SPATIAL PROFILING OF MALARIA EPIDEMIOLOGY IN A HIGHLAND AREA OF LOW ENDEMICITY: TRANSMISSION, DIAGNOSIS AND MALARIAL ANAEMIA IN NANDI COUNTY-KENYA

By

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Philosophy in Epidemiology

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DECLARATION

I declare that the work presented in this thesis is my original work and has not been previously submitted or presented for the award of a degree in any university.

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I dedicate this work to Robert Sambuli Luchu & Dorothy Waeni Kakuti.

May your souls rest in eternal peace.

ABSTRACT

Malaria remains prevalent in many parts of Kenya but areas in the highlands of Nandi North sub-county have reported declining incidences of malaria infection and disease burden, and between April 2007 and March 2008 a possible interruption of transmission was reported. Given the low transmission intensity in the current study sites, it is not clear if fever and/or a combination of other symptoms are accurate predictors of malaria diagnosis. Relationship between malaria and anaemia is well documented. However, spatial autocorrelation of malaria and anaemia before and after reported interruption of transmission is not well understood. After a reported interruption of transmission in the current study sites between April 2007 and April 2008, new malaria cases have been documented. It is not clear if these cases are newly imported or are representative of an existing transmission reservoir. The study objectives were to: determine the predictive value of fever and/or other malaria symptoms for *P. falciparum* parasitemia among individuals seeking treatment in highland areas of very low transmission; determine spatial association between anaemia prevalence and previous malaria incidence before and after reported interruption of transmission and; determine if new malaria cases reflect existence of transmission hotspots in highland areas of very low malaria transmission. The study design used both retrospective (2003-2009) and prospective (2010-2012) review and monitoring of clinical, climatic, demographic and entomologic data respectively. A total of 3420 asymptomatic individuals and 1682 households were sampled. Sensitivity, specificity likelihood ratios were analyzed using STATA and R, spatial autocorrelation was determined using ArcGIS and SaTScan while correlation was determined using STATA. Malaria hotspots were determined using ArcGIS and SaTScan. $P<0.05$ was considered significant for all analysis. The findings of the study show that in areas of low, unstable malaria transmission, fever and/or combination of symptoms is not sensitive for clinical diagnosis of malaria in children <5 years, and in individuals ≥5 years. Fever had 55.8% sensitivity and 54.4% specificity for parasitaemia for < 5 years. The addition of headache increased sensitivity for parasitemia to 94.4% in children <5 years, and to 96.8% in individuals ≥5 years but decreased specificity to 9.9% and 11.6%, respectively. For children < 5 years, malaria incidence spatially correlated with anemia prevalence in Kapsisiywa only ($p < 0.001$) but not for Kipsamoite. For individuals ≥5 years, malaria incidence spatially correlated with anemia prevalence both in Kapsisiywa ($p = 0.001$) and Kipsamoite ($p < 0.001$). SaTScan analysis detected one hotspot in children < 5 years both in Kipsamoite ($p< 0.001$) and Kapsisiywa ($p = 0.003$). For individuals ≥ 5 years, Kipsamoite had 2 significant hotspots accounting for 9% of all households and 45% of total malaria cases ($p < 0.001$ and $p = 0.002$) while in Kapsisiywa, 3 significant hotspots covering 42% of all households and accounted for 71% of total malaria cases ($p < 0.001$, $p < 0.001$ and $p = 0.003$). The study concludes that screening for symptoms in addition to fever does not accurately capture all cases of clinical malaria in < 5 years and individuals ≥5 years old in areas of low malaria transmission; malaria and anemia are spatially correlated for individuals ≥5 in both sites but only spatially correlated in Kapsisiywa for children < 5 years and that there exist malaria hotspots in both sites. It does not conclusively determine if new malaria cases after reported interruption of transmission are a reflection of new infections or existence of malaria transmission reservoirs. The study recommends parasitological diagnosis, use of spatial approaches for malarial-anaemia interventions and parasite genotype confirmation to ascertain new malaria cases.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACT - Artemisinin Combination Therapy

AU - African Union

CBR - Crude Birth Rate

CDR - Crude Death Rate

DHS – Demographic Health Survey

DNA – Deoxyribonucleic Acid

DOMC- Division of Malaria Control

EC - European Commission

GIS – Geographic Information System

GPS – Geographic Positioning System

iCCM- Integrated Community Case Management

IMAI- Integrated Management of Adolescent and Adult Illness for adolescents and adults

IMCI - Integrated Management of Childhood Illness for children under five years of age

IMR - Infant Mortality Rat

IPTi- Intermittent preventive treatment strategies infants

IPTp-Intermittent preventive treatment strategies in pregnant women

IRS –Internal Residual Spraying

ITN – Insecticide Treated Bednets

KMIS-Kenya Malaria Indicator Survey

KNMS-Kenya National Malaria Strategy

malERA - Malaria Eradication Research Agenda

MDGs-Millenium Development Goals

NCAPD - National Coordinating Agency for Population and Development

NMCP-National Malaria Control Programme

PCR- Polymerase Chain Reaction

RBM -Roll Back Malaria

RDTs Rapid Diagnostic Tests

RS – Remote Sensing

WHO -World Health Organization

Definition of terms

Clinical malaria: A clinical malaria case was defined as an individual with malaria-related symptoms (fever, vomiting, headache, chills, loss of appetite, jaundice, backache, joint pains, nausea and malaise) and a positive blood smear for *P. falciparum* parasitaemia.

Sensitivity: The probability that a test will indicate 'positive for disease' among those with the disease

Specificity: The probability that a test will indicate "negative for disease" among those without disease

GIS System: A geographical information system (GIS) constitutes a system of hardware and software used for storage, management, retrieval, manipulation, analysis, modeling, and mapping of geographical data

Malaria risk: Exposure to possible infection with malaria parasite through bites of mosquitoes infected with P. falciparum and individuals with little or no immunity to malaria (young children and pregnant women or travelers coming from areas with no malaria).

Malaria hotspot: A small area with a significantly elevated malaria incidence in comparison to its surroundings.

Malaria elimination: The interruption of local transmission of a specified malaria parasite in a defined geographical area as a result of deliberate efforts. Continued measures are required to prevent re-establishment of transmission.

Malaria eradication: The permanent reduction to 0 of the worldwide incidence of malaria infection caused by human malaria parasites as a result of deliberate efforts. Once eradication has been achieved, intervention measures are no longer needed.

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CHAPTER ONE:

1 INTRODUCTION

1.1 Background

Malaria disease burden, trends in interventions' scale up, surveillance, progress towards global targets and inadequate funding have been the top key malaria-related issues in the World Health Organization's (WHO) malaria fact sheets and reports from 2011, 2012 and 2013, 2014 and 2015 (WHO, 2012; WHO, 2013; WHO, 2014, WHO, 2015). All the 2012, 2013, 2014 and 2015 WHO reports present data by children under the age of 5 years and individuals 5 years and above due to the natural immunity against malaria infants acquire from their mothers and lasts until they are 5 years old.

Over 40% of the world's population lived in malaria endemic countries with 247 million cases and nearly one million deaths in 2008 (WHO, 2010). According to the WHO 2013 fact sheet, 97 countries had ongoing malaria transmission with an estimated 3.4 billion people at risk of malaria with a further estimated 207 million cases of malaria in 2012 and 627 000 deaths (WHO, 2013). It also reported that 90% of all malaria deaths occur in sub-Saharan Africa with an estimated 482 000 deaths to children under five years of age. The report also indicated that expanded malaria interventions between 2000 and 2013 had led to a reduction of malaria incidence by 30% globally, and by 34% in Africa while malaria mortality rates in children had decreased by an estimated 53% worldwide and by 58% in Africa. Overall, despite the heavy burden of malaria morbidity and mortality, a steady trend of decline in malaria is being witnessed.

The WHO Malaria Report of 2015 estimated that half the world's population (3.2 billion people) from 95 countries were at risk of contracting malaria with sub-Saharan Africa region bearing the largest disease burden. Specifically, the region was home to the region was home to 88% of malaria cases and 90% of malaria deaths (WHO, 2015). According to the 2010 Kenya Malaria Indicator Survey (KMIS) (DOMC, 2011), malaria remains the leading cause of death among young children and also one of the most serious threats to the health of pregnant women and their newborns in Kenya with approximately 28 million of Kenya's 43 million population at risk of being affected by malaria. It also accounts for 34% of outpatient visits and 15% of all hospital admissions resulting in a significant health and economic burden and remains an impediment to Kenya's quest to reach the Millennium Development Goals (MDGs) as well as meet its Vision 2030 objectives.

Due to their high accuracy in malaria detection and easy availability and relatively low costs, the WHO recommends that quality assured rapid diagnostic tests (RDTs) and microscopy be used as the primary diagnostic tools for confirmation of suspected clinical malaria both in areas of and low transmission intensity (WHO, 2014). Microscopic assessment of stained blood films remains the standard method to confirm clinical episodes of malaria (Périssé & Strickland, 2008). Before the advent of confirmatory diagnosis, the WHO had recommended that since children usually have symptomatic clinical malaria in high malaria endemicity areas, they should be provided with anti-malarial therapy for any children with fever or a history of fever while in very low malaria transmission settings, the WHO recommended parasitic confirmation before

treatment and better training of health care workers for prompt identification of exposure to malaria (WHO, 2006).

In their argument in support for a shift from "presumptive malaria treatment to laboratory-confirmed diagnosis and treatment in African children with fever", D'Acremont *et al.,* (2009), questioned the then guidelines that recommended that all fever episodes in African children be treated presumptively with antimalarial drugs whilst there was evidence pointing towards declining malarial transmission as well as declining proportions of fevers due to malaria in parts of sub-Saharan Africa. In most settings, fever still remains the universal screening symptom for malaria infection especially in malaria endemic areas. Unfortunately, the areas with the highest malaria burden are in resource limited settings and therefore laboratory support is lacking and malaria diagnosis and treatment depends only on clinical diagnosis (Font *et al*., 2001; malERA, 2011, D'Acremont & Bosman, 2013).

It has been shown that clinical manifestation of malaria is influenced by transmission intensity (seasonal vs sporadic), biological factors of the host (genetic and immunological protection), host behavior (quality of housing, knowledge and use of ILLNs/IRS/ACT, treatment seeking behavior) and risk of co-morbidity (Ross & Smith 2010). While malaria intervention successes show that anti-malaria efforts are finally bearing fruit, questions are arising as to whether the changes in malaria transmission may be accompanied by changes in clinical manifestations of malaria. It is hypothesized that clinical manifestation of malaria changes as the immune responses associated with protection against clinical malaria wanes (Hafalla *et al.*, 2011). Even though the use of fever for clinical diagnosis of malaria has been widely explored (Muhe *et al., 1999*;

D'Acremont *et al.,* 2009), there still exists knowledge gaps in whether using a combination of some or all clinical symptoms of malaria (i.e. fever, vomiting, headache, chills, loss of appetite, jaundice, backache, joint pains, nausea and malaise) can increase accuracy (sensitivity and specificity) of clinical diagnosis of malaria especially in highland areas of low transmission intensity.

Anaemia is due to both a great increase in clearance of uninfected cells and a failure of adequate bone marrow response (Kai *et al.,* 1999; Kai & Roberts, 2008). Malarial anaemia is a common cause of morbidity and mortality especially among children in sub-Saharan Africa (Haldar & Mohandas, 2009). With a multifactor etiology, anaemia which is common in developing countries is usually caused by parasitic infections, HIV infection, chronic inflammatory disorders, micronutrient deficiencies and genetic disorders (Tatem *et al*., 2010). It has been reported that iron deficiency accounts for 50% of all anaemia cases while malaria, infection with HIV and with bacteremia-causing organisms accounts for the rest (Crawley, 2004). Malaria risk is associated with elevation and land surface temperature which are linked to distance to a perennial water body, land surface temperature and the normalized difference vegetation index (Ernst *et al.*, 2006). The severity of malarial anaemia is dependent on patient-specific characteristics like age, innate acquired immunity and comorbid features as well as parasite-specific characteristics species and drug-resistance phenotype (Odhiambo *et al.*, 2008). However, increased destruction and reduced production of red blood cells (RBCs) are also associated with malarial anaemia (Menendez *et al.*, 2000; Phillips *et al.*, 1986).

Anaemia interventions, particularly in children below 5 years of age have been have scaled up significantly in the last couple of decades (WHO, 2011; WHO, 2008). Global

estimates have shown that prevalence of anaemia in sub-Saharan Africa is highest in preschool-age children at 67.6% and pregnant women at 57.1% (WHO, 2008). The 1993-2005 World Prevalence of Anemia Report (WHO, 2008) also showed that the use of national prevalence estimates of anaemia (usually with a lot of variability) hinders the efficient delivery of control programmes and propose geographical quantification of anaemia variation. The prevalence of anaemia has been used as a proxy indicator for evaluating control programmes for malaria (WHO, 2006). In this regard, the World Health Organization (WHO) and Roll Back Malaria (RBM) Partnership recommend that anaemia be used as an extra indicator to monitor the burden of malaria especially in areas of high transmission (MalEra, 2011). Anaemia prevalence has been shown to correspond to malaria incidence even in areas of low endemicity (Noland *et al.,* 2011).

Studies conducted to investigate geographic relationships between malaria and anaemia have ranged from simple descriptive mapping of spatial distribution patterns (Magalhãesa & Clementsa, 2011; Kreuels *et al.,* 2007) to more complex statistical models that incorporated environmental factors such as seasonal temperature and rainfall (Aimone *et al.,* 2013; Carneiro *et al.,* 2006; Ernst *et al.,* 2006). These studies examined overall anaemia prevalence and malaria incidences in a given geographical area and the possible factors that contributed to the relationship but not household and/or individual specific relationships.

Informed by results of national and regional surveys, the planning of resources required for anaemia control is based on prevalence data which are mostly extrapolated to the country-level estimates. This has led to inefficient allocation of health interventions to control anaemia as geographical differences in the causes of anaemia which are

attributable to large-scale variability in environmental factors like nutritional and infectious causes were not considered during such interventions.

There exists a knowledge gap between spatial correlation of malaria and anaemia as no deliberate effort has been made to spatially determine if malaria and anaemia are occurring in the same geographical space and if observed decrease or increase in malaria that coincides with decrease or increase in anaemia respectively is happening in the same households or even among the same individuals. Specifically, there is little literature to show that prior malaria incidence correlates with anaemia prevalence. Further, if indeed there is a correlation between prior malaria incidence and anaemia prevalence, no study has shown if this relationship persists during periods of both high and low (up to interrupted) transmission.

Malaria elimination is the reduction to zero of the incidence of infection caused by human malaria parasites through deliberate interventions in a defined geographical area (WHO 2014). Malaria has been eliminated from over 40 countries with an additional 32 currently planning for, or committed to elimination (Gatton & Cheng 2010; WHO, 2014) either by committing to nationwide or spatially progressive within border elimination strategies (Reid *et al.,* 2010). Further, Namibia and Saudi Arabia have also been earmarked for malaria elimination (WHO, 2014). During the launch of its "Africa Malaria Elimination Campaign", the Africa Union conceded that despite being both preventable and curable, malaria remains one of the most serious public health problems in Africa with considerable burden and benefits in terms of human and economic development, if it were to be controlled (African Union, 2007). Similarly, in its 2014-2015 World Malaria Day Theme of "Invest in the future. Defeat malaria", the World

Health organization (WHO), launched a guide titled "From malaria control to malaria elimination: a manual for elimination scenario planning"- a manual geared towards helping countries assess their technical, operational and financial feasibility of moving towards malaria elimination. While the optimal strategy for elimination is still subject of debate, possible distinctions between control and elimination efforts lie in the geographical scale and intensity of key intervention approaches (Sabot *et al.,* 2009).

John *et al.,* (2009) reported interruption of transmission after widespread use of indoor residual sprays (IRS) and artemisinin combination therapy (ACT) in a highland area of very low transmission. Similar reports have come from other areas in East Africa (Jaenisch *et al.,* 2010). To counter the malaria burden, different interventions have been implemented: among them long-lasting insecticide treated bed nets (LLINs), IRS, ACT, intermittent preventive treatment strategies in pregnant women (IPTp) or infants (IPTi) (Senn *et al.,* 2009) as well as the use of new diagnostic tests using rapid diagnostic kits (RDTs) that allow prompt treatment of confirmed cases and reduce mistreatment (WHO, 2010).

Sustaining interruption of transmission or elimination of malaria remains a key challenge especially in resource limited settings. Several countries with historically medium-to-high transmission intensities, have reported approaching a state of controlled low-endemic malaria re-igniting the debate of whether policy makers should accept low rates of malaria transmission with a strategy of consolidating interruption of transmission or aiming for elimination altogether (WHO, 2007; Cohen *et al.,* 2008; Cohen *et al.,* 2010). Further, there are still challenges in areas of very low transmission in establishing whether ongoing transmission is attributable to new infections or due to presence of

transmission reservoirs in the population and hence a need to establish if in a homogenous environment, there could be certain hotspots for which malaria reemerge. No study has been done in an area that reported interruption of malaria transmission before and experienced malaria reemergence to establish if the malaria cases were due to an existing infection reservoir or new infections.

1.2 Statement of the Problem

The WHO has recently developed algorithms for the management of fevers within communities at peripheral health facilities for children under five (integrated community case management-iCCM) in addition to the already existing integrated management of childhood illness (IMCI) for children under five years of age and integrated management of adolescent and adult illness (IMAI) for adolescents and adults. However, following many years of practice of treating fever as assumed malaria, there is a running risk that health workers may continue using fever for clinical diagnosis especially in the absence of guidance and medicines for the management of non-malaria febrile illnesses. Most clinicians in remote settings where parasitological confirmation of malaria is hindered by resource constraints, use fever or a history of fever as a screening symptom for malaria. While fever is widely used in high transmission settings, in areas of low transmission intensity, a combination of fever and some or all malaria symptoms and their respective sensitivity and specificity to clinical diagnosis of malaria has not been explored. The sensitivity and specificity of symptoms in areas that have had historically low malaria incidence to the point of interruption of transmission is also poorly understood. This scenario calls for a better understanding of presentation symptoms or combination of

symptoms (beyond fever alone) of *P. falciparum* parasitemia and their sensitivity and specificity to reduce misdiagnosis and or subsequent drug resistance.

There have been many attempts made to reduce the burden of malaria and anaemia in vulnerable groups especially children less than 5 years of age. The relationship between malaria and anaemia continues to be a big public health problem especially in children. The nature and mechanism of these relationship, especially in areas of very low malaria transmission remains largely unresolved. Further, in areas of very low transmission, it has not been demonstrated that anaemia prevalence correlates with prior malaria infection. It is also not clear how long such a correlation lasts, if it indeed it exists. Specifically, it has not been established if anaemia persists even during transitional periods between high and low transmission intensity times. If the spatial correlation of malaria and anaemia during both high and low intensity periods is not well understood, it is possible that interventions may not only reach the targeted individuals but may end up being ineffective (costly and time consuming).

To sustain malaria interruption, appropriate treatment of clinical and asymptomatic infections as well as developing malaria prevention strategies is vital. After the reported possible interruption of transmission within the current study site-a highland area of very low transmission-isolated cases of malaria have been documented in the same area since then. However, it is not known if the new malaria cases are emanating from specific geographical hotspots/households hence pointing to existing transmission reservoirs or are new and are randomly and evenly occurring within the study area. The uncertainty as to whether reemerging malaria cases are new infections or resultants of existing transmission reservoirs especially in very low malaria transmission settings can lead to

ineffective interventions that may not sustain interruption or elimination efforts. Areas that have attained interruption or very low malaria intensity are best placed to maintain this status through focused control programs that target individuals and specific geographic points within a community.

1.3 Objectives of the study

1.3.1 General objective

The overall objective of this study was to spatially characterize malaria epidemiology in a highland area of low endemicity in terms of transmission, diagnosis and malarial anaemia.

1.3.2 Specific objectives

i. To determine the predictive value of fever and / or other malaria symptoms for *P. falciparum* parasitemia among individuals seeking treatment in highland areas of very low transmission

ii. To determine spatial association between anaemia prevalence and previous malaria incidence in highland areas of very low malaria transmission

iii. To determine spatial association between anaemia prevalence and malaria incidence after reported interruption of transmission in highland areas of very low malaria transmission.

iv. To determine if new malaria cases reflect existence of transmission hotspots in highland areas of very low malaria transmission.

1.4 Null Hypotheses

i. H_o: Fever and or other malaria symptoms are not sufficient predictor(s) of *P. falciparum* parasitemia in an area of low endemicity.

ii. H_o: There is no spatial correlation between anaemia prevalence and prior malaria incidence.

iii. H_o: There is no spatial correlation between anaemia prevalence and malaria incidence after reported interruption of transmission.

iv. H_o: New malaria cases after reported interruption of transmission do not reflect existing hotspots in highland areas of very low malaria transmission.

1.5 Significance of the study

Many of malaria's signs and symptoms are indistinguishable from those of other febrile diseases (European Commission *et al.,* 2010). Since peripheral health facilities in areas where malaria burden or risk of malaria transmission is high tend to lack microscopy for the diagnosis of malaria it is vital to identify clinical symptom(s) that are both sensitive and specific for the diagnosis of malaria by health workers in such circumstances for improved malaria case detection. In areas of interrupted malaria transmission, diagnosis of clinical malaria is a challenge due to changing transmission intensity. As transmission decreases and individuals are less exposed to malaria, they lose acquired immunity with a higher proportion of infections presenting with symptoms (malERA, 2011). Helping clinicians and other health workers in peripheral health facilities to standardize clinical malaria diagnosis especially in resource limited settings like the tropics of Africa would result in reduced misdiagnosis, accurate case management and by extension reduced emergence of malaria drug resistance. This study will also add valuable knowledge to the

non-exhaustively explored area of combination of malaria symptoms for clinical diagnosis in areas of very low transmission intensity.

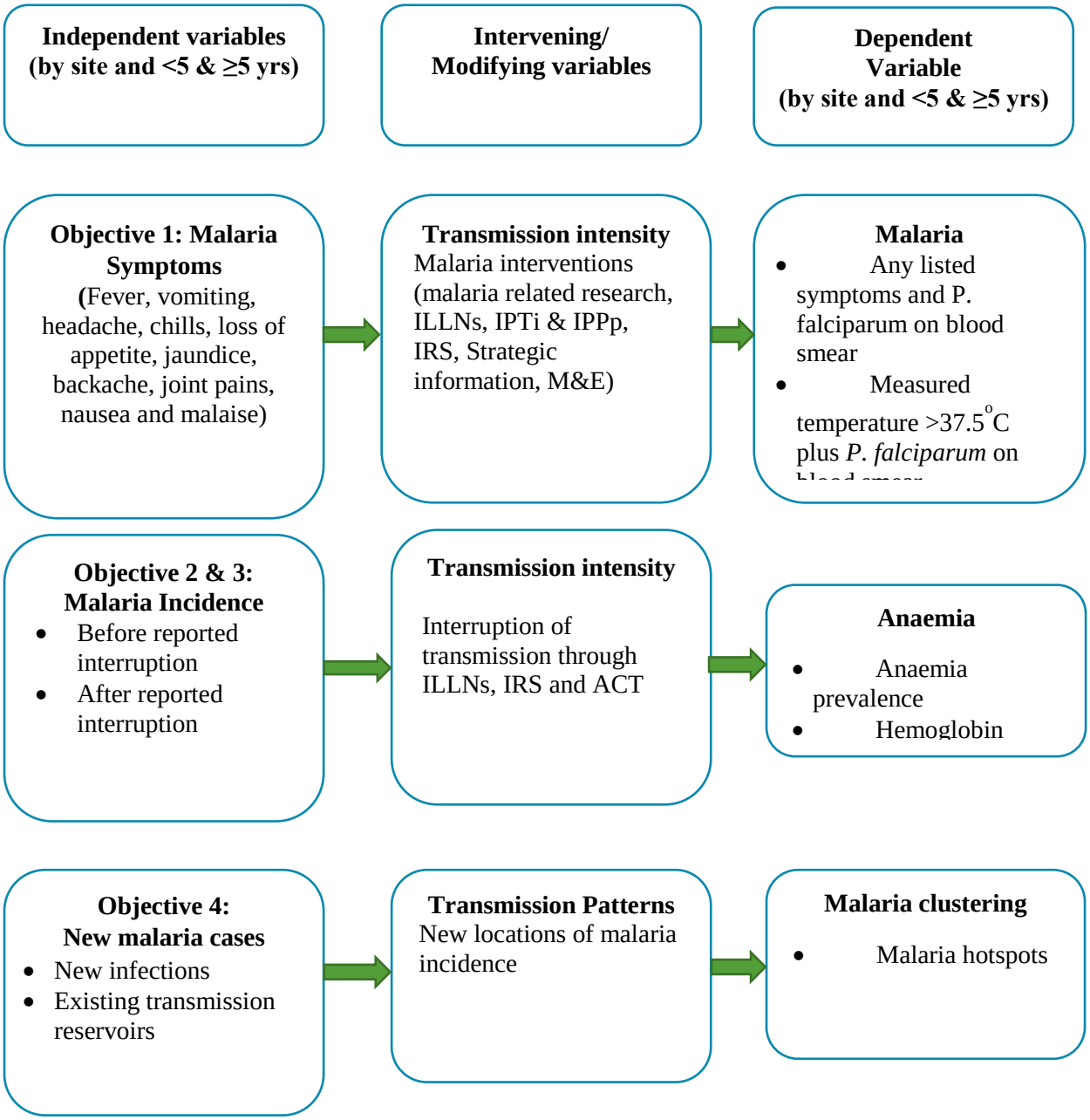
Anaemia has been suggested as a surrogate measure of effectiveness of malaria interventions over time (Mathanga *et al.,* 2010). If indeed it is true that anaemia prevalence correlates with prior incidences of malaria, then periodic surveys of hemoglobin levels could be used to provide estimates of an area's transmission intensity and the heterogeneity of transmission in an area. Using GIS techniques to investigate the malaria-anaemia disease to disease interaction could contribute important new information for developing effective and efficient anaemia and malaria interventions especially in an area of very low malaria transmission like the current study sites. Targeted malaria-anaemia interventions would significantly reduce intervention costs, morbidity associated with adoption of an integrated approach and deaths especially in children under 5 years.

Measuring effectiveness of intervention strategies is vital and remains a key challenge in low transmission areas where malaria control programs are implemented. In settings of very low or interrupted malaria transmission, sustaining interest in elimination is very important. It is important to determine if transmission remains interrupted, and malaria cases occasionally occur because of existence of hotspots within an area and hence supporting ongoing transmission. Targeted malaria control interventions are likely to yield better results for both individuals included in control efforts as well as at community level since there is reduced risk of multiple transmission of parasites. These targeted interventions are effective, efficient and a vital approach in malaria elimination

efforts especially in areas where transmission has decreased tremendously and may be transmitted through existing hotspots.

1.6 Conceptual framework

Figure 1: Conceptual Framework



All the independent and dependent variables in the current study were measured, analyzed and reported by site and age. Results from previous studies conducted at the two study sites-which even though adjacent to each other-have shown different geographical features like proximity to swamps and forests as well as malaria incidence. On the other hand, malaria in children under 5 and individuals 5 years and above is a widely used desegregation during analyses and reporting. Therefore, for each objective, where feasible analyses has been done for each site, and for under 5 years and 5 years and above separately.

