A comprehensive look at malaria in an epidemic-prone area of highland Kenya using a traditional, spatial, and immuno-epidemiologic approach

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Contents

Acknowledgements i
Dedication iii
List of Tables viii
List of Figures x

1 Malaria Overview 1
  1.1 Epidemiology .................................................. 1
  1.2 Life Cycle ....................................................... 5
  1.3 Symptoms, Diagnosis, Treatment, and Prevention ............... 7
  1.4 Global Milestones ................................................. 9
  1.5 Control and Elimination ....................................... 11
  1.6 Malaria in Kenya ................................................ 14

2 Study Description 19
  2.1 Kenya Medical Research Institute-University of Minnesota Malaria Research Project .......................... 19
  2.2 Study Area Location and Population ............................. 20
2.3 Methods

2.3.1 Demography Surveillance

2.3.2 Malaria Surveillance

2.3.3 Climate and Entomology Surveillance

2.3.4 Human Subjects Protection

2.4 Period of Interruption

2.5 Dissertation Focus

3 Evaluating the implementation of the Kenya Ministry of Health National Malaria Strategy in highland Kenya with a focus on the impact of a mass bed net distribution campaign

3.1 Objectives

3.2 Background

3.3 Methods

3.3.1 Surveillance

3.3.2 ITN campaign logistics survey

3.3.3 Statistical Analysis

3.3.4 Barriers to ITN use post-hoc survey

3.4 Results

3.4.1 Indoor residual spraying

3.4.2 Insecticide-treated bed nets

3.4.3 Malaria Incidence

3.4.4 ITN distribution campaign logistics

3.4.5 Impact of mass ITN campaign

3.4.6 Impact of individual ITN use

3.4.7 Bed net ownership and barriers to use
### Spatial analysis of malaria incidence before and after a 13-month period of interruption of clinical malaria

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Objectives</td>
<td>51</td>
</tr>
<tr>
<td>4.2 Background</td>
<td>51</td>
</tr>
<tr>
<td>4.3 Methods</td>
<td>53</td>
</tr>
<tr>
<td>4.4 Results</td>
<td>58</td>
</tr>
<tr>
<td>4.4.1 Descriptive spatial analysis</td>
<td>58</td>
</tr>
<tr>
<td>4.4.2 Kernel density estimation - household level</td>
<td>60</td>
</tr>
<tr>
<td>4.4.3 Kernel density estimation - individual level</td>
<td>70</td>
</tr>
<tr>
<td>4.5 Discussion</td>
<td>82</td>
</tr>
</tbody>
</table>

### Correlates of protection from clinical *Plasmodium falciparum* malaria in an area of low and unstable transmission

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Objectives</td>
<td>86</td>
</tr>
<tr>
<td>5.2 Background</td>
<td>86</td>
</tr>
<tr>
<td>5.3 Methods</td>
<td>87</td>
</tr>
<tr>
<td>5.3.1 Study Site, Participants, Data and Sample Collection</td>
<td>87</td>
</tr>
<tr>
<td>5.3.2 Antibody Testing</td>
<td>89</td>
</tr>
<tr>
<td>5.3.3 Statistical Analysis</td>
<td>90</td>
</tr>
<tr>
<td>5.4 Results</td>
<td>91</td>
</tr>
<tr>
<td>5.4.1 Incidence of source population</td>
<td>91</td>
</tr>
<tr>
<td>5.4.2 Study population characteristics</td>
<td>92</td>
</tr>
<tr>
<td>5.4.3 Analysis of antibody responses</td>
<td>94</td>
</tr>
<tr>
<td>5.4.4 Combinations of antibody responses</td>
<td>95</td>
</tr>
<tr>
<td>5.5 Discussion</td>
<td>98</td>
</tr>
</tbody>
</table>
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Descriptions of endemicity classifications</td>
<td>4</td>
</tr>
<tr>
<td>3.1</td>
<td>Percentages of study participants reporting sleeping under a bed net during 2011 and 2012 demography surveillance, pre- and post-mass bed net distribution campaign, respectively, by village.</td>
<td>36</td>
</tr>
<tr>
<td>3.2</td>
<td>Reasons provided for lack of bed net ownership among surveyed heads of households who were aware of the mass distribution campaign but did not own a bed net.</td>
<td>39</td>
</tr>
<tr>
<td>3.3</td>
<td>Impact of mass ITN campaign on malaria incidence in the study area.</td>
<td>42</td>
</tr>
<tr>
<td>3.4</td>
<td>Association between individual ITN use and malaria incidence in the study area</td>
<td>43</td>
</tr>
<tr>
<td>3.5</td>
<td>Number of bed nets owned per household.</td>
<td>45</td>
</tr>
<tr>
<td>3.6</td>
<td>Reasons provided for not sleeping under bed net.</td>
<td>45</td>
</tr>
<tr>
<td>4.1</td>
<td>Overall cases of clinical malaria from the number of unique individuals residing in the number of unique households, by time period.</td>
<td>57</td>
</tr>
<tr>
<td>4.2</td>
<td>Estimated risk and relative risk ranges for the household-level and individual-level analysis, by time period.</td>
<td>79</td>
</tr>
<tr>
<td>5.1</td>
<td>Evaluation of balance of age between cases and controls after matching, by age category.</td>
<td>94</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>5.2</td>
<td>Associations between dichotomized antibody responses to <em>Plasmodium fal-ciparum</em> antigens and developing clinical malaria.</td>
<td>96</td>
</tr>
<tr>
<td>5.3</td>
<td>Associations between continuous antibody levels to <em>Plasmodium falciparum</em> antigens and developing clinical malaria.</td>
<td>97</td>
</tr>
<tr>
<td>5.4</td>
<td>Area under the receiver operating characteristic curve (AUROC) of predicted probabilities of developing clinical malaria for single antibody responses to 11 <em>Plasmodium falciparum</em> antigens and combination antibody responses to select antigens CSP, GLURP-R0, GLURP-R2, and LSA-1.</td>
<td>99</td>
</tr>
<tr>
<td>5.5</td>
<td>Characteristics of studies of correlates of protection from <em>Plasmodium falciparum</em> malaria, by endemicity (high transmission-low transmission).</td>
<td>105</td>
</tr>
</tbody>
</table>
# List of Figures

1.1 Global distribution of *Plasmodium falciparum* and *Plasmodium vivax* ........................................... 2  
1.2 Epidemiologic triad for malaria ................................................................. 5  
1.3 Life cycle of *Plasmodium falciparum* malaria ........................................ 6  
1.4 Malaria program transition phases ............................................................... 12  
1.5 World map categorizing countries as eliminated, eliminating, or controlling malaria ........................................... 14  
1.6 Malaria endemicity map of Kenya, 2009 ......................................................... 17  
2.1 Location of the study area, Kipsamoite and Kapsisiywa, Nandi County, Kenya .................. 21  
2.2 Detailed map of the study area, Kipsamoite and Kapsisiywa, Nandi County, Kenya .................................................................................................................................................. 21  
2.3 KEMRI-UMN Malaria Research Project schedule of activities, 2003–2013 .... 23  
3.1 Percentages of households reporting indoor residual spraying, by year, 2003–2013 ................................................................. 35  
3.2 Percentages of study area participants reporting sleeping under a bed net, by year, 2003–2013 ................................................................. 35  
3.3 Malaria incidence per 1,000 persons and Ministry of Health indoor residual spraying and insecticide treated bed net campaigns in Kipsamoite and Kapsisywa, April 2003–December 2013 ........................................................................................................... 37
4.1 Global distribution of malaria from 1900 to 2002

4.2 Kernel estimation of a point pattern

4.3 Household locations of the last 10 cases before and first 10 cases after a period of possible interruption of malaria transmission in Kipsamoite and Kapsisiywa.

4.4 Estimated probabilities of clinical malaria among households before and after a period of interruption of clinical malaria.

4.5 Estimated probabilities of clinical malaria among households during peak periods of malaria transmission in 2004 and 2005.

4.6 Estimated probabilities of clinical malaria among households during peak periods of malaria transmission in 2007 and 2009.

4.7 Estimated probabilities of clinical malaria among households during peak periods of malaria transmission in 2011 and 2013.

4.8 Estimated log relative risks comparing after vs. before a period of interruption of clinical malaria, and the 2005 vs. 2004 peak periods.


4.11 Estimated probabilities of clinical malaria among individuals before and after a period of interruption of clinical malaria.


4.15 Estimated log relative risks comparing cases after vs. before a period of interruption of clinical malaria, and the 2005 vs. 2004 peak periods.

4.16 Estimated log relative risks of clinical malaria comparing cases during peak periods of malaria transmission (2007 vs. 2005, 2009 vs. 2007).


4.18 Histograms of the estimated relative risks of clinical malaria (before and after the period of interruption, peaks 2005 vs. 2004, and peaks 2007 vs. 2005), by household or individual-level analysis.

4.19 Histograms of the estimated relative risks of clinical malaria (peaks 2009 vs. 2007, peaks 2011 vs. 2009, and peaks 2013 vs. 2011), by household or individual-level analysis.

5.1 Clinical malaria incidence per 1,000 persons, Kipsamoite and Kapsisiywa, June 2007–June 2013.

5.2 Clinical malaria incidence per 1,000 persons, Kipsamoite and Kapsisiywa, June 2007–June 2013, by age group.

5.3 Four diagnostic plots to describe discrimination for the fitted model of the combination of CSP, GLURP-R0, GLURP-R2, and LSA-1 as continuous measures.
Chapter 1

Malaria Overview

1.1 Epidemiology

Recent World Health Organization (WHO) estimates indicate that 3.2 billion people world-
wide inhabit areas where malaria is transmitted and hence are at risk. Populations in sub-Saharan Africa are most at risk for acquiring the disease with 80% of the estimated 198 million annual cases and 90% of the resulting 584,000 deaths occurring in this region, primarily among children under age 5.

Malaria is an infectious disease caused by protozoan parasite species of the *Plasmodium* genus that affects vertebrates including birds, reptiles, and mammals. Over 150 types of malaria parasite species exist and each is highly host-specific. Four species are transmitted among humans including *falciparum, malariae, ovale* and *vivax*. A fifth emerging human parasite species, *knowlesi*, primarily known to infect macaque monkeys, has recently infected and caused fatalities in humans in Southeast Asia. *P. falciparum* is the most severe and can be fatal; it is the only type that can attack the brain. *P. vivax* is the most geographically widespread and it, along with *P. ovale*, can lie dormant in the liver causing a relapse of infection weeks or even years after initial infection. *P. malariae*
is the slowest to develop, and while generally not severe, if left untreated, can persist in humans for decades. Figure 1.1 illustrates the global geographic distribution of the two most abundant and significant types, *P. falciparum* and *P. vivax*. Over 90% of malaria in sub-Saharan Africa is caused by *P. falciparum*, responsible for the majority of morbidity and mortality due to malaria worldwide. [50]

![Figure 1.1: Global distribution of *Plasmodium falciparum* and *Plasmodium vivax*. Source: Reprinted from The Lancet, Vol. 376, Feachem, R.G., Philips, A.A., Hwang, et. al., Shrink- ing the malaria map: progress and prospects., pp. 1566–78, Copyright © 2009, with permission from Elsevier.](image)

Over 3,500 species of mosquitoes exist; about 430 are *Anopheles*, the genus responsible for transmitting malaria between humans. [18] They have a near world-wide presence. The behaviors of *Anopheles* are generally species-specific; some feed on humans only. They also have preferences for whether they feed, rest, or lay eggs indoors or outdoors during daytime or night. Over 70 species of *Anopheles* are capable of transmitting malaria to humans and roughly 40 do it well. [103][140] *A. gambiae* is the most predominant and efficient vector in sub-Saharan Africa. It feeds on humans, is nocturnal, and prefers feeding and resting indoors. [140]
Only female *Anopheles* mosquitoes transmit malaria between humans. Male mosquitoes have a life span of about one week and do not feed on vertebrates but rather survive on the juices of plants. Females also feed on plants primarily as a source of energy, yet require a blood meal for egg development. They typically mate once, prior to their first blood meal, and store sperm for future egg batches, each of which requires a new blood meal for maturation. Blood meals generally occur every 2–3 days until death. Once infected with the malaria parasite, they can transmit malaria for the duration of their life span, generally 1\(\frac{1}{2}\)–4 weeks, largely depending upon the climate and existing mosquito control measures. The malaria parasite is harmless to the mosquito.

After a bite from an infected mosquito, the human immune system is highly stimulated to attack the parasite at multiple stages throughout the body. However, through time, malaria parasites have evolved means of evading the immune responses. About 20% of infectious bites lead to human infection. After years of repeated exposure, protective immunity to malaria is built up. In *stable* transmission settings, where year-round infection occurs and the entomological inoculation rate (EIR) (i.e., number of infectious bites per given time period, often year) is high, the majority of clinical symptomatic cases are in children under age 5 since older children and adults have acquired immunities that are maintained with continuous inoculations. In areas of *unstable* transmission with low EIR, symptomatic disease occurs at all ages since immunities are not built or sustained with limited or sporadic exposure.

Transmission patterns are often used to describe and compare settings, which may include a classification of endemicity. Spleen rates and parasite rates in children or EIR serve as markers of endemicity class ranging from holo-, hyper-, meso-, to hypo-endemic in order of decreasing transmission intensity. Stable transmission patterns reflect hyper- and holo-endemic areas, whereas unstable transmission settings include meso- and hypo-endemic areas which are prone to epidemics. Table 1.1 describes the endemicity classifications
by spleen rate, parasite rate, and EIR.  

Table 1.1: Descriptions of endemicity classifications.

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
<th>Spleen rate/Parasite rate(^a)</th>
<th>EIR(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holoendemic</td>
<td>Intense perennial transmission</td>
<td>≥ 75%</td>
<td>&gt; 140</td>
</tr>
<tr>
<td>Hyperendemic</td>
<td>Intense seasonal transmission</td>
<td>≥ 50%</td>
<td>11–140</td>
</tr>
<tr>
<td>Mesoendemic</td>
<td>Regular low-level transmission</td>
<td>11–&lt; 50%</td>
<td>0.25–10</td>
</tr>
<tr>
<td>Hypoendemic</td>
<td>Limited transmission</td>
<td>&lt; 10%</td>
<td>&lt; 0.25</td>
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\(^a\) Spleen and parasite rates refer to children ages 2–9, except the parasite rate in holoendemic areas reflect infants.

\(^b\) EIR = entomological inoculation rate.

Climate plays a large role in transmission patterns as ambient temperature, humidity, altitude, and rainfall can affect the survival of *Anopheles*. They are abundant in tropical settings but when the temperature exceeds 35°C or humidity drops below 50%, their life span is greatly reduced. *Anopheles* are typically not found at altitudes above 2,500 meters, likely due to the correlation between temperature and altitude (for every increase in elevation of 100 meters there is a drop in temperature of about 0.5°C). Rainfall is important in the proliferation of *Anopheles* as they lay their eggs on surface water, so generally more rains yield more potential breeding sites. Most *Anopheles* are found within 2–3 km of their breeding sites.

When a mosquito takes a blood meal from an infected human, the parasite it ingests is not in its infective stage. Further transmission is dependent upon the mosquito living long enough for the parasite to develop into this stage and taking another blood meal. This process, which takes place within the mosquito, depends on the ambient temperature, with optimal conditions ranging between 25–30°C. For *P. falciparum*, at 28°C the process takes 9–10 days, but increases to over 3 weeks at 20°C. Species preferences and climate are important factors to consider while devising control strategies for particular
settings.

Figure 1.2 depicts the Epidemiologic Triad for malaria commonly used to describe infectious diseases which require a disease agent (malaria parasite), a susceptible host (human), and an environment (temperature, altitude, and breeding sites) that brings the agent, host, and — in vector-borne diseases — vector together. The female *Anopheles* mosquito is the vector of transmission for human malaria.

![Epidemiologic Triad for Malaria](image)

**Figure 1.2: Epidemiologic triad for malaria.**

### 1.2 Life Cycle

Figure 1.3 illustrates the complex life cycle of *Plasmodium falciparum* in the mosquito vector and human host. When an infected mosquito takes a blood meal, malaria parasites in the form of sporozoites enter into the human blood stream from the mosquito’s saliva and travel to the liver. Sporozoites can be found in the blood stream for up to an hour; some are ingested by the body’s protective white blood cells, while others may reach the liver in as little as two minutes. Once a sporozoite reaches the liver, its surface
proteins bind with those on the surface of hepatocytes and this interaction results in an invasion of the liver cell. Once inside, the sporozoite, which loses its exoskeleton during invasion, undergoes repeated nuclear division developing into a hepatic schizont containing tens of thousands of merozoites. This replication process takes about 5.5–7 days until the schizont swells and eventually ruptures the host liver cell, releasing these merozoites back into the blood stream where they in turn invade healthy red blood cells. Once inside, each merozoite transforms into the ring-shaped trophozoite that ingests hemoglobin and similarly undergoes division multiplying into 8–24 merozoites contained in an erythrocytic schizont. The schizont ultimately swells and ruptures the infected red blood cell releasing more merozoites into the blood stream capable of infecting and ultimately destroying additional red blood cells. This cycle in the red blood cell takes approximately
After several cycles, some of the infected red blood cells develop into sexual gametocytes, which can be ingested by another mosquito during a blood meal. Once ingested, the male and female gametocytes fertilize to form zygotes that develop into ookinetes which penetrate the mosquito gut and form oocysts. Inside each oocyst, over 10,000 sporozoites develop until it bursts, releasing the infective sporozoites to migrate from the mosquito gut to the salivary glands. The life cycle of the malaria parasite continues during the mosquito’s next blood meal.

The life cycle of *P. falciparum* is described here as this species is responsible for most morbidity and mortality due to malaria worldwide and is the predominant species in sub-Saharan Africa including Kenya, the study location for this dissertation. However, there are slight differences in the life cycles of the differing species of *Plasmodium* primarily with respect to the development time and numbers of parasites in each stage. For example, gametocytes develop earlier for *P. vivax*, often prior to the host exhibiting symptoms. Additionally, *P. vivax* and *P. ovale* may develop a hypnozoite form, capable of lying dormant in the liver for weeks or even years, which is responsible for relapses in infection specific to these species.

### 1.3 Symptoms, Diagnosis, Treatment, and Prevention

Malaria shares flu-like symptoms including abdominal discomfort, headache, fatigue, joint pain, nausea, and vomiting with other infectious diseases like pneumonia and typhoid fever. Its primary distinguishing characteristic is intermittent fever and chills, which is a result of the human’s response to the simultaneous destruction of multiple red blood cells and release of toxic heme into the blood stream. For *P. falciparum* malaria, symptoms appear 9–14 days after an infectious mosquito bite. Malaria disease
is distinguishable from malaria infection, which is asymptomatic and often detected during routine surveillance activities. However, it is important to note that these asymptomatic hosts are still reservoirs for transmission. \[169\]

The gold standard for diagnosing malaria is by microscopic evaluation of thick and thin blood smears. Thick smears are generally used for detection of parasitemia and to quantify parasite density, while thin smears are used to identify parasite species. \[68\] Thin smears have also been shown to be more sensitive than thick smears at detecting parasitemia at low densities. \[115\] Alternative methods to diagnosing malaria include rapid diagnostic tests (RDT) which detect parasite-specific antigens or enzymes. \[169\] RDT cannot quantify parasitemia but are advantageous in settings where skilled technicians or an energy source are lacking. Currently no diagnostic tool exists to directly detect latent liver-stage parasites. \[154\] In the absence of parasitological evidence, presumptive diagnosis based on clinical features, primarily fever, is currently recommended. \[169\] However, attempts should first be made to rule out other infectious diseases with shared symptoms.

