

Examining Mosquito Biting Patterns and the Efficacy of Insecticide-Treated Bed Nets in Preventing Mosquito Bites in Webuye, Kenya.

by

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Thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in the Duke Global Health Institute
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ABSTRACT

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Abstract

Background: Despite widespread access and use of insecticide-treated bed nets (ITN) in Bungoma County in Kenya, there has been little reduction in malaria infection rates. It has been hypothesized that this gap between theoretical and actual ITN efficacy is caused by improper use of ITNs, poor physical condition of ITNs, or insecticide resistance in local mosquitos. This study aims to examine potential factors that affect the efficacy of the ITNs in Western Kenya.

Methods: In order to assess the aforementioned aim, a longitudinal observational study was conducted. The study enrolled 9 households and performed weekly data and mosquito collections. Data and sample collection was conducted over an 8-week duration, from June 2016 to July 2016.

Results: The study found high ITN usage in the study households (99.3% coverage), a negative association between the number of mosquitoes collected and time, a high proportion of blood fed mosquitoes (0.409), and statistically significant associations with the proportion of blood fed mosquitos and twelve different predictor variables. Conclusion: This study shows that it is feasible to examine factors reducing ITN efficacy in the area and lays down a potential template to be scaled up to examine these factors more specifically.

Dedication

I would like to dedicate this thesis to my parents who took care of me every time I got malaria as a child and have sacrificed so much for my education.

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I would also like to thank Dr. Larry Park for his help with the data management and statistical analysis as well as Betsy and Brandt for their support throughout my two years in Taylor Lab.

1. Introduction

1.1 Burden of Malaria

In 2005, the United Nations (UN) released a formalized list of global development goals called the Millennium Development Goals, with combatting malaria number 6 on the list.¹ Malaria is a mosquito-borne parasitic infection that affected 214 million people and caused close to 450,000 deaths in 2015.² The disease accounts for approximately 1.5% of the global mortality rate and approximately 2.7% of the global disability-adjusted life years (DALYs).³ Almost half of the world population is at risk for malaria infection with an estimated 3.2 billion people living in transmission zones.² The infection affects people of all ages, but children under the age of five are at the greatest risk from malaria,^{2,4} with 70% of malaria deaths occurring in this age range.⁵ The malaria burden disproportionately affects Africa, specifically the sub-Saharan region.⁴ Sub-Saharan Africa contains almost all of the global malaria burden, containing 90% of malaria cases and 92% of the malaria deaths.⁵ Malaria accounts for approximately 9.42% of DALYs and 7.83% of all the deaths in Africa.³ This concentration of the malaria burden in SSA has resulted in 70% of global malaria funding going to Africa.⁵ As a result of this funding, the malaria burden is being reduced and continental insecticide-treated bed net (ITN) coverage has risen to 53%.⁵

Like many countries in sub-Saharan Africa, malaria is a major public health issue in Kenya. The Kenyan Ministry of Health (MOH) reports that malaria accounts for 18% of outpatient doctor visits and 6% of the hospitalizations.⁶ Further, the disease causes 2.24% of deaths and 2.47% of DALYs in the country.³ Even though 80% of the population is at risk of malaria infection, the level of risk varies according to location.⁸ Areas around Lake Victoria and the coast are considered endemic and report very high rates of malaria while areas in central Kenya along the Rift Valley are considered malaria-free.^{8,9} The estimated nightly ITN coverage in Kenya is 48%, with 63% of households owning at least one net and 40% having enough nets to cover the entire household.¹⁰ Net coverage for at-risk populations is higher than the average, with 56% of children under age five and 58% of pregnant women sleeping under bed nets.¹⁰

1.2 Transmission of Malaria

Malaria infections are caused by the *Plasmodium* protozoa.² *Plasmodium falciparum* is the deadliest species of *Plasmodium* and the most prevalent in Africa.⁴ Mosquitoes become infected when they bite an infected host and ingest *Plasmodium* gametocytes.¹¹ Inside the mosquito the gametocytes go through the sporogonic cycle to become sporozoites.¹² *Plasmodium* sporozoites are transmitted to a human host via a bite from an infected female mosquito.¹³

Almost immediately after introduction to the hosts blood stream the sporozoites invade hepatocytes where the parasite matures.¹⁴ The hepatocytes eventually rupture releasing merozoites that infect erythrocytes.¹² Once inside the erythrocytes the merozoites can mature into gametocytes that can infect another mosquito that bites the human host thus continuing the infection cycle.¹²

1.3 Insecticide Treated Bed Net Efficacy and Implementation in Kenya

The World Health Organization (WHO) recommends vector control methods to prevent malaria, specifically insecticide-treated bed nets.² This is due to their low cost, ease of distribution, cost-effectiveness, and scalability.¹⁵ Insecticide-treated bed nets (ITNs) or long-lasting insecticide-treated nets (LLINs) are nets that have been treated with pyrethroid class insecticides and are hung over sleeping spaces.¹⁶ ITNs are inexpensive and cost effective, costing between \$4-10 per DALY averted.¹⁷ These nets act as both physical and chemical barriers to mosquitoes and provide the additional benefit of reducing vector populations.^{16,18} ITNs also have exito-repellent properties as they deter mosquitoes from entering houses and cause an increased rate of mosquito exophily (leaving the house).¹⁹ These factors make them very effective at preventing malaria, providing a 45% protective efficacy in randomized control trials.²⁰ The protective effects extend outside the individual household, providing

additional protective effects for the entire community.²¹ Studies have shown that the higher the communal ITN coverage, the lower the communal parasite prevalence, parasitemia, and anemia,²⁰ even in households without ITNs.²¹

ITN efficacy can be measured by calculating the human biting index (HBI), the proportion of mosquito blood meals that contain human DNA, in randomized control trials.²² To calculate the human biting index a genetic analysis is done on the mosquito blood meals to determine if they contain human DNA.²²

In the early 2000's, Kenya implemented nationwide programs and policy changes to decrease the malaria mortality and morbidity rates.¹⁰ Following WHO recommendations, Kenya attempted to increase ITN ownership,¹⁰ which was at 6% at the time.²³ In order to do so, ITN prices were subsidized, large scale ITN distribution initiatives occurred in malaria endemic regions, and free ITNs were dispensed to pregnant women at antenatal check ups.¹⁰ Additionally, free artemisinin combination therapy was made available at all public health centers.^{10, 23} Distribution campaigns are still ongoing with close to 11.6 million ITNs being distributed in 2015.⁵ These efforts have been successful, increasing ITN ownership from 6% at implementation to 44% in 2010 and 63% in 2015.¹⁰

The efforts to reduce malaria were initially successful, reducing the numbers of cases and deaths significantly between the years 2005 to 2010.⁵ These

reductions were not nationwide, as certain areas have shown little to no change in response to the vector control methods.²⁴ Malaria is now back on the rise in Kenya, with the number of cases rising from 3.3 million in 2010 to 6.5 million in 2015.⁵ The resurgence of malaria is being seen in Western Kenya,²⁵ where infection rates are highest.⁹

There are a variety of factors that could be contributing to this resurgence of malaria. Human noncompliance and misuse of ITNs,²⁶ vector biting behavior changes,²⁷ and poor ITN condition are all potential factors that decrease the efficacy of ITNs.²³ Another key factor is the increased insecticide resistance observed in western Kenya.²⁸ Only one class of insecticides (pyrethroids) are approved by the WHO for use in ITNs,²⁹ making them more vulnerable to resistance. Insecticide resistance significantly reduces ITN efficacy,²⁴ and can occur via a variety of mechanisms: target-site resistance, metabolic resistance, and cuticular resistance.¹⁸

1.4 Entomology of *Anopheles* spp. and *Culex* spp.

Malaria in sub-Saharan Africa is transmitted by *Anopheles* mosquitoes, but different species of *Anopheles* transmit the parasite at different rates with the subspecies *Anopheles gambiae* being the primary malaria vector.³⁰ Mosquito populations are dependent on rainfall and temperature, normally peaking in Kenya in the middle of the rainy season around June.³¹ Different species of

Anopheles peak at different times due to different larval site preference.³² The *A. gambiae* ssp. is the most efficient at transmitting malaria in part due to the *Plasmodium falciparum* parasite influencing the mosquito's biting patterns and increasing the frequency of multiple host feeding.³³ Understanding vector density and what factors such as this affect mosquito biting is integral to combating malaria.

While the *Anopheles* species have some biting behaviors in common such as feeding primarily at night³² and feeding at low levels close to the ground,³⁴ these behaviors also vary greatly across the different species of *Anopheles*. Biting behavior and species role in malaria transmission vary across locations depending on available hosts, host density, and vector species densities.³¹ *A. gambiae* ssp. is highly anthropophilic even in areas with high ITN coverage whereas *A. arabiensis* and *funestus* ssp. are more zoophilic, especially in areas of high ITN coverage.^{30, 31} *A. arabiensis* ssp. primarily bite outdoors while other species bite indoors.^{31, 34} The use of ITNs and indoor residual spraying (IRS) is also affecting mosquito biting behaviors, resulting in a shift towards higher outdoor biting rates not previously seen in certain species as well as higher exophily.^{30, 34}

Although *Culicine* mosquitoes do not transmit malaria, they can be useful for studying ITN efficacy. *Culex* mosquitoes, like *Anopheles*, feed between dusk

and dawn,³⁵ bite at low levels close to the ground,³⁴ and display location dependent biting behaviors according to similar locational characteristics.³⁶ However, *Culex* mosquitoes are less anthropophilic than *Anopheles* and are considered more opportunistic feeders, which results in their biting behavior being heavily dependent on the host availability and density.³⁷ This difference become less pronounced in areas of human density where human biting rates between *Anopheles* and *Culex* are comparable.³⁵ These similarities make *Culex* a sympatric species that can be studied alongside *Anopheles*.³⁷

1.5 Rationale and Study Aims

The resurgence or complete resistance of malaria incidence rates to vector control methods in western Kenya raise serious questions, specifically why vector control methods that are successful in other areas are not working. Determining the reason for the gap between the expected ITN efficacy and actual ITN efficacy is essential in order to reduce malaria in the area.