Table 1: Operationalization of variables

	Variables	Measurement of indicators
Objective 1:	Malaria symptoms: Fever, vomiting, headache, chills, loss of appetite, jaundice, backache, joint pains, nausea and malaise	Number and proportion of individuals with symptom or combination of symptoms and also testing positive for malaria. Analysis for sensitivity, specificity, positive and negative predictive values of symptoms and malaria outcome will be done Chi Square tests for differences in presence and/or absence of symptoms and malaria outcome. Logistic regression tests to determine odds of presence and/or absence of symptom and malaria outcome.
	Malaria	Malaria is defined as: • Any listed symptoms and P. falciparum on blood smear • Measured temperature >37.5oC plus P. falciparum on blood smear
Objective 2 & 3	Malaria incidence	Malaria incidence will be measured: • Before reported interruption of transmission (before April 2007) • After reported interruption of transmission (after April 2008)
	Anaemia	Anaemia will be measured: • By prevalence before and after reported interruption of transmission. • By the hemoglobin levels of individuals before and after reported interruption of transmission
Objective 4	New malaria cases	New malaria cases will be classified as a result of new infections from outside of the study sites and/or infections that stem from existing transmission reservoirs within the sites.
	Malaria hotspots	Malaria hotspots will be measured by establishing malaria location patterns. Consistent locations with elevated malaria incidences before and after interruption would be indicative of existing malaria hotspots while malaria locations with high disease concentration before interruption but none after interruption would not be indicative of malaria hotspots

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Malaria

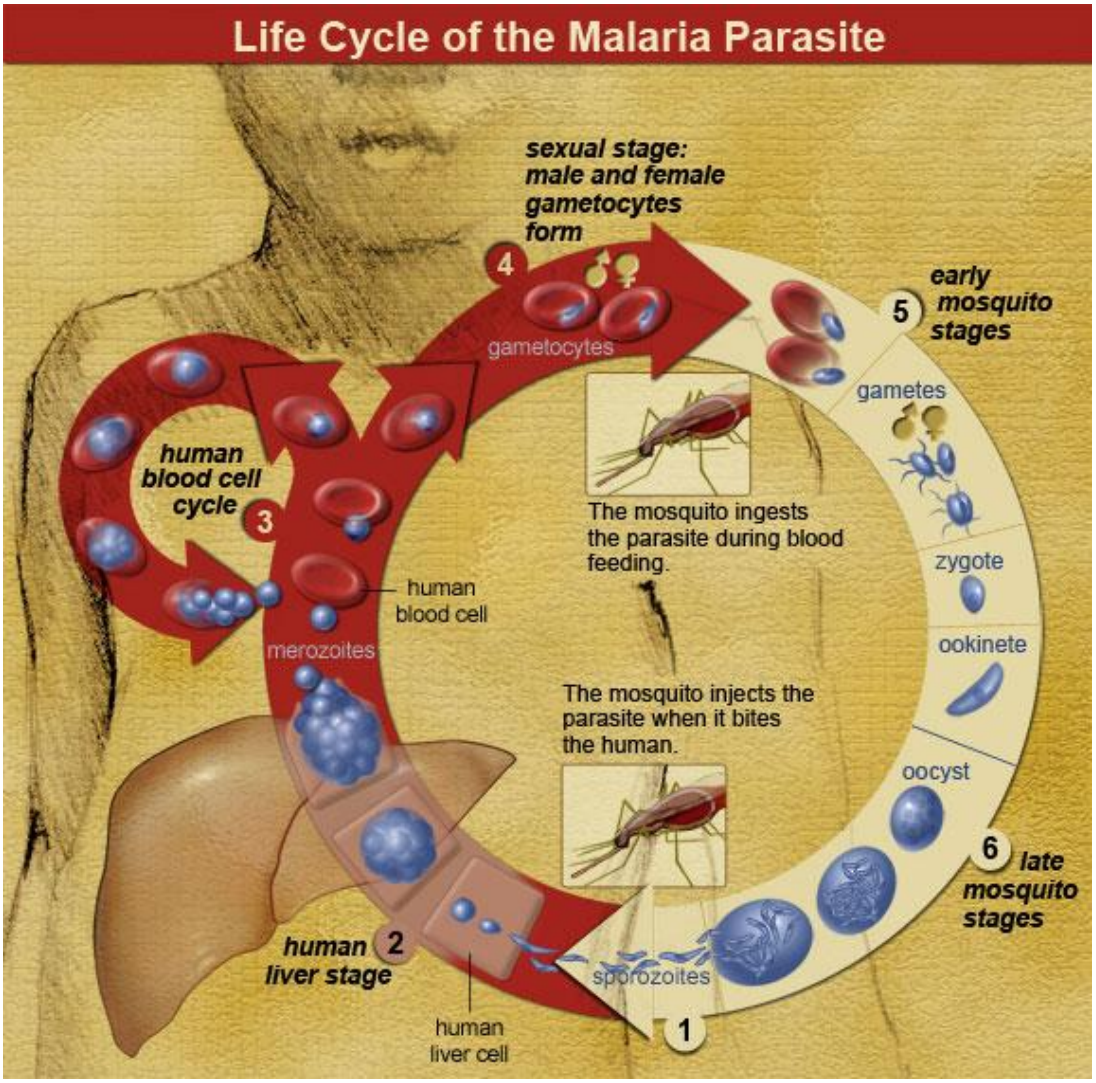
Malaria is derived from 18th century Italian *mala* meaning "bad" and *aria* meaning "air" when it was initially thought that the disease was caused by foul air in marshy areas. However, in 1880, it was discovered that it was a parasitic disease and by the end of the 18th century, scientists had confirmed that it could be transmitted by the bite of the female *Anopheles* mosquito, (Medical News, 2014).

The malaria parasite, Plasmodium, is a unicellular protozoan that causes malaria in humans. Four primary species of malaria parasites infect humans: P. falciparum, P. ovale, P. malariae and P. vivax with an additional species P. knowlesi, a malaria parasite that typically is found in monkeys as reservoirs (Sabbatani *et al*., 2010). Of the five species, P. falciparum is greatly responsible for malaria's global morbidity and mortality (Hay *et al*., 2004; WHO, 2008). P. falciparum is also the most widely distributed Plasmodium species in tropical Africa, eastern Asia, Oceania and the Amazon (Lysenko & Beljaev, 1969). .

Infection of human hosts occur when a previously infected female *Anopheles* mosquito bites an individual. Through the mosquito's saliva and into the human blood, the parasite-called sporozoite at this stage-is injected into the human body. The parasites then multiply in the liver, and enter the blood stream again through the red blood cells (RBCs) as merozoites. The merozoites then develop into the trophozoite stage and then divide/multiply through a process called schizogony to produce more merozoites which

attack non-infected blood cells. Some merozoites develop into new trophozoites while others develop into male microgametocytes or female macrogametocytes. At this stage, an uninfected *Anopheles* mosquito can get infected if they bite an individual with mature gametocytes in their peripheral blood. Inside the mosquito, the microgametocytes exflagellate into gametes before fertilizing the macrogametocytes to form zygotes. The zygotes change into an ookinete and later into an oocyst generally found in the mid-gut wall of the mosquito. The sporozoites leave the oocyst and get into the mosquito's salivary glands from where they infect humans through a blood meal (Cowman *et al.*, 2012; Gilles & Warrel,1993).

Figure 2: Life cycle of malaria parasite



National Institute of Allergy and Infectious Diseases (NIAID)

The first symptoms usually occur 10-28 days after infection, though they can appear as early as 8 days or as long as a year after infection while the symptoms occur in cycles of 48 to 72 hours (MedLine Plus, 2014). Some of the common symptoms of malaria are fever, appetite loss, headache, vomiting, chills, malaise, diarrhea, nausea, joint pains and

jaundice. Even though malaria is a preventable and treatable disease, when left untreated, malaria patients may develop severe complications that may lead to death.

According to the December 2013 WHO malaria estimates, there were about 207 million cases of malaria worldwide in 2012, and an estimated 627 000 deaths with malaria mortality rates falling by 42% globally since 2000, and by 49% in the WHO African Region (WHO, 2014). According to WHO (2009), the social and economic burden of malaria in Kenya has been huge with an estimated 15 million malaria cases in 2006 and approximately 40,000 malaria related deaths while funding for malaria control increased from less than US$ 1 million in 2003 to approximately US$ 62 million in 2008, mainly from the Global Fund based on the Presidential Malaria Initiative (PMI) Operational Plan for 2011 (PMI, 2011). Malaria epidemiology in Kenya is varied based on geographical transmission intensities, and is classified into four zones:

Endemic zones: zones like areas around Lake Victoria in western Kenya with high and intense malaria transmission throughout the year with a *P. falciparum* malaria prevalence between 20% and 40%. Even though some parts of Coastal region have experienced a decrease in malaria- estimated malaria risk classification of less than 5%, the DOMC still classifies the region as endemic as this reduction is not yet stable and the risk for a resurgence of malaria burden in the area exists.

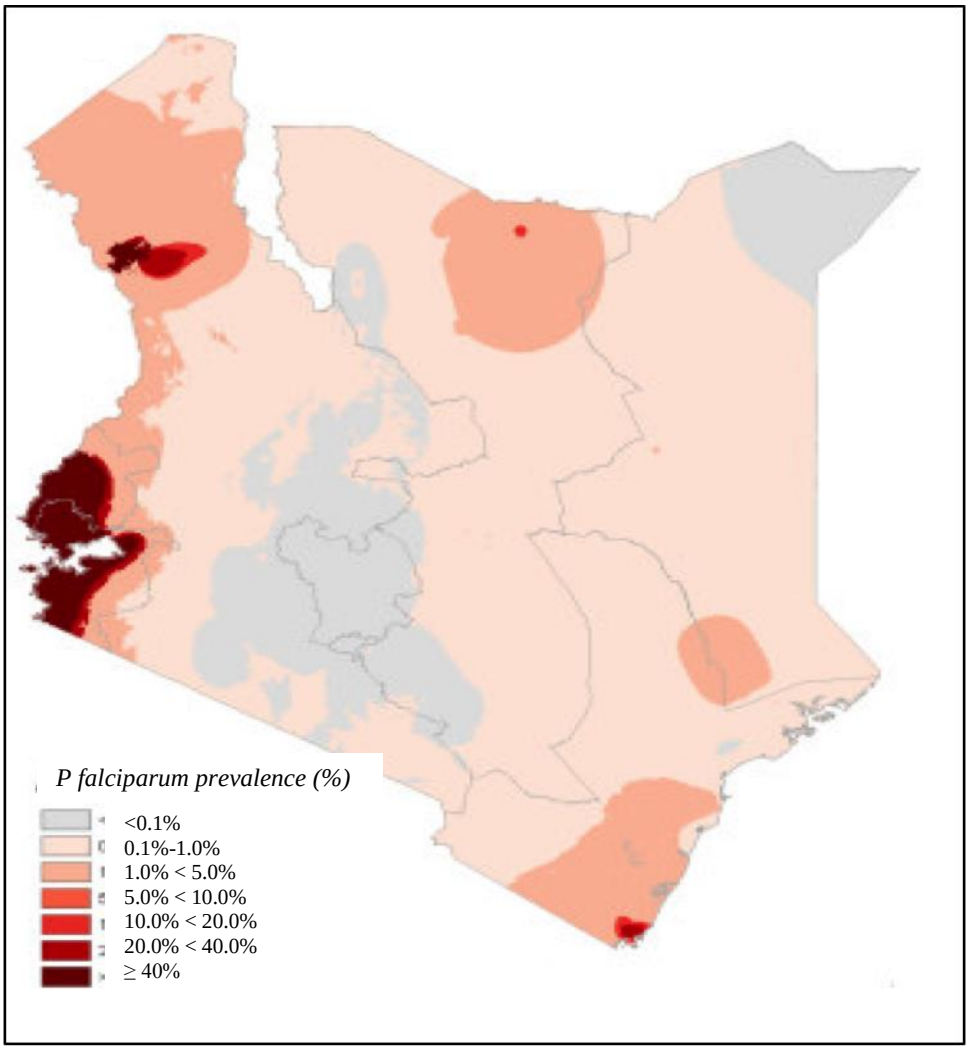
Epidemic-prone: areas around western highlands where malaria transmission is seasonal with considerable year-to-year variation. They are considered epidemic-prone areas because temperature increases and rainfall variation can impact vector breeding and malaria transmission. Due to dense highland population (Hurni, 1990), approximately

20% of Kenyans live in these areas and have a malaria prevalence ranging from 1% to less than 5% but with occasional prevalence between 10% and 20%.

Seasonal transmission: Areas covering the arid/semi-arid zone in Northern and South Eastern parts which experience short periods of malaria transmission of less than 5% at any time during the rainfall seasons and account for approximately 21% of the Kenyan population.

Low-risk: These are mainly the central highlands of Kenya where 30% of Kenyans live with little to no malaria transmission.

Figure 3: Map of malaria risk-Kenya



Noor *et al. (2009)*

2.2 Epidemiology of malaria in the highlands

African highlands are described to be of altitudes approximately higher than 1500 meters elevation above sea level and or with daily mean temperatures of below 20°C. Kenya, Tanzania, Rwanda, Burundi, Uganda, Ethiopia and Madagascar constitute about 82.4% of the African highlands which is approximately 4% of the total land area of sub-Saharan region (Hurni, 1990). Malaria appears to have been introduced by rail and travel of persons from endemic populations to highland areas for tea planting and harvesting in the early 1900's (Matson, 1957; Campbell 1929). According to Matson (1957), the first malaria epidemic 1919 in western Kenya highlands was documented following the influenza pandemic during troop demobilization and resettlement after World War I in 1918 while according to (Fontaine *et al*., 1961) between the 1920s and the 1950s infrequent malaria epidemics were reported in eastern Africa highlands.

Thus, families in this area have not had generations of exposure to malaria, as have families in nearby areas such as lowland areas of Nyanza Province (Lindsay & Snow, 1988). After extensive malaria eradication campaigns, no malaria epidemics were reported between the 1960s and the early 1980s (Roberts, 1964). This is supported by the low prevalence of sickle cell trait and G6PD deficiency in individuals in the highland areas as compared to individuals in lowland areas (Moormann *et al.*, 2003). Thus symptoms and immunity in this population may differ from those in populations with a long history of exposure (e.g., low land areas bordering the lake) and in populations with no exposure for decades (e.g., the United States and Europe).

The Kenyan highlands have traditionally been characterized as prone to unstable and limited malaria transmission occasioned by the average annual low temperatures. Malaria transmission has been attributed to short spells of warm periods which generate epidemic conditions in immunologically naive human populations living at high altitudes (Hay *et al*., 2004; Alonso *et al.,* 2011) while low temperatures reduce transmission intensity through effects on the population growth of the mosquito vector and on parasite development within the vector (Siraj *et al*., 2014).

Development of immune responses and clinical immunity to *P. falciparum* have been shown to directly correlate with malaria transmission intensity (Baird, 1998). It has also been shown that immunity to *P. falciparum* malaria is developed as a result of long term exposure to the parasite and depends on immunological memory since individuals suffering from chronic exposure to malaria are able to slowly develop a type of non-sterile or semi-immunity that eventually protects against high parasitemia, severe clinical syndromes and malaria-related deaths (Lusingu *et al.*, 2004).

MacDonald (1957) describes a malaria epidemic as a sharp rise in the incidence of malaria out of proportion to the normal incidence that a community is normally subject to. Epidemics can therefore occur in areas of unstable malaria which have experienced a modification in any of the transmission factors that "upset" the existing equilibrium and where communal immunity is absent. Epidemics have also been attributed to temporary disturbances to stable hypo-endemic equilibrium, such as those resulting from abnormal meteorological conditions and major changes in the eco-epidemiological system like environmental modifications for purposes of economic development and interruptions to anti-malarial intervention measures which have kept malaria under control (Nájera *et al.,*

1998). Other factors more likely to be associated with the rise in epidemics include: rise in resistance to antimalarial drugs, population migration and breakdown of health service provision. In Africa, there have been spates of malaria epidemics described as 'highland malaria' (Lindblade *et al*., 2000). Even though these epidemics occurred at altitudes previously assumed to be 'safe' from malaria, there occurrence is not a new phenomenon as 'highland' malaria epidemics were observed in the 1940-1950's in Kenya and Ethiopia (Matson, 1957; Fontaine, 1961; Alonso *et al.*, 2011).

2.3 Clinical Diagnosis of Malaria

Early diagnosis of malaria serves the dual purpose of mitigating the suffering of individuals as well as reducing transmission of the parasite in the community. Prompt and accurate diagnosis of malaria is therefore needed for implementation of appropriate treatment to reduce associated morbidity and mortality as well as developing drug-resistance. There are a number of malaria diagnostic techniques: clinical criteria, microscopy, rapid diagnostic tests and molecular diagnostic techniques with most cases of uncomplicated malaria in the tropics of Africa diagnosed clinically on the basis of fever and or history of fever (WHO, 2000).

Methods for measuring transmission are central to any elimination agenda. Current methods for measuring transmission that may be applied in endemic areas are time-consuming, expensive and too insensitive for use in conditions of low and non-uniform infection (Bell *et al.*, 2006). The very low levels of transmission now being attained in many countries focusing on elimination present new challenges that demand new diagnostic tools and strategies, like change from passive case detection to ''active'' case detection (malERA, 2011). While reviewing malaria diagnosis, (Tangpukde *et al*., 2009)

say that as much as malaria diagnosis involves a ‘‘simple’’ procedure of identifying malaria parasites or antigens/products in patient blood, the diagnostic efficacy is subject to many other factors like: the different malaria species and their endemicity; the life cycle stage of the disease, external factors like levels of transmission, imported and or exported malaria, population immunity, clinical presentation; drug resistance; recurrent malaria and persisting viable or non-viable parasitemia can all influence the identification and interpretation of malaria parasitemia in a diagnostic test.

Microscopy is regarded as the most suitable diagnostic instrument for malaria control because it is relatively inexpensive in cost and can differentiate malaria species and as well as quantify parasites (Barcia, 2007). However, it has been demonstrated that this technique requires experience and intensive training of the microscopists, it also requires rigorous maintenance and functional infrastructures for effective quality control and quality assurance, its sensitivity for detecting low level parasitaemia is poor and accessibility is low (Craig & Sharp, 1997) in resource limited settings like sub-Saharan Africa. In their study conducted between 2010 and 2013 to monitor implementation of the “test and treat” case-management policy for malaria in 172-176 health facilities in Kenya, Zurovac *et al.,* (2014) found that 91% of all facilities were using parasitological malaria diagnosis (a 35% increase from the baseline information).

Rapid diagnostic tests (RDTs) that detect malaria antigens in blood are easy to perform and do not require trained personnel or special equipment. However, they are relatively expensive and, although it can diagnose *P. falciparu*, it cannot distinguish between *P. ovale, P. malariae* and *P. vivax* from one another. RDTs do not require microscopy or specialized laboratory skills. RDT results are obtained in less than 15 minutes, usually

indicated as a colored line on a strip, are subjected to less investigator variations compared to microscopy, are easy to interpret, do not require capital investment and advanced infrastructure (WHO, 2010, Erdman & Kain, 2008). Zurovac *et al* (2014) also document that in 2013, RDTs were available for use in 70% of the facilities compared to 8% in 2010 showing a marked uptake of RDTs as a malaria diagnosis technique.

In his review of malaria diagnostic techniques, (Igbinosa *et al*., 2010) say that molecular diagnosis by parasite nucleic acids detection using polymerase chain reaction (PCR) yields more accurate results than microscopy since low parasitemia and or other unique clinical symptoms can impair malaria diagnosis due to drug resistance or misuse. PCR is therefore a superior technique with regard to monitoring of response to antimalarial treatment since PCR allows accurate species identification and can detect low level parasitaemia. They also argued that while conventional PCR assays demonstrated increased sensitivity and specificity, they remain slow and labor intensive as well as vulnerable to potential contamination problems. They further argued that in most malaria incidence settings of limited resources-where timely and accurate diagnosis is a priority-the lead time between sample collection, transportation, processing and dissemination of results back to clinicians, negate the usefulness of PCR in routine clinical practice settings. It is therefore not a suitable method for routine clinical management of malaria even in settings without resource barriers.

Serological diagnostic methods are based on detection of antibodies against asexual blood stage malaria parasite. Antibody detection using indirect fluorescent antibody immunoassays (IFAT) is regarded as the gold standard for malarial serology (Igbinosa *et al*., 2010) and have a sensitivity and specificity of 98% and 99.5% respectively (Sulzer &

Mariann, 1971). However, in routine diagnosis and malaria management, serologic approaches are not feasible due to: the long turn-around times involving antibody development, they are difficult to automate and IFAT reagents are not standardized yet hence difficulty in comparing results from different laboratories (Igbnosa *et al*., 2010).

Clinical diagnosis of malaria is essentially deduced from symptomatic features by individuals presenting for treatment. In most of the resource limited settings where malaria is prevalent, clinical diagnosis is the most commonly used technique, since it is not only inexpensive but also requires no special infrastructure while it is based on the symptoms the patient presents with, physical findings at examination and a high health worker suspicion index (Mwangi *et al*., 2005)..With the overlapping of malaria symptoms with other febrile illnesses, clinical diagnosis has led the indiscriminate use of antimalarial drugs caused by a 40%-80% range of over-diagnosis (Mwangi *et al*., 2005) based on studies conducted among individuals of different ages in Philippines, Sri lanka, Thailand, Tanzania, Chad Mali and Kenya. In a study conducted by Zurovac *et al.* (2014), to monitor implementation of the "test and treat" case-management policy for malaria in Kenya, 9% of the between 172-176 health facilities were using clinical diagnosis to detect malaria.

Before parasitological diagnosis and rapid diagnostic tests were adopted, the WHO program for Integrated Management of Childhood Illness (IMCI) had outlined that in resource limited settings where over 5% of fever in children is caused by malaria then fever should be adopted as the primary clinical diagnosis of malaria and consequently such places be classified as high malaria zones (WHO, 2005). The IMCI approach was to enable health workers in resource limited areas to identify severely ill children using

simple evidence-based clinical algorithms while acting as the most risk-adverse approach to malaria management across Africa.

With a general consensus that *Plasmodium falciparum* is not the only cause of febrile illnesses especially in children, the etiology of fever in malaria endemic areas has been the subject of considerable health research for many years as it scores highly on sensitivity for diagnosis while suffering from poor specificity (Okiro & Snow, 2010). However, due to overlap of malaria symptoms with other febrile illness, fever is subjective in nature and its specificity is impaired increasing the likelihood of indiscriminate use of anti-malarial drugs for managing febrile conditions in endemic areas (Igbinosa *et al.* 2010). However, it has been shown that the use of fever and other malaria symptoms as a clinical algorithm in low-to-moderate transmission settings with limited resources, supports the screening process for further parasitic identification (Perise and Trickland, 2008).

The clinical utility of diagnosis is dependent upon 4 building blocks with the following scenarios:

True positive: an individual has the disease and the test is positive.

False positive: an individual does not have the disease but the test is positive.

True negative: an individual does not have the disease and the test is negative

False negative: an individual has the disease but the test is negative.

Sensitivity can be defined as the probability that a test will indicate 'positive for disease' among those with the disease while specificity is the fraction of individuals whose test indicates ‘negative for disease’ and they have no disease. In the current study, sensitivity was described as the total number of malaria cases (as defined) attributable to a symptom (or

combination of symptoms) divided by the total number of malaria cases. Specificity was defined as the total number of cases without a symptom (combination of symptoms) and did not have malaria divided by the total number of cases without malaria. Simply put, sensitivity is the ability of a test to correctly identify individuals with a disease while specificity is the ability of a test to identify individuals without a disease.

Positive predictive value (PPV) is the probability that subjects with a positive test during screening truly have the disease (measured by number who are true positives divided by total of both true and false positives) while negative predictive value (NPV) is the probability that subjects with a negative test during screening truly don't have the disease (measured by number of true negatives divided by total of true and false negative). The PPV and NPV are dependent on the population of subjects being tested and the prevalence of the disease.

Likelihood ratio is a measure of how much more likely an individual testing positive with disease actually has the disease compared to an individual who tests negative. In the current study, likelihood ratio of a symptom(s) was measured by sensitivity of the symptom(s) divided by 1 less the specificity of the symptom(s).

Sensitivity, specificity, positive predictive values and negative predictive values have been used in clinical diagnosis to test how symptoms can be used for predicting presence or absence of malaria. Ideally, a symptom(s) should have a sensitivity of 100% in order to correctly identify all individuals who have malaria. The WHO recommends an acceptable minimum sensitivity value of 95% for clinical diagnosis of malaria (Bisoffi *et al*., 2010). It is documented that the specificity of clinical diagnosis is 20-60%

(depending on level of endemicity, malaria season and age group) compared with microscopy (Van der *et al.*, 1998).

In their review of malaria-anaemia relationship and the scientific advances made in pathophysiology, epidemiology, management and prevention of anaemia from *P. falciparum* malaria, (Menendez *et al*., 2000) say that malaria infection in humans by *Plasmodium* species-especially *falciparum* has often led to anaemia through reduction in haemoglobin levels. Anaemia is a common manifestation of all types of malaria and poses more morbidity and mortality risk for pregnant women and children below 5 years (Haldar & Mohandas 2009). In developing countries of the tropics, pre-existing anaemia, most commonly due to nutrition and helminthes infections, compounds the problem (Menendez *et al.*, 2000). The incidences of severe and age specific prevalence rates of anaemia have been shown to strongly correlate with *Plasmodium falciparum* transmission intensity. It has also been demonstrated that chronic anaemia may impair cognition, school performance, motor and behavioral development among school attending children (Crawley, 2004). The causal mechanisms between anaemia and malaria are known to be complex but well understood: malaria causes anaemia both by rupturing red blood cells and by suppressing the production of new red blood cells.

The WHO describes severe malarial anaemia as a haemoglobin (Hb) concentration <50 g l^{-1} or a haematocrit (Hct) <0.15 in the presence of a *Plasmodium falciparum* parasitaemia >10 000 parasites μl^{-1}, with a normocytic blood film (Warrell *et al*., 1990). Malarial anaemia is multi-factorial, involving both the destruction of RBC and the decreased production of RBC based on an individual's age, pregnancy state, antimalarial immune

status and genetic constitution of infected individuals, and malaria endemicity (Menendez *et al*, 2000) as described in table 2 below.