Fortunately, when treated early, malaria is effectively cured by means of antimalarial medications. During the past decade, new artemisinin-based combination therapies (ACT), proven highly effective against \textit{P. falciparum}, have been implemented worldwide, replacing many drugs to which the parasites developed resistance. \[12, 171\] Combination therapies are more effective than mono-therapies and if a parasite is resistant to one of the drugs, often the other will kill it. \[169\] Unfortunately, since presenting flu-like symptoms can be attributable to many other diseases, medical attention may be sought for patients only after complications develop. Most malaria-related morbidity and mortality are due to complicated forms of \textit{P. falciparum} infections that are left untreated for 24 hours or more after the first signs of symptoms. \[171\] Cerebral malaria (CM) manifests as convulsions and unarousable coma with no other plausible clinical diagnosis (e.g., meningitis). \[72\] Patients with severe malarial anemia (SMA) have hemoglobin levels $<5$ g/dL and require blood
transfusions. Due to the potential for rapid progression to severe disease, it is important 
to promptly diagnose and treat \textit{P. falciparum} malaria. \cite{171}

Malaria is not only treatable but it is also preventable. Prevention is primarily through 
vector (i.e., mosquito) control measures including the use of insecticide treated bed nets 
(ITN) and indoor residual spraying (IRS). Bed nets act as physical barriers to the mosquito 
and offer protection from malaria to the person(s) under them. \cite{167} When they are treated 
with insecticides, mosquitoes that come in contact with the nets often die, which reduces 
the mosquito population and thus can prove beneficial in settings with high coverage even 
to those in the community who are not sleeping under nets. \cite{11,60,167} IRS involves spray-
ing insecticides on the inside walls of structures to target resting indoor mosquitoes after 
a blood meal in an effort to kill them before they can transmit malaria to others. \cite{159} 
Other prevention measures include wearing protective clothing, applying repellents, or 
through environmental management of breeding sites (e.g., larvicidal application, filling, 
drainage). \cite{5} For pregnant women and infants, WHO recommends at least two doses of in-
termittent preventive treatment (IPT), where asymptomatic women and infants regardless 
of their malaria infection status are given antimalarial medications. \cite{169} Treatment with 
ACT combined with prevention measures have proven successful as evidenced by decreasing 
incidence and transmission in several areas of sub-Saharan Africa. \cite{25,74,100,119}

\subsection*{1.4 Global Milestones}

Malaria parasites, humans, and mosquitoes have been living and evolving together for 
centuries. Reports of fever epidemics and enlarged spleens, characteristic of malaria, date 
back to 2700 B.C. in the Chinese Nei Ching (\textit{The Canon of Medicine}). \cite{21} By 400 B.C., 
Hippocrates, the “Father of Medicine”, distinguished intermittent malarial fevers from 
continuous fevers of other infectious diseases. \cite{21} He also attributed its appearance to
settings close to stagnant marshy waters during certain times of the year. Miasma theory dominated the belief systems with respect to disease transmission into the 18th century, and malaria was no exception—“mala-aria” translates from Italian to English as “bad air”.

Following the paradigm shift from miasma to germ theory as a means for transmitting infectious diseases, scientists accepted the plausibility that an alternative besides noxious air could be the source of malaria infection. In 1880, French army surgeon Alphonse Laveran was the first to discover protozoan parasites in the blood of a malaria patient. In 1897, English physician Ronald Ross effectively discovered the role of mosquitoes in transmission when he dissected an *Anopheles* and found parasites in its gut after feeding on a malaria patient. Pharmacologists at Bayer in Germany discovered chloroquine as an effective antimalarial drug in 1934. Five years later, the insecticidal properties of dichlorodiphenyl-trichloroethane (DDT) were discovered by Swiss chemist Paul Müller. Laveran, Ross, and Müller were all awarded Nobel Prizes for their discoveries.

In 1942, the United States established the Office of Malaria Control in War Areas (OMCWA) to control vector-borne diseases around military training bases and prevent reintroduction of disease to surrounding areas from returning soldiers during World War II. The Office was located in Atlanta, as opposed to Washington, D.C., because the southern States had the most malaria transmission. After the war, the Communicable Disease Center – now known as the Centers for Disease Control and Prevention (CDC) – was established in 1946 to essentially replace OMCWA but continue its efforts. Over 50% of CDC’s initial work focused on control and elimination of malaria in the U.S., which was realized in 1951.

With an effective drug, insecticide, and proven success in regional elimination efforts as in the U.S., the international community felt it had the tools necessary to eradicate malaria worldwide. In 1955 WHO introduced plans for the Global Malaria Eradication Program (GMEP) at the World Health Assembly. GMEP was successful at eliminating malaria
from many areas of Europe, Asia, North America, and Latin America, but less successful in tropical countries of Asia and South America. Very little was attempted in Africa due to logistical complexities of dealing with weak infrastructures. While some pilot projects were conducted, including in Kenya, national strategies to eliminate malaria were lacking. During the GMEP campaign, parasite resistance to antimalarials and mosquito resistance to insecticides emerged, and the feasibility of global eradication weakened as resurgence of disease occurred in areas where malaria had been in decline. In 1969, with waning financial support from funders, GMEP formally ended with a call from WHO for improved surveillance and further research into alternative drugs, insecticides, and malaria control strategies.

1.5 Control and Elimination

Over the past few decades, advancements in tools and technology have included ACT, RDT, ITN, and ongoing vaccine development. With new innovations and control strategies comes renewed interest. In 1998, WHO announced the Roll Back Malaria Partnership as a systematic approach to coordinating malaria control efforts. Their vision is “a world free from the burden of malaria.” World leaders recognized the importance of combating malaria by the commitments set forth during the establishment of what has become known as the United Nations Millennium Development Goals (MDG) in 2000. Specifically, MDG 6 targets to halt and begin to reduce the incidence of malaria by 2015. In 2007 Bill and Melinda Gates named malaria eradication the goal of their Foundation, and in 2008 WHO backed their philanthropic vision. Since then, elimination and the potential for worldwide eradication have again been widely discussed among the malaria community.

WHO provides a guideline for nations considering malaria elimination and categorizes
malaria programs into four transition phases (Figure 1.4). The goal of malaria control programs is to reduce transmission to where it is no longer a public health problem (i.e., reduce morbidity and mortality). Treatment and prevention primarily involve case detection by passive surveillance and vector control strategies. During this phase, countries focus on improving their surveillance systems and treatment and prevention coverage. Interventions like IRS and ITN distribution are targeted to broad areas nationally. When the diagnostic test positivity rate for fevers drops below 5%, programs may start to realistically think about committing to elimination. During this pre-elimination phase, committed countries must reorient their malaria control programs to focus on the interruption of transmission. Interventions are generally intensified and focal with the aid of geographic spatial data for both cases and vectors. Active surveillance joins the existing passive efforts already in place. If successful, with adequate political and economic support, programs will transition into the elimination phase. WHO places this milestone when about < 1 case per 1,000 persons are at risk per year.

![Figure 1.4: Malaria program transition phases.](image)

To meet the goal of interrupting local transmission nationwide, the reproductive number under control \( R_c \), defined as the number of new cases produced from each case in a setting where control measures are in place, must be less than 1. Identifying all infections (e.g., asymptomatic, low parasite density, and imported) is a key component...
to successful elimination campaigns. Laboratory techniques such as parasite detection by polymerase chain reaction (PCR) and distinguishing indigenous from imported cases via parasite genotyping are integral to this process. These techniques combined with spatial data improves surveillance and allows for intensifying focal vector control strategies that are required to achieve the goal of zero locally acquired cases.

Surveillance is key to the success of elimination strategies. It aids in identifying trends in incidence and epidemics. When incidence reaches low levels, it also serves as a tool to provide evidential support for allocating resources efficiently to target those most at risk. When programs achieve zero locally acquired cases, another program reorientation is warranted to prevent the reintroduction of malaria to the area. This program reorientation must be considered during the initial planning stages of committing to eliminate malaria as this may prove to be the most challenging phase for some countries. Preventing reintroduction relies on strong surveillance systems and continued vector control strategies which may prove long-term. The emphasis on the importance of preventing reintroduction of transmission was largely ignored during the GMEP campaign.

WHO grants “elimination” status to countries that have interrupted malaria transmission and successfully prevented reintroduction for at least three years with surveillance systems in place to support that finding. To date, 111 countries have successfully eliminated malaria. Data suggest incidence is declining in several African countries where the burden is most prominent, primarily due to ITN and IRS campaigns. Of the 98 remaining malaria-endemic countries, 64 are controlling malaria (including Kenya) while 34 are focused on eliminating it. Figure illustrates the map of the world and malaria control status for each country. It is important to note that while “elimination” status is granted at the national level, countries may wish to target elimination efforts in regional areas considering the variable epidemiology of malaria within their borders.
1.6 Malaria in Kenya

Kenya is currently categorized as “controlling” malaria. However, in 2009 the Kenya Ministry of Health (MOH) announced its Vision 2030 plan which includes “A Malaria Free Kenya” indicating malaria elimination is an eventual goal. The Division of Malaria Control (DOMC) in the Ministry of Public Health and Sanitation manages Kenya’s National Malaria Control Program. The most recent DOMC National Malaria Strategy (NMS) 2009–2017 acknowledges the shortcomings of its inaugural NMS 2001–2010 in terms of organization and implementation and seeks to scale up malaria control interventions and strengthen capacity and surveillance.

Kenyans had been implementing malaria control measures for nearly a century before their formal strategy was introduced. In the 1920s, environmental measures aimed
at reducing vector breeding sites were implemented in more populated areas like Mombasa and Nairobi. \[113\] Then, mass drug administrations were implemented in isolated areas primarily to control epidemics using quinine. \[52, 63, 113\] Following their discovery in the 1940s, insecticides were used in addition to new drugs like chloroquine and pyrimethamine. \[53, 113, 142\] Each of these control measures was effective in its targeted setting. As indicated above, during the WHO-led GMEP, little was attempted in Africa due to weak infrastructures. However, WHO did fund some pilot projects in Kenya during this time primarily on the effectiveness of specific insecticides and drugs. \[62, 133–135\]

Over a decade ago, African nations committed to improving the health of their people and strengthening their health infrastructures by signing the Abuja Declaration, where they committed to allocate 15% of their overall budget to the health sector. \[1, 170\] Most nations, including Kenya, have yet to reach their commitment and rely on partnerships to assist in their efforts. The major partners that contribute to Kenyan malaria control efforts include the Global Fund to Fight AIDS, Tuberculosis, and Malaria; British Department for International Development; Population Services International; United Nation’s Children’s Fund; United States President’s Malaria Initiative; United States Agency for International Development; and the World Health Organization. \[85\]

With the exception of \textit{P. knowlesi}, all human malaria parasite species are found in Kenya, although \textit{P. falciparum} accounts for over 98% of all infections. \[85\] Currently, malaria is second only to acute respiratory illnesses in terms of outpatient morbidity accounting for up to 30% of attendance and 19% of admissions to health facilities. \[85\] It is also the leading cause of mortality in children under age 5. \[85\]

Kenya is located in equatorial East Africa and borders Tanzania, Uganda, South Sudan, Ethiopia, Somalia, Lake Victoria, and the Indian Ocean. It rises from sea level to 5,199 meters at Mt. Kenya, its highest peak. Kenya experiences two rainy seasons described
as the long rains from April to June and the short rains from October to December. The geography, topography, and climate are conducive to four major malaria transmission epidemiological zones: 1) endemic, 2) seasonal transmission, 3) highland epidemic prone, and 4) low risk. Approximately 29% of Kenyans live in areas considered endemic, where annual stable transmission occurs, specifically around Lake Victoria or in the coastal regions where incidence has reduced but a risk for resurgence remains. The arid and semi-arid regions of northern and southeastern Kenya, where about 21% of Kenyans reside, experience intense seasonal transmission over short periods during the rainy seasons. The western highlands of Kenya are epidemic prone due to variation in minimum temperatures and rainfall which affects vector breeding and can result in intense malaria transmission seasons. About 20% of Kenyans live in these highland epidemic prone settings. Areas of Kenya considered low risk include the central highlands regions where temperatures are simply too low for the infective stage of the parasite to develop in the mosquito, essentially limiting transmission. Almost 30% of Kenyans live in low risk areas which includes the capital city of Nairobi.

Kenya’s malaria endemicity map was updated in 2009 using \( P. falciparum \) parasite rate in children ages 2 to under 10 (\( Pf \text{ PR}_{2-10} \)) as a marker of risk. Figure 1.6 illustrates transmission intensities in Kenya, where more intense regions are represented by darker shades. Noteworthy is that over 94% of Kenya’s surface area has a \( Pf \text{ PR}_{2-10} \) under 5%, meaning targeted campaigns and a long-term goal of malaria elimination are feasible with the right commitments.

As mentioned, approximately 20% of a total estimated 45.9 million Kenyans live in highland settings prone to epidemics. Highland areas (>1,500m above sea level) are targeted for elimination due to their unstable transmission patterns. Unlike malaria holoendemic regions, where partial immunities to malaria are built up and sustained through years of repeated bites from infectious mosquitoes, populations of all ages in highland areas are
Figure 1.6: Malaria endemicity map of Kenya, 2009.

susceptible to epidemics as their immune responses wane due to the highly seasonal and sporadic nature of transmission. 45 Specifically, in the dry season, malaria incidence is extremely low. Over recent decades, P. falciparum malaria epidemics in the highlands of East Africa have occurred with increasing frequency and have resulted in significant morbidity and mortality. An outbreak in 2002 was estimated to cause over 200 deaths and more than 500 hospitalizations in a single region of western Kenya. 145

Malaria is believed to have been introduced to the Nandi plateau (and Nandi people) in highland western Kenya, where the study area for this dissertation is located, as reference to an illness resembling it is entirely lacking from early tribal communications and stories from elders. 92 It may have been introduced in the late 1890s when the British moved into the area to establish military posts and could have brought infected people into the area, although the Nandi generally did not interact with the British. It may also have been introduced during the building of the Uganda Railway in the early 1900s, which brought both thousands of people and a new means of transportation to south Nandi, including potentially infected residents from the holoendemic regions near Lake Victoria. 92 But again, the Nandi tribe did not interact much with outsiders before World War I, when several hundred enlisted as soldiers and scouts. Nandi elders believe malaria was introduced to the highlands by returning soldiers as evidenced by the 1918–1919 epidemic, the first documented among this population, where about 25% of the population were infected with the disease. 87,92

Chapter 2 describes the Kenya Medical Research Institute-University of Minnesota (KEMRI-UMN) Malaria Research Project, a long-standing malaria surveillance study in the highland areas of Kipsamoite and Kapsisiywa, Nandi County in western Kenya, and the source of the data and samples for this dissertation.
Chapter 2

Study Description

2.1 Kenya Medical Research Institute-
University of Minnesota Malaria Research Project

This thesis work used and tested existing data and samples from three consecutive studies led by Principal Investigator Dr. Chandy John of Indiana University, formerly of the University of Minnesota (UMN) and Case Western Reserve University (CWRU), in collaboration with researchers at the Kenya Medical Research Institute (KEMRI) in Kisumu, Kenya. The three studies — 1) Early Warning System for Malaria in Highland Kenya, 2) Malaria Transmission and Immunity in Highland Kenya, 3) How does reduction of malaria transmission affect immune responses to \textit{Plasmodium falciparum} and prevalence of anemia? — will collectively be referred to for the purposes of this dissertation as the “KEMRI-UMN Malaria Research Project.”
2.2 Study Area Location and Population

The study population consists of men, women, and children from the Kipsamoite and Kapsisiywa areas of Nandi County in western Kenya. Kipsamoite (Kip) comprises seven villages — Chepyewet, Kabuson, Kapkweino, Kipsagat, Kipsamoite, Kwindich, and Morongen. Kapsisiywa (Kap) comprises ten villages — Birei, Chepkober, Chemase, Emgwen, Kabasgei, Kapsile, Kamagande, Kimondi, Mateget, and Tiriin. The study population grew from 6,752 in April 2003 to 9,186 in December 2013 with the total number of households increasing from 1,309 to 1,799. Children under age 5 accounted for 17.9% (n=1,217) of the overall population in April 2003 and 12.1% (n=1,108) in December 2013. The vast majority of individuals who live in these areas are of the Nandi ethnic group and speak Kalenjin. The main occupations are animal husbandry and subsistence farming of maize, sugarcane, tea, and vegetables. [45] The study sites are located approximately 20 kilometers apart and share similar ranges of altitudes (Kip: 1940–2108m; Kap: 1892–2065m). The western edge of Kip borders the Nandi North Forest and the large Kimondi swamp borders the eastern edge of Kip and western edge of Kap. [45,46] Both sites experience unstable malaria transmission patterns and their populations are at risk for malaria epidemics. Figure 2.1 illustrates the study locations in Nandi County, western Kenya. [35–38,45,106,107,110,173] Figure 2.2 details the study area, including the locations of households and health clinics.

2.3 Methods

Data have been collected during a series of surveillance activities in Kipsamoite since 2001 and Kapsisiywa since 2003. This dissertation uses data starting in April 2003, when data were being collected at both study sites, through 2013. Figure 2.3 depicts the timeline of surveillance activities from 2003–2013. Note: Due to changes in studies, forms, and clinic
Figure 2.1: Location of the study area, Kipsamoite and Kapsisiywa, Nandi County, Kenya.

Figure 2.2: Detailed map of the study area, Kipsamoite and Kapsisiywa, Nandi County, Kenya.
personnel, surveillance activities were not conducted during brief periods of 2005 and 2006.

2.3.1 Demography Surveillance

During the study period, field assistants (FA) enumerated households in the entire study area and recorded data on births, deaths, and migrations of occupants. At the time of initial enrollment, the FA collected household data including roof material and number of rooms, and also used global positioning systems (GPS) to map household coordinates and elevation. From 2003–early 2005, these data were collected every 3–4 months. In 2005, the data collection instrument was modified to add assessment of travel, use of ITN, and IRS treatment of houses. From late 2006–2008, these data were collected every 4–6 months and starting in 2009 an annual round of demography surveillance was implemented. As such, the population of the area at any given moment can be well estimated.

2.3.2 Malaria Surveillance

Clinical malaria was evaluated through ongoing passive surveillance efforts at the Kipsamoite Health Center and Kapsisiywa Health Center, both of which are Ministry of Health (MOH) dispensaries and are the only healthcare facilities in the study area. Study participants that presented with symptoms of malaria, including fever, backache, chills/shivering, diarrhea, headache, jaundice, joint pains, loss/lack of appetite, nausea, severe malaise, or vomiting, were evaluated for malaria by microscopy. Malaria case status was determined by two independent evaluations by trained microscopists, with a third and final assessment by a third microscopist if the first two results were discordant. Free diagnosis and treatment of malaria was provided per MOH guidelines. A series of active surveillance efforts supplemented the ongoing passive surveillance where FA from the villages of active cohort members conducted weekly visits to inquire about symptoms, travel, and ITN use. Members of the active cohort were referred to the Health Centers when feeling ill with
Figure 2.3: KEMRI-UMN Malaria Research Project schedule of activities, 2003–2013.
(D=Demography, SW=Site-wide blood collection, B=Active cohort blood collection)
symptoms of malaria and the same procedures at the clinic were followed for diagnosis and treatment.

Asymptomatic parasitemia in the study area was also assessed through scheduled active cohort and site-wide blood sample collections. In May 2007, a site-wide blood collection of all consenting study participants was conducted, and in July 2008 approximately \( \frac{1}{3} \) of these individuals had a repeat blood sample taken.

Blood samples were collected by finger prick at the Health Centers, and by venipuncture in the field during active cohort and site-wide collections. All thick and thin blood smears, filter-paper samples, and plasma were stored for future use.

