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Characteristics of Bed Net Use and Malaria Incidence During Sick Visits in a Cohort of Malawian Children Living in an Area of Insecticide Resistance

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An abstract of
a thesis submitted to the Faculty of the
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Abstract

Characteristics of Bed Net Use and Malaria Incidence During Sick Visits in a Cohort of Malawian Children Living in an Area of Insecticide Resistance
By Barbara A. Nagy

Background: Insecticide treated bed nets (ITN) are one of the main pillars of efforts to control malaria, but the emergence of insecticide resistance threatens to reverse gains made in malaria control over the past decade. This study examines the effectiveness of ITNs in an area with known pyrethroid resistance.

Methods: A cohort of 1201 Malawian children aged 6-59 months, living in an area of high pyrethroid resistance, was cleared of parasitemia at baseline, given deltamethrin impregnated ITNs and followed at sick visits for one year. Rapid diagnostic tests for *P. falciparum* malaria were done at each visit. Data from 806 sick visits were analyzed using multivariate logistic regression to examine the relationship between net use last night and malaria parasitemia, controlling for potential confounders. Odds ratios, confidence intervals and p values were calculated to create a final model generating adjusted odds ratios and confidence intervals.

Results: The final model contained four covariates significantly associated with malaria parasitemia: net use (best, good or worst quality nets); age; caregiver's education; and season; and demonstrated that best quality ITNs (less than 36 months of age, without holes) exerted a significant protective effect against malaria incidence (aOR 0.4, 95% CI 0.2, 0.7) compared with no bed net. Children 48-59 months of age had increased odds of malaria compared with 6-11 month olds (aOR 1.67, 95% CI 0.95, 2.91). Maternal completion of primary school greatly increased the odds that a child used a bed net on the night before sick visit (OR 10.3, 95% CI 1.4, 75.4). Distribution of enough ITNs to cover all household members increased the percentage of children below five years using ITNs by 14.7%, to 93.7%.

Conclusions: ITNs retained their protective effectiveness in an area of Malawi with high levels of pyrethroid resistance as long as they were newer and had few holes, because physical barriers of intact nets and irritant properties of pyrethroids remained important. Age shift in malaria incidence indicates that malaria control interventions should be extended to older age groups. Distribution of sufficient ITNs to cover all household inhabitants significantly increases use by young children. Maternal primary education strongly favors ITN use by children.

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Chapter 1. Introduction

Background and Rationale

Malaria, in spite of prodigious international efforts at control, remains the most deadly and costly human parasitic disease in the world. The World Health Organization's World Malaria Report 2013 catalogues 97 countries in which malaria is still present, with 3.4 billion people at risk, 207 million cases and 627,000 deaths recorded in 2012 [1]. Because 90 percent of those deaths occur in sub-Saharan Africa and 77 percent of the deaths are in children under the age of five, 1300 child deaths per day, Africa has been the focus of much of the control effort. In 2013 alone, malaria cost Africa over 12 billion dollars in direct costs, and much more in terms of human capital and lost economic growth [2]. The Roll Back Malaria Partnership has coordinated international efforts to eradicate malaria, whose main strategies include: provision of insecticide treated bed nets (ITN); support of regular indoor residual spraying (IRS); intermittent prophylaxis of pregnant women with appropriate antimalarials (IPTp); and appropriate malaria case management including microscopy or rapid diagnostic tests (RDT), with artemisinin based combination therapy (ACT) for cases of *Plasmodium falciparum* malaria.

As part of this strategy, the appropriate use and effectiveness of ITNs have been extensively researched. World Health Organization (WHO) recommendations for malaria control have included ITNs since 1998. More than 290 million ITNs have been distributed in sub-Saharan Africa since 2008, and 53% of households reported owning at least one ITN

[1, 31]. Meta-analysis of ITNs in children [4] and pregnant women [5] has shown them to be safe and effective means of disease prevention, as well as economically feasible adjuncts to other malaria control strategies [23, 24].

Challenges to effective ITN use at the individual level and reports of insecticide resistance have been of grave concern to malaria control strategists. Documented widespread multiple insecticide resistance has raised the possibility that ITNs would fail as malaria control strategies, erasing important gains made against the disease in the past decade, and fueling research into combination insecticide treated bed nets.

Problem Statement

Whereas ITNs constitute a significant pillar in the fight against malaria, their effectiveness in areas of insecticide resistance has been called into question as the extent of insecticide resistance worsens worldwide. Issues surrounding ITN bioefficacy and survivorship have not been completely explored in the setting of pyrethroid resistance. Some studies have been limited by reliance upon historical data for insecticide resistance which changes significantly over time and location, by coexistence of indoor residual spraying in study catchment areas that might confound results, and by incomplete descriptions of net conditions and age.

Research Questions, Purposes and Aims

The objective of this project was to evaluate the effectiveness of ITN for the prevention of malaria in an area of high resistance to pyrethroids, using an incidence cohort of Malawian children aged 6-60 months, cleared of parasitemia at baseline, and followed at sick visits. Malaria incidence was measured with *P. falciparum* malaria rapid diagnostic tests. Pyrethroid resistance was measured during the study using WHO cone tests. The null hypothesis is that ITN use will have no significant effect on malaria parasitemia as measured by malaria rapid diagnostic test.

In addition to this question the following secondary research questions will be addressed:

1. What is the contribution of bed net holes and age on ITN effectiveness in an area of pyrethroid resistance?
2. What socio-demographic and other factors influence ITN use?

The results of this study will inform public policy decisions about the continued effectiveness, survivorship and utilization of ITN at the household level in an area of documented pyrethroid resistance.

Definition of Terms

Insecticide Treated Bed Net (ITN): An ITN is any insecticide impregnated net designed to be hung over a sleeping space to repel, disable and/or kill mosquito vectors.

Long Lasting Insecticide Treated Bed Net (LLIN): A LLIN is an ITN that will retain its effective biological activity against mosquitoes for at least 20 WHO standard washes and at least three years of use.

Untreated Bed Net (UTN): An UTN is any net designed to be hung over a sleeping space to prevent mosquito feeding upon net users.

Chapter 2. Review of the Literature

Insecticide Treated Bed Nets

Bed nets have been used for centuries for prevention of malaria, but in the 1980's research on pyrethroids, the only chemical now approved for use in ITNs, showed them to be safe and effective at killing malaria transmitting mosquitoes [3]. Long lasting insecticide treated bed nets (LLIN) have been used for the past ten years to eliminate burdensome retreatment of ITNs with insecticides. ITNs act in three ways to reduce malaria: bed nets act as physical barriers to prevent blood feeding of vectors which is required for malaria transmission, insecticides kill mosquitoes, and pyrethroids deter mosquitoes from entering areas where ITNs are hanging because they act as irritants. Only the killing of mosquitoes is impacted by the presence of pyrethroid resistance.

A 2004 meta-analysis of insecticide treated bed nets and curtains in areas of stable (>1 infective bite per year) and unstable (<1 infective bite per year) malaria reviewed fourteen cluster randomized controlled trials and eight individually randomized controlled trials for endpoints of child mortality, severe malaria, uncomplicated malarial episodes, parasite prevalence, hyperparasitemia, splenomegaly and anemia, and found ITNs to be highly efficacious for all outcomes. Thirteen of the trials were done in Africa in areas of stable endemnicity, including five trials reporting child mortality from all causes. ITNs provided 17% protective efficacy for mortality compared with no bed nets (relative rate 0.83, 95% CI 0.76, 0.90) and 23% protective efficacy compared with untreated bed nets (relative rate

0.77, 95% CI 0.63, 0.95). ITNs reduced severe malaria in stable transmission areas by 45% (95%CI 0.2, 0.63) compared with no bed nets, and increased the average hematocrit by 1.7 percent. In stable malaria areas ITNs reduced parasite prevalence by 13%, hyperparasitemia by 29% and splenomegaly by 29%, markers of severe or chronic malaria infection, compared with children not using bed nets [4]. Across all studies in the meta-analysis, use of ITNs saved 5.5 lives (95%CI 3.4, 7.7) per 1000 children protected. If the impact on low birth weight infants born to ITN protected mothers were factored in, this figure would be higher [5], because placental malaria is a main cause of premature and low birth weight infants, and these deaths are often overlooked in child mortality data.

All but one study considered in the above meta-analysis were settings in which ITNs were distributed and used with high degrees of supervision and support. The remaining study, done within the framework of a national malaria control program, was a cluster randomized controlled trial of ITNs versus untreated bed nets conducted in 25,000 Gambian children aged 1-60 months in an area hyperendemic for P. falciparum malaria. Children were followed for one year after bed nets in the treatment areas were impregnated with 200 mg/m^2 of permethrin by village health workers and community volunteers. Distribution of bed nets reached only 70% of households, yet there was an improvement in all causes child mortality from 24.3/1000 children/year in untreated bed net areas to 18.7/1000 children/year in ITN areas, a protective efficacy of 23% (95% CI 5, 37). Weight-for-height z scores, a marker of acute malnutrition, were better in ITN villages (z=-0.98) than in villages with untreated bed nets (z=-1.13; p=0.001 after adjustment for

area, age, differential bed net use and gender), demonstrating a significant impact of ITNs on child morbidity and development.

Lim et al. [30] evaluated the impact of ITNs on all-cause child mortality (1-59 months) across 29 Demographic and Health Surveys (DHS) from 22 African countries conducted since 2000, using a matched logistic regression model exactly pairing children who lived in households owning an ITN or slept under an ITN on the night prior to the survey with children not living in households owning an ITN or not sleeping under an ITN on the night prior to the surveys. Children were matched for covariates of maternal age, parity and birth interval, sex of the child, single or multiple birth, maternal education, household wealth quintile, urban versus rural residence, skilled birth attendance and immunization coverage at the primary sampling units, malaria transmission intensity and wet or dry season. Household ownership of at least one ITN was associated with a pooled relative reduction in all-child mortality of 23% (95% CI 13-31%, $p>0.05$). Lim et al. constructed a similar matched logistic regression of Malaria Indicator Survey (MIS) data from six African countries showing that children aged 1-59 months sleeping under ITNs had a 20% relative reduction in parasitemia prevalence (95% CI 3-35%, $p<0.01$) compared to children not sleeping under ITNs.

Insecticide Resistance

Reports of dramatic increases in pyrethroid resistance in malaria transmitting mosquitoes in the past decade have caused major concern in malaria control organizations, especially as no new classes of insecticides for ITNs have been developed over the past 30 years [8].

Two primary types of resistance have been described, target site, or knock-down resistance (*kdr*) and metabolic resistance, both of which may be present in the same vector population at any given time. The *kdr* mechanism works through genetic modification of insecticide target sites so that the insecticide no longer 'fits' mosquito neural receptors and blocks neural pathways. This mutation can be tracked genetically, which has become standard practice for some national malaria control programs. The metabolic resistance mechanism is more complex –numerous cytochrome P 450 enzyme genes may be simultaneously altered or hyper-expressed so that pyrethroids are detoxified before they kill the vectors. Both types of resistance are dynamic and often fluctuate widely in a single population of mosquitoes throughout a single malaria season. Experts are concerned that resistance may become fixed at a high level, such that strategies to overcome it may not be effective [9]. Two additional vector characteristics may impact insecticide resistance patterns: behavioral changes such as altered feeding or resting behavior may reduce mosquito contact with insecticides, and cuticular changes may reduce insecticide levels *in vivo* if mosquito appendages become thicker or less lipophilic.

Insecticide resistance can be determined using WHO tube bioassays using offspring of wild-caught mosquitoes of appropriate species from study sites, raised in insectaries. Non-blood-fed female mosquitoes aged 2-5 days from the insectaries are introduced into WHO test cylinders for three minutes, and then blown back into holding chambers. Assessment of a total of 50 mosquitoes per sample yields mortality (after 24 hours) results for pooled analysis. WHO considers resistance to be present when the mortality of the tested vector falls below 90% for the insecticide being assessed [18].