Table 2: Mechanisms for malarial anaemia

Mechanism	Comment	Reference
Increased RBC destruction		
Rupture of PBRC	The severity of early anaemia in acute malaria correlates with density of parasitaemia and schizontoemia	Phillips & Pasvol (1992)
Phagocytosis of PBRC	By proliferating and hyperactive macrophages in the RES	Davis *et al*. (1990)
Phagocytosis of unPBRC	Removed by macrophages due to several factors including reduced deformability and membrane binding of parasite components	Dondorp, A.M. *et al*. (1999)
Hypersplenism	The spleen pools parasitized and unparasitized RBCs from the circulation, haemolyzes them and expands the plasma volume	Angus *et al*. (1997)
Autoimmune (extravascular) haemolysis	Deposition of immunoglobulin and complement component on the surface of unparasitized RBCs	Kai *et al*. (1999)
Hapten-induced intravascular haemolysis	Quinine acts as a hapten combining with an RBC protein to become antigenic	Van-den-Ende, *et al*. (1998)
Decreased RBC production		
Inflammation-induced erythroid hypoplasia	An autologous serum factor may suppress the growth of early precursors of RBC, the BFU-E and the CFU-E	Phillips *et al*. (1986)
Suppression of erythropoietin synthesis	EPO synthesis is suppressed in some individuals with malaria	El Hassan *et al*. (1997)
Dyserythropoiesis	Mainly observed in individuals with recurrent falciparum infections. It is related to malaria itself and not to micronutrient deficiencies	Clark and Chaudhri (1988)
Imbalance of cytokines	Low IL-10 concentration has been found to be associated with severe malarial anaemia. Genetic factors may induce this imbalance	Kurtzhal *et al*. (1998)
Concomitant infections	The interaction between malaria and bacterial and viral secondary infections 5 on the severity of anaemia is increasingly recognized	Warrell *et al*. (1990)

Abbreviations: PRBC-parasitized red blood cells; RES-reticulo-endothelial system; BFU-E-burst-forming unit-erythron; CFU-E-colony-forming unit-erythron; EPO-erythropoietin; IL-10-interleukin 10.

Further, after reviewing published literature of randomized controlled trials on malarial anaemia, (Korenromp *et al.,* 2004) concluded that the impact of ITNs on anaemia in moderate-to-severe anaemia settings was more pronounced than on parasitaemia prevalence or clinical malaria because anaemia responds more quickly than mortality to changes in malaria exposure with increased coverage of malaria interventions.

2.4 Malaria mapping

Malaria, an environmental disease, largely distributed and determined by rainfall and temperature (Craig *et al*., 1999), is variant as smaller geographic units are broken down in combination with other small area variables (Booman *et al.* 2000). Suitable conditions for the survival of the *Anopheles* mosquito in terms of habitat and breeding ground as well as appropriate temperatures that favor the parasite life cycle are at the heart of malaria transmission and intensity. For instance, increased rainfall and vegetation density favor malaria transmission through provision of breeding sites for the vector while raised temperatures accelerate transmission through shortened sporogony (Le sueur *et al.* 1997; Craig *et al*., 1999). The use of geographic information system (GIS) techniques for health issues at both macro- and micro-levels can aid planning of health infrastructure, mapping disease distribution, investigating the spatial dynamics of communicable, environmental and infectious disease transmissions (e.g. malaria and anaemia) and modeling health service utilization and disease control intervention (Le sueur *et al.* 1997). The emergence of spatial techniques like GIS through tools like spatial database management systems, mapping and geo-processing tools, distance and inverse weighted calculations, digital elevation models (DEM), buffer zone computations and geo-statistical analysis have

helped different health specialists to make statistical inferences between the environment and disease occurrence and distribution (epidemiology).

GIS is an efficient information management system in malaria eradication processes and programmes. It aids in quick retrieval of information and dynamic generation of maps to highlight hot spots of malaria for formulating prompt and focused malaria control strategy. GIS mapping has made it easier to update information instantly and to identify "malaria-specific" hot spots at given locations and times. GIS has contributed immensely to malaria control programmes. It is now a valuable tool in the operations and implementation strategies of malaria intervention programmes. It has additional capabilities of being an evaluation tool for providing spatially analyzed outputs generated by health information systems in graphic visual formats which can be readily understood by field workers and program managers. Financial constraints, however, limit the widespread use of the GIS technology. GIS and Remote Sensing have fueled a renaissance in malaria risk mapping in Africa using climate data. Such climate and environmental data enable the stratification of the varied epidemiology of malaria across the areas of sub-Sahara Africa where the disease is endemic (Hay *et al.*, 2004). Maps are heuristic models, used to communicate, interpret, and explain data. They aid in the visualization of differences, clustering, heterogeneity, or homogeneity within data. Spatial patterns can be determined and correlations visualized through the use of maps.

The powerful tools of spatial technology have revolutionized the way epidemiological researches are conducted. Symbols and colors can communicate detail or the relative importance of certain features (Cuzick & Elliot, 1992). Spatial technology can be used to identify the spatial limits of disease prevalence and overlay disease information with

mapping of relevant risk factors like vector distribution (Saxena *et al.,* 2009). This highlights the need to collect, manage, display and analyze malaria data at the micro-level in order to understand the factors that result in the small scale, non-uniform transmission intensities even in homogenous populations. Malaria risk mapping aids in the development of community-based malaria control programs by accumulating past experiences and solutions to different factors associated with malaria outbreaks (Wimberly *et al.,* 2012). Stratification can also point to the existing clusters of incidence, prevalence or factors associated with malaria, anaemia or both allowing for more focused and customized interventions since malaria occurrence is influenced by numerous phenomena outside the habitual framework of health systems e.g. population movements, environmental changes and agricultural practices (Gething *et al,.* 2010). GIS techniques and its functionalities can therefore support malaria research and control through integrated use of remote sensing mapping of malaria risk, surveillance activities, and monitoring breeding habitats and analysis of areas of high disease prevalence.

In their review of spatial cluster analysis techniques for point-event data (Fritz *et al*., 2013) postulate that based on John Snow's foundational investigation in 1849 in London-where cholera cases were visually clustered around a water pump suspected as the source of disease (at a time when it was strongly believed that cholera transmission was airborne)-epidemiologists have continued to develop a keen interest in understanding disease patterns in both space and time. This interest has led to advancement in contemporary methods in spatial epidemiology with continued multi-disciplinary growth in the spatial statistics field. As a result, there is increased knowledge translation and sharing between

public health specialists, spatial statisticians and geographical information systems (GIS) developers leading to better understanding of concepts of spatial epidemiology

Maps resulting from GIS data can play an important role in formulating malaria intervention policies, providing appropriate information to communities, planning for resources and infrastructural developments, evaluating changes in malaria transmission and anaemia prevalence over time and allocating resources for control. They may further provide decision support platforms for both regional and national malaria control initiatives through the specialization and standardization of control and intervention strategies.

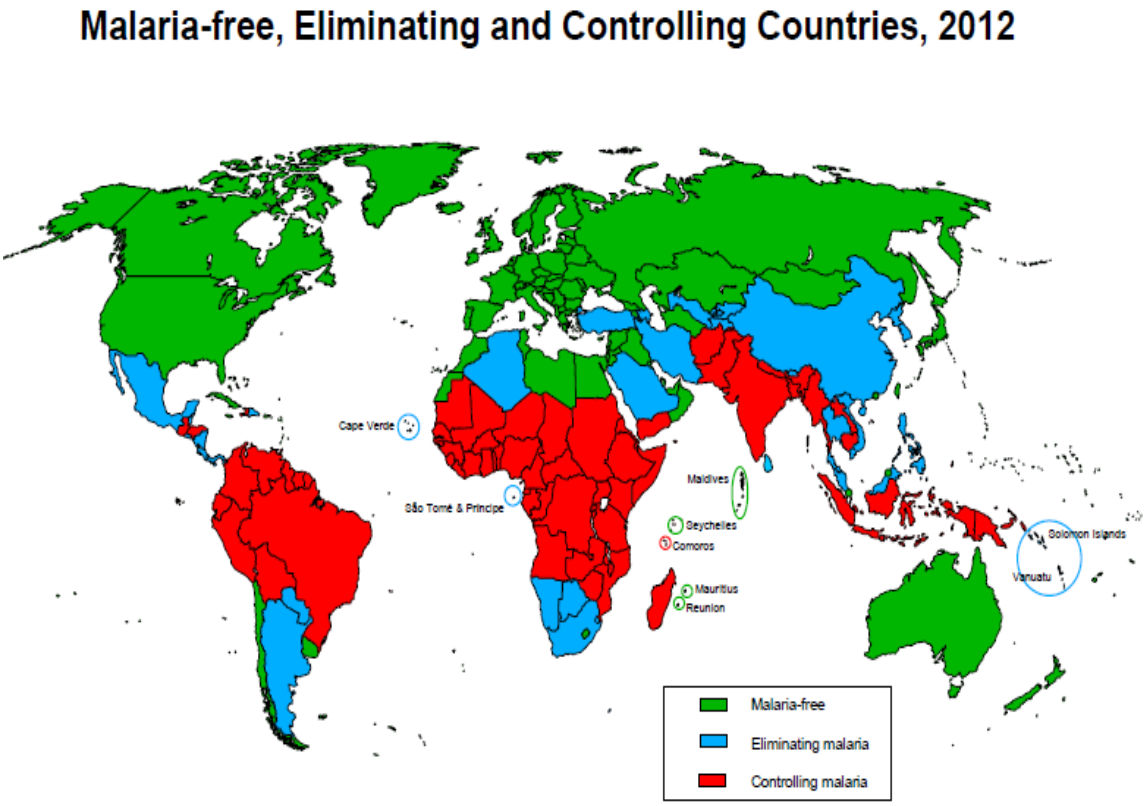
2.5 Malaria elimination/interruption of transmission

Even though the knowledge of malaria parasite's etiology has been greatly advanced through complex research findings-(malERA, 2011) and that malaria is a treatable and preventable disease, malaria eradication still remains a challenge. An evaluation of the evolution, implementation and outcome of the Global Malaria Eradication Program (GMEP) provides vital lessons for current elimination/eradication campaigns (Na'jera *et al.,* 2010). Drawing from mixed successes and failure of the WHO/GMEP of 1955-1969 (malERA, 2011), the Malaria Eradication Research Agenda (malERA) initiative was convened in 2008 to define the knowledge base, strategies and tools required to eradicate malaria from the human population. The main failure of WHO/GMEP project can be attributed to lack of recognition of the heterogeneity of malaria transmission like the combination of biological, vector, social and environmental factors like use of DDT, resistance to chloroquine, resistant parasites and human movements (Mendis *et al.* 2009; Na´jera *et al.,* 2010).

Malaria interventions like ITNs, IRS, ACT, IPTp or IPTi have been successfully implemented in areas of both high and low transmissions in East Africa. Greenwood (2008) noted that as malaria prevalence declines, remaining transmission is increasingly restricted to small specific geographical strata thus calling for higher precision in the application of interventions, coupled with intense identification and elimination of remaining points of transmission and/or resistance. Measuring the impact of such interventions is vital in documenting levels of success in the fight against malaria. However, the challenge of assessing effects of malaria prevention strategies in low transmission settings still exists with the currently available tools such as microscopy lacking optimal sensitivity (Bousema *et al.,* 2008).

John *et al.,* (2009) have shown that malaria elimination can be targeted at highland areas where malaria transmission is unstable because transmission decreases to very low levels during the dry season. Their study site in Nandi County had reported widespread implementation of prevention strategies; IRS, IPTp, IPTi, and artemether/lumefantrine (AL) as first-line therapy for uncomplicated malaria as per the Ministry of Health of Kenya guidelines (John *et al.,* 2009). The global "Atlas Project"-a joint University of California, San Francisco and University of Oxford project has developed maps of prospects for malaria elimination and/or decline from 1900 to 2012. The chart provides a visual highlight of countries that have successfully eliminated malaria, those moving towards elimination and those still controlling for malaria with a view of helping to inform where resource focus should be channeled.

Figure 4: Malaria-free, eliminating and controlling countries



In Figure 4 above, countries in green are malaria free, those in blue are in malaria elimination stage while those in red are still controlling for malaria. Moonen *et al.* (2010) advise that most malaria-endemic countries considering elimination should aim to prevent importation of new infections through proactive case detection at their borders, screening of high-risk migrants and implementation of cross-border and regional initiatives that can reduce transmission at the migration source. Lack of consideration of imported infections has been cited as one of the reasons the GMEP strategy to eradicate malaria did not realize its objectives (Lysenko & Beljaev, 1969). Endemic transmission should also be stopped and any transmission reservoirs drained while reducing the basic reproduction number (R_0) to less than one (Tatem *et al.,* 2010). Interruption of

transmission requires additional measures including identification of infections even at asymptomatic stage leading to effective treatment of infections before onward transmission while intensifying and adapting focal vector control activities (ITNs), indoors residual spraying (IRS), artemisinin combination treatments (ACT), intermittent preventive treatment strategies in pregnant women (IPTp) or infants (IPTi) (Moonen *et al.*, 2010).

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Study design

The current study used both retrospective and prospective methods to examine its objectives. A retrospective review of clinical, climatic, demographic and entomologic data for the period 2003-2009 was done while prospective monitoring and reporting of the same indicators for data from 2010 to 2012 was carried out. Study participants were drawn from asymptomatic individuals from a highland area of very low malaria intensity. To determine if fever and or any other symptom(s) can be used as predictors of malaria, clinical records' data from 2003-2007 was used. To determine the spatial correlation of anaemia prevalence and prior malaria incidence before interruption of transmission, hemoglobin data from 2007 and malaria incidence data from 2003-2005 were used. To determine the spatial correlation of anaemia prevalence and malaria incidence after reported interruption of transmission, hemoglobin data from 2010 and malaria incidence data from 2008-2010 were used. To determine if their exist hotspots for malaria transmission, malaria incidence data before reported interruption (2003-2007) and after reported interruption of transmission (2008-2010) were used.

3.2 Study site

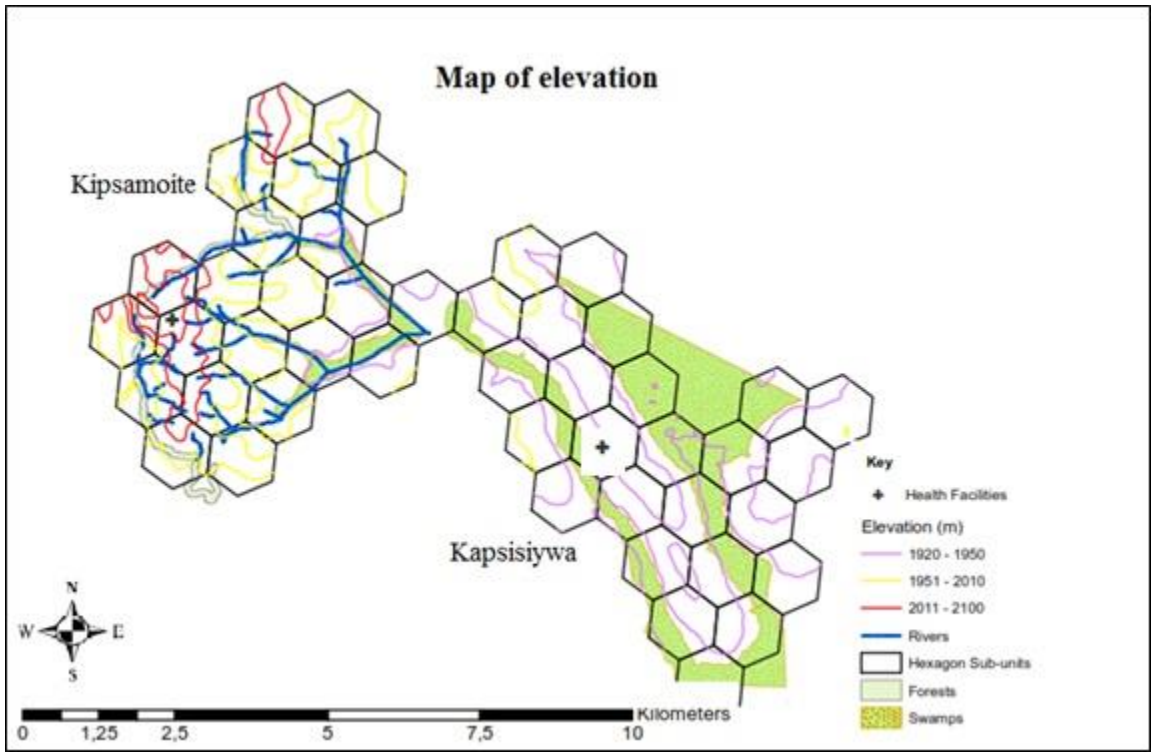
The study was done in the Kenyan highland sites of Kipsamoite and Kapsisiywa locations, both in Nandi County, which have similar-sized populations (Kipsamoite, ~4800, Kapsisiywa~4000). The two locations are from the original Nandi District of Rift

Valley Province. Both sites lie between 0°16'55.64° N to 0°21'52.40° N latitude and 34°59'7.17" E to 35°5'19.90" E longitude. Kapsisiywa has a lower altitude ranging from 1,887 m to 1,982 m, while Kipsamoite varies between 1,950 m and 2,100 m (figure 5). Kipsamoite's western side is bordered by a hilly and rocky forest while a swamp borders the eastern part of the study area (Ernst *et al.*, 2006).

Subsistence farming (maize, beans and vegetables), cash crop farming (sugar cane and tea) and animal husbandry (chickens, cattle, sheep and goats) are the major occupation. The area is epidemic prone with sporadic and unstable malaria transmission. Malaria incidence is highly seasonal in both sites, with heavy rains experienced between late March and early May, often followed by a peak in malaria cases during June or July (Cohen *et al*., 2008). *Anopheles gambiae s.l* is the most abundant vector (97.5%) while *Anopheles funestus* is 2.5% (Ernst *et al.*, 2006). *P. falciparum* is the predominant malaria species. Between April 2007 and March 2008, the study area experienced a reported interruption of malaria transmission after a targeted Ministry of Health indoor residual spraying and distribution of ITNs to pregnant women and children <5 years in 2006 (John *et al.*, 2009).

Malaria is the leading cause of mortality and morbidity in Nandi district with other health indicators as follows: crude birth rate (CBR) of 50/1000; crude death rate (CDR) 12/1000; life expectancy 50 years; infant mortality rate (IMR) 66/1000 and under 5 mortality rate of 111/1000 (NCAPD 2005). Within the study sites, there are only two health centers which are both supported by the study. Treatment at the health centers is free and no patient is charged for services offered.

Figure 5: Map of study site topography



3.3 Study population and recruitment

Study participants were residents of all the seven and ten villages of Kipsamoite and Kapsisiywa locations respectively.

3.3.1 Inclusion criteria

To be considered for participation, individuals of all ages had to be permanent residents of the two study sites with no intention of relocating permanently before the end of the study. They were also required to willingly sign consent forms for themselves and/or for their children.

3.3.2 Exclusion criteria

Non-residents at the time of recruitment were not considered for participation in the study.

3.4 Sampling procedure

This study used data from the same population followed over a period from 2003 to 2012 during two consecutive prospective observational researches: the first a malaria early warning system study (2002-2005) whose aim was to ascertain the correlation of rainfall, temperature & vector density and assessment of the specificity of a malaria epidemic early warning system that used vector density to indicate risk of an epidemic. The second source of data was a malaria transmission and immunity study (2006-2012) whose objectives were to determine the effect of *P. falciparum* transmission on antigen-specific immunity and associated protection from infection; identify antigen-specific immune responses protective against *P. falciparum* disease and elucidate the relationship of parasite genotypes to *P. falciparum* disease.

Due to low malaria transmission, both studies adopted a site-wide (census) sampling approach to ensure that accurate measures of malaria incidence could be carried out over time and for obtaining representative random samples for prospective monitoring of transmission and interventions given the low malaria prevalence rates. Initially, public *barazas* were organized through the local chief and sub-chief's offices to meet residents and introduce the study. Their concerns and suggestions were addressed. Later, using the field assistants from within the villages, each household was visited and any individual issues not addressed during the public forum were resolved. Individuals who consented were enrolled and data collected using interviewer administered questionnaires to obtain demographic information.

3.5 Sample size

This study used all individuals who accepted to participate in the study from all the villages (including minors whose parents or guardians consented to their participation) since 2003 and at each subsequent annual follow-up data collection exercises till 2012. Table 3 below shows the number of study participants recruited against the total population assessed from 2003 to 2012:

Table 3: Population and samples enrolled for study participation

	Kipsamoite			Kapsisiywa		
Year	**Population**	**Enrolled**	**% enrollment**	**Population**	**Enrolled**	**% enrollment**
2003	3856	3728	97%	3653	3477	95%
2004	4086	3846	94%	3929	3545	90%
2005	4236	3983	94%	4020	3610	90%
2006	4796	4069	85%	4457	3757	84%
2007	5113	4267	83%	4716	3869	82%
2008	5398	4336	80%	5001	3876	78%
2009	5497	4528	82%	5131	3969	77%
2010	5945	4714	79%	5433	4057	75%
2011	6097	4842	79%	5497	4059	74%
2012	6370	4967	78%	5514	4135	75%

In table 3 above, population represents all individuals in all households residing within the study sites during data collection whilst enrolled are total number of individuals who accepted to participate in the study during the specified time. Overall, whilst the proportion of individuals who were enrolled declined due to drop-outs, decline to continue participation within the study and new bornes not enrolled; the actual number of individuals in the study increased as the population of the study sites grew over the 10 years.

3.6 Data collection methods

This study used both primary and secondary data. All the different datasets (clinical, climatic, demographic and entomologic) were linked by the unique study number assigned to an individual at the time of enrollment. The unique number comprised of a village code, household code and an individual code. Therefore, the final dataset comprised of demographic details (personal biodata e.g. age, sex, marital status, ITN and ITN use, travel information etc), clinical data (malaria symptoms and Hb levels), entomologic data (IRS, presence or absence of vectors), geospatial data (longitude, latitude and altitude) and climatic data (rainfall, temperature).

3.6.1 Demography and surveillance for clinical malaria and asymptomatic parasitemia

Demographic information for all households in the study area was collected from 2003 and every 4-6 months thereafter. From 2005, demographic information collected included household births and/or deaths, use of insecticide-treated bed nets, resident travel assessments and IRS treatment of resident houses. Clinical malaria surveillance was initiated in 2003 at both the health facilities and at home. Passive surveillance for clinical malaria was conducted at each of the 2 health facilities-which are the only health care facilities within the area.

Trained local field assistants were trained in malaria surveillance and tasked with making weekly visits to each enrolled household within their village. Study participants were monitored and detected for malaria in 3 different ways: a) self-reporting of current or illnesses within the past week; b) reaching out to the village field assistants incases of illness and; c) study participants directly reporting to the local health facility. The project

supported free malaria diagnosis using microscopy. Passive surveillance included all individuals living in the two sites with symptoms of malaria that went to the dispensary for evaluation and were tested for malaria.

Table 4: Demography and surveillance data

	Kipsamoite		Kapsisiywa	
Year	**<5 yrs**	**≥ 5 yrs**	**<5 yrs**	**≥ 5 yrs**
2003	744	2984	571	2906
2004	758	3088	568	2977
2005	787	3196	554	3055
2006	789	3280	588	3170
2007	805	3462	585	3283
2008	732	3602	570	3306
2009	700	3826	555	3413
2010	690	4028	550	3506
2011	663	4176	501	3561
2012	676	4244	493	3674

Individuals with malaria-like symptoms were asked to attend either health facility for evaluation, and encouraged to go to the clinics at any time between visits if they had symptoms of malaria.

Individuals with symptoms consistent with malaria who did not have a clear alternative diagnosis were tested for *Plasmodium* species by microscopic examination of blood smears. The symptoms were reviewed by local clinical officers and included fever, chills, headache, loss of appetite, vomiting, jaundice, diarrhea, backache, joint pains, nausea, and severe malaise. Microscopy was performed as previously described (John *et al*., 2005) with 2 independent readings. A third and final reading for slides with results that

did not match was also done and its result was used to break the tie. All persons with positive blood smears for *P. falciparum* or *P. malariae,* the only *Plasmodium* species documented in this area, were treated with artemether-lumefantrine (Coartem©).

3.6.2 Secondary data

Demographic information of all households was collected annually (2003-2011). Clinical malaria surveillance data from 2007-2012 was accessed from the central database at Kisumu data office and was used to profile malaria manifestations. From 2005, assessment of ITN use and IRS treatment of houses was systematically conducted.

Blood (0.5 mL) collected by finger prick was used for on-the-spot measurement of haemoglobin concentration.

A total of 1682 households in the study area were mapped (latitude, longitude and elevation) in Path-finder using a Trimble GPS (GPS, Trimble Navigation, Sunnyvale, CA), with a corrected resolution of 1 m. GPS data for 2003 and 2012 mapping was collected and geo-referenced to contain household locations, natural (wells, swamps, forests, hills, rivers etc) and man-made (church, market place, hospital etc) landmarks.

3.7 Clinical and laboratory procedures

3.7.1 Blood collection

Finger prick blood samples of approximately 0.5mls were collected into microtainer tubes from all consenting study participants. The samples collected in the field were kept in a cool box and brought to the laboratory. On the same day of sample collection, they were centrifuged at 10,000 revolutions/minute for 5 minutes and stored at -30ºC.

3.7.2 Recording of clinical manifestation

Clinical officers at the two health facilities in the study area used clinical records form to record all clinical manifestations by individuals presenting at the health centers. They also referred individuals to the laboratory for microscopy tests.

3.7.3 Hemoglobin testing

Blood samples from asymptomatic individuals were collected for hemoglobin testing during 2 household surveys: the 1st survey was conducted in May 2007 and 5753 samples were collected while the 2nd survey was conducted in July 2008 where approximately one third of randomly selected individuals (from those tested for Hb in 2007) were tested for hemoglobin. A cohort of 1697 individuals (910 from Kapsisiywa and 787 from Kipsamoite) had hemoglobin tested at both time points. In 2012, 745 (583 from Kapsisiywa and 162 from Kipsamoite) samples from randomly selected active cohort individuals were collected and tested for hemoglobin. Hemoglobin levels were determined by skilled microscopists using photometry (HemoControl, EKF Diagnostics, Barleben, Germany) immediately after blood sample collection. The following procedure was used: A drop of blood was placed in the hemocue cuvette and excess blood outside wiped off. The cuvette was checked to make sure it was full with blood and contained no air bubbles. The cuvette was put into the cuvette holder on the hemocue photometer and carefully pushed inside into the measuring position for approximately 30 seconds to display the Hb reading on the hemocue photometer screen. The cuvette was then disposed of after measurement.

3.7.4 Anaemia determination

After determining Hb levels, they were adjusted for sex, pregnancy status, age and altitude (WHO 2001). For individuals residing in altitude ranges of ≥1500 and < 2000 m, a Hb adjustment of -0.5 g/dl was done while those at altitudes of ≥ 2000 m above sea level had a -0.8 g/dl Hb adjustment. Based on the adjustments individuals were considered anemic if their Hb levels were below the stated g/dl in table 5 below:

Table 5: Anaemia cut-off points

Age or gender	Hemoglobin (g/dl)
Children 6 – 59 months	< 11.0
Children 5 -11 years	< 11.5
Children 12 -14 years	< 12.0
Non-pregnant women (above 15 years)	< 12.0
Pregnant women	< 13.0
Men (above 15 years of age)	< 13.0

3.7.5 Testing for *P. falciparum* using microscopy

The following procedure was used to identify *P. falciparum* by microscopy: dry thick and thin blood smears were stained for 30-40 minutes using Giemsa. The stained smears were cleaned and rinsed using clean water. The slides were then dried with films facing downwards. The slides were placed on the microscope and examined using oil immersion (X100). The detailed procedure is as explained in Appendix 4.