2.3.3 Climate and Entomology Surveillance

Field assistants recorded daily cumulative rainfall using standard metal rain gauges and minimum and maximum temperatures using min-max mercury thermometers in the study area. These data were initially collected at one location in each of the 17 villages, but starting in February 2010, climate surveillance data were collected from one location in Kip and one in Kap.

To track the distribution and density of the mosquito population in the study area, households were randomly selected for vector surveillance. The indoor resting mosquitoes were captured using pyrethrum spray collection techniques. Namely, after removing humans and animals from the home and covering all food, a sheet was placed on the floor of the house and a trained FA sprayed pyrethrum towards the ceiling and around the doors, windows, and eaves of each room. After waiting outside for at least 10 minutes, the knocked-down mosquitoes were counted and collected. A laboratory technologist identified the species of each mosquito.
2.3.4 Human Subjects Protection

Separate written consent forms were collected from heads of households during initial enrollment for participation in demography and passive clinical malaria surveillance, from active cohort subjects during periods of active surveillance, from subjects for participation in the site-wide blood collection, and from subjects presenting to the clinics with symptoms of malaria for blood collection. Verbal consent was obtained for vector surveillance. Ongoing epidemiologic investigations like this dissertation were anticipated in these initial protocols. As such, the consent forms addressed storing samples for future testing, which study participants had the option to decline. The KEMRI-UMN Malaria Research Project’s “Study 1” was approved by the CWRU Institutional Review Board (IRB) and “Studies 2 & 3” have been approved by the UMN IRB. All three studies were approved by the KEMRI Ethical Review Committee. The UMN IRB Human Subjects Committee determined this dissertation work was exempt from full review under Federal guidelines 45 CFR Part 46.101(b) category #4 “Existing Data: Records Review, Pathological Specimens.”

2.4 Period of Interruption

Evidence of malaria transmission interruption in the study area was reported from April 2007–April 2008 after the Kenya Ministry of Health (MOH) implemented annual IRS campaigns and switched to first-line artemisinin-combination therapy (ACT) antimalarial drugs for intermittent preventive treatment in pregnancy (IPTp) and treatment of uncomplicated malaria. Interrupting local transmission is the first step towards the elimination stage. One may assume as the Kenyan government strives toward its Vision 2030, that elimination efforts will commence in the highland areas of Nandi due to its epidemiological profile.
2.5 Dissertation Focus

This dissertation takes a comprehensive look at malaria in this epidemic prone area of highland Kenya using a traditional, spatial, and immuno-epidemiologic approach. Investigating the epidemiology of malaria in this study area is especially important with the increased interest among the international community to once again attempt to eradicate malaria worldwide, as malaria transmission patterns in areas of stable transmission will likely convert to patterns with prolonged periods of low transmission leaving large new populations living in unstable transmission conditions similar to those of this highland Kenya study area.

Chapter 3 Evaluating the implementation of the Kenya Ministry of Health National Malaria Strategy in highland Kenya with a focus on the impact of a mass bed net distribution campaign describes the incidence of malaria and intervention coverage levels from the period of possible interruption through 2013. Then, generalized estimating equations were used to evaluate the impact of the campaign on malaria incidence, and multi-level mixed effects logistic regression models were used to evaluate whether individual bed net use was associated with incidence.

Chapter 4 Spatial analysis of malaria incidence before and after a 13-month period of interruption of clinical malaria uses kernel density estimation methods for analyzing spatial point patterns to evaluate the spatial variation of clinical malaria during periods from 2003–2013, before and after interruption, and for periods of peak incidence. Areas with significantly elevated risk of malaria were identified and compared.
Chapter 5  Correlates of protection from clinical Plasmodium falciparum malaria in an area of low and unstable malaria transmission reports results from a nested matched case-control study designed to assess correlates of protection from clinical malaria in a low transmission setting, which is inherently difficult. Conditional logistic regression was used to evaluate the associations of antibody responses to 2 pre-erythrocytic and 9 blood-stage antigens with the odds of developing clinical malaria.
Chapter 3

Evaluating the implementation of the Kenya Ministry of Health National Malaria Strategy in highland Kenya with a focus on the impact of a mass bed net distribution campaign
3.1 Objectives

#1: Describe the intervention coverage levels and incidence of malaria in the study area from the period of possible interruption in malaria transmission through 2013.

#2: Evaluate the impact of the Kenya Ministry of Health mass insecticide treated bed net (ITN) distribution campaign on malaria incidence in the study area.

#3: Determine the relationship between individual ITN use and malaria incidence in the study area, in the absence of other interventions.

3.2 Background

Malaria is the leading cause of morbidity and mortality in Kenya. In unstable transmission settings like the highland areas of western Kenya, malaria occurs among individuals of all ages since immunities are not built or sustained with limited exposure. With little immunity, these populations are susceptible to epidemics; about 20% of an estimated 45.9 million Kenyans live in highland settings.

The Kenya Ministry of Health (MOH) first formalized a National Malaria Strategy (NMS) in 2001 and revised it in 2009. The approaches for reaching many objectives are specific to the country’s defined epidemiologic zones, one of which is the “epidemic prone areas of the western highlands.” In line with the NMS, the MOH conducted independent campaigns of either indoor residual spraying (IRS) of households or delivering insecticide-treated bed nets (ITN) to individuals in the Kipsamoite (Kip) and Kapsisiywa (Kap) areas of highland western Kenya from 2005–2012. Both are effective prevention tools that have been shown to reduce morbidity and mortality in various transmission settings. The class of insecticides used in the MOH IRS campaigns was pyrethroids, which have
residual effects lasting up to six months. We have previously reported that widespread (>85%) IRS coverage contributed to possible interruption of malaria transmission in the study area from April 2007–April 2008. IRS ceased in the area after 2010 following the guidelines set forth in the revised NMS, which called for two years of IRS in this epidemiologic zone transitioning to focused IRS in response to epidemics thereafter. IRS ceased in the area after 2010 following the guidelines set forth in the revised NMS, which called for two years of IRS in this epidemiologic zone transitioning to focused IRS in response to epidemics thereafter. Mass ITN distribution campaigns targeting 100% population coverage were carried out in Kip in November 2011 and Kap in June 2012. Universal coverage, defined by the World Health Organization as one net for every two household members, was used as the operational definition in the MOH campaign. Prior to this, government-led bed net distributions were targeted to the high-risk population of pregnant mothers and infants. Kip and Kap were under ongoing study surveillance activities for the KEMRI-UMN Malaria Research Project from April 2003 throughout the period of intervention delivery and beyond.

This project seeks to build on what we have already learned about the effectiveness of the MOH-led IRS interventions on malaria incidence in the study area and focus on the effectiveness of the change in strategy of reduced IRS coverage and increased ITN distribution. The goals of this project, specific to the study area, are to report intervention coverage levels and malaria incidence after the reported period of interruption through 2013, describe the impact of the mass ITN campaign on malaria incidence, and evaluate whether individual bed net use, in the absence of other interventions, is associated with malaria incidence. With prospective data collected on the study population cohort before, during, and after the implementation of the MOH interventions, we are distinctively positioned to evaluate and provide feedback on the effectiveness of these efforts specific to this epidemic prone highland Kenya setting.
3.3 Methods

3.3.1 Surveillance

Using data previously collected for the KEMRI-UMN Malaria Research Project, we determined the percentages of annual IRS coverage and ITN use, and the monthly incidence of malaria in the study area, from 2003–2013. Recrudescent cases in individuals, defined as a case occurring within 30 days of another case without a negative blood smear in between visits, were excluded. Monthly averages of daily cumulative rainfall, minimum temperature, and maximum temperature were calculated and used during analysis.

3.3.2 ITN campaign logistics survey

We randomly sampled 5 households from each of the 17 villages to survey their respective heads of households to better understand the logistics of the MOH ITN distribution campaign. We wanted to know whether they knew about the campaign, and if so, how they learned about it, when and where the nets were distributed, and for whom the nets were intended. For those who did not own a net, we further wanted to know the reasons why. The survey is shown in Appendix A.1 which was conducted by Field Assistants (FA) in September 2013 after the ITN distribution campaigns were completed.

3.3.3 Statistical Analysis

To evaluate the impact of the mass ITN campaign on malaria incidence in the study area, we used generalized estimating equations (GEE) on a dataset in which an observation corresponded to an individual for a month. In our dynamic cohort, an individual was included in any given month if they lived in the study area at least one day during that month, as malaria cases can be reported any day. We created a dichotomous variable to indicate
whether the record was before or after the mass ITN campaign, our predictor, taking into account the different time frames of distribution for Kip and Kap. We used GEE with a binomial distribution and logit link, with clusters being individuals, and an autoregressive of order one (AR-1) working correlation; the analysis used robust standard errors. Similarly, an alternative GEE defined clusters to be households and used an exchangeable (EXC) working correlation.

We used the same dataset to evaluate whether an individual’s ITN use was associated with a reduction in that individual’s risk of malaria. Specifically, we estimated odds ratios (OR) using multi-level mixed effects logistic regression, including random effects for individuals, and individuals nested within households.

For both GEE and multi-level analyses, individual ITN use as reported during each annual demography survey was considered the status for the respective calendar year. The data analyzed here covered 2011–2013, after any potential effects from the last round of IRS conducted in the study area in 2010 had worn off.

We considered the potential confounding effects of age, village, average monthly rainfall, average monthly minimum and maximum temperatures, household elevation, roof material, and distances to nearest clinic, forest, and swamp, which were selected a priori. We also considered controlling for annual seasonal effects using a general sinusoidal pattern, including covariates for both the sine and cosine of \([(t - 1)/12]/2\pi\), where \(t\) is the month during the study period, ranging from 1–36 accounting for the 36-month time period of analysis.

We employed the purposeful selection of covariates method, with consideration of the correlated nature of these data, to determine the final adjusted models; used fractional polynomials to evaluate the linearity in the logit of each continuous variable; and tested for statistical interaction of ITN use with age, and elevation with the weather patterns of rainfall and temperatures.  

\[69\, 71\]
Statistical analyses were performed using Stata SE version 14. $P<0.05$ was considered statistically significant.

3.3.4 Barriers to ITN use post-hoc survey

As a post-hoc analysis to examine barriers against bed net use, we identified all households that reported >50% bed net use among its members in the 2012 demography survey but <50% use in 2013 (N=61). FA surveyed the heads of these households in April 2014 to obtain information on the number of ITN owned versus the number used among members of their respective households. Reasons the heads of households thought bed net use declined in their households from 2012 to 2013, and reasons members in their households did not sleep under bed nets were also collected. In addition, the FA indicated whether, during their visit, they observed bed nets hanging over sleeping areas or used for other purposes. Appendix A.2 shows the post-hoc survey that was administered to these heads of households. The results of the survey are presented as frequency distributions.

3.4 Results

3.4.1 Indoor residual spraying

Figure 3.1 shows the percentages of households in Kipsamoite and Kapsisiywa with reported indoor residual insecticide spraying, by year, from 2003–2013. The MOH-led IRS campaigns that started in 2005 increased to widespread (>85%) coverage in 2007, which contributed to possible local transmission interruption in the study area. In subsequent years, MOH targeted fewer study area homes and focused efforts near potential breeding sites in surrounding areas. IRS was conducted in 71.6% of households in the study area in July–August 2008, 88.8% in April–May 2009, and 53.6% in April–June 2010. The 2008
and 2009 IRS campaigns commenced after cases were already being reported. Through 2013, IRS was not again conducted in the study area.

3.4.2 Insecticide-treated bed nets

Reported ITN use by study participants was consistently low (<25%) for years 2006–2011, but increased from 17.1% in 2011 to 51.7% in 2012 followed by 35.8% in 2013. The substantial increase in bed net use from 2011 to 2012 corresponds to the MOH ITN distribution campaigns. While percentages for the entire study population are reported by year in Figure 3.2, variability among the villages was large. Table 3.1 shows the percentages of study participants sleeping under a bed net, by village, as reported in the nearest demography survey preceding the ITN campaign in 2011, and the nearest after the campaign in 2012. Bed net use by village ranged from 1.6% to 69.6% before the campaign, and 2.9% to 98.4% after the campaign.

3.4.3 Malaria Incidence

Figure 3.3 illustrates the monthly incidence of clinical malaria from April 2003–December 2013, with an emphasis on the incidence after the period of possible transmission interruption when the MOH NMS changed, which is the focus of this chapter. Post-interruption, incidence of clinical malaria was low, but malaria was reported each year. Between the period of interruption and ITN distribution in the respective study sites, 182 cases were reported, and 107 cases were reported after ITN distribution through the end of 2013. While low incidence of clinical malaria has been noted in all subsequent years since interruption, there have been peaks in incidence after long rainy seasons. In May 2011, prior to mass ITN distribution, incidence peaked at 4.9 per 1,000 persons, and in June 2013, after ITN distribution, incidence reached 5.6 per 1,000 persons.
Figure 3.1: Percentages of households reporting indoor residual insecticide spraying, by year, 2003–2013. *In 2008 and 2009, spraying commenced after malaria cases were being reported.

Figure 3.2: Percentages of study participants reporting sleeping under a bed net during demography surveillance, by year, 2003–2013.
Table 3.1: Percentages of study participants reporting sleeping under a bed net during 2011 and 2012 demography surveillance, pre- and post-mass bed net distribution campaign, respectively, by village. Villages are listed in order of increasing bed net use as reported in 2012.

<table>
<thead>
<tr>
<th>Village</th>
<th>2011</th>
<th>2012</th>
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</thead>
<tbody>
<tr>
<td>Kwindich</td>
<td>6.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Birei</td>
<td>1.6</td>
<td>22.1</td>
</tr>
<tr>
<td>Kipsaget</td>
<td>9.6</td>
<td>28.3</td>
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<td>Morongen</td>
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<tr>
<td>Mataget</td>
<td>13.2</td>
<td>66.6</td>
</tr>
<tr>
<td>Kapkwenio</td>
<td>1.8</td>
<td>68.6</td>
</tr>
<tr>
<td>Kamagande</td>
<td>17.6</td>
<td>78.9</td>
</tr>
<tr>
<td>Kabasgei</td>
<td>31.4</td>
<td>80.3</td>
</tr>
<tr>
<td>Kimondi</td>
<td>32.4</td>
<td>82.4</td>
</tr>
<tr>
<td>Chemase</td>
<td>10.5</td>
<td>93.6</td>
</tr>
<tr>
<td>Kapsile</td>
<td>15.5</td>
<td>98.4</td>
</tr>
</tbody>
</table>
Figure 3.3: Malaria incidence per 1,000 persons and Ministry of Health indoor residual spraying (IRS) and insecticide treated bed net (ITN) campaigns in Kipsamoite (Kip) and Kapsisywa (Kap), April 2003–December 2013.
3.4.4 ITN distribution campaign logistics

The overall response rate for the logistics survey was 71/85 (83.5%) with every village represented. All respondents were aware the mass bed net distribution campaign took place. We learned from the surveyed heads of households that village elders announced the mass campaign by visiting individual households with a public health officer to enumerate members and ultimately register them for ITN distribution. Those registered were given a later date to visit a stated Health Center to pick up their nets from the public health officer. Household members in villages closer to the clinics were enumerated first, and assigned earlier dates for collection. The distribution of nets at the clinics took place in our study area over the course of 3 days. The nets were intended for use by each household member inclusive of men, women, and children of all ages, although the number of bed nets allotted per member of each household was not 1:1. Fifteen heads of households we surveyed that received ITN during the distribution campaign commented that they were provided 2–3 bed nets for their entire household, which had from 3 to 9 members at the time of registration. One head of household with 7 members indicated they were not given enough bed nets during the campaign, and therefore not all family members used them. Nine of the 71 (12.7%) respondents indicated that no one in their household owned a bed net, even though all respondents were aware of the campaign. Reasons provided for lack of ownership included learning about the mass distribution campaign after registration took place, arriving too late to the Health Center on the designated date of pick-up, being absent the day of ITN distribution, believing there were no mosquitoes in the home so bed nets were not needed, and misunderstanding the intended distribution population, believing it was solely children under age 5 rather than men, women and children of all ages (see Table 3.2).
Table 3.2: Reasons provided for lack of bed net ownership among surveyed heads of households who were aware of the mass distribution campaign but did not own a bed net.

<table>
<thead>
<tr>
<th>Reason for lack of bed net ownership</th>
<th>N=9</th>
<th>n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Learned about campaign after registration</td>
<td>1</td>
<td>(11.1)</td>
</tr>
<tr>
<td>Late to Health Center for pick-up on day of distribution</td>
<td>3</td>
<td>(33.3)</td>
</tr>
<tr>
<td>Absent day of distribution</td>
<td>3</td>
<td>(33.3)</td>
</tr>
<tr>
<td>Belief: Do not need bed net due to no mosquitoes in home</td>
<td>1</td>
<td>(11.1)</td>
</tr>
<tr>
<td>Misunderstanding about intended distribution population</td>
<td>1</td>
<td>(11.1)</td>
</tr>
</tbody>
</table>

3.4.5 Impact of mass ITN campaign

Initially, it appeared that the impact of the mass ITN campaign on malaria incidence approached, but did not reach, statistical significance. In the crude GEE model taking into consideration the correlation of individuals within households, during the study period (2011–2013), the odds of developing clinical malaria after the campaign was 24% less than before the campaign (OR=0.76, 95% confidence interval (CI): 0.56 to 1.04, P=0.085). The estimated working correlation in the EXC model with clustering by household is 0.0008, suggesting the correlation within households is small, and justification for use of traditional logistic regression models assuming independence (IND) could be made. A similar small working correlation starting at 0.00037 was estimated for the AR-1 model with clustering by individuals. The quasi-likelihood information criteria (QIC) are nearly identical, although slightly smaller, for the independence working correlation models as compared to the other models (QIC = 3068.92 IND vs. 3068.96 EXC for correlation of individuals within households models, and 3068.40 IND vs. 3068.80 AR-1 for correlation within individuals models). Subsequent adjusted GEE models could not converge, hence we report crude and adjusted results from traditional logistic regression models alongside the crude GEE model estimates in Table 3.3 for comparison purposes. Conceptually, the crude model
should be all that is necessary to report for this population-level question, as the mass ITN campaign targeted 100% population coverage of the study area, and risk factors for malaria should inherently be unrelated to the campaign itself. However, we know that the distribution of ITN during the campaign was not uniform and universal coverage was not achieved. Hence, in our adjusted model, we evaluated plausible risk factors for malaria as potential confounders. The final model adjusted for age, elevation, and distance to nearest clinic. The fractional polynomial analysis indicated that no transformation of elevation or distance to nearest clinic was better than the model treating each covariate as linear in the logit. However, fractional polynomial transformations for age were indicated and used. No statistical interactions (i.e., effect modifiers) were observed. Results of the adjusted model still suggest the ITN campaign reduced incidence of malaria for this study population, however, it lacks statistical significance (OR=0.80, 95% CI: 0.59 to 1.07, P=0.133).

### 3.4.6 Impact of individual ITN use

The odds of developing clinical malaria among individuals who slept under ITN in Kip and Kap were 26% less than the odds among study area individuals who did not sleep under an ITN, a trend that approaches but does not reach statistical significance (OR=0.74, 95% CI: 0.52 to 1.04, P=0.084). Table 3.4 presents the results from crude and adjusted multi-level mixed effects logistic regression models, which included random effects for individuals, and individuals nested within households. The fractional polynomial analysis indicated that no transformation of elevation, distances to nearest clinic or swamp, or average monthly minimum temperature was significantly better than the model treating each covariate as linear in the logit. However, fractional polynomial transformations for age, distance to nearest forest, average monthly rainfall, and average monthly maximum temperature were warranted. The statistical interaction between elevation and average monthly rainfall was significant (i.e., elevation modified the effect of rainfall). The adjusted model controls for
age, village, elevation, distance to nearest clinic, average monthly total rainfall, average monthly maximum temperature, and the significant interaction terms. We also fit a model replacing the rainfall and temperature covariates with a general seasonal sinusoidal pattern, but elected to present the adjusted model using the climate data collected in our study area, as this corresponds to the specifics of this setting and will account for seasonal variations across years. The model fit with the general seasonal pattern had similar results (OR=0.75, 95% CI: 0.53 to 1.05, P=0.092).