The entomological and epidemiological consequences of these resistance patterns remain uncertain. Examples of loss of insecticide effectiveness with high degrees of resistance for IRS have been documented, but ITNs have continued to show protective efficacy in spite of documented resistance of local mosquito vectors. Maharaj et al. [10] reported on entomological surveillance in a malaria endemic area of KwaZulu Natal where routine DDT indoor residual spraying took place until 1995, replaced by deltamethrin. Over the next three years, pyrethroid spraying was associated with a rapid rise in resistance to pyrethroids in *Anopheles gambiae* and *Anopheles funestus*, the two main vectors, and an over eight-fold increase in malaria cases reported by area health centers. DDT spraying was resumed in 2000, correlated with a significant drop in clinical malaria back to incidences seen prior to the transition to pyrethroids. Conversely, Henry et al. [11] reported on a randomized controlled trial of ITNs in the Gambia in an area with 90% *An. gambiae kdr* resistance and a secondary vector, *An. funestus*, remaining susceptible. Protective efficacy of ITNs was maintained at 56% (95% CI 25, 75) compared with no bed nets. Damien et al. [26] reported an observational study of ITNs in Benin with the *kdr* mutation present in 47-61% of *An. gambiae* and *An. arabiensis*. ITN use was associated with a 26% decreased prevalence of malaria in spite of the resistance.

Strode et al. [37] recently completed a meta-analysis of 36 laboratory and 24 field studies conducted between Jan 1980 and December 2013 on the impact of insecticide resistance on African Anopheline mosquitoes. They stratified studies into low insecticide resistance (>80% vector mortality), moderate resistance (25-80% vector mortality) or high resistance

groups (<25% vector mortality) and considered the variables of mosquito mortality, blood feeding, induced exophily, deterrence, and knock down using WHO cone tests, tunnel tests and hut tests. Comparing ITNs with untreated bed nets (UTN) their meta-analysis suggested a stepwise relationship between resistance and the risk difference (RD) for mosquito mortality across the studies. For hut studies the low resistance RD was 0.56 (95% CI 0.43, 0.68), the moderate resistance RD was 0.39 (95% CI 0.16, 0.61) and the high resistance RD was 0.35 (95% CI 0.27, 0.43). Their analysis was limited by a high degree of heterogeneity observed between studies and the lack of consistent reporting of resistance testing, however they concluded that ITNs are still effective against African Anopheline mosquitoes despite the presence of insecticide resistance.

Mosquito age and condition are important considerations because younger, better fed mosquitoes manifest more insecticide resistance than older mosquitoes in the same vector population. Jones et al. [16] tested 3-5 day or 17-19 day old *An. gambiae* mosquitoes with deltamethrin and found a loss of insecticide resistance with the 17-19 day old mosquitoes compared with those which were 3-5 days old (OR 5.28, 95% CI 2.81, 9.92). The rate of deterioration of insecticide resistance in the vector and the time required after hatching for acquisition of malaria infectivity may at present be preserving a window of effectiveness for insecticide treated bed nets that could prove difficult to sustain, although the physical barrier of the ITN may remain intact.

Durability of Long-Lasting Insecticide Treated Bed Nets

Longevity of LLIN is an important question because it informs malaria control programs and donors of how often bed nets need to be replaced and it gives insight into when and why users discard bed nets. Three factors determine bed net durability: the development of holes, retention of insecticidal activity (bioefficacy) and bed net diversion to other uses (survivorship). Most users consider bed nets to be a means of eliminating nuisance biting rather than malaria prevention [12]. Therefore, when this benefit is lost as holes develop, bed nets with effective levels of insecticide are likely to be discarded by their owners, or deferred to other uses.

All LLIN currently approved by WHO have manufacturer predicted lives of four to five years and at least 20 washes under perfect conditions, but experience in communities has shown a 36 month lifespan to be more realistic [13]. In a study of Olyset® bed nets in western Kenya, only 15% of bed nets were found to be in good condition five years after distribution. Bed nets with more than 100 cm^2 of holes (bed net surface area) were less effective at protecting users from malaria. Households with a bed net in moderate or poor condition were 17.6% (95% CI 4, 30.5) more likely to have a family member with a positive blood smear for *P. falciparum* malaria than those with bed nets in good condition [10].

The bioefficacy of bed nets is affected by the brand of bed net, the washing habits of owners and insecticide resistance patterns in target communities. Lindblade et al. studied five types of LLIN in western Kenya over two years, with periodic WHO cone bioassays using susceptible *An. gambiae*. Any bed net with two consecutive bioassay mortality rates of

<50% was considered to have failed. The average interval between washes was between 2.6 months and 8.3 months based on bed net type, thus the average number of washes was far short of the WHO limit of twenty. Of 314 bed nets distributed between June and July 2002, 62.4% failed during the first two years of monitoring, although one bed net tested, the Permanet® 1, retained 82.2% of its bioefficacy throughout the testing period [14].

Rehman et al. [15] compiled observational data to assess the impact of ITNs in different conditions on preventing malaria infections in children in Equatorial Guinea and Malawi. They divided bed nets into five categories: best nets (LLIN or pre-treated ITN with no holes), second best nets (LLIN or pre-treated ITN with small holes), middle quality nets (untreated bed net with no holes), second worst nets (LLIN or pre-treated ITN with big holes) and worst nets (untreated bed net with holes, untreated bed net of unknown condition, or unknown type of bed net or condition), and were able to show a significant protective effect that deteriorated as the condition of the bed nets worsened. Best nets reduced odds of *P. falciparum* infection in Equatorial Guinea (OR 0.65, 95% CI 0.55, 0.77) and Malawi (OR 0.81, 95% CI 0.56-1.18), and this effect did not change with the presence of small holes (those smaller than a D size battery). Tables 1 and 2 below illustrate the protective effect of classes of bed nets in Equatorial Guinea and Malawi (taken from Rehman, et. al., 2011).

Table 1. Odds Ratios of infection with *P. falciparum* among 1-14 year olds in Equatorial Guinea [15]

Covariate	% Infected (n)	Crude OR	95% CI	Adjusted OR	95% CI
Protection from bed net[1]					
Worst and no bed net	40 (10,814)	1			
Second worst	33 (508)	0.73	(0.56-0.96)	0.95	(0.71-1.28)
Middle	37 (783)	0.85	(0.66-1.10)	0.85	(0.71-1.01)
Second best	30 (765)	0.62	(0.50-0.77)	0.65	(0.54-0.79)
Best	34 (1937)	0.77	(0.58-1.02)	0.65	(0.55-0.77)

[1] Crude test for linear trend in bed net condition p=0.013 OR 0.92 (0.86-0.98). Adjusted test for linear trend in bed net condition p<0.001, OR 0.89 (0.86-0.93) per increase in condition category.

Table 2. Odds Ratios of infection with *P. falciparum* among 1-14 year olds in Malawi [15]

Covariate	% Infected (n)	Crude OR	95% CI	Adjusted OR	95% CI
Protection from bed net[1]					
Worst and no bed net	52 (2140)	1		1	
Second worst	53 (288)	1.05	(0.74-1.48)	0.98	(0.75-1.26)
Second best	48 (610)	0.86	(0.66-1.70)	0.84	(0.72-0.98)
Best	45 (475)	0.77	(0.53-1.13)	0.81	(0.56-1.18)

[1] Crude test for linear trend in bed net condition p=0.083 OR 0.92 (0.84-1.01). Adjusted test for linear trend in bed net condition p=0.067, OR 0.93 (0.86-1.01) per increase in condition category.

In spite of the well documented adverse effects of poorly maintained bed nets, most authors note that owners are not making repairs of holes nor being taught to do so, and that recipients need more information about bed net washing frequency and techniques, so that insecticidal activity may be maintained and holes prevented [15, 19].

Qualitative Evaluation of Bed Net Use

Having grappled with the technical problems of bed net longevity and insecticide bioefficacy, we are faced with yet another major problem in the effort to eradicate malaria, that many bed net owners don't use their bed nets, use them irregularly, or fail to use them for those highest at risk–pregnant women and young children. DHS, MIS, and Multiple Indicator Cluster Surveys (MICS), across 15 countries in sub-Saharan Africa from 2003-2006 have reported significantly lower bed net use than bed net ownership. The proportion of children <5 years covered by an ITN the previous night in households owning

at least one ITN ranged from 27.28%- 69.38%, whereas within houses where children <5 did not sleep under bed nets, the proportion of adults sleeping under bed nets ranged from 17.8-69.49%. The proportion of pregnant women using ITN within houses owning ITNs ranged from 29.1%-82.3%, and the proportion of households owning at least one ITN where no one slept under a bed net ranged from 8.7% to 52.2%. The mean ratio of household residents per ITN ranged from 3.7-6.79, and regression analysis demonstrated that child ITN use increased as family access to ITNs increased ($p=0.02$, $R^2=0.404$) [21]. When household access to ITNs was included in multivariate analysis, caregiver's education, family's socioeconomic status, gender of child and urban/rural residence were not found to be significant in determining whether children <5 slept under available ITNs.

Korenromp, et al [22] highlighted bed net condition and seasonality as important determinants of whether available bed nets were used. They noted that national surveys tend to avoid rainy seasons when respondents were likely to use their ITNs more frequently, and that responses to 'bed net use last night' during hot dry seasons might differ significantly during rainy seasons. These data underscore the importance of physically checking reported use of ITNs as part of data validation, and of working with communities to determine which parameters determine bed net preference and correct use.

Diagnosis and Treatment of Malaria

WHO recommendations for the diagnosis of malaria include examination of blood films by light microscopy for the presence of malaria parasites, or chromatography based rapid

diagnostic tests (RDT) to detect malaria antigens. Most malaria diagnosis now relies on RDTs for the diagnosis of malaria because microscopy is technically demanding and time consuming, often yielding inaccurate results. RDTs use fingerstick blood samples placed in wells on plastic test strips, with positive or negative results available in fifteen minutes. The most common RDTs presently in use in Africa use histidine-rich protein II (HRPII) tests specific to *P. falciparum* malaria, with sensitivities and specificities of >99% for WHO approved products. RDTs will be positive in asymptomatic persons with circulating malaria parasites as well as those ill from malaria, and after treatment for malaria will remain positive for approximately two weeks [27]. The first choice treatment for uncomplicated *P. falciparum* malaria in Malawi follows the WHO recommendation of an artemisinin based compound (ACT), presently artemether-lumefantrine tablets.

Chapter 3. Methods

Study Design

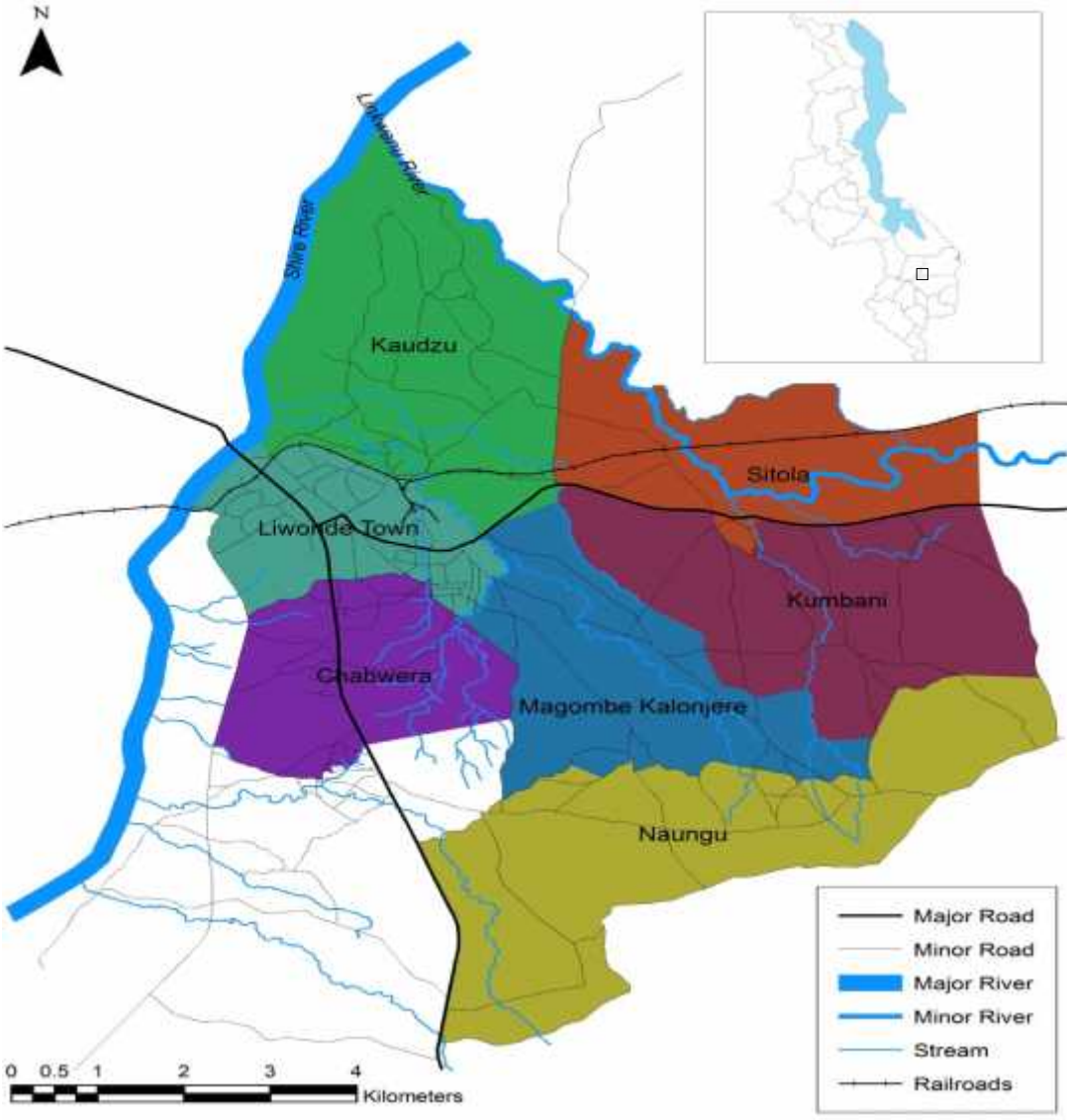
In order to measure the ability of ITNs to prevent malaria in an area with documented insecticide resistance [33], the study from which these data were taken enlisted a cohort of Malawian children aged 6-59 months, cleared them of parasitemia at baseline by administration of treatment doses of artemether-lumefantrine (LA) as per standard Malawi malaria protocol, and followed them at sick visits for 12 months. All enlisted children received LA at enrollment, regardless of their infection status. Study children also had to be >5 kg, reside in study villages for >1 month after enrollment, and not be taking cotrimoxazole prophylaxis (CPT), effectively excluding children with known HIV infection. They were followed at routine and sick visits to determine the presence of malaria.