3.8 Data management

All study participants were assigned a unique identification number which was a combination of site number, village number, household number and individual number within the household. Data on new births, deaths, in-migrations, out-migrations and even new households was continuously collected during the subsequent demography surveys

conducted. Data entry for primary data was done as per the project's data entry standard operating procedure (SOP)-see Appendix 3.

Secondary data that was already entered in the Filemaker Pro database was reviewed and any errors that were detected were validated against hard copy results. Where hard copies did not provide enough proof field visits were made to verify missing or unverifiable information. Old households that had not been previously mapped and new households (of new study participants) were mapped and geo-referenced continuously.

Data entry for laboratory, clinical, demographic and geographic information was done continuously at the central data office by scanning forms from the field using a Cardiff Teleform Software (as explained in Appendix 3 of the data entry SOP). Thereafter the data was exported to an existing Filemaker Pro database. Prior to scanning, the forms were always subjected to quality control checks like completeness, accuracy and validity.

3.9 Data analysis

STATA 12 (Stata Corporation, College Station, TX) and R (R Foundation, Wien, Austria) were used for statistical analysis while ArcGIS 10.0 (ESRI, New York, 2007-2010) and SaTScan (SaTScan, 9.3. Boston, MA) were used for spatial analysis.

3.9.1 Determining suitability of fever and/or other symptoms as a predictor of presence of *P. falciparum*

To respond to the 1st research question which sought to determine if fever and or other symptoms(s) is a sufficient predictor of presence of *P. falciparum* parasitemia in a highland population or if other factors should be assessed when looking for clinical malaria, sensitivity and specificity, Chi-square and ANOVA tests were used to determine

differences between symptoms and clinical malaria outcome. The exposure variables were any malaria symptom (fever, vomiting, headache, chills, loss of appetite, jaundice, backache, joint pains, nausea and malaise). The primary outcome was any of the listed symptoms plus *P. falciparum* on blood smear while clinical malaria (measured temperature > 37.5^{o}C plus *P. falciparum* on blood smear) was the secondary outcome. Age was the major confounder that was controlled for. Since clinical immunity to malaria differs in most populations in children <5 years of age as compared to individuals ≥5 years of age, analyses were done separately for individuals in these two age groups. Frequency of symptoms in individuals with parasitemia versus without parasitemia was compared by χ^2 testing. Logistic regression was conducted to compare odds of having *P. falciparum* parasitemia or *P. falciparum* parasitemia with a measured temperature ≥37.5°C with a specific symptom.

3.9.2 Spatial correlation of anaemia prevalence and prior malaria incidence

The 2nd research question sought to establish if anaemia prevalence spatially correlated with prior malaria incidence before reported interruption of transmission and if therefore anaemia can be used as a marker of prior malaria prevalence in a highland area of very low transmission. The exposure variable in this objective was annual malaria incidence while the outcome variable was anaemia. The secondary outcome was hemoglobin levels. Malaria incidence data for 2003, 2004, 2005 and 2003-2005 combined and anaemia prevalence data for 2007 were mapped. The 2007 data is drawn from 1697 randomly selected passive cohort individuals whose Hb was collected while the 2003, 2004 and 2005 data is for the whole population. Analysis was done separately for children <5 years of age and individuals ≥5 years of age and for study sites due to differences in malaria

incidence in Kipsamoite and Kapsisiywa. The following steps were used to carry out the spatial and correlation analysis:

1. Annual household malaria incidence data was mapped using ArcGIS (each year separately then the combined 2003-2005).
2. Repeated hexagonal units of approximately 0.62 km2 were overlaid on the malaria maps. Hexagon shapes were used in order to reduce sampling bias from edge effects of the study area grid because of their high perimeter to area ratios. These hexagons were given unique IDs (from 1 to 47). The hexagons in all the years were similar in size.
3. Annual malaria incidence for each hexagon by <5 and ≥5 age categories was then calculated (i.e. number of individuals who were P. falciparum positive in each hexagon divided by the total number of individuals per hexagon and multiplied by 1000)
4. Using the incidences in iii above, malaria intensity within the two study sites was established by assigning each polygon a color intensity code (the darkest shade of color implied the highest proportion of malaria or anaemia intensity). The color coding and proportions were not equal throughout the years for ease of comparison.
5. All hexagons that had less than 5 individuals were removed to adjust for false significance due to low malaria incidences.
6. Steps "i – v" above were repeated for anaemia data in 2007 as well.
7. Spatial association between malaria and anaemia was determined using Spearman's Correlation in STATA. Annual malaria incidence and anaemia

prevalence per hexagon were tabulated, scatter plots drawn and coefficient of correlation determined.

3.9.3 Spatial correlation of anaemia prevalence and malaria incidence after reported interruption of malaria transmission

In the 3rd objective, this study sought to establish if anaemia prevalence spatially correlated with prior malaria incidence after reported interruption of transmission. Due to the low number of malaria cases, the exposure variable in this objective was combined malaria incidence for 2010-2012 while the outcome variable was anaemia. The secondary outcome was hemoglobin levels. Combined malaria incidence data for 2010-2012 and anaemia prevalence data for 2012 were mapped. The 2012 anaemia data was drawn from 744 randomly selected individuals from the active cohort. Analysis was done separately for children <5 years of age and individuals ≥5 years of age and by study sites.

The following steps were used to carry out the spatial and correlation analysis:

1. Combined household malaria incidence data for 2010-2012 was mapped using ArcGIS.
2. Repeated hexagonal units of approximately 0.62 km2 were overlaid on the malaria maps. The hexagons were of similar size.
3. Malaria incidence for each hexagon by <5 and ≥5 age categories was then calculated.
4. Malaria intensity was established by assigning each polygon a color intensity code (the darkest shade of color implied the highest proportion of malaria or anaemia intensity). The color coding and proportions were not equal throughout the years for ease of comparison.

5. All hexagons that had less than 5 individuals were removed to adjust for false significance due to low malaria incidences.
6. Steps “i – v” above were repeated for anaemia data as well.
7. Association between malaria and anaemia was determined using Spearman’s Correlation in STATA. Annual malaria incidence and anaemia prevalence per hexagon were tabulated, scatter plots drawn and coefficient of correlation determined.

3.9.4 Cluster analysis to determine existence of malaria hotspots

Analysis for objective 4 was to determine whether new malaria cases reflect existence of transmission hotspots. According to Tobler's "first law of geography", "everything is related to everything else, but objects nearer to each other are more related than distant ones" (Tobler, 1979). Spatial clustering is the process of grouping a set of objects into classes (or clusters) in such a way that objects within a cluster have high similarity in comparison to one another but are not similar to objects in other clusters (Han & Kamber, 2003). Hot Spot Analysis is a spatial cluster detection method which identifies statistically significant spatial concentrations of high and/or low values associated with a set of geographic features. Spatial cluster analysis was used to determine if there existed malaria hotspots before and after interruption of transmission.

The following procedure was used to determine existence of hotspots:

1. Household malaria incidence data (2003, 2004, 2005, 2010-2012) was mapped using ArcGIS.
2. Analysis of spatial clustering was conducted for each year. For each time period, data was converted into 3 text files: cases, population and coordinates.

The “cases file” contained household ID (HHID), number of cases per household and the year; the “population file” contained HHID, year and the total number of individuals in the household and the “coordinates file” contained the HHID, the latitude and longitude of the household.

3. “Cases”, “population” and “coordinates” data for each year was uploaded onto SaTScan by age and site.

4. SaTScan was used to examine the data at the household level and to isolate malaria clustering at both sites. Using the Poisson cluster analysis method in SaTScan, malaria clusters for 2003, 2004, 2005, 2010-2012 were developed. The likelihood ratios were tested for significance using the Monte Carlo method. For this analysis, due to low malaria incidences, 10% of the total population was assumed to be at risk of malaria and 999 Monte Carlo replications were permitted, the maximum cluster circle was for a radius of 1 km (households that were 1 km from each other) and measured for significance at p-value less than 0.05.

5. The relative risk for each significant cluster was calculated using the logistic regression model in STATA.

In summary, spatial analysis was performed to assess possible clustering of P. falciparum-positive individuals within households as the unit of analysis. A spatial scan statistic was obtained using the Bernoulli model in SaTScan. The analysis involved application of multiple circular windows, which are not fixed in both position and size, across the two study sites separately due to the difference in malaria incidence. Each distinct circle represented a possible cluster. For each circle, the number of observed and

expected malaria-infected individuals was counted, while the expected numbers were calculated assuming an even distribution of infections across the households. A likelihood ratio test was used to compare the prevalence of infection within the cluster (circle) to that outside it to identify significant clusters of higher than expected (hotspot) prevalence. The statistical significance of this hotspot was evaluated taking into account the multiple tests for the many potential cluster locations and sizes evaluated as well as the distribution of the households. Due to the low incidence of malaria within the study sites, the maximum proportion of the population that a cluster could contain was set at 10%. The 1km distance was used to align the cluster analysis to a relatively similar analysis area like that used for the hexagons in objectives 2 and 3 for consistency of results.

3.10 Ethics consideration

The malaria early warning systems study (2003-2005) which generated part of the data for this study was cleared and approved by the Kenya Medical Research Institute National Ethical Review Committee and the Institutional Review Board for Human Studies at Case Western Reserve University (SCC letter of approval number 707). The malaria transmission and immunology study 2006-2013 was reviewed and approved by the Kenya Medical Research Institute National Ethical Review Committee and the Institutional Review Board at the University of Minnesota (SCC letter of approval number 939). This study proposal was also submitted to the KEMRI Ethics Review Board for approval. (Appendix 1)

Voluntary participation by the study participants required signing of a consent form, which was administered in duplicate; one copy remaining with study participant (or parent or guardian where minors were involved) while the other being taken to the central data centre (Appendix 2). Individuals who do not know how to sign were always required to give thumbprints in the presence of a literate witness who signed a witness form. Study

participants were informed and were free to discontinue their participation at their own choice.

CHAPTER FOUR

4. RESULTS

This chapter presents the results of analyses conducted in the methods described in chapter 3. It is organized by the study objectives. Summaries of the results are generally presented in tables and figures. In all the analyses, significant results are presented for values that are $P < 0.05$.

4.1 Objective 1: Predictive value of fever and / or other malaria symptoms for *P. falciparum* parasitemia among individuals seeking treatment in highland areas of very low transmission

4.1.1 Sensitivity and specificity of specific symptoms for *P. falciparum* parasitemia

During the period 2007-2011, only 3/3,420 smears done were positive for *P. malariae* (0.09%), other individuals were positive for P. falciparum. This study therefore focused on *P. falciparum* for the analysis. From 2007 to 2011, 3,420 symptomatic individuals in the passive surveillance cohort were screened for malaria at the two dispensaries. *P. falciparum* parasitemia was detected in 224 individuals (6.5%), including 36/634 children <5 years (5.7%) and 188/786_individuals ≥ 5years (6.7%, P=0.33). The median parasite density was 30,700 per ul of blood (IQR 10,770 - 135390), and did not differ significantly in children <5 years (768 [IQR 295-3202]) compared to individuals ≥5 years (19,420 [IQR 3230-56,920], *P*=0.32).

The frequency of individual symptoms for clinical malaria, defined as *P. falciparum* parasitemia in a person with any of the eleven symptoms, along with the sensitivity and specificity of each symptom, are presented in Table 6 for children <5 years and in Table 7 for individuals ≥5 years.

Table 6: Frequency, sensitivity and specificity of symptoms for clinical malaria (symptomatic Plasmodium falciparum parasitaemia) in children <5 years of age

Symptoms	Pf pos,[a]	Pf neg,[b]	*P value*	Sensitivity	Specificity
	n (%) N=36	n (%) N=598		(%)	(%)
Fever	32 (89)	506 (85)	0. 49	88. 9	15. 4
Appetite loss	25 (64)	323 (54)	0. 07	69. 4	46
Headache	19 (53)	191 (32)	0. 01	52. 8	68. 1
Vomiting	11 (31)	237 (40)	0. 28	30. 6	60. 4
Chills	10 (28)	56 (9)	<0. 001	27. 8	90. 6
Malaise	7 (19)	53 (9)	0. 04	19. 4	91. 1
Diarrhea	5 (14)	121 (20)	0. 35	13. 9	79. 8
Nausea	3 (8)	30 (5)	0. 38	8. 3	95
Joint pains	1 (3)	12 (2)	0. 75	2. 8	98
Jaundice	0 (0)	7 (1)	0. 51	0	98. 8
Backache	0 (0)	8 (1)	0. 49	0	98. 7

Abbreviations: *Pf P. falciparum*; *pos* positive; *neg* negative.

Results in Table 6 above show that for children < 5 years, fever (88.9%) and loss of appetite (69.4%) had high sensitivity but suffered from poor specificity of 15.4% and 46% respectively. All the remaining symptoms exhibited a higher specificity than sensitivity with headache scoring a modest sensitivity of 52.8% and specificity of 68.1%. Due to low frequency, jaundice and backache had a sensitivity of 0%.

Table 7: Frequency, sensitivity and specificity of symptoms for clinical malaria (symptomatic Plasmodium falciparum parasitaemia) in individuals ≥ 5 years of age

Symptoms	Pf. pos,[a]	Pf. neg,[b]	*P value*	Sensitivity	Specificity
	n(%) N=188	n(%) N=2598		(%)	(%)
Headache	167 (89)	2055 (79)	0. 001	88. 8	20. 9
Fever	105 (56)	1185 (46)	0. 01	55. 9	54. 4
Appetite loss	69 (37)	834 (32)	0. 19	36. 7	67. 9
Chills	68 (36)	457 (18)	<0. 001	36. 2	82. 4
Joint pains	57 (30)	641 (25)	0. 08	30. 3	75. 3
Vomiting	48 (26)	460 (18)	0. 01	25. 5	82. 3
Malaise	36 (19)	325 (13)	0. 01	19. 1	87. 5
Backache	35 (19)	494 (19)	0. 89	18. 6	81
Nausea	26 (14)	447 (17)	0. 23	13. 8	82. 8
Diarrhoea	6 (3)	323 (12)	<0. 001	3. 2	87. 6
Jaundice	2 (1)	25 (1)	0. 89	1. 1	99

Of particular interest, among individuals ≥ 5 years, was that fever was neither sensitive (56%) nor specific (54%). Headache had an elevated sensitivity of 88.8% compared to the < 5 age group but at the expense of a decreased specificity of 20.9%. Loss of appetite also had a reversed sensitivity (36.7%) and specificity (67.9%) scores respectively. The remaining symptoms all had high sensitivities compared to low specificity.

Findings from analysis for clinical malaria defined as asymptomatic *P. falciparum* parasitemia with measured fever (axillary temperature≥37.5°C) are summarized in Table 8 and table 9 below. Negative and positive predictive values are not shown, because the low frequency of *P. falciparum* parasitemia in participants seen at these clinics which led

to high negative predictive values (all >96%) and low positive predictive values (all <16%) for all symptoms assessed.

Table 8: Frequency, sensitivity and specificity of particular symptoms for symptomatic Plasmodium falciparum parasitemia with plus a measured axillary temperature ≥37.5°C in children <5 years of age.

Symptoms	Pf. pos,[a] n (%) N=31	Pf. neg,[b] n (%) N=254	*P value*	Sensitivity (%)	Specificity (%)
Fever	29 (94)	228 (90)	0.5	93.5	10.2
Appetite loss	22 (71)	134 (53)	0.05	71.0	47.2
Headache	17 (55)	83 (33)	0.02	54.8	67.3
Vomiting	6 (19)	48 (19)	0.95	32.3	61.8
Chills	10 (32)	31 (12)	0.003	32.3	87.8
Malaise	7 (23)	29 (11)	0.08	22.6	88.6
Diarrhea*	4 (13)	39 (16)	0.72	12.9	84.6
Nausea	2 (6)	14 (6)	0.83	6.5	94.5
Joint pains	1 (3)	6 (2)	0.77	3.2	97.6
Jaundice	0 (0)	3 (1)	0.54	0	98.8
Backache	0 (0)	3 (1)	0.54	0	98.8

Abbreviations: Pf., *P. falciparum*; pos, positive; neg, negative; * lack of diarrhea

Sensitivity of fever was higher (93.5%) for children with asymptomatic *Plasmodium falciparum* parasitemia and a measured axillary temperature ≥37.5°C while headache had a sensitivity of 54.8% and specificity of 67.3% respectively. However, for children who lacked diarrhea had a specificity of 84.6% and a low sensitivity of 12.9%.

Table 9: Frequency, sensitivity and specificity of particular symptoms for symptomatic Plasmodium falciparum parasitemia plus a measured axillary temperature ≥37.5°C in individuals≥5 years of age.

Symptoms	Pf. pos,[a] n (%), N=135	Pf. neg,[b] n (%), N=538	*P value*	Sensitivity %	Specificity %
Headache	120 (89)	439 (82)	0.04	89.1	18.4
Fever	83 (61)	326 (61)	0.85	61.3	39.5
Appetite loss	51 (38)	196 (36)	0.77	38	63.7
Chills	49 (36)	125 (23)	0.002	35.8	76.7
Joint pains	42 (31)	135 (25)	0.16	30.7	74.7
Vomiting	34 (25)	114 (21)	0.32	26.3	81.2
Malaise	28 (21)	74 (14)	0.04	20.4	86.2
Backache	24 (18)	71 (13)	0.17	17.5	86.8
Nausea	18(13)	67 (12)	0.78	13.9	87.7
Diarrhea*	6 (4)	86 (16)	<0.001	4.4	84
Jaundice	2 (1)	5 (1)	0.57	1.5	99.1

Abbreviations: Pf., P. falciparum; pos, positive; neg, negative;

Overall, the findings in tables 8 to 9 above show that symptoms with the greatest specificity lacked sensitivity (e.g., chills in children<5 years had a specificity 91% but a sensitivity of only 28%), and those with the greatest sensitivity lacked specificity (e.g., fever in children <5 years had a sensitivity of 89% but a specificity of only 15%) and vice versa.

4.1.2 Odds of *P. falciparum* parasitemia with specific symptoms

Table 10 below summarizes the odds ratios for presence of each symptom for clinical malaria in children <5 years and individuals ≥ 5 years. Individuals <5 years and ≥5 years with *P. falciparum* parasitaemia were significantly more likely to have headache,

malaise, and chills than those without parasitaemia. Individuals ≥5 years with *P. falciparum* parasitaemia were also significantly more likely to have fever and vomiting, and significantly less likely to have diarrhoea, than those without parasitaemia. Findings for the outcome of *P. falciparum* parasitaemia with measured fever (axillary temperature ≥37.5°C) were very similar and are summarized in table 11. In table 11, all non-febrile persons were excluded and then those with fever were tried for any other symptom to check if such a combination would increase prediction.

Table 10: Odds ratios of symptoms for prediction of clinical malaria (symptomatic Plasmodium falciparum parasitaemia)

Predictor	**<5 years**			**≥5 years**		
	OR	**95% CI**	**P**	**OR**	**95% CI**	**P**
Fever	1. 96	0. 59–6. 53	0. 27	1. 47	1. 09–1. 97	0. 01
Headache	2. 54	1. 28–5. 04	0. 01	2. 04	1. 3–3. 21	0. 002
Appetite loss	2. 14	1. 01–4. 53	0. 05	1. 21	0. 89–1. 65	0. 22
Vomiting	0. 7	0. 34–1. 46	0. 34	1. 74	1. 25–2. 43	0. 001
Chills	3. 88	1. 77–8. 49	0. 001	2. 63	1. 93–3. 51	<0. 001
Jaundice	1	-	-	1. 08	0. 25–4. 60	0. 92
Diarrhoea	0. 5	0. 18–1. 46	0. 21	0. 23	0. 1–0. 52	<0. 001
Backache	1	-	-	0. 95	0. 65–1. 38	0. 78
Joint pains	1. 44	0. 12–11. 39	0. 73	1. 29	0. 93–1. 78	0. 13
Nausea	1. 78	0. 52–6. 14	0. 36	0. 79	0. 52–1. 2	0. 27
Malaise	2. 58	1. 07–6. 18	0. 03	1. 67	1. 15–2. 44	0. 01

Table 11: Odds ratios of particular symptoms for prediction of symptomatic P. falciparum parasitemia with a measured axillary temperature ≥ 37.5°C.

Predictor	<5 years			≥5 years		
	OR	95% CI	P	OR	95% CI	P
Fever	1.65	0.37-7.33	0.51	1.04	0.70-1.53	0.85
Headache	2.50	1.18-5.32	0.02	1.8	1.01-3.22	0.05
Appetite loss	2.19	0.97-4.94	0.06	1.06	0.72-1.56	0.77
Vomiting	1.03	0.40-2.65	0.95	1.25	0.81-1.94	0.32
Chills	3.43	1.48-7.95	0.004	1.88	1.26-2.82	0.002
Jaundice	1	-	-	1.6	0.31-8.35	0.58
Diarrhea	0.82	0.27-2.46	0.72	0.24	0.10-0.57	0.001
Backache	1	-	-	1.42	0.86-2.36	0.17
Joint pains	1.38	0.16-11.84	0.77	1.35	0.89-2.04	0.16
Nausea	1.18	0.26-5.46	0.83	1.08	0.62-1.89	0.73
Malaise	2.26	0.90-5.72	0.08	1.64	1.01-2.66	0.04

4.1.3 Sensitivity and specificity of combinations of symptoms for *P. falciparum* parasitemia

The study analyzed for four possible outcomes: (1) clinical malaria (which was considered as temperature>37.5 + *P falciparum* positive); (2) *P falciparum* positive, *(3) P falciparum* density ≥ 1000, and (4) *P falciparum* density ≥ 4000.

To assess whether any combination of symptoms could improve on fever for sensitivity and specificity of detection of clinical malaria, a three-step process was undertaken: (1) candidate subsets of symptoms to consider were selected; (2) for each candidate subset of symptoms, a logistic regression model was fitted including those symptoms; (3) the logistic regression results were used to define rules and estimate their sensitivity and specificity.

Selecting candidate subsets of symptoms.

A stepwise forward selection approach was used where no predictors was considered, then all 11 symptoms were considered as individual predictors. Then symptom with the smallest P-value was added while comparing the remaining 10 symptoms according to their P-values in a model that also included the first-selected symptom. This process was repeated while adding a symptom at a time and stopped when all remaining variables had P-values of 0.10 or greater if added to the already-included symptoms. For instance (for all the 4 outcomes, for both <5 and ≥ 5 years age groups) chills was selected first since it had the smallest P-value (and as a predictor, chills had bad sensitivity of 20-30% but excellent specificity 80-90% for all outcomes and both age groups).

For children with age < 5 years, headache and malaise were selected second and third respectively for all 4 outcomes while appetite or fever were neither significant not non-significant. For age ≥ 5 years, symptoms selected after chills were always diarrhea, vomiting, headache, malaise and fever, even though the order of selection varied between the 4 outcomes.

Four symptoms were never selected: backache, jaundice, joint pains and nausea since they all did not have any good predictive power.

Finally, after selecting candidate symptoms, the top 3 symptoms (for age group and outcomes) were checked for any two-way interactions-results showed that none had an interaction of $P < 0.05$.

Fitting logistic regression models with candidate subsets of symptoms.

For each selected candidate subset of symptoms, a logistic regression without any interactions was fitted using the below logistic regression estimate which estimated the probability of interest:

Recall, logit(Pr[Malaria]) = ln{Pr[Malaria]/(1-Pr[Malaria])}, as a linear function of the predictors. Thus, for instance, a fitted logistic regression of clinical malaria (ClinMal) on two select symptoms (chills and fever), was described as in table 12 below:

Table 12: Fitting logistic regression model

Row	Chills?	Fever?	logit(Pr[Clinical Malaria])			Pr[ClinMal]
1	N	N	–2.946	– 0.493	– 0.429	0.020
2	Y	N	–2.946	+ 0.493	– 0.429	0.047
3	N	Y	–2.946	– 0.493	+ 0.429	0.053
4	Y	Y	–2.946	+ 0.493	+ 0.429	0.117

In the above estimate, any rule is based on Pr[ClinMal] or its logit: if Pr[ClinMal] > K given an individual's symptoms, then they were declared to have malaria, otherwise they were declared no to have malaria. For instance, based on table 12 above, using both chills and fever, the two possible rules were to declare that a person had malaria:

i. if either symptom is present (rows 2, 3 and 4 are declared to have malaria); or

ii. if both symptoms are present (row 4 is declared to have malaria).

In cases where a rule could declare that rows 3 and 4 as having malaria, then the rule ignored chills and split the population based on the presence or absence of fever. Under such circumstances, then step (c) below would be applied:

Use of logistic regression results to define rules and estimate their sensitivity and specificity.

Under this step, the objective was to establish if any rules could better than fever alone as a predictor of malaria i.e.:

- For age < 5: Compared to fever alone, a rule with better specificity and improved sensitivity (since fever alone had high sensitivity and poor specificity).
- For age ≥ 5: Compared to fever alone, better sensitivity and better specificity.

Overall, from the computations, rules that involved 4 or 6 symptoms were unlikely to be better than simpler rules (of 2-3 symptoms) by large percentage points on sensitivity or specificity. The results of this 3-step assessment are summarized in Table 13 (for individuals <5 years) and Table 14 (for individuals ≥ 5 years).

The abbreviations used for computations were:

A = Appetite; C = Chills; F = Fever; H = Headache; M = Malaise; V = Vomiting; ~D = No Diarrhea.

Table 13: Sensitivity and specificity of combinations of symptoms for different clinical malaria definitions in children <5 years of age

A. Outcome 1. Symptomatic *P. falciparum* parasitaemia			
Predictors	Rule	Sensitivity (%)	Specificity (%)
Fever	Fever	88. 9	15. 4
F, C	F or C	88. 9	13. 4
F, H	F or H	94. 4	9. 9
F, C, H	Any of F,C,H	94. 4	8. 9
F, C, H, M, A	Any of F,C,H,M,A	97. 2	5. 5
F, C, H, M	Any of F,C,H,M	94. 4	8. 0
B. Outcome 2. Symptomatic *P. falciparum* parasitaemia plus T ≥ 37. 5°C			
Predictors	Rule	Sensitivity	Specificity
Fever	Fever	92. 0	11. 7
F, C, H, M, A	Any of F,C,H,M,A	100. 0	5. 6
C, H, M, A	Any of C,H,M,A	96. 0	25. 6
C, H, M	Any of C,H,M	80. 0	55. 8
F, H	F or H	100. 0	6. 5
C. Outcome 3. Symptomatic *P. falciparum* parasitaemia ≥1,000parasites/μL plus T ≥ 37. 5°C			
Predictors	Rule	Sensitivity	Specificity
F	Fever	100. 0	19. 3
F, H	F or H	100. 0	13. 3
D. Outcome 4. Symptomatic *P. falciparum* parasitaemia ≥4,000 parasites/μL plus T ≥ 37. 5°C			
Predictors	Rule	Sensitivity	Specificity
F	Fever	100. 0	19. 5
F, H	F or H	100. 0	13. 3

For children < 5 years, in outcome 1, the first rule used fever only which had a high sensitivity (88.9%) and poor specificity (15.4%). The second rule added chills (since it

was the best single predictor in terms of P-value) and declared a child to have malaria if either symptom were present. The combination was not any better than fever alone (sensitivity-88.9% and specificity-15.4%). The third rule used fever or headache, declaring a child to have malaria if she/he has either fever or headache. This rule improved on sensitivity (94.4%) but lost some specificity (9.9%). Compared to fever alone, it gained on sensitivity but lost on specificity. The 5th rule used any of the 5 symptoms F, C, H, M, A which was not any better than using fever or headache.

