

**PREVALENCE OF *PLASMODIUM* SPECIES INFECTION AMONG PRIMARY
SCHOOL CHILDREN AND PERFORMANCE OF MALARIA RAPID DIAGNOSTIC
TEST KITS IN BARINGO COUNTY, KENYA.**

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DECLARATIONS

Declaration by candidate

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

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DEDICATION

This thesis is dedicated to my lovely parents Jacob Midigo and Elizabeth Midigo; they are my strength in everything I do.

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ACRONYMS AND ABBREVIATIONS

ACT	Artemisinin-based Combination Therapies
CI	Confidence Interval
ELISA	Enzyme-linked Immunosorbent Assay
HRP	Histidine Rich Protein
IFA	Immunofluorescence Antibody Assay
IRS	Indoor Residual Spray
IPT	Intermittent Preventive Treatment
ITNs	Insecticide Treated Nets
MOH	Ministry of Health
LR	Likelihood Ratios
MOPHS	Ministry of Public Health and Sanitation
NPV	Negative Predictive Value
pLDH	Parasite Lactate Dehydrogenase
PPV	Positive Predictive Value
RDT	Rapid Diagnostic Kits
SD	Standard Diagnostic
WHO	World Health Organization

DEFINITION OF TERMS

Asymptomatic	Infected individuals with no clinical signs and symptoms
Incidence	The rate of newly diagnosed cases of a disease
McNemar	A test of difference between two paired or repeated samples
Negative predictive value	The proportion of patients who test negative who are actually free of the disease
Prevalence	The actual number of cases alive with the disease during a period of time
Positive predictive value	The proportion of patients who test positive who actually have the disease
Sensitivity	The ability of the test to identify correctly those who have the disease
Specificity	The ability of the test to identify correctly those who do not have the disease
Symptomatic	Infected individuals with signs and symptoms

ABSTRACT

Malaria causes the greatest public health burden in sub-Saharan Africa where high mortality mainly occurs in children under five years of age and pregnant women. Majority of Kenyan population are at risk of malaria infection. Traditionally, malaria has been studied mainly in Western and Coastal Kenya while the rift valley especially Baringo County few malaria studies have been conducted indicating seasonal transmission. This has resulted in scanty information on actual malaria prevalence and transmission patterns which may hinder setting up of proper control strategies. Moreover, primary school children seem to be endangered due to minimal protection by insecticide treated nets. The purpose of this study was to determine the prevalence of *Plasmodium* species infection among primary school children and to evaluate the performance of malaria rapid diagnostic test kits in diagnosis of malaria in Baringo County, Kenya. One thousand six hundred and sixty eight (1668) children from fifteen primary schools located in 4 ecological zones (lowlands, midlands, highlands and riverine) of three sub-Counties of Baringo County were recruited into the study. Finger prick blood sampling was done every four months (during the dry season in January/ February, during the long rains in June/July, short rains in November 2015 and during dry season in January/ February 2016). *Plasmodium* species infection was tested using three rapid diagnostic test kits (CareStart Pf, SD Bioline Ag Pf and SD Bioline Ag P.f/ Pan). Microscopic examination was done on all RDT positive and 10% of negative cases. A total of 268 (16.1%), out of 1668 pupils tested positive for *P. falciparum* by RDT; 78% had a single episode of infection, 16.8% had 2 episodes, 4.9% had 3 episodes and 0.4% had 4 episodes of infections. *Plasmodium* species infection varied within local ecological zones. For instance, the riverine zone had the highest cases of *Plasmodium* species infection compared to lowland, highland and midland (Fisher's exact test = 0.005). More cases of *Plasmodium* species infection (10.7 and 6.2%) were reported in dry season compared to 2.6% in the long rains (Fisher's exact test= 1.000) and 5.7% in short rains (Fisher's exact test= 0.197). Risk of *Plasmodium* species infection was significantly higher in riverine zone compared to midland, lowland and highland (IRR= 40.24165 (95% CI: 7-1623). Infection rate for males and females was similar (IRR = 1.02 (95% CI: 0.55-1.88). The study also established that children aged between 10 – 15 years were at higher risk of *Plasmodium* species infection than those aged between 5- 9 (IRR 1.6 (95% CI: 0.9- 3.1). The kits performed relatively well in the diagnosis of malaria using microscopy as reference. SD Bioline Ag-Pf/ Pan RDT kit had a higher sensitivity (90%) compared to that of CareStart Pf (70%) (McNemar's $\chi^2 = 0.5$, df = 1, p-value = 0.4795). Similarly, the sensitivity of SD Bioline Pf was the same as that of CareStart Pf (82.4%). The kits can therefore be used to guide treatment of febrile illness within Baringo County. In addition, present study findings, indicate that *Plasmodium* species infection was relatively low within Baringo County compared to endemic regions of Kenya; however, there is a need for continued monitoring of transmission dynamics under changing climatic conditions as well as establishing expanded malaria control strategies especially within the riverine zone.

CHAPTER ONE

INTRODUCTION

1.1 Background information

In spite of substantial build up in malaria control in many endemic countries, the disease continues to be an important vector-borne parasitic disease worldwide (Shiff *et al.*, 2011). Globally, 3.2 billion people are estimated to be at risk of malaria while 214 million cases resulted in 438, 000 deaths in the year 2015 (WHO, 2015b). About 88% of these deaths occurred in sub-Saharan Africa where young children are the most affected (WHO, 2013, 2015b). In Kenya, malaria is the leading cause of death in children under five years of age and pregnant women (Mohajan, 2014). For instance, more than 46,000 people including 34,000 aged below five years died of malaria in the year 2013 (Mohajan, 2014). Transmission pattern in Kenya is quite diverse with endemic regions (Western part of the country and the Coastal region) experiencing continuous transmission throughout the year. Highlands of Kenya experience both seasonal and epidemic malaria cases during long rains while arid and semi arid regions including Baringo County experience seasonal malaria transmission which intensifies during and just after the rains (Mohajan, 2014).

Effective management of malaria requires accurate diagnosis accompanied by prompt treatment (Wongsrichanalai *et al.*, 2007). Microscopy is the gold standard and in remote settings where adequate expert performance is needed, this method is a challenge due to few trained personnel to carry out microscopy (Amexo *et al.*, 2004). Application of other malaria diagnostic tests such as Rapid Diagnostic Tests (RDT) to circumvent the drawbacks is therefore necessary. Kits from different companies may differ in sensitivity and specificity. It is therefore imperative to

conduct field evaluation of kits from different manufacturers before they are recommended for widespread use in diagnosis of malaria (Fancony *et al.*, 2013). This is important since their performance may be impacted by extreme temperatures and high humidity (Maltha *et al.*, 2013). Studies on performance of malaria rapid diagnostic test kits have been conducted in various parts of Kenya and findings indicated better sensitivities and specificities (Wanja *et al.*, 2016; Osanjo *et al.*, 2017).

Understanding the current incidence and transmission patterns of malaria can positively influence the choice of control tools deployed in different epidemiological zones. Baringo County is considered a low malaria transmission area, with seasonal patterns (Snow *et al.*, 2009; Mohajan, 2014). Such areas are mostly characterized by pockets of transmission which may be intense in nature due to absence of acquired immunity against malaria (Hay *et al.*, 2008). These features necessitate extensive malaria screening to map out the possible existence of foci with prolonged transmission, necessary for the estimation of the malaria burden (Fancony *et al.*, 2013). Unfortunately, few studies are usually conducted in low transmission areas leading to scarcity of information, hence no interventions or inappropriate ones are put in place to curb the malaria transmission (Silal *et al.*, 2013).

Information on *Plasmodium* species infection risks and clinical epidemiology in Baringo County is limited and only documented as exhibiting seasonal transmission which intensifies during the short rains and just after the long rains (Snow *et al.*, 2009). This may not have taken into account the existence of hotspots that may be experiencing malaria transmission throughout the year. The purpose of this study was therefore to determine malaria prevalence

within three sub-counties (Marigat, Baringo Central and Baringo North) of Baringo County, with a view of identifying probable transmission hot spots, useful in mounting focused interventions. The study also evaluated the performance of Standard Diagnostic (SD) Bioline kits compared to CareStart RDT kits that are normally provided by the Ministry of Health for malaria Diagnosis in endemic areas.

1.2. Statement of the problem

Arid and semi-arid regions considered to experience low transmission rates face limited disease surveillance leading to poor knowledge on infection risks and clinical epidemiology of malaria (Snow *et al.*, 2009). These low transmission areas may also contain pockets of transmission which may fuel transmission to the wider community (Hay *et al.*, 2008; Bousema *et al.*, 2012). Moreover, due to climate change, malaria epidemics have been reported in areas initially considered free of malaria infection (Lindsay *et al.*, 1998; Kurane, 2010; Tonnang *et al.*, 2010; Lotfy, 2014; Wu *et al.*, 2016). It is therefore imperative to continuously monitor transmission dynamics to identify emerging transmission areas requiring efficient integrated control measures. Baringo County being mainly arid and semi arid region, information on infection risks is limited (Snow *et al.*, 2009). Poor knowledge on infection risk and epidemiology of malaria may hinder establishment of proper control strategies putting the lives of people at great risk. This necessitated an extensive malaria survey to generate detailed information on infection status among primary school children in Baringo County and most importantly, to map out probable transmission hotspots.

1.3. Justification of the study

There have been reported reductions in severe malaria and mortality in children under the age of five years in the coastal region of Kenya (Okiro *et al.*, 2007; Snow *et al.*, 2009). These reductions have been linked to wide access to malaria control strategies (Snow *et al.*, 2009). To maximize the impacts of control interventions in different epidemiological zones, the study of prevalence and incidence of malaria transmission is of utmost importance. Due to scanty information on transmission dynamics within Baringo County, detailed malaria survey was conducted to guide on future control strategies.

Parasite-based diagnosis has been recommended by World Health Organization especially in areas which exhibit low malaria transmission (WHO, 2015a). In low transmission areas such as Baringo County, some clinical signs like fever may not automatically mean *Plasmodium* infection hence confirmation is important to avoid inappropriate treatment (Snow *et al.*, 2009). Due to challenges in implementing microscopy-based diagnosis especially in resource-poor areas, malaria rapid diagnostic test have been proposed (WHO, 2011). These kits are manufactured by different companies and may have different sensitivity and specificity. In addition, their performance may be affected by excessive temperatures and humidity which vary from place to place (Maltha *et al.*, 2013). Due to these, it is important to conduct field evaluation before their deployment in different areas.

Previous studies have also indicated that primary school children are less likely to sleep under bed nets hence mostly infected with malaria parasites (Clarke *et al.*, 2008). The present study therefore recruited primary school children to determine malaria transmission pattern.

1.4 Research questions

- i) What is the prevalence of *Plasmodium* species infection among primary school children in different ecological zones in Baringo County?
- ii) What is the incidence of *Plasmodium* species infection among primary school children in different ecological zones in Baringo County?
- iii) What is the sensitivity and specificity of SD Bioline RDT kits compared to CareStart kits in diagnosis of malaria in Baringo County?

1.5. Hypotheses

- i. There is no difference in prevalence of *Plasmodium* species infection among the four ecological zones of Baringo County.
- ii. There is no difference in incidence of *Plasmodium* species infections among the four ecological zones of Baringo County.
- iii. There is no difference in sensitivity and specificity between SD Bioline and CareStart RDT kits in the diagnosis of *Plasmodium* spp.

1.6. Objectives of the study

1.6.1. General objective

To determine the prevalence of *Plasmodium* species infection among primary school children and compare performance of malaria SD Bioline to CareStart RDT kits in Baringo County, Kenya

1.6.2. Specific objectives

- i. To determine the prevalence of *Plasmodium* species infection among primary school children in four ecological zones of Baringo County of Kenya.
- ii. To determine the incidence of *Plasmodium* species infections among primary school children in Baringo County, Kenya
- iii. To determine the sensitivity and specificity of SD Bioline and CareStart RDT kits in the diagnosis of *Plasmodium* spp. infections among primary school children in Baringo County.

1.7. Significance of the study

The study provides valuable information on the prevalence and incidence of *Plasmodium* species infections in the four different ecological zones within three sub-counties (Baringo North, Baringo Central and Marigat) of Baringo County. The findings identified riverine zone as an area that required expanded control interventions by the Ministry of Health and other stakeholders. The study further revealed high asymptomatic cases within riverine zone which may act as a reserve for malaria parasites promoting continuous transmission. These asymptomatic cases always remain silent within the population and may render designed control strategies ineffective if not detected and treated. Information on the performance of both the SD Bioline and CareStart RDT kits indicated that they can be used in remote setting for guiding patients' treatment of febrile illness within Baringo County.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malaria

Malaria is a worldwide health problem where Sub-Saharan Africa being the worst hit (WHO, 2013). In Africa, malaria is not only a health problem but also a development problem (Gallup and Sachs, 2001). Studies within malaria endemic countries further suggest that economic growth is likely to go down by at least one percent due to this disease (Malaney *et al.*, 2004). This has pushed countries to allocate resources towards controlling or eliminating malaria. Concerted efforts that have been put in place include the use of insecticide-treated bed nets, indoor residual spraying for vector control, chemotherapy and epidemic preparedness (Kokwaro, 2009; MOPHS, 2010).