This study aims to examine potential factors that affect the efficacy of the ITNs in Western Kenya. We hypothesized that the number of people sleeping in each sleeping space will have the highest correlation with the proportion of blood fed and half gravid mosquitoes. This is surmised due to the increasing attractive affects more people would have as well as the increased potential for

through the net biting due to reduced sleeping space area and the high levels of insecticide resistance in the area.

This study is unique in its approach of examining the correlations between the proportion of blood fed mosquitoes and household demographics/ITN characteristics. Findings from the study have the potential to provide invaluable information that could inform future studies and policies to more effectively study and reduce malaria in western Kenya.

2. Methods

2.1 Setting

The study was conducted in Bugoma East sub-county in Western Kenya, approximately 50 kilometers east of the Ugandan border. The sub-county has an estimated population of 230,000 approximately 40,000 of whom live in a small town center called Webuye.³⁸ The area has a relatively low socioeconomic status, shown by approximately 61% of the population living below the poverty line.³⁸ Educational attainment is low, demonstrated by only 21% of residents having a secondary education or above.³⁹ Most houses in the area are made of mud and wood with corrugated iron roofing sheets, use firewood as a cooking fuel, and use tin kerosene lamps for lighting.³⁹ A majority of the population practice farming as their occupation.³⁹ The people are primarily of the Luhya ethnic group⁴⁰ and children aged 0-14 account for almost half the population.³⁹ Bungoma East has endemic malaria, exhibiting seasonal peaks from March to June following the rainy season.⁴¹ ITN coverage is estimated at 67%.²³

All field mosquito sorting took place at the Webuye Health and Demographic Surveillance Site (HDSS).

2.2 Participants

Areas with high malaria transmission rates were identified using information from previous malaria studies²³ in the area and local community

health workers. Three villages within these areas were selected and one household within each village was enrolled. Once the primary household had been identified, two further households at each village were enrolled in the study.

Households were eligible for enrollment if they were in one of the areas identified with high biting rates, had at least four members, and had a household head over 18 years of age. Households were excluded if the household head was mentally handicapped and/or unable to give consent.

To enroll households, a study team traveled to each potential household to perform a house visit. Household consent was garnered from the household head or secondary household head. A research assistant explained the project in detail to the household and performed a demonstration mosquito collection. Additionally, the households were informed that they could ask the collections to cease at anytime during the study. A copy of the study protocol in both Swahili and English were left with each household along with the contact information of the study coordinators.

2.3 Procedures

2.3.1 Sample Collection and Assessment

Sample collection commenced the week after households were enrolled in the study and were collected over the course of 7 weekly collections. The day

before a collection, the household head was notified via a telephone call that the collection would be taking place the next day. They were requested to keep all doors and windows closed as well as leave all ITNs down until the collection had taken place to keep mosquitoes from escaping from the house.

All sample collection was conducted between the hours of 7 AM and 9 AM. The study team traveled to the sites via vehicle or motorbike. Upon arrival at a study household, the household members exited the house and the mosquito collections were conducted using Prokopack mosquito aspirators. The Improved Prokopack Aspirator Model 1419 is a mosquito aspirator used for indoor resting mosquito collections. The aspirator was designed to be small, lightweight, cost effective, and easily maneuverable while providing the same aspiration capacity as the CDC Backpack Aspirator.^{42, 43} The prokopack was found to be more effective at collecting resting mosquitoes than the CDC Backpack Aspirator.⁴²

Households were aspirated systematically by room. Collection cups being changed for each room and were labeled by household, room, date, and time. The collections cups were then placed in coolers with ice packs in order to slow digestion of the blood meal. Samples were transported back to the Webuye Health and Demographic Surveillance Site and immediately placed in a freezer in order to kill the mosquitoes.

Collection cups were removed by household from the freezer after roughly 30 minutes. If the mosquitoes inside the collections cups were still alive after being removed from the freezer, they were asphyxiated by covering the collection cups in cotton wool with chloroform. When all the mosquitoes inside the collection cups were dead, the cups were emptied out onto pieces of paper where a trained research assistant separated them according to sex and species using forceps. Female mosquitoes were further sorted according to blood meal status.

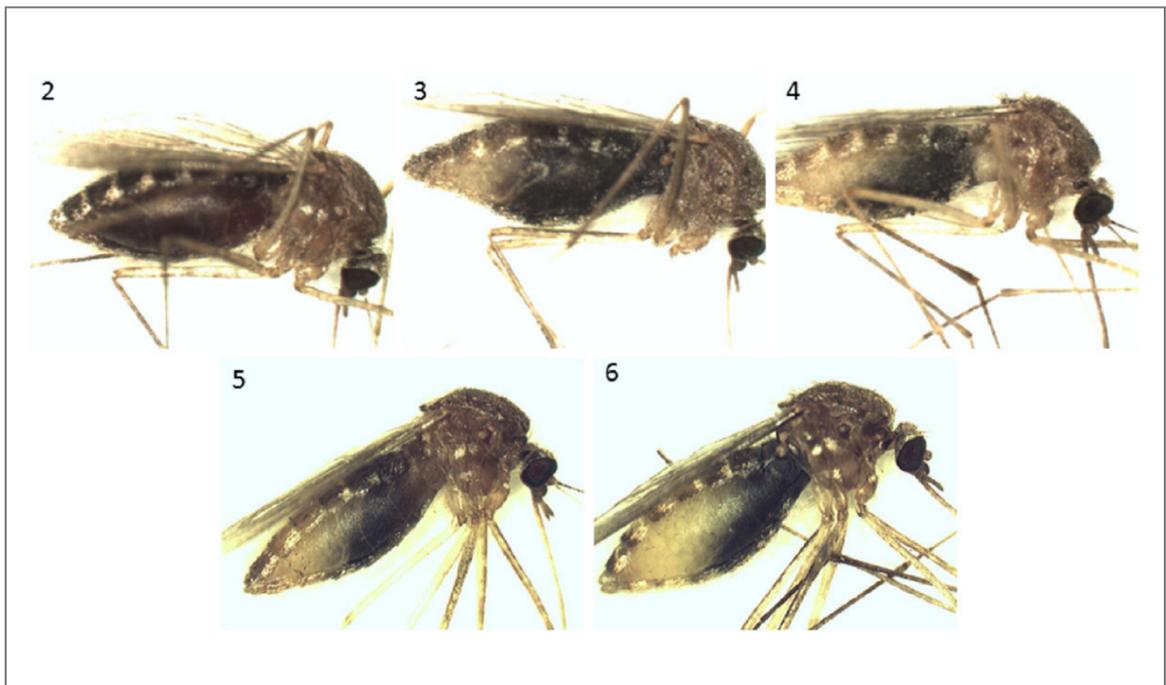


Figure 1: Female *Culex pipiens* sorted according to blood meal status. The numbers indicate the sequential progression of the blood meal digestion with 2 being blood fed and 6 approaching fully gravid.⁴⁴

The numbers of each species, sex, and blood meal status were counted and recorded. Mosquito species was determined by differences in coloration on wings and palp morphology.⁴⁵ Mosquito sex determination is possible with the naked eye due to males possessing antennal flagellum on their antennae.⁴⁵ Blood meal status was assessed via visual inspection and estimation of proportion of the blood meal that had been turned into eggs. A mosquito was considered fed if the abdomen was less than 10% gravid, half gravid if the abdomen was between 10% and 66% gravid, and gravid if more than 66% gravid. These proportions were chosen to select mosquitoes that had fed within the last day. It takes approximately two days for a female *Anopheles* to digest a blood meal and become fully gravid.⁴⁵

All *Anopheline* females and fed or half gravid female *Culex* were individually logged. All male mosquitoes were discarded due to the fact that they do not consume blood.

2.3.2 Data collection

Study data was collected via two surveys and weekly collection questionnaires. The first survey was conducted as soon as the household head had consented to participate in the study. This survey recorded household member information, the number of sleeping spaces, the presence of bed nets, and structural data on the house. The second survey was conducted after the

second collection. Household heads or secondary heads were asked to set up the sleeping spaces as they were every evening. The survey then recorded extensive variables about the bed nets. Weekly collection questionnaires were also completed. These questionnaires recorded the collection times, who slept in each sleeping space, and how many nights the previous week the bed net was used.

2.3.3 Ethical Considerations

Ethical clearance was obtained from the Duke Medicine Institutional Review Board for Clinical Investigations (IRB#: Pro00071523) and the Moi University Institutional Research and Ethics Committee (IRB#: IREC 1641). Data was stored in a secure box folder and all paper copies kept in a locked cabinet. At the end of the study, bed nets in poor condition were replaced and participating households were thanked with a gift of school exercise books, pens, and pencils for all the school-age children.

2.4 Analysis

The data was initially recorded on paper forms then entered into a Microsoft Excel spreadsheet stored in a secure Box folder. Data was analyzed using Stata 14 (Version 14.2). Descriptive statistics were used to describe household and household head demographics as well as ITN characteristics. The descriptive statistics are the predictor variables. Due to the observational nature of the study and small number of households no statistical tests were performed

to compare characteristics of households or villages. Missing data was omitted from analysis.