For outcome 2 (symptomatic *P. falciparum* parasitaemia plus T ≥ 37. 5°C) fever or headache had a perfect sensitivity (100%) and specificity (6.5%). The same rule (fever or headache) had perfect sensitivity for outcomes 3 (symptomatic *P. falciparum* parasitaemia ≥1,000parasites/μL plus T ≥ 37.5°C) and 4 (symptomatic *P. falciparum* parasitaemia ≥4,000 parasites/μL plus T ≥ 37. 5°C). Outcomes 3 and 4 also had perfect sensitivity due to the low frequency of children with *P. falciparum* parasitaemia ≥1,000 and ≥4,000 parasites/μL respectively.

Negative predictive values (NPV) and positive predictive values (PPV) have not been included in table 13 above and table 14 below since there were very few true cases i.e. for age < 5 years, the NPV would be 609/634 = 96.1% (total children = 634, negative= 609, positive= 25). This implies that none of the rules would give a NPV of less than 96.1%.

Table 14: Sensitivity and specificity of combinations of symptoms for different clinical malaria definitions in individuals ≥5 years of age

A. Outcome 1. Symptomatic *P. falciparum* parasitaemia

Predictors	Rule	Sensitivity	Specificity
F	Fever	55. 2	54. 4
C, F, M, H, V, ~D	Any of F,C,~D,V,H	100. 0	2. 4
C, F, M, ~D	Any of F,C,~D	98. 5	5. 6
F, ~D	Either of F,~D	98. 5	6. 6
~D	~D	96. 9	12. 5
C, F, M, ~D	≥ 2 of C,F,M,~D	73. 7	43. 5
F, H	F or H	96. 8	11. 5

B. Outcome 2. Symptomatic *P. falciparum* parasitaemia plus T ≥ 37. 5°C

Predictors	Rule	Sensitivity	Specificity
F	Fever	64. 7	54. 5
C, F, M, H, V, ~D	Any of C,F,M,H,V,~D	100. 0	2. 1
C, F, M, ~D	Any of C,F,M,~D	98. 3	4. 9
F, ~D	F,~D	98. 3	6. 5
~D	~D	96. 6	12. 2
C, F, M, ~D	≥ 2 of C,F,M,~D	82. 8	40. 9
F, H	F or H	96. 6	7. 6

C. Outcome 3. Symptomatic *P. falciparum* parasitaemia ≥1,000 parasites/μL plus T ≥ 37. 5°C

Predictors	Rule	Sensitivity	Specificity
F	Fever	69. 6	58. 4
F, H	F or H	100. 0	23. 0

D. Outcome 4. Symptomatic *P. falciparum* parasitaemia ≥4,000 parasites/μL plus T ≥ 37. 5°C

Predictors	Rule	Sensitivity	Specificity
F	Fever	64. 8	54. 2
C,F,M,V,~D,H	Any of C,F,M,V,~D,H	100. 0	2. 0
C,F,M,V,~D	Any of C,F,M,V,~D	97. 7	4. 8
~D	~D	95. 5	12. 1
C, F, M, V	Any of C,F,M,V	84. 1	34. 8

F, H	F or H	100. 0	22. 9

In all the outcomes in table 14 above, diarrhea presented a statistical oddity i.e. it was a backward symptom: people with diarrhea are less likely to have malaria, by definition of all the 4 outcomes, than people without diarrhea. Thus, the symptom "~D" ("no-diarrhea") means if an individual did not have diarrhea they were more likely to have malaria (the symptom predicting malaria here is not having diarrhea).

As shown in Tables 13 and 14 above, no combination of symptoms had higher sensitivity than presence of fever or headache for detection of clinical malaria, although this combination had poor specificity. Some symptom groupings had improved specificity, but always at the cost of sensitivity. A combination of fever or headache achieved a sensitivity of 94% and 97% for individuals <5 years and ≥5 years respectively (Tables 13 and 14), but at the cost of greatly decreased specificity (6.5%) in individuals ≥5 years. The addition of headache increased the number of children <5 years who would be tested for *P. falciparum* infection by only two persons (6%), but increased the number of individuals ≥5 years who would be tested by 1,190 persons (92%) from 1290 to 2480.

To assess whether more stringent criteria for a definition of clinical malaria would result in different findings, sensitivity and specificity of symptom combinations was assessed for a number of stricter definitions of clinical malaria, as noted in Tables 6 and 7. Similar patterns were seen for all alternate definitions of clinical malaria. Finally, measured fever was incorporated in the clinic into the "symptom" predictor variable. For the primary definition of clinical malaria (symptomatic *P. falciparum* parasitaemia), having a history of fever *or* measured fever in the clinic had a sensitivity and specificity of 94% and 11%,

respectively, for children <5 years, and 78% and 44%, respectively, for persons ≥5 years. In summary, adding more symptoms to the rules increased sensitivity while decreasing specificity or increased specificity at a reversed expense of sensitivity.

4.1.4 Prevalence of asymptomatic parasitemia

The active surveillance cohort of 600-650 individuals was assessed for presence of asymptomatic parasitemia at ten time points from 2007-2010 (July and November 2007, March, August and October 2008, February, April, August and October 2009, and May 2010). Due to movement of individuals during the study period, different numbers were tested at each time point, but during the 3 year period, only 5 persons out of 5195 tested had asymptomatic parasitemia (0.1%), with a median parasite density of 240 parasites per ul of blood (interquartile range [IQR], 200-2,560).

4.2 Objective 2: Spatial correlation of prior malaria incidence and anaemia prevalence before reported interruption of transmission

4.2.1 Population and annual malaria profiling before and after reported interruption of transmission

Table 15: Population and annual malaria profiling by age and siteSite

	Year	Total <5 yrs	Total ≥ 5 yrs	Total *Pf* positive	Total *Pf* no duplicates	*Pf* +ve no duplicates	
						Total <5 yrs	*Total* ≥5 yrs
Kipsamoite	2003	744	2984	128	118	23	95
Kipsamoite	2004	758	3088	170	161	28	133
Kipsamoite	2005	787	3196	79	77	15	62
Kipsamoite	2006	789	3280	17	17	0	17
Kipsamoite	2007	805	3462	25	25	6	19
Kipsamoite	2008	732	3602	8	8	2	6
Kipsamoite	2009	700	3826	35	35	3	32
Kipsamoite	2010	690	4028	16	16	1	15
Kipsamoite	2011	663	4176	27	27	4	23
Kipsamoite	2012	676	4244	8	8	2	6
Kapsisiywa	2003	571	2906	426	395	106	289
Kapsisiywa	2004	568	2977	419	387	80	307
Kapsisiywa	2005	554	3055	63	62	9	53
Kapsisiywa	2006	588	3170	6	6	1	5
Kapsisiywa	2007	585	3283	28	26	4	22
Kapsisiywa	2008	570	3306	6	6	1	5
Kapsisiywa	2009	555	3414	29	27	2	25
Kapsisiywa	2010	550	3506	11	11	3	8
Kapsisiywa	2011	501	3561	50	48	7	41
Kapsisiywa	2012	493	3674	9	9	1	8

Table 15 above shows the annual malaria incidence by age, site, by cohort (active or passive) and annual incidence without duplicates before reported interruption of transmission (2003-2005) and after reported interruption of transmission (2010-2012). Individuals with malaria were considered duplicates if more than one episode was recorded within a 30 day period. A total of 1406 malaria cases with duplicates were reported while 94% (1319) malaria cases without duplicates from both sites were recorded between 2003-2005 and 2010-2012. The active cohort constituted 23% (299) of the total cases while the passive cohort accounted for 77%. Malaria in children under 5 years represented 21% (279) of the total malaria cases.

The highest number of malaria cases reported in Kipsamoite (161) was in 2004 while for Kapsisiywa, 2003 had the highest cases of malaria (395). In both sites, total malaria cases dropped by 56% for Kipsamoite and 84% for Kapsisiywa between 2004 and 2005. Similarly, between 2011 and 2012, a 70% and 81% drop was witnessed between Kipsamoite and Kapsisiywa respectively. No single individual in the active cohort (both <5 and ≥ 5 years) tested positive to *P. falciparum* between 2010 and 2012 (the period after reported interruption of transmission).

4.2.2 Monthly malaria incidence

Figures 7 and 8 below show the monthly malaria incidence in the two study sites. The downward arrows indicate the start and end of the reported interruption of malaria period (April 2007 to April 2008).

Figure 6: Kipsamoite monthly malaria incidence 2003-2012

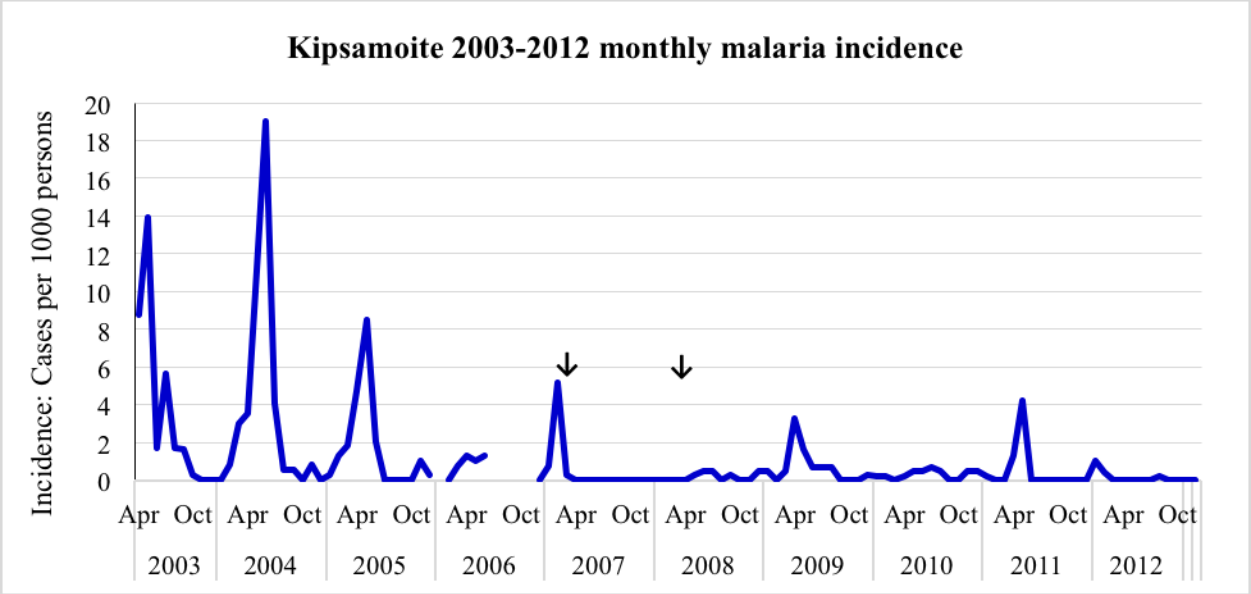
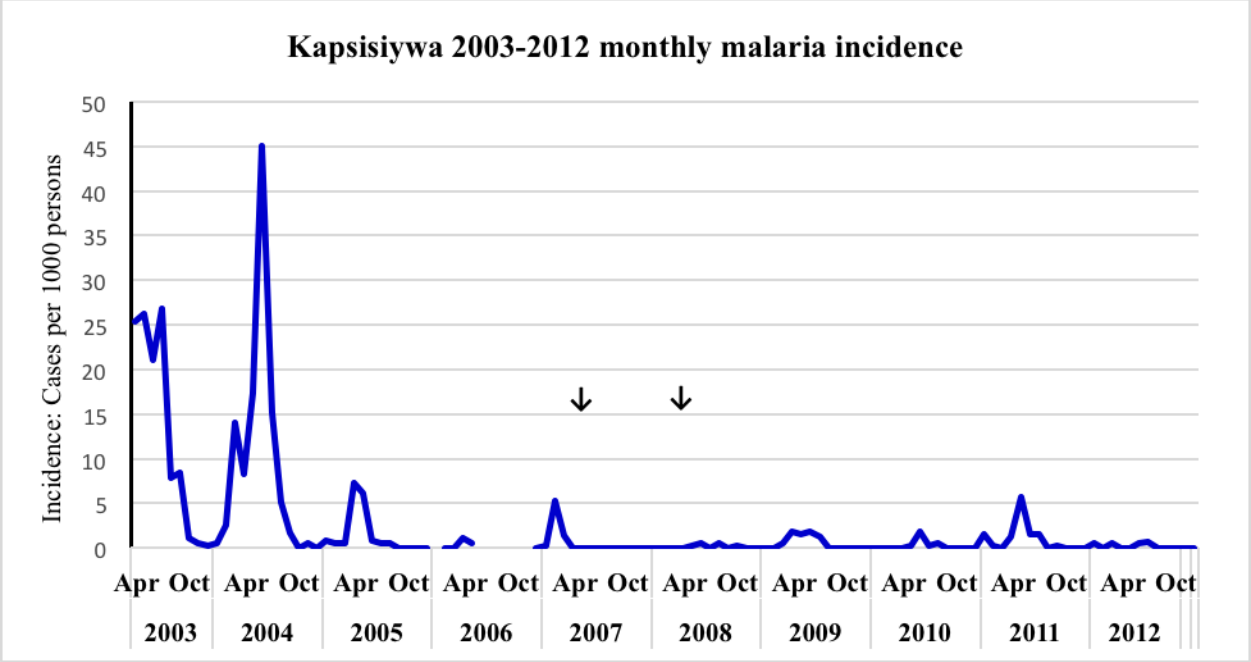


Figure 7: Kapsisiywa monthly malaria incidence 2003-2012



In June 2004, both Kapsisiywa and Kipsamoite recorded the highest monthly malaria incidences over the entire study period with 45 and 19 cases per 1000 persons

respectively. Figures 7 and 8 also show consistent seasonal variation in malaria albeit Kapsisiywa having higher incidence. The graphs show a similar peak in malaria cases every April, May and June and a drop every October to February. Due to change of study protocols from the "malaria early warning study" which ended in 2005 and start of "malaria transmission and immunity study" in 2006, incidence data for January and July-November 2006 was not collected.

Table 16: Annual malaria incidence: distribution by age and site

Year	Kipsamoite per 1,000 persons		Kapsisiywa per 1,000 persons		*P* value		Rate Ratio by site	
	<5	≥5	<5	≥5	<5	≥5	<5	≥5
2003	31	32	106	289	< 0.001	< 0.001	6	3.1
2004	37	43	80	307	< 0.001	< 0.001	3.8	2.4
2005	19	19	9	53	0.841	0.61	0.9	0.9
2010	1	4	3	8	0.999	0.357	3.8	0.6
2011	6	6	7	41	0.431	0.005	2.3	2.1
2012	3	1	1	8	0.999	0.59	0.7	1.5

Malaria incidence was calculated by age and site. Rate ratios were computed as the ratio of the incidence rate in Kipsamoite divided by the incidence rate in Kapsisiywa (which was considered comparison or reference group). The rate ratio was computed to determine if there was a difference in the rates of malaria in the two sites. In 2003 and 2004, statistically significant rate ratios are noted by both site and age groups. For instance, in 2003 and 2004, children < 5 were from Kapsisiywa were 6 times and 3.8 times more likely to have malaria respectively compared to those from Kipsamoite while adults from Kapsisiywa were 3.1 times and 2.4 times more likely to have malaria respectively during

the same period. The rate ratios between the two sites and the age categories were not significantly different for the subsequent years (2005, 2010 to 2012).

4.2.3 Anaemia prevalence

Annual samples for hemoglobin testing from asymptomatic individuals were measured in two separate cross-sectional survey periods: 2007-2008 a period before interruption of malaria transmission; and 2010-2012 a period after reported interruption of malaria transmission. Table 17 below shows anaemia prevalence for the periods 2007-2008, 2010, 2011 and 2012. Owing to reported interruption of malaria in the study site between April 2007 and April 2008, anaemia could not be measured for 2009 due to the few reported malaria cases in 2008. Hemoglobin level was determined by photometry, values adjusted for altitude and pregnancy status and WHO cut offs applied as described in section 3.7.4 above.

Table 17: Anaemia prevalence by site and age

Year	Kipsamoite		Kapsisiywa	
	<5	≥5	<5	≥5
2007	77 /163 (47.2%)	115 /624 (18.4%)	100/174 (57.5%)	164 /736 (22.3%)
2008	51/163 (31.3%)	113/624 (18.1%)	66/174 (37.9%)	104/736 (14.1%)
2010	18 /114 (15.7%)	85 /471 (18.1%)	65 /165 (39.4%)	133 /735 (18.1%)
2011	20 /36 (55.6%)	67 /173 (38.7%)	39 /122 (31.7%)	74 /461 (16.1%)
2012	2 /16 (12.5%)	55 /145 (37.9%)	39 /123 (31.7%)	64 /461 (13.9%)

At both sites in 2007, 456 of 1697 (26.9%) individuals tested for hemoglobin had anaemia. However, significant anaemia prevalence differences were noted between the age categories: 52.5% of children < 5 years tested had anaemia compared to 20.5% prevalence among individuals of 5 years and above meaning children < 5 years were 4.3 more times likely to have anaemia (OR 4.3, $p<0.001$). In 2008, 334 of 1697 (19.7%) individuals

tested for hemoglobin had anaemia. Similarly, significant anaemia prevalence differences were noted between the age categories: 34.7% of children < 5 years tested had anaemia compared to 16.0% prevalence among individuals of 5 years and above implying children < 5 years had a 2.8 more likelihood of having anaemia compared to individuals with 5 years or more (OR 2.8, $p<0.001$). In 2010, after reported interruption of transmission, significant anaemia prevalence differences were noted both between the age categories (29.7% for < 5 years and 18.1% for ≥ 5 years, $p<0.001$) and between the sites within the < 5 years age category (15.7% in Kipsamoite and 39.4% in Kapsisiywa, $p< 0.001$). Significant anaemia prevalence differences were also noted in 2011 between age categories (< 5 years-37.3% and ≥ 5 years-22.2%, $p< 0.001$) and within the age categories between the sites: for < 5 years (55.6% Kipsamoite and 31.7% Kapsisiywa, $p = 0.012$) and for ≥ 5 years (38.7% Kipsamoite and 16.1% Kapsisiywa, $p < 0.001$). Due to very low numbers, site prevalence differences were not computed for < 5 years. However, for ≥ 5 years individuals, a significant prevalence difference was established with individuals in Kipsamoite (anaemia prevalence 37.9%) 3.8 times more likely to have anaemia than their Kapsisiywa (anaemia prevalence 13.9%) counterparts, $p < 0.001$) even though this may not be attributable to malaria only.

4.2.4 Spatial autocorrelation of prior malaria and anaemia

Spatial association between malaria and anaemia was computed in STATA using Spearman's Correlation statistic. Correlation coefficient statistic was used to measure the strength (if any) of spatial relationship between malaria and anaemia and the direction of the relationship (positive or negative). Age and site specific analysis was done for all the units (hexagons) that had more than 5 persons, the number of malaria and anaemia cases.

Figure 11 below shows that children from Kipsamoite under 5 years, there was no significant spatial correlation between prior malaria (2003, 2004, 2005 and combined 2003-2005) and anaemia (2007). However in the same age group for Kapsisiywa, with the exception of 2005, prior malaria and anaemia exhibited weak positive but significant spatial correlation: (R^2= 0.22, p = 0.01 in 2003 and R^2= 0.39, p = 0.002 for 2004). The combined cases of malaria for 2003-2005 showed a moderate positive significant spatial correlation with anaemia (R^2= 0.48, $p < 0.001$). These results show that whilst there is no relationship between prior malaria and anaemia in Kipsamoite, anaemia in Kapsisiywa could be used as a moderate proxy measure of malaria intensity in children under 5 years.

Figure 8: Spatial autocorrelation of prior malaria and anaemia for <5 years (n = number of units with at least 5 persons)

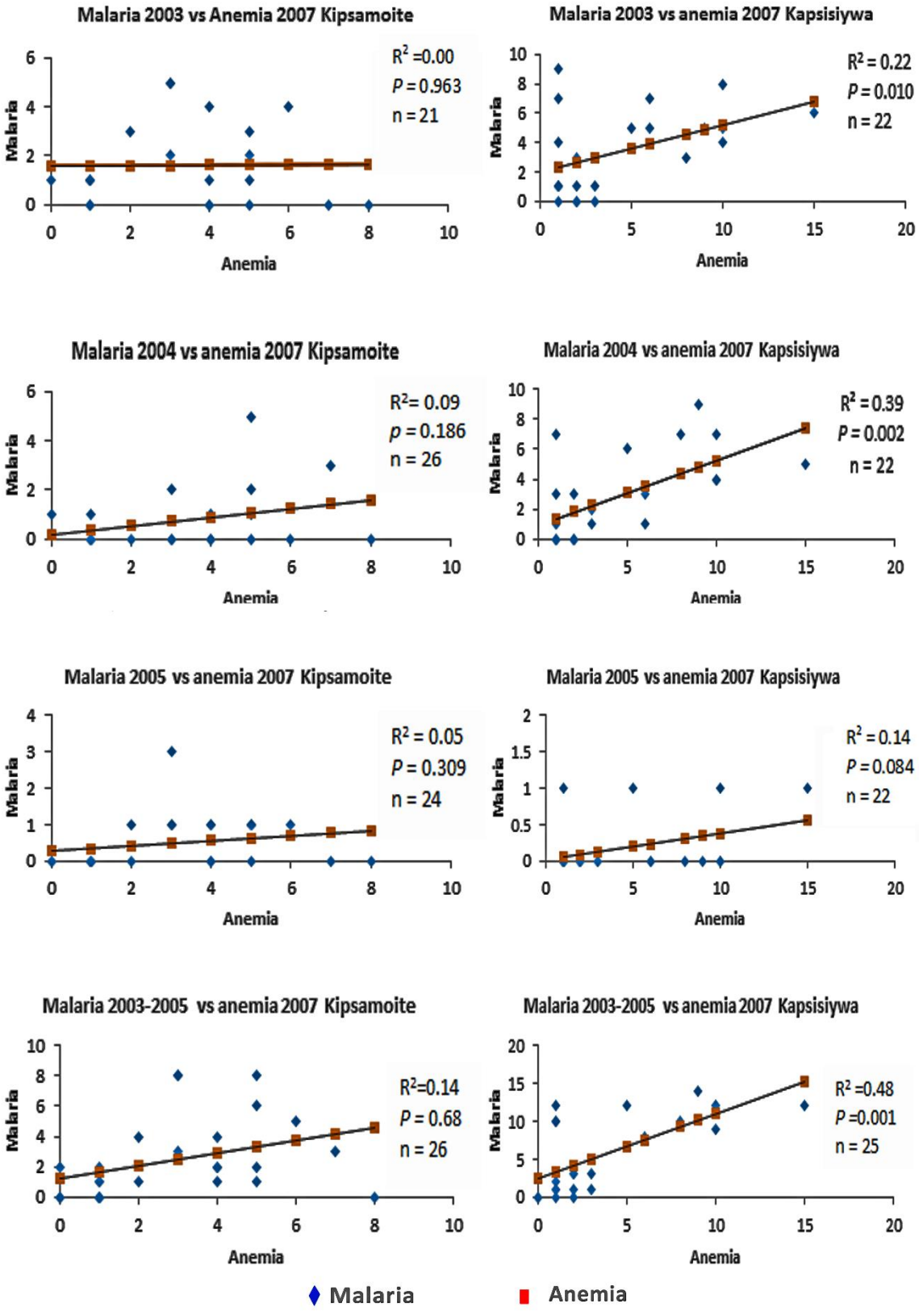
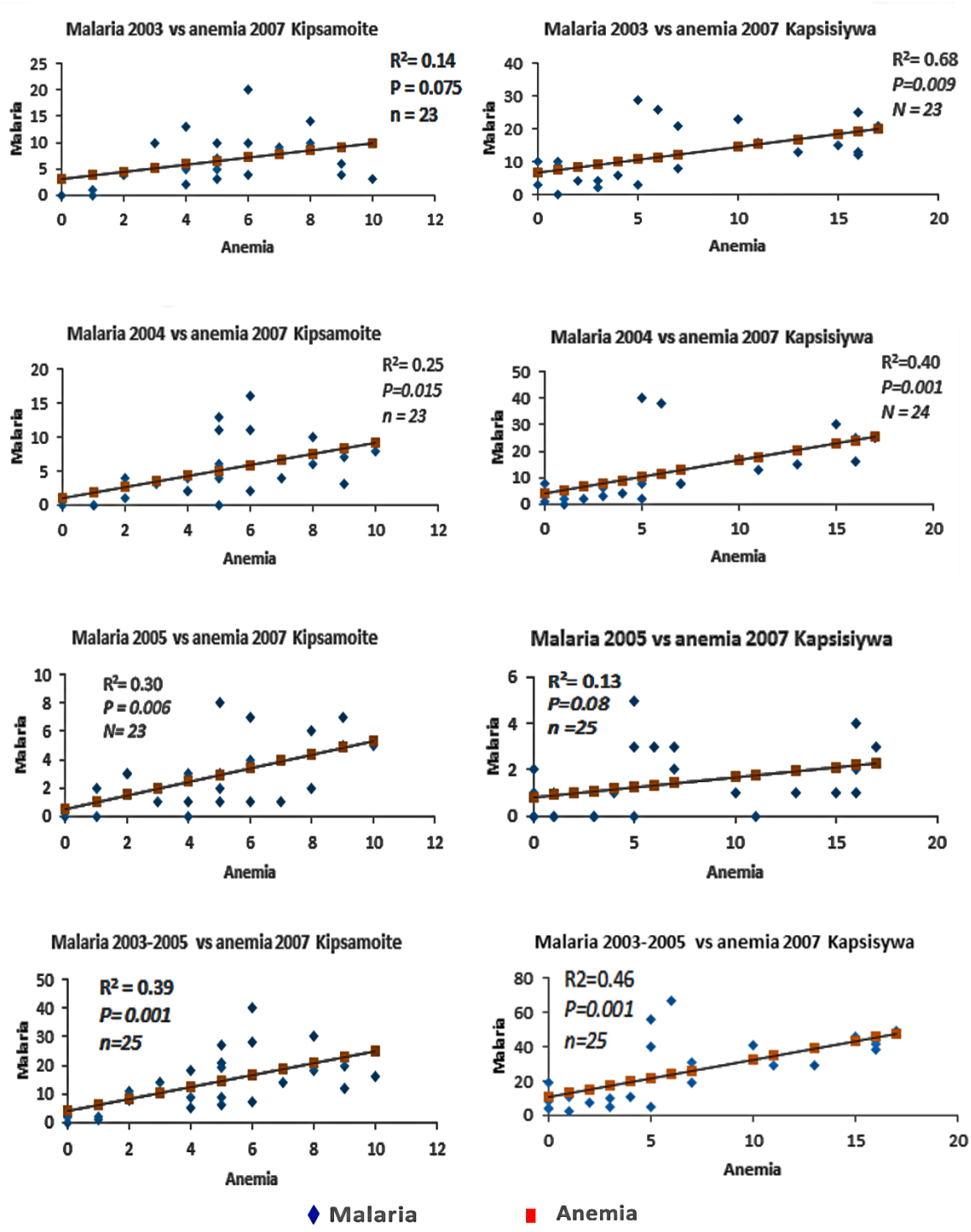

Malaria 2003 vs Anemia 2007 Kipsamoite
R² =0.00
P = 0.963
n = 21
Malaria
Anemia
Malaria 2003 vs anemia 2007 Kapsisiywa
R² = 0.22
P = 0.010
n = 22
Malaria
Anemia
Malaria 2004 vs anemia 2007 Kipsamoite
R²= 0.09
p = 0.186
n = 26
Malaria
Anemia
Malaria 2004 vs anemia 2007 Kapsisiywa
R² = 0.39
P = 0.002
n = 22
Malaria
Anemia
Malaria 2005 vs anemia 2007 Kipsamoite
R² = 0.05
P = 0.309
n = 24
Malaria
Anemia
Malaria 2005 vs anemia 2007 Kapsisiywa
R² = 0.14
P = 0.084
n = 22
Malaria
Anemia
Malaria 2003-2005 vs anemia 2007 Kipsamoite
R²=0.14
P = 0.68
n = 26
Malaria
Anemia
Malaria 2003-2005 vs anemia 2007 Kapsisiywa
R² =0.48
P =0.001
n = 25
Malaria
Anemia
Malaria
Anemia

Figure 9: Spatial autocorrelation of prior malaria and anaemia for ≥ 5 years (n = number of units with at least 5 persons)



There is spatial correlation between prior malaria and anaemia in individuals≥ 5 years. Figure 12 above shows that whereas no spatial correlation is observed in 2003 in Kipsamoite, weak positive correlations are noted in 2004 and 2005 (R^2= 0.25, p = 0.015 and R^2= 0.30, p = 0.006) respectively with a weak positive correlation for the combined 2003-2005 period (R^2= 0.39, p < 0.001). In Kapsisiywa, strong and moderate spatially significant correlations are noted for 2003 and 2004 (R^2= 0.68, p = 0.009 and R^2= 0.40, p < 0.001) respectively. Malaria incidence in 2005 is not spatially correlated with anaemia in 2007. The overall malaria incidence for 2003-2005 shows a moderate positive spatial correlation (R^2= 0.46, p = 0.001). These results in Figure 12 show that for individuals who are ≥ 5 years, anaemia prevalence was spatially correlated with previous malaria incidence.