Almost three percent of ITN data were missing in the dataset due to missed demography surveys or people who left or entered the study area before or after the demography survey took place in a given year. We fit our models using imputed data for ITN use at both extremes (i.e., all “yes” or all “no”). The model where missing ITN values were treated as “yes” enhanced the effect of individual ITN use on developing clinical malaria (OR=0.68, 95% CI: 0.48 to 0.95, P=0.024), whereas the opposite was true when missing ITN values were treated as “no” (OR=0.79, 95% CI: 0.56 to 1.11, P=0.181). We reported the results from the dataset that leaves these ITN data missing for our final model so as not to draw any conclusions from imputed data.

These mixed effects models have small random effects variances, and coupled with the results from the likelihood tests comparing the mixed effects model with the logistic model, suggest justification for presenting the results using a traditional logistic regression model under the independence assumption. However, we prefer to present the model taking into account the correlation, even though it is small, as we know it conceptually exists.
Table 3.3: Impact of mass ITN campaign on malaria incidence in the study area, evaluated using generalized estimating equations and traditional logistic regression.

<table>
<thead>
<tr>
<th>Method</th>
<th>Cluster</th>
<th>Working Correlation</th>
<th>Crude OR (95% CI)</th>
<th>P-value</th>
<th>Adjusted(^a) OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEE</td>
<td>Individuals</td>
<td>AR-1</td>
<td>0.77 (0.57, 1.04)</td>
<td>0.088</td>
<td>Did not converge</td>
<td></td>
</tr>
<tr>
<td>GEE</td>
<td>Households</td>
<td>EXC</td>
<td>0.76 (0.56, 1.04)</td>
<td>0.085</td>
<td>Did not converge</td>
<td></td>
</tr>
<tr>
<td>LR</td>
<td>None</td>
<td>IND</td>
<td>0.74 (0.55, 0.999)</td>
<td>0.049</td>
<td>0.80 (0.59, 1.07)</td>
<td>0.133</td>
</tr>
</tbody>
</table>

Abbreviations: GEE = generalized estimating equations, LR = logistic regression, AR-1 = autoregressive of order 1, EXC = exchangeable, IND = independent, CI = confidence interval.

\(^a\)Adjusted for age, elevation, and distance to nearest clinic. Two transformed terms for age were included, \(ln(age)\) and \(1/\sqrt{age}\).
Table 3.4: Association between individual ITN use and malaria incidence in the study area, evaluated using multi-level mixed effects logistic regression.

<table>
<thead>
<tr>
<th>Method</th>
<th>Levels</th>
<th>Crude OR (95% CI)</th>
<th>P-value</th>
<th>Adjusted* OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multi-level mixed effects logistic regression</td>
<td>Individuals</td>
<td>0.88 (0.64, 1.21)</td>
<td>0.415</td>
<td>0.74 (0.52, 1.04)</td>
<td>0.084</td>
</tr>
<tr>
<td></td>
<td>Households</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI = confidence interval.

*Adjusted for age, village, elevation, distance to nearest clinic, average monthly total rainfall, average monthly maximum temperature, and the interactions between elevation and transformed rainfall terms. Transformed terms for age \( \ln(\text{age}) \) and \( 1/\sqrt{\text{age}} \), average total monthly rainfall \( \text{rain}^2 \) and \( \text{rain}^2 \times \ln(\text{rain}) \), and average monthly maximum temperature \( \text{maxtemp}^3 \) and \( \text{maxtemp}^3 \times \ln(\text{maxtemp}) \) were included in the model.
3.4.7 Bed net ownership and barriers to use

Concerned with the decrease in ITN use from 2012 to 2013 so soon after the mass distribution campaign, we sought to understand barriers to ITN use in the study area. Three of the 61 households meeting the criteria to be surveyed had moved out of the study area by the time the survey commenced. In the remaining 58 households, 53 households (91.4%) still owned at least one net. The 5 households with no nets had 2 to 6 members each. Three of these households were present during the mass distribution campaign but did not receive nets for undisclosed reasons, 1 was absent during the campaign, and 1 used the nets provided during the campaign as planting ropes. The 53 households owning at least one net owned 1 to 8 nets for households ranging in size from 1 to 9 members. About 67% of the households surveyed owned either 2 or 3 bed nets. Table 3.5 lists the number of nets owned per reporting household. Bed net usage among households with ITN ownership at the time of survey, defined as the proportion of household members that slept under a bed net each night, ranged from 0–100% (mean: 68.3%, median: 75%). Five households that owned ITN reported no usage for varying reasons: 1) one household with 2 members did not believe there were mosquitoes in the house; 2) two households, with 3 and 4 members each, indicated their nets had worn out; and 3) two households, with 6 and 8 members each, indicated they had not yet hung them since the time they were issued during the mass distribution campaign.

Overall, respondents reported a variety of perceived reasons for the decrease in household ITN use from 2012 to 2013. A majority (74.1%) believed there were not many mosquitoes in the area. Some attributed the increased use of ITN after the distribution campaign to a (perceived) decline in numbers of mosquitoes. Others shared this belief as they commented that they did not see mosquitoes in their houses. Nearly 14% reported that bed nets were uncomfortable to sleep under and 8.6% reported that the rectangular
ITNs that were distributed were difficult to hang in the typical round thatched roof homes found in the area. Almost 7% reported using the nets for other domestic purposes, including as ropes for washing, as nursery beds to protect crops, to confine their chickens, or for use in children’s games. Table 3.6 provides a comprehensive list of reasons provided by the heads of households for why some household members did not sleep under a bed net.

Table 3.5: Number of bed nets owned per household.

<table>
<thead>
<tr>
<th>Number of bed nets owned</th>
<th>N=58 n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5 (8.6)</td>
</tr>
<tr>
<td>1</td>
<td>5 (8.6)</td>
</tr>
<tr>
<td>2</td>
<td>22 (37.9)</td>
</tr>
<tr>
<td>3</td>
<td>17 (29.3)</td>
</tr>
<tr>
<td>4</td>
<td>4 (6.9)</td>
</tr>
<tr>
<td>5</td>
<td>2 (3.5)</td>
</tr>
<tr>
<td>6</td>
<td>1 (1.7)</td>
</tr>
<tr>
<td>8</td>
<td>2 (3.5)</td>
</tr>
</tbody>
</table>

Table 3.6: Reasons provided for not sleeping under bed net.

<table>
<thead>
<tr>
<th>Reason for not sleeping under bed net (select all that apply)</th>
<th>N=58 n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No mosquitoes in area</td>
<td>43 (74.1)</td>
</tr>
<tr>
<td>Uncomfortable/irritating</td>
<td>8 (13.8)</td>
</tr>
<tr>
<td>Difficult to hang</td>
<td>5 (8.6)</td>
</tr>
<tr>
<td>Other domestic purposes</td>
<td>4 (6.9)</td>
</tr>
<tr>
<td>Forget to use them</td>
<td>4 (6.9)</td>
</tr>
<tr>
<td>Other members in household do not have them/not enough for everyone</td>
<td>2 (3.5)</td>
</tr>
<tr>
<td>Worn out</td>
<td>2 (3.5)</td>
</tr>
<tr>
<td>Work at night</td>
<td>2 (3.5)</td>
</tr>
<tr>
<td>Someone else uses it</td>
<td>1 (1.7)</td>
</tr>
</tbody>
</table>
The FA reported visually seeing bed nets hung in 24.5% (13/53) of the households they surveyed that owned at least one net. One net was visually seen hung in 4 households, 2 nets in 7 households, and 3 nets in 2 households. For 4 households, the FA documented that the survey was administered outside (i.e., away from the sleeping area), so they were unable to assess whether any nets were hung inside the home. There were 3 households where the FA visually saw the nets being used for other purposes (2 to confine chickens and 1 for children’s games); only 1 of these same households reported using their nets for other domestic purposes when asked to provide reasons household members did not sleep under bed nets. One reported that nets were uncomfortable to sleep under, while the other indicated they worked at night.

3.5 Discussion

ITN use at the rates used in this population appeared to provide some benefit, but not enough to interrupt local transmission. Interruption of malaria in areas of low transmission will likely require a high-level of IRS or ITN coverage. The MOH change in strategy outlined in 2009 to phase out routine IRS and implement mass ITN distribution campaigns, with IRS reserved solely in response to epidemics, may not be sufficient to interrupt local transmission in this epidemiologic zone. However, universal coverage of ITN, while targeted in the evaluated mass ITN distribution campaign, was not achieved in the study area so it is unknown whether truly complete coverage and usage of ITN in the area would have led to a greater decrease in transmission or interruption. Other evaluations of bed net usage post-mass ITN distribution campaigns in sub-Saharan Africa share this finding. [4, 9, 126, 149, 163, 174] In Tanzania, after a universal coverage campaign, only 58.4% of households reported owning enough ITN to cover all of their sleeping places. [163] In a separate survey in western Kenya among respondents from several locations in various
transmission settings, almost 41% of those at risk for malaria needed additional ITN to achieve universal coverage. Bed net usage, an indicator of coverage, was lower in our study area both before and after the mass distribution campaign as compared to these studies.

The reported barriers to ITN use from the survey, albeit from a small sample, overlap with those of other studies, although the predominant factors vary. In contrast to another study in western Kenya where technical factors such as inaccessibility and loss of physical integrity of the nets were the primary reasons for not sleeping under them, results from this study are related to environmental factors, such as a perception of no mosquitoes in the area, or the nets being uncomfortable to sleep under. As malaria transmission occurs year-round in the study area, there is a need for educational campaigns that focus on the importance of daily ITN use even in cooler and drier months.

Misuse of ITNs was infrequently reported in our study as well as others. However, what people report and what they do may differ, as evidenced by 2 of the 3 households surveyed not reporting that they used their nets for other purposes which the FA observed. Any misuse, even small, is cause for concern and highlights the need for educational campaigns promoting ITN use.

As suggested elsewhere, “hang-up campaigns” where assistance is provided to physically hang nets in households should complement mass distribution campaigns to ultimately alleviate barriers to use due to the difficulty in hanging them, which was also reported in our study area. Having a net hung has been shown to be associated with higher ITN use. Additionally, round nets as opposed to rectangular nets may be more appropriate in settings where round thatched roof homes are common, and should be considered during the planning stages of future campaigns.

A proposed recommendation from the Kenya MOH to increase ITN coverage is to conduct a “follow-up/mop up campaign” after initial mass distributions, which would target
households that did not achieve universal coverage. Results from the survey support the need for implementing this recommendation in the future, as over 75% of those surveyed who did not own bed nets either missed the campaign’s registration or distribution.

In this study, bed net use status collected during annual demography surveys may be misclassified due to a self-reporting social desirability bias: bed nets are known to protect against malaria so people may self-report their use if they own them, even when they do not truly sleep under them. Underreporting of bed net use is also a possibility if a participant thought they might receive an ITN by responding “no” to current use. We do not suspect this is the case however, as bed net distribution has never been a part of the study protocols.

There may, however, be evidence of self-reporting bias regarding bed net use collected during the post-hoc survey. Each of the 58 households surveyed had reported <50% bed net usage among its members during the July 2013 demography survey, but only 18 reported <50% use during the April 2014 post-hoc survey. Respondents were made aware of the selection criteria for being surveyed, as its main purpose was to better discern the reason why bed net usage declined from the 2012 demography survey (the first survey after the mass campaign) to the 2013 demography survey. Hence, they may have felt inclined to report higher use when faced with the intended reason for being selected for participation in the follow-up survey.

Certainly, usage among these respondents may have truly been higher in April 2014 compared to July 2013 due to changes in behavior related to climate. In general, Kenya experiences two rainy seasons described as the long rains from April to June and the short rains from October to December. In our study area, April is generally the wettest month of the year, while July is drier and often the coolest. Rain increases the number of vector breeding sites and cooler temperatures reduce the mosquito life span. As has been shown elsewhere, people may sleep under bed nets during times of the year when they
perceive more risk of malaria. However, our study rainfall data suggest the long rains were delayed in 2014 and did not start until May, so this may not fully explain the reported differences in ITN use between these two time points in this subset population that was surveyed.

Still, an inherent limitation is that bed net use data were collected during cross-sectional demography surveys, and may not reflect variability of use among individuals throughout the year. This has been reported in other studies in highland areas where unstable transmission occurs. The higher reported bed net usage among the entire study population in the 2012 demography survey could be attributed to more people sleeping under nets during rainy seasons as the survey was conducted in October in 2012, while the 2010, 2011, and 2013 surveys were conducted in July. (In the study area, October is a wetter month than July.) However, bed net usage reported in the 2013 demography survey was still higher than all years prior to the mass ITN distribution campaign, so even when potential climate-driven behavior is taken into consideration, the campaign likely influenced the increased usage reported after the mass campaign.

The trend toward a decrease in transmission after ITN coverage was enhanced suggests that truly universal coverage might lead to a decrease in transmission. While the survey results suggest that the goal of universal coverage might be improved with supplemental educational, “follow-up/mop up,” and “hang-up” campaigns to help alleviate potential barriers to ITN use post-distribution, they also highlight that universal coverage and use in areas of low transmission, with prolonged periods where there are few cases of malaria and few mosquitoes, may be difficult to achieve even with these campaigns. It is likely that in these settings, additional measures like mass treatment, identification and treatment of asymptomatic carriers, or vaccination may be needed to achieve malaria elimination.
Chapter 4

Spatial analysis of malaria incidence before and after a 13-month period of interruption of clinical malaria
4.1 Objectives

#1a: Determine whether cases of malaria re-emerged in the same areas where they were last reported just before the period of possible interruption of clinical malaria reported in the study area.

#2a: Determine whether areas of elevated risk of malaria incidence existed before, after, and during peak periods before and after the period of interruption.

#2b: If areas of elevated risk existed, determine whether they were in the same or different regions.

4.2 Background

The value of maps to inform intervention strategies was established during the first epidemiological investigation linking cholera to the Broad Street pump in London by John Snow in 1854. 151 With advancements in technology, the collection of spatial data has become an integral piece of current malaria elimination strategies where cases, vectors, parasite isolates, and interventions are all mapped. 16 The Roll Back Malaria (RBM) Partnership recognized the importance of using spatial data in malaria elimination strategies and has specifically called for shrinking the malaria map from the endemic margins inward in its Global Malaria Action Plan. 136, 153 This practical strategy builds on the successes of the past century as illustrated in the global distribution of malaria from 1900 to 2002 shown in Figure 4.1 61 A blanket approach to interventions is not sustainable, and incorporating spatial data into investigations can inform targeted prevention and control strategies to maximize effectiveness with limited financial resources.
Local interruption is the first step toward elimination. As reported, a period of possible local interruption in transmission was experienced in the KEMRI-UMN Malaria Research Project study area of Kipsamoite and Kapsisiywa from April 2007–April 2008. An important phase in malaria programs is preventing the reintroduction of disease after interruption has been achieved (see Section 1.5). Identifying and then targeting clusters of malaria transmission when incidence reaches low levels is one strategy employed as programs transition to this phase. Once this phase has been reached, these targeted interventions are continued as elimination status is sought. This strategy relies on the assumption that malaria will re-emerge where it faded away. As our study area experienced a period of possible interruption, we have the unique opportunity to use our data to test this assumption by exploring whether cases did re-emerge in the same locations where they were last reported. Another goal is to evaluate whether clusters of malaria existed before and after the period of interruption, and if so, whether they were in the same regions.
4.3 Methods

Spatial data previously collected for the KEMRI-UMN Malaria Research Project were imported into ArcGIS version 10.1 and projected to the WGS 84 / UTM zone 36N coordinate system. Household coordinates for the last 10 reported clinical malaria cases preceding the period of possible interruption and first 10 cases after this period were mapped along with characteristics of the study area including the locations of the Health Centers and forest and swamp borders. A shapefile of the polygonal boundaries of the study area was created in ArcGIS and exported for use in R.

Kernel density estimation (KDE) methods for analyzing spatial point patterns were used to evaluate the spatial variation of clinical malaria in the study area. KDE is a smoothing technique applied to spatial point data where a moving three-dimensional function of a given bandwidth visits each location and weights events within the area defined by the bandwidth according to their distance from the location being considered. The size of the bandwidth largely influences the KDE. A bigger bandwidth will produce more smoothing and tend to be more biased whereas a smaller bandwidth will tend to be less biased but more noisy, with more peaks, most of which are spurious. Adaptive smoothing techniques have been developed to allow the bandwidth to be different in different parts of the map based on the amount of information available at a given location. In low density areas, the bandwidth is larger to allow greater smoothing whereas areas with more densely packed events will have smaller bandwidths. This allows greater sensitivity to these events and ultimately avoids smoothing out important detail in areas with enough information to support more detailed estimates. Edge correction methods have also been developed so that bias is not introduced to the density estimates for locations near the borders of the study area. Figure 4.2 graphically illustrates the pieces of the standardized KDE in
Equation (4.1):

$$
\hat{\lambda}_\tau(s) = \sum_{i=1}^{n} \frac{1}{\tau^2} k \left( \frac{s - s_i}{\tau} \right)
$$

(4.1)

where $\hat{\lambda}_\tau(s)$ is the estimated probability density at location $s$

$s$ is a location in the study area

$s_i$ is the location of a typical event

$n$ is the number of locations

$\tau$ is the bandwidth smoothing parameter

$k()$ is the kernel weighting function in standardized form

Figure 4.2: Kernel estimation of a point pattern.

KDE requires no more than one event per spatial location, so we created an indicator variable to identify whether a household had at least one case of clinical malaria reported during each of the two time periods being considered in any given analysis. We then used KDE to estimate the probability of developing clinical malaria in our study area as a function of location. To do this, we first computed the probability density of malaria case households at each location \( s \), \( f(s) \), and multiplied this by the total number of households with malaria cases, \( n \). Similarly, we computed the probability density of all households at each location \( s \), \( g(s) \), and multiplied this by the total number of households (case and non-case), \( m \). Next, we took the natural log of the ratio to estimate the logarithm of the probability of clinical malaria in our study area and used the results to create heat maps (see Equation 4.2). To evaluate whether peaks in the estimated surface identify regions with significantly elevated risk of malaria in the study area, we estimated one-sided asymptotic tolerance contours (i.e., \( P \)-value contours at a significance level of 0.05) and overlaid these on the maps. \[ 34 \]

\[
\log((n \ast f(s))/(m \ast g(s))) = \log(f(s)/g(s)) + \log(n/m) \tag{4.2}
\]

In 2006, a gap in data collection occurred due to study and staff transitions. No distinguishable peak periods were observed in 2008, 2010, or 2012, where 14, 27, and 17 annual cases were reported, respectively (see malaria incidence Figure 3.3).