Adoption of national malaria strategy in Kenya in the year 2001 emphasized the distribution of insecticide treated bed nets and recommended selective indoor residual spraying for epidemic control and also prompt treatment of diagnosed cases (Abuja+12, 2013). However, the country is still far from eliminating the disease due to inadequate diagnostic equipment in health facilities, improper or non-use of insecticide treated nets (ITNs) and non-compliance with treatment regimes (Schantz-Dunn and Nawal, 2009). For example, in malaria endemic areas especially around the lake and Coastal regions, three out of five children under five years of age sleep under ITN while majority (9 out of 10) of these children in the endemic lake region sleeps in a home that has not been sprayed (Pathania, 2014b). Currently, early treatment with effective antimalarial drugs is the main life-saving intervention but treatment is threatened by the increasing resistance of parasites to the existing drugs (Laxminarayan *et al.*, 2006). The

reason surrounding intensification of drug resistance emanates from inappropriate use of antimalarial drugs (WHO, 2015a).

2.2 Etiology of malaria

Human malaria is caused by five parasites namely; *Plasmodium falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* (WHO, 2013). These parasites are transmitted to human host by female anopheles species mosquitoes. *Anopheles gambiae*, *An. arabiensis* and *An. funestus* are the major malaria vectors (Githeko *et al.*, 2000; Githeko *et al.*, 2012). High malaria transmission usually occurs in places where both *An. gambiae* and *An. funestus* are present due to their ability to inhabit different breeding habitats and increase in population in different seasons (Kelly-Hope *et al.*, 2009). For instance, *An. gambiae* increases in population during rainy season and tends to inhabit humid environment while *An. funestus* are most abundant at the end of long rain and inhabit swampy areas (Coetzee *et al.*, 2000; Coetzee, 2004; Coetzee and Fontenille, 2004; Minakawa *et al.*, 2005). In addition, *An. arabiensis* prefer arid areas and peak during dry season (Lindsay *et al.*, 1998).

The global distribution of malaria parasites varies with *P. falciparum* and *P. vivax* being the most widely distributed. *Plasmodium falciparum* is found mainly in the hotter and more humid regions of the tropical and sub-tropical areas while *P. vivax* has a wide distribution in the tropical, sub-tropical and temperate areas due to its ability to develop in mosquitoes at lower temperatures (Cheesbrough, 2009). Both *P. malariae* and *P. ovale* have low prevalence whereby, *P. malariae* accounts for about 25% of the infections in tropical/subtropical regions and about 10% of *P. ovale* infections in some parts of Africa but mostly in West Africa (Cheesbrough, 2009).

Four species of *Plasmodium* have been detected in Kenya with exception of *P. knowlesi*, but *P. falciparum* which causes the severest form of the disease accounts for 98.2% of all malaria infections. *Plasmodium falciparum* causes severe malaria due to its ability to reproduce rapidly within the blood (Githeko *et al.*, 2000; Githeko *et al.*, 2012). Some studies also suggest that *P. vivax* and *P. knowlesi* may cause severe and fatal malaria (Bartoloni and Zammarchi, 2012). *Plasmodium malariae*, *P. ovale* and *P. vivax* often occur as mixed infections and account for 1.8% (Pathania, 2014a). The first symptoms such as fever, headache and chills which appear one to four weeks after infection are mild but if not treated patients end up developing severe and even fatal disease (WHO, 2013).

2.3. Life cycle of *Plasmodium*

The life cycle of malaria parasite is complicated due to the parasite's ability to switch its cellular and molecular composition and to develop both within and outside the cell niches in the human host and mosquito vector (Aly *et al.*, 2009). The life cycle of all species of human malaria parasites is divided into two major categories; the sexual and asexual stages which take place in the mosquito vector and human host, respectively. The parasites undergo development within the mosquito midgut then migrate to haemolymph and finally to the salivary glands. It's during this point that the mosquito is able to transmit the parasites to the new human host (Cator *et al.*, 2013; Paaijmans *et al.*, 2013; Smallegange *et al.*, 2013; Cator *et al.*, 2014).

The asexual phase takes place in the liver and red blood cells of the human host. The sporozoites are released into the blood stream when infected female anopheles mosquito bites and this marks the onset of asexual phase (Amino *et al.*, 2006; Yamauchi *et al.*, 2007). The

injected, sporozoites enter the liver cells where they start the asexual division known as pre-erythrocytic schizogony (Antinori *et al.*, 2012). The development and multiplication within the liver cells take approximately 5 days (Prudencio *et al.*, 2006). This multiplication leads to production of several merozoites which are later released into the blood stream after about 5 - 14 days (Mazier *et al.*, 2009). This is different for both *P. vivax* and *P. ovale* which instead of developing directly into schizonts, some of their sporozoites differentiate into hypnozoites which remain dormant in the hepatocytes for a period of time but when reactivated, they undergo asexual division and cause clinical relapse some weeks, months or years after the initial infection (Garnham, 1988; Galinski *et al.*, 2013). Once the merozoites are in the blood stream, they invade the red blood cells leading to appearance of clinical signs. They develop into trophozoites then schizonts while the rest develop directly into male and female gametocytes which are ingested by mosquitoes when they take a blood meal and the sexual phase begins (Garnham, 1988).

The length of development of the malaria parasite within the mosquito depends on *Plasmodium* species, environmental temperatures and mosquito host species which may range from 10 to 14 days (Garnham, 1988; Killeen *et al.*, 2000). This means that the mosquitoes are unable to transmit the parasite to the next host immediately after acquisition (Cator *et al.*, 2014). Malaria transmission intensity depends on the length of time the parasite takes within the mosquito to complete development to the time it can be transmitted to another host Figure 2.1 is an illustration of *Plasmodium* life cycle (<http://www.cdc.gov/malaria/about/biology/index.html>)

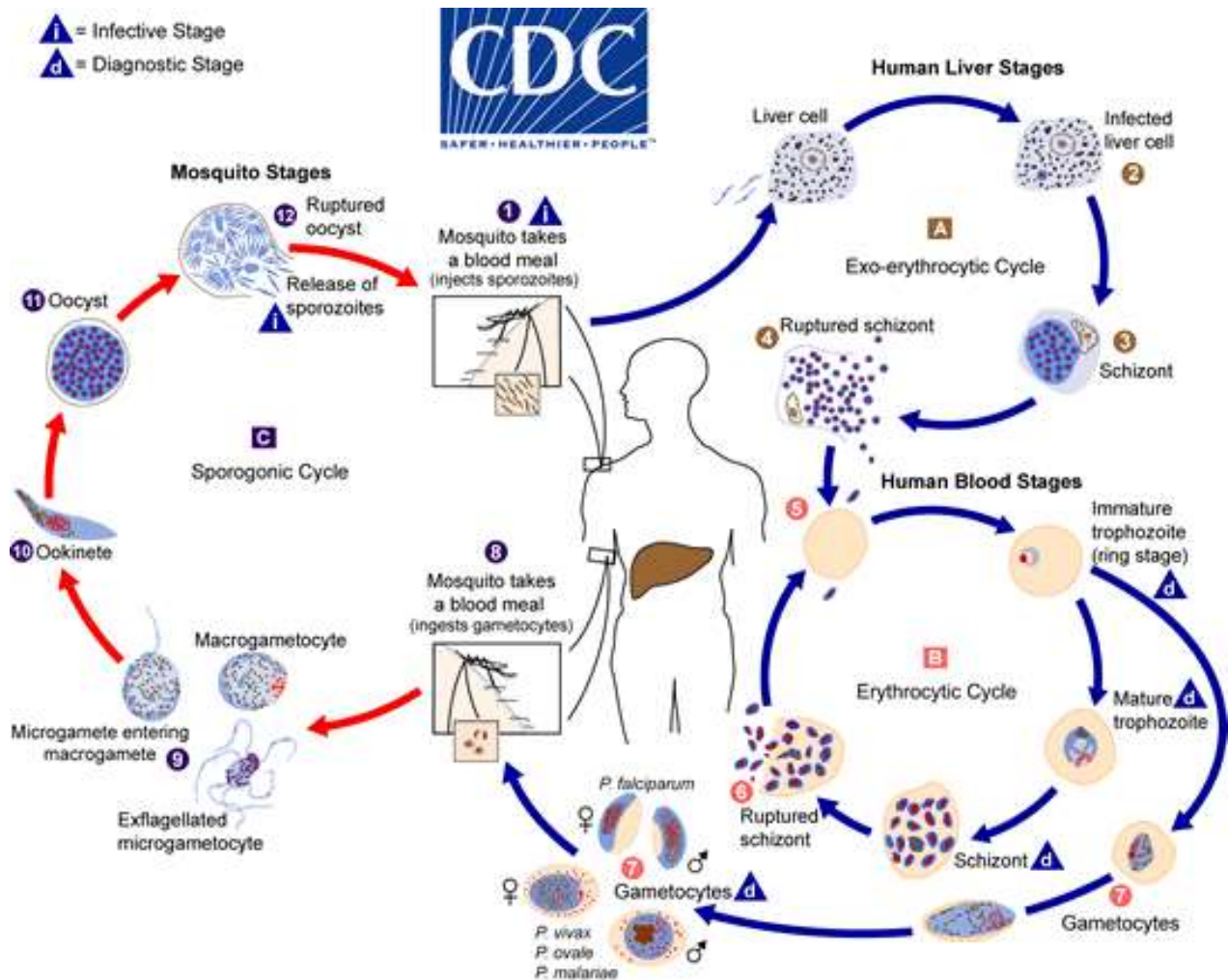


Figure 2.1. Life cycle of *Plasmodium*

(Source: Centers for Disease Control and Prevention, <http://www.cdc.gov/malaria/about/biology/index.html>).

2.4. Risks of *Plasmodium* species infection in Kenya

Understanding the nature and degree of malaria transmission is important when designing an effective strategy to manage the disease (Hay and Snow, 2006). Maps that can accurately outline the underlying patterns of disease transmission are needed to guide the selection of

appropriate interventions (Hay and Snow, 2006; Hay *et al.*, 2009). For instance, control strategies such as indoor residual spraying (IRS) may be more effective when carried out just before the start of malaria season (Roca-Feltrer *et al.*, 2009). In addition, other control interventions such as Intermittent Preventive Treatment (IPT) given to pregnant women or infants may be timely if the start and duration of malaria transmission is well documented (Roca-Feltrer *et al.*, 2009).

Due to difference in altitude, temperature intensity and rainfall patterns within the Kenya, many regions experience varying rates in parasite transmission (Mohajan, 2014). For example, in regions around the lake such as Nyanza and Western, malaria transmission is usually intense throughout the year with prevalence rates of 20-40 % while in the Coastal region, it is perennial but prevalence rate is below 5% (Mohajan, 2014). In these endemic malaria transmission regions, the life cycle of mosquito is relatively short but the survival rate is high due to high temperatures and availability of water for breeding (MOPHS, 2009, 2010; Mohajan, 2014). In the Western highlands and the arid and semi-arid regions, parasite prevalence is usually less than 5%. However, during long rains, these regions often experience seasonal transmission which at times can be intense leading to epidemics (MOPHS, 2009; Mohajan, 2014). In addition, most parts these regions such as Turkana, Marasabit, Mandera, Garissa and Wajir, unanticipated pockets of transmission are likely to emerge (Snow *et al.*, 2009). Unfortunately, little is known about the factors that may influence such transmission. Similarly, in arid and semi-arid parts of North Western Kenya, little is known about infection risks and clinical epidemiology is not well documented (Snow *et al.*, 2009).

2.5 Climate change and malaria transmission

Climate change is currently a global concern and despite continuing controversy about its cause and the magnitude of its effects, it is likely that it will continue to affect the incidence and prevalence of infections in the tropics (Koenraadt *et al.*, 2004). Human life is greatly affected by increased vector borne diseases due to changing climatic conditions (Githeko *et al.*, 2000). Malaria, Rift Valley fever and Dengue are important mosquito borne diseases whose distribution and seasonal activity are sensitive to climatic factors (Caminade *et al.*, 2014). Due to possible sensitivity of mosquitoes to climate changes (Gage *et al.*, 2008), it is likely that the distribution of a number of mosquito species may have already changed or will change under climate projections (Postigo *et al.*, 2007).

Change in climate is therefore an important factor since it directly affects disease transmission by shifting the vector's geographic range. For instance, change in precipitation in an area, may strongly influence the creation of aquatic habitats for mosquitoes (Gage *et al.*, 2008). Temperature, rainfall and humidity are other important climatic factors that are capable of influencing parasite development within the vector, vectors survival and biting rate hence may lead to increased parasite transmission (Githeko *et al.*, 2000; Gage *et al.*, 2008). For instance, the aquatic stages of anopheline mosquito ceases to develop or breed below 16⁰C (Bayoh, 2001) while temperatures below 18⁰C inhibits the development of *P. falciparum* within the mosquito vector (Githeko *et al.*, 2000; Aly *et al.*, 2009; Bousema and Drakeley, 2011). Other environmental factors such as land forms, water, topography and vegetation cover have been shown to influence malaria transmission, species abundance and distribution (Kelly-Hope *et al.*, 2009; Cottrell *et al.*, 2012; Githeko *et al.*, 2012). Changes in environmental factors can

positively or negatively influence establishment of breeding habitats for malaria vectors, thereby affecting transmission rate or species abundance (Kelly-Hope *et al.*, 2009).