Description of the Study Population

The study took place in six villages of Sitola Traditional Authority (TA), Machinga District, southern Malawi, between January 2012 and April 2013. Sitola TA has a population of approximately 39,000. Machinga District is bounded by the Shire River to the west, Liwonde National Park to the north, and other TA's to the east and south (Figure 1). The elevation ranges from 450-550 meters above sea level.

Residents in Sitola TA are predominantly of the Yao tribe, but Chichewa and Chiyao are spoken. The primary religion is Islam. Polygamy is practiced in seven percent of southern households, and this has been associated with slightly higher use of bed nets [26].

Figure 1. Map of Machinga District, Malawi, 2012

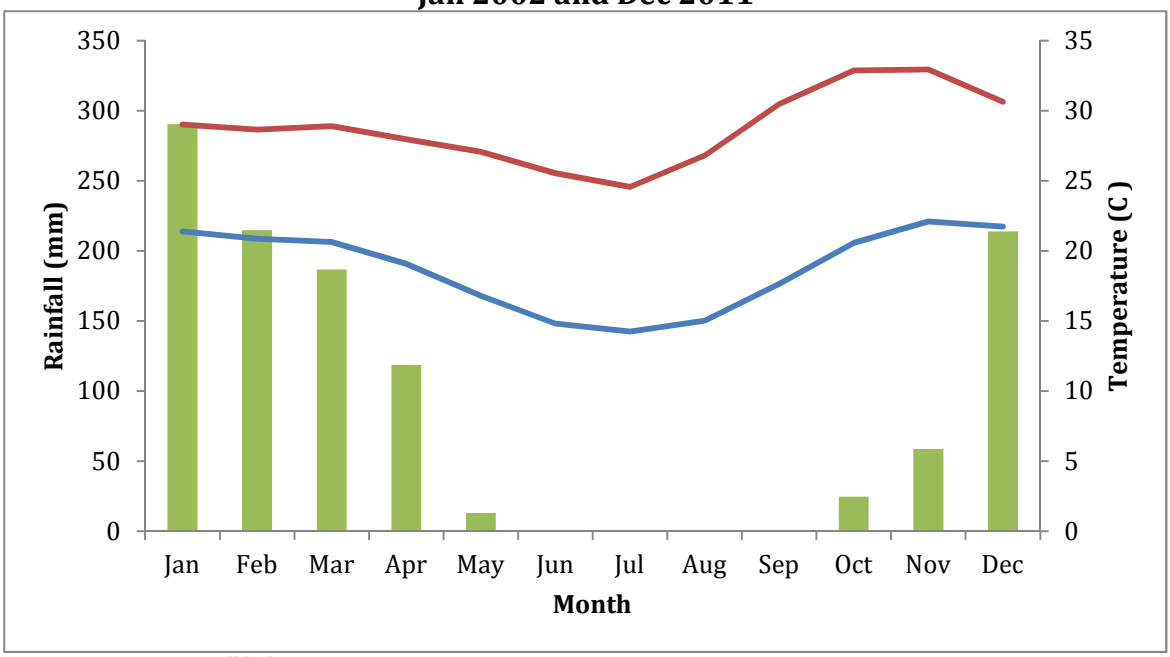


Courtesy of Gerard Lopez

Livelihoods are mostly agriculture (subsistence farming), fishing and small businesses such as tailoring, bicycle repair or market vending. Malawi 2010 DHS data [25] for the southern region describe that 69% of men and 68% of women have attended primary school which includes eight grades in Malawi, however only 39% of respondents could read a whole

sentence in their primary language. Primary education in Malawi is now free, and 78.5% of 6-15 year old children in the southern region were reported to be attending school. Malaria transmission is high (> 100 infective bites per year) and perennial in Machinga District [29], but peaks during and after the rainy season, which usually occurs between November and April (Figure 2). *P. falciparum* is the predominant species of malaria seen in Malawi, although *P. malariae* and *P. ovale* have also been found with much lower frequencies. The main malaria vector is *An. funestus,* but *An. gambiae* is also common. In 2011, tube assays with *An. funestus* exposed to 0.05% deltamethrin showed between 67-100% resistance [28].

Figure 2. Average Rainfall and Temperature in Machinga District, Malawi, between Jan 2002 and Dec 2011



Courtesy Dr. Kim Lindblade, CDC

Surveyed before the last national LLIN distribution exercise in 2012, 59% of people in the southern region had at least one ITN, and 41% of children <5 had slept under ITN the night before the survey was conducted. The average number of bed nets per household was 1.0,

and this did not differ from other regions of Malawi. As DHS data does not include information about age or condition of bed nets, many of these may have been in poor condition.

The under 5 mortality rate for Malawi southern region in 2010 was 127/1000 live births and the neonatal mortality rate was 31/1000 live births [25]. Forty seven percent of children were stunted and four percent were wasted. Among children less than 5 years, 34.5% were reported to have had a fever in the two weeks preceding the survey, which took place during the dry season. Of those with fever, 29% received ACT within 24 hours of fever onset.

Children in Machinga District had a 73% incidence of anemia at baseline (44.8% moderate or severe), which is associated with poor development and school performance. Twenty one percent of study participants were stunted (height for age z-score <-2) and eight percent were wasted (weight for height z-score <-2). Five percent of children presenting for enrollment in the ITN study were rejected because they were on cotrimoxazole therapy as prophylaxis against opportunistic infections associated with HIV [33]. The prevalence of HIV infection in 1119 children admitted to nearby Queen Elizabeth Hospital from 1996-2005 was 16% [34].

Study Procedures

Consultations with the National Malaria Control Programme, Machinga District Health Office, the District Development Committee and Sitola TA were undertaken during study

development, and the study was launched with their approval. A series of community meetings explained the study purpose, procedures and eligibility criteria to people living in the study area. Initially, a bed net census was taken on all households in the study area to determine ownership, type, age, condition and use of ITN at baseline.

Regardless of previous ownership of ITNs, new LLIN (Permanet®2 Vestergaard-Frandsen, Switzerland, which are treated with deltamethrin 0.05%) were distributed to all households in the study area with a minimum of one LLIN for each sleeping space or one LLIN for every two household members plus an extra bed net for households with odd numbers of inhabitants. Twice during the initial study period, a validation exercise compared caregivers' responses about bed net use with observations about bed net use during home visits. Mosquitoes were collected for insecticide resistance testing to deltamethrin and permethrin at intervals during the study, using pyrethrum spray catches and WHO tube assays. Deltamethrin was the insecticide found on bed nets distributed by the study protocol. Permethrin was the insecticide present on most bed nets in the study area at the time the study began.

Cohort children were instructed to attend the study clinic at Machinga District Hospital if they fell ill. Transportation to and from the facility was reimbursed for study participants, although after December 2012 this was limited to one visit per month. Weights and heights were measured twice, and if the results indicated a greater than 10% disparity, a third 'tiebreaker' measurement of weight and height was done. Each enrollee had a fingerstick blood sample drawn for malaria polymerase chain reaction (PCR) with species

differentiation for *P. falciparum, P. malariae, P. ovale and P. vivax* at the beginning of the study period. During each sick visit, updated information about bed net use and condition was collected, and children were examined. Blood was collected by fingerstick for thick and thin blood film malaria testing and SD Bioline® RDT for *P. falciparum*. All final results in the analyses presented here were based on the results of SD Bioline® RDT. Hemoglobins were determined using Hemocue® Hb201+ Analyzers (Hemocue Inc., Cypress, CA, USA). Children with anemia were prescribed iron syrup, and any child not having documentation of antihelminthics within six months was given an antihelminthic [28]. Children with positive RDTs were given treatment for malaria in accordance with Malawi Integrated Management of Childhood Illness guidelines (usually ACT) and all children received other necessary outpatient treatments free of charge. Table 3 provides a timeline of study interventions and goals.

Table 3: Malawi LLIN effectiveness study activities and timeline 2012-2013 [28]

		Study Month and Season															
		Rainy				Dry							Rainy				
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A
1	Household, LLIN and sleeping space registration/Make appointments for enrollment		X														
2	Incidence cohort enrollment			X													
1	Permanet distribution, centralized area			X	X												
2	LLIN census		X									X					
2	Incidence cohort monthly surveillance visit				X	X	X	X	X	X	X	X	X	X	X	X	X
	Anthropometric measurements[1]			X					X						X		
2	Passive health facility surveillance (sick visits)			X	X	X	X	X	X	X	X	X	X	X	X	X	X
2	Validation exercise										X	X					
2	LLIN integrity					X		X		X	X	X	X	X			
3	Pyrethrum Spray Catches		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Resistance testing		X					X					X				

1 = As part of incidence cohort measures. 2 = Incidence cohort-related measures. 3=Insecticide resistance measures.

Statistical Analysis

Data were analyzed using Epi Info 7.0 and OpenEpi with 95% confidence intervals, except for logistic regression, which was done with SAS 9.3 using a stepwise selection process for variables with $p<0.1$ association with RDT (Table 5). Initially, all relevant variables were subjected to univariate analysis to determine their association with RDT positivity. The variables net age, net holes, large holes and extra large holes were used to create a net quality variable which met the $p<0.1$ inclusion criterion. The tiered net quality variable was then compared with no net use to predict the primary outcome of malaria incidence as measured by RDT positivity (Table 4).

Table 4. Quality of Net Used Last Night

Category Name	Composition
No Net	Response to 'Net Use Last Night' = No
Worst Quality Net	Response to 'Net Use Last Night' = Yes, **and** Net Age was ≥36 months, **and either** 'Reported Extra Large Holes' = Yes **or** 'Reported Number of Holes' = >10
Good Quality Net	Response to 'Net Use Last Night' = Yes, **and** 'Reported Extra Large Holes' = No, **and** 'Reported Number of Net Holes' = 1-10
Best Quality Net	Response to 'Net Use Last Night' = Yes, **and** Net Age was <36 months, **and** Reported Number of Net Holes = None

Age group at enrollment was constructed by reassigning children's months of age at enrollment into categories corresponding to years. Those aged 6-11 months became year 1; 12-23 months, year 2; 24-35 months, year 3; 36-47 months, year 4; 48-60 months, year 5; and 61-66 months became year 6. Household wealth index was constructed using a contextually appropriate standardized catalogue of assets, with cohort households divided into five categories based on asset ownership: poorest (1) to least poor (5). Hemoglobin at enrollment (grams/dl) was converted into categories of moderate (<11 g/dl) and severe (<7 g/dl) anemia. Sick visit dates were converted into categories of those occurring during

rainy season (December through June) and dry season (July through November). Clinical malaria was a combination of fever and positive RDT. Anthropometric data were converted into stunting (height for age z score < -2) and wasting (weight for height z score < -2) yes/no variables using WHO growth parameters [32]. Use of the full sick visit data set with repeated measures on some of the children would have necessitated accounting for this correlation, which was not possible using Epi Info. First sick visits were therefore selected for analysis, but the direction and strengths of associations between ITN use and malaria RDT positivity were similar when the full data set was used.