To calculate the household ITN coverage over the course of the study, the study person days spent sleeping under a net was calculated using data collected in the collection form. The number of person days under a net was then divided by the total number of person days in the study to yield the proportion of nights spent under an ITN. Missing data was omitted from analysis.

Descriptive results of the mosquito collections were also compiled using Stata 14 (Version 14.2). The proportion of fed and half gravid females (BFP) was calculated for each species and the aggregate total. This was calculated by dividing the number of fed and half gravid by total number of females caught. The blood fed proportion (BFP) was calculated for each collection giving a total of 63 collection proportions. Males were not included in analysis. Missing data was omitted from analysis.

$$\text{Blood fed proportion (BFP)} = \frac{\text{Number of fed and half gravid females}}{\text{Number of females collected}}$$

Due to the continuous nature of the BFP and a non-normal distribution of data it was necessary to use a regression model to evaluate the correlation between the outcome and predictor variables. The possibility of unknown correlation between collection BFPs causes the potential for bias. To address this, a generalized estimating equation (GEE) regression was used. GEE is an

appropriate model to address the potential correlation due to its ability to calculate accurate regression coefficients and standard errors without a specified covariance structure.⁴⁶

To assess the change in number of mosquitoes collected by collection week, a GEE regression was used. The outcome variables were the numbers of mosquitoes captured at each collection (male, female, fed/half gravid, and BFP) and the predictor variable was the number of weeks since the commencement of the study. Three separate models were run using the numbers of *Culex*, *Anopheles*, and aggregate totals as continuous outcomes. An independent correlation structure was used when running the GEE. Missing data was omitted from analysis. Associations were considered statistically significant if $p < 0.05$.

A GEE regression was also used to examine the association between BFP and household, household head, and ITN characteristics. The outcome variable was the BFP and household, household head, and ITN characteristics were the predictor variables. Three separate models were run using *Anopheline*, *Culex*, and aggregate BFP as continuous outcome variables. An independent correlation structure was used when running the GEE. Missing data was omitted from analysis. Associations were considered statistically significant if $p < 0.05$.

3. Results

3.1 Household Descriptive Statistics

The nine study households were divided into clusters of three in three different locations. There were a total of 53 household members. The mean household size was 5.84, the mean number of children per household was 4, a third of whom were under the age of five. The mean age of the household members was 16.52 and 58.5% (31/53) of the members were female.

Each household had a designated household head and eight had a secondary head. The mean age of the household heads was 42.4 years old. Household heads were 77.8% (7/9) male and 22.2% female (2/9). Secondary heads were spouses or adult children of elderly household heads. The mean age of the secondary heads was 30.3 years old and all of them were female (8/8).

Seven collections took place at each household, resulting in 21 at each location and 63 total. Households contained an average of 2.1 sleeping spaces per household and an average of 2.98 people per sleeping space. At the commencement of the study, every sleeping space had an ITN (18/18). One sleeping space was created mid-study and did not have an ITN. The sleeping space's ITNs were an average of 23.17 months old, had an average area of holes of 244.85 square centimeters, and were washed in 55.6% (5/6) of the households.

Table 1 summarizes the household demographics and Table 2 summarizes the characteristics of the households and sleeping spaces.

Table 1: Household Descriptive Statistics

	<i>Total, Percent</i>	Total
Households		9, 100%
Household Size: Mean (SD)		5.84, 1.65
Number of Children in Household: Mean (SD)		4, 1.50
Age of Children: Mean (SD)		6.61 (2.09)
Number of Children 5 or younger		1.33 (0.67)
Participants		53, 100%
Age: Mean (SD)		16.528 (16.12)
Sex		
	<i>female</i>	31, 58.49%
	<i>male</i>	22, 41.51%
Education Level		
	<i>none</i>	19, 35.85%
	<i>some primary</i>	22, 41.51%
	<i>completed primary</i>	7, 13.21%
	<i>some secondary</i>	2, 3.77%
	<i>completed secondary</i>	3, 5.66%
Employment		
	<i>none/farmer</i>	13, 24.53%
	<i>self employed</i>	3, 5.66%
	<i>job</i>	1, 1.89%
	<i>na</i>	36, 67.92%

Table 2: House Structure and ITN Descriptive Statistics.

	<i>Total, Percent</i>	Total
Number of Collections		63, 100%
Sleeping Spaces (SS)		
Number of SS		19 ,100%
Average Number of SS per Household: Mean (SD)		2.11 (0.61)
Average Number of People per SS: Mean (SD)		2.98 (0.66)
Average SS Area Per Person: Mean (SD)		0.71 (0.15)
Insecticide Treated Nets (ITN)		
Average Area of Holes in ITN (sq. cm): Mean (SD)		244.85 (426.53)
Total household ITN hole area (sq. cm): Mean (SD)		491.25 (852.22)
Average Age of ITN (months): Mean (SD)		23.17 (9.66)
Household washes ITN		
	<i>yes</i>	5, 55.56%
	<i>no</i>	4, 44.44%
Structural		
	<i>open eaves and uncovered windows</i>	4, 44.44%
	<i>closed eaves and uncovered windows</i>	4, 44.44%
	<i>closed eaves and covered windows</i>	1, 11.11%

3.2 Mosquito Descriptive Statistics

Table 3 describes the results from the mosquito collections. Seven collections took place at each household, resulting in 21 at each location and 63 total. A total of 294 *Anopheles* were collected, representing 5.25% of the total mosquitoes collected. Females accounted for 40.5% (119/294) of the collected *Anopheles*. The proportion of anopheline females that were blood fed or half gravid was 0.513 (61/119).

A total of 5309 *Culex* were collected representing 94.75% of the total mosquitoes collected. Females accounted for 42.6% (2260/5309) of the collected *Culicine*. The proportion of anopheline females that were blood fed or half gravid was 0.404 (912/5309).

A total of 5603 mosquitoes were captured over the course of the study. Females accounted for 42.3% (2379/5603) of them. The proportion of female mosquitoes that were fed or half gravid was 0.409. The BFP was negatively associated with an increase in the number of mosquitoes caught in a collection (Figure 2).

Table 3: Mosquito Descriptive Statistics

	<i>Total (Collection Mean, SD)</i>	Total
Anopheles		294 (4.667, 7.40)
Male		175 (2.778, 5.20)
Female		119 (1.889, 2.76)
Fed/Half Gravid		61 (0.968, 1.31)
<i>Proportion of Females Fed/Half Gravid</i>		0.513 (0.60, 0.35)
Culex		5309 (84.269, 104.65)
Male		3049 (48.397, 72.81)
Female		2260 (35.873, 37.58)
Fed/Half Gravid		912 (14.476, 17.49)
<i>Proportion of Females Fed/Half Gravid</i>		0.404 (0.28, 0.22)
Total Mosquitos		5603 (88.967, 104.45)
Male		3224 (51.17, 72.21)
Female		2379 (37.76, 38.15)
Fed/Half Gravid		973 (15.444, 18.01)
<i>Proportion of Females Fed/Half Gravid</i>		0.409 (0.44, 0.22)

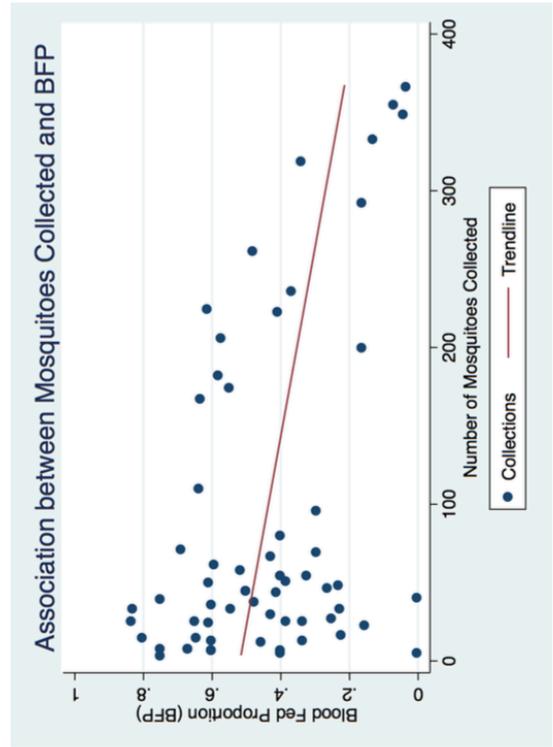
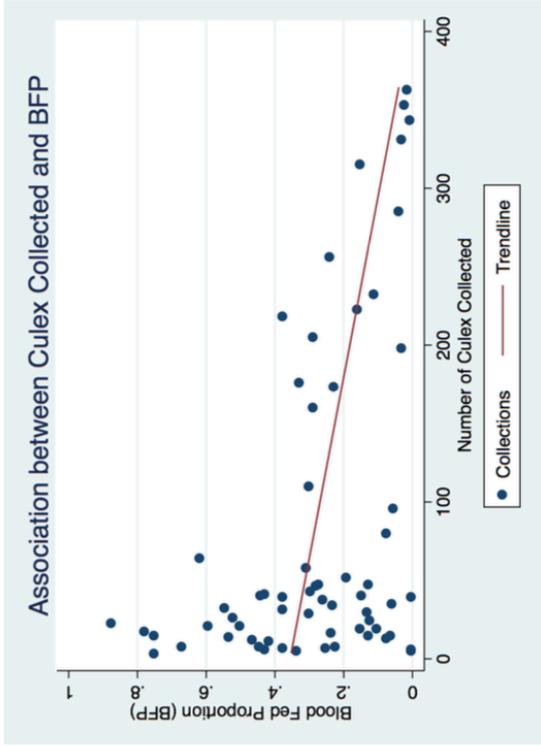
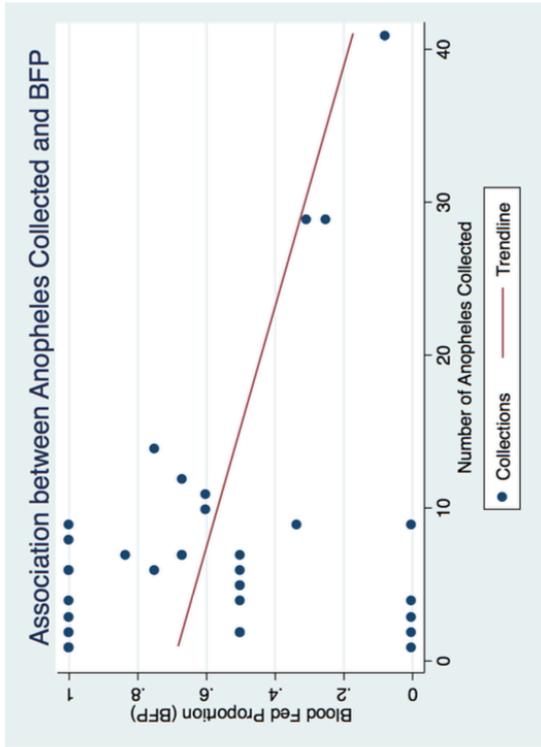