To evaluate whether the spatial distribution of malaria cases differed between two time periods, we applied the same KDE technique to estimate the log relative risk of the corresponding two probability estimates of case households in each time period. We did this under the assumption that the population locations experienced little change between the time periods compared. With this assumption, for example, when we compared the probability of malaria during the time periods after vs. before interruption, \( g(s_{after}) = g(s_{before}) \) (see Equation 4.3). After we estimated the log relative risks, we used the same methods as described above to create heat maps with overlaid tolerance contours, to identify regions with significantly elevated risk as compared to other regions in the study area. Taking the log of the risk ratios sets the reference for the null hypothesis of no change in risk between time periods at 0, which aids interpretation of the heat maps. \[ \text{[14]} \]

\[
\begin{align*}
\log \left( \frac{f(s_{after})}{g(s_{after})} \right) + \log \left( \frac{n_{after}}{m_{after}} \right) \\
- \left( \log \left( \frac{f(s_{before})}{g(s_{before})} \right) + \log \left( \frac{n_{before}}{m_{before}} \right) \right) \\
= \log \left( \frac{f(s_{after})}{f(s_{before})} \right) - \log \left( \frac{n_{after}}{n_{before}} \right) + \log \left( \frac{n_{after}}{m_{after}} \right) - \log \left( \frac{n_{before}}{m_{before}} \right)
\end{align*}
\]

under the assumption that \( g(s_{after}) = g(s_{before}) \)

Many epidemiologic studies do not enumerate every person in every household in a given study area, so that a sample-level analysis like the household one described above is the only option available to researchers. However, the KEMRI-UMN Malaria Research Project did enumerate every person, so that the underlying population can be truly well-estimated. Table 4.1 shows the number of overall cases from the number of unique individuals residing in the number of unique households for each time period of analysis.
Table 4.1: Overall cases of clinical malaria from the number of unique individuals residing in the number of unique households, by time period.

<table>
<thead>
<tr>
<th>Time period</th>
<th>Cases</th>
<th>Individuals</th>
<th>Households</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before interruption (Apr 2003–Mar 2007)</td>
<td>956</td>
<td>833</td>
<td>498</td>
</tr>
<tr>
<td>After interruption (May 2008–Dec 2013)</td>
<td>281</td>
<td>277</td>
<td>234</td>
</tr>
<tr>
<td>Peak 2004 (May–Jul)</td>
<td>319</td>
<td>311</td>
<td>231</td>
</tr>
<tr>
<td>Peak 2005 (Apr–Jun)</td>
<td>85</td>
<td>84</td>
<td>73</td>
</tr>
<tr>
<td>Peak 2007 (Jan–Mar)</td>
<td>51</td>
<td>51</td>
<td>45</td>
</tr>
<tr>
<td>Peak 2009 (Apr–Jun)</td>
<td>42</td>
<td>42</td>
<td>36</td>
</tr>
<tr>
<td>Peak 2011 (Apr–Jun)</td>
<td>60</td>
<td>59</td>
<td>58</td>
</tr>
<tr>
<td>Peak 2013 (Jun–Jul)</td>
<td>71</td>
<td>71</td>
<td>67</td>
</tr>
</tbody>
</table>

Note: Peak periods for 2003, 2006, 2008, 2010, and 2012 are not reported. In 2003, data collection commenced during the period of peak incidence. In 2006, a gap in data collection occurred due to study and staff transitions. In 2008, 14 clinical malaria cases total were reported and none prior to May. In 2010 and 2012, no distinguishable peak periods were observed, with total annual cases of 27 and 17 reported, respectively.

To enable us to use this individual-level information (instead of reducing it to a household-level summary), we repeated the spatial point pattern analysis using jittered household coordinates for individuals who shared the same household so that no two individuals shared exact coordinates (i.e., instead of using identical coordinates for all individuals in a household, we added some random noise to each individual’s coordinates to spread them out a bit). This allowed each individual to contribute to the analysis using their respective case status during the time period of analysis. No individual had a jittered coordinate assigned more than 10 meters in either latitude or longitude from the initial household coordinate recorded during demography surveillance.

All analyses excluded potential recrudescent cases in individuals, defined as a case
occurring within 30 days of another case without a negative blood smear in between visits. Trans-migration information captured during demography surveys was evaluated to account for individuals who moved households within the study area since initial enrollment. The \textit{sparr} package in R version 3.2.1 was used to do all computations presented here. [129,157]

4.4 Results

4.4.1 Descriptive spatial analysis

Figure 4.3 displays the locations of the households corresponding to the last 10 cases before the period of possible interruption and the first 10 cases after it. Households labeled with negative values represent cases before the period of interruption, increasing in order from the tenth-to-last case (-10) to the last case (-1) identified at the Health Centers. Households labeled with positive values represent cases after the period of interruption, increasing in order from the first case (1) to the tenth case (10) identified at the Health Centers. Households that share values on the map had cases of clinical malaria reported on the same day. While both the last and first cases adjacent to the period of interruption were reported in Kipsamoite, there is no clear spatial pattern of disease distribution among these 20 mapped cases. Eight of the last ten cases before interruption were reported in Kapsisiywa, including three reported on the same day, while half of the first ten cases after interruption were experienced in each of Kipsamoite and Kapsisiywa. Two cases in Kipsamoite after the period of interruption were from members of the same household.
Figure 4.3: Household locations of the last 10 cases before and first 10 cases after a period of possible interruption of malaria transmission in the Kipsamoite and Kapsisiywa study area. Negative values indicate the order the cases were reported for the last cases prior to interruption, with increasing values nearer to this period. Positive values indicate the order the cases were reported after the period of interruption, with increasing values further from this period. Cases reported on the same day share values.
4.4.2 Kernel density estimation - household level

Risk. After excluding potential recrudescent cases, from April 2003–March 2007 before the period of possible interruption, clinical malaria cases were reported among individuals from 498 households as compared to 234 households from May 2008–December 2013 after the period of possible interruption. Figure 4.4 shows heat maps of the estimated values of the risks (or probabilities) of clinical malaria before and after interruption. Darker shades of gray represent regions with higher probability of malaria. In this household-level analysis, before interruption, significantly elevated risk was primarily seen in Kapsisiywa, with a few small regions of significantly elevated risk in northern Kipsamoite. After interruption, significantly elevated risk was again more prominent in Kapsisiywa, especially eastern Kapsisiywa, but more and different areas in Kipsamoite also experienced significantly elevated risk of clinical malaria.

During the peak period of malaria transmission from May–July 2004, clinical malaria was identified at the Health Centers among study area individuals from 231 households. After indoor residual spraying (IRS) commenced in the area in 2005, incidence was reduced (see Figure 3.3). During the peak April–June 2005 and January–February 2007 transmission seasons, cases were reported among individuals from 73 and 45 households, respectively. After interruption, cases among individuals from 36, 58, and 67 households were reported during the peak April–June 2009, April–June 2011, and June–July 2013 transmission seasons, respectively. Figures 4.5, 4.6, and 4.7 illustrate the estimated probabilities of clinical malaria during these peak periods before and after interruption in the study area. During the peak 2004 transmission season, significantly elevated risk was predominant in Kapsisiywa and northern Kipsamoite. In 2005, significantly elevated risk of clinical malaria was reported in fewer areas than seen in 2004, primarily in northern Kapsisiywa and eastern and north-central Kipsamoite. During the nearest peak before interruption in 2007,
the only region with significantly elevated risk was reported in a central area close to the Kapsisiywa Health Center. During the nearest peak after interruption in 2009, no areas of significantly elevated risk were reported. During the 2011 peak transmission season, significantly elevated areas of risk were again reported, primarily in northern Kapsisiywa. Interestingly, in 2013, the areas of significantly elevated risk differed from those in 2011 with the exception of one small area in eastern Kapsisiywa. Instead, southern and eastern Kapsisiywa and areas of north-central Kipsamoite experienced areas with significantly elevated risk of clinical malaria.

**Relative risk.** The probability of clinical malaria was also compared before and after interruption of clinical malaria and between adjacent peak transmission seasons. Figures 4.15, 4.16, and 4.17 show heat maps of the estimated values of the log relative risks comparing after vs. before interruption and the peak periods before interruption (2005 vs. 2004, 2007 vs. 2005), the nearest peaks before and after interruption (2009 vs. 2007), and after interruption (2011 vs. 2009, 2013 vs. 2011). A blue to red diverging color scale was used to illustrate the log relative risks, with blue hues representing areas with reduced relative risks (logRR < 0) and yellow-red hues representing areas with increased relative risks (logRR > 0).

The probability of clinical malaria in the eastern areas of Kapsisiywa and eastern and northern areas of Kipsamoite were significantly elevated after vs. before the period of possible interruption. Both of the before interruption peak transmission period comparisons (2005 vs. 2004, 2007 vs. 2005) showed reduced risk in the later period as compared to the most recent adjacent peak period of transmission. This makes sense since incidence decreased in the years leading up to interruption. When the probability of malaria in the nearest peak period after interruption (2009) was compared with the nearest peak period before interruption (2007), little spatial variation was seen. Both of the after interruption
peak transmission period comparisons (2011 vs. 2009, 2013 vs. 2011) showed areas with increased risk in the later period as compared to the most recent adjacent period, which also is intuitive since incidence has increased in the years after interruption.

Before interruption, only two regions in Kipsamoite were significantly elevated in the 2005 vs. 2004 comparison. In the after interruption comparisons, only one region in each comparison was significantly elevated; in northern Kapsisiywa for the 2011 vs. 2009 comparison, and in north-central Kipsamoite in the 2013 vs. 2011 comparison. This is not surprising as the 2009 peak transmission season experienced no regions with significantly elevated risk, but the 2011 peak transmission season did, in northern Kapsisiywa. Similarly, the 2013 peak period reported significantly elevated risk in Kipsamoite, but none were reported in Kipsamoite in the peak 2011 season. These results are also not surprising because power to detect differences has been reduced by using a subset of the available data, with a smaller sample size.
Figure 4.4: Estimated probabilities of clinical malaria among households before and after a period of interruption of clinical malaria. Areas with significantly elevated risk are indicated by overlaid red tolerance contours (P<0.05).
Figure 4.5: Estimated probabilities of clinical malaria among households during peak periods of malaria transmission in 2004 and 2005. Areas with significantly elevated risk are indicated by overlaid red tolerance contours ($P < 0.05$).
Figure 4.6: Estimated probabilities of clinical malaria among households during peak periods of malaria transmission in 2007 and 2009. Areas with significantly elevated risk are indicated by overlaid red tolerance contours (P<0.05).
Figure 4.7: Estimated probabilities of clinical malaria among households during peak periods of malaria transmission in 2011 and 2013. Areas with significantly elevated risk are indicated by overlaid red tolerance contours (P<0.05).
Figure 4.8: Estimated log relative risks comparing after vs. before a period of interruption of clinical malaria, and the 2005 vs. 2004 peak periods. Areas with significantly elevated risk are indicated by overlaid black tolerance contours (P<0.05).
Figure 4.9: Estimated log relative risks (logRR) of clinical malaria comparing case household peak periods of malaria transmission before (2007 vs. 2005) and the nearest peaks before vs. after (2009 vs. 2007) the period of interruption. Areas with significantly elevated risk are indicated by overlaid black tolerance contours (P<0.05).
Figure 4.10: Estimated log relative risks (logRR) of clinical malaria comparing case household peak periods of malaria transmission after (2011 vs. 2009, 2013 vs. 2011) the period of interruption. Areas with significantly elevated risk are indicated by overlaid black tolerance contours (P<0.05).
4.4.3 Kernel density estimation - individual level

**Risk.** The population-level analysis of individuals with jittered household coordinates provided more detailed maps of the risk of clinical malaria in the study area than the sample-level analysis using households. This is not surprising because the power of the analysis comes from events, and the individual-level analysis has more events to consider. Clinical malaria cases were reported from 833 and 277 individuals before and after the period of interruption, respectively. The Health Centers identified 311, 84, 51, 42, 59, and 71 individuals with clinical malaria during the defined peak periods in 2004, 2005, 2007, 2009, 2011, and 2013. Figures 4.11, 4.12, 4.13, and 4.14 emulate those of Figures 4.4, 4.5, 4.6, and 4.7 replacing the household-level analysis with an individual-level approach.

Similar to the household-level analysis, significantly elevated risk of clinical malaria was predominant in Kapsisiwywa before interruption, although many more areas in Kipsamoite experienced elevated risk than the small regions in Kipsamoite identified in the household-level analysis. The overall risk of malaria was lower throughout the study area when the individual-level analysis was considered (i.e., there are more areas with lighter shades of gray in the individual-level analysis than the household-level analysis). This was also true for the period after interruption.

During the 2004 peak transmission period, significantly elevated risk was experienced in multiple regions scattered throughout the study area. During the 2005 peak period, significantly elevated risk was seen predominately in eastern and northern Kipsamoite. More areas in Kapsisiwywa experienced significantly elevated risk during the peak 2007 period, primarily centrally located near the Health Center. Also, like the 2005 peak period, eastern and northern Kipsamoite had significantly elevated risk in 2007, but in fewer areas. Only one small area of significantly elevated risk was identified in northern Kipsamoite during the nearest peak period after interruption in 2009. During the 2011 peak period after
interruption, significantly elevated risk was identified in northern and eastern Kapsisiywa, and in 2013, more areas of significantly elevated risk were identified, including in southern Kapsisiywa and central Kipsamoite.

**Relative risk.** Unlike the household-level analysis, when the comparisons in probability between the two time periods were made, there were many areas where the risk after vs. before was significantly elevated, including in eastern, northern, and southern Kapsisiwya and varying regions spanning Kipsamoite except the north-central region. This indicates the probability of clinical malaria did differ in regions between time periods. In contrast to the after vs. before comparison map, the peak period comparison maps are more similar between the individual-level and household-level analyses, with more regions appearing with significantly elevated risk in the individual-level analysis, which can be expected with more data.

The same trends identified in the household-level analysis are true in this individual-level analysis. Overall a reduced risk in the later period as compared to the most recent adjacent peak period of transmission is seen before the period of possible interruption, with little spatial variation in the nearest peak after vs. before interruption. Overall increased risk is predominant in the peak period comparisons after interruption.

Before interruption, two regions in Kipsamoite were significantly elevated in the 2005 vs. 2004 comparison, and one region was significantly elevated in south-central Kapsisiywa in the 2007 vs. 2005 comparison. In the after interruption comparisons, one small region in northern Kapsisiywa was significantly elevated for the 2011 vs. 2009 comparison. More regions were identified for the 2013 vs. 2011 peak period comparison in south-eastern Kapsisiywa and various regions in Kipsamoite, including the south, west, north-central, and north regions.
Figure 4.11: Estimated probabilities of clinical malaria among individuals before and after a period of interruption of clinical malaria. Areas with significantly elevated risk are indicated by overlaid red tolerance contours (P<0.05).
Figure 4.12: Estimated probabilities of clinical malaria among individuals during peak periods of malaria transmission in 2004 and 2005. Areas with significantly elevated risk are indicated by overlaid red tolerance contours (P<0.05).
Figure 4.13: Estimated probabilities of clinical malaria among individuals during peak periods of malaria transmission in 2007 and 2009. Areas with significantly elevated risk are indicated by overlaid red tolerance contours (P<0.05).
Figure 4.14: Estimated probabilities of clinical malaria among individuals during peak periods of malaria transmission in 2011 and 2013. Areas with significantly elevated risk are indicated by overlaid red tolerance contours (P<0.05).
Figure 4.15: Estimated log relative risks comparing cases after vs. before a period of interruption of clinical malaria, and the 2005 vs. 2004 peak periods. Areas with significantly elevated risk are indicated by overlaid black tolerance contours (P<0.05).
Figure 4.16: Estimated log relative risks (logRR) of clinical malaria comparing cases peak periods of malaria transmission before (2007 vs. 2005) and the nearest peaks before vs. after (2009 vs. 2007) the period of interruption. Areas with significantly elevated risk are indicated by overlaid black tolerance contours (P<0.05).
Figure 4.17: Estimated log relative risks (logRR) of clinical malaria comparing cases peak periods of malaria transmission after (2011 vs. 2009, 2013 vs. 2011) the period of interruption. Areas with significantly elevated risk are indicated by overlaid black tolerance contours (P<0.05).
The range of estimated risks and relative risks for the household and individual analyses illustrated in the figures above are reported in Table 4.2. The distribution of these relative risks are displayed in the histograms in Figures 4.18 and 4.19. These distributions indicate that there was an overall reduction in risk before vs. after interruption (i.e., most of the study area had a RR<1). This trend is true for the before adjacent peak comparisons and the nearest after vs. nearest before peak comparisons. The after interruption peak comparisons indicate there was an overall increased risk of clinical malaria in the later periods as compared with the most recent adjacent peak periods (i.e., most of the study area had a RR>1).

Table 4.2: Estimated risk and relative risk ranges for the household-level and individual-level analysis, by time period.

<table>
<thead>
<tr>
<th>Time period</th>
<th>Household (min, max)</th>
<th>Individual (min, max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before interruption (Apr 2003–Mar 2007)</td>
<td>(0.027, 0.683)</td>
<td>(0.014, 0.637)</td>
</tr>
<tr>
<td>After interruption (May 2008–Dec 2013)</td>
<td>(0.014, 0.477)</td>
<td>(0.003, 0.157)</td>
</tr>
<tr>
<td>Peak 2004 (May–Jul)</td>
<td>(0.016, 0.402)</td>
<td>(0.006, 0.377)</td>
</tr>
<tr>
<td>Peak 2005 (Apr–Jun)</td>
<td>(0.006, 0.121)</td>
<td>(0.002, 0.034)</td>
</tr>
<tr>
<td>Peak 2007 (Jan–Mar)</td>
<td>(0.004, 0.055)</td>
<td>(8.6e-4, 0.040)</td>
</tr>
<tr>
<td>Peak 2009 (Apr–Jun)</td>
<td>(0.003, 0.041)</td>
<td>(6.8e-4, 0.009)</td>
</tr>
<tr>
<td>Peak 2011 (Apr–Jun)</td>
<td>(0.004, 0.111)</td>
<td>(6.8e-4, 0.025)</td>
</tr>
<tr>
<td>Peak 2013 (Jun–Jul)</td>
<td>(0.005, 0.153)</td>
<td>(0.001, 0.066)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relative risk</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>After vs. Before interruption</td>
<td>(0.23, 1.49)</td>
<td>(0.02, 2.36)</td>
</tr>
<tr>
<td>Before peaks (2005 vs. 2004)</td>
<td>(0.12, 0.65)</td>
<td>(0.02, 0.86)</td>
</tr>
<tr>
<td>Before peaks (2007 vs. 2005)</td>
<td>(0.30, 0.86)</td>
<td>(0.24, 1.45)</td>
</tr>
<tr>
<td>After vs. Before peaks (2009 vs. 2007)</td>
<td>(0.48, 0.89)</td>
<td>(0.20, 1.32)</td>
</tr>
<tr>
<td>After peaks (2011 vs. 2009)</td>
<td>(0.95, 4.19)</td>
<td>(0.78, 3.75)</td>
</tr>
<tr>
<td>After peaks (2013 vs. 2011)</td>
<td>(0.62, 3.28)</td>
<td>(0.68, 7.82)</td>
</tr>
</tbody>
</table>
Figure 4.18: Histograms of the estimated relative risks of clinical malaria for time periods before and after interruption, peaks 2005 vs. 2004, and peaks 2007 vs. 2005, by household or individual-level analysis.
Figure 4.19: Histograms of the estimated relative risks of clinical malaria for time period comparisons of peaks 2009 vs. 2007, peaks 2011 vs. 2009, and peaks 2013 vs. 2011, by household or individual-level analysis. Note: The x-axis for the 2013 vs. 2011 comparison is scaled differently than the other histograms due to its wider distribution.
4.5 Discussion

In this study, we identified areas of significantly elevated risk of clinical malaria in Kipsamoite and Kapsisywa before and after a 13-month period in which clinical malaria was absent in the study area. However, the regions of elevated risk were not consistent during the periods before and after interruption of clinical malaria cases. In particular, during peak periods after interruption (2009–2013) the sites of elevated incidence were not the same as those with elevated incidence during peak periods before this period of possible interruption (2003–2007). Hence, no patterns were identified that would warrant targeting intervention and control strategies to sub-locations in the study area.