Ethical Considerations

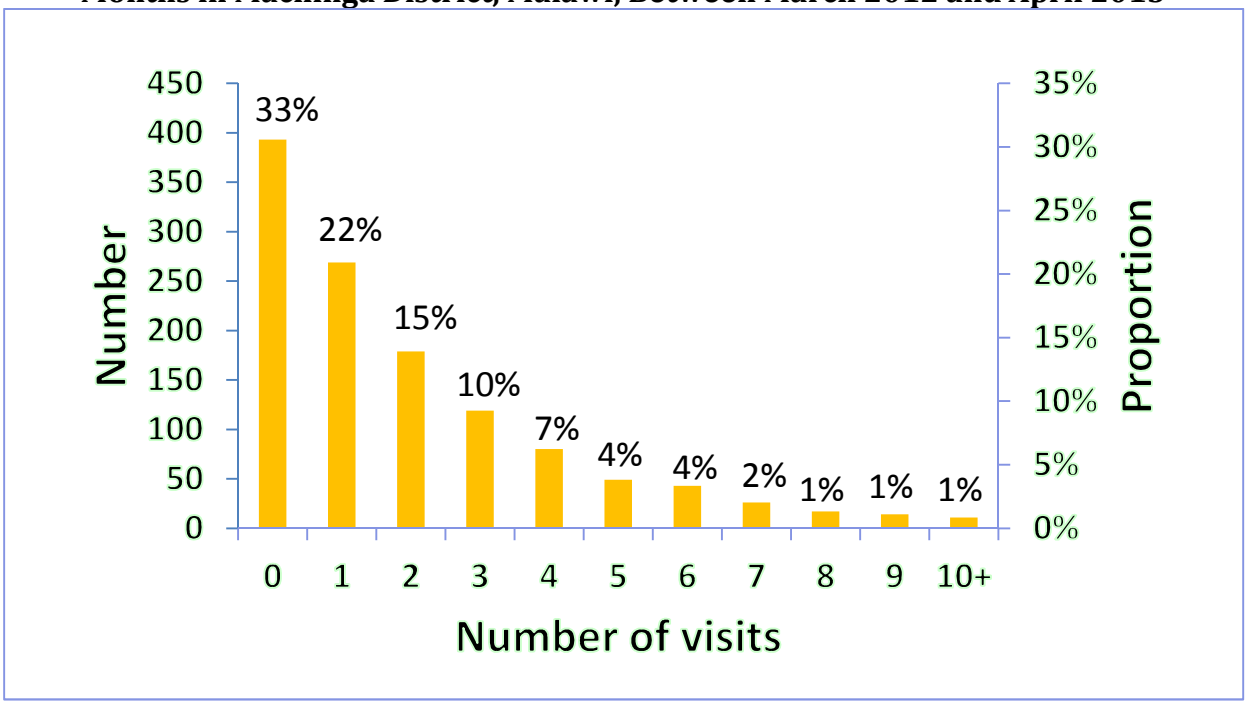
This study was submitted to and approved by institutional review boards at the College of Health Sciences, University of Malawi (Blantyre, Malawi, Approval # P.01/13/1332), and the US Centers for Disease Control and Prevention (Atlanta, GA, USA, Approval #6227). Verbal consent in Chichewa was obtained from all households in the study area for the ITN and sleeping space census, follow-up censuses, and distribution of Permanet® 2. Written informed consent forms in Chichewa were read to caregivers of all study eligible children, and consent was indicated by signature or fingerprint of caregivers. Caregivers were informed that they were free to withdraw their children from the study at any time without penalty. Risks to study children were possible bruising and pain at sites of fingerstick blood draws (Appendix 1). Benefits included improved access to care, reinforcement of the importance of ITNs for malaria prevention and the satisfaction of contributing to the well-being of their communities. Participants did not receive any compensation other than the above.

Results

Descriptive

In total, 806 children made at least one sick visit out of the group of 1201 enrolled in the first cohort of children (Figure 3, Table 5, and Table 6).

Figure 3. Numbers and proportions of sick visits in a Cohort of Children Aged 6-60 Months in Machinga District, Malawi, Between March 2012 and April 2013



At the study enrollment visit, 79.9% of children had slept under an ITN the previous night, while during sick visits (after ITN distribution), 93.7% of children were reported to have slept under an ITN on the previous night, an improvement of 14.7%. At enrollment 56.6% of children had bed nets <36 months old (generally associated with adequate levels of insecticide on the ITNs) compared to 91.6% at sick visit follow-up. Holes were present in

28.9% of bed nets at sick visit follow-up, with 5% having extra large holes and 5.7% of bed nets having more than 10 holes per net.

Overall, 35.3% of children were positive for *Plasmodium* by PCR at enrollment. Of these, 96.1% had *P. falciparum* infections and 6.0% had *P. malariae*. None of the children had *P. vivax or P. ovale*. At sick visits 48.2% of children were positive for *P. falciparum* by RDT. These results would not be comparable to PCR results at enrollment, because at that time children were not acutely ill, and the RDT will remain positive for several weeks after malaria is treated. A large proportion of children were anemic upon enrollment (73.3%) and 12.0% were severely anemic, which improved during the study period. A two sample paired t-test comparing mean hemoglobin results at enrollment (9.9 g/dl, σ 1.7) and sick visits (10.5 g/dl, σ 1.5) yielded $t=9.43$, $p<0.001$ (Table 7). Most of the sick visits were during the rainy season (86.4%).

Age distribution across the sample was fairly even, except for a very few children in the >60 months group. Males and females were equally represented. Stunting was found in 20.1% of children at enrollment and wasting was present in 7.3%. The distribution of households from poorest to least poor was comparable (20.3%-21.6%) except for slightly fewer families at the least poor level (16.9 %). Slightly more boys than girls presenting for sick visits had been sleeping under bed nets (OR 1.6, 95% CI 0.9, 2.9). Seventeen percent of caregivers completed primary school, compared to 33.8% (95% CI 30.5, 37.2) of partners of caregivers completing primary school.

Bed net verification exercises done from December 2012through January 2013 showed 99% accuracy in reporting bed net use (95% CI 98-100%) and a second exercise done from September through October 2013 showed a 91% reporting accuracy (95% CI 88-94) as shown in Table 7 [35].

Insecticide resistance testing done during the study [35] revealed that 29% (95%CI 16, 42) of *An. funestus*, the primary vector in the area, were killed by deltamethrin and 57% were killed by permethrin (95% CI 35, 80). Deltamethrin killed 53% (95% CI 24, 81) of *An. gambiae*, the area's second most frequent malaria vector and permethrin killed 53% (95% CI 24-81).

Table 5. Enrollment Characteristics of a Cohort of Children Aged 6-60 Months Making Sick Visits in Machinga District, Malawi, Between March 2012 and April 2013 (N=806)

Covariate	Covariate Level	Number (Percentage)	Mean (Standard Deviation)
Net Use Last Night/Enrollment	Yes	630 (79.9)	
	No	159 (20.1)	
Net Age	<36 months	356 (56.6)	
	≥36 months	273 (43.4)	
Net Holes	Holes Present	218/754 (28.9)	
	Large Holes[1]	111/750 (14.8)	
	Extra Large Holes[2]	37/747 (5.0)	
	1-10 Holes/Net	168/743 (22.6)	
	>10 Holes/Net	42/743 (5.7)	
	No Holes	536/754 (71.1)	
Plasmodium PCR[3]	Positive	284 (35.3)	
	Negative	520 (64.7)	
Multispecies RDT	*P. falciparum* +	273/284 (96.1%)	
	P. malariae +	17/284 (6.0%)	
	P. vivax +	0/283 (0%)	
	P. ovale+	0/283 (0%)	
Bedtime Hour	<7 pm	185 (23.4)	
	7-8 pm	597 (75.4)	
	9-10 pm	10 (1.3)	
Age Group at Enrollment	6-11 months	102 (12.7)	
	12-23 months	203 (25.2)	
	24-35 months	179 (22.2)	
	36-47 months	162 (20.1)	
	48-60 months	147 (18.3)	
	>60 months	13 (1.6)	
Gender	Male	409 (50.7)	
	Female	397 (49.3)	
Wasting[4]	Yes	57 (7.3)	
	No	722 (92.7)	
Stunting[5]	Yes	149 (20.1)	
	No	594 (80.0)	

[1]A large hole would permit a fist to pass. [2]An extra large hole would permit a head to pass. [3]*P. falciparum* RDT, SD Bioline® [4]Weight for height Z score < -2 [5]Height for age Z score < -2.

Table 5. continued, Enrollment Characteristics of a Cohort of Children Aged 6-60 Months Making Sick Visits in Machinga District, Malawi, Between March 2012 and April 2013 (N=806)

Covariate	Covariate Level	Number (Percentage)	Mean (St. Deviation)
Altitude[1]		591	502.3 (23.1)
Hemoglobin at Enrollment (g/dl)		799	9.9 (1.7)
Anemia at Enrollment	Moderate	585 (73.3)	
	Severe	96 (12.0)	
Household Wealth Index	0 (Poorest)	161 (20.3)	
	1	171 (21.6)	
	2	164 (20.7)	
	3	163 (20.6)	
	4 (Least Poor)	134 (16.9)	
Caretaker Finished Primary School	Yes	135 (17.0)	
	No	658 (83.0)	
Partner Finished Primary School	Yes	268 (33.8)	
	No	525 (66.2)	

[1] In meters above sea level.

Table 6. Characteristics of a Cohort of Children Aged 6-60 Months at Sick Visits in Machinga District, Malawi, Between March 2012 and April 2013 (N=806)

Covariate	Covariate Level	Number (Percentage)	Mean (St. Deviation)
Net Use Last Night at Sick Visit	Yes	755 (93.7)	
	No	51 (6.3)	
Net Age	<36 months	675 (91.6)	
	≥36 months	62 (8.4)	
P. falciparum RDT[1]	Positive	371 (48.2)	
	Negative	399 (51.8)	
Season of Visit	Rainy Season	687 (86.4)	
	Dry Season	108 (13.6)	
Hemoglobin at Sick Visit (g/dl)			10.5 (1.5)

*

Table 7. Results of a Bed net Verification Survey in Machinga District, Malawi [35]

December to January 2013			
	ITN observed		
Caregiver report of ITN use	Yes	No	Total
Yes	207	2	209
No	3	1	4
Total	210	3	213

September to October 2013			
	ITN observed		
Caregiver report of ITN use	Yes	No	Total
Yes	268	25	293
No	5	4	9
Total	273	29	302

Univariate Analysis

The primary outcome variable, RDT positivity, was compared to sixteen other variables, including the constructed variable 'net quality', which combined net use, net age, reported number of net holes, reported large hole and reported extra large hole (Table 8). Four variables were found to be associated with RDT result at $p<0.1$ level. These were age group at enrollment, caregiver completing primary school, season of visit, and best quality nets.

Net use on the night prior to sick visit had a significantly protective effect compared with non-use of bed nets (OR 0.44, 95% CI 0.24, 0.8). Children sleeping under best quality nets had the greatest protective effect from their use, OR 0.37 (95% CI 0.20, 0.69), better quality net users had an OR of 0.59 (95% CI 0.30, 1.18) of malaria, and worst net users had an OR of 0.8 (95% CI 0.22, 3.0) of malaria versus children not using a bed net. Sick visit children in the rainy season had an OR of 2.8 (95% CI 1.8, 4.5) of malaria compared to those visiting during the dry season, and those whose caregivers completed primary school had an OR of 0.6 (95% CI 0.4, 0.8) of having malaria, which correlates with higher use of bed nets. Bed net use last night was found to be strongly associated with whether caregivers had completed primary school (OR 10.3, 95% CI 1.4, 75.4, $p<0.005$).