Figure 2: This figure examines the association between mosquitoes collected and the collection BFP. The first panel examines the association with the collection *Anopheles* BFP and number of *Anopheles* collected. The second panel examines the association with the collection *Culex* BFP and number of *Culex* collected. The third panel examines the association with the collection aggregate BFP and total number of mosquitoes collected. All three associations are statistically significant negative associations.

3.3 ITN Coverage

All the household in the study owned at least one ITN giving the study 100% household coverage. Sleeping space coverage was high as well with only one sleeping space out of the 19 in the study did not have an ITN. The study households had very high rates of ITN compliance. There were a total of 2527 of participant person days in the study, including 2464 (97.5%) person days under an ITN. Two of the three locations/villages in the study had 100% ITN compliance.

3.4 Association between number of mosquitoes collected and time.

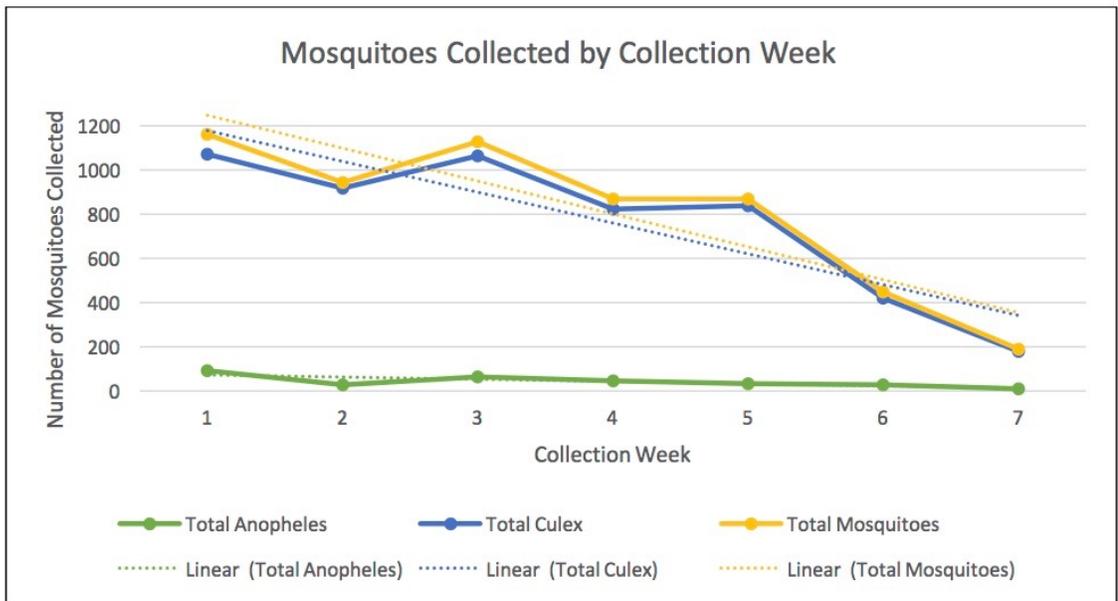
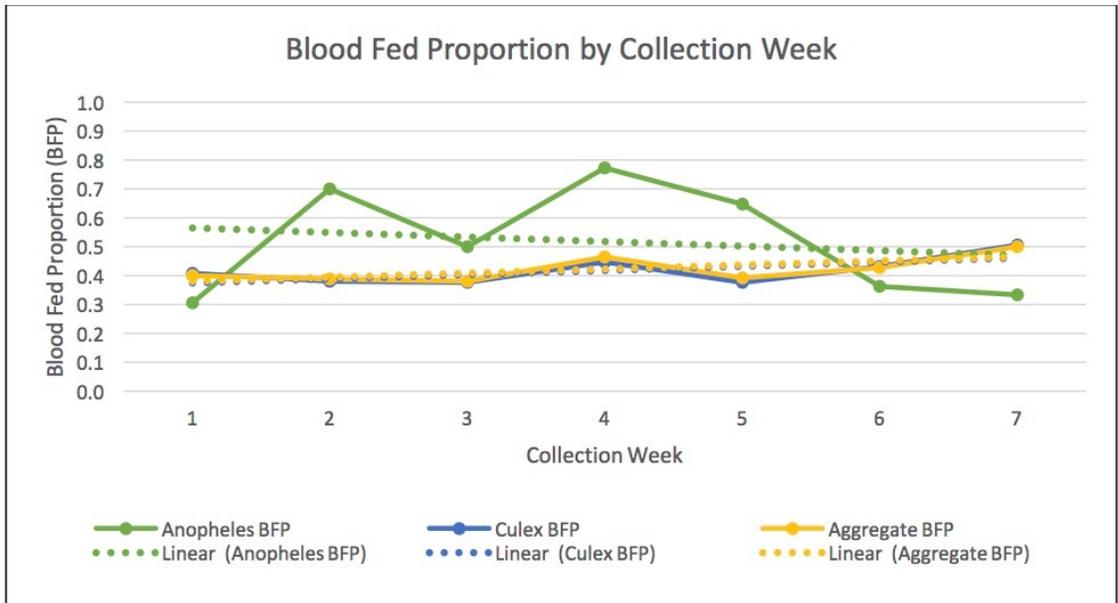
Collection totals for *Anopheles*, *Culex*, and the aggregate total of both species were significantly negatively associated with time (Figure 3). *Anopheles* collection totals were associated with a 1.091 (Coef = -1.091, 95% CI = -1.956, -0.226) decrease in mosquitoes collected per week. *Culex* were associated with a 15.476 (Coef = -15.476, 95% CI = -27.710, -3.242) decrease in mosquitoes collected per week. The aggregate total of the two species was associated with a 16.567 (Coef = -16.567, 95% CI = -28.689, -4.446) reduction in mosquitoes collected per week.

In addition to the associations to the total number of mosquitoes, negative associations were also found in various subpopulations of the collection totals. In collected *Anopheles*, significant negative associations were also observed in the number of males (Coef = -0.694) and females (Coef = -0.397). Among collected *Culicine*, significant negative associations were observed in the number of females collected (Coef = -7.294) and the total number of females that were fed or half gravid (Coef = -2.738). Significant negative associations were observed in in the aggregate total number of males (Coef = -8.877), females (Coef = -7.690), and fed or half gravid females (Coef = -2.877).

Appendix A Table 4 summarizes the numbers of mosquitoes collected by week (Appendix A Table 4a) and the results of a GEE regression examining the

association between the number of mosquitoes collected and time (Appendix A Table 4b).

Figure 3:



This figure examines the correlation between mosquito collection statistics and time. The first panel shows the correlation between the BFP and collection week. There was no significant statistical association between BFP and collection week. The second panel shows the correlation between the number of mosquitoes collected and collection week. There is a statistically significant reduction in the number of mosquitoes collected by collection week.

3.5. Blood Fed Proportion Regression Results

A GEE regression results revealed statistically significant associations between the proportion of blood fed and half gravid females (BFP) and various household demographic and sleeping space characteristics. The analysis was done for both species using the appropriate BFP as well as the aggregate total and aggregate BFP.

The regression results rejected the study hypothesis that the number of people within a sleeping space would be associated with a higher blood fed proportion. An increase in the number of people in a sleeping space was significantly associated with a decrease in blood fed proportion for both the *Culex* (Coef = -0.126) and aggregate BFP (Coef = -0.120). These coefficients indicate that there was a decrease of 0.126 and 0.120 in *Culex* and aggregate BFP for each additional member of a sleeping space.

3.5.1 *Anopheles* BFP

There were no significant associations found between the *Anopheles* BFP and the household demographic or sleeping space characteristics.

3.5.2 *Culex* BFP

Culex BFP was significantly associated with six of the household demographic variables collected. Average household age (Coef = -0.0153),

average age of adults in the household (Coef = -0.0058), age of the household head (Coef = -0.0049), and being employed outside the home (Coef = -0.2503) were associated with a statistically significant decrease in BFP. An increase in BFP was significantly associated with being self employed (Coef = 0.3415) and the number of years of education of both the household head (Coef = 0.0242) and secondary head (Coef = 0.0599). The associations with household and secondary household head were no longer significant when the education level was made categorical.