4.3 Objective 3: Spatial correlation of malaria incidence and anaemia prevalence after reported interruption of transmission.

4.3.1 Spatial malaria incidence variation (cases/1000 persons/year)

A total of 47 repeated analysis units (hexagons) of approximately 0.62 km^2 were overlaid on the malaria incidence (2010-2012) points. Age and site-controlled spatial incidence for malaria was computed for each analysis unit by dividing number of individuals who were *P. falciparum* positive in each unit with the total number of individuals in the unit and multiplying by 1000. Only units with more than 5 individuals were considered for analysis. The color intensity assigned to each analysis unit was based on malaria incidence within each unit-the darkest shade of color symbolizes the highest proportion of malaria or anaemia intensity.

Figure 10: Spatial malaria incidence variation (cases/1000 persons/year) for under 5 years

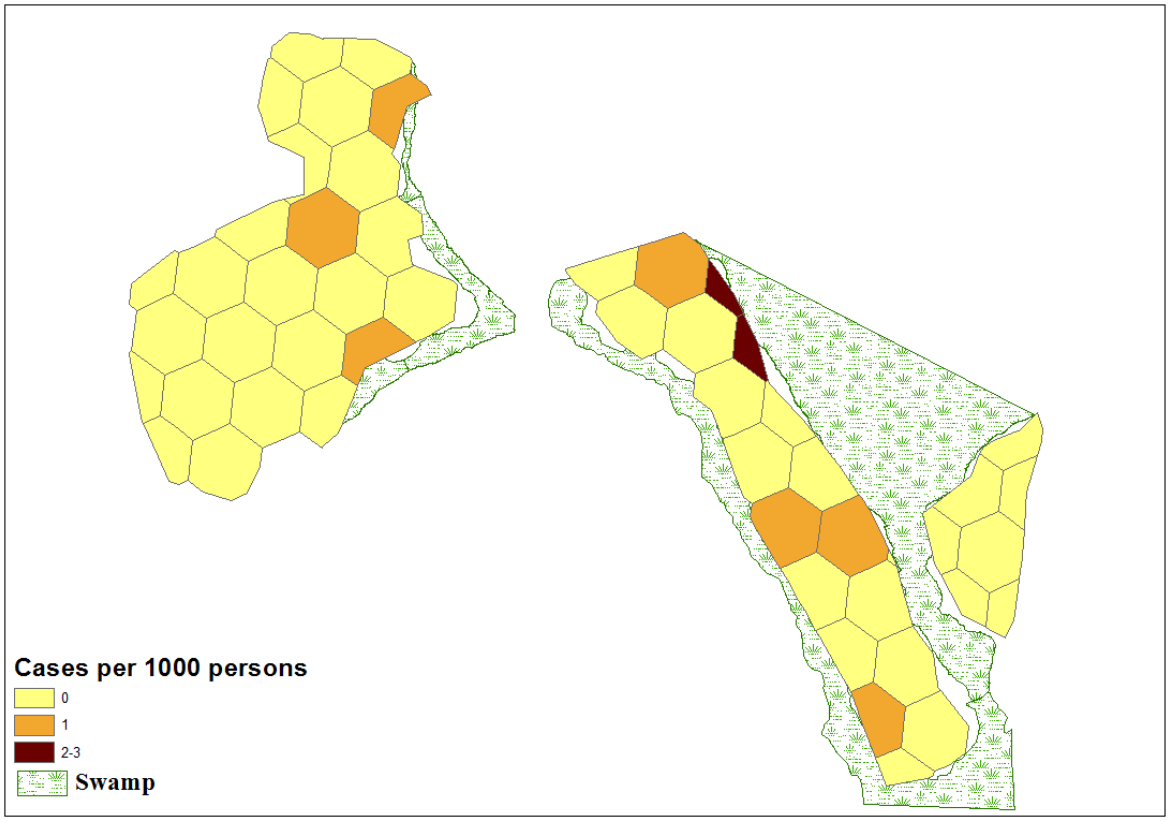


Figure 13 above shows spatial malaria incidence for the combined period 2010-2012 in children under 5 years. During this period, the highest unit of incidence of malaria had 2-3 cases/1000 persons/year while most of the units had 0 cases/1000 persons/year. This highest incidence occurred on the right hand side (Kapsisiywa) of the map where malaria can visually be seen as concentrated or forming a focus around the northwest units. Overall, the map also shows that malaria incidence from 2010-2012 was much lower in both sites compared to the incidence for the period 2003-2005.

Figure 11: Spatial malaria incidence variation (cases/1000 persons/year) in individuals 5 years and above

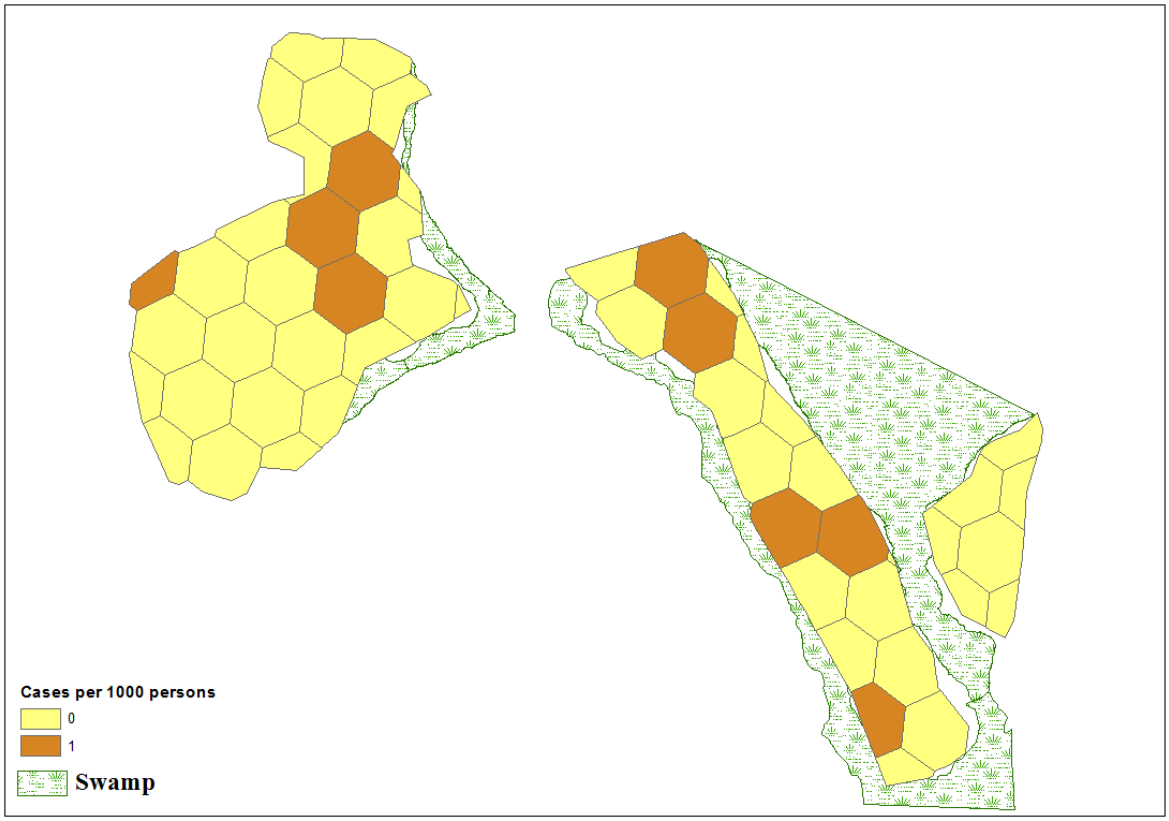


Figure 14 above shows spatial malaria incidence for the combined period 2010-2012 in individuals 5 years and above. The highest unit of incidence of malaria had 2-3 cases/1000 persons/year while most of the units had 0 cases/1000 persons/year. This highest incidence occurred on the right hand side (Kapsisiywa) of the map where malaria can visually be seen as concentrated or forming a focus around the northwest units. Overall, the map also shows that malaria incidence from 2010-2012 was much lower in both sites compared to the incidence for the period 2003-2005

4.4 Objective 4: Malaria hotspot cluster analysis

In order to determine if new malaria cases after reported interruption of transmission were a reflection of existing malaria transmission hotspots or just random occurrence, malaria clusters for the periods before (2003-2005) and after (2008-2012) reported interruption of transmission were calculated using Poisson cluster analysis in SaTScan and likelihood ratios tested for significance (at $p < 0.05$) using the Monte Carlo method at 999 replications within a radius of 1 km. The relative risk for each significant cluster was calculated using logistic regression models in STATA.

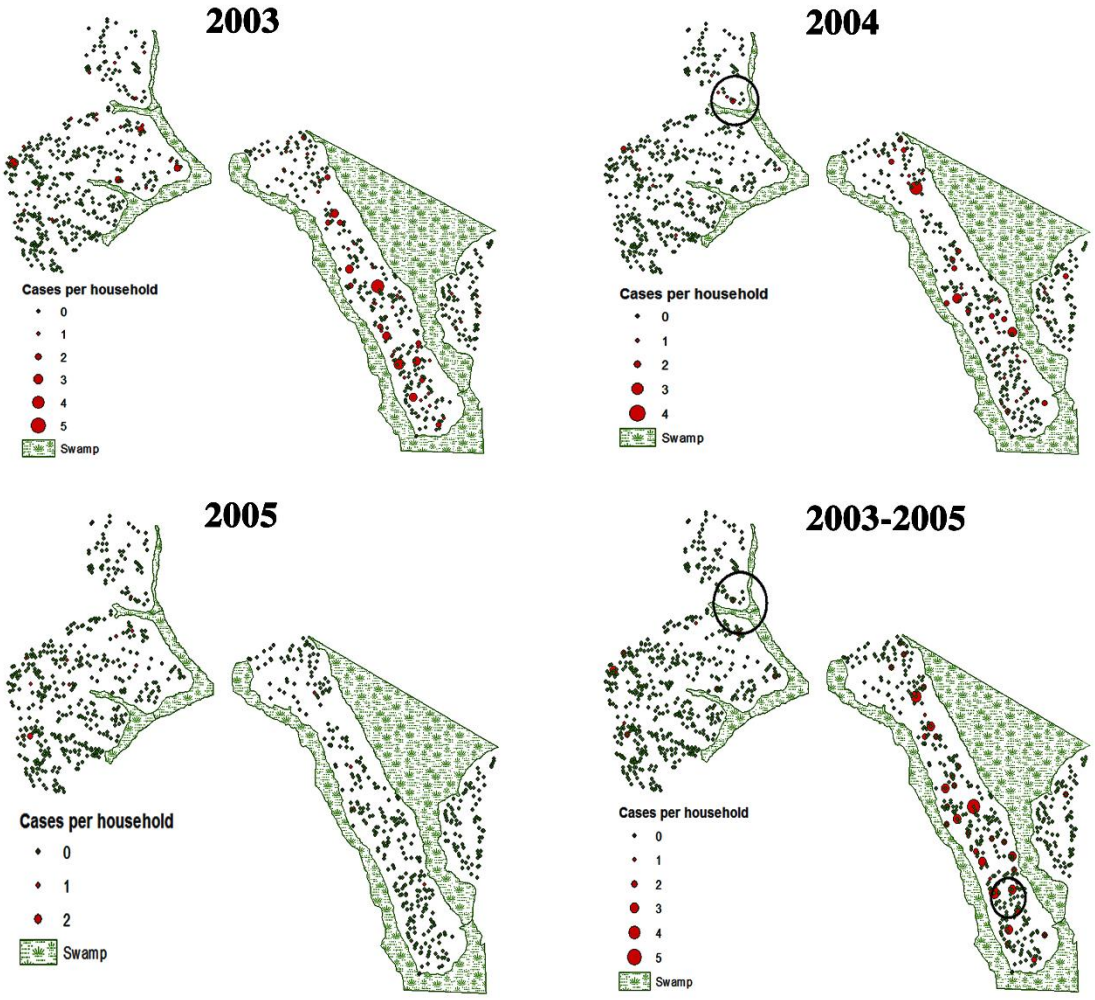
4.4.1 Malaria hotspot cluster under 5 years

Table 18 shows relative risk of malaria in significant clusters for Kapsisiywa and Kipsamoite; Figure 15 is a representation of the clusters detected by Kuldorff Scan in SaTScan before reported interruption of transmission (2003-2005); Figure 16 represents clusters detected by Kuldorff Scan in SaTScan after reported interruption of transmission (2010-2012); and Figure 17 depicts the malaria hotspots before and after reported interruption of transmission.

Table 18: Household and malaria case frequency and relative risk of malaria in clusters (hotspots) of malaria incidence for under 5, 2003–2005

Year	Kipsamoite			Kapsisiywa		
	No. of HHs in cluster (% of total HHs)	**No. of malaria cases in cluster (% of total malaria cases)**	**Relative risk in cluster (Log likelihood statistic, P-value)**	**No. of HHs in cluster (% of total HHs)**	**No. of malaria cases in cluster (% of total malaria cases)**	**Relative risk in cluster (Log likelihood statistic, P-value)**
2003	-	-	-	-	-	-
2004	12 (3%)	7 (25%)	21.7 (LLR=13.2, $p < 0.001$)	-	-	-
2005	-	-	-	-	-	-
2003-2005	20 (4%)	17 (26%)	21.7 (LLR=17.2, $p < 0.001$)	8 (2%)	8 (4%)	10.1 (LLR=11.1, $p = 0.003$)
2010-2012	1 (0.2%)	4 (33%)	100.5 (LLR=13.6, $p = 0.001$)	1 (0.2%)	3 (17%)	49 (LLR=8.5, $p = 0.024$)
	1 (0.2%)	3 (25%)	83.8 (LLR=9.9, $p = 0.021$)			

Figure 12: Significant malaria clusters 2003-2005 as detected by Kuldorff Scann for under 5 years



SaTScan analysis detected only one cluster in Kipsamoite in 2004 (log likelihood ratio = 13.2, $p < 0.001$) and a significant cluster in both Kipsamoite (log likelihood ratio = 17.2, $p < 0.001$) and Kapsisiywa (log likelihood ratio = 11.1, $p = 0.003$) for the combined period 2003-2005 as shown in Table 18 above and visually depicted in Figure 12. In the period 2003-2005, children who were within the spatially significant clusters were at 22 (Kipsamoite) and 10 (Kapsisiywa) fold greater risk of contracting malaria than children who lived outside the clusters (Table 18). In the combined period 2003-2005, 4% of households (in

Kipsamoite) were in the significant cluster and accounted for 17 (26%) of the total malaria cases while 2% of households in the significant cluster accounted for 4% of total malaria cases in Kapsisiywa.

Figure 13: Significant malaria clusters 2010-2012 as detected by Kuldorff Scan for under 5 years

During the period 2010-2012, SaTScan analysis detected two clusters in Kipsamoite (log likelihood ratio = 13.6, p = 0.001 and log likelihood ratio = 9.9, p = 0.021) while Kapsisiywa had one cluster (log likelihood ratio = 8.5, p = 0.024). Two households (0.4%) in Kipsamoite were in the significant clusters and accounted for 7 (58%) of the total malaria cases while 1 household (0.2%) in the significant cluster accounted for 3 (17%) of total malaria cases in Kapsisiywa.

Figure 14: Under 5 years malaria hotspots 2003-2005 and 2010-2012

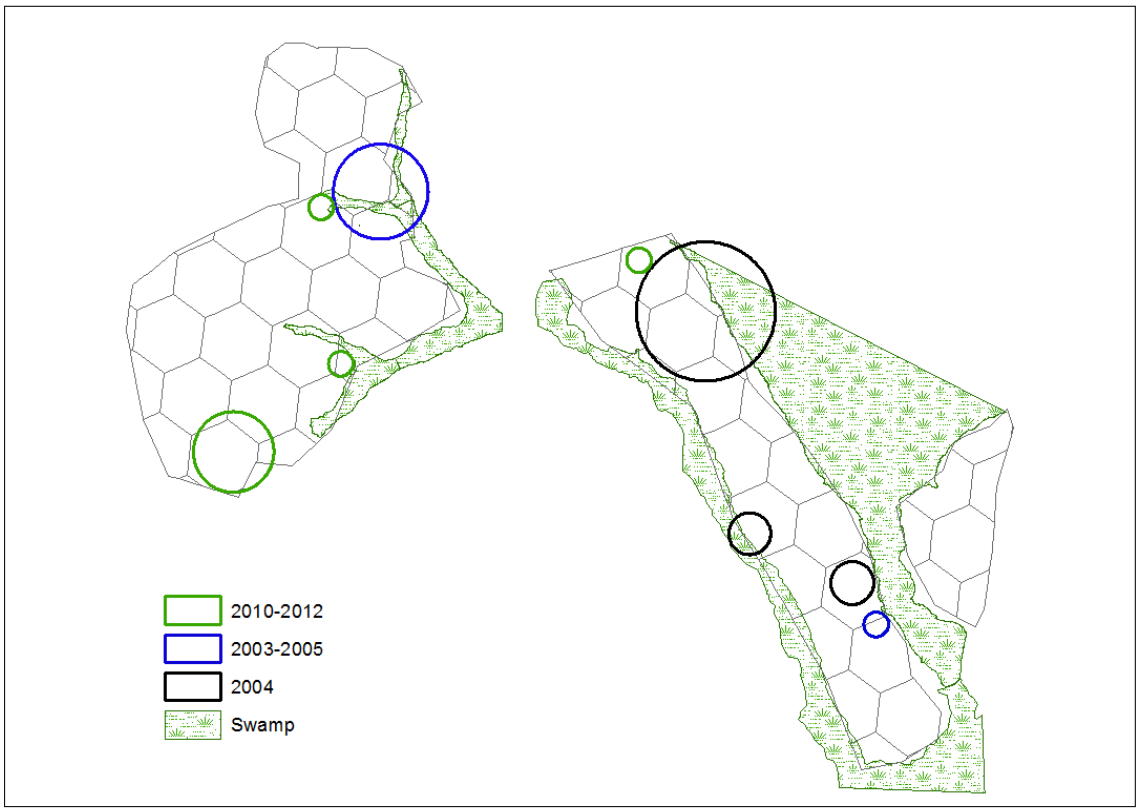


Figure 17 above does not show any overlap of malaria clusters between 2003-2005 (in blue) and 2010-2012 (in green). This lack of overlap is an indication that there does not exist malaria transmission reservoirs within Kipsamoite and Kapsisiywa for children under 5 years even though the clustering could be partly due to environmental factors based on a study done by Ernst *et al*, (2006).

4.4.2 Malaria hotspot analysis 5 years and above

Hotspot analysis for individuals 5 years and above are presented in: Table 19 which shows significant clusters of malaria for Kapsisiywa and Kipsamoite; Figure 18 represents the clusters detected by Kuldorff Scan in SaTScan before reported interruption

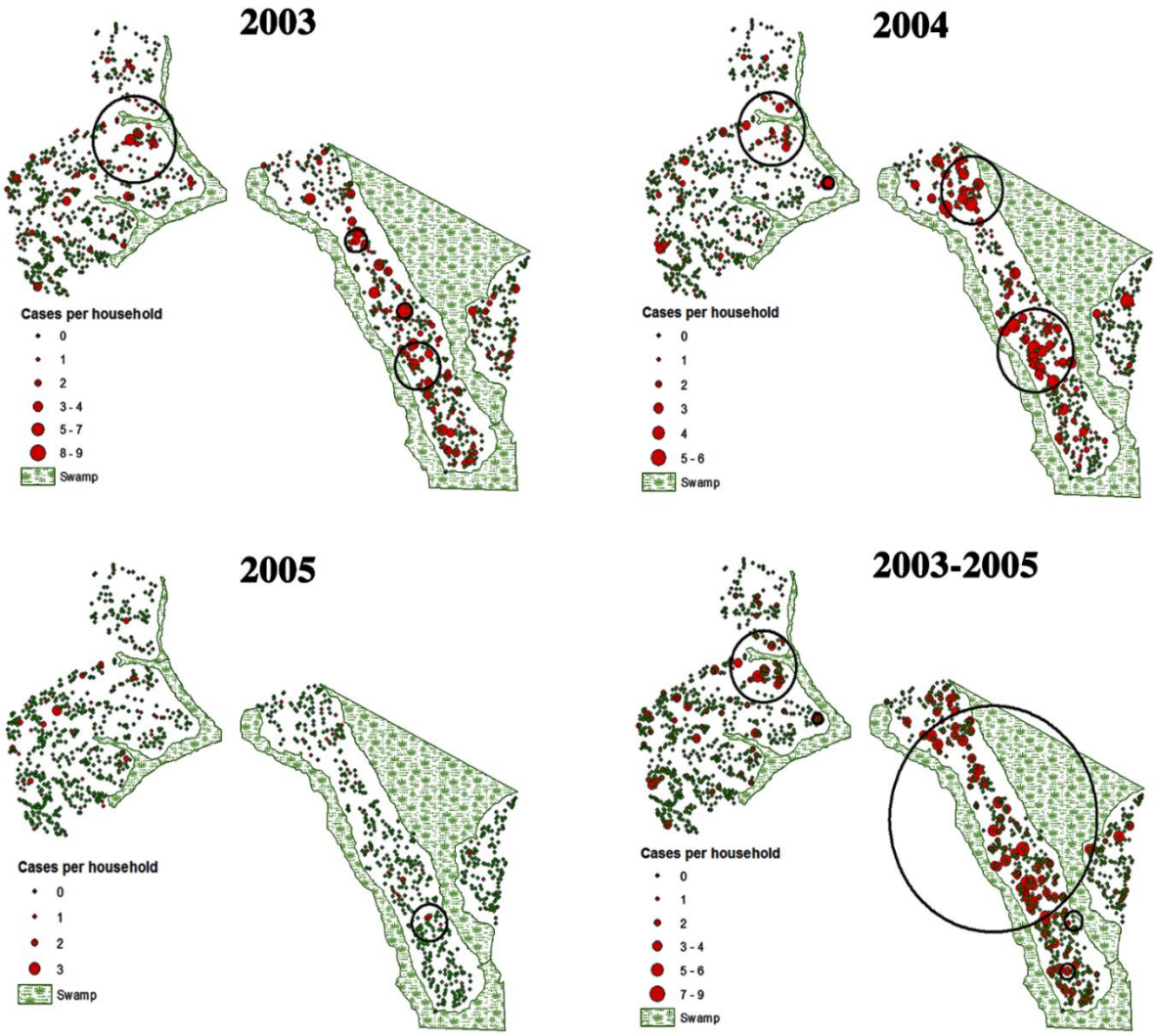
of transmission (2003-2005); Figure 19 represents clusters detected by Kuldorff Scan in SaTScan after reported interruption of transmission (2010-2012); and Figure 20 depicts malaria hotspots before and after reported interruption of transmission

Table 19: Household and malaria case frequency and relative risk of malaria in clusters (hotspots) of malaria incidence for 5 years and above, 2003–2005

Year	Kipsamoite			Kapsisiywa		
	No. of HHs in cluster (% of total HHs)	**No. of malaria cases in cluster (% total malaria cases)**	**Relative risk in cluster (Log likelihood statistic, P-value)**	**No. of HHs in cluster (% of total HHs)**	**No. of malaria cases in cluster (% total malaria cases)**	**Relative risk in cluster (Log likelihood statistic, P-value)**
2003	77 (12%)	53 (56%)	3.4 (LLR=22.3, $p < 0.001$)	14 (2%)	23 (8%)	3.4 (LLR=11.5, $p = 0.004$)
				37 (6%)	41 (14%)	2.4 (LLR=11.2, $p = 0.011$)
				1 (0.2%)	9 (3%)	8.1 (LLR=10.8, P p= 0.011)
2004	22 (4%)	38 (29%)	3.4 (LLR=23.2, $p < 0.001$)	99 (16%)	61 (20%)	2.4 (LLR=15.8, $p < 0.001$)
	3 (1%)	7 (5%)	10.1 (LLR=5.5, $p = 0.018$)	8 (1%)	102 (33%)	2.3 (LLR=20.6, $p < 0.001$)
2005	-	-	-	2 (0.3%)	4 (8%)	10.5 (LLR=38.4, $p = 0.013$)
2003-2005	52 (8%)	114 (39%)	4.1 (LLR=66.7, $p < 0.001$)	237 (38%)	409 (63%)	1.8 (LLR=32.2, $p < 0.001$)
	5 (1%)	16 (6%)	4.8 (LLR=12.3, $p = 0.002$)	12 (2%)	41 (6%)	3.4 (LLR=20.6, $p < 0.001$)
				1 (0.2%)	10 (2%)	7.8 (LLR=11.8, $p = 0.003$)
2010-2012	44 (5%)	30 (25%)	6.7 (LLR=28.4, $p < 0.001$)	24 (3%)	20 (13%)	5.0 (LLR=15.4, $p < 0.001$)
	13 (1%)	18 (15%)	8.5 (LLR=21.4, $p < 0.001$)	9 (1%)	12 (8%)	6.1 (LLR=12.1, $p = 0.002$)
	22 (2%)	17 (14%)	8.6 (LLR=20.6, $p < 0.001$)	12 (2%)	12 (8%)	4.7 (LLR=8.8, $p = 0.046$)