Age group at enrollment showed a higher risk of malaria for older children, with OR 1.7 (95% CI 1.0, 2.8) for those 36-47 months, OR 1.5 (95% CI 0.9, 2.5) for those 48-60 months and OR 4.1 (95% CI 1.1, 16.2) for those >60 months, as contrasted to OR 1.1 (95% CI 0.7, 1.8) for those 12-23 months, and OR 1.1 (95% CI 0.7, 1.9) for those 24-35 months. This is

consistent with what other studies have observed in eastern Africa [38] as malaria control interventions have progressively decreased the numbers of infective bites per person per year, and the peak incidence of malaria has shifted to older children [3].

Contrary to other studies of ITN use and effectiveness, education of the caregivers' partners was not associated with RDT results of children (OR 0.9, 95% CI 0.7, 1.3, $p<0.66$). Wasting ($p<0.74$), household wealth index ($p<0.33$) and bedtime hour ($p<0.36$) were also not significantly associated with RDT results in children at sick visit follow-up.

Table 8. Odds of having malaria at sick visits, children aged 5-60 months, Machinga District, Malawi, between Jan 2012 and Mar 2013 (N=806)

VARIABLE	N (%) RDT+	ODDS RATIO (95% CI)	Prevented Fraction in Population (95% CI)	Prevented Fraction in the Exposed (95% CI)
Net Use Last Night				
No	32 (66.7)	1		
Yes	339 (47.0)	0.44 (0.24, 0.8)	27.7 (10.1, 39.6)	29.6 (12.7, 43.2)
Net Quality				
No Net	32 (66.7)	1		
Worst Quality Net	10 (71.4)	0.8 (0.22, 3.0)	16% (-29.2, 58.6)	20.0% (-195.2, 78.3)
Better Quality Net	77 (54.2)	0.59 (0.30, 1.18)	32.7% (17.8, 55.9)	40.8% (-17.5, 70.1)
Best Quality Net	212 (42.6)	0.37 (0.20, 0.69)	59.6% (-74.1, 70.8)	62.9% (30.7, 80.2)
Net Age				
No Net	32 (66.7)	1		
<12 months	247 (43.0)	0.38 (0.2, 0.7)	59.5% (-74.7, 70.8)	62.4% (29.8, 79.8)
No Holes	203 (42.0)	0.36 (0.19, 0.68)	60.3% (-81.4, 71.0)	63.8% (32.2, 80.6)
Net Holes	44 (47.8)	0.46 (0.22, 0.95)	40.6% (13.7, 60.4)	54.2% (5.3, 77.8)
12-23 months	35 (56.5)	0.65 (0.30, 1.42)	22.1% (-44.8, 46.7)	35.2% (-41.7, 70.4)
No Holes	9 (56.3)	0.64 (0.20, 2.04)	10.9% (-30.2, 32.2)	35.7% (-104.2, 79.8)
Net Holes	26 (56.5)	0.65 (0.28, 1.5)	19.4% (-42.0, 43.77)	35.0% (-50.1, 71.8)
24-35 months	25 (65.8)	0.96 (0.4, 2.4)	1.7% (-63.9, 29.8)	3.8% (-136.4, 60.9)
≥36 months	20 (69.0)	1.1 (0.41, 3.0)	3.8% (-31.2, 38.9)	10% (-100, 66.5)
Caregiver Finished Primary School				
No	47 (37.0)	1		
Yes	320 (50.6)	0.6 (0.4, 0.8)	8.7% (2.5, 14.2)	42.7% (15.2, 61.3)

Table 8, continued. Odds of having malaria at sick visits, children aged 5-60 months, Machinga District, Malawi, between Jan 2012 and Mar 2013 (N=806

VARIABLE	N (%) RDT+	N (%) RDT-	ODDS RATIO (95% CI)	Etiologic Fraction in Population (95% CI)	Etiologic Fraction in the Exposed (95% CI)
Age Group at Enrollment					
6-11 months	42 (42.0)	58 (58.0)	1		
12-23 months	83 (43.9)	106 (56.1)	1.1 (0.7, 1.8)	5.0% (-25.6, 35.6)	7.5% (-51.0, 43.4)
24-35 months	78 (45.4)	94 (54.7)	1.1 (0.7, 1.9)	8.3% (-20.8, 37.3)	12.7% (-43.6, 47.0)
36-47 months	85 (54.8)	70 (45.2)	1.7 (1.0, 2.8)	27.0% (4.2, 49.8)	40.4% (0.9, 64.1)
48-60 months	74 (52.1)	68 (47.9)	1.5 (0.9, 2.5)	21.3% (-2.8, 45.5)	33.5 (-11.5, 60.3)
>60 months	9 (75.0)	3 (25.0)	4.1 (1.1, 16.2)	13.4% (1.3, 25.5)	75.9% (5.4, 93.8)
Season of Visit					
Dry (July-Nov)	28 (27.2)	75 (72.8)	1		
Rainy (Dec-Jun)	337 (51.4)	319 (48.6)	2.8 (1.8, 4.5)	59.7% (43.2, 76.2)	64.6% (44.0, 77.7)
Bedtime Hour					
Early (≤7 pm)	93 (25.6)	84 (21.3)	1		
Late (>7 pm)	270 (74.4)	310 (78.7)	1.27 (0.91, 1.78)	2.9** (-1.2, 7.0)	11.4** (-4.5, 24.9)

Multivariate Analysis

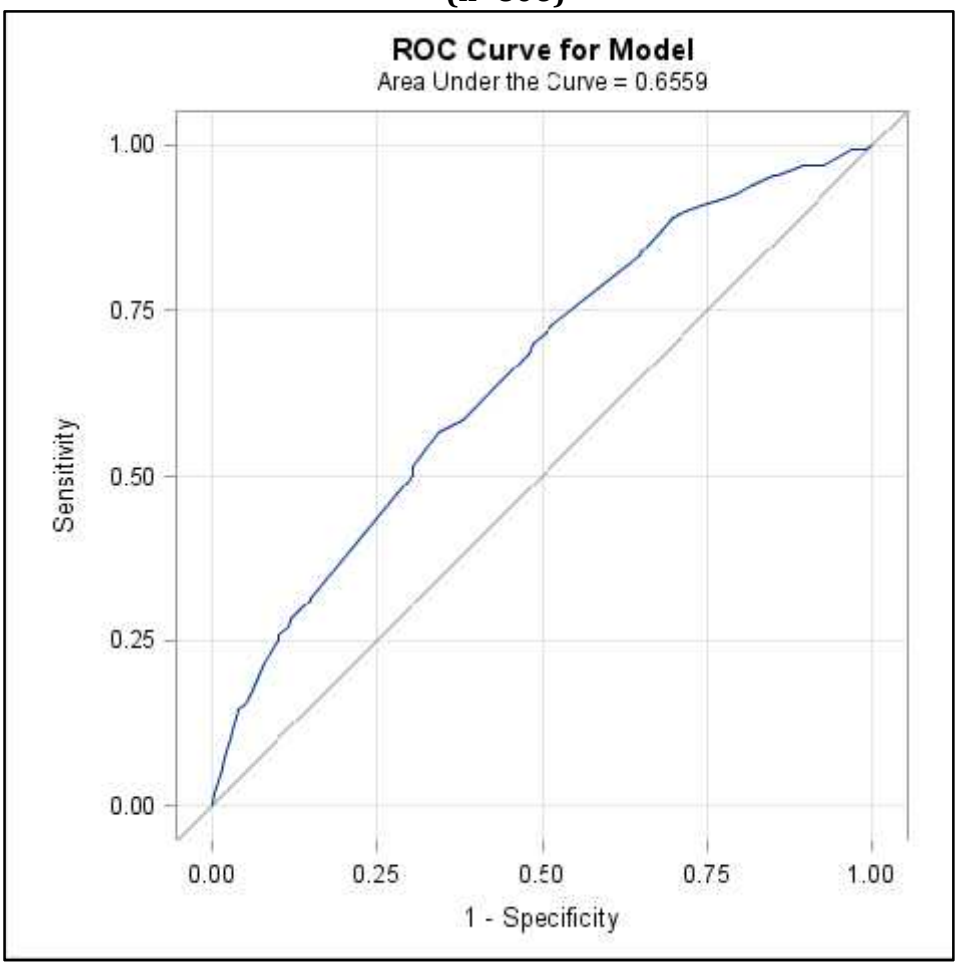
The final multivariate model consisted of four variables: age group at enrollment, season, caregiver completing primary school, and bed net quality (Table 8). Other covariates of gender, altitude, household wealth index, sleeping room type, bedtime hour, stunting, wasting and partner completing primary were found not to contribute to the model, and were therefore excluded. The ROC curve demonstrated an area under the curve of 0.6559 (Figure 4). There was a protective effect for best quality nets (OR 0.4, 95% CI 0.2, 0.7) compared with no bed net. The best quality nets would have prevented 59.6% of malaria

incidence in the entire population of <5 children (95% CI -74.1, 70.8) and 62.9% of malaria incidence in the population of children who slept under best quality nets (95% CI 30.7, 80.2). The odds of malaria rose progressively with age, from 12-23 months (OR 1.05, 95% CI 0.62, 1.80) through >59 months (OR 6.73, 95% CI 1.62, 28.01).

Table 9. Logistic Regression Analysis of Children Seen at Sick Visit Follow-up for Incidence of *P. falciparum* Malaria Rapid Diagnostic Test Positive, Machinga District, Malawi, between March 2012 and April, 2013 (n=806)

Predictor	Adjusted Odds Ratio	95% CI
Net Quality		
No Net	**1**	
Worst Quality Net	**0.8**	**(0.3-1.9)**
Good Quality Net	**0.5**	**(0.3-1.1)**
Best Quality Net	**0.4**	**(0.2-0.7)**
Age Group at Enrollment		
6-11 months	**1**	**1**
12-23 months	**1.05**	**(0.62-1.8)**
24-35 months	**1.27**	**(0.74-2.17)**
36-47 months	**1.67**	**(0.97-2.87)**
48-59 months	**1.67**	**(0.95-2.91)**
>59 months	**6.73**	**(1.62-28.01)**
Season		
Dry	**1**	**1**
Rainy	**2.75**	**(1.69-4.46)**
Caregiver Finished Primary School		
No	**1**	**1**
Yes	**0.65**	**(0.43-0.99)**

Figure 4. ROC Curve for a Model of ITN Use as a Predictor of *P. falciparum* Rapid Diagnostic Test Results When Adjusted for Age, Season, and Education of Caregiver, (n=806)



Limitations of the study

This data set included 2460 sick visits for 1201 children enrolled in the study cohort, however the analysis was restricted to first visits to circumvent the problem of correlated data due to repeated measures and utilize Epi Info for analysis. This approach meant that more of the data in this analysis was collected early in the study period, which may have

weakened the magnitude of the findings observed. For example, hemoglobin level at the conclusion of the study might have been higher than what was measured earlier on if ITNs proved effective at preventing malaria. One month of data was lost, October 2012, due to a problem with the recording system. This likely resulted in the loss of <5% of entries, since most first sick visits were made early in the study period and we would not have expected this to have biased our results.

The use of RDT results to define malaria has benefits and limitations in a setting where many individuals will have malaria parasitemia without being clinically ill. In our sick visit children 71.7% (95%CI 68.4, 74.8) with positive RDT results had temperatures ≤37.5 C, yet caregivers judged them to be sick as evidenced by their presence in sick clinic. The use of RDT results is a more precise measure of the incidence of malaria than clinical diagnosis, and is not subject to as many technical limitations malaria microscopy, therefore it is a robust tool in terms of determining the outcomes of malaria interventions.

Limitations in recall may have made some historical data such as bed net age less precise. Bed net verification exercises were conducted during daytime hours when staff would not actually observe children sleeping under ITNs, yet the degree of accuracy as verified by ITNs hanging was very high, suggesting that participants were giving accurate histories of use. Finally, the distribution of ITNs at the beginning of the study in numbers sufficient to cover every individual in target villages might have blunted the measured effects of ITNs because of the herd benefits of the presence of so many bed nets. If this were true, the impact of ITNs on malaria RDT results would have been stronger than we reported.