Five of the examined structural and sleeping space characteristics had statistically significant associations with *Culex* BFP. The sleeping spaces being located in a building with closed eaves and covered windows (Coef = -0.2054) and the number of people in a sleeping space (Coef = -0.1257) were associated statistically significant reduction in BFP. A statistically significant increase in BFP was correlated with average sleeping space area per person (Coef = 0.6325) and both average hole area in household ITNs (Coef = 0.0231) and total area in household ITNs (Coef = 0.0116).

3.5.3 Aggregate BFP

Aggregate BFP was significantly associated with six of the household demographic variables collected. Average household age (Coef = -0.0150, average age of adults in the household (Coef = -0.0046), average age of children in the

household (Coef = -0.0429), age of the household head (Coef = -0.0043), and the household head being employed outside the home (Coef = -0.3640) were associated with a statistically significant decrease in BFP. A statistically significant increase in BFP was correlated with secondary household head education level in years (Coef = 0.050) and households with household heads that were self employed (Coef = 0.1575).

Only one sleeping space characteristic had a significant statistical correlation with the aggregate BFP. The observed association was a decrease in BFP with an increase in the number of people per sleeping space (Coef = -0.1202). Two of the three categories of three categorical classification of average sleeping space area, 0.6-0.69 square meters (Coef = 0.2331) and greater than 0.8 square meters (Coef = 0.1497), were associated with an increased BFP.

The GEE regression results for the comparison of the proportion of blood fed and half gravid females (BFP) and various household demographic and sleeping space characteristics is shown in more detail in Appendix A Table 5.

4. Discussion

4.1. Main Findings

The study found high ITN usage in the study households, a negative association between the number of mosquitoes collected and time, a high proportion of blood fed mosquitoes, and statistically significant associations with BFP and twelve different predictor variables. ITNs were used for 97.5% of the person time in the study. A statistically significant negative association was found between the number of mosquitoes collected and the collection week, and no association was observed between BFP and the collection week. 40.9% of the female mosquitoes captured were fed or half gravid. Age predictor variables were negatively correlated with BFP while years of education were positively correlated with BFP.

4.1.1. Net Coverage

The household ITN coverage found in the study was much higher than the estimated national coverage. ITN ownership in Kenya is estimated at 63% of households, with 40% of households having enough nets to cover all members of the household.¹⁰ Estimated national nightly ITN utilization is 48%.¹⁰ ITN coverage in the study was approaching 100%, with all study households owning at least one ITN and having an ITN for every sleeping space. ITN nightly utilization during the study was 97.5%. This difference between the national average and

study average could be explained by several factors. First, the households were in an area where ITNs are distributed for free. This is supported by the fact that none of the households paid for any of their ITNs. Second, since the study used data from the Obala et al.²³ study to select areas of high transmission, a number of the households participated in both studies. The Obala et al.²³ study replaced/distributed ITNs for the households participating in the study thereby increasing the coverage above the national average. The lack of households without ITNs resulted in there being no households to use as negative controls to compare how ITN coverage affects BFP.

4.1.2. Mosquito Data

This study used the proportion of blood fed and half gravid mosquitoes to estimate the feeding success rate of the female mosquitoes. This method introduces potential error as mosquitoes feed on cattle and other household animals as well. Studies often use a human biting index (HBI) to estimate the proportion of blood meals that are from a human host. This was not done for this study but previous studies found *Anopheles*' HBIs of 98.29% and 95.9%,^{47,48} with the *Anopheles* HBI expected range being 80%-100%.³⁶ This proportion changes according to location, density of animals and humans, and mosquito species densities.³⁶ *Culex* are less anthropophilic than *Anopheles* and considered more of an opportunistic feeder resulting in highly variable HBIs depending heavily on

density of animals and humans.³⁷ Entomological studies in Kenya have estimated *Culex* HBIs to be between 12-88%.^{37, 49, 50}

The number of females per collection and proportion of blood fed or half gravid mosquitoes was 1.89 females per collection (51.3% fed/half gravid) for *Anopheles* and 35.873 females per collection (40.4% fed/half gravid) for *Culex*. These values both fall in the range of number of females per collection from previous studies 0.1 – 62.^{24, 36, 48} This variability is highly dependent on household proximity to breeding grounds.³⁶ The BFP of the mosquitoes captured in this study was much higher than previous studies in the same area, with previous BFPs ranging from 0.09 to 0.253.^{23, 26, 51} This higher proportion could be due to using a prokopack to perform collections. Previous studies used light traps, human landing catches, and permethrin spray catches. Prokopack aspiration catches select for blood fed females resting on walls,⁴² potentially causing this difference. Other entomological factors like mosquito density or exophily, as well demographic factors and household/human density could also play a role in this difference.

4.1.3. Temporal Regression Data

There was a statistically significant negative association between the number of mosquitoes collected and the date of the collection. This is likely caused by the seasonal differences in mosquito density caused by the rainy and

dry seasons in Kenya. The collections commenced in mid-June, which is towards the end of the long rains and right at the peak of mosquito density.³⁶ No statistically significant association was observed between the BFP and the date of the collection, inferring that even though mosquito populations are diminishing, their biting success remains the same.

4.1.4. Regression Results

Anopheline BFP was not significantly associated with any of the household demographic or sleeping space characteristics. The small numbers of female *Anopheles* collected in addition to numerous collections where no *Anopheles* were collected resulted in missing data. Due to the missing data, the GEE regression was only performed on 57.1% (36.63) of the collection BFPs resulting in little to no statistical power.

Six different household demographic variables were significantly associated with both *Culex* BFP and aggregate BFP. Household age variables accounted for 3 of the significant *Culex* BFP associations and 4 of the significant aggregate BFP associations. All the household age variables regardless of mosquito species showed decreased BFP with increased age. The protective effects of increased age of households, household heads, adults, and children could be due to a combination of factors associated with increased age such as higher socio-economic status (SES).

SES is a critical factor for malaria risk, with increased SES being associated with reduced infection rates and lower biting rates.^{52, 53} This association is observed in two of the three household demographic variables tied to SES with improved housing structure and employment showing negative associations with BFP. This is in agreement with not just the studies that show the protective effects of increased SES, but also with other studies which show that poor housing structure increased odds of malaria infection²³ and biting rate.⁵² Interestingly, this study found that BFP was positively associated with the number of years of education of both the household head (*Culex* only) and the secondary head (both *Culex* and aggregate). This association is contrary to most other studies which find that increased caregiver education is associated with decreases in malarial infection rates.⁵³ This association could possibly be explained by the small sample size and the majority of the household heads and secondary heads having low education levels.

Four sleeping space variables were significantly associated with *Culex* BFP and one was associated with aggregate BFP. Lower numbers of people per sleeping space and increased sleeping space area per person were associated with an increase in BFP. This association contradicts not only the proposed hypothesis but findings from other studies showing that increased numbers of people in the same sleeping space result in higher numbers mosquitoes captured

in the room.^{54, 55} Additionally, studies have shown that increased numbers of people in houses also increases the number of mosquitoes caught.⁵⁶ This is believed to be the case due to the increased attractive effects of humans and would therefore increase mosquito density in the house.

The condition of an ITN plays an important role in its ability to protect the people who sleep under it. While the insecticidal properties still play a protective role even when the ITN is in poor physical condition, insecticide resistance nullifies these protective effects. The study showed associations between increased hole area in household ITNs and increased *Culex* BFP. This association is congruent to results from other studies, including the Obala et al.²³ study that preceded this one.

4.2 Study strengths and limitations

One of the study's main strengths was the use of an effective collection method for mosquitoes. The prokopack is designed for cost effectiveness and mobility, which are fundamental for the study setting and methods. Additionally, product assessments have shown it to be more effective than the CDC backpack aspirators at collecting resting mosquitoes,⁴² making it better suited for this study than the CDC standard. The study was also successful at collecting large amounts of data that can be used as both predictor and outcome variables in a short amount of time with limited funding.

This study was constrained by limited funding, time, and modest sample size. Additionally, the observational nature of the study in combination with the lack of controls means that the study cannot prove causative relationships between BFP and predictor variables. The study start date was delayed due to school scheduling, resulting in the study commencing past the anopheline vector density peak. This delay resulted in too low a number of Anopheline BFPs to statistically analyze. Further, past participation of households in previous studies is a potential confounder for ITN coverage results.

4.3 Implications for policy and practice

Kenya's efforts to increase ITN coverage has been crucial to decreasing the country's burden of malaria but more needs to be done in areas where ITNs are not working. Current practices and policies for resistant areas need to be changed in order to appropriately address the issue.

The theoretical efficacy of ITNs can be undermined by a multitude of factors, ranging from communal adherence/coverage, household compliance, and ITN condition to vector susceptibility and breeding ground proximity.²³ Therefore, increasing coverage is simply the first step in the fight against malaria. Once coverage has been increased, policies and interventions should be implemented that confront the issues that reduce ITN efficacy. Potential interventions or policies can approach resistance from multiple angles. Policies

that identify and research resistant areas can be enacted. Information campaigns that educate people on proper ITN use or how to identify and destroy potential breeding grounds can be run. Additional vector control methods that include indoor residual spraying can be implemented in tandem with the ITN distributions in highly resistant areas to reduce the malaria burden. By addressing these factors ITN efficacy will be improved and malaria rates should be reduced as a result.