Our individual-level evaluation did identify more detailed regions of significantly elevated risk as compared to the household-level analysis, but the latter did capture many of the regions of elevated risk, just at a more smoothed scale. The biggest difference between the analyses was before interruption. This is primarily driven by the much higher incidence experienced in the study area during 2003 and 2004 before the MOH-led IRS campaigns were initiated in the study area in 2005 (see Figure 3.3). When transmission, and subsequently incidence, is higher, individuals from the same household are more likely to experience cases. A wider time interval also increases the chances that individuals from the same household will experience cases during the defined time period. In the household-level analysis only 498 household locations were evaluated to represent the 956 cases before interruption, whereas the individual-level analysis evaluated 833 individuals to represent the 956 cases.

Limitations of this study include the observational nature of data collection, gaps in data collection during brief periods of 2005 and 2006, and the kernel density estimation techniques requiring zero or one events at a given spatial location. To address the potential methodology issue, given that we had population (i.e., individual) level rather than strictly
sample (i.e., household) level data, we jittered the coordinates for members of shared households so they each had unique locations and repeated the spatial point pattern analysis. Information was still lost for individuals with multiple cases during a specified time period, but this alternative analysis did provide more data for analysis than strictly evaluating case status at the household level. While evaluating the spatial distribution of cases to potentially detect clusters (i.e., elevated-risk regions) of disease is of primary interest, solely considering case locations as used in alternative methods may only reflect the distribution of the population. \[14\] KDE is a superior method to alternative cluster detection methods that only consider cases and do not take into consideration the underlying population distribution of the study area and use traditional shape boundaries to identify regions of elevated risk. \[104\] Major strengths of the KDE approach are the adaptive bandwidth technique, which provides flexibility in modeling heterogeneous spatial distributions, and edge-correction methods, which accounts for the portion of the kernel that lies outside the defined study boundary such that a negative bias is not introduced.

The period of possible interruption of transmission was reported by the KEMRI-UMN Malaria Research Project's study surveillance data, rather than the Kenya Ministry of Health (MOH) surveillance data. We now know asymptomatic transmission occurred during the reported period of possible interruption; a series of asymptomatic blood sample collections was taken during this time frame in which 15 asymptomatic cases were detected by microscopy and 2 smear negative samples tested positive by a more sensitive polymerase chain reaction (PCR) method. \[77\] After the period of possible interruption of clinical malaria was reported, 14 additional samples from symptomatic individuals that were initially smear negative were later detected to be PCR-positive (unpublished data). Hence, transmission likely continued in the study area, but at levels undetectable by our passive surveillance system.

Certainly the goal of any intervention is to reduce transmission, but there was no
programmatic strategy in place to commit resources to maintain interruption status with the goal of eventual elimination during the MOH-led IRS campaigns implemented in the study area. Maintaining interruption of transmission requires operational and financial commitment, and its likelihood of success should be evaluated before devoting resources to that objective. Future research should evaluate the spatial distribution of the MOH-led intervention campaigns implemented in the study area to determine whether areas of elevated coverage existed in the study area, and whether they were associated with the spatial distribution of malaria incidence (see Figures 3.1 and 3.2).

When elimination does become the goal in epidemic-prone highland Kenya, we know our study area can achieve undetectable levels of transmission. Performing spatial epidemiology near the time of intervention implementation may increase the effectiveness of a planned intervention strategy as well, such that the current (or most recent) areas of elevated risk can be identified. The study area may also benefit from additional active case detection methods, where persons with asymptomatic infections are targeted for testing and treatment. Under these conditions, targeted interventions may be most appropriate. A combination of strategies is likely required to achieve interruption and eventual elimination of malaria in our study area.
Chapter 5

Correlates of protection from clinical *Plasmodium falciparum* malaria in an area of low and unstable transmission
5.1 Objectives

#1: Evaluate correlates of protection to clinical malaria in a low transmission setting.

#2: Evaluate whether antibody responses in combination predict clinical malaria more accurately than single antibody responses.

5.2 Background

In malaria-endemic settings, repeated exposure to bites from mosquitoes infected with human \textit{Plasmodium} species leads to naturally acquired immunity to clinical disease. \cite{42, 91} This helps explain why 78\% of the 584,000 deaths from an estimated 198 million malaria cases worldwide occurred in children under age 5, as older children and adults living in stable transmission settings have acquired immunity to symptomatic infections that are maintained with continuous inoculations. \cite{172} While it is known that immunity can develop naturally over time, its mechanism remains poorly understood. Previous studies have demonstrated that antibodies play a role in protection from clinical malaria, which triggered vaccine development research. \cite{27, 29, 95, 138} Current vaccine candidates largely target \textit{P. falciparum}, which is responsible for the majority of morbidity and mortality worldwide. \cite{118, 131, 172} Efficacy in trials of the most advanced vaccine candidates has been low and of limited duration, prompting calls to explore an expanded pool of candidate antigens in varying malaria transmission settings, as well as multiple antigens in combination, including antigens from different stages of the parasite life cycle. \cite{6, 7, 131}

In unstable transmission settings, symptomatic disease is seen among individuals of all ages because limited exposure does not permit naturally acquired immunity to accumulate. With more rapid diagnostics, effective treatments, and use of prevention measures
such as bed nets and indoor residual spraying, more people will be living in areas of unstable transmission with less naturally acquired immunity. However, few studies have assessed whether antibody-associated protection from clinical malaria occurs in settings of low transmission. Using a novel nested case-control design matched on age and village, with prolonged follow-up for malaria over 6 years in a large study population, we were able to assess risk of clinical malaria in a population with highly seasonal malaria transmission that recently experienced interruption of clinical malaria for 13 months, and maintained low transmission during the subsequent follow-up period. We hypothesized that correlates of protection against clinical malaria identified in such a less-immune population may identify different yet potentially important markers suitable for new research into an efficacious long-lasting vaccine. It is inherently difficult to predict correlates of protection in low transmission settings due to the limited number of cases, but our longitudinal prospective study allowed testing of antibody responses to 11 different antigens (2 pre-erythrocytic and 9 blood-stage); 10 were tested simultaneously using a newly developed multiplex cytometric bead assay (CBA). This study’s objective was to evaluate correlates of protection to clinical malaria in a low transmission setting to better inform vaccine candidate choices.

5.3 Methods

5.3.1 Study Site, Participants, Data and Sample Collection

We performed a nested case-control study of the men, women, and children from the 17 villages comprising the Kipsamoite and Kapsisiywa study areas in western highland Kenya, which experiences unstable malaria transmission patterns.

The current study examines subjects who participated in a site-wide blood sample collection that targeted the entire cohort, performed from April–June 2007, and developed clinical malaria (cases, defined as having a measured fever, reported fever, or headache in
the presence of *P. falciparum*) or did not (controls), during clinical follow-up from June 2007–June 2013. The study area population in 2007 was about 8,000. In low transmission settings where disease burden transcends age, fever alone is not sufficient in screening persons for malaria. Among individuals ages 5 and above, screening persons presenting with fever or headache increased sensitivity of detecting clinical malaria in this setting. [105] Three controls per case were selected from among participants who had the same follow-up time as cases but for whom clinical malaria was not detected; controls were matched with cases on village and age (controls in age categories 0–4, 5–14, and 15–64 were matched to cases in the same categories and differing in age by no more than 2 years, and controls of age 65 and older were matched to cases in the same category and differing in age by no more than 5 years, due to low numbers.)

At the time of initial enrollment, the FA collected household data including roof material and number of rooms, and they used global positioning systems (GPS) to map household coordinates and elevation. The study areas Health Centers, forest edge, and swamps were also previously mapped using GPS. [45] The Euclidean distance from each subject's household at the time of the site-wide blood collection to the nearest of each of these study area attributes was calculated in ArcGIS version 10.1. [48] Individual use of bed nets (yes or no), individual travel outside the study area (yes or no), and household treatment by indoor residual spraying (yes or no) were collected during demography surveys, which were conducted every 4–6 months during 2007 and 2008 and annually starting in 2009. Bed net use was also ascertained at the time of the site-wide blood collection. We considered as adjusters bed net use, travel, and indoor residual spraying defined for each subject as a fraction, the number of “yes” responses divided by the total number of responses from 2007 to the year corresponding to the case date, which for controls was the year of the case to which they were matched.
5.3.2 Antibody Testing

Stored plasma samples from the site-wide blood collection were used to test human immunoglobulin G (IgG) antibody responses to 11 antigens, including 2 pre-erythrocytic stage antigens: circumsporozoite protein (CSP) (NANP)\textsubscript{5} repeat peptide and liver-stage antigen 1 (LSA-1) C-terminal region 3D7 strain; and 9 blood-stage antigens: apical membrane antigen 1 (AMA-1) FVO strain, AMA-1 3D7 strain, erythrocyte-binding antigen 175 (EBP-175), erythrocyte-binding protein 2 (EBP-2), merozoite surface protein 1 (MSP-1\textsubscript{42}) FVO strain, MSP-1\textsubscript{42} 3D7 strain, merozoite surface protein 3 (MSP-3) FVO strain, glutamate rich protein N-terminal non-repetitive region (GLURP-R0), and glutamate rich protein C-terminal repetitive region (GLURP-R2). IgG antibodies to CSP (NANP)\textsubscript{5} peptide were measured by an enzyme-linked immunosorbent assay (ELISA) as previously described.\cite{76} Responses to the 10 recombinant antigens were simultaneously measured by a multiplex cytometric bead assay (CBA) previously optimized and described with the additional inclusion of EBP-2 (coating concentration of 2\textmu g) and LSA-1 (coating concentration 2.5mg).\cite{120,121} Recombinant AMA-1 was expressed in \textit{Escherichia coli} (\textit{E. coli}), provided by Sheetij Dutta, Walter Reed Army Institute for Research.\cite{43} David Narum, National Institutes of Health, provided recombinant EBA-175 and EBP-2 expressed in \textit{Pichia pastoris} and also recombinant MSP-1\textsubscript{42} and MSP-3 expressed in \textit{E. coli}. Recombinant GLURP was expressed in \textit{E. coli}, provided by Michael Theisen, Statens Seruminstitut, Copenhagen, Denmark.\cite{156} Recombinant LSA-1 was expressed in \textit{E. coli}, provided by David Lanar, Walter Reed Army Institute for Research. Ten percent of samples received duplicate testing. To aid in evaluation and analysis, each plate also included negative controls consisting of nine samples tested in duplicate from North Americans never exposed to malaria, as well as two positive control samples consisting of a plasma pool from 30 Kenyans living in a lowland area where malaria is endemic, and four blank sample wells.
containing phosphate buffered saline diluents.

5.3.3 Statistical Analysis

Antibody levels were expressed in arbitrary units (AU). For ELISA, these were calculated for a particular plate as the test sample optical density (OD) value divided by a quantity derived from the ODs for the North American controls on the same plate, namely the mean OD plus 3 standard deviations. For CBA, AU were calculated using median fluorescence intensity (MFI) values in lieu of OD. The antibody levels were used two ways in the analyses: first, dichotomizing them as positive or negative, where AU ≥ 1 was considered a positive response; and second, using the common log (log to base 10) of the antibody level as a measurement on a continuous scale.

We described characteristics of cases and controls using frequencies, means, or medians, as appropriate. We evaluated the balance of age between cases and controls after matching using the standardized difference in means. To assess whether associations exist between antibody responses to the antigens tested and clinical malaria, we estimated odds ratios (ORs) using conditional logistic regression, taking into account the matching of age and village in the study design. The crude associations were used to compare cases and controls in antibody responses dichotomized as positive or negative, and median antibody levels as a continuous measure, as we employed 1:many matching, namely 1 case to 3 controls, in the study design. During final model selection, we considered the potential confounding effects of household elevation; treatment by indoor residual spraying; roof material; number of rooms; distances to nearest forest, swamp, and health clinic; individual bed net use; and travel, selected a priori. To determine the final adjusted models, we employed the purposeful selection of covariates method; used fractional polynomials to evaluate the linearity in the logit of each continuous variable; tested for statistical interaction of both antibody response with age and bed net use with age; calculated diagnostic statistics of
leverage, lack of fit (change in Pearson’s chi-square), and influence (Cook’s distance) for the matched data; and evaluated the sensitivity of the model fits when excluding data for the matched groups that were potentially poorly fit or influential. 69,71

To evaluate whether antibodies in combination predicted clinical malaria more accurately than antibodies alone, we used the predicted probability generated from the adjusted conditional logistic regression models for single antibodies as well as antibodies in combination and compared their respective areas under the receiver operating characteristic curve (AUROC). Single antibody responses that predicted protection at P<0.1 were explored in every possible combination.

Statistical analyses were performed using Stata SE version 12. 147 P<0.05 was considered statistically significant. To acknowledge the antibody responses to multiple antigens that we evaluated, we also considered the Bonferroni correction P-value of 0.0045 (= significance level / # tests, or 0.05/11) for discussion purposes.

5.4 Results

5.4.1 Incidence of source population

Monthly incidence of clinical malaria from June 2007–June 2013 is shown in Figure 5.1, which illustrates the highly seasonal nature of the disease in the study area. Incidence of clinical malaria was variable during the study period with an annual incidence per 1,000 person-years ranging from 1.7 (or 0.002 clinical episodes per person per year) in 2008 to 10.3 (or 0.01 clinical episodes per person per year) in 2013. The month with the highest incidence of clinical malaria during the study period was June 2013, with 5.6 cases per 1,000 persons. Figure 5.2 depicts monthly incidence by age categories. During periods of peak transmission, incidence was highest among children ages 5–14 years.
5.4.2 Study population characteristics

Of 257 clinical malaria cases with a measured fever, reported fever, or headache from June 2007–June 2013, 154 (59.9%) lived in the study area between April–June 2007, participated in the site-wide blood collection during that time period, and had a stored plasma sample to test. The mean age of case subjects at the time of the site-wide blood collection was 15.3 years (standard deviation (SD), 14.7 years). The mean temperature of these subjects at case detection was 38.2°C (SD, 1.2°C) and median parasite density was 19,220/µl (interquartile range (IQR), 3,840/µl to 53,860/µl). The mean age of the 462 control subjects at the time of the site-wide blood collection was 15.2 years (SD, 14.7 years). Table 5.1 shows the mean ages of cases and controls, and the standardized differences, by the age categories in which they were matched. The standardized difference in mean ages overall in
Figure 5.2: Clinical malaria incidence per 1,000 persons, Kipsamoite and Kapsisiywa, June 2007–June 2013, by age group.

cases and controls was 0.006, which indicates that the selection of age ranges for matching within each age category produced a balanced distribution of age between cases and controls after matching. However, among children under age 5 at the time of the site-wide blood collection, the mean age of cases was 3.0 years (SD, 1.3 years), and of controls was 2.7 years (SD, 1.2 years), with a standardized difference in means of 0.257, which suggests a potential for residual confounding in age even after matching.
Table 5.1: Evaluation of balance of age between cases and controls after matching, by age category.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Cases Mean (SD&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>Controls Mean (SD&lt;sup&gt;a&lt;/sup&gt;)</th>
<th>Standardized difference&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5</td>
<td>3.0 (1.3)</td>
<td>2.7 (1.2)</td>
<td>0.257</td>
</tr>
<tr>
<td>5–14</td>
<td>9.3 (2.5)</td>
<td>9.4 (2.5)</td>
<td>-0.034</td>
</tr>
<tr>
<td>15–64</td>
<td>32.3 (11.5)</td>
<td>32.2 (11.6)</td>
<td>0.006</td>
</tr>
<tr>
<td>65+</td>
<td>72.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.7 (4.1)</td>
<td>0.053</td>
</tr>
<tr>
<td>All ages</td>
<td>15.3 (14.7)</td>
<td>15.2 (14.7)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

<sup>a</sup> SD = standard deviation.

<sup>b</sup> Standardized difference = (sample mean of cases - sample mean of controls) / \(\sqrt{(\text{sample variance of cases}^2 + \text{sample variance of controls}^2)/2}\)

<sup>c</sup> Age of the single case in this age category.

5.4.3 Analysis of antibody responses

The frequency of positive responses to each antigen tested and their median levels are presented in Tables 5.2 and 5.3, respectively. Among cases, frequencies ranged from 38.3% to 89.0% and median levels ranged from 0.4 to 18.7 AU. Controls elicited higher positive responses for every antigen except EBP-2 (cases 38.3%, controls 34.0%) with frequencies ranging from 34.0% to 92.2% and median levels ranging from 0.5 to 15.4 AU.

All analyses of associations of antibody responses to *P. falciparum* antigens and clinical malaria used conditional logistic regression, which accounts for the matching on age and village done during the design and thus implicitly adjusts for age and village. To account for the potential residual confounding in age in the under age 5 category after matching, we added age as a continuous measure to the adjusted models. The fractional polynomial analysis conducted for each continuous variable indicated that no transformation of elevation, distance to nearest forest, bed net use, or indoor residual spraying was significantly better than the model treating each covariate as linear in the logit. Similarly, treating antibody levels (after the common log transformation) as a continuous measure did not violate linearity assumptions. No significant statistical interactions were observed.
Conditional logistic regression performed on laboratory results of 616 tested plasma samples (154 cases, 462 controls) identified positive antibody responses to two antigens that were statistically significantly associated with protection from clinical malaria. Table 5.2 shows that after adjusting for bed net use, household treatment by indoor residual spraying, roof material, distance to nearest forest, elevation, and potential residual confounding by age, subjects who elicited a positive response to GLURP-R2 in the baseline period from April–June 2007 had a 44% decrease in odds of developing clinical malaria between June 2007 and June 2013 (the follow-up period), compared to subjects who did not elicit a positive response to GLURP-R2 during the baseline period (OR = 0.56, 95% CI: 0.36 to 0.89, P=0.013). Similarly, subjects who elicited a positive response to LSA-1 also had a 44% decrease in odds of developing clinical malaria (OR= 0.56, 95% CI: 0.36 to 0.87, P=0.010). Subjects who elicited a positive response to GLURP-R0 had a 33% decrease in odds of developing clinical malaria, which approached but did not reach statistical significance (OR= 0.67, 95% CI: 0.43 to 1.02, P=0.060). When continuous antibody levels were assessed (Table 5.3), responses to three antigens, LSA-1, GLURP-R2, and CSP, were significantly associated with clinical malaria during follow-up: for every 10-fold increase in antibody response levels, a 39% (OR = 0.61, 95% CI: 0.41 to 0.89, P=0.011), 40% (OR= 0.60, 95% CI: 0.43 to 0.84, P=0.003), and 51% (OR= 0.49, 95% CI: 0.24 to 0.99, P=0.046) decrease in odds, respectively, was found. Antibody response to GLURP-R2 treated as a measure on the continuous scale was the only statistically significant association using the Bonferroni adjusted p-value of 0.0045 for multiple comparisons.

5.4.4 Combinations of antibody responses

To allow comparison of individual antigens and combinations of antigens, predictive accuracy of antibody responses to protection from clinical malaria was measured by the area
Table 5.2: Associations between dichotomized antibody responses to *Plasmodium falciparum* antigens and developing clinical malaria over a 6-year time period (June 2007–June 2013).