Chapter 4: Discussion and Conclusions

Discussion

The results of this study clearly demonstrate that ITNs retained their protective effectiveness against malaria infections in an area with known high levels of pyrethroid resistance in the two principal malaria vectors, if bed nets were relatively new with few holes. ITNs' continued effectiveness in the face of insecticide resistance is consistent with the intact physical barrier of the net and the persistent irritant properties of pyrethroids, which repelled mosquito vectors from sleeping areas. As bed nets aged they developed holes which were directly related to their loss of protective effect.

The bed net quality scale attempted to explore the lifespan of LLIN under real world conditions. Although insecticide remains effective for 36 months, almost half of the ITNs in our study developed holes by 12 months of age, and were either less effective at preventing malaria, or less used by owners because of the entrance of nuisance insects. The effective life of unrepaired ITNs is likely much less than 36 months unless steps are taken to correct the problem of holes. Holes could easily be limited by including inexpensive repair kits with each bed net. At least one manufacturer has considered this. The problem could also be addressed by appointing and equipping individuals at the community level to make repairs to bed nets for minimal prices, yet enough to keep those individuals interested in making bed net repairs. Labels sewn into each bed net indicating net age would further aid the surveillance of ITN condition.

Compared with other similar studies, this study contributed information about the effectiveness of ITN alone in an area in which pyrethroid resistance was measured during the study, and compared the preventive effectiveness of different qualities of ITN. Although malaria microscopy is still considered the gold standard of malaria diagnosis, the use of RDT eliminated many of the practical limitations of microscopy in field settings. (Table 10)

Table 10. Comparison of Studies of Effectiveness of ITNs in Areas of Insecticide Resistance

Study/Year	Country	EIR	Ages of Enrollees	Comparison	Malaria Diagnostic	Resistance	Malaria Incidence
Henry, 2005	Cote d'Ivoire	High	0-59 months	ITN/No Net	Microscopy	Historical, *kdr* 90%	ITN reduced malaria incidence by 12%
Rehman, 2009-2010	Equatorial Guinea, Malawi	High High	1-14 years	ITN/UTN/No Net and variable IRS	RDT	Historical	Best ITNs reduced malaria incidence 35% in EG, 19% in Malawi
Damien, 2010	Benin	High	0-59 months	Correct use ITN/all other	Microscopy	Measured, *kdr* 47% and 61% in two main vectors	Correct use ITN reduced malaria incidence by 26%
This study, 2012-2013	Malawi	High	0-59 months	ITN/UTN/No Net	RDT	Measured, *An. funestus* 71% resistant to deltamethrin, 43% resistant to permethrin *An, gambiae* 47% resistant to deltamethrin, 47% resistant to permethrin	Best nets reduced malaria incidence by 37% compared with no nets.

The increased risk of RDT positivity with advancing age in our cohort verifies what other studies in Africa have observed [38], and indicates that malaria control interventions should now extend to older age groups. Distribution of an adequate number of ITN to cover all household residents was also associated with a significant increase in young children sleeping under ITN, and would allow boarding students and travelers to take ITNs with them without decreasing coverage of other members of their households.

Maternal (caregiver) primary school graduation increased the chances that a child slept under an ITN by tenfold, and is tied to other important factors such as the caregiver's understanding of health messages, whether her view of illness encompasses current preventive care strategies, her role in the family regarding health decision making, and her ability to afford preferential treatment for high risk family members such as <5 children . The absence of significant gender bias in use of ITN and presentation for sick care is encouraging, including the understanding that this will be a factor in enabling girls to access education more equitable.

Stunting in cohort children was significantly lower (20.1%) than that reported in 2010 DHS data [25] for the southern region (47.6%), and wasting was significantly higher (7.3%) than that reported in 2010 DHS data (4%). We did not have an explanation for this, as our data set did not explore many issues necessary to fully evaluate nutrition in the study areas. We were able to exclude stunting and wasting as factors in ITN use and malaria incidence in our sample population.

The developmental impact of improved hemoglobin concentration, though not a primary study endpoint, is important evidence of the power of ITNs to not only prevent malaria, but to improve child wellness, child development, educational potential, and ultimately the wellbeing of society as a whole. When considered in these terms, the benefits of ITNs become even more compelling.

Conclusions

In areas of Malawi with demonstrated high level resistance to insecticides in major mosquito vectors, insecticide treated nets retain their protective effectiveness when they are relatively new and with few holes. In this study, ITNs developed significant numbers of holes by 24 months, limiting their effectiveness. Providing ITNs to every one to two household residents significantly increased the percentage of children under five using bed nets and covered older children, who were at higher risk of malaria parasitemia. However, the strongest factor affecting use of ITN in children under 5 that we identified was maternal education. In the world of malaria control and eradication, we wish to have a magic bullet, whereas in fact we have a chess game. Insecticide treated nets, although threatened by cost, insecticide resistance, poor health literacy and simple neglect, remain one of the most important pieces on the board.

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Appendix A.

Title: Insecticide-treated net effectiveness in a setting of significant pyrethroid resistance: an observational cohort study of malaria incidence in children in Malawi

Protocol v. 7

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Abbreviations and acronyms

ACT - artemisinin combination therapy
AL - artemether-lumefantrine
DHS - demographic and health survey
EIR - entomologic inoculation rate
GEE - generalized estimating equations
Hb - hemoglobin
HSA - health surveillance assistant
IRS - indoor residual spraying
kdr - knockdown resistance gene
LLIN- long-lasting insecticidal mosquito net
MAC - Malaria Alert Centre
MUAC - mid upper arm circumference
NMCP - National Malaria Control Programme
PCR - polymerase chain reaction
PDA - personal digital assistants
PE - protective effectiveness
PSC - pyrethrum spray catch
RDT - rapid diagnostic test
SD - secure digital
TA – traditional authority

Executive Summary

Given their proven efficacy through multiple randomized controlled trials (RCTs) over the last few decades, long-lasting insecticidal mosquito nets (LLINs) have become one of the primary strategies used to reduce the malaria burden in endemic countries. There has been increasing concern, however, about the effectiveness of LLINs in the face of increasing resistance of malaria vectors to pyrethroid insecticides. Pyrethroids are currently the only class of insecticide that is approved for use on LLINs due to their low mammalian toxicity. Although several studies have examined the effect of pyrethroid resistance on entomological indicators using experimental huts, few epidemiological studies have assessed LLIN effectiveness for malaria control in settings of pyrethroid resistance, with somewhat conflicting results.

Given the dependence on LLINs for malaria control in Malawi and other malaria-endemic countries, it is important to determine whether LLINs are still effective at reducing malaria transmission. Since it would be unethical to conduct a RCT of LLINs, given their status as a "proven" malaria control intervention, an appropriate study design is an observational cohort study that carefully measures use of LLINs and accounts for potential confounders to try and isolate the effect of LLINs in the population.

The proposed study will assess the effectiveness of LLINs on malaria incidence in an area of documented high resistance to pyrethroids in Machinga District, Malawi. As an expected national, universal LLIN coverage campaign has not yet gotten underway, we will provide full LLIN coverage in the study area using LLINs approved by the World Health Organization and used widely in Malawi and other African countries. Using a cohort approach, the study will measure monthly malaria incidence—defined by new episodes of asexual parasitemia—using both active and passive surveillance over an 21-month period and capture information on LLIN use and important confounding factors. Concurrent entomologic monitoring will document the nature and mechanisms of insecticide resistance in the study area.

The study will consist of several components, primarily an incidence cohort of children aged 6–59 months, cleared of parasitemia at baseline, and followed monthly for 21 months using active surveillance for parasitemia and anemia. The cohort will be replaced after first 12 months to ensure representativeness of the cohort to the population aged 6-59 months in the population. In addition, children in the cohort will be encouraged to visit the study clinic at Machinga District Hospital, the main health facility serving the study area, if they become ill in between the monthly monitoring visits. At all visits, the caregivers of cohort children will be asked for a two-week illness history along with a thorough LLIN use history; a blood sample will be taken for thick and thin blood smears, and anemia testing; filter paper blood samples will be taken at enrollment to detect subpatent infections using molecular assays. At the beginning, middle and end of the survey, an LLIN census will be carried out on all households in the study area to identify household ownership of various types of LLINs. During the 21-month period, several measurements will be taken to validate LLIN use data and test insecticide concentration on LLINs. At baseline, six months and 12 months, measurement of pyrethroid availability on the surface of LLINs will be

conducted. Finally, entomologic monitoring of vector density will be conducted with pyrethrum spray catches (PSCs) and phenotypic resistance testing will be done using the CDC bottle and WHO tube assays during the 21 month study period.

Standard laboratory techniques will be followed for analyzing blood samples for malaria parasitemia and anemia. Children found to be positive for malaria during the monthly monitoring visits (active surveillance) or during a sick visit to Machinga Hospital (passive surveillance) will be treated with a weight-appropriate course of the first-line antimalarial in Malawi according to national guidelines. Children who are found to be anemic will receive a supply of iron syrup; anemic children 12 months and older who have not received an antihelminthic in the past six months will receive appropriate treatment for deworming.

We will use generalized estimating equations approaches that take into account the correlated nature of the longitudinal data. The primary outcome of interest is the protective effectiveness of LLINs in the study area, assessed by comparing the incidence of malaria among LLIN users to non-users in the study population. Potential confounders of LLIN use, including socioeconomic status and maternal education, in addition to LLIN type, age and condition, will be assessed and included in the model if appropriate. Data will be analyzed separately for each cohort of children. Study findings will be disseminated to the National Malaria Control Program and other partners working in malaria control in Malawi, and will be written up for publication in a peer-reviewed journal. We will make a specific effort to present the findings to the Machinga District public health community.

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1. Background

Given their proven efficacy through multiple randomized controlled trials (RCTs) over the last few decades, long-lasting insecticidal mosquito nets (LLINs) have become the cornerstone of global malaria control to reduce the malaria burden in endemic countries. LLINs serve as a physical barrier to night biting mosquitoes that are prevented from transmitting malaria to sleepers. The addition of a pyrethroid insecticide to the net fabric enhances the efficacy of LLINs by repelling mosquitoes from entering houses, causing them to exit before feeding and by killing mosquitoes that come into contact with the nets [1-3].

Multiple RCTs have demonstrated the efficacy of LLINs on reducing child mortality and morbidity, including parasite prevalence, and the incidence of clinical malaria, anemia and child sick visits. A 2004 systematic review found that LLINs reduce all-cause child mortality by 18% [4], the incidence of parasitemia and clinical malaria by 50% [4] and the prevalence of parasitemia by 13% compared to no nets [4]. Analysis of observational data from seven national cross-sectional surveys found similar reductions in parasitemia of 20% among children under five who used LLINs compared to those who did not [5], suggesting that LLINs have a "similar and sizeable effect on health outcomes under routine use compared to that seen in efficacy trials" (p. 11).

However, given the recent increases in insecticide resistance among malaria vectors in Africa, there is increasing concern about the continued effectiveness of LLINs [6]. Pyrethroids are currently the only class of insecticide that is approved for use on LLINs due to their low mammalian toxicity. As malaria endemic countries strive for universal coverage of LLINs, pyrethroid resistance among malaria vectors represents a potential threat to the long term sustainability of malaria control programs. However, while pyrethroid resistance has been reported from many African countries, there is a dearth of data on the impact of resistance on the effectiveness of LLIN programs. One experimental hut trial in an area of Benin, where mosquitoes had high resistance to pyrethroids due to the presence of the knockdown resistance gene (*kdr*), found that lambda-cyhalothrin-treated nets with holes had no effect on human blood feeding and killed only 30% of mosquitoes compared to 96% inhibition of feeding and 98% mosquito mortality rates in an area with susceptible mosquitoes. Although the results still indicated some personal protection (~50%) relative to untreated nets, the insecticidal effect was more than 95% lost, undermining the potential community effect of LLINs [3]. There is only very limited epidemiological evidence on the effect of pyrethroid resistance. One study in Cote d'Ivoire, in an area with high *kdr* resistance, found continued high efficacy of LLINs, which reduced parasite prevalence by 12% and malaria incidence over one year by 56% ($p<0.001$ for both), similar to the effects of LLINs in nearby areas with no documented insecticide resistance [7]. Another study from north Cameroon, however, where *Anopheles gambiae* demonstrate elevated oxidase enzyme activity that can detoxify pyrethroids, showed that children randomized to sleep under LLINs had significant reductions in parasitemia prevalence after three months compared to children randomized to sleep under untreated nets, but this protective effect disappeared by six months [8]. More recently, a study in Senegal reported a surge in malaria incidence that coincided with an increase in the

frequency of the *kdr* allele, which has been associated with resistance to DDT and pyrethroid insecticides [9].