4.4 Implications for further research

The implications for further research are extensive. This study is in essence a pilot study for a larger scale study and was designed to inform future research on this issue. A larger study could run similar methods on a larger scale in order to better examine the issue and account for the weaknesses in this study. Namely, a larger study population would allow for increased statistical power and stronger conclusions.

In order to more accurately assess BFP, genetic analysis should be done on the mosquito blood meal. This genetic analysis should include a polymerase chain reaction (PCR) assay to assess the source of blood meal thereby enabling a HBI to be calculated. Additionally, short tandem repeat analysis (STR) could be run on the blood meals in order to obtain a genetic fingerprint of each blood meal. The STR results from the blood meals could then be used to match them to

the household member bitten and thereby calculate an accurate BFP per household member. Individual household member BFP would allow an individual biting risk to be calculated and increase the specificity of the analysis of associations. Using this method, associations between predictor variables for individual members of the study and the BFP or biting risk can be assessed. This larger study should also cover a longer period of time, which would allow for the season trends in vector density and biting risk between the rainy and dry season to be assessed.

5. Conclusion

The study found high ITN usage in the study households, a negative association between the number of mosquitoes collected and time, a high proportion of blood fed mosquitoes, and statistically significant associations with BFP and twelve different predictor variables. The study hypothesis that an increase in persons per sleeping space would increase BFP was rejected.

This study shows that it is feasible to examine factors reducing ITN efficacy in the area and lays down a potential template to be followed. Further, limitations of this study can be successfully accounted for in a scaled up study, especially if genetic analysis is used to determine a more specific biting risk. Future research is crucial for assessing the ITN efficacy gap, especially as insecticide resistance increases and threatens to undo the efforts made to combat malaria in sub-Saharan Africa.

Appendix A

Table 1: DESCRIPTIVE STATISTICS					
Household Statistics					
	<i>Total, Percent</i>	Village L	Village M	Village K	Total
Households		3, 33.33%	3, 33.33%	3, 33.33%	9, 100%
Household Size: Mean (SD)		6.19, 2.06	5.33, 0.97	6, 1.67	5.84, 1.65
Number of Children in Household: Mean (SD)		4.33, 1.93	3.33, 0.97	4.33, 1.28	4, 1.50
Age of Children: Mean (SD)		6.76 (2.95)	6.08 (1.15)	6.97 (1.75)	6.61 (2.09)
Number of Children 5 or younger		1.33 (0.97)	1.33 (0.48)	1.33 (0.48)	1.33 (0.67)
Participants		19, 35.85%	16, 30.19%	18, 33.96%	53, 100%
Age: Mean (SD)		17.105 (18.51)	15.75 (13.16)	16.61 (16.69)	16.528 (16.12)
Sex					
	<i>female</i>	10, 52.63%	10, 62.50%	11, 61.11%	31, 58.49%
	<i>male</i>	9, 47.37%	6, 37.50%	7, 38.89%	22, 41.51%
Education Level					
	<i>none</i>	6, 31.58%	5, 31.25%	8, 44.44%	19, 35.85%
	<i>some primary</i>	7, 36.84%	6, 37.50%	9, 50.00%	22, 41.51%
	<i>completed primary</i>	3, 15.79%	3, 18.75%	1, 5.56%	7, 13.21%
	<i>some secondary</i>	0, 0%	2, 12.50%	0, 0%	2, 3.77%
	<i>completed secondary</i>	3, 15.79%	0, 0%	0, 0%	3, 5.66%
Employment					
	<i>none/farmer</i>	5, 26.32%	5, 31.25%	3, 16.67%	13, 24.53%
	<i>self employed</i>	1, 5.26%	1, 5.25%	1, 5.56%	3, 5.66%
	<i>job</i>	0, 0%	0, 0%	1, 5.56%	1, 1.89%
	<i>na</i>	13, 68.42%	10, 62.50%	13, 72.22%	36, 67.92%
Relationship to Household Head					
	<i>household head</i>	3, 15.79%	3, 18.75%	3, 16.67%	9, 16.98%
	<i>spouse</i>	2, 10.53%	3, 18.75%	2, 11.11%	7, 13.21%
	<i>child</i>	10, 52.63%	10, 62.50%	10, 55.56%	30, 56.60%
	<i>grandchild</i>	3, 15.79%	0, 0%	3, 16.67%	6, 11.32%
	<i>neice/nephew</i>	1, 5.26%	0, 0%	0, 0%	1, 1.89%
Household/Secondary Head Statistics					
	<i>Total, Percent</i>	Total			
Household Heads		9, 100%			
Age: Mean (SD)		42.44 (14.73)			
Sex					
	<i>female</i>	2, 22.22%			
	<i>male</i>	7, 77.78%			
Average years of education: Mean (SD)		6.56 (2.78)			
Employment					
	<i>none/farmer</i>	7, 77.78%			
	<i>self employed</i>	1, 11.11%			
	<i>employed outside the home</i>	1, 11.11%			
Secondary Heads		8, 100%			
Age: Mean (SD)		30.28 (4.53)			
Sex					
	<i>female</i>	8, 100%			
	<i>male</i>	0, 0%			
Average years of education: Mean (SD)		9.09 (2.26)			
Employment					
	<i>none/farmer</i>	6, 75.00%			
	<i>self employed</i>	2, 25.00%			
	<i>employed outside the home</i>	0, 0%			
Relationship to Household Head					
	<i>spouse</i>	7, 87.50%			
	<i>child</i>	1, 12.50%			
Structural and Bednet Statistics					
	<i>Total, Percent</i>	Village L	Village M	Village K	Total
Number of Collections		21, 33.33%	21, 33.33%	21, 33.33%	63, 100%
Sleeping Spaces (SS)					
Number of SS		8, 42.11%	6, 31.58%	5, 26.32%	19, 100%
Average Number of SS per Household: Mean (SD)		2.66 (0.58)	2, (0)	1.67 (0.58)	2.11 (0.61)
Average Number of People per SS: Mean (SD)		2.60 (0.34)	2.67 (0.48)	3.67 (0.48)	2.98 (0.66)
Average SS Area Per Person: Mean (SD)		0.79 (0.11)	0.75 (0.18)	0.61 (0.09)	0.71 (0.15)
Insecticide Treated Nets (ITN)					
Average Area of Holes in ITN (sq. cm): Mean (SD)		9.50 (3.83)	548.96 (606.67)	142.48 (103.24)	244.85 (426.53)
Total household ITN hole area (sq. cm): Mean (SD)		24.13 (13.48)	1097.91 (1213.35)	284.96 (206.48)	491.25 (852.22)
Average Age of ITN (months): Mean (SD)		22.19 (3.96)	22.17 (9.30)	25 (13.04)	23.17 (9.66)
ITN Washed					
	<i>yes</i>	1, 33.33%	2, 66.66%	2, 66.66%	5, 55.56%
	<i>no</i>	2, 66.66%	1, 33.33%	1, 33.33%	4, 44.44%
Structural					
	<i>open eaves and uncovered windows</i>	2, 66.66%	0, 100%	2, 66.66%	4, 44.44%
	<i>closed eaves and uncovered windows</i>	0, 100%	3, 100%	1, 33.33%	4, 44.44%
	<i>closed eaves and covered windows</i>	1, 33.33%	0, 100%	0, 100%	1, 11.11%