Intergovernmental Panel on Climate Change (IPCC) further anticipates that an increase in both temperature and rainfall are likely to have great impact on prevalence of vector borne diseases especially malaria in regions which were traditionally considered to experience no cases of malaria (Kovats *et al.*, 2001; McMichael, 2001b, 2001a). Studies further suggest resurgence of malaria in the East African highlands due to changing climatic conditions (Pascual *et al.*, 2006; Himeidan and Kweka, 2012); Martens, 1995; Lindblade *et al.*, 2000). Climate change is also likely to alter land use patterns, potentially influencing the mosquito species composition and population size, resulting in changes in malaria transmission (Kovats *et al.*, 2001; McMichael, 2001b, 2001a). In areas where transmission is intense and occurs throughout the year, pregnant women with reduced immunity and children under the age of five who have not yet developed immunity against malaria are the most affected. However, in areas where transmission is epidemic, both young and old are equally at risk (Feachem *et al.*, 2010).

2.6. Diagnosis of malaria

Accurate and timely diagnosis accompanied with immediate treatment is an important aspect in malaria control and management (Endeshaw *et al.*, 2008). Unfortunately, due to reliance on clinical diagnosis, treated confirmed cases in Africa are comparatively lower than other regions of the world (WHO, 2008). This shows that majority of treated cases are only clinically diagnosed yet the latter can be considerably unreliable due to the non-specific nature of signs and symptoms of malaria (Bardaji *et al.*, 2008; Endeshaw *et al.*, 2008; Juma and Zurovac,

2011). Treatment based on such diagnosis may also lead to indiscriminate use of antimalarials drugs and may impair the required quality care for patients suffering from other diseases displaying similar signs and symptoms (Reyburn *et al.*, 2004; Mwangi *et al.*, 2005; McMorrow *et al.*, 2008). In Kenya, the new National Malaria Strategy (2009-2017) which emphasized on treatment of only confirmed positive cases across all age groups was launched in 2009 (MOPHS, 2009) Before the launch, only a few (34%) health facilities offered parasitological-based diagnosis (microscopy) and less than 7% conducted RDT testing for malaria (Snow *et al.*, 2009).

After the launching of this malaria strategy, there have been improvements on treatment of confirmed cases either by microscopy or use of RDT kits. For instance, a study conducted by Zurovac *et al.* (2014) to assess the milestones in the quality of malaria case-managements due to the Kenyan policy of ‘test and treat’, reported significant scale up of parasitological diagnosis by health facilities at about 91%. Another study also reported further improvements in application of RDTs (70%) and other equipments for malaria diagnosis (90%) by Kenyan health facilities (KMOH, 2015). Various parasite-based diagnostic methods such as flow cytometry, ELISA and indirect immunofluorescence antibody assay (IFA) have been developed to help in reduction of morbidity and mortality associated with malaria. However, owing to requirement of highly trained personnel and sophisticated equipment, most of them are not frequently used in diagnosis of malaria in Kenyan hospitals.

2.6.1. Microscopy

Microscopy was introduced in 1904 by a microscopist known as Gustav Giemsa (Fleischer, 2004) and subsequently became the gold standard of malaria diagnosis. The accepted laboratory practice for the diagnosis of malaria is the preparation and microscopic examination of blood films stained with Giemsa, Wright's or Field's stain (Warhurst and Williams, 1996). This is regarded as the most suitable diagnostic tool for malaria control because it is inexpensive to perform, able to differentiate malaria species and quantify parasites (McKenzie *et al.*, 2003). However, light microscopy faces a number of challenges that occasionally lead to inaccurate results. First, it requires well trained and competent microscopists. Such microscopists are scarce even in some developed countries (Johnston *et al.*, 2006). Microscopic examination of a single blood sample can miss parasites especially in low parasitaemia thus resulting in false negative outcome (Bell *et al.*, 2005). In addition, patients with *P. falciparum* malaria may have parasites sequestered in the capillaries of visceral organs leading to false negative results by microscopy. As a result, a *P. falciparum* infection may be missed in the blood films (Anstey *et al.*, 2007; Amante *et al.*, 2010; Lacerda *et al.*, 2012; Manning *et al.*, 2012; Brugat *et al.*, 2014).

Identification of mixed species is a major concern in light microscopy. Most microscopists might not look for other species after identifying one (Zakeri *et al.*, 2010). This may lead to misclassification of malaria parasites in an area. Parasite density of infections by *P. vivax*, *P. malariae* or *P. ovale* species is usually low compared to *P. falciparum*. Therefore, other *Plasmodium* species are easily missed, particularly in the absence of symptoms (Tajebe *et al.*, 2014; Patel *et al.*, 2015). Moreover, in mixed infections, the background of large numbers of *P.*

falciparum parasites makes the observation difficult to differentiate other species (Fancony *et al.*, 2012). Treatment of only *P. vivax* in a mixed-species infection, may lead to build up of *P. falciparum* parasitaemia (Mason and McKenzie, 1999). Moreover, mixed-species infection may increase the possibility of anti-malarial drug resistance. (Lee *et al.*, 2011). Therefore, accurate diagnosis capable of identifying different species in a mixed-species infection for instance microscopy and multi-species rapid diagnostic test kits may play a significant role towards therapeutic decisions (Lee *et al.*, 2011; Obare *et al.*, 2013).

2.6.2. Malaria rapid diagnostic tests

Malaria rapid diagnostic tests (RDTs) are a relatively new and evolving technology that is becoming popular in various endemic settings especially in areas with inadequate number of experienced microscopists (Lubell *et al.*, 2007). They are becoming an essential tool in malaria management or eradication campaign (Bisoffi *et al.*, 2009). In Kenya, ministry of health launched widespread use of malaria RDT kits in 2009 (MOPHS, 2009). CareStart was recommended after various field evaluations indicated better performance. SD Bioline has not been recommended for mass diagnosis of malaria in Kenyan hospitals or health facilities. This could be due to inadequate studies to ascertain their performance in the field. A number of malaria RDTs have been developed which vary in formats such as plastic cassettes, dipstick or cards (Moody, 2002). They detect specific antigens or proteins such as histidine-rich protein-2 (HRP-2), a water-soluble protein produced by asexual stages and young gametocytes of *Plasmodium falciparum*. Another antigen group, parasite lactate dehydrogenase (pLDH), is an enzyme in the glycolytic pathway with distinct isomer for each *Plasmodium* spp. and is produced by all blood-stage parasites (Moody, 2002). These antigens are present in the blood of

infected individuals and can therefore be used as markers of infection. Parasite lactate dehydrogenase is usually depleted in the blood after clearance of all malaria parasites in the blood while HRP-2 remains active for days after the successful clearance of parasites by antimalarials (Endeshaw *et al.*, 2008). The third antigen used in RDTs is *Plasmodium* aldolase which is an enzyme of the glycolytic pathway produced by all four species (Moody, 2002).

The recommended sensitivity of all RDTs should be 95% and above at 100 parasites per μl for *P. falciparum* (WHO, 2004). However, this sensitivity may vary depending on certain factors. For instance, high temperature and humidity can adversely affect their sensitivity hence storage and transport should be well managed (WHO, 2006). Even though recent reviews have indicated that kits detecting HRP-2 may have greater sensitivity compared to those detecting pLDH, rate of false positive results with HRP-2 based kits may be more due to its ability to persist in blood even after clearance of parasites by antimalarials (Humar *et al.*, 1997; Mharakurwa *et al.*, 1997; Wongsrichanalai *et al.*, 1999; Mayxay *et al.*, 2001; Huong *et al.*, 2002; Marx *et al.*, 2005; Wongsrichanalai *et al.*, 2007; Hopkins *et al.*, 2008). It is therefore important to take note of any recent treatment. The persistence of HRP2 may be advantageous especially in detecting low-level or fluctuating parasites in chronic malaria (Bell *et al.*, 2005).

2.7 Treatment of malaria

Chloroquine was the first-line treatment of malaria for many decades but due to widespread drug resistance by the parasite, efficacy of the drug diminished (Shujatullah *et al.*, 2012; Wongsrichanalai and Sibley, 2013). Sulphadoxine-Pyrimethamine (SP) which replaced

chloroquine was also rendered less effective due to increased resistance by malaria parasites to this drug (Nkhoma, 2007; Mwai *et al.*, 2009).

Later, Second and third-line treatments were introduced which included Amodiaquine and Quinine (Achan *et al.*, 2011). Quinine remained the drug of choice for complicated manifestations of the disease such as cerebral malaria. However, due to growing resistance to the drug and its challenge of high toxicity when administered intravenously, the preference of the drug is decreasing (Trampuz *et al.*, 2003; Achan *et al.*, 2011).

Currently, artemisinin are the most effective antimalarial drugs still remaining. They can speedily clear a broad range of asexual blood stages of the malaria parasite as well as *P. falciparum* gametocytes at manageable concentrations (Maude *et al.*, 2010). Due to increasing drug resistance by malaria parasites, artemisinin now forms an essential element of recommended first-line antimalarial worldwide (WHO, 2015a). To minimize the risk of resistance, they have been recommended for treatment of uncomplicated malaria in combination with other antimalarials (WHO, 2015a). The five combinations which are currently recommended include; artemether–lumefantrine, artesunate–amodiaquine, artesunate–mefloquine, artesunate–sulfadoxine–pyrimethamine and dihydroartemisinin–piperaquine. Their rollout has resulted in documented reductions in malaria prevalence in a number of African and Asian countries (WHO, 2015b), unfortunately, there are already some early signs of artemisinin resistance appearing in western Cambodia and if this resistance were to spread, it would be disastrous for malaria control efforts worldwide (Maude *et al.*, 2010). This is the major reason why strongly insists that confirmation before treatment of malaria cases is important.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted in three sub Counties of Baringo County namely; Marigat, Baringo North and Baringo Central between January 2015 and February 2016. Baringo County is located in the former Rift Valley Province and lies between 35.602 E, 0.541 N and 36.277 E, 0.723 N at altitudes ranging between 870 to 2499 meters above sea level (Figure 3.1). The study area represents highland, arid and semi-arid parts of Baringo County and was sub-divided into four ecological zones based on soil, land form, altitude and climatic characteristics (Kipruto *et al.*, 2017) (Figure 3.2). The four zones include; lowlands (900-1000 meters above sea level), midlands (1000-1500m meters above sea level), highlands (1500-2300m meters above sea level) and riverine (1000-1200m meters above sea level).

The lowland zone lies to the east of the study area and receives mean annual rainfall of about 650mm with temperatures ranging between 30⁰C to 37⁰C. The zone is characterized by both lakes (Lake Baringo and Lake Bogoria) and perennial rivers (Perkerra and Molo) with poor drainage soil types. The main vegetation covering the area is *Prosopis juliflora*. Other key features in the low altitude zone include Perkerra irrigation scheme.

The midland zone is characterized by well drained soil types and is interspersed with dry riverbeds that flow only after heavy rains. The main vegetation cover is *Acacia/ Commiphora* bushes. The existing water points in this area were constructed to serve the community and

their livestock. They include; Kimau dam, Kipcherere spring, Kimalel dam and Chebarsiat dam.

Highland zone receives an average rainfall that ranges between 1000- 1500mm per year with well drained soil types. Most parts of the highland have thick indigenous forests as well as exotic forests.

The riverine zone borders the Kerio valley to the west of the study area. The major lake and river within this zone is Lake Kamnarok and river Barwessa. The soils are poorly drained and the area is prone to flooding. Baringo County has two distinct weather patterns with the cold months (June and July) and the hot months (January and February). The region experiences two rainy seasons; March to June (long rains) and October to November (short rains). Rainfall is between 1000 and 1500mm annually in the highlands and 600mm in the lowlands. The temperatures range from a minimum of 10⁰C to a maximum of 35⁰C in different parts of the county. According to 2009 Kenya National Population Census, Baringo County which covers an area of 11,075 km² has a population of 555,561. Malaria transmission in this region is seasonal which rises during rainy season. The current incidence of malaria is about 12% based on outpatient visits to local health facilities (Open data, 2012).

