Treated Bed Nets on Malaria Prevalence in School Children in Kasipul, Homa-Bay County, Kenya".

Any assistance given will be highly appreciated.

Yours faithfully,

for MRS. LUCY N. MBAABU
FOR: DEAN, GRADUATE SCHOOL

APPENDIX X. MINISTRY OF EDUCATION AUTHORIZATION LETTER

MINISTRY OF EDUCATION, SCIENCE & TECHNOLOGY
State Department of Education

E-mail: rachuonyosoutheduc.office@gmail.com
Telephone 05931267

when replying please quote

REF: RACH/ADM/18/VOL.II/210

SUB COUNTY EDUCATION OFFICE,
RACHUONYO SOUTH SUB COUNTY
P.O. Box 178,
OYUGIS

25/02/2016

All Head Teachers,
Primary Schools,
Kaipul.

RE: RESEARCH AUTHORIZATION

Reference is made to National Commission for Science, Technology and Innovation letter Ref. No. NACOSTI/P/16/64632/9312 dated 23/2/2016 authorizing **Omondi Robert Omballa** of Kenyatta University to carry out research on **"Effects of long-lasting insecticide treated bed nets on malaria prevalence in school children in Kaipul, Homa-Bay County, Kenya"** from the month of March, 2016 to February, 2017

The purpose of this letter is therefore to request you to give Mr. Robert Omondi Oballa maximum cooperation while carrying out research in your schools.

Thank you.

SUB COUNTY DIRECTOR OF EDUCATION
RACHUONYO SOUTH
25.2.2016

M. A. MIRUKA,
FOR: SUB-COUNTY DIRECTOR OF EDUCATION,
RACHUONYO SOUTH SUB COUNTY.

APPENDIX XI. RESEARCH PERMITS AND HOSPITAL AUTHORIZATION LETTER

THIS IS TO CERTIFY THAT:
MR. OMONDI AUGUST OMBALLA
of KISII UNIVERSITY, 159-40200
KISII,has been permitted to conduct
research in Homa Bay County

on the topic: EFFECTS OF
LONG-LASTING INSECTICIDE TREATED
BED NETS ON MALARIA PREVALENCE IN
SCHOOL CHILDREN IN HASPUL,
HOMA BAY COUNTY, KENYA

for the period ending:
23rd February,2017

Applicant's
Signature

Permit No : NACOSTI/P/16/4833/9513
Date Of Issue : 23rd February,2016
Fee Received :Ksh 1000

The Director General
National Commission for Science,
Technology & Innovation

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before commencing on your research. Failure to do that may lead to the cancellation of your permit
2. Government Officers will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Prevention, Heating and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one(1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

National Commission for Science,
Technology and Innovation

RESEARCH CLEARANCE
PERMIT

Serial No.A 7851

CONDITIONS: see back page

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471,
2241349,310571, 2219420
Fax: +254-20-318245, 318249
Email: secretary@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/16/64632/9312**

Date:
23rd February, 2016

Omondi Robert Omballa
Kenyatta University
P.O. Box 43844-01000
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Effects of long-lasting insecticide treated bed nets on malaria prevalence in school children in Kasipul, Homa-Bay County, Kenya"*** I am pleased to inform you that you have been authorized to undertake research in **Homa Bay County** for a period ending **23rd February, 2017.**

You are advised to report to **the County Commissioner and the County Director of Education, Homa Bay County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

DR. S. K. LANGAT, OGW
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Homa Bay County.

The County Director of Education
Homa Bay County.

COUNTY GOVERNMENT OF HOMABAY

MINISTRY OF HEALTH

KOKWANYO HEALTH CENTRE

P.O. BOX 189 ,

KADONGO.

21ST March, 2016

TO WHOM IT MAY CONCERN:

RE: ROBERT OMONDI

This is to certify that the above named was hereby authorized to carry out a research project at our facility on **"EFFECTS OF LONG-LASTING INSECTICIDE BED NETS ON MALARIAL PREVALENCE IN KABONDO –KASIPUL HOMA-BAY COUNTY."** From 15th March, 2016 to 17th March,2016 and was successful.

PATRICIAH ONGAKI

0728757157

OFFICER INCHARGE

MINISTRY OF HEALTH
KOKWANYO HEALTH CENTRE
DATE

KOKWANYO HEALTH CENTRE