	Village L					Village M					Village K					Total
	A	B	C	Village L Total	(Collection Mean, SD)	K	L	M	Village M Total	X	Y	Z	Village K Total			
Anopheles	2	8	2	12		100	85	38	223	27	21	11	59	294		
	(0.286, 0.49)	(1.143, 0.90)	(0.286, 0.49)	(0.571, 0.75)		(14.286, 14.99)	(12.143, 8.19)	(5.429, 2.57)	(10.619, 10.22)	(3.857, 2.79)	(3.358)	(1.571, 1.81)	(2.810, 2.50)	(4.667, 7.40)		
Male	2	4	1	7		72	48	26	146	7	8	7	22	175		
	(0.286, 0.49)	(0.571, 0.53)	(0.143, 0.38)	(0.333, 0.48)		(10.286, 11.34)	(6.857, 4.71)	(3.714, 2.43)	(6.952, 7.39)	(1, 0.82)	(1.143, 1.46)	(1, 1.29)	(1.048, 1.16)	(2.778, 5.20)		
Female	0	4	1	5		28	37	12	77	20	13	4	37	119		
	(0, 0)	(0.571, 0.79)	(0.143, 0.38)	(0.238, 0.54)		(4, 4.40)	(5.286, 3.90)	(1.714, 1.50)	(3.667, 3.65)	(2.857, 2.19)	(1.857, 2.04)	(0.571, 0.79)	(1.762, 1.95)	(1.889, 2.76)		
Fed/Half Gravid	0	2	1	3		7	18	7	32	15	8	3	26	61		
	(0, 0)	(0.286, 0.49)	(0.143, 0.38)	(0.143, 0.36)		(1, 1)	(2.571, 1.40)	(1, 1)	(1.524, 1.33)	(2.143, 1.57)	(1.143, 1.77)	(0.429, 0.53)	(1.238, 1.51)	(0.968, 1.31)		
BFP	.	0.500	1.000	0.6		0.250	0.486	0.583	0.416	0.750	0.615	0.750	0.703	0.513		
	(,.)	(0.50, 0.50)	(1.00, .)	(0.63, 0.48)		(0.49, 0.45)	(0.52, 0.33)	(0.53, 0.36)	(0.51, 0.36)	(0.82, 0.21)	(0.47, 0.30)	(0.83, 0.29)	(0.70, 0.30)	(0.60, 0.35)		
Culex	83	266	98	447		145	189	278	612	1166	1817	1267	4250	5309		
	(11.857, 8.71)	(38, 17.79)	(14, 11.37)	(21.286, 17.43)		(20.714, 9.16)	(27, 9.27)	(39.714, 17.30)	(29.143, 14.36)	(166.571, 74.18)	(259.571, 133.88)	(181, 94.01)	(202.381, 106.93)	(84.269, 104.65)		
Male	21	114	40	175		20	88	60	168	558	1320	828	2706	3049		
	(3, 2.89)	(16.286, 6.97)	(5.714, 5.82)	(8.333, 7.86)		(2.857, 3.53)	(12.571, 7.44)	(8.571, 4.39)	(8, 6.54)	(79.714, 34.21)	(188.571, 93.65)	(118.286, 58.32)	(128.857, 78.33)	(48.397, 72.81)		
Female	62	152	58	272		125	101	218	444	608	497	439	1544	2260		
	(8.857, 6.72)	(21.714, 11.57)	(8.286, 7.18)	(12.952, 10.47)		(17.857, 8.80)	(14.429, 3.31)	(31.143, 15.53)	(21.143, 12.39)	(86.857, 43.20)	(71, 45.39)	(62.714, 43.33)	(73.524, 42.97)	(35.873, 37.58)		
Fed/Half Gravid	36	62	30	128		87	21	119	227	347	37	173	557	912		
	(5.143, 4.18)	(8.857, 6.18)	(12.429, 3.59)	(6.095, 4.97)		(12.429, 6.70)	(3, 1.83)	(17, 10.95)	(10.810, 9.27)	(49.571, 23.66)	(5.286, 3.64)	(24.714, 16.34)	(26.524, 24.43)	(14.476, 17.49)		
BFP	0.581	0.408	0.517	0.471		0.571	0.208	0.546	0.511	0.571	0.074	0.394	0.361	0.404		
	(0.41, 0.25)	(0.21, 0.11)	(0.29, 0.17)	(0.31, 0.19)		0.696(0.61, 0.21)	(0.11, 0.04)	(0.43, 0.12)	(0.38, 0.25)	(0.29, 0.05)	(0.02, 0.02)	(0.16, 0.11)	(0.16, 0.13)	(0.28, 0.22)		
Total Mosquitos	85	274	100	459		245	274	316	835	1193	1838	1278	4309	5603		
	(12.143, 8.97)	(39.143, 17.98)	(14.286, 11.37)	(21.857, 17.82)		(35, 22.52)	(39.143, 16.33)	(45.143, 19.31)	(39.762, 19.03)	(170.429, 75.50)	(262.57, 135.29)	(182.571, 95.40)	(205.191, 108.10)	(88.967, 104.45)		
Male	23	118	41	182		92	136	86	314	565	1328	835	2728	3224		
	(3.29, 3.20)	(16.86, 7.08)	(5.86, 5.70)	(8.67, 8.015)		(13.14, 13.35)	(19.43, 10.95)	(12.29, 6.40)	(14.95, 10.60)	(80.71, 34.20)	(189.71, 94.19)	(119.29, 59.29)	(129.90, 78.78)	(51.17, 72.21)		
Female	62	156	59	277		153	138	230	521	628	510	443	1581	2379		
	(8.86, 6.72)	(22.29, 11.80)	(8.43, 7.30)	(13.19, 10.71)		(21.86, 11.25)	(19.71, 7.16)	(32.86, 16.45)	(24.81, 13.01)	(89.71, 44.32)	(72.86, 46.34)	(63.29, 49.98)	(75.29, 44.04)	(37.76, 38.15)		
Fed/Half Gravid	36	64	31	131		94	39	126	259	362	45	176	583	973		
	(5.143, 4.18)	(9.143, 6.39)	(4.429, 3.78)	(6.238, 5.13)		(13.429, 7.59)	(5.571, 2.44)	(18, 11.68)	(12.333, 9.36)	(51.714, 24.45)	(6.429, 5.00)	(25.143, 16.72)	(27.762, 25.16)	(15.444, 18.01)		
BFP	0.581	0.410	0.525	0.473		0.614	0.283	0.549	0.497	0.576	0.088	0.397	0.369	0.409		
	(0.52, 0.27)	(0.38, 0.14)	(0.54, 0.11)	(0.48, 0.20)		(0.63, 0.19)	(0.28, 0.09)	(0.55, 0.12)	(0.49, 0.20)	(0.59, 0.05)	(0.10, 0.10)	(0.42, 0.18)	(0.37, 0.24)	(0.44, 0.22)		

Table 3: ITN Coverage				
<i>Village:</i>	<i>L</i>	<i>M</i>	<i>K</i>	<i>Total</i>
Number of person days in study	910	784	833	2527
Number of person days under ITN	847	784	833	2464
Number of person days without ITN	63	0	0	63
ITN Coverage	93.08%	100%	100%	97.51%
*42 person days lost due to attrition				

	1	2	3	4	5	6	7	Total
<i>Total</i> (Collection Mean, SD)								
Anopheles	90	27	63	46	32	28	8	294
	(10, 14.65)	(3, 4.30)	(7, 8.86)	(5, 11.1, 4.70)	(3, 5.56, 2.51)	(3, 11.1, 4.54)	(0, 8.89, 1.36)	(4, 6.67, 7.40)
Male	54	17	43	24	15	17	5	175
	(6, 9.64)	(1, 8.89, 2.93)	(4, 7.78, 7.84)	(2, 6.67, 3.39)	(1, 6.67, 1.5)	(1, 8.89, 2.62)	(0, 5.56, 1.01)	(2, 7.78, 5.20)
Female	36	10	20	22	17	11	3	119
	(4, 5.29)	(1, 1.11, 1.96)	(2, 2.22, 2.05)	(2, 4.44, 2.30)	(1, 8.89, 2.03)	(1, 2.22, 1.99)	(0, 3.33, 0.5)	(1, 8.89, 2.76)
Fed/Half Gravid	11	7	10	17	11	4	1	61
	(1, 2.22, 1.30)	(0, 7.78, 1.30)	(1, 1.11, 1.05)	(1, 8.89, 1.90)	(1, 2.22, 1.30)	(0, 4.44, 1.014)	(0, 1.11, 0.33)	(0, 9.68, 1.31)
Proportion of Females Fed/Half Gravid	0.306	0.700	0.500	0.773	0.647	0.364	0.333	0.513
	(0.51, 0.31)	(0.79, 0.25)	(0.55, 0.38)	(0.74, 0.36)	(0.69, 0.25)	(0.37, 0.32)	(0.33, 0.58)	(0.60, 0.35)
Culex	1072	916	1064	823	835	420	179	5309
	(119, 111, 119.50)	(101, 77.8, 127.18)	(118, 22.2, 141.49)	(91, 4.44, 96.94)	(92, 7.78, 117.32)	(46, 6.67, 40.21)	(19, 8.89, 14.71)	(84, 2.69, 104.65)
Male	630	516	544	432	556	265	106	3049
	(70, 91.01)	(57, 33.3, 84.51)	(60, 4.44, 88.84)	(48, 6.67, 8.84)	(61, 7.78, 96.80)	(29, 4.44, 30.20)	(11, 7.78, 11.18)	(48, 3.97, 72.81)
Female	442	400	520	391	279	155	73	2260
	(49, 111, 36.99)	(44, 4.44, 44.65)	(57, 7.78, 57.05)	(43, 4.44, 34.59)	(31, 2.5, 90)	(17, 2.22, 15.84)	(8, 1.11, 6.27)	(35, 8.73, 37.58)
Fed/Half Gravid	180	152	196	175	105	67	37	912
	(20, 18.96)	(16, 8.89, 18.89)	(21, 7.78, 26.38)	(19, 4.44, 17.73)	(11, 6.67, 14.10)	(7, 4.44, 10.30)	(4, 1.11, 4.88)	(14, 4.76, 17.49)
Proportion of Females Fed/Half Gravid	0.407	0.380	0.377	0.448	0.376	0.432	0.507	0.404
	(0.26, 0.19)	(0.33, 0.24)	(0.33, 0.21)	(0.37, 0.29)	(0.27, 0.19)	(0.15, 0.11)	(0.26, 0.25)	(0.28, 0.22)
Total Mosquitos	1162	943	1127	869	868	448	187	5603
	(129, 111, 115.07)	(104, 77.8, 125.86)	(125, 22.2, 139.89)	(96, 4.44, 118.40)	(96, 4.44, 118.40)	(49, 7.78, 39.19)	(20, 7.78, 15.14)	(88, 9.67, 104.45)
Male	684	533	587	456	571	282	111	3224
	(76, 87.84)	(59, 22, 83.16)	(65, 2.2, 87.27)	(50, 6.7, 65.95)	(63, 4.4, 97.61)	(31, 3.3, 29.30)	(12, 3.3, 11.45)	(51, 1.7, 72.21)
Female	478	410	540	413	296	166	76	2379
	(53, 11, 36.33)	(45, 5.6, 44.67)	(60, 5.7, 57.28)	(45, 8.9, 35.61)	(32, 8.9, 27.31)	(18, 4.4, 15.80)	(8, 4.4, 6.50)	(37, 7.6, 38.15)
Fed/Half Gravid	191	159	206	192	116	71	38	973
	(21, 22.2, 19.36)	(17, 6.67, 18.71)	(22, 8.89, 27.09)	(21, 3.33, 18.34)	(12, 8.89, 15.22)	(7, 8.89, 10.22)	(4, 2.22, 4.99)	(15, 4.44, 18.01)
Proportion of Females Fed/Half Gravid	0.400	0.388	0.381	0.465	0.392	0.428	0.500	0.409
	(0.39, 0.16)	(0.51, 0.23)	(0.46, 0.22)	(0.52, 0.22)	(0.46, 0.22)	(0.33, 0.17)	(0.45, 0.28)	(0.44, 0.22)

Variable:	Anopheles			Culex			Total				
	Value	Coefficient	P Value	Value	Coefficient	CI	P Value	Value	Coefficient	CI	P Value
Total Number	294	-1.091	*0.013	5309	-15.476	-27, 710, -3, 242	*0.013	5603	-16.567	28, 689, -4, 441	*0.007
Male	175	-0.694	*0.027	3049	-8.183	-16, 869, 0.504	0.065	3224	-8.877	17, 446, -0.301	*0.042
Female	119	-0.397	*0.016	2260	-7.294	-11, 529, -3, 058	*0.001	2379	-7.690	11, 960, -3, 421	*0.000
Fed or Half Gravid Females	61	-0.139	0.082	912	-2.738	-4, 770, -0, 706	*0.008	973	-2.877	4, 965, -0, 789	*0.007
Proportion Fed/Half Gravid	0.513	-0.024	0.455	0.404	-0.015	-0.041, 0.012	0.275	0.409	-0.007	-0.034, 0.020	0.615

* = p-value is less than 0.05

Bolded red font denotes a statistically significant coef.