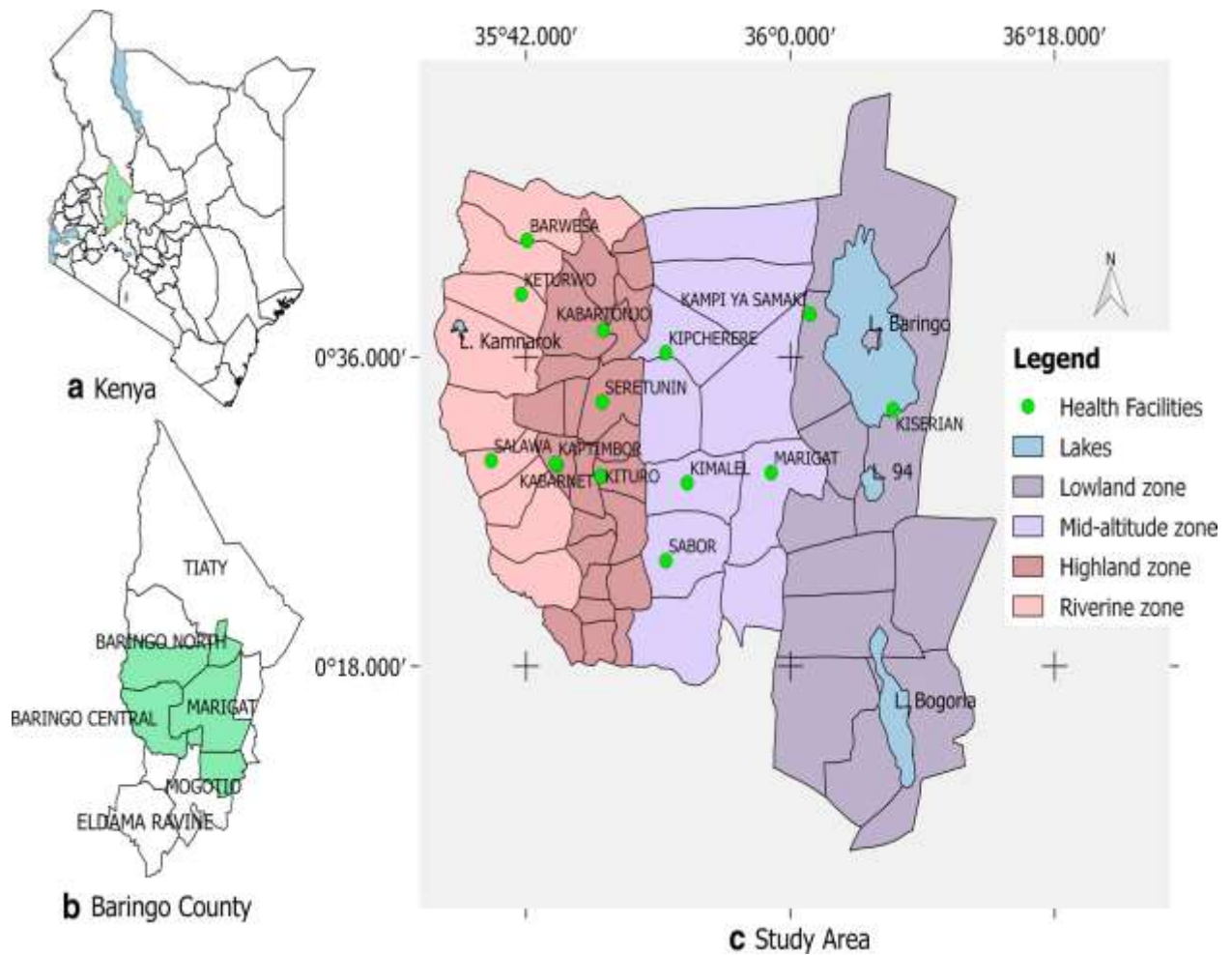


Figure 3.1. Map of Kenya, Baringo County and study area

a Map of Kenya showing the location of Baringo County in (shaded green), **b** the sub-county administrative units within Baringo County with the study area shaded out *green*, and **c** the four ecological zones within the study area

3.2 Study design

This study was both longitudinal and cross-sectional comparative survey. The study was conducted between January 2015 and February 2016. It comprised a baseline survey (January-February) during the dry season and three follow-up studies, the first in the long rainy season (June-July), the second in the short rainy season (October-November) and the third in the dry season (January-February 2016). During baseline and each of the three follow-up studies, primary school children aged between 5 and 15 years were examined for infections with *Plasmodium* spp. using SD Bioline RDT kits, Care Start RDT kits and microscopy.

3.3 Sampling technique

Systematic random sampling was applied to identify schools and simple random sampling to recruit study participants. Fifteen public primary schools were identified within the study area based on the proximity to mosquito breeding (aquatic) habitats. These breeding sites were equitably spatially distributed across the study area. From these, the school closest to a breeding site included in the study was conveniently selected and based on the populations of the respective schools; fifteen schools were adequate for the desired sample size. From these schools, 1,668 pupils were randomly recruited (Table 3.1). Pupils aged between 5 -15 were targeted. Population size in each school, acceptance of parents or guardians to sign a written consent and willingness of pupils to participate in the study guided the selection. A complete list of all willing pupils per each school were then prepared. Considering the desired minimum sample size (1218), random numbers were developed which helped to select study participants.

Table 3.1. Location of primary schools enrolled for the study and pupils tested

Ecological zones	Name of Primary school	School code	Latitude	Longitude	School Population	No. consented and tested
Riverine	LakeKamnarok	P1	0.632158	35.63598300	300	207
Riverine	Litein	P2	0.64919500	35.66384800	380	268
Riverine	Barwessa	P3	0.71376200	35.69900700	500	288
Highlands	Talai	P4	0.58701000	35.81288000	312	100
Highlands	Tandui	P5	0.37665100	35.79000700	491	95
Highlands	Kaptimbor	P6	0.49670000	35.73352000	694	58
Highlands	Borowanin	P7	0.44183600	35.77961400	224	31
Midlands	Kimao	P8	0.44964900	35.83329700	94	50
Midlands	Sabor	P9	0.40782100	35.86160700	228	83
Lowlands	Kapkuikui	P10	0.36974900	36.04301500	237	70
Lowlands	Loboi	P11	0.35602200	36.06090100	350	97
Midlands	Kipcherere	P12	0.59782000	35.85527000	360	46
Lowlands	Salabani	P13	0.54555900	36.02163400	215	119
Lowlands	Perkerra	P14	0.46915200	36.03177400	277	40
Lowlands	Loitip	P15	0.44817000	36.07968300	370	116
Total		15			5032	1668

3.3.1 Inclusion criteria

Pupils aged between 5 and 15 years were randomly recruited following parental or guardian's written informed consent (Appendix IV).

3.3.2 Exclusion criteria

Children below 5 years and above 15 years, or those whose parents did not consent were excluded from the study.

3.3.3 Sample size determination

To determine malaria prevalence and incidence within the study area, the current malaria incidence in Baringo County estimated to be about 12% (Open data, 2012) based on outpatients visits to local health facilities was used to calculate the minimum number of primary school children required for the study. It was calculated using the formula given below by Kirkwood and Stern (2003).

$$N = \frac{(u + v)^2 (\mu_1 + \mu_2)}{(\mu_1 - \mu_2)^2}$$

Where;

N- Desired sample size

μ - One sided percentage point of the normal distribution corresponding to 100% minus the power (90%). Hence, $\mu = 1.28$.

V- Percentage of the normal distribution corresponding to the (two-sided) significance level (5%) hence $V = 1.96$.

μ_1 - The current malaria incidence (12%)

μ_2 - New rate of malaria corresponding to 5% increase or decrease= 17% or 7%;

$$N = \frac{(1.28 + 1.96)^2 (0.12 + 0.17)}{(0.12 - 0.17)^2}$$

$$N = \frac{3.044304}{0.0025}$$

$$N = 1218$$

Due to possible loss during follow-ups, a minimum sample size of 1218 was exceeded by 450 to 1668

In addition, to determine the performance of RDT kits, sample size was calculated using the formula given by Naing *et al.* (2006). Briefly, using the prevalence data 12%, precision 0.05 and level of confidence 1.96, the minimum number of pupils needed for this study was 162. This was calculated as follows;

$$N = \frac{Z^2 P(1 - P)}{d^2}$$

Where Z= Level of confidence (1.96)

P= Prevalence (12%)

d= Precision (0.05)

$$N = \frac{1.96^2 \times 0.12(1 - 0.12)}{0.05^2}$$

$$N = 162$$

The total number of pupils screened in the first and second surveys was 261 and 300 respectively.

3.4 Familiarization and advocacy

In January 2015, just before the commencement of the study, meetings were held in the identified schools with teachers, parents and pupils to explain the objectives of the study and procedures involved. It was during these meetings that written consent to participate in the study was requested. Only children, whose parents or guardians signed the written consent

were recruited. Participation was voluntary and children who were not willing to participate were free to dropout. Meetings were also held with Director of Medical Services Baringo County, Pharmacists in charge and some of the county medical personnel (trained nurses and medical laboratory technicians) who helped in distribution of drugs, testing and treatment of infected pupils..

3.5. Data collection

Data collection was conducted during dry season (January/February, long rainy season (June/July) and short rainy season (October/ November). Rapid Diagnostic Test kits were used to detect the presence of malaria parasite's antigen while microscopy was used to detect the presence of malaria parasites in blood. Structured questionnaires were administered to pupils to collect information on health status, symptoms, history of medication and place where medication was taken (Appendix v). Geopositioning equipment (GPS) was used to locate and map the primary schools and major water bodies within the study area. The average monthly rainfall data was downloaded from climate data library (IRI/LDEO, 2016).

3.6 Mapping of major aquatic habitat

A total of 18 major water bodies within the study area were mapped using Geographical positioning system (GPS). They guided in the selection of Primary schools. Their coordinates, altitude, habitat type, turbidity and vegetation types were recorded (Table 3.2).

Table 3.2 Distribution of major aquatic habitats within the study area

Point ID	Coordinates	Altitude	Habitat type	Turbidity	Vegetation
Lowland zone					
Salabani	0.5453N, 36.0488E	999m	Lake margin	Medium	Dry- no vegetation
Kapkuikui	0.369N, 36.0465E	1015m	Swap, old fish pond	Low, high in pond	Reeds, grass, algae
Loboi	0.3586N, 36.0494E	1019m	Drain/ trench in swamp	Medium	Grass, reeds
L. 94 (Sirata)	0.4634N, 36.0934E	983m	Marshy lake margin	Low	Succulent water plants
Roberts camp	0.6130N, 36.0062E	982m	Inland lake extension- stagnant	Clear	Algae
Nteppes/ Pekerra	0.4699N, 36.0359E	987m	Overflow from irrigation canal	Clear	Small floating plants
Midland zone					
Kipcherere	0.5980N, 35.8544E	1450m	Spring, river fringe	Clear	Algae
Kimao dam	0.4504N, 35.8358E	1485m	Seasonal river bed	Medium	Reeds, grass, algae
Kabeswa , Sabor	0.40565N, 35.86364E	1334m	Seasonal river bed	Clear	Algae
Highland zone					
Kurget, Talai	0.41687N, E 035. 69375	2140m	Spring	Low	None
Kiplewa (Airstrip)	0.52352N, E 035.73151	1837m	Stagnant water margin	High	Papyrus reeds, grass
Kaptimbor dam-Z City	0.49212N, E 035.73233	1925m	Swamp at margin of river	Medium	Papyrus reeds, grass
Kiptagich, Sacho	0.35137N, 35.79517E	2179m	Water pit	Medium	None
Riverine zone					
Barwessa River	N 00.70512, E035.70098	1141m	Perennial river	Clear	Grass&othe r plants
Barwessa Secondary	N0.71755, E 035.69978	1145m	Water pit	Clear	None
Enot (Kapchore)	N 00.66706, E 035.67719	1103m	Spring	Medium	Grass,algae ,reeds,others
Litein (Kapchore)	N 00.64636, E 035.66613	1061m	Drain/trench	Very high	Reeds,grass, algae
Lake Kamnarok	N 00.63547, E 035.62564	1117m	Lake margin	Clear	Water plants present

3.7. Collection of blood sample

Physical examination of the pupils was done by a trained nurse before collection of blood. Medical status (fever, headache, chills and joint pains) and history of medication was recorded. Both the body temperature and weight of the children were taken using clinical thermometers and weighing machine respectively. Finger prick blood sample (20µl) was collected by a phlebotomist. (Appendix V).

3.8. Examination of blood samples

About 5 µl of the blood sample was examined for the presence of malaria parasite antigens using SD Bioline kit and another 5 µl for Care start kit. The other 10 µl was used to prepare a thin and thick smear which was stained with Giemsa and examined for malaria parasites under a light microscope.

3.8.1 Rapid diagnostic test

CareStart Ag-P.f Cat No. G0140 (Access Bio Inc, USA) RDT kits were used in the entire study area while SD Bioline (Standard Diagnostic Inc, Korea) Ag-P.f Cat No. 05FK50C and SD Bioline Ag-P.f/Pan Cat No. 05FK60 were only used in Riverine zone for comparison purposes. The kits were used to test for the presence of parasite antigen in whole blood, according to the manufacturer's instructions. The middle finger of the pupils was cleaned with 70% alcohol swab, allowed to dry and then pricked with a sterile lancet. Thereafter one capillary blood sample (5µl) was collected from each child using a 5µl capillary pipette up to the black line and transferred into the round sample well of each kit. Two to three drops of the assay diluent were added into the square assay diluent well of each kit and the test read after 15 minutes. When

initial result was negative after 15 minutes, reading was repeated at 30 minutes. The presence of two color bands (“PF” test line and “C” control line) within the P.f test window was considered *P. falciparum* positive results. The presence of one colour band at “C” control line was considered negative while the presence of two colour bands both at Pan and C line was considered Pan (*P. vivax*, *P. ovale* and *P. malariae*) positive. The presence of three color bands at C, Pan and P.f lines was considered malaria mixed species infection. The number of pupils who were positive by each of the CareStart and/ or SD Bioline kits was recorded.