In Malawi, household ownership of an LLIN as of the last malaria indicator survey in 2010 was 58%. A national LLIN universal coverage campaign was supposed to have launched in November 2011 but was delayed due to organizational challenges. This campaign should resume between February and April 2012 and will boost LLIN coverage significantly. In seven districts, Malawi has conducted indoor residual spraying (IRS); two districts used organophosphates and five used pyrethroid insecticides. Pyrethroid resistance has been reported from other countries neighboring Malawi, including Mozambique and Zambia, as well as from Likoma Island in Malawi [10-12]. In 2010 in Nkhotakota District, *An. funestus* was observed resting on walls in houses that had recently been sprayed with a pyrethroid (T. Mzilahowa, J. Gimnig, unpublished data). It was later determined that this population of mosquitoes was resistant to three different pyrethroid insecticides (M. Coleman, T. Mzilahowa, unpublished data). In 2011, pyrethroid resistance in *An. funestus* was observed in five districts in Malawi. In Machinga District in 2011, mortality of *An. funestus,* the major malaria vector, was between 0 and 33% after exposure to 0.05% deltamethrin in a standard WHO tube assay. Typically, WHO considers resistance to an insecticide to be present when mortality falls below 80%.

This observed pyrethroid resistance forced a switch in Malawi in the insecticides used for IRS in 2010 in the two districts sprayed using funds from the President's Malaria Initiative, from a pyrethroid to an organophosphate. Preliminary data suggest the entomological impact of IRS was greater in districts sprayed with a non-pyrethroid than in districts sprayed with a pyrethroid insecticide (T. Mzilahowa, unpublished data). Given the impact of pyrethroid resistance on the effectiveness of IRS with pyrethroids, the question of how much pyrethroid resistance impacts the effectiveness of LLINs becomes paramount. This study is designed to assess the effectiveness of LLINs in Machinga District, where high levels of pyrethroid resistance have been observed, using an observational cohort study combined with entomological monitoring. If LLINs remain effective, net users should experience less malaria than non-users. If pyrethroid resistance is compromising the effectiveness of LLINs, then the incidence of malaria is expected to be similar among both users and non-users.

If the results of this study suggest no significant added benefit of LLINs due to insecticide resistance, we will recommend use of IRS with non-pyrethroids in malarious areas with significant pyrethroid resistance. However, there are large parts of Malawi as well as other countries in Africa that have significant pyrethroid resistance, and the cost and availability of non-pyrethroid insecticides will determine whether there are sufficient funds for IRS. Additionally, we will await results from a companion, entomology-based protocol on the use of LLINs combined with a synergist (a chemical that renders resistant mosquitoes susceptible to pyrethroids) to determine whether combination nets might be useful in reducing malaria transmission. If combination nets prove effective in pilot testing on entomological outcomes, we will develop a protocol to determine whether they have an impact on epidemiologic outcomes in the area whether this study is conducted.

1.1 Justification for study

LLINs are one of the principal malaria control strategies used in Africa. In Malawi, the National Malaria Control Program (NMCP) plans to achieve universal coverage with LLINs by the end of 2012. However, mortality of *An. funestus* exposed to pyrethroids has fallen below 80% in all pyrethroids tested in five districts where entomologic resistance monitoring has taken place over the last three years. Given the dependence on LLINs for malaria control in Malawi and other malaria-endemic countries, it is important to investigate whether LLINs are still effective at reducing malaria. Because RCTs of LLINs compared to no LLINs would not be considered ethical, an observational cohort comparing those who choose to use LLINs and those who do not is the best study design to determine whether LLINs continue to be effective. Because use of LLINs is a personal or familial choice, there is substantial opportunity for confounding of the association between LLIN use and malaria incidence. The measurement of potential confounders, and the validity of LLIN use variables, are of primary concern in this study.

2 Study objectives

The proposed study will assess the effectiveness of LLINs on malaria incidence in an area of documented high resistance to pyrethroids. Using a cohort approach, the study will measure monthly malaria incidence—defined by asexual parasitemia—in the study cohort using active and passive surveillance over a 21-month period and will capture information on LLIN use and important confounding factors. The study will also use entomologic monitoring to characterize vector populations and susceptibility in the area over the study period.

2.1 Primary objective

Measure the protective effectiveness (PE) of LLINs on the monthly incidence of malaria infections (both symptomatic and asymptomatic) over a 21-month period among children 6-59 months old in an area of high mosquito resistance to pyrethroids.

2.2 Secondary objectives

1. Measure the PE of LLINs on monthly incidence of clinical malaria among children 6–59 months old over a 21-month period;
2. Measure the PE of LLINs on the first or only episode of moderate to severe anemia (hemoglobin [Hb]<8 g/dl) among children 6–35 months old over a 21-month period;
3. Measure the PE of LLINs on all-cause sick visits over a 21-month period based on passive health facility-based surveillance;
4. Characterize malaria vector species and density in the study area through twice-monthly pyrethrum spray catches in selected households in each study area;
5. Assess insecticide resistance in the study area using both the WHO tube assay and CDC bottle assay;

6. Assess the use of cell phones by residents of the area to determine whether they could serve as a channel of communication for malaria interventions.

3 Methods

3.1 Study design

We will conduct a cohort study of children 6-59 months of age, cleared of parasitemia at baseline, who will be followed using active and passive surveillance for 21 months to measure the PE of LLINs in reducing malaria. The cohort will be replaced after first 12 months to ensure representativeness of the cohort to the population aged 6-59 months in the population. A planned national LLIN universal coverage campaign was to have provided LLINs to all study area households but did not launch in November 2011. Therefore, we will provide WHO-approved LLINs (Permanet 2.0, Vestergaard-Frandsen, Lausanne, Switzerland) to all houses in the study area, including households that do not have eligible children or choose not to participate in the study. We will attempt to cover all sleeping spaces with an LLIN but at the minimum we will provide one LLIN for every two residents plus an extra LLIN to cover odd numbered residents. At the beginning of the study, an LLIN census will be carried out on all households in the study area to determine the household ownership and use of LLINs, along with the type, age and condition of each net (these will be existing LLINs). In the middle and the end of the study, the community census will be repeated to monitor the number and type of LLINs used in the study area. Use of LLINs by participating children will be monitored monthly. At one to two points during the study, an LLIN validation exercise will be conducted to compare self-reported LLIN use with observed use. At six and 12 months, pyrethroid availability on the surface of LLINs will be measured. On a monthly basis, PSCs will be conducted to monitor mosquito density, sporozoite rates and entomologic inoculation rates along with vector species composition. Mosquitoes will be collected for resistance testing at the beginning and end of the study in four to five locations distributed throughout the study area. Resistance testing using the WHO tube assay, CDC bottle assay as well as polymerase chain reaction (PCR) analysis of mosquito DNA will be implemented.

Table 1 below provides an overview of the various study components and the timing of each.

Table 1: Malawi LLIN effectiveness study activities and timeline, **2012—2013**

		Study Month and Season																							
		Rainy						Dry						Rainy						Dry					
		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D
1	Household, LLIN and sleeping space registration/Make appointments for enrolment		X																						
2	Incidence cohort enrolment			X																					
1	Permanet distribution, centralized area			X	X																				
2	LLIN census		X									X						X							
2	Incidence cohort monthly surveillance visit				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Anthropometric measurements*			X					X						X						X				X
2	Passive health facility surveillance (sick visits)			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2	Validation exercise										X	X							X	X					X
2	LLIN integrity					X		X		X	X	X	X	X											X
2	LLIN insecticide concentration									X															X
3	Pyrethrum Spray Catches		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	Resistance testing		X					X					X												X
4	Cell phone assessment											X													

*** As part of incidence cohort measures.**

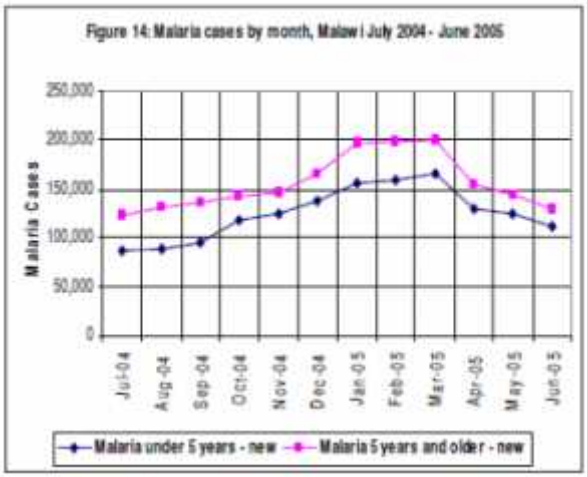
1 = All household in study area (sample for LLIN bioassays)

2 = Incidence cohort-related measures 3 = Entomologic measures (on a subset of households and LLINs)

3.2 Study setting and population

This study will be conducted in six to nine villages of the Sitola Traditional Authority (population approximately 39,000) in Machinga District in southern Malawi. This site has been used previously by CDC for *in vivo* efficacy studies of several antimalarials. Residents of Machinga are mainly of the Yao ethnic groups, who earn their living through subsistence farming, fishing, and small businesses. Chichewa is the main language spoken in the study area. Malaria transmission is high and perennial, with peak transmission during and after the rains, November through April. *Plasmodium falciparum* is the dominant malaria species in Malawi although *P. malariae* and *P. ovale* are also present. According to the 2010 Malawi Demographic and Health Survey (DHS), 72.3% of children in Machinga were anemic (44.8% moderate or severe anemia), comparable to the national averages of 63.5% anemic (39.7% moderate or severe) [13]. Among children less than five years of age who had fever in the previous two weeks, 36.2% received an artemesinin-combination therapy (ACT) from any source, and 23.9% received an ACT within 24 hours of fever onset [13]. In Machinga, 53.2% of children with fever in the last two weeks were taken to a health facility or provider (compared to 59.4% nationally), according to DHS 2010 data [13]. The July 2004–June 2005 Health Management Information System report indicates that malaria incidence rates in Machinga District (384 per 1000 per year) were somewhat higher than the nationwide malaria incidence rate (282 per 1000 per year). Using data from the same source, Figure 1 graphs malaria cases by month for the country from July 2004 to June 2005.

Figure 1. Malaria cases by month, Malawi July 2004–June 2005



DHS data indicate that 56.8% of households in Malawi owned an LLIN, and 39.5% of children under five slept under an LLIN the night before the survey, although the DHS was conducted during the dry season when use is lowest [13]. According to the 2010 MIS report, which was conducted during the rainy season, household LLIN ownership is 58.2%, and use among children <5 years old is 55.4%. ACTs (artemether-lumefantrine, or AL) were introduced as first-line treatment of uncomplicated malaria and are provided in government and publicly-supported Christian Association of Malawi health facilities since

2007. National guidelines require that children <5 years old with reported fever be treated presumptively for malaria if parasitologic (rapid diagnostic tests or microscopy) diagnosis is unavailable. Those ≥5 years old should be evaluated with a diagnostic test and treated for malaria according to diagnostic test results. Rapid diagnostic tests were rolled out to health facilities in Malawi in mid to late 2011, although currently demand is greater than the supply.

In the study area, the only health care facility is Machinga District Hospital. Currently, there is no community-based case management for malaria.

Entomological indicators
The main malaria vectors are *An. gambiae* s.s., *An. funestus*, and *An. arabiensis* with *An. funestus* predominant in areas of perennial transmission along the lakeshore and the Shire river valley. Although measurements of the entomologic inoculation rate (EIR) are unavailable for Machinga, it is expected to be over 100 infective bites per year (W. Hawley, unpublished data). Data from standard WHO tube assays show *An. funestus* mortality when exposed to deltamethrin 0.05% of between 0 and 33% (T. Mzilahowa, unpublished data).

Study setting within Machinga
The study area in the Traditional Authority (TA) Sitola consists of approximately 2500 households in the rural areas of the TA on the east bank of the Shire River near Machinga District Hospital. The area consists of nine villages, and all households within these study area villages will be provided with LLINs free of charge by the project. We will attempt to distribute one LLIN (Permanet 2.0) per sleeping space, but at a minimum we will provide one LLIN per two household residents, with an extra LLIN for odd-numbered households.