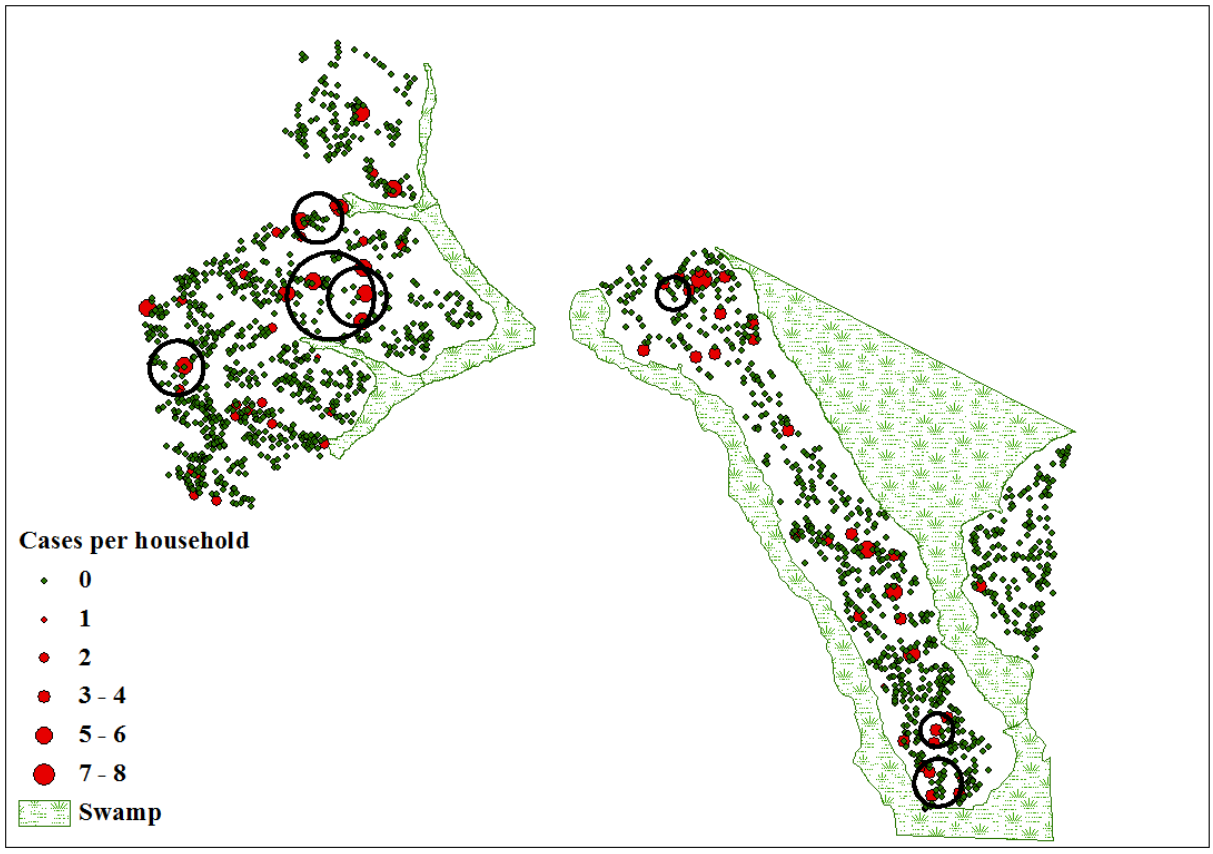
	19 (2%)	16 (13%)	7.0 (LLR=16.5, $p < 0.001$)			

Figure 15: Significant malaria clusters 2003-2005 as detected by Kuldorff Scan for individuals 5 years and above



In Kipsamoite, 1 and 2 significant clusters were detected in 2003 and 2004 respectively while none was detected in 2005. Over the combined period 2003-2005, 2 significant clusters with 9% of total households accounted for 45% of total malaria cases (log likelihood ratio = 66.7, $p < 0.001$ and log likelihood ratio = 12.3, $p = 0.002$). Individuals in these Kapsisiywa clusters had an average 4.5 fold greater risk of having malaria than those who were not within the clusters (Table 19 above). In Kapsisiywa, 3, 2 and 1 significant clusters were detected in 2003, 2004 and 2005 respectively. Over the combined period 2003-2005, 3 significant clusters covering 42% of total households accounted for 71% of total malaria cases (log likelihood ratio = 32.2, $p < 0.001$, log likelihood ratio = 20.6, $p < 0.001$ and log likelihood ratio = 11.8, $p = 0.003$). Individuals in these clusters had an average 4.3 fold greater risk of having malaria than those who were not within the clusters (Table 19). The significant clusters in the 2 sites are presented in Figure 18

Figure 16: Significant malaria clusters 2010-2012 as detected by Kuldorff Scan for individuals 5 years and above



After reported interruption, 2010-2012, there were 4 clusters in Kipsamoite which consisted of 10% all households and accounted for 67% of all malaria cases while in Kapsisiywa, 3 clusters consisting of 6% of total households accounted for 29% of total malaria cases. Individuals from Kipsamoite who were in the malaria clusters had an average of approximately 8 fold higher risk of having malaria than those who were not within the clusters while those in clusters from Kapsisiywa had an average 5 fold higher risk of having malaria.

Figure 17: Malaria hotspots 2003-2005 and 2010-2012 5 years and above

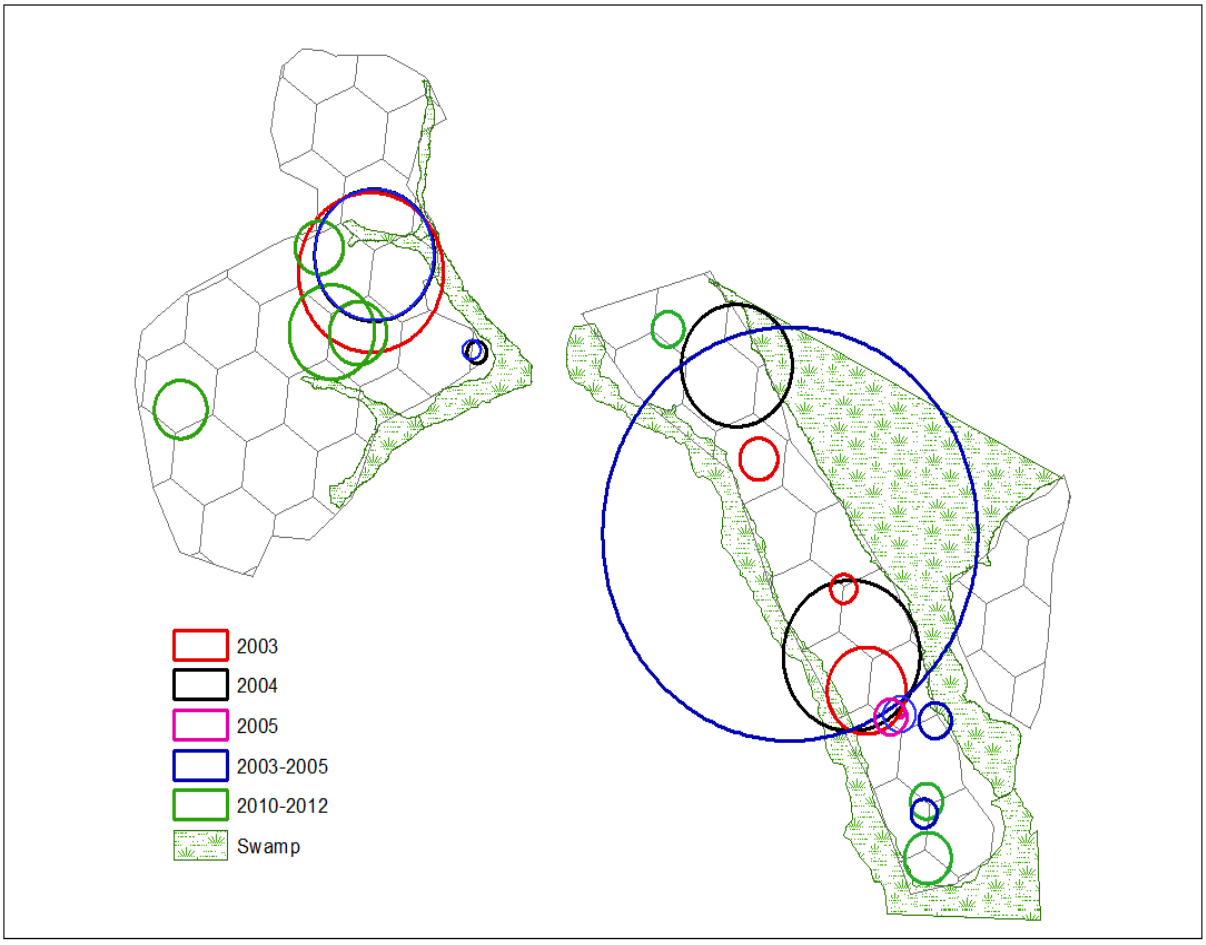


Figure 20 above shows an overlap of 2003-2005 (in blue) and 2010-2012 (in green) clusters in Kipsamoite while no cluster overlap is seen in Kapsisiywa. The hotspot noted at the north west in Kipsamoite is consistent with the annual malaria incidences seen in previous graphs.

CHAPTER FIVE

5. DISCUSSION

This chapter discusses the results of the study findings presented in Chapter 4. It also compares the findings with results from similar studies on the subject.

5.1 Malaria diagnosis in areas of low transmission

Fever is the universal screening tool for clinical malaria in research studies. This is likely appropriate in children <5 years, who constitute the majority of malaria cases in highly endemic areas, because they are more likely to develop fever when they have infection than are older individuals. However in areas such as the highland area of the present study, a substantial proportion of clinical malaria occurs in individuals ≥5 years (Rolfes *et al*., 2012) and local clinicians state that fever alone does not detect all cases of malaria in these individuals. The present study supports this position. Almost half of individuals ≥ 5 years who came to the clinic with symptoms of illness and who had *P. falciparum* parasitaemia would not have been captured as malaria cases had history of fever been the sole screening criterion. A recent study by D'Acremont *et al.* (2014) found that in one area of Tanzania, malaria was a less common cause of fever than other infections, notably respiratory viral infection. The present study answers the inverse question (does fever as a symptom or any other combination of symptoms capture all cases of clinical malaria?), and finds that in areas of low malaria transmission, fever alone or any combination of symptoms may not be an adequate screening criterion for malaria.

A history of fever may be absent in individuals ≥5 years with parasitaemia for several reasons. Fever may be less evident in older children and adults than in younger children,

whose temperature may be checked or noted by their parent. This is supported by the only modest increase in sensitivity of a history of fever among individuals ≥5 years who had a measured fever at the clinic. In areas of higher transmission, parasitaemia could be incidental rather than causative of symptoms. However in this area, based on the surveys done, one would expect <0.2% of individuals to have asymptomatic parasitaemia, so incidental parasitaemia should account for only a tiny fraction of the parasitaemia in symptomatic individuals seen in the clinic. In addition, history of fever had a sensitivity of only 65% even for clinical malaria defined as parasitaemia > 4,000 parasites/μL and a measured fever at the clinic, individuals who in this context indisputably have clinical malaria. The Nandi words for each symptom, including fever, were carefully reviewed with our study field assistants, and verified by the clinical officers and the local advisory group, and the symptom of fever was highly sensitive for detection of parasitaemia in young children, so confusion about the term is unlikely. The most likely explanation seems to be that other symptoms precede fever in older individuals. These symptoms bring them to the dispensary, at which time some have measured fever but others remain afebrile. Given the clinical course of malaria, all of these individuals were likely to have a fever eventually, but in a dispensary setting, particularly in an area of low transmission that is working toward elimination, one wants to detect malaria early and accurately. Individuals with malaria who have symptoms other than fever, but are not tested or treated because fever is the sole screening criterion, are likely to go outside the health system, and potentially receive inappropriate treatment.

Unfortunately, no symptom combination emerged that would allow us to detect >90% cases of parasitaemia in individuals ≥5 years without screening many more uninfected

individuals. A previous study in highland Uganda found that a history of fever or presence of measured fever at the clinic had a sensitivity of 95.7% and a specificity of 25.5% (Lindblade *et al*., 2001), but the findings were not separated by age. In the present study, reported or measured fever had a sensitivity and specificity of only 78% and 44%, respectively, for individuals ≥5 years, so reported or measured fever alone would not be adequate for screening in older individuals. The simplest combination of symptoms that achieved approximately 95% sensitivity in individuals ≥ 5 years was fever or headache (sensitivity 96. 8%), but screening with these two symptoms increased the number of individuals ≥ 5 years to be screened to almost twice what was required with fever (from 1,290 to 2,480). Headache has been noted in the travel medicine literature to be a frequent complaint in adult travellers with malaria, though most travellers with malaria also have fever (Pherez & Cunha, 2009). The presence of fever early in travellers, but not in this highland population, may be due to partial immunity from prior exposure in this population, resulting parasitaemia that does not produce immediate fever but does produce other lower-grade symptoms such as headache.

Definitions that relied on more symptoms were more complex, but did not have better sensitivity than asking about fever or headache, so in the present study site, fever or headache will be used as screening symptoms because they are the best single set of screening symptoms that will capture ≥95% individuals with clinical malaria. An alternative would be to wait for individuals to come in for a repeat visit, since malaria likely will eventually result in fever in all individuals, but this would lead to missed chances to diagnose malaria, to prolonged parasitaemia in symptomatic individuals, and potentially to lost confidence in the health dispensary. The findings of the present study

may have applicability to other sites of unstable transmission, and in these areas analysis of symptoms should be done to determine if screening for fever alone can detect >95% of individuals who have clinical malaria.

In areas of high malaria transmission, malaria occurs most often in children and is deadliest in children (Rowe *et al*., 2006; Kleinschmidt *et al*., 2009; Korenromp *et al*., 2003; WHO, 2013; Snow *et al*., 2003), so a history of fever, which detects almost all cases of malaria in young children, is a reasonable screening criterion. However, as more areas become areas of low transmission, disease in adults and adolescents may form a larger part of the clinical burden, and additional symptoms may be needed to identify all potential cases. Individuals in areas of unstable, low transmission differ from individuals in areas of high transmission, who have some clinical immunity and travellers from non-malaria endemic areas, who have little or no immunity. The intermediate initial presentation of low-grade symptoms, such as headache, may reflect their partial clinical immunity.

An interesting incidental finding is that the *absence* of diarrhoea had 96% sensitivity for detection of clinical malaria in individuals ≥5 years. The finding probably reflects a combination of the low incidence of clinical malaria at this site, the frequency of diarrhoea as an illness, the multiple other causes of diarrhoea in this area, and the relatively low frequency of diarrhoea as a symptom of malaria. The study findings contrast with those of Coldren *et al*., who found a modest association between *P. falciparum* parasitaemia and a history of diarrhoea in adults from three areas of Kenya with differing malaria transmission intensity (Coldren *et al*., 2006). The differences may in part reflect different populations: the present study was restricted to an area of very low endemicity and included children as well as adults.

5.2 Spatial malaria-anaemia correlation

The etiology of anaemia is complex and determined by several factors like micronutrient deficiencies like iron deficiency; parasitic infections like malaria and intestinal nematodes such as hookworms; infection with human immunodeficiency virus (HIV), chronic inflammatory disorders, and hemoglobinopathies (Menendez *et al*, 2000; Nussenblatt and Semba, 2002, Nagel, 2002). Several studies have shown that in areas of high malaria transmission intensity, there is a relationship between malaria and anaemia (Knoblauch *et al.* 2004) and malarial anaemia has been identified as the most common clinical manifestation of severe malaria especially in infants and young children (Breman *et al*, 2001; Lusingu *et al*, 2004; Perkins *et al*, 2011; Choge *et al*, 2014). In the current study-done in an area of very low malaria transmission intensity, malaria and anaemia have been examined to determine if they are spatially correlated in aggregate areas (hexagons of approximately 0.62km^2). Results from correlational analysis of anaemia prevalence and malaria incidence in each spatial unit show that for both children <5 and individuals ≥5 years and in both sites, malaria incidence and anaemia prevalence had a statistically significant spatial correlation. Further, spatial units with higher incidences of malaria recorded relatively lower HB compared to those that had few cases of malaria. This study's results are consistent with findings from a study conducted on children <5 years in Butare highlands in Rwanda that found 82% of asymptomatic children had anaemia (Gatuhu *et al*, 2011) and that of Noland *et al* (2011)-conducted on the same population as that of the current study-which found a decrease in anaemia prevalence in all age groups after 1 year of reported interruption of malaria transmission. A recent analysis of the same population as that of the current study established that iron deficiency prevalence in children decreased significantly after the interruption of malaria transmission while

correlating with an increase in hemoglobin levels (Frosch *et al,* 2014). In this study, spatial correlation of malaria and mean haemoglobin levels per hexagon for children <5 was not significant while correlation for persons ≥5 years exhibited significant spatial association. This could be attributed to the few <5 children whose haemoglobin levels were analyzed. However, when haemoglobin levels were dichotomized into anaemic and non-anaemic persons, both <5 and ≥5 age groups showed significant spatial correlation between prior malaria and anaemia prevalence. The difference between the current study and that of Noland *et al* (2011) and (Frosch *et al,* 2014) is that while this study looked at whether malaria and anemia occurred in similar geographic areas (spatial autocorrelation), the other two looked at overall reduction of anemia with reduced malaria transmission.

While spatial analysis of malaria transmission intensity (Ernst *et al,* 2006; Gaudart *et* al, 2006; Kazembe *et al,* 2006; Yeshiwondim *et al,* 2009; Bejon *et al,* 2010; Reid *et al,* 2010; Gething *et al,* 2010) and anaemia prevalence (Kreuels *et al.* 2008; Gayawan *et al,* 2014) have been conducted separately in both high and low malaria endemicity settings, the findings of this study differ from them in the sense that, data for both anaemia and malaria from the same population households was used to assess for spatial autocorrelation. A study conducted to establish spatial heterogeneity of haemoglobin concentration in preschool-age children in sub-Saharan Africa concluded that even though there were different environmental and epidemiological drivers of anaemia in West and Eastern Africa, anaemia occurred in clusters (Magalhães & Clements, 2011). That study did not relate to malaria specifically but demographic health survey (DHS) data in 3 West African countries (Ghana, Burkina Faso and Mali) while the current study has used individual household level data from a relatively homogenous population.

A study done in the neighboring highlands (altitude 1,450–1,580 meters above sea level) of Kakamega county to assess for spatial patterns of infection rates of *P. falciparum* established clustering of sexual parasite density near major vector breeding habitats (Munyekenye *et al.* 2005). The results from Munyekenye and colleagues are similar to those of Cohen *et al* (2008) done in the current study area and showed that proximity to areas with high water concentration significantly and consistently led to increased household-level malaria incidence, even in a small geographical area with homogenous topography and led them to conclude that high wetness indices were not only related to valleys or water flow pathways but also a good starting point for developing models for predicting malaria risk in highland regions. In their cross-sectional assessment of the epidemiology of malaria and anaemia in 2 small mining communities in central Côte d'Ivoire with small-scale spatial and temporal heterogeneity, Knoblauch *et al.* (2014) reported *P. falciparum* infection to be significantly associated with anaemia. Their cross-sectional study which was conducted in a highly endemic area postulated that the malarial-anaemia relationship might be influenced by human activities, such as mining and related disturbances of the environment.

This study's findings differ with findings of Gayawan *et al.* (2014) which showed significant geographical variation in the Hb concentration level as well as anaemic status of children in Nigeria. The Gayawan *et al* study analysed data from cross-sectional 2010 Nigeria Malaria Indicator Survey which also looked at other risk factors like household wealth indices, while the current study data was derived from individual household longitudinal data over a 3 year period.

The results of the current study show a strong spatial correlation of prior malaria incidences and anaemia prevalence, and concur with the suggestion that malaria-related interventions may result in a significant direct impact on anaemia prevalence within a population (Noland *et al.,* 2011; Korenromp *et al,* 2003) hence provide vital programmatic intervention information on malaria-anaemia relationship in highland areas of very low transmission. Most importantly. The fact that anaemia is still persistent up to 4 years after malaria incidence shows that the impact of malaria may take several years to be totally resolved. However, in their review of community-based studies of malaria interventions, Korenromp *et al* postulated that in areas of low unstable transmission, like our study sites, malaria control measures may not have a great impact on anaemia outcomes. The current study shows that anaemia was more likely to occur in same geographic units as malaria while mean haemoglobin levels increased with decrease in malaria incidence in the same units within the study sites (Figures 8-10) even though it could not conclusively determine if all the anaemia was due to malaria or other factors.

5.3 Malaria Hotspots

The WHO (2013) World Malaria Report shows a 25% global and 31% African region malaria incidence decrease between 2000 and 2012. Several high burden African countries like Eritrea, Rwanda and Zanzibar (WHO, 2009) have also recorded a 50% incidence reduction in the last decade. With the right strategies, this decline means that more countries will transition from a malaria control phase to malaria elimination phase. The documented decline in malaria incidence also means that the geographical distribution of malaria is less dense pointing to need for geostatistical approaches to determine transmission hotspots which could be surrogates for unobserved or unknown risk factor for

malaria. Malaria clusters exist at all transmission levels (Bousema *et al.,* 2012). However, in low transmission settings, it is possible that continuous infections are attributable to hotspot clusters that serve as transmission reservoirs (Bousema *et al,* 2012; Rulisa *et al,* 2013) calling for more hotspot foci targeted interventions (WHO, 2013). The settings of the current study (very low malaria transmission), therefore call for use of geospatial approaches to determine malaria incidence and patterns.

In the present study, the results show considerable spatial variation in malaria incidence between the two study sites and age categories. In children under 5, single clusters were observed before (2003-2005) and after (2010-2012) reported interruption of transmission. However, none of these clusters overlapped completely indicating that the malaria cases after reported interruption did not signify an existence of transmission reservoirs (Figure 16). The results in children under 5 further demonstrate that there may be some mitigating factors that preclude re-establishment of malaria from occurring in the areas that might be more environmentally suitable. For individuals who were 5 years and above, several clusters were observed in both Kipsamoite and Kapsisiywa. Unlike the overlap of clusters in Kapsisiywa which are not very distinct, the clusters in Kipsamoite show clear overlaps before (2003-2005) and after (2010-2012) reported interruption of transmission (Figure 19). These findings are consistent with those of Ernst *et al.* (2006) which found a significant cluster around the northwest foci of Kipsamoite. The current study also shows that even though the clusters were slightly variant in location, the general areas of occurrence seemed the same. Spatial analysis studies conducted at the Kenyan coast (Bejon *et al,* 2010) and Sudan (Mirghani, *et al.,* 2010) also recorded clusters over periods of 12 and 11 years respectively just like the current study which recorded clusters over 3

year periods before and after interruption of transmission. Studies within the same settings by Cohen *et al.* (2008) demonstrated that use of spatial techniques for malaria surveillance could help to identify households at higher risk and help focus interventions and Ernst *et al.* (2006) showed that specific ecological risk factors were consistent in malaria risk areas both during epidemic and non-epidemic years hence concluding that sustained interventions in areas with identified ecological risk factors were cost-effective methods of reducing malaria incidence and or preventing epidemics even in situations where there existed no malaria early warning systems. The consistency of the spatial analysis results in this study with such other geospatial techniques point to its validity in low malaria transmission settings.

In their study to assess spatial-temporal clustering of *P. falciparum* infection in Gezira State-Sudan, Mirghani, *et al.* (2010), found that 49% of positive individuals

were located within a spatio-temporal cluster. The current study also found malaria cases to be concentrated within the clusters. For instance, Kipsamoite data for 2010-2012 shows that, in children below 5 years, 0.4% of households accounted for 58% of malaria cases and for individuals 5 years and above, 10% of households accounted for 67% of all malaria cases. The findings are consistent with those of previous studies that showed for spatial-temporal approaches to stratify malaria risk zones and clusters at community levels (Gaudart *et al.,* 2006; Wimberly *et al.,* 2010) can successfully isolate malaria cases even in areas of low transmission.

This study recorded unstable clusters over the years especially in children under 5 before and after reported interruption of malaria transmission both in Kipsamoite and Kapsisiywa.

The instability of the malaria clusters observed in this study over the years may not have been due to variations in age or site only (Figures 15 and 18). In their study of "Stable and Unstable Malaria Hotspots in Longitudinal Cohort Studies in Kenya" Bejon *et al.* (2010) also observed that the position of hotspots of febrile malaria changed annually in their study areas while hotspots for asymptomatic parasitaemia were more stable over time. They further noted that overall; the distribution of homesteads was more strongly predictive of hotspots for the following year. This study did not consider the various factors that could have contributed to the malaria clusters like water bodies and vegetation. However, previous studies done in the study area and other variant settings have shown that malaria transmission intensity varies by geographical features such as altitude, distance to swamp, cultivation practices, type of house, ITN use, socioeconomic factors (Ernst *et al.*, 2006; Baragatti *et al*, 2009; Lindsay & Mertens, 1998). An earlier analysis of data from 2001-2004 in one of the study sites had shown existence of malaria hotspots (Ernst *et al.*, 2006) while a study done on the same subjects by Cohen *et al.* (2008) concluded that elevation through its contribution on transmission through effect on temperature, land-cover, agricultural practices and household proximity to non-moving water bodies were useful parameters to measure malaria risk in the highland areas. The results of the present study strengthen the theory of existing malaria hotspots within Kipsamoite area only despite very low malaria transmission intensity (This study was done in two sites covering 17 villages with uniformly distributed very low malaria transmission intensity). From their observed proximity in time and space between malaria clusters Gaudart *et al.* (2006) also noted that mean transmission changes over time at the level of an entire village form low surveillance resolution areas whereas identifying high-risk

individuals in clusters makes it possible to find a suitable interpretation for spatial and temporal changes in *P. falciparum* infection.

While previous highland malaria studies have tended to concentrate on early warning systems through large scale assessments using spatial-temporal trends and not geo-referenced and localized spatial patterns or clusters (Yeshiwondim *et al*., 2009), the identified hotspots and differential risks in the study can be targeted for specific interventions in resource-constrained countries of sub-Saharan Africa. However, in the current study scenario, it is possible that the drivers of malaria transmission in Kipsamoite (which shows consistent hotspots all through the years) may be different from those of Kapsisiywa which did not have any specific hotspot points (but visually resembled a caterpillar lying on the ground). This can be explained by the distinct topographical features: while Kipsamoite is surrounded by a forest with one particular low-lying area which had malaria clusters, Kapsisiywa lies on a "relatively low area" and is surrounded by swamps. In his review of differences in remotely sensed environmental and geographic features associated with malaria clusters, Larsen (http://syr.academia.edu/DavidLarsen) assessed for malaria clusters using kernel intensity functions in ArcGIS for identifying points where increased or decreased risk were no longer statistically significant and the Sparr extension in R to estimate the log-relative risk function of malaria. Larsen argues that the kernel intensity function is an improvement of spatial scan in categorizing a household within or outside of a given cluster. This study used Kuldorff scan in SaTScan, a solid spatial statistical approach that employs heterogeneous Poisson processes which offer flexible models of determining spatial distribution of point-locations of malaria and non-overlapping characteristics.