Variable:	Anopheles			Culex			Total		
	Coefficient	CI	P Value	Coefficient	CI	P Value	Coefficient	CI	P Value
Number of people in household	-0.038	-0.128, 0.052	0.412	-0.0036	-0.036, 0.029	0.831	-0.0251	-0.057, 0.007	0.121
Average age of household members	-0.012	-0.046, 0.022	0.485	-0.0153	-0.028, -0.002	*0.020	-0.0150	-0.028, -0.002	*0.023
Average age of adult household members	0.001	-0.009, 0.01	0.908	-0.0058	-0.010, -0.002	*0.003	-0.0046	-0.008, -0.001	*0.021
Average age of child household members	-0.047	-0.109, 0.015	0.139	-0.0245	-0.049, 0.001	0.055	-0.0429	-0.067, -0.019	*0.000
Number of children 5 and under	0.072	-0.120, 0.264	0.464	-0.022	-0.101, 0.058	0.597	0.011	-0.069, 0.090	0.793
Age of household head	-0.001	-0.010, 0.008	0.823	-0.0049	-0.008, -0.001	0.005	-0.0043	-0.008, -0.001	0.014
Under 35	REF	REF	REF	REF	REF	REF	REF	REF	REF
35 - 49	-0.140	-0.383, 0.102	0.257	-0.226	-0.330, -0.122	*0.000	-0.2733276	-0.373, -0.173	*0.000
50 - 70	0.194	-0.212, 0.599	0.349	-0.247	-0.400, -0.094	*0.002	-0.1511236	-0.297, -0.005	*0.042
70 or older	-0.139	-0.545, 0.266	0.500	-0.194	-0.347, -0.041	*0.013	-0.1952448	-0.341, -0.049	*0.009
Sex of household head									
Female	REF	REF	REF	REF	REF	REF	REF	REF	REF
Male	-0.083	-0.385, 0.219	0.589	0.1236	-0.001, 0.248	0.052	0.0536	-0.073, 0.180	0.406
Employment status of household head									
Unemployed/Subsistence Farmer	REF	REF	REF	REF	REF	REF	REF	REF	REF
Self Employed	-0.162	-0.462, 0.138	0.290	0.3415	0.212, 0.471	*0.000	0.1575	0.024, 0.291	*0.021
Employed outside the home	-0.183	-0.506, 0.140	0.267	-0.2503	-0.380, -0.121	*0.000	-0.3640	-0.498, -0.230	*0.000
Employed outside the household head									
Education level of household head									
Some Primary	REF	REF	REF	REF	REF	REF	REF	REF	REF
Completed Primary School	NR	NR	NR	0.0031	-0.125, 0.131	0.962	-0.0263	-0.155, 0.102	0.688
Completed Secondary School	-0.105	-0.342, 0.131	0.384	0.1448	-0.024, 0.314	0.093	0.0795	-0.090, 0.249	0.358
Years of education of household head	-0.054	-0.11, 0.001	0.057	0.0242	0.006, 0.043	*0.010	0.0070	-0.012, 0.026	0.473
Years of education of secondary household head	-0.007	-0.066, 0.051	0.803	0.0599	0.038, 0.081	*0.000	0.0500	0.027, 0.073	*0.000
House and Window Structure									
Open Eaves and Uncovered Windows	REF	REF	REF	REF	REF	REF	REF	REF	REF
Closed eaves but uncovered windows	NR	NR	NR	-0.0826	-0.251, 0.086	0.336	-0.0508	-0.224, 0.122	0.565
Closed eaves and covered windows	0.090	-0.137, 0.318	0.435	-0.2054	-0.374, -0.037	*0.017	-0.1229	-0.297, 0.051	0.166
Average number of people per sleeping space	0.025	-0.151, 0.201	0.781	-0.1257	-0.202, -0.050	*0.001	-0.1202	-0.196, -0.044	*0.002
Average sleeping space area per person (sq. m)	-0.386	-1.194, 0.422	0.349	0.6325	0.304, 0.961	*0.000	0.3263	-0.025, 0.678	0.069
< 0.6 (sq. m)	REF	REF	REF	REF	REF	REF	REF	REF	REF
0.6 - 0.69 (sq. m)	0.204	-0.129, 0.536	0.230	0.1568	-0.023, 0.337	0.087	0.2331	0.051, 0.415	*0.012
0.7 - 0.79 (sq. m)	-0.097	-0.364, 0.170	0.475	0.1622	0.033, 0.292	*0.014	0.0587	-0.073, 0.191	0.384
> 0.8 (sq. m)	-0.088	-0.421, 0.245	0.604	0.2442	0.104, 0.384	*0.001	0.1497	0.008, 0.291	*0.038
Average area of holes in bed nets (sq. dm)	0.000	0.000, 0.000	0.337	0.0231	0.011, 0.035	*0.000	0.0106	-0.002, 0.023	0.102
Total area of holes in bed nets (sq. dm)	0.000	0.000, 0.000	0.337	0.0116	0.006, 0.017	*0.000	0.0053	-0.001, 0.0117	0.100
Average age of bed nets (months)	0.007	-0.004, 0.017	0.203	-0.0028	-0.009, 0.003	0.330	0.0024	-0.003, 0.008	0.415
Net Washed?									
No	REF	REF	REF	REF	REF	REF	REF	REF	REF
Yes	0.188	-0.043, 0.420	0.111	-0.0730	-0.184, 0.038	0.196	0.0620	-0.049, 0.173	0.272

* = p-value is less than 0.05
Bolded red font denotes a statistically significant coef.

Appendix B

Household Form V1- STRAMB Form 2						
Household Study ID:						
Date		Interviewer Name				
Location		Sublocation			Village	
Number of buildings in the compound						
Number of buildings with sleeping spaces						
HOUSEHOLD MEMBERSHIP TABLE						
	One name	Age	Sex	Education level completed	Employment	Relation-ship to head
Household Head (1)						Head
Spouse of head (if applicable)(2)						Spouse
Member 3						
Member 4						
Member 5						
Member 6						
Member 7						
Member 8						
Member 9						

SLEEPING SPACES AND NETS						
Building _	ROOF	(check)	FLOOR	(check)	WALLS	(check)
	Thatched		mud/earth		mud/earth	
	Mabati		cement		cement	
	Cement		wooden planks		stone	
	Other:		tiles		mabati	
	No roof/open		other:		wood	
Gap between wall and roof (eaves)?			Netting or screen on windows? (All, Some, None, Not applicable because no windows)			
Number of sleeping spaces in building 1			Description:			
Sleeping Space:	Who sleeps in this space (first names)					
	Is there a net for this space? Y/N			Is the net hanging now? Y/N		
	Was the net used over this space last night?			How many nights in the last week was the net used over this space?		
	Are there times when the net is not used? If yes, describe					
	Have you ever treated it with chemicals since you got it?			Describe the net hanging		
	When did you acquire this net?			Was it treated when you got it?		
	Did you pay for it? Y/N			If yes, how much?		
	Describe the sleeping space:					
	What room is the sleeping space in?					
	Do you wash the net? If yes, how often? (weekly, monthly, several times per year, once per year)					
	Does the net have holes:					
	None	<5	5 to 10	>10		
	Size of the holes:					
	Smaller than a coin	Larger than a coin		Larger than a hand		
				REPEAT FOR ALL SLEEPING SPACES IN ALL BUILDINGS		

STRAMB – Supplementary Sleeping Space Survey									
Household ID& SS#:			Date:			Interviewer:			
Height of the net above the sleeping space									
Is the net tucked in?								Yes	No
Is there bedding? If yes, record type of bedding.								Yes	No
Mattress		Sleeping matt		Clothes/Blankets		Grass/Straw		Other	
Sleeping space raised up on bed?								Yes	No
Number of holes in the net?									
Size of the holes in the net? (Diameter for round, dimensions for square, length of rips)									
Location of the holes in the net?					Top	Sides	Tucked edges		
How is the net stored during the day?					Left tucked in	Left Open	Put up		
Dimensions of Sleeping Space						Length:		Width:	
Type of net?				Square	Round		Other		
How many places is the net secured to?									
Where is the net hung from?									
Roof		Rafters		Wall		String across room		Other	
Does the net reach the ground?								Yes	No
Brand and date of the net?									

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