3.8.2 Microscopy examination

Microscopy examination was carried out at Marigat Division of vector borne diseases laboratory. Thin and thick blood films were prepared on the same labeled slides using peripheral blood samples in the field. The slides were transported to laboratory, fixed in methanol and stained using 10% Giemsa stain solution for ten minutes. Giemsa stock solution was diluted with distilled water of pH 7.2 to make the 10% which was then filtered. For quality control of the stain, it was first used to stain a known positive slide. The stain was washed off gently and slides air-dried before being examined under $\times 100$ oil immersion lens (Cheesbrough, 2009). The slide examination was done by two microscopists who were blinded from the RDT results. Where there was disagreement on the reading between the two microscopists, a third microscopist was involved. For positive slides, number of parasites was counted against 200 leucocytes and quantification of parasite density estimated by assuming 8,000 leucocytes/ μ l of blood. Slides were considered negative when no parasite was detected after examining 100 microscopic fields.

3.9. Statistical analysis

Data collected was entered in excel spread sheet, cleaned and malaria incidence, prevalence, sensitivity and specificity of the kits analyzed using STATA version 12 (Stata Corporation, College station, Texas, USA, 2013). Inferential statistics (Pearson χ^2) was used to compare proportions of infection and to determine possible significant differences. Fisher's exact test was performed to determine the difference in *Plasmodium* species infection in 4 ecological zones. Percentage of Episodes of *Plasmodium* species infection was determined based on the total number of those who tested positive during the entire period of study. Since treatment was administered to all detected positive cases and given the long duration between surveys (4 months), a positive test on the same pupil during subsequent follow-ups was considered as an episode.

Incidence rate ratio was applied to determine association between risk of infection and ecological zones. Incidence was estimated as the number of new cases of malaria per 1000 person-months (Persons per month) at risk. To calculate the new cases, once the pupil tested positive or lost to follow-up due to transfer to other school or absence from school on the testing day, the pupil was considered out of the study and was not followed. Time at risk for each pupil was then calculated based on how long the pupil was followed during the entire study period. Those who tested positive at baseline survey were not included when calculating time at risk since they all had time zero at risk. The incidence rate was calculated at an interval of four months twice. Those who tested positive during the first follow up had two months time at risk each, while in the second follow up, each had 6 months at risk. Those who were followed throughout the study period each had 8 months time at risk. Date of testing during

baseline and subsequent follow up was recorded to determine time interval between each surveys (Figure 3.3).

The diagnostic performance characteristics such as sensitivity, specificity and positive & negative predictive value (PPV & NPV) were calculated against microscopy results by the formula given below according to Cochrane and Holland (1971);

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}}$$

$$\text{Positive Predictive Value} = \frac{\text{TP}}{\text{TP} + \text{FP}}$$

$$\text{Negative Predictive Value} = \frac{\text{TN}}{\text{TN} + \text{FN}}$$

Where, TP- True Positive-

TN- True Negative

FN- False Negative

FP- False Positive

The results were interpreted at 95% confidence interval (CI). Since both diagnostic test kits were performed on each pupil (paired data), the McNemar' test statistic was performed to determine if the sensitivities of the two diagnostic test kits were statistically different. Since b +

$c < 25$, the following formula (McNemar's test with continuity correction) given by Edwards

(1948) was used; $\chi^2 = \frac{(|b-c|-1)^2}{b+c}$

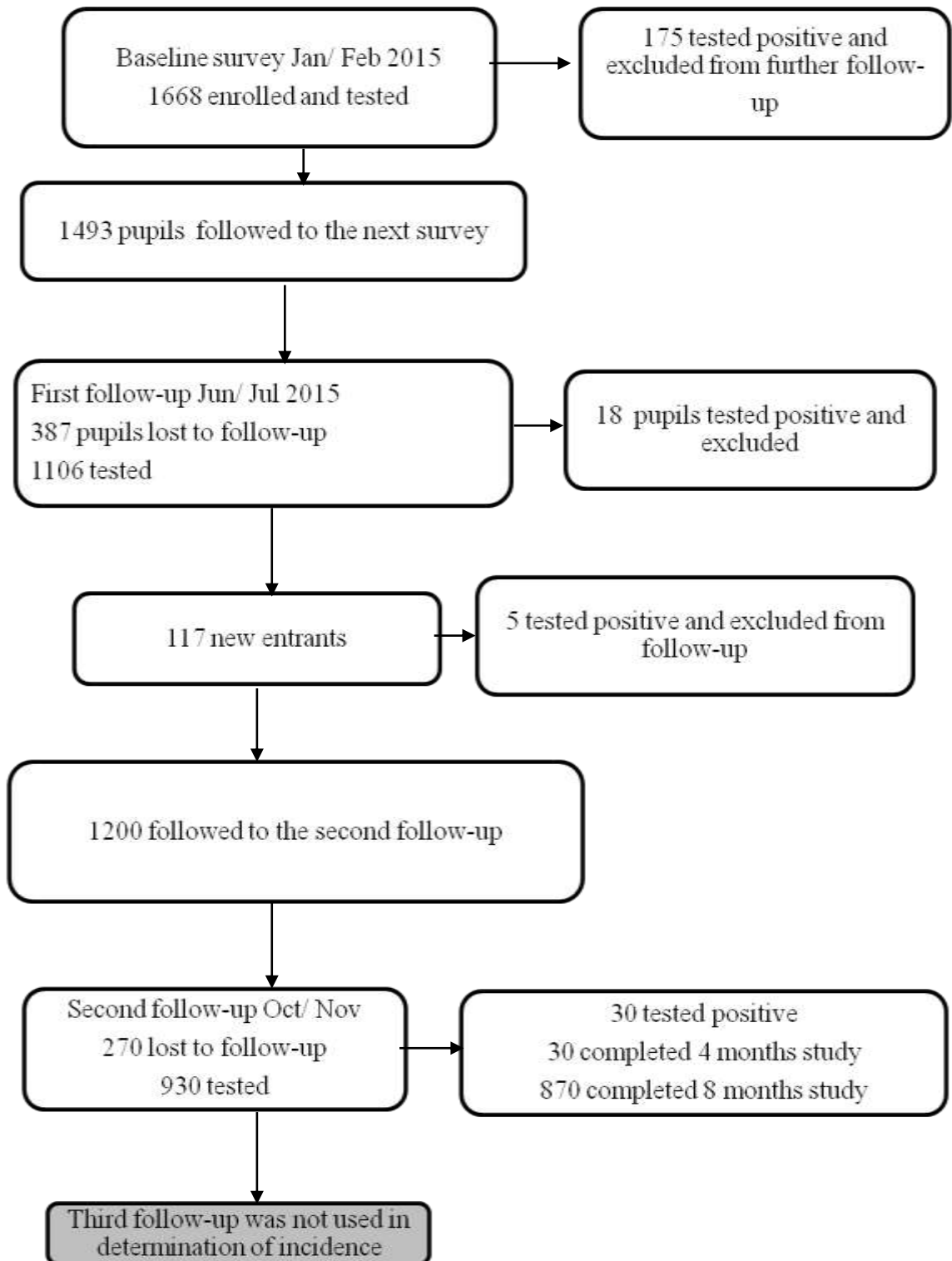


Figure 3.3 Distribution of study participants from enrollment to end of 8 months follow-up period

3.10 Ethical consideration

The study was part of a larger study on “Early warning systems for improved human health and resilience to climate sensitive vector-borne diseases in Kenya” whose ethical review and approval was obtained from Kenyatta National Hospital and University of Nairobi Ethics and Research Committee (Appendix I). Permission to conduct the study was also obtained from department of Health Services, Baringo County Government (Appendix II). Written informed consent was obtained from the parents or guardians of the pupils and assent was obtained from the pupils themselves before enrolment into the study. Participation was voluntary and confidentiality was maintained. Positive individuals were treated for malaria using Artemether Lumefantrine (AL) tablets by the nurse and instructed to comply with treatment regulation.

CHAPTER FOUR

RESULTS

4.1. Prevalence of *Plasmodium* species infection

Determination of prevalence was based on RDT results. During the baseline survey carried out in the dry season (January/February 2015). Of the total number tested, 175 (10.5%) pupils were positive for *P. falciparum* infection. The highest prevalence was recorded in the Riverine Zone (22.8%) followed by the Lowlands (0.2%). Both Midlands and Highland zones had no cases of malaria (Table 4.1).

During the first follow-up conducted towards the end of long rains (June/July 2015) the overall prevalence dropped from 10.5% to 2.6% however the difference was not statistically significant (Fisher's exact test= 1.000). The number of pupils who were tested also dropped from 1668 to 1372 (17.7% lost to follow up). All positive cases were from the Riverine zone giving a prevalence of 6.5%.

The second follow up was carried out during the short rains (October/November 2015). The prevalence of *P. falciparum* infection rose to 5.5% from 2.6% (Fisher's exact test = 0.551). The prevalence of *P. falciparum* infection in the Riverine Zone was 11.4%, while that in the Lowland Zone was 0.6%. No positive cases were reported in the Highland and Midland zones.

The third follow up was conducted immediately after the end of the *El Niño* rains (January/February 2016). The Riverine Zone recorded the highest prevalence of *Plasmodium* species infection (12.7%) compared to Highlands (1.9%) and the Lowlands with 0.3%. The

overall point prevalence of *Plasmodium* species infection was 6.2% where 2 (0.2%) were non-*P. falciparum* infection from Riverine Zone. The overall prevalence slightly increased to 6.2 from 5.5% (Fisher's exact test = 0.057). The Midland Zone had no malaria cases during the entire period of the study (Table 4.1).

Table 4.1 Number of pupils tested per zone

ZONES	Baseline study Jan/Feb		First follow up Jun/July		Second follow up Oct/Nov		Third follow up Jan/Feb	
	No. Tested	Positive no./ %	No. Tested	Positive no./ %	No. Tested	Positive no./ %	No. tested	Positive no./ %
Riverine	763	174 (22.8)	560	35 (6.3)	594	68 (11.4)	582	74 (12.7)
Lowland	442	1 (0.2)	396	0 (0)	319	2 (0.6)	321	1(0.3)
Highland	284	0 (0)	256	0 (0)	224	0 (0)	257	5 (1.9)
Midland	179	0 (0)	160	0 (0)	141	0 (0)	131	0 (0)
Total	1668	175 (10.5)	1372	35 (2.6)	1278	70 (5.5)	1291	80 (6.2)

In general, prevalence varied with ecological zones where riverine recorded highest prevalence followed by the lowland the highland and midland. Midland and highland zones recorded less than five cases of *P. falciparum* infection during baseline survey and subsequent follow-ups. The results showed that *Plasmodium* species infection within riverine was significantly higher compared to lowland, highland or midland (Fisher's exact test = 0.005) (Table 4.2).

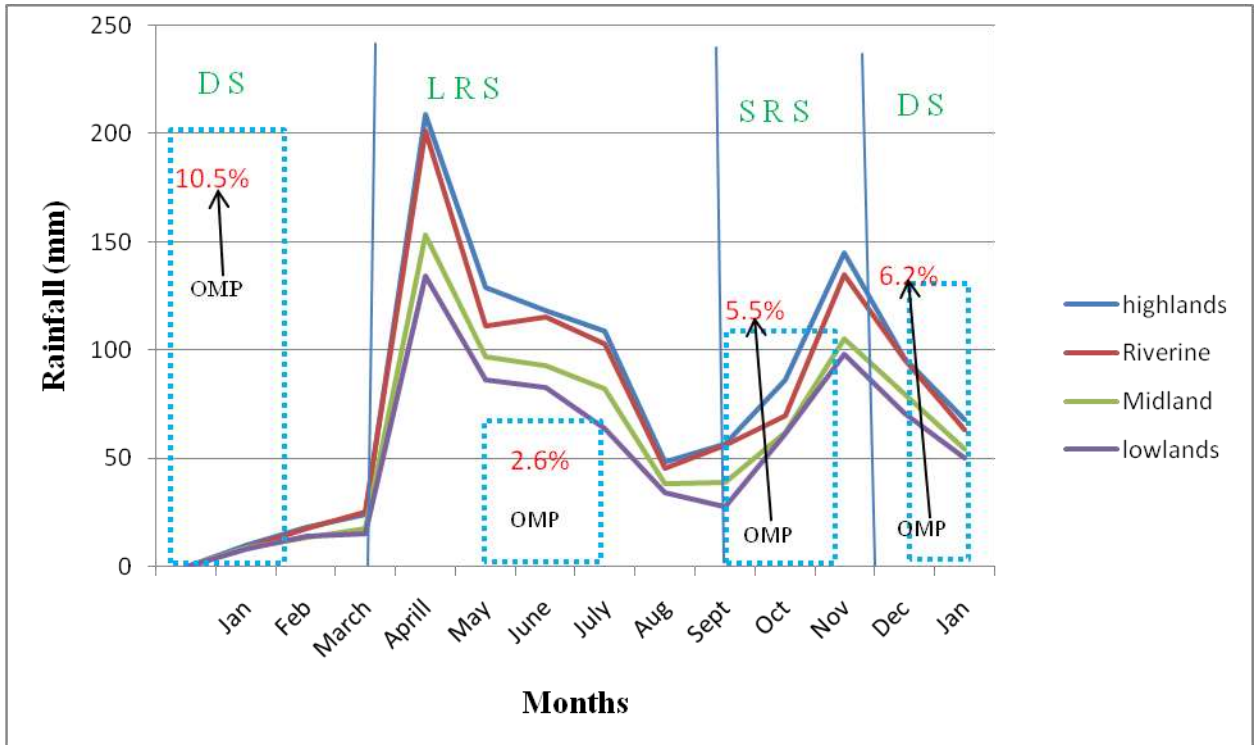
Table 4.2 Positive malaria cases per zone

Zones	Baseline	First follow- u	Second follow- u	Third follow-up	Total
Riverine	174	35	68	74	351
Lowland	1	0	2	1	4
Highland	0	0	0	5	5
Midland	0	0	0	0	0
Total	175	35	70	80	360

Fisher's exact test = 0.005

4.1.1 Prevalence of *Plasmodium* species infection during different seasons

The study was longitudinal where baseline and subsequent follow-ups were conducted in different seasons during the study period. It was therefore necessary to assess rainfall pattern and relate it with malaria prevalence data. Rainfall data retrieved from IRI/LDEO climate data library revealed that Baringo County had a bimodal rainfall pattern with the long rains falling between April and July, the short rains between August and November while dry season occurred between December and March. Malaria transmission was highest during dry season (10.5% and 6.2%) followed by Short rain season (5.5%) and lowest during long rain season (2.6%) (Figure 4.1).