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Cases (N=154)</th>
<th>Controls (N=462)</th>
<th>Dichotomized response&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted&lt;sup&gt;c&lt;/sup&gt;</td>
<td>P-value&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AMA-1 3D7</td>
<td>137 (89.0)</td>
<td>426 (92.2)</td>
<td>0.63 (0.32, 1.24)</td>
</tr>
<tr>
<td>AMA-1 FVO</td>
<td>134 (87.0)</td>
<td>413 (89.4)</td>
<td>0.73 (0.38, 1.41)</td>
</tr>
<tr>
<td>EBA-175</td>
<td>81 (52.6)</td>
<td>243 (52.6)</td>
<td>1.00 (0.63, 1.57)</td>
</tr>
<tr>
<td>EBP-2</td>
<td>59 (38.3)</td>
<td>157 (34.0)</td>
<td>1.32 (0.83, 2.10)</td>
</tr>
<tr>
<td>GLURP-R0</td>
<td>83 (53.9)</td>
<td>285 (61.7)</td>
<td>0.67 (0.44, 1.01)</td>
</tr>
<tr>
<td>GLURP-R2</td>
<td>77 (50.0)</td>
<td>275 (59.5)</td>
<td>0.58 (0.37, 0.90)</td>
</tr>
<tr>
<td>LSA-1</td>
<td>87 (56.5)</td>
<td>308 (66.7)</td>
<td>0.58 (0.38, 0.88)</td>
</tr>
<tr>
<td>MSP-1&lt;sub&gt;42&lt;/sub&gt; 3D7</td>
<td>108 (70.1)</td>
<td>326 (70.6)</td>
<td>0.97 (0.57, 1.62)</td>
</tr>
<tr>
<td>MSP-1&lt;sub&gt;42&lt;/sub&gt; FVO</td>
<td>117 (76.0)</td>
<td>369 (79.9)</td>
<td>0.60 (0.39, 1.21)</td>
</tr>
<tr>
<td>MSP-3</td>
<td>123 (79.9)</td>
<td>384 (83.1)</td>
<td>0.79 (0.49, 1.28)</td>
</tr>
<tr>
<td>CSP&lt;sup&gt;d&lt;/sup&gt;</td>
<td>78 (50.6)</td>
<td>252 (55.4)</td>
<td>0.77 (0.51, 1.15)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Association between case/control status and dichotomized antibody response where an antibody response of arbitrary unit (AU) ≥ 1 was considered a positive response. <sup>b</sup>All analyses used conditional logistic regression that implicitly adjusted for age and village by the matched case-control design that was used. <sup>c</sup>Adjusted for bed net use, household treatment by indoor residual spraying, roof material, distance to nearest forest, elevation, and potential residual confounding on age. <sup>d</sup>N=455 controls measured by ELISA.
Table 5.3: Associations between continuous antibody levels to *Plasmodium falciparum* antigens and developing clinical malaria over a 6-year time period (June 2007–June 2013).

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Cases (N=154) Median (IQR)</th>
<th>Controls (N=462) Median (IQR)</th>
<th>OR (95% CI) Crude</th>
<th>P-value&lt;sup&gt;c&lt;/sup&gt;</th>
<th>OR (95% CI) Adjusted&lt;sup&gt;d&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMA-1 3D7</td>
<td>12.3 (2.4–42.6)</td>
<td>11.5 (3.2–43.1)</td>
<td>0.83 (0.57, 1.22)</td>
<td>0.351</td>
<td>0.73 (0.49, 1.09)</td>
<td>0.129</td>
</tr>
<tr>
<td>AMA-1 FVO</td>
<td>10.1 (2.7–37.1)</td>
<td>9.6 (2.4–33.2)</td>
<td>1.02 (0.69, 1.50)</td>
<td>0.922</td>
<td>0.92 (0.62, 1.37)</td>
<td>0.681</td>
</tr>
<tr>
<td>EBA-175</td>
<td>1.1 (0.4–9.9)</td>
<td>1.1 (0.4–8.8)</td>
<td>0.88 (0.61, 1.27)</td>
<td>0.498</td>
<td>0.83 (0.57, 1.21)</td>
<td>0.336</td>
</tr>
<tr>
<td>EBP-2</td>
<td>0.4 (0.2–2.1)</td>
<td>0.5 (0.2–2.0)</td>
<td>0.83 (0.56, 1.24)</td>
<td>0.362</td>
<td>0.78 (0.52, 1.17)</td>
<td>0.237</td>
</tr>
<tr>
<td>GLURP-R0</td>
<td>1.1 (0.7–3.7)</td>
<td>1.4 (0.7–4.0)</td>
<td>0.72 (0.46, 1.10)</td>
<td>0.127</td>
<td>0.70 (0.45, 1.09)</td>
<td>0.115</td>
</tr>
<tr>
<td>GLURP-R2</td>
<td>0.9 (0.4–6.2)</td>
<td>1.6 (0.5–8.0)</td>
<td>0.62 (0.45, 0.86)</td>
<td>0.004</td>
<td>0.60 (0.43, 0.84)</td>
<td>0.003</td>
</tr>
<tr>
<td>LSA-1</td>
<td>1.5 (0.54.3)</td>
<td>1.8 (0.79.4)</td>
<td>0.60 (0.41, 0.89)</td>
<td>0.010</td>
<td>0.61 (0.41, 0.89)</td>
<td>0.011</td>
</tr>
<tr>
<td>MSP-1&lt;sub&gt;42&lt;/sub&gt; 3D7</td>
<td>5.6 (0.8–16.2)</td>
<td>3.6 (0.8–15.1)</td>
<td>1.11 (0.77, 1.60)</td>
<td>0.588</td>
<td>1.02 (0.70, 1.50)</td>
<td>0.915</td>
</tr>
<tr>
<td>MSP-1&lt;sub&gt;42&lt;/sub&gt; FVO</td>
<td>18.7 (1.2–55.9)</td>
<td>15.4 (1.4–54.6)</td>
<td>0.98 (0.73, 1.32)</td>
<td>0.907</td>
<td>0.90 (0.66, 1.24)</td>
<td>0.528</td>
</tr>
<tr>
<td>MSP-3</td>
<td>2.4 (1.3–5.4)</td>
<td>2.3 (1.2–6.1)</td>
<td>0.92 (0.63, 1.36)</td>
<td>0.681</td>
<td>0.89 (0.60 1.33)</td>
<td>0.576</td>
</tr>
<tr>
<td>CSP&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.1 (0.6–1.8)</td>
<td>1.1 (0.7–1.7)</td>
<td>0.51 (0.26, 1.02)</td>
<td>0.058</td>
<td>0.49 (0.24, 0.99)</td>
<td>0.046</td>
</tr>
</tbody>
</table>

<sup>a</sup> Association between case/control status and antibody response as a continuous measure in arbitrary units (AU); the odds ratio is for a 10-fold increase in antibody level. <sup>b</sup>IQR = interquartile range. <sup>c</sup>All analyses used conditional logistic regression that implicitly adjusted for age and village by the matched case-control design that was used. <sup>d</sup>Adjusted for bed net use, household treatment by indoor residual spraying, roof material, distance to nearest forest, elevation, and potential residual confounding on age. <sup>e</sup>N=455 controls measured by ELISA.
under the receiver operating characteristic curve (AUROC) using the true case status (Table 5.4); an AUROC of 1 represents perfect prediction and a value of 0.5 is no better than a random coin flip. GLURP-R2 and LSA-1 were the best single predictors of protection considering antibody response as dichotomous and as a continuous measure (AUROCs 0.6385, 0.6428; and 0.6521, 0.6472, respectively). In general, treating antibody response as a continuous measure provided better predictions than treating it as dichotomous. Nine different combinations of antibody responses to CSP, GLURP-R0, GLURP-R2, and LSA-1, each with predicted protection at $P < 0.1$, performed better than any single predictor, with the combination of all four doing best (AUROC 0.6664). Figure 5.3 illustrates four diagnostic plots to describe discrimination from this combination model. These plots show that the estimated probability of clinical malaria is higher in cases than controls, but there is considerable overlap in their distributions; the model’s ability to distinguish between those with versus without clinical malaria is about 67%.

5.5 Discussion

Our nested matched case-control study tested antibody responses to 11 *P. falciparum* antigens and found that the pre-erythrocytic antigens CSP and LSA-1, and blood-stage antigen GLURP-R2, measured on the continuous scale, were associated with statistically significant protection from clinical malaria in an unstable transmission setting over a period of 6 years, while the blood-stage antigen GLURP-R0, dichotomized as positive or negative, showed a non-significant trend. All four (CSP, GLURP-R0, GLURP-R2, LSA-1) in combination predicted protection from clinical malaria better than any single response or any other combination evaluated. These findings are consistent with those of earlier studies, which found that combinations of antibody responses predicted protection from malaria better than a single response. We similarly demonstrated that combinations of
Table 5.4: Area under the receiver operating characteristic curve (AUROC) of predicted probabilities of developing clinical malaria for single antibody responses to 11 *Plasmodium falciparum* antigens and combination antibody responses to select antigens CSP, GLURP-R0, GLURP-R2, and LSA-1. Rows are sorted in decreasing order of AUROC treating antibody response as a continuous measure.

<table>
<thead>
<tr>
<th>Antigens</th>
<th>AUROC (dichotomous)</th>
<th>AUROC (continuous)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSP, GLURP-R0, GLURP-R2, LSA-1</td>
<td>0.6605</td>
<td>0.6664</td>
</tr>
<tr>
<td>CSP, GLURP-R2, LSA-1</td>
<td>0.6586</td>
<td>0.6655</td>
</tr>
<tr>
<td>GLURP-R0, GLURP-R2, LSA-1</td>
<td>0.6528</td>
<td>0.6617</td>
</tr>
<tr>
<td>CSP, GLURP-R0, GLURP-R2</td>
<td>0.6505</td>
<td>0.6610</td>
</tr>
<tr>
<td>CSP, GLURP-R2</td>
<td>0.6468</td>
<td>0.6600</td>
</tr>
<tr>
<td>GLURP-R2, LSA-1</td>
<td>0.6531</td>
<td>0.6592</td>
</tr>
<tr>
<td>CSP, GLURP-R0, LSA-1</td>
<td>0.6551</td>
<td>0.6548</td>
</tr>
<tr>
<td>CSP, LSA-1</td>
<td>0.6495</td>
<td>0.6548</td>
</tr>
<tr>
<td>GLURP-R0, GLURP-R2</td>
<td>0.6417</td>
<td>0.6527</td>
</tr>
<tr>
<td>GLURP-R2</td>
<td>0.6385</td>
<td>0.6521</td>
</tr>
<tr>
<td>LSA-1</td>
<td>0.6428</td>
<td>0.6472</td>
</tr>
<tr>
<td>GLURP-R0, LSA-1</td>
<td>0.6451</td>
<td>0.6463</td>
</tr>
<tr>
<td>CSP, GLURP-R0</td>
<td>0.6373</td>
<td>0.6396</td>
</tr>
<tr>
<td>CSP</td>
<td>0.6281</td>
<td>0.6351</td>
</tr>
<tr>
<td>GLURP-R0</td>
<td>0.6296</td>
<td>0.6294</td>
</tr>
<tr>
<td>AMA-1 3D7</td>
<td>0.6254</td>
<td>0.6287</td>
</tr>
<tr>
<td>EBP-2</td>
<td>0.6297</td>
<td>0.6223</td>
</tr>
<tr>
<td>MSP-142 FVO</td>
<td>0.6307</td>
<td>0.6206</td>
</tr>
<tr>
<td>AMA-1 FVO</td>
<td>0.6228</td>
<td>0.6204</td>
</tr>
<tr>
<td>EBA-175</td>
<td>0.6183</td>
<td>0.6203</td>
</tr>
<tr>
<td>MSP-3</td>
<td>0.6244</td>
<td>0.6197</td>
</tr>
<tr>
<td>MSP-142 3D7</td>
<td>0.6208</td>
<td>0.6194</td>
</tr>
</tbody>
</table>
Figure 5.3: Four diagnostic plots to describe discrimination for the fitted model of the combination of CSP, GLURP-R0, GLURP-R2, and LSA-1 as continuous measures, area under the receiver operating characteristic curve (AUROC) = 0.6664, N=616 (154 cases, 462 controls).
antibody levels treated as continuous measures predicted protection better than combinations of dichotomized antibody responses. Furthermore, our results lend support to the belief expressed by others that an efficacious vaccine may require a combination of pre-erythrocytic and blood-stage antigens, the former to prevent clinical disease altogether and the latter to reduce disease severity.

Several previous studies have also found that antibody responses to CSP, GLURP-R0, GLURP-R2, and LSA-1 confer protection from clinical malaria, either alone or in combination with other antigens, while other studies have not. Antibody responses to the other blood-stage antigens we tested, which did not show protection in our study, have produced conflicting results in the literature. The contrasting conclusions may be due to differing study protocols (see Table 5.5). Most tested antibody responses to a single antigen or limited number of antigens using ELISA, which we used to test CSP, but our study was able to test responses to a larger number of antigens, as compared to other studies (11 total; 10 simultaneously using the multiplex CBA). Also, the ages of study participants and duration of follow-up time vary across studies, with our study including all ages for a 6-year follow-up period, which is pertinent to the development of a long-lasting vaccine. The different transmission settings of the study populations could also influence results. The majority of studies tested samples collected from populations inhabiting malaria-endemic settings where transmission is year-round, while our study tested samples from an unstable transmission setting with highly seasonal or sporadic transmission. A limited number of studies have tested associations of antibodies with protection from clinical malaria in areas of highly seasonal transmission. These studies found protection associated with AMA-1, GLURP-R0, MSP-1, MSP-2 (not tested in this study), and MSP-3. However, even in these studies, rates of clinical malaria were much higher than in the present study.
The difficulty of studying malaria in areas of low transmission is that a large sample size is required to detect associations with protection from clinical disease. Our study addressed this issue with a matched case-control design, nested into our prospective cohort study, with assessment over a long follow-up time, and provides new information about protection from clinical malaria in an area of low transmission. The study demonstrates that with relatively low levels of exposure, antibodies to specific antigens are associated with protection from clinical malaria over a long follow-up period. As malaria elimination moves many areas to transmission conditions like those in the present study, these data will be highly relevant to considerations of vaccine implementation and assessments of vaccine efficacy.

Vaccines incorporating CSP, LSA-1, and GLURP-R0 have been tested in human clinical trials to varying degrees. RTS,S is the most advanced malaria vaccine with Phase III trials completed. The European Medicine Agency recently supported implementing its use in malaria-endemic settings in combination with continued use of established control measures, like bed net use. RTS,S, branded as Mosquirix™, incorporates CSP but is not long-lasting; efficacy after 4 years was 16.8%. Improving the vaccine’s efficacy is a goal that could be approached by developing a new vaccine based on RTS,S that includes other antigens, such as LSA-1. A recent vaccine incorporating LSA-1 proved safe but did not elicit protection from infection. Phase I trials of vaccines incorporating GLURP-R0 alone and in combination with MSP-3 proved safe and well-tolerated. The latter combination vaccine, GMZ2, has progressed to a multi-center Phase IIB trial in Africa. To the author’s knowledge, thus far, GLURP-R2 has not been tested in any vaccine. The present study’s results support the further use of these antigens in vaccines and we recommend exploring adding GLURP-R2 to potential novel combinations.

This study was not designed to address the mechanism of the immune responses, so no causal conclusions regarding antibody responses and protection from clinical malaria can
be drawn. Since our goal was to identify potential markers of protection, rather than draw causal conclusions, we interpreted results primarily based on the standard significance threshold rather than the Bonferroni-corrected threshold, which increases Type II error (i.e., false negatives, the chance of missing something that could be important). Our design also took advantage of the capability of the CBA to test 10 antigens simultaneously, and we felt that omitting antigens for the purpose of preserving Type I (i.e., false positive) error rates was not warranted. We tested antibody responses from samples collected in April–June 2007 and defined cases and controls using clinical malaria through June 2013; we cannot be certain that antibody levels at one time point can predict clinical malaria up to 6 years later. In low transmission settings, case numbers are a limiting factor in this type of research; our prospective data on a large cohort allowed for this testing in a nested matched case-control design. When we diagnosed the quality of the matching, the ages of cases and controls overall was well-balanced, although in the under age 5 category, after matching controls to cases within 2 years, controls were slightly younger than cases, so the adjusted analyses controlled for potential residual confounding of age. Our design was limited to the stored samples already collected, and there were insufficient potential control samples to match within a tighter range. Clinic-based surveillance can misclassify individuals as controls if they sought care outside the study clinics. However, this is unlikely as the Kipsamoite and Kapsisiywa Health Centers are the only Ministry of Health clinics in the study area, where free diagnostics and treatment have always been provided per study protocols, and travel is infrequent for members of the study cohort. The case-control study was retrospective, although this study was nested in a prospective cohort study so the samples tested for exposure and covariate data were collected prospectively and not subject to recall bias.