CHAPTER SIX

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The present study documents that in a highland area of unstable transmission, where symptomatic parasitaemia is rare, the symptom of fever alone is not adequate for detection of clinical malaria in both children <5 years and in individuals ≥5 years. No single symptom or combination of symptoms had a sensitivity and specificity of 95% and above for clinical malaria hence did not meet the WHO threshold of acceptance. Adding headache to fever as screening symptom increases sensitivity of detection in individuals ≥5 years old but at the cost of decreased specificity. Screening for symptoms in addition to fever did not accurately capture all cases of clinical malaria in individuals ≥5 years old in areas of low malaria transmission either. The study therefore concludes that parasitic confirmation of malaria is the most accurate malaria detection method in an area of very low malaria intensity.

The study findings show differences in spatial autocorrelation between malaria and anemia between the sites and age categories. Before reported interruption of transmission, for individuals < 5 years, anemia (2007) was found to be spatially correlated with previous malaria incidences (2003-2005) in Kapsisiywa but not Kipsamoite. However, in individuals ≥5 years, previous malaria and anemia were found to be spatially correlated. The findings show that even in areas of very low transmission intensity, malaria and

anaemia can be spatially correlated suggesting that control programs for malaria can be used to target anaemia prevalence reduction.

After reported interruption of malaria transmission, no autocorrelation is documented between malaria and anaemia. The study concludes that in cases where very low malaria and anaemia cases have been reported, there does not exist a spatial relationship between malaria and anaemia.

The study shows existence of one malaria hotspot for children under 5 years which could be a random occurrence given the low numbers of children < 5 years within the analysis units. But it reveals existence of malaria hotspots before and after reported interruption of transmission especially in individuals ≥5 years. These hotspots could be indicative of existence of local reservoirs of very low incidence of malaria transmission. The result showed considerable spatial variation in malaria clusters between Kipsamoite and Kapsisiywa which could not be detected using aggregated data analysis methods. The approach of identifying clusters is a useful tool for detecting malaria hotspots especially in areas of low endemicity to support foci targeted interventions. The study findings give a visual and quantitative description of differences in geographical profiles of malaria hotspots in two relatively homogenous sites adding to the need for context-adapted intervention and treatment strategies.

6.2 Recommendations

Early and accurate diagnosis using microscopy or malaria rapid diagnosis test is the most effective way for effective malaria disease management and surveillance. This will also reduce misdiagnosis which can lead to morbidity and mortality.

The study recommends inclusion of anemia reduction interventions in malaria control programs even in areas of very low malaria intensity as malaria intensity correlates with anemia prevalence especially in children and adults above 5 years. It also recommends use of spatial techniques for malaria and anemia interventions.

In light of existence of malaria hotspots before and after reported interruption of transmission, the study recommends use of spatial approaches to identify malaria incidences not only as techniques and tools for isolating and providing foci targeted interventions but also for identifying transmission patterns over time. The study further recommends conducting the same spatial techniques in similar settings (low transmission) to validate the current findings. The study also recommends the use of immunological techniques to confirm if new malaria cases are indeed an indicator of existing malaria reservoirs or new infections.

6.3 Suggestions for future research

Even though the study identifies existing malaria hotspots, it does not ascertain if the new malaria cases at the hotspots are from transmission reservoirs within the sites or if new cases are from outside the study area and only concentrated at certain hotspots. The researcher recommends a study to determine parasite genotypes of the new malaria cases to conclusively determine if new cases of malaria after reported interruption of transmission are from within (suggesting possible transmission reservoirs) or are being imported by any possible mechanisms.

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8. APPENDICES

8.1 Appendix 1: KEMRI Ethical Approval

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **October 27, 2014**

TO: **CHANDY C. JOHN,**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. STEPHEN MUNGA,**
ACTING DIRECTOR, CGHR,
KISUMU

Dear Sir,

RE: SSC PROTOCOL No. 939 (*RESUBMISSION2-REQUEST FOR ANNUAL RENEWAL*): MALARIA TRANSMISSION AND IMMUNITY IN HIGHLAND KENYA

Reference is made to your letter dated 17th October, 2014 of which the ERC Secretariat acknowledges receipt 22nd October 2014.

This is to inform you that the Committee determined that the issues raised at the 226th ERC meeting are adequately addressed. Consequently, the study is granted approval for continuation effective this **October 27, 2014** through to **October 26, 2015**. Please note that authorization to conduct this study will automatically expire on **October 26, 2015**.

If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the ERC secretariat by **14 September, 2015.**

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.

You may continue with the study.

Yours faithfully,

PROF. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

8.2 Appendix 2: UMN/KEMRI Consent for Human Investigational Studies

Study Title: Malaria transmission and immunity in highland Kenya

Consent form: Site-wide blood sample collection

Purpose: This is a malaria immunology study looking at the various methods the body uses to defend itself against malaria. Dr. Chandy John from University of Minnesota (UMN) in the USA and his colleagues at the Kenya Medical Research Institute (KEMRI) are asking your permission for you/your child to participate in this research study. This study is sponsored by the National Institute of Allergy and Infectious Diseases, USA. We believe that the body has ways to protect itself against malaria. The goal of this research study is to understand how the body protects itself against malaria. We can do this by examining the blood from you/your child in the laboratory. We are collecting samples from all consenting people in the entire community (Kipsamoite and Kapsisiywa). Information gained from this study will help researchers design laboratory tests that measure malaria protection. This information may be helpful for future malaria vaccine studies that may be conducted in this area.

Procedure: We request a small blood sample from you/your child. We would like obtain a single sample from you this year. If we test again in future years, we will again request consent from you. We will be obtaining blood by finger prick sampling, and we will obtain approximately 10-20 drops of blood (0.5 -1.0 mL). We will use a sterile lancet to prick your/your child's finger after clearing it with an alcohol swab, and drip the blood into a sterile tube. Taking this amount of blood from you/your child should not cause any harm to you/your child. Blood will be transported to the UMN/KEMRI laboratory in Kisumu. Tests done in the laboratory will tell us how well your/your child's body is protecting itself against malaria (i.e. cytokine & antibody responses). There are no expenses involved in

participating in the study. Your participation can end if you decide to end it or if the study decides to end it. We anticipate the participation of ~5800 people in Kipsamoite and Kapsisiywa in this study. You will be informed promptly if information becomes available that may be relevant to your willingness to participate.

Genetic studies: We also would like to store part of your/your child's blood at the UMN/KEMRI laboratory to do genetic studies. The studies we will do currently include only testing for genes belonging to the malaria parasite. This is important to see what strains of malaria parasite are in the community. Future studies may include genes belonging to you/your child, such as HLA type or other genes that are thought to protect you against malaria. If we get any results from the lab studies that may affect the health of you/your child, we will inform you. We will request permission for any genetic studies not described here from the Institutional Review Board (IRB) at UMN and the KEMRI Scientific Steering Committee (SSC) and Ethical Review Committee (ERC).

If you do not agree to have your/your child's blood stored for genetic testing, we will destroy the sample after we have tested it for the type of malaria parasite. If, at any time you wish to withdraw your agreement, please contact Mr. Joseph A. Okweso and we will destroy the sample. If you do not wish to have your/your child's blood stored for genetic testing, you/your child may still participate in this study. You/Your child will still be examined for defenses against malaria. If you/your child has malaria, we will still treat you/your child.

Long-term storage and future studies: I agree for UMN/KEMRI to store my/my child's blood for futures studies of factors that may protect against malaria. I understand that if any test results are found that are important for my/my child's health, UMN/KEMRI will

try to report this to me, if possible. This testing may be done in UMN labs in Kisian, Kenya or in the labs of UMN in Minneapolis, Minnesota. I understand that I have the right to withdraw my agreement to use my/my child's blood for future research anytime and for any reason. If I withdraw my agreement to use my/my child's blood for future research testing, the samples will be destroyed. I may also ask that my/my child's blood not be used for certain types of testing. To do this, I may tell Mr. Peter Odada Sumba, the UMN/KEMRI field supervisor, of my request and he will tell the study people at UMN/KEMRI. I understand that the UMN IRB and KEMRI SSC and ERC must approve any future testing not described here.

If you agree, circle "YES". If you do not agree, circle "NO" YES NO

Participant/Parent or Guardian Signature*_____ Date _____

Witnessed by_____ Date_____

*A parent can sign, or verbally state his/her consent in the presence of a witness who will then sign.

Risks and Benefits: There are minimal risks to having your/your child's blood drawn. The risks include bleeding, pain, bruising and possible infection. All of these are uncommon events that have occurred in very few adults or children previously studied by this research group. If you are pregnant, there are no additional risks to your fetus. The benefits of having your/your child's blood drawn are free testing for malaria parasites. If malaria parasites are found and you/your child has malaria symptoms, such as fever and headache, you/your child will be offered the drugs currently recommended by the Kenya Ministry of Health for the treatment of uncomplicated malaria. The Clinical Officer at the

health centre will give you the appropriate amount of medication to treat you/your child, and the study will cover payment of any clinic treatment expenses for malaria. A few days after treatment, the field assistant assigned to your area will inquire about your/your child's recovery progress to make sure the drugs are working properly.

During the course of this study, if you or your child develops disease from malaria, you or your child should be seen at the health center, where you will be provided with treatment following the Kenya National Health Guidelines for the treatment of uncomplicated malaria. For adults and children greater than 5 kg, Artemether-Lumefantrine (CoArtem®) is the current treatment. Artemether-Lumefantrine has been shown to be very effective for treating uncomplicated malaria even if the parasites are resistant to other antimalarial drugs. The side effects of this drug may include dizziness and fatigue, loss of appetite, nausea, vomiting, abdominal pain, myalgia, racing heart beat, trouble sleeping headache, rash and aching joints. These side effects are extremely rare. For children weighing less than 5 kg, the first line treatment is Amodiaquine. The side effects of this drug may include nausea, vomiting, skin rash or itching. If CoArtem® has been approved for treatment of children under 5 kg, this option will be provided. Neither of these drugs should be taken by women who are pregnant or nursing. If you are pregnant, you will be given oral quinine, as per Kenya National Guidelines. The side effects of quinine include ringing in the ears, dizziness, nausea and vomiting. These side effects are fairly common but stop after the medication is stopped. Rare side effects from oral quinine include low blood sugar and heart rhythm abnormalities.

Confidentiality: The results of the studies using your/your child's blood will be assigned a study number to preserve confidentiality. A database linking you or your child's personal

identifiers to the study number will be kept by the principal investigator and relevant key personnel. Only study personnel and study monitors, auditors and institutional review boards will be allowed access to the medical information collected in this study.

Summary of your rights as a participant in a research study: Your/your child's participation in this research study is voluntary. Refusing to participate will not alter your/your child's usual health care or involve any penalty or loss of benefits to which you or your child are otherwise entitled. If you decide to enroll yourself or your child in the study, you may withdraw yourself or your child at any time and for any reason. If information generated from this study is published or presented, your and your child's identity will not be revealed. Under some circumstances, the sponsor of the study will pay for injuries resulting directly from being in the study. If you want information about those circumstances or if you think you have suffered a research related injury, let the study physicians know right away. If you or your child experiences physical injury or illness as a result of participating in this research study, contact The Director of the Center for Vector Biology and Control Research (CVBCR) at KEMRI in Kisumu at PO Box 1578 at 057-2022924/22923 or Mr. Joseph A. Okweso at 057-2022989, 0721-257220 or 0733-280116.

Contact Information: _____ has described to you what is going to be done, the risks, hazards, and benefits involved, and can be contacted at _____. Further information with respect to illness or injury resulting from a research procedure as well as a research subjects' rights is available from KEMRI/National Ethical Review Committee (ERC), PO Box 54840, Nairobi at 020-722541 or The Director of KEMRI, PO Box 54840, Nairobi at 020-722541. If you have

any questions about this study, you may also speak to The Director of CVBCR, KEMRI in Kisumu at 057-22924. Dr. John's designate, Mr. Joseph A. Okweso can be contacted at CVBCR, KEMRI, PO Box 1578 in Kisumu at 057-2022989, 0721-257220 or 0733-280116.

Signature: Signing below indicates that you have been informed about the research study in which you voluntarily agree to enroll yourself/your child; that you have asked any questions about the study; and that the information given to you has permitted you to make a fully informed and free decision about your/your child's participation in the study. By signing this consent form, you do not waive any legal rights, and the investigators are not relieved of any liability they may have. You can withdraw yourself/your child from this study at any time. You will be offered a copy of this consent form, and it will be provided to you if you would like one.

Printed Name of Participant

_____

Date_____

Signature of Participant or Child's Signature if this form is used to obtain assent

If Participant is a minor or legally incompetent adult.

_____ Date_____

Parent or Legal Guardian signature

Relationship to Participant _____

_____ Date_____

Signature of Person Obtaining Consent

_____

Printed Name of Person Obtaining Consent

(Must be study investigator or individual who has been designated in the Checklist to obtain consent.)

_____ Date_____

Signature of Principal Investigator (Affirming subject eligibility for the Study and that informed consent has been obtained.)

SOP Author / Institution	SOP Date	SOP Approved By	Approval Date	Version No.
GS Park, GS Noland / UMN	02 July 2009	GS Noland	02 July 2009	3

8.3 Appendix 3: UMN / KEMRI SOP_LAB_010 NOTEBOOK AND DATA ENTRY

TABLE OF CONTENTS

- **OBJECTIVE**

  This SOP describes how hard-copy lab notebooks are to be organized and how experimental soft-copy computer files are to be handled. This protocol also explains how to properly use experiment numbers on samples and files.

- **RESPONSIBLE PERSONNEL**

  All laboratory personnel

- **PROCEDURES**

A. Lab Notebooks

i. Each project scientist should maintain their own lab notebook (even if the information is derived from a team effort).
ii. Each person's notebook should be numbered consecutively beginning with 01.
iii. Before any work is recorded, ALL pages in the notebook should be numbered (both front and backs sides of a page are included in numbering).
iv. All daily lab activities must be recorded in notebook listed along with the date. This includes sample processing, sample analysis, organization of samples within freezers, inventory counting, etc.—essentially everything you do must be recorded.
v. **ALL** experimental details, including methodology (or reference to existing SOP), aberrations from standard methodology, results, and interpretations should be included.
vi. Notebooks are subject to audit by the Lab Director and Project Manager at any time.
vii. Lab notebooks are property of UMN-KEMRI Project, and must remain in the lab when scientists depart from the lab.
viii. Experiment numbers should be used for labeling all solutions, samples, files, etc. in addition to dates.

B. Pre- and Post-Experiment Summary Sheets

i. Before any experiment is initiated, the "Pre-Experiment Summary Sheet" must be filled out and submitted to the Lab Director, Project Manager, and Project PI.
ii. A copy of the approved "Pre-Experiment Summary Sheet" for each experiment must be included in the laboratory notebook.
iii. Likewise, within one day of completion of an experiment, a "Post-Experiment Summary Sheet" must be completed and submitted for review with Lab Director, Project Manager, and Project PI.
iv. Approved hard copy version should be printed and saved in notebook on pages associated with the experiment; soft-copy versions should be saved in scientist's "Experiments" folder on local HD, as well as on server.

C. Computer Files (Excel Workbooks, Bioplex and Softmax files, etc)

Naming of Files:

- Every file generated by the Project should now adhere to the following format in order to more easily trace data back to the source information in lab notebooks: "##AAA_pp_XXXXXXXX_DDMMMYYYY.doc"
    - ## is the notebook number where the data is recorded
    - AAA is the initials of the individual in whose notebook the data is recorded
    - pp is the page number in the notebook where the experiment is initiated (e.g.001).
    - XXXXXXXX is the name of the file (no explicit limit on length)
    - DDMMMYY is the date the experiment was initiated.

    Notes:

    - The pp must be three digits (e.g. "001", not "1")
    - Use two digits for day even during first nine days of the month (e.g. "03", not "3");
    - Do not use letters in dates (e.g. "03", not "3rd")
    - Use three and only three letter abbreviations for month (e.g. "Apr", not "April")
    - Use four digit year (e.g. "2009", not "09")
    - Make sure that all files include the 3-letter (4-letter in MS 2008) file extension name—e.g. ".xls" (Excel), ".doc" (Word), ".ppt" (PowerPoint), ".pdf" (Abode file).
    - Do not use special characters in the name of the file except for underscores ("_")

I. Subsequent modifications to the primary data file should be performed in a new worksheet <u>WITHIN THE ORIGINAL EXCEL WORKBOOK.</u>

II. Any template file should be named "XXXXXXXX_template_date.doc"

III. Subsquent versions of template files should be renamed "XXXXXXX_DDMMMYYYY.doc", where XXXXXXXX is the name of the file

and DDMMMYYYY is the current date. *Note*: the word "template" should be removed from working copy documents.

Excel Workbooks

- Most data will be collected in Excel Workbooks. Each experiment or plate should have its own worksheet in these workbooks, so that we can go back to primary data. If you correct errors, add numbers or alter data in these primary sheets in any way, you MUST make a new worksheet, so that the primary data remains unaltered.
- **The final worksheet should always be the summary "Log" worksheet.** This worksheet should contain the following column headings: "Date", "User", and "Summary". The summary column should list:
    1. What the workbook contains (e.g., "IFN-g testing on PBMC samples from R56" or "Retesting of IFNg samples from R56")
    2. An explanation, if needed, of why the testing was done (e.g. "This was retesting because the first set of samples had standard values that were lower than expected [see file ###AAA_pp_XXXXXXXX_DDMMYYYY]. New standards were used for this testing and tested on trial plates [Worksheet plate1]"),
    3. Dates of testing
    4. Changes/modification to information on the worksheets and/or between versions of worksheets (e.g., "Plate A-J is orig data from first plate. Plate Acorr is a revised data set that includes sample ID numbers, removes blank lines and corrects/removes outlier standards").

D. Experiment Numbers and Notebook Organization

Use of experiment numbers with samples:

1. Each experiment number is derived from the notebook number, initials of the individual, and the page number on which the experiment information is initiated. (e.g. 01GSN_001)
2. Experiment numbers should be written on all samples that are generated in that experiment.

3. In cases where a "team" effort produces samples, the senior researcher (or alternatively a designated researcher) should label samples with his/her experiment number. Thus, that person will also have the "official" record of the samples. Other members of the team must note who has the "official" record.
4. All sample identifying labels must be recorded within the experiment of the notebook. Thus, any sample can be tracked back to a particular notebook.
5. All relevant information should be recorded with the sample identifier within the experiment. (e.g. DNA in PBS at a concentration of X mg/ml from this StudyID, stored at this temperature at this location on this date)
6. The experiment number must be written on the page where samples labels are recorded.

Use of experiment numbers with computer folders/files:

- Each research scientist must have a folder labeled "Experiments"
- Each research scientist must keep folders within the "Experiments" folder labeled as per each individual experiment number.
- Each research scientist must keep all relevant files of an experiment within the individual experiment number folder.
- "Hard copy" printouts of all raw data must be attached to pages within an experiment.

Notebook Organizaton

7. Experiments that are broken up among many different pages, must have the experiment number labeled at the top of each page used.
8. It is preferred if the page 1 is reserved for a table of contents.
9. The Table of Contents should contain a list of experiment numbers, the major goal of each experiment, and the page numbers used in each experiment (very important).

E. Data Handling:

File Handling/Storage:

1. All files generated from an individual's desktop must also be saved on the server.
2. If major modifications need to be made to the file, the file can be downloaded to a local computer. Once changes have been made, and the file renamed according to the procedure above, the updated file should be saved back to the server
3. All data files must include a worksheet (placed in front of the other worksheets) titled "Log", which list all details relevant to the data set—what the source of the data was, what calculations were made, what changes were made from previous versions, etc—anything that would be useful to someone looking at the data for the first time and for those who return to the data months later and completely forgot what they did.

SOP Author / Institution	SOP Date	SOP Approved By	Approval Date	Version No.
Jackson Abuya / DVBD/UMN-KEMRI	16 May 2008			2

8.4 Appendix 4: Microscopy testing for *Plasmodium* species

TABLE OF CONTENTS

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1. **Goal:** To provide guidelines for the proper receiving, reading, identification and quantification of malaria parasites in Giemsa stained slides**.**

2. **Principles**

a. In a thick malaria blood film (MBF) the red blood cells (RBCs) are lysed and dehaemoglobinized while the malaria parasites are left intact and concentrated. This eases detection and identification of malaria parasites.

b. The thin (MBF), when fixed with absolute methanol, enables the RBC's to retain their original morphology with malaria parasites, visible inside the cells.

3. **Equipment/Supplies**
    a. Binocular microscope
    b. Tally counter
    c. Initial reading forms for both sites
    d. Pen
    e. Lens paper
    f. Slide boxes

4. **Reagents**
    a. Immersion oil
    b. Lens cleaning solution.

5. **Responsible Personnel**
    a. Laboratory Technologists/Microscopists
    b. Study Supervisor
    c. Study Data Manager

6. **Abbreviations**
    a. SOP-Standard Operating Procedure
    b. RBC- Red blood cells
    c. MBF-Malaria Blood Film
    d. QA-Quantity Assurance
    e. PF- Plasmodium Falcipurum

f. WBC-White blood cells

7. **Procedure**

a. The thick and thin blood smears are performed and Giemsa stained by the technologist at each health centre Kapsisiywa and Kipsamoite (see Field Clinic Microscopy SOP).

b. The technologist at each Health Centre do the 1st reading and place them into slide boxes for transport.

c. The study supervisor will collect the slide boxes weekly from each health center, verify the presence of all slides, and transport them to Kisumu, where he will deliver them to the Laboratory personnel in the KEMRI/UMN laboratory, Kisumu. Receipt of forms and slides will be recorded on a weekly log kept by the study supervisor, and signed by the health center microscopists, the Kisumu microscopists and the study supervisor weekly.

d. The data manager and data clerks will scan all forms and generate pre-printed microscopy forms for the study microscopists. These forms will be delivered to the microscopists the day they are completed, and no later than one week after they are delivered to the data office.

e. A second slide reading will be done by microscopists at the main laboratory in Kisumu (blinded to the first reading). Microscopy will be performed according to the principles specified in the DVBD microscopy training and the Walter Reed course on microscopy. The microscopy form will be filled out according to the Microscopy Form SOP.

f. The second readings will be entered via Teleforms software, and a new table generated which compares the two readings and generates a list of slides with discordant readings. Any slides in which one reader disagrees with the other on the presence of any Plasmodium species or gametocytes are considered to have discordant readings.

g. This list will be similar to the regular microscopy forms in that all areas except the person's study ID and date of slide will be left blank. The lists will be generated

according to reader, so that those slides read by reader 1 will now be read by reader 2 and vice versa.

h. The final reading is that of the third reader. This reading is provided to the data office, and entered into the DB via Teleforms as the final reading. The species, gametocyte count and parasite count on this reading are the final readings of these values.

i. The data manager will generate a sensitivity and specificity reading for each of the health center microscopists for each of the parasite species monthly. Sensitivity = number of positives read by HC microscopist/number of final positives x 100. Specificity = number of negatives read by HC microscopist/number of final negatives x 100. This data will be generated in a FMP table from the data entered monthly, and will be reviewed monthly by the study supervisor. Sensitivity or specificity falling <90% for any microscopist requires re-training.

8. **References**

a. DVBD Microscopy Manual/SOP.

b. Walter Reed Microscopy Training Course.

8.5 Appendix 5: Sensitivity and Specificity computations

Outcome clinical malaria, U5s				
Rule	n tested	% tested	sens (%)	spec (%)
Fever	538.0	88.5	92.0	11.7
Any of F,C,H,M,A	600.0	94.6	100.0	5.6
Any of C,H,M,A	477.0	75.2	96.0	25.6
Any of C,H,M	289.0	45.6	80.0	55.8
F or H	573.0		100.0	
Outcome clinical malaria, O5s				
Rule	n tested	% tested	sens (%)	spec (%)
Fever	1290.0	46.3	64.7	54.5
Any of C,F,M,H,V,~D	2731.0	98.0	100.0	2.1
Any of C,F,M,~D	2653.0	95.2	98.3	4.9
F,~D	2611.0	93.7	98.3	6.5
~D	2457.0	88.2	96.6	12.2
≥ 2 of C,F,M,~D	1673.0	60.1	82.8	40.9
F or H	2480.0		96.6	
Outcome Pf positive, U5s				
Rule	n tested	% tested	sens (%)	spec (%)
Fever	538.0	84.9	88.9	15.4
Any of F,C,H,M,A	600.0	94.6	97.2	5.5
Any of F,C,H,M	584.0	92.1	94.4	8.0
Any of F,C,H	579.0	91.3	94.4	8.9
Either of F,C	550.0	86.8	88.9	13.4
F or H	573.0	90.4	94.4	
Outcome Pf positive, O5s				
Rule	n tested	% tested	sens (%)	spec (%)
Fever only	1290.0	46.3	55.2	54.4
Any of F,C,~D,V,H	2723.0	97.7	100.0	2.4
Any of F,C,~D	2638.0	94.7	98.5	5.6
Either of F,~D	2611.0	93.7	98.5	6.6
~D	2457.0	88.2	96.9	12.5
Either of F,C	1608.0	57.7	73.7	43.5
F or H	2480.0	89.0	96.8	
Of the 631 U5s who have credible temp data:				

Of the 2768 O5s who have credible temp data:				
New outcome				
Clin Malaria				
F or H	573.0	90.4	100.0	
Rule	n tested	% tested	sens (%)	spec (%)
Fever	1284.0	46.4	64.8	54.2
Any of C,F,M,V,~D,H	2714.0	98.0	100.0	2.0
Any of C,F,M,V,~D	2637.0	95.3	97.7	4.8
~D	2441.0	88.2	95.5	12.1
Any of C,F,M,V	1822.0	65.8	84.1	34.8
F or H	2480.0	89.0	96.6	
For Pf4000 alone				
F or H	2480.0	89.0	97.1	

Abbreviations: sens-sensitivity, spec-specificity, O5s-over 5 years, U5s-under 5 years,

8.6 Appendix 6: List of 35 malaria eliminating countries

Asia Pacific	Latin America and Caribbean	North Africa, Europe, Middle East, Central Asia	Sub-Saharan Africa
1. Bhutan	1. Argentina	1. Algeria	1. Botswana
2. China	2. Belize	2. Azerbaijan	2. Cape Verde
3. Democratic People's Republic of Korea	3. Costa Rica	3. Iran	3. Namibia
3. Malaysia	4. Dominican Republic	4. Kyrgyzstan	4. São Tomé and Príncipe
4. Philippines	5. El Salvador	5. Saudi Arabia (*coming soon*)	5. South Africa
5. Republic of Korea	6. Guatemala	6. Tajikistan	6. Swaziland
6. Solomon Islands	7. Mexico	7. Turkey	
7. Sri Lanka	8. Nicaragua	8. Uzbekistan	
8. Thailand	9. Panama		
9. Vanuatu	10. Paraguay		
10. Vietnam			

Malaria Elimination Group: http://www.malariaeliminationgroup.org/resources/elimination-countries