OMP- Overall malaria prevalence, DS- Dry season, LR- Long rain, SR- Short rain

Figure 4.1 Rainfall pattern for the year 2015 (IRI/LDEO, 2016)

4.1.2 Prevalence of asymptomatic *Plasmodium* species infection

Asymptomatic *Plasmodium* species infection was determined using both RDT and Microscopy. During baseline survey, 61.1% (95% CI: 0.53-0.68) of the positive cases by RDT were asymptomatic. The proportion of asymptomatic cases were relatively high in both first follow up, 65.7% (95% CI: 0.48-0.8) and second follow up 62.9% (95% CI: 0.5-0.7). During the third follow up, 48.9% of the positive cases were asymptomatic (Figure 4.2). Microscopy also indicated that 55.6% of the confirmed cases during the entire period of study had no clinical symptoms.

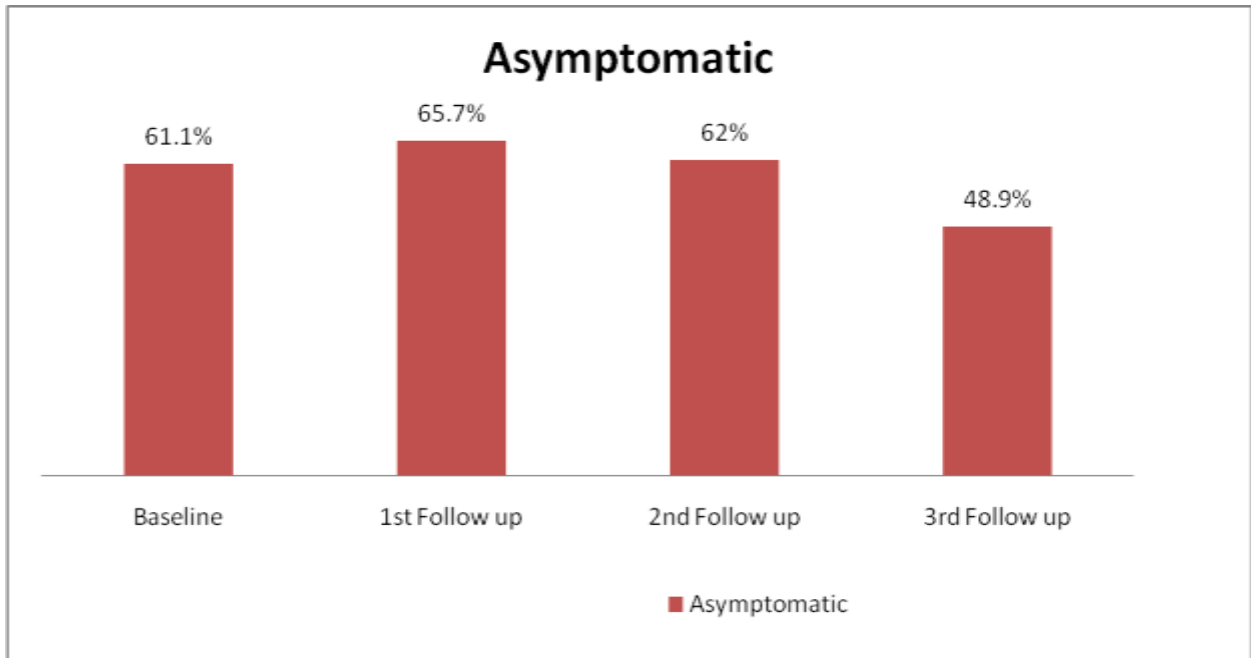


Figure 4.2 Asymptomatic cases

Males were slightly more asymptomatic than females, however not significant (Pearson $\chi^2 = 2.8885$, $df = 3$, $p = 0.409$). Similarly, there was no significant difference of asymptomatic *Plasmodium* species infection between children aged 5 - 9 and 10 - 15 years (Pearson $\chi^2 = 0.6746$, $df = 3$, $p = 0.879$) (Table 4.3).

Table 4.3 Cases of asymptomatic by gender and age group

Gender	Baseline	First Follow-up	Second Follow-up	Second Follow-up	Total	p-value
Females	44	12	13	18	87	0.409
Males	60	11	27	20	118	
Total	104	23	40	38	205	
Age group						
5 – 9	50	11	19	21	101	0.879
10 – 15	54	12	21	17	104	
Totals	104	23	40	38	205	

4.1.3 *Plasmodium* species infection episodes per child

During the baseline survey and the three subsequent follow-ups, a total of 268 (16.1%) pupils tested positive for *Plasmodium* species. infection by RDT. Out of 268 positive cases, 209 (78.0%) tested positive once (single episode) and negative during other three surveys, 45 (16.8%) tested positive twice (2 episodes), 13 (4.9%) had 3 episodes while 1 (0.4%) had 4 episodes of *Plasmodium* species infection (Table 4.4).

Table 4.4. Episodes of *Plasmodium* species infection among primary school pupils

Sample size (n) 1668	No Pf infection episodes	Single episode of Pf infection	2 episodes of Pf infection	3 episodes of Pf infection	4 episodes of Pf infection
No of pupils	1426	209	45	13	1
%	83.9%	78%	16.80%	4.9%	0.4

Pf- *Plasmodium falciparum*

4.2. Malaria Incidence

Incidence study was conducted at four months interval twice. A total of 48 new cases of *Plasmodium* species infection were recorded with 18 cases in the first follow-up and 30 in the second follow-up. During the first and second follow-ups, 387 and 270 study participants were lost to follow-up respectively. Out of 930 tested in second follow-up, 30 pupils tested positive, Eight hundred and seventy completed the eight months study while the remaining 30, were in the study for 4 months. The incidence rate was calculated by dividing the number of new cases by the total amount of time at risk (Person-month) for all the study participants. The incidence rate for 8 months period was calculated to be 5/1000 persons per month (Table 4.5).

Table 4.5 Time at risk per study participant

First follow-up			
	Time at risk (months)	Number of pupils	Total time at risk (Months)
New cases	2	18	36
Lost to follow-up	2	387	774
Second follow-up			
New cases	2	1	2
	6	29	174
Lost to follow-up	2	77	154
	6	193	1158
Completed the study without <i>P. falciparum</i> infection	4	30	120
	8	870	6960
Total	8	1605	9378

Plasmodium falciparum infection incidence varied significantly within geographical zones. The riverine zone had the highest incidence at 14/1000 person-months followed by lowland with an

incidence rate of 0.5/ 1000 person-month. Both highland and midland zones recorded zero incidences during the eight months study period (Table 4.6). Incidence rate ratio was used to determine the association between rate of infection and ecological zones. The incidence rate ratio (RR) between riverine and lowland zone was 40.2 (95% CI: 7- 1623). Since the null value (RR= 1.0) was not contained within the 95% confidence interval, the finding was statistically significant.

Table 4.6. Incidence rate of *Plasmodium* species infection by ecological zone

Zone	Person-month	New cases	Incidence rate/ 1000	95% Conf. Interval		RR
				Lower limit	Upper limit	
Highland	1938	0	0	.	.	
Lowland	2870	1	0.35	0.0676	3.4065	40.24
Midland	1218	0	0	.	.	
Riverine	3352	47	14.0	10.6686	18.8986	
Total	9378	48	5.1	4.3155	7.599	

Rate Ratio 40.24165(95% CI: 7-1623)

The risk *Plasmodium* species infection among those aged between 10- 15 was 1.6 higher than those aged between 5- 9. The difference was not statistically significant since null value (RR= 1.0) was included in 95% confidence interval, IRR 1.6 (95% CI: 0.9- 3.1). Similarly, the rate of *Plasmodium* species infection between males and females was not significantly different within the study area, Incidence Rate Ratio= 1.02 (95% CI: 0.55-1.88) (Table 4.7).

Table 4.7. Incidence rate of *Plasmodium* species infection by age bracket and gender

Age bracket	PM	Cases	Incidence rate/ 1000	95% confidence interval		RR
				Lower limit	Upper limit	
5-9	4849	19	3.9	2.798	6.876	-
10-15	4529	29	6.4	4.969	10.29	1.63
Total	9378	48	5.1	4.312	7.594	
Gender						
Males	4773	24	5.0	3.798	8.453	-
Females	4605	24	5.2	3.874	8.624	1.02
Total	9378	48	5.1	4.312	7.594	

PM- Person-month, RR- Rate Ratio, *P* – P-value

There were no deaths among the study participants and majority of the pupils who were lost to follow-up, were as a result of being transferred to other schools or absent from school on the day of screening.

4.3. Comparison of CareStart Ag-P.f and SD Bioline Ag-P.f

Out of 261 blood samples from primary school children, 35 (13.4%) samples tested positive for *P. falciparum* infections by both RDT kits while 7 (2.7%) were confirmed as positive by microscopy. The test results of both CareStart and SD Bioline Ag-P.f were similar (Table 4.8).

Table 4.8. Comparative performance of CareStart Ag-P.f and SD Bioline P.f against Microscopy

RDT- Care Start/SD Bioline	Microscopy		Total
	Positive	Negative	
Positive	TP = 6	FP=29	35
Negative	FN= 1	TN=225	226
Total	7	254	261

TP= True Positive, FP= False Positive, FN= False Negative, TN= True Negative

The sensitivity and specificity of both kits was 85.7% (95% CI: 42.1- 99.6) and 88.6% (95% CI: 84- 92.2) respectively. The positive and negative predictive values (PPV and NPV) of both kits for detection of *P. falciparum* were 17.1% (95% CI: 6.56-33.6) and 99.6% (95% CI: 97.6- 100) respectively. Sensitivity, specificity and predictive values of both kits for detection of *P. falciparum* are shown in table 4.9.

Table 4.9. Comparative performance indicators of SD Bioline P.f and CareStart Ag-P.f RDT kits (First survey)

Attribute	Test results	95% Confidence interval	
		Lower limit	Upper limit
Sensitivity	85.7%	42.1%	99.6%
Specificity	88.6%	84%	92.2%
Likelihood ratio(+)	7.51	4.75	11.9
Likelihood ratio(-)	0.161	0.0263	0.99
Odd ratio	46.6	7	-
Positive predictive value	17.1%	6.56%	33.6%
Negative predictive value	99.6%	97.6%	100%

4.3.1. Comparison between CareStart Ag-P.f and SD Bioline Ag P.f/ Pan

SD Bioline Pan is a multi-species RDT kit which is capable of detecting occurrence of four species of *Plasmodium*: *P. vivax*, *P. falciparum*, *P. malarie* and *P. ovale*. Unlike CareStart Pf which detects Histidine Rich Protein 2 (HRP-2) which is specific for *P. falciparum*, SD Bioline Ag-Pf/ Pan detects *Plasmodium* lactate dehydrogenase (pLDH) specific for the four *Plasmodium* species. Out of the 300 blood samples tested in second follow-up (October/ November 2015), SD Bioline Pan detected 60 (20%) samples as positive for *Plasmodium* species infection of which 45 (15%) were positive on the *P. falciparum* band, 13 (4.3%) positive on both *P. falciparum* and Pan band while 2 (0.7%) were positive only on Pan band. CareStart diagnosed 50 (16.7%) as positive for *P. falciparum* and 2 invalid test results. Microscopy confirmed 10 (3.3%) as positive for *P. falciparum* infection (Table 4.10).