In conclusion, we evaluated correlates of protection from clinical \textit{P. falciparum} malaria in an unstable transmission setting, which is inherently difficult and which we believe to be
novel. We identified both pre-erythrocytic and blood-stage antigens with the property that the antibodies to them, in combination, provided more protection than any single antigen that we tested. The current direction of future research is to consider incorporating novel combinations of multi-stage antigens into a single vaccine. Identifying markers of protection in less-immune individuals is important for future vaccine development, because as malaria control efforts increase with renewed calls to eradicate malaria worldwide, transmission will continue to decrease, leaving many populations living in transmission settings similar to our highland Kenya cohort.
Table 5.5: Characteristics of studies of correlates of protection from *Plasmodium falciparum* malaria, by endemicity (high transmission-low transmission).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Location</th>
<th>Transmission setting</th>
<th>Antigens tested</th>
<th>Ages</th>
<th>Study design</th>
<th>N</th>
<th>Follow-up period</th>
</tr>
</thead>
<tbody>
<tr>
<td>John, 2005</td>
<td>Kanyawegi, Nyanza Province, Kenya</td>
<td>Holoendemic</td>
<td>AMA-1, CSP, EBA-175, LSA-1, MSP-1, TRAP</td>
<td>≥ 15 years</td>
<td>Cohort</td>
<td>68</td>
<td>14 weeks</td>
</tr>
<tr>
<td>John, 2008</td>
<td>Kanyawegi, Nyanza Province, Kenya</td>
<td>Holoendemic</td>
<td>CSP, LSA-1, TRAP</td>
<td>&gt;3 months &amp; &lt; 8 years</td>
<td>Cohort</td>
<td>86</td>
<td>1 year</td>
</tr>
<tr>
<td>McCanna, 2011</td>
<td>Kanyawegi, Nyanza Province, Kenya</td>
<td>Holoendemic</td>
<td>AMA-1, EBA-175, MSP-1</td>
<td>3 months–8 years</td>
<td>Cohort</td>
<td>87</td>
<td>1 year</td>
</tr>
<tr>
<td>Hoffman, 1987</td>
<td>Saradidi, Kenya</td>
<td>High transmission</td>
<td>CSP</td>
<td>20–45 years, one age 63</td>
<td>Cohort</td>
<td>83</td>
<td>98 days</td>
</tr>
<tr>
<td>Hogh, 1992</td>
<td>Grand Gedeh County, Liberia</td>
<td>Holoendemic</td>
<td>GLURP</td>
<td>30 days–78 years</td>
<td>Cross-sectional</td>
<td>423</td>
<td>N/A</td>
</tr>
<tr>
<td>Oeuvray, 2000</td>
<td>Dielmo, Senegal</td>
<td>Holoendemic</td>
<td>GLURP-R0, GLURP-R1, GLURP-R2</td>
<td>All ages</td>
<td>Cohort</td>
<td>214</td>
<td>1 year</td>
</tr>
<tr>
<td>Lusingu, 2005</td>
<td>Tanga region, Tanzania</td>
<td>Holoendemic</td>
<td>GLURP-R0</td>
<td>0–19 years</td>
<td>Cohort</td>
<td>171</td>
<td>6 months</td>
</tr>
<tr>
<td>Stanisic, 2009</td>
<td>Mugil and Megiar, Madang Province, Papua New Guinea</td>
<td>Highly endemic</td>
<td>AMA-1, MSP-119, MSP-2</td>
<td>5–14 years</td>
<td>Cohort</td>
<td>206</td>
<td>6 months</td>
</tr>
<tr>
<td>Richards, 2010</td>
<td>Mugil and Megiar, Madang Province, Papua New Guinea</td>
<td>Highly endemic</td>
<td>AMA-1, EBA-175, MSP-110, MSP-2</td>
<td>5–14 years</td>
<td>Cohort</td>
<td>206</td>
<td>6 months</td>
</tr>
<tr>
<td>Al-Yaman, 1996</td>
<td>Kunkingini and Apusit, Papua New Guinea</td>
<td>Highly endemic</td>
<td>MSP-142</td>
<td>6 months–15 years</td>
<td>Cohort</td>
<td>230</td>
<td>1 year</td>
</tr>
<tr>
<td>Migot-Nabias, 2000</td>
<td>Dienga, Gabon Pouma, Cameroon</td>
<td>Dienga: Highly endemic, rainforest Pouma: Perennial</td>
<td>LSA-1</td>
<td>Children (Dienga mean: 10.6, Pouma mean: 8.8)</td>
<td>Cohort</td>
<td>119</td>
<td>1 year</td>
</tr>
<tr>
<td>Migot-Nabias, 1999</td>
<td>Dienga, Gabon Pouma, Cameroon</td>
<td>Dienga: Highly endemic, rainforest Pouma: Perennial</td>
<td>MSP-1</td>
<td>Children (Dienga mean: 10.6, Pouma mean: 8.8)</td>
<td>Cohort</td>
<td>119</td>
<td>1 year</td>
</tr>
<tr>
<td>Reference</td>
<td>Location</td>
<td>Transmission setting</td>
<td>Antigens tested</td>
<td>Ages</td>
<td>Study design</td>
<td>N</td>
<td>Follow-up period</td>
</tr>
<tr>
<td>---------------</td>
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</tr>
<tr>
<td>Osier, 2007</td>
<td>Chonyi, Kilifi District, Kenya</td>
<td>High transmission, EIR: 20-100</td>
<td>MSP-3</td>
<td>All ages</td>
<td>Cohort</td>
<td>536</td>
<td>6 months</td>
</tr>
<tr>
<td>Polley, 2004</td>
<td>Chonyi, Kilifi District, Kenya Ngerenya, Kilifi District, Kenya</td>
<td>High transmission Chonyi EIR: 20-100 Ngerenya EIR: 10</td>
<td>AMA-1</td>
<td>All ages</td>
<td>Cohort</td>
<td>1,071</td>
<td>24 weeks</td>
</tr>
<tr>
<td>Dodoo, 2000</td>
<td>Dodowa, Ghana</td>
<td>Hyperendemic, seasonal, EIR: 20</td>
<td>GLURP-R0, GLURP-R1, GLURP-R2</td>
<td>3–15 years</td>
<td>Cohort</td>
<td>115</td>
<td>8 months</td>
</tr>
<tr>
<td>Dodoo, 2008</td>
<td>Dodowa, Ghana</td>
<td>Moderate and stable transmission with a seasonal peak</td>
<td>AMA-1, GLURP, MSP-1, MSP-3</td>
<td>3–10 years</td>
<td>Cohort</td>
<td>352</td>
<td>9 months</td>
</tr>
<tr>
<td>Theisen, 2001</td>
<td>Dodowa, Ghana</td>
<td>Hyperendemic, seasonal, EIR: 20</td>
<td>GLURP-R0, GLURP-R2</td>
<td>3–15 years</td>
<td>Cohort</td>
<td>104</td>
<td>9 months</td>
</tr>
<tr>
<td>Webster, 1988</td>
<td>Ubon, Thailand</td>
<td>Endemic (study period during rainy season/peak transmission)</td>
<td>CSP</td>
<td>18–35 years</td>
<td>Cohort</td>
<td>135</td>
<td>5 months</td>
</tr>
<tr>
<td>Wongsrichanalai, 1991</td>
<td>Baan Phluang, Chanthaburi Province, Thailand</td>
<td>Endemic</td>
<td>CSP</td>
<td>≥ 15 years</td>
<td>Cohort</td>
<td>300</td>
<td>1 year</td>
</tr>
<tr>
<td>Nebie, 2008a</td>
<td>Balonghin, Burkina Faso</td>
<td>Stable but seasonal, Balonghin EIR: 224</td>
<td>CSP, GLURP-R0, GLURP-R2, MSP-3</td>
<td>6 months–10 years</td>
<td>Cohort</td>
<td>Balonghin: 196–244 Tensobentenga: 116–142</td>
<td>4 months</td>
</tr>
<tr>
<td>Nebie, 2008b</td>
<td>Balonghin, Burkina Faso</td>
<td>Stable but seasonal, EIR: 0.3 during dry season, 44.4 during rainy season</td>
<td>AMA-1, GLURP, MSP-1, MSP-3</td>
<td>6 months–15 years</td>
<td>Cohort</td>
<td>286</td>
<td>4 months</td>
</tr>
<tr>
<td>Meraldi, 2004</td>
<td>Balonguen, Burkina Faso</td>
<td>Seasonal, EIR: 129 during high season, EIR: near 0 during low season</td>
<td>CSP, GLURP, MSP-3</td>
<td>6 months–9 years</td>
<td>Cohort</td>
<td>293</td>
<td>7 months</td>
</tr>
<tr>
<td>Soe, 2004</td>
<td>OoDo, Myanmar</td>
<td>Hyperendemic, seasonal, EIR: 10</td>
<td>GLURP-R0, GLURP-R1, GLURP-R2, MSP-1, MSP-3</td>
<td>All ages</td>
<td>Cohort</td>
<td>116</td>
<td>1 year</td>
</tr>
<tr>
<td>Dziegieł, 1993</td>
<td>Fula and Kataba, The Gambia</td>
<td>Seasonal</td>
<td>GLURP</td>
<td>3–8 years</td>
<td>Cohort</td>
<td>385</td>
<td>6 months</td>
</tr>
<tr>
<td>Reference</td>
<td>Location</td>
<td>Transmission setting</td>
<td>Antigens tested</td>
<td>Ages</td>
<td>Study design</td>
<td>N</td>
<td>Follow-up period</td>
</tr>
<tr>
<td>-----------</td>
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<td>------------</td>
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</tr>
<tr>
<td>Gray, 2007</td>
<td>Farafenni, The Gambia</td>
<td>Seasonal, EIR: 1-5</td>
<td>AMA-1, MSP-1, MSP-2, MSP-3</td>
<td>3–9 years</td>
<td>Cohort</td>
<td>189</td>
<td>6 months</td>
</tr>
<tr>
<td>Okenu, 2000</td>
<td>Farafenni, The Gambia</td>
<td>Stable but seasonal</td>
<td>EBA-175</td>
<td>3–9 years</td>
<td>Cohort</td>
<td>284</td>
<td>5 months</td>
</tr>
<tr>
<td>Polley, 2007</td>
<td>Basse, The Gambia</td>
<td>Endemic</td>
<td>MSP-3</td>
<td>3–7 years</td>
<td>Cohort</td>
<td>319</td>
<td>5 months</td>
</tr>
<tr>
<td>Osier, 2008</td>
<td>Chonyi, Kilifi District, Kenya</td>
<td>Endemic, EIR: 20-100</td>
<td>AMA-1, EBA-175, MSP-11, MSP-1, MSP-2, MSP-3</td>
<td>All ages</td>
<td>Cohort</td>
<td>119</td>
<td>6 months</td>
</tr>
<tr>
<td>Zhou, 2002</td>
<td>Asembo Bay, Kenya</td>
<td>Hyperendemic, EIR: 2–10</td>
<td>CSP, LSA-1, MSP-2</td>
<td>Infants</td>
<td>Cohort</td>
<td>28</td>
<td>1 year</td>
</tr>
<tr>
<td>Marsh, 1988</td>
<td>Kataba, The Gambia</td>
<td>Highly seasonal, Malaria incidence: 0.31 cases/child/yr</td>
<td>CSP</td>
<td>1–11 years</td>
<td>Cohort</td>
<td>126</td>
<td>6 months</td>
</tr>
<tr>
<td>Greenhouse, 2011</td>
<td>Kampala, Uganda</td>
<td>Mesoendemic, 2 seasonal peaks</td>
<td>AMA-1, CSP, LSA-1, MSP-1, MSP-3</td>
<td>1–10 years</td>
<td>Cohort</td>
<td>414</td>
<td>1 year</td>
</tr>
<tr>
<td>Pang, 1988</td>
<td>Mae Thawaw, Karen village, Thai-Myanmar border</td>
<td>Unstable, monthly incidence 5–25%</td>
<td>CSP</td>
<td>5–15 years</td>
<td>Nested case-control</td>
<td>51</td>
<td>25 weeks</td>
</tr>
<tr>
<td>Iriemenam, 2009</td>
<td>Gedaref town, eastern Sudan, El-Obied, western Sudan</td>
<td>Gedaref: Highly seasonal El-Obied: Unstable and seasonal</td>
<td>AMA-1 (3D7), GLURP-R0, GLURP-R1, GLURP-R2, MSP-2, MSP-3</td>
<td>All ages</td>
<td>Cross-sectional</td>
<td>545</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviations: AMA = apical membrane antigen; CSP = circumsporozoite protein; EBA = erythrocyte binding antigen; EIR = entomological inoculation rate (# infectious bites per year); ELISA = enzyme linked immunosorbant assay; GLURP = glutamate rich protein; LSA = liver stage antigen; MSP = merozoite surface protein; TRAP = thrombospondin-related adhesive protein.
Chapter 6

Summary

Malaria is a vector-borne disease caused by parasites transmitted to humans by mosquitoes. It is completely preventable and treatable, yet an estimated 198 million annual cases resulting in 584,000 deaths occur worldwide, primarily among children under age 5, because in stable transmission settings, the human immune system builds up protective immunity after years of repeated exposure to bites from infected mosquitoes. Malaria is the leading cause of morbidity and mortality in Kenya. In unstable transmission settings like the highland areas of western Kenya, malaria occurs among individuals of all ages since immunities are not built or sustained with limited exposure. With little immunity, these populations are susceptible to epidemics; about 20% of an estimated 45.9 million Kenyans live in highland settings.

The source of data and samples for this dissertation was the Kenya Medical Research Institute-University of Minnesota (KEMRI-UMN) Malaria Research Project, which is a long-standing malaria surveillance project in the highland areas of Kipsamoite and Kap-sisiywa, western Kenya that has collected prospective data on the population cohort since 2003. A 13-month (April 2007–April 2008) period of possible interruption of clinical malaria
was reported in this area after the Kenya Ministry of Health (MOH) implemented indoor residual spraying (IRS) campaigns and switched to first-line antimalarial drugs for treatment.

We were able to evaluate the implementation of the revised MOH National Malaria Strategy (NMS) specific to this highland epidemic prone zone setting, essentially at a population-level. Other evaluations of mass bed net distribution campaigns have occurred in sub-Saharan Africa, but these have primarily been conducted among a sample of survey respondents, and often are focused on bed net use, a marker for coverage, only after the campaign itself. Hence, these evaluations focused on whether the campaign itself achieved its targeted coverage levels. We were able to report this and also report on its actual impact on malaria incidence as we had prospective data already being collected on ITN use and incidence before and after the campaign.

When the NMS shifted its approach to reduced IRS and increased bed net use, following a mass insecticide-treated bed net (ITN) distribution campaign that targeted 100% coverage, bed net usage levels increased to over 50% and incidence declined in the year after the campaign. However, this increased usage was not sustained. Generalized estimating equations were used to evaluate the impact of the campaign itself on malaria incidence, and multi-level mixed effects logistic regression models were used to evaluate whether individual bed net use was associated with incidence. Both analyses showed some benefit that approached, but did not reach statistical significance. However, universal coverage of ITN, while targeted in the mass campaign, was not achieved in the study area so it is unknown whether truly complete coverage of ITN in the area would have led to a greater decrease in transmission. Some of the reasons the targeted coverage was not reached may have to do with the logistics of the campaign itself, as distribution was not uniform.

To truly evaluate whether bed net use alone is sufficient to interrupt transmission, we
need increased coverage, and to increase coverage, we need populations to both have access to and be willing to sleep under their nets. One easy recommendation would be to consult the communities in advance to learn of their bed net structure preference. In this community, it was clear that round nets would have been more appropriate to deliver than the rectangular ones that were distributed. The goal of universal coverage may also be improved with “follow-up/mop up” campaigns which seek to distribute nets to households that did not receive them (e.g., they were out of town during the initial distribution), or through “hang-up” campaigns, which is when assistance is provided to physically hang the nets in the households during the distribution campaign. Incorporating these recommendations into the planning phase of future campaigns may increase coverage and alleviate potential barriers to use.

Next, kernel density estimation methods for analyzing spatial point patterns were used to evaluate the spatial variation of clinical malaria during periods from 2003–2013, before, after, and for periods of peak incidence before and after the period of possible interruption. Heat maps were created to illustrate the estimated probabilities and relative risks, and were overlaid with asymptotic tolerance contours to identify areas with significantly elevated risk of malaria. The regions of elevated risk were not consistent during peak periods before and after interruption of clinical malaria cases. Hence, no sub-locations were identified that would warrant targeting intervention and control strategies in the study area. This was likely due to asymptomatic cases still being transmitted in the study area. Still, we do know that reaching levels of clinical malaria undetectable by surveillance is possible in the study area, so essentially elimination in this highland setting is also possible. It may just likely require a multi-stage approach. One recommendation would be to implement active case detection methods when incidence reaches low levels, which is when persons with asymptomatic infection, who still serve as reservoirs for transmission, are targeted for testing and treatment. We could do this in a mass manner, or we could essentially
“follow the case” where a symptomatic case identified at the clinic is followed home by healthcare workers who would then test members of the same household and potentially their neighbors, and treat any detected cases in the community.

Finally, a nested matched case-control study was designed to assess correlates of protection from clinical malaria in a low transmission setting, which is inherently difficult because you need a large sample size to detect associations with protection from disease. We were able to design a study to evaluate these markers in a (very) low transmission setting; it was the lowest of a comprehensive literature review conducted. Using the multiplex assay also enabled us to test antibody responses to 10 antigens simultaneously. Nearly all the studies that report correlates of protection use enzyme-linked immunosorbent assays (ELISA) and only look at up to a few antigens because it is not logistically or financially feasible to test so many using this method. Conditional logistic regression was used to evaluate the associations of antibody responses to 2 pre-erythrocytic and 9 blood stage antigens with the odds of developing clinical malaria. Positive antibody responses to two antigens, one pre-erythrocytic, liver-stage antigen-1 (LSA-1), and one blood stage, glutamate rich protein-R2 (GLURP-R2), were found to be each independently associated with a statistically significant 44% decrease in odds of developing clinical malaria for up to 6 years of follow-up. Antibody levels to LSA-1, GLURP-R2, and circumsporozoite protein (CSP) measured as a continuous outcome were also associated with a statistically significant decrease in odds of developing clinical malaria over the study period. Antibody responses to antigens in combination were more protective than antibody responses to single antigens alone. Based on a comprehensive literature review, no vaccine under development has ever incorporated GLURP-R2. Vaccines containing these antigens may be worthwhile to explore in potential novel vaccine candidate combinations.
With renewed interest among the international community to eradicate malaria, intervention measures will increase and stable transmission patterns will likely convert to unstable, leaving large new populations living in transmission intensities and having similar immunologic profiles as our highland Kenya cohort. This dissertation took a comprehensive look at the epidemiology of malaria in an epidemic prone area of highland western Kenya using a traditional, spatial, and immunological approach. It is likely that in these settings, a multi-stage approach may be required to interrupt local transmission, and that in addition to IRS and ITN campaigns, the identification and treatment of asymptomatic carriers, or mass vaccinations, may be needed to achieve malaria elimination.
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Appendix A

Appendix for Chapter 3

A.1 ITN campaign logistics survey
Form F14 - Bednet

1. Do you or anyone in your household currently own a bednet?
   - Yes
   - No  → If "No", turn page over and Skip to Q12

2. Do you currently sleep under a bednet?
   - Yes
   - Sometimes
   - No

3. Does anyone in your household currently sleep under a bednet?
   - Yes
   - Sometimes
   - No

4. When did you receive your bednet?
   - Month/Year: [ ] / [ ]

5. Who or what organization provided you with your bednet?
   - Kenya Ministry of Health
   - Other organization - specify: [ ]
   - I bought it myself
   - Other specify: [ ]

6. Where did you receive your bednet?
   - Kipsamaote Health Center
   - Kapsisiywa Health Center
   - Kapsabet Hospital
   - Someone brought it to my house
   - Other specify: [ ]

7. Who were the bed nets available to? (Mark all that apply)
   - Pregnant women
   - Children under age 5
   - Children age 5 and above
   - Women of all ages
   - Men of all ages
   - Other specify: [ ]

8. How did you learn that bed nets were available to you?
   - Announcement at Kipsamaote Health Center
   - Announcement at Kapsisiywa Health Center
   - Other announcement - specify: [ ]
   - Word of mouth
   - Other specify: [ ]

9. Was there a mass bed net distribution campaign in your community?
   - Yes
   - No  → Skip to Q11

10. When was the mass bed net distribution campaign?
    - Month/Year: [ ] / [ ]

11. Please elaborate on details regarding how and when you obtained your bed net and about any bed net distribution campaigns that have occurred in your community:

END
12. Why don’t you or anyone in your household sleep under a bed net? (Mark all that apply.)
   - I don’t own one
   - No one has offered me one
   - I don’t think malaria is a serious risk
   - Other reason(s)-specify:

13. Have there been any mass bed net distribution campaigns in your community?
   - Yes
   - No—Skip to Q20

14. When was the mass bed net distribution campaign?
   Month/Year
   M M / Y Y Y Y

15. Who or what organization was providing the bed nets?
   - Kenya Ministry of Health
   - Other organization - specify:
   - Other-specify:

16. Where did you need to go to get a bed net?
   - Kipsamoite Health Center
   - Kapsisiywa Health Center
   - Kapsabet Hospital
   - Someone would bring it to my house
   - Other specify:

17. Who were the bed nets available to? (Mark all that apply)
   - Pregnant women
   - Children under age 5
   - Children age 5 and above
   - Women of all ages
   - Men of all ages
   - Other-specify:

18. How did you learn that bed nets were available?
   - Announcement at Kipsamoite Health Center
   - Announcement at Kapsisiywa Health Center
   - Other announcement- specify:
   - Word of mouth
   - Other specify:

19. Why didn’t you receive a bed net?
   Write the reason below:

20. Please elaborate on details regarding why you do NOT use a bed net and any bed net distribution campaigns that have occurred in your community:

END
A.2 Barriers to ITN use post-hoc survey
During the 2012 demography survey, after the mass bed distribution at the Health Centre, over 50% of members in your household reported sleeping under a bed net, but during the 2013 demography survey, less than 50% reported sleeping under a bed net. We are trying to understand the reason(s) why bed net usage declined in your household and why less than 100% of members in your household sleep under a bed net.

5. Why do you think bed net usage declined between 2012 to 2013 in your household?

6. Why do you think some members in your household don't sleep under a bed net? (select all that apply)
   - They believe it is uncomfortable to sleep under a bed net (e.g., too hot)
   - They believe there are not many mosquitoes in the area (e.g., perception of low mosquito density)
   - The bed net was difficult to hang or keep in place
   - Someone else is/was using their bednet
   - They work at night
   - We use them for other domestic purposes (e.g., to confine chickens or seedlings)
   - Other reason(s) for not using bed net:

For Field Assistant to Answer:

7. Do you see the bed net(s) hanging over the sleeping areas(s)?
   - Yes How many do you see hanging?
   - No Do you see them being used for a different purpose
     - Yes if yes, for what purpose?
     - No

Comments

<table>
<thead>
<tr>
<th>Field Asst ID</th>
<th>Visit Date (dd/mm/yyyy)</th>
<th>Study ID</th>
<th>Household ID</th>
</tr>
</thead>
<tbody>
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1. How many bed nets do you currently own?

2. Are all the bed nets that you own currently being used?
   - Yes
   - No If no, why

3. How many members are in your household?

4. How many members of your household sleep under bed net each night?