Table 4.10 Malaria diagnosis by SD Bioline Malaria Ag P. f/ Pan and CareStart Ag P. f

SD Bioline Malaria Ag P. f/ Pan (05FK60)					
	Negative	Positive			
	(Control band only)	<i>P. falciparum</i> band only	Pan band only	<i>P. f</i> and Pan band	Total positive
Number	240	45	2	13	60
%	80%	15%	0.7%	4.3%	20%
CareStart Ag P. f (G0140)					
	Control band	Control and <i>P. f</i> band	Invalid result	Total positive	
Number	248	50	2	50	
%	82.7%	16.7%	0.7%	16.7%	

It was not possible to tell the two positive cases detected on Pan band only since the slides prepared from those two samples were read as negative by microscopy. Nine out of Sixty (10%) samples detected by SD Bioline as positive were confirmed by microscopy while only seven samples (14%) were confirmed from the 50 positive samples by CareStart (Table 4.11).

Table 4.11 Performance of SD Bioline P.f/ Pan and CareStart Ag P.f against microscopy

		Microscopy		
		Positive	Negative	Total
SD Bioline	Positive	TP = 9	FP = 51	60
	Negative	FN = 1	TN = 239	240
Total		10	290	300
CareStart	Positive	TP = 7	FP = 43	50
	Negative	FN = 3	TN = 247	250
Total		10	290	300

TP= True Positive, FP= False Positive, FN= False Negative, TN= True Negative

The overall sensitivity for detection of *P. falciparum* antigen by the SD Bioline P.f/Pan was 90% (95% CI: 55.5- 99.7) while that of CareStart Ag-P.f was 70% (95% CI: 34.8- 93.3). The specificity for the SD Bioline P.f/ Pan and CareStart Ag-P.f was 82.4% (95% CI: 77.5- 86.6) and 85.2% (95% CI: 80.6- 89.1) respectively. The study findings show that the sensitivity of SD Bioline was slightly higher than that of CareStart Ag-P.f. However, the specificity of CareStart Ag-P.f was slightly higher than that of SD Bioline. The positive predictive value (PPV) for detection of *P. falciparum* antigen by SD Bioline Pf/ Pan and CareStart P.f was 15% (95% CI: 7.1- 26.6) and 14% (95% CI: 5.82- 26.7) respectively while the negative predictive value (NPV) was 99.6% (95% CI: 97.7- 100) and 98.8% (95% CI: 96.4- 99.7) respectively. The

likelihood ratios (LR) of the two kits were also determined by STATA version 12. The SD Bioline had a positive LR of 5.12 (95% CL: 3.7- 7.07) and a negative LR of 0.121 (95% CL: 0.0189- 0.78) while that of CareStart Ag-Pf had LR of 4.72 (95% CL: 2.89- 7.71) and a negative LR of 0.352 (95% CL: 0.136- 0.909) (Table 4.12).

Table 4.12 Performance indicators of CareStart Ag-P.f and SD Bioline P.f/ Pan

	CareStart Pf	SD Bioline Pf/ Pan	McNemar p value
Sensitivity	70%	90%	0.4795
(95% CI)	34.8- 93.3	55.5 - 99.7	
Specificity	85.2%	82.4%	
(95% CI)	80.6 - 89.1	77.5 - 86.6	
Positive Predictive Value	14%	15%	
(95% CI)	5.82 - 26.7	7.1 - 26.6	
Negative predictive Value	98.8%	99.6 %	
(95% CI)	96.5 - 99.8	97.7 – 100	
Likelihood ratio (+)	4.72	5.12	
(95% CI)	2.89 - 7.71	3.7 - 7.07	
Likelihood ratio (-)	0.352	0.121	
(95% CI)	0.136 - 0.909	0.0189 - 0.78	

n= 300

4.3.2Parasite density

Parasite density ranged between 160 to 32800 parasites/ μ l of blood. The first follow-up was occasioned by relatively low parasite density of 160- 3040 compared to second follow-up (280- 32800)/ μ l of blood. Of the 18 positive blood slides, 8 had parasite density of less than 500/ μ l, 5 had density below 6050/ μ l while the remaining 5 blood slides had parasite density ranging between 12520/ μ l to 32800/ μ l of blood (Table 4.13).

Table 4.13 Parasite density

Number/ % of positive blood slides	Parasite/ μ l
8 (44.4)	< 500
5 (27.8)	1600- 6040
5 (27.8)	12520- 32800

4.3.3 McNemar's test of sensitivity

The sensitivity of SD Bioline Ag-P.f/Pan (90%) and that of CareStart Ag-P.f (70%) was tested to find out if they were significantly different with respect to sensitivity. Out of 300 blood samples 10 were confirmed as positive by microscopy. Out of these 10 confirmed cases, SD Bioline detected 9 as positive and 1 as negative while CareStart Ag-.Pf detected 7 as positive and 3 as negative. The seven samples detected by CareStart Ag-Pf as positive were equally detected by SD Bioline as shown in Table 4.14. The sensitivities of SD Bioline P.f/ Pan and CareStart P.f kits were not significantly different ($\chi^2= 0.5$, $df = 1$, p - value = 0.4795). This indicates that their performance is relatively similar.

Table 4.14 McNemar's statistical test of sensitivity of RDT kits

		CareStart		
		Positive	Negative	Total
SD BiolinePf/Pan	Positive	a=7	b=2	9
	Negative	c=0	d=1	1
Total		7	3	10

McNemar's $\chi^2 = 0.5$, $df = 1$, p -value = 0.4795

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Prevalence of *Plasmodium* species infection

The findings of the present study revealed the presence of *Plasmodium* species infections in children in parts of the Rift Valley highlands not previously reported, particularly in the lower altitude areas, although highland malaria has widely been reported (Brooker *et al.*, 2004; John *et al.*, 2004; Ernst *et al.*, 2006; John *et al.*, 2009). This study found that *Plasmodium* species infections are mainly restricted to the riverine zone, with *P. falciparum* as the main species. While it is not possible for this study to describe the reasons for the observed distribution, it is likely that ecological and environmental factors, including altitude, vegetation, terrain, water bodies, rainfall, temperature, humidity and vector abundance could have played a role, as previously reported (Lindblade *et al.*, 1999, 2000; Pascual *et al.*, 2008; Peterson *et al.*, 2009; Omukunda *et al.*, 2013). Human activity, especially land use, has also been reported to influence malaria transmission patterns (Lindblade *et al.*, 2000), which is a key consideration for the interpretation of these results given the diversity in land use across the four zones in the study area.

Plasmodium species infection within lowland was lower than that of the riverine zone, although both zones bear nearly similar environmental characteristics, which are different from the midland and highland zones. This is likely due to different control strategies reportedly used within these two zones. The lowland zone had more interventions, especially the use of

insecticide treated bed nets and indoor residual spray of houses to control leishmaniasis (Ngure *et al.*, 2015). In addition, there was apparently much lower access to health services in the riverine zone due to the few, distant and ill-equipped health facilities which are not connected to proper road networks. Due to lower access to health services in the riverine zone relative to the other zones, malaria transmission could have been sustained in the riverine zone due to limited control measures, diagnosis and treatment. In the absence of proper diagnosis and prompt treatment, the buildup of parasites within infected individuals increases the efficiency of malaria transmission due to rise in gametocyte densities (Churcher *et al.*, 2015) hence every bite by mosquito is likely to pick the parasites which are transmitted eventually to healthy individuals.

The present study findings indicate higher *Plasmodium* species infection during the dry season compared to the wet season. This is similar to other study findings which linked high transmission during dry season to risk associated to peri-domestic crop production and household levels (Bigoga *et al.*, 2012; Townes *et al.*, 2013). The lowest prevalence was recorded towards the end of long rains while during short rainy season, the prevalence slightly increased. Low malaria cases during long rains is due to flushing off of malaria vector breeding sites which may further lead to a decrease in larval population (Edillo *et al.*, 2004; Mutuku *et al.*, 2006).

Most of the pupils who tested positive in the highland zone reported to have travelled outside their villages to malarious zones in western Kenya. The probability of finding infections in highlands due to travel concurs with other study findings reported in Ethiopia and Western

highlands of Kenya (Arness *et al.*, 2002; Yukich *et al.*, 2013). (Edillo *et al.*, 2004; Mutuku *et al.*, 2006).

Although malaria prevalence within Baringo County is low (2.6- 10.5%) compared to endemic regions of Kenya where prevalence is greater than 20% (KMOH, 2015), riverine zone exhibited higher rates of malaria transmission throughout the study period with dry seasons recording the highest cases followed by short rain season. This might point the possible emergence of a hot spot within the County. The findings of this study are consistent with a study conducted by Snow *et al.* (2009) which also indicated the possible emergence of pockets of transmission within some arid and semi-arid regions in Kenya such as Turkana, Mandera and Garissa. Other studies also suggest that as malaria transmission rate declines, possible emergence of pockets of transmission is high and may likely fuel transmission to neighboring communities (Hay *et al.*, 2008; Bousema *et al.*, 2012). Transmission in both dry and wet season might also be indicative of a perennial pattern. This contrasts previous study findings which classified malaria transmission within Baringo County as seasonal (Snow *et al.*, 2009; Mohajan, 2014). Moreover, higher rate of transmission during dry season in this study is similar to other study findings conducted in Malawi and South Region of Cameroon (Bigoga *et al.*, 2012; Townes *et al.*, 2013).

5.1.2 Prevalence of asymptomatic *Plasmodium* species infection

High proportion of asymptomatic *Plasmodium* species infections in the present study was consistent with findings from previous studies which reported that school-age children represent the group with high cases of asymptomatic malaria (Clarke *et al.*, 2008; Noor *et al.*,

2009). Asymptomatic cases are usually common in high transmission areas where continuous exposure leads to development of partial immunity in children (Kun *et al.*, 2002). Baringo county malaria transmission is considered to be seasonal and parasite prevalence is usually below 5% (MOPHS, 2009; Mohajan, 2014). However, the presence of asymptomatic individuals particularly within riverine zone may point a continuous malaria transmission rather than seasonal.

Asymptomatic infected individuals may be potential challenge to various malaria control interventions as they act as reservoirs within the community (Singh *et al.*, 2014). Therefore, mass screening to identify and treat the asymptomatic individuals may lead to reduction of gametocytes hence interruption of continuous transmission. Treatment of asymptomatic infected individuals is also important in reduction of severe malaria cases which often develop due to non- treatment of uncomplicated malaria.

5.1.3 Malaria incidence

Quantification of malaria transmission rates within regions characterized by low transmission is of utmost importance for proper planning and appropriate intervention. This study sought to determine the risks of malaria infection among primary school children aged between 5-15 years in Baringo County. This age group is considered as asymptomatic carriers and may consequently act as reservoirs of malaria parasites within the community (Nzobo *et al.*, 2015). The entire study area indicated low risk of *Plasmodium* sp. Infection, but the risk greatly varied with ecological zones. The riverine zone recorded the highest risk while lowlands, highlands and midlands recorded least or no risk of infection. These findings are consistent with other

studies which demonstrated micro-epidemiological variations in malaria exposure especially in low transmission areas (Clark *et al.*, 2008; Snow *et al.*, 2009; Bousema *et al.*, 2012).

Similarly, a study conducted in Kampala, Uganda on factors determining the heterogeneity of malaria incidence also reported similar findings (Clark *et al.*, 2008). These results indicated a continuous trend of malaria transmission rather than seasonal within the riverine zone. The results further indicated that children aged 10 – 15 years were slightly more at risk than those aged 5 – 9 years old, although not statistically different, but points to agreement with other studies which have reported more susceptibility among the age group of 10 – 15 years than those aged 5 – 9 years (Brenyah *et al.*, 2013; Sena *et al.*, 2014). According to the present study, those aged between 5 – 9 years were reported to be still sleeping in the same bed with their parents hence were protected by the family bed nets. Infection rate for males and females was similar, in contrast to other studies which indicated that females were more at risk for malaria infection (Karunamoorthi and Bekele, 2009; Alemu *et al.*, 2012; Sena *et al.*, 2014). Therefore, intensive surveillance to map these areas is likely to guide on appropriate control interventions to protect the larger community.

5.1.4 Performance of CareStart Ag-Pf and SD Bioline Ag-Pf Kits

The sensitivity of CareStart Ag-Pf (85.7%) was comparable to that reported in other studies such as 89.68% in China-Myanmar (Xiaodong *et al.*, 2013), 88.8% in Belgium (Maltha *et al.*, 2010) and 89.1% in Myanmar (Ashley *et al.*, 2009). The present sensitivity was however slightly higher than that reported from an endemic region in Nigeria at 78.4% (Sheyin and Bigwan, 2013). Moreover, the sensitivity of 85.7% for SD Bioline Pf reported in this study was

similar to a study conducted in Central African Republic where the SD Bioline Pf RDT reported a sensitivity of 85.4% (Djalle *et al.*, 2014).

The specificity of 88.6% obtained in the present study for diagnosing *P. falciparum* was quite high indicating a specific test kit. Such specificity is important for detecting malaria parasite antigens with low parasite densities which might be easily missed out through microscopy (Guthmann *et al.*, 2002). This is particularly important in remote settings where adequate expert performance of microscopy may be a challenge. The high specificity findings of this study were consistent to specificity findings reported in endemic areas in Nigeria (Sheyin and Bigwan, 2013), 96.21% in Karachi (Harani *et al.*, 2006), 96% in Sierra Leone (Gerstl *et al.*, 2010) and 94.2% in north-west Ethiopia (Moges *et al.*, 2012), but relatively higher than 72% reported in Uganda (Kyabayinze *et al.*, 2008). The likelihood ratio of a test kit combines both the sensitivity and specificity into a single figure and usually indicates how the test result can reduce the uncertainty of a given diagnosis. For instance, a positive likelihood ratio of > 5 and a negative likelihood ratio of < 0.2 indicate high chances of a test result of a kit reporting a positive or negative outcome when given a positive screening or negative screening, respectively (Akobeng, 2007; Ashton *et al.*, 2010). Both CareStart and SD Bioline Pf kits had a positive LR of 7.51 and a negative LR of 0.161. This indicated a better performance in malaria diagnosis.

There was relatively low parasite detection by microscopy in the present study compared to previous findings in Northwest Ethiopia (50.5%) and Wondo Genet in southern Ethiopia (47%) (Sharew *et al.*, 2009; Hailu and Kebede, 2014). The current findings further contradict previous

studies conducted in China-Myanmar border (Xiaodong *et al.*, 2013) and North-West Ethiopia (Moges *et al.*, 2012) which reported more malaria cases detected by microscopy as compared to RDTs. The low detection of malaria parasites by microscopy in this study could have been due to low parasite density as observed during the first survey of the study. The effect of low parasite density in detection of malaria parasites by microscopy was also reported by McManus and Bowles (1996) and Snounou *et al.* (1993). Another explanation of low detection of malaria parasites in this study could be due to auto fixation of thick smears due to long storage time before staining occasioned by the expansive nature of the study area. This could have hindered the identification of parasites in thick smears which usually provide enhanced detection of parasites in blood film for easy quantification especially in cases of low level of parasitaemia (Moody, 2002).

5.1.5 Performance of SD Bioline Pf/ Pan and CareStart Kits

During the second survey, the sensitivity and specificity of CareStart kits dropped. This could have been due to few cases of invalid results by CareStart which were positive by SD Bioline kits. The sensitivity of 90% reported in this study for SD Bioline Pf/Pan to detect *Plasmodium* species infection was closely similar to other studies which evaluated the performance of the same RDT kit, for instance, a sensitivity of 88.2% reported in the Central African Republic (Djalle *et al.*, 2014) and 97.4% reported in Greece (Tseroni *et al.*, 2015). There were generally high negative predictive values for SD Bioline Pf/ Pan and CareStart Ag-Pf. The high NPV for the kits indicate that they can reliably rule out *Plasmodium* species infection. The kits however, had low positive predictive values of 15% and 14% for SD Bioline Pf/ Pan and CareStart Pf

respectively. The low PPV means that some individuals may be falsely diagnosed as positive hence confirmation of the positive cases by microscopy before treatment can be very important.

Two samples with high parasite density of 29,840 and 32,800 parasite/ μl of blood were only detected as positive by SD Bioline Pf/Pan. Failure to detect these two samples could have been due to prozone effect to the sensitivity of CareStart kits. For instance, higher parasite densities may lead to excess antigen or antibody in blood hindering the efficacy of some kits (Gillet *et al.*, 2009). Previous studies conducted to determine prozone effect on malaria HRP-2 and pLDH RDT kits reported more prozone effects on HRP-2 based RDT and not on pLDH based RDT (Gillet *et al.*, 2009). Other studies also reported that sensitivity of HRP-2 based RDT kits were more affected by high parasite densities ranging between 10,000 parasite/ μl of blood to 100,000 parasite/ μl of blood (Pieroni *et al.*, 1998; Forney *et al.*, 2001; Marx *et al.*, 2005; Ohrt *et al.*, 2007; Kyabayinze *et al.*, 2008; Van der Palen *et al.*, 2009). The present study further reported 2 (0.7%) cases of non *P. falciparum* infection within the riverine zone which were not confirmed through microscopy as they all read negative. Most of the slides which were positive on both *P. falciparum* and Pan band were positive by microscopy indicating active infection unlike HRP-2 which persists in blood long after clearance of parasites (Endeshaw *et al.*, 2008).

5.2 Conclusions

- i. The overall prevalence of *Plasmodium* species infection in Baringo County during baseline study, first, second and third follow-ups were 10.5%, 2.6%, 5.5% and 11.4% respectively. Higher prevalence (10.5%) was recorded during the dry season (January to March) which coincided with the baseline survey and which was preceded by short rains in the months of

October, November and part of December. The riverine ecological zone recorded highest overall prevalence (22.8%) followed by highland (1.9%), lowland (0.6%) and lastly by midland which had no cases of infection. *Plasmodium* species infection within riverine zone seemed to be perennial and not seasonal with highest prevalence (22.8%) occurring during dry season followed by short rainy season in October/ November (11.4%) and lastly during long rainy season in June/ July (6.5%). The proportion of asymptomatic *P. falciparum* infections was relatively higher within the riverine zone compared to other zones.

- ii. The overall risk of *Plasmodium* species infections in the study area was 5 in every 1000 persons per month. However, the risk of infection in riverine zone was 14 per 1000 persons per month.
- iii. The study showed that the performance of the three RDT kits in diagnosis of *P. falciparum* infections was relatively adequate and can be used in guiding treatment of febrile illness in remote settings especially where microscopy may be a challenge. SD Bioline Ag-Pf/Pan performed slightly better than CareStart Pf however, the difference was not statistically significant. Both SD Bioline Pf and CareStart Pf had similar test results. The three RDT kits had high negative predictive values indicating they can be reliably rule out the possible *Plasmodium* species infection. However, the low positive predictive values shows that individuals may be falsely diagnosed as positive by RDTs hence confirmation of the positive tests by microscopy to avoid unnecessary treatment.

5.3 Recommendations

- i. There is need for expanded malaria control strategies especially within the riverine zone which had the highest *P. falciparum* infections.
- ii. Mass screening within the riverine zone should be considered to target the asymptomatic individuals for treatment in order to limit development of severe malaria. This would also lower transmission rates by reducing parasite load in the population. This can be done by Ministry of Health and other stakeholders.

5.4 Suggestion for further Research

- i. There is need to determine anemia among school going children to indicate the level of morbidity as a result of asymptomatic or clinical malaria.
- ii. There is need to conduct a comparative study of these RDT kits in malaria hyper endemic regions to compare with the current study findings.
- iii. Further study on molecular diagnosis is needed to determine the other *Plasmodium* species responsible for mixed species infection particularly in the riverine zone.

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APPENDICES

Appendix I Ethical clearance form



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

Ref. No.KNH/ERC/R/68

KNH/UON-ERC
Email: uonkih_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link: uonbi.ac.ke/activities/KNHUoN



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

21st May 2014

Prof. Benson Estambale
Principal Investigator
Research, Innovation and Outreach
Jaramogi Oginga Odinga University of Science and Technology

Dear Prof.Estambale

Re: Approval of annual renewal – Early warning systems for improved Human Health and Resilience to climate sensitive Vector-Borne Disease in Dryland Areas of Kenya (P70/02/2013)

Refer your communication of May 4, 2014.

This is to acknowledge receipt of the study progress report and hereby grant you annual extension of approval for ethical research Protocol **P70/02/2013**.

The approval dates are 15th April 2014 to 14th April 2015.

This approval is subject to compliance with the following requirements:

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



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Ref: KNH-ERC/ R/75

4th May, 2015

Prof. Benson B.A. Estambale
Deputy Vice-Chancellor
Principal Investigator
Jaramogi Oginga Odinga University of Science and Technology
Bondo

Dear Prof. Benson

Re: Approval of annual study renewal - Early Warning Systems for Improved Human Health and Resilience to Climate Change Sensitive Vector-Borne Diseases in Dryland Areas of Kenya (P70/02/2013)

Your communication of 10th April 2015 refers.

This is to acknowledge receipt of the study progress report and hereby grant you annual extension of approval for ethical research protocol.

The study renewal dates are 15th April, 2015 to 14th April 2016.

This approval is subject to compliance with the following requirements:

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

Protect to discover

Appendix II Research approval by County Government

REPUBLIC OF KENYA

BARINGO COUNTY GOVERNMENT

Tel: 0720945345/0722661669
Email: cdhbaringo@gmail.com



County Director, Health Services
P.O. BOX 21-30400,
KABARNET

DEPARTMENT OF HEALTH SERVICES

REF: BCG/CDH/GEN/VOL.II/2015

DATE: 16th June, 2015

The Deputy Vice Chancellor
Research Innovation and Outreach
Jaramogi Oginga Odinga University
BONDO

Dear Sir/Madam,

RE: AUTHORITY TO CONDUCT RESEARCH

I acknowledge receipt of your Research request REF: No. JOUST/DUC-R10/FP/F2/001- WHO dated 16th June 2015 to collect blood samples from patients attending local health facilities and primary school children in Baringo County to be tested for exposure to RVF and malaria parasites, respectively.

You are therefore granted permission for the above mentioned activities.

We hope your researchers and research personnel will handle the research subjects ethically and uphold confidentiality as required.

I also request the staff in the concerned health facilities and schools to assist your staff as necessary.

Thank you.

An official circular stamp from the County Director of Health Services, Baringo County, dated 15 JUN 2015. The stamp contains the text 'COUNTY DIRECTOR OF HEALTH SERVICES', 'BARINGO COUNTY', and 'KABARNET'. A handwritten signature is written over the stamp.

Dr. Abakalwa M.S.G
County Director
Health Services
BARINGO

Appendix III Informed consent

Prevalence of *Plasmodium* species infection among Primary school children and performance of Rapid Diagnostic Test kits in Baringo County, Kenya

Consent to participate in this study

My name is **Collince J. Omondi** an MSc. (Applied Parasitology) student at Kenyatta University. I am conducting a study on Malaria Prevalence among Primary school children in Baringo County, Kenya and performance of Rapid Diagnostic Test kits

The purpose of the study

The study aims to determine the prevalence and incidence of *Plasmodium* species infection among primary school children in Baringo County of Kenya and to determine the performance of Rapid Diagnostic Test kits in malaria diagnosis. I will be visiting primary schools in Baringo County. Working with the local medical staff, I will be taking finger prick blood samples from the primary school children. The samples will be examined for malaria parasites in the field using rapid diagnostic kits and microscopy in the laboratory.

Why your child/ children have been chosen

As your child/ children are residents of this area where malaria is endemic, I need your permission and collaboration to carry out this study. The information generated will be useful for making decisions about malaria control in your community and the country at large.

Risks and benefits

There are no more risks involved in participation in this study other than those in routine medical examination. No drugs will be administered in order to take samples. Minimum invasive procedures (finger prick) will be used to collect blood samples from your child/ children. If you or your children are found infected they will be treated for free. If your child/ children present with other ailments, they will be referred to the relevant medical facility for treatment.

Confidentiality

The information documented from this work will not be divulged to anybody, and will be used by the study investigator only for purposes of report writing. No information that can identify you or your children will be used in the reports. After reports have been written the information collected will be kept private, for reference by the investigator only.

Conditions for participation

I have selected your child/ children for the study, but the consent for your child/children to participate is absolutely voluntary. You are free to accept or reject participation of your child/ children in the study. If you accept your child/ children to participate, you remain free to withdraw your child/ children from the study at any time. Your rejection will not affect you or your child's/ children's access to any public health service.

If you have any questions

I will be readily available to answer any questions, OR you may contact the **Principal Investigator: Prof. Benson Estambale, Telephone: 0713464619.**

Appendix IV Informed consent form

I,.....Id.No.....,
 from.....(village and county) being of 18 years or older and
 having full legal capacity to consent for my child/ children (named below), have been informed
 about the study entitled:

Title: “Prevalence of *Plasmodium* species infection among Primary school children and Performance of Rapid Diagnostic Test kits in Baringo County, Kenya”, headed by the Study Investigator Prof. Benson Estambale, Telephone: 0713464619.

The nature, duration, purpose, voluntary nature and inconveniences or hazards that may reasonably be expected have been fully explained to me. I have understood the information regarding the study, and what will happen. I have been given the opportunity to ask questions concerning this study and these (if any) have been answered to my satisfaction.

I understand that I may at any time during the study, withdraw the consent in the best interest of my children without any loss or penalty. My refusal of the subject to participate will involve no penalty or loss of benefits to which my family are otherwise entitled.

Tick (√) only one box per individual

Participants’ name	Age (yrs)	I <u>do</u> consent	I <u>do not</u> consent
1.			
2.			
3.			
4.			

Parents’ signature or left thumb print:..... Date.....

Witness: I hereby confirm that the study has been explained to the parent. All questions (if any) have also been answered to his/ her satisfaction, and he/ she, of his/ her own free will, has consented for his/ her child/ children to take part in the study.

Name of witness			
Signature of witness		Date:	

Name of person explaining the study:

6. General health condition

a) Normal () (b) Fair () (c) Severe ()

7. *Plasmodium falciparum* infection status (RDT)?

Negative () Positive ()

8. Treatment given by nurse present

Anti-malarials () Pain killers () others ()

Appendix VI Photos showing Clinical examination, collection of blood sample and testing



Physical examination by the nurse



Blood collection



Clinical diagnosis



Slide preparation



Slide examination