3.3 Study period

The cohort study will last for 21 months in total, with an additional month of data collection during baseline. A cohort of children ages 6–59 months will be followed monthly for a 21-month period. The cohort will be replaced after the first 12 months to ensure representativeness of the cohort to the population aged 6-59 months in the area. The baseline LLIN censuses will take place at the beginning of the study. Entomological monitoring and passive surveillance of sick child visits at Machinga District Hospital will take place during the 21 months of the study.

3.4 Inclusion and exclusion criteria

<u>LLIN census</u>
Inclusion criteria

- Living in a household in study area
- Verbal consent

<u>Incidence cohort study</u>
Inclusion criteria

- Living in a household in study area

- Child aged 6–59 months at beginning of cohort study
- Written, informed parental consent

Exclusion criteria

- Children weighing < 5 kg
- Severely ill and unlikely to be able to complete study
- Taking cotrimoxazole prophylaxis for HIV infection
- Intending to stay in the study area less than one month

Enrollment in the incidence cohort study covers passive surveillance of sick child visits and every two month visits to measure LLIN integrity.

3.5 Sample size

Incidence cohort study

The study sample size is based on a generalized estimating equations (GEE) modeling approach that takes into account the correlated nature of the longitudinal measurements within study children. A Poisson distribution will be used to model the incidence of malaria in study children over the 12-month study period, with the aim of isolating the effect of recent LLIN use, controlling for other important confounding variables and predictors.

To calculate the sample size needed, we used a program developed based upon a GEE approach to longitudinal data analysis [14]. Assumptions made for key parameter inputs into the model are summarized in Table 2.

Table 2: Key parameters used in sample size calculation[14]

Parameter	**Value used in calculation**
Power	70%
Alpha (Type 1 error rate)	5%
Malaria incidence among non-LLIN users	1 episode/child/year
Malaria incidence among LLIN users	0.7 episodes/child/year
LLIN use in study area among cohort children	70%
Phi (within-child correlation)	0.25
Type of GEE correlation structure	Exchangeable
Dispersion parameter (Psi)	1 (model not over- or under-dispersed)

Inputting the parameters specified above in the sample size program yields a total of 684 LLIN users and 297 non-LLIN users (total = 981 cohort study children) required to demonstrate at least a 30% reduction in incidence between the two groups. Assuming a 5% refusal rate, a 15% loss-to-follow-up rate across the study, and a mortality rate among the cohort of 65 per 1,000 live births (~1.5% per year), a total of 1,335 children need to be enrolled at baseline. Under the assumption that in the study area, 60% of households have at least one child aged 6–59 month, a total of 2,225 households would need to be approached for cohort recruitment. Given that 2500 households have been mapped in the study area, all households will be approached at baseline and again at 12 months during the LLIN census for recruitment of children into the cohort study.

For the second cohort beginning in 2013, the parameters to be used are as above. The length of time the cohort is to be followed is decreased from 12 to 9 months, but this only increases the sample size by 6% to 1043. In the first cohort, we estimated a 5% refusal rate, but actually had a 12.4% refusal rate. An additional 5.3% of children were ineligible due to use of cotrimoxasole in HIV-exposed children, 3.4% had moved out of the study area by the time of enrollment, and 6.8% could not be located. Once enrolled, loss to follow-up was within the expected 15%. During the past year, community relations have improved substantially and we expect refusal to be significantly lower. Some of the children currently in the cohort may decide to not participate in the renewal, but we feel this will be minor. Given a shorter time frame, we estimate loss to follow-up to be on the order of 10%, requiring a sample size of 1159. We therefore estimate overall non-participation to be 20%, requiring a total sample size of 1449. We registered 2727 households in the last census and 1644 children who will be aged 6-59 months at the start of the new cohort. Given that our sample size is close to the total number of children in the study area, we will again approach all households to recruit them for the new cohort.

PSCs

Sample sizes for PSCs were estimated using the mean difference option in OpenEpi (http://www.openepi.com/OE2.3/Menu/OpenEpiMenu.htm). It is estimated that an effective net will reduce susceptible mosquito populations by 50% or more and that mosquito numbers before the net distribution will be 20 *An. funestus* per house. It was assumed that the standard deviation of mosquito numbers in houses would be equal to the mean and that 70% of households would use nets on any given night. To detect a difference in 10 mosquitoes per house in any given month at α=0.05 with 80% power, a total of 73 houses would need to be sampled. To ensure adequate sampling of the entire study area, we will sample from 10 houses per village per month giving a total of 110 houses. This sample size exceeds the estimated number required but will account for under-sampling that may occur on some days and will allow for village level clustering to be taken into account in the analysis.

Study procedures

3.6 General community sensitization

Initial study approval will be sought from the National Malaria Control Programme, followed by the District Health Management Team of Machinga District, the District Development Committee and the TA in the study area. Before beginning the LLIN census and the incidence cohort study, study coordinators will hold meetings with leaders within the mapped communities to explain the intended purpose and methods of the study. Study staff will initially meet with district administrative and health authorities to obtain permission to carry out the study in Machinga. Staff will also meet with traditional authorities, community leaders, and health surveillance assistants (HSAs) in the target communities. Once leaders grant their permission, study staff will hold a series of general community meetings to explain the study purpose, eligibility criteria, study procedures, and discuss the informed consent process.

3.7 Household, LLIN and sleeping spaces registration

Data from each household in the study area will be collected at baseline about the household's occupants, ownership and use of existing LLINs, and the number of sleeping spaces. The purpose of this exercise is to: a) identify eligible children, b) document existing LLINs as baseline data and c) obtain the number of sleeping spaces to inform the free LLIN distribution we will undertake.

All households within the study area will be visited. Verbal permission will be obtained from a household respondent who is a usual member of the household at least 18 years of age. For those households that will be headed by a person under-18 years of age, we have defined them in this protocol as matured/emancipated minors. They will provide all informed consents for the enrollment of their household and their under five children. At least three attempts will be made to interview a suitable respondent if the survey team finds no one at home initially. If the household respondent grants verbal consent (see Appendix 1 for consent script), the survey team will administer a brief questionnaire that asks about: age and sex of all members of the household and guests who stayed the previous night; the number, type and age of all LLINs owned by the household; and whether each LLIN owned was used the previous night (and by which household members), and number of sleeping spaces in the household (see Bednet Census Questionnaire in the questionnaire files).

Households reporting resident children 6 to 59 months old as of March 1, 2012, will be left with an enrollment appointment card to attend the enrollment activity approximately one month later. In 2013, households from the last 2012 census reporting resident children who would be aged 6 to 59 months as of March 1, 2013, will be requested to attend enrollment in late March/early April 2013.

The household LLIN census will be repeated at the middle and end of the study. The information from the census will be used to define LLIN coverage overall and within certain distances from cohort participants. We will also use LLIN census data to examine

the impact of a mass distribution for universal coverage on the LLIN use patterns in the area.

3.8 Incidence cohort

All households found to have a child between 6 and 59 months of age during the LLIN censuses will be eligible for participation in the incidence cohort study. During the initial registration exercise, households with children in this age range will be invited to the study enrollment during the following month.

Cohort enrollment

Study enrollment will take place in small groups at multiple locations throughout the study area, chosen to be close to the homes of all participants. On enrollment day, all households with children 6 to 59 months of age will be invited to attend. The study team will provide a brief introduction to the study, ask for verbal consent to conduct a brief screening (see Appendix 2 for the verbal consent script) and then apply a short screening form to determine the child's eligibility (the screening form is included as the first part of the cohort enrollment form). If the child is eligible, the study teams will read or allow the child's caregiver to read the consent form (see Appendix 3 for the consent form) and explain any questions they may have. After written, informed consent is obtained from a child's parent or caregiver, the child will be enrolled in the cohort study and assigned a study id number.

During the study enrollment, a questionnaire will be administered to the caregiver of enrolled children including an illness history for the previous two weeks, history of antimalarial medications taken, and LLIN use in the previous night and the previous two weeks (see questionnaire titled Cohort Enrollment Questionnaire). The questionnaire will also include questions on potential confounders of LLIN and malaria incidence, including: wealth, maternal and paternal education, caregiver's knowledge about malaria, and other protective practices in the household against mosquitoes (e.g., coils, sprays). The child's axillary temperature will be taken, and data on the child's height (recumbent if unable to stand), weight, and middle-upper arm circumference (MUAC) will be collected. Trained study nurses will take finger-prick blood samples on enrolled children to collect enough blood for a thick and a thin blood smear, filter paper sample (for subsequent analysis by PCR to detect sub-patent levels of parasitemia) and hemoglobin measurement. Children found to be anemic according to national guidelines in Malawi (Hb<10.5) will be given a two-week supply of iron syrup at recommended dosages according to age. Anemic children >12 months of age who have not taken an antihelminthic in the last six months will be given appropriate antihelminthic treatment. The caregivers of anemic participants will be instructed to visit a local health facility after two weeks for re-assessment. Blood smears will be transported back to the Machinga District Hospital lab for staining and reading.

During the study enrollment, all children enrolled in the cohort who have not taken an antimalarial in the previous seven days will be given a weight-appropriate course of AL to clear any parasitemia. The first dose of AL will be given by the study team (and will be re-administered if the child vomits within 30 minutes). Subsequent dose administration will be conducted by the caregiver at home. If a child has taken AL within the previous seven

days, a weight-appropriate dose of the second line antimalarial, artesunate-amodiaquine, will be given.

Malawi has a high rate of use of health passports. The health passports of enrolled children will have a sticker placed in them identifying children as participating in the LLIN effectiveness study. They will be asked to show their passport and the sticker for any sick visits to the study clinic at Machinga District Hospital. Village field assistants will be recruited from every village as part of the study team; they will be given a list of all children enrolled in the cohort and will be responsible for reminding caregivers to bring their children to the selected location for the next monthly visit.

Monthly cohort surveillance visits
Each month, caregivers will be asked to bring enrolled children to the same central location, within walking distance of their homes. The study team will administer a brief questionnaire to the parent/guardian about the child's illnesses and symptoms in the previous two weeks, any antimalarial medications taken, and LLIN use the previous night and in the previous two weeks by the enrolled child.

The child's axillary temperature will be taken. (Anthropometric measurements will only be repeated at six months and 12 months.) Trained study nurses will perform finger sticks on enrolled children to collect enough blood for a thick and a thin blood smear, and hemoglobin measurement. Blood from the sample will be placed in a rapid diagnostic test (RDT) for immediate parasitemia. RDTs will be read after 15 minutes, and children with positive RDTs who have not received AL in the previous 28 days will be given a weight-appropriate dose of AL. The first dose of AL will be given by the study team (and will be re-administered if the child vomits within 30 minutes). Subsequent administration of doses will be given by caregivers at home. If a child has had a full course of AL in the last 28 days, they will be treated with a weight appropriate dose of the second line antimalarial, artesunate-amodiaquine.

Children found to be anemic according to national guidelines in Malawi (Hb<10.5) will be given a two-week supply of iron syrup at recommended dosages according to age. Anemic children $\geq$12 months of age who have not taken an antihelminthic in the last six months will be given appropriate dosage of an antihelminthic. The caregivers of anemic participants will be instructed to visit a local health facility after two weeks for re-assessment.

Blood smears will be transported back to the study lab for staining and reading. A prioritization algorithm for reading the blood smears will be followed to expedite the receipt of AL by parasitemic children who were RDT negative. Slides will therefore be read in the following order: 1) slides from children who are febrile but who had a negative RDT; 2) slides from afebrile children who had a negative RDT; 3) slides from children who had a positive RDT. All children whose blood smear shows asexual parasites and who have not yet been given AL by study staff (e.g., RDT was negative) and had not previously taken AL in the 28 days before the blood sample was taken will be given a full course of ACT by the village field worker within 48 hours. If a child has had a full course of AL in the last 28 days,

they will be treated with a weight appropriate dose of the second line antimalarial, artesunate-amodiaquine.

3.9 Permanet distribution and LLIN census

Permanets will be distributed to all households in the study area based on the number of sleeping spaces identified in the registration activity (or as one LLIN per every two residents plus one net for odd-numbered households, depending on the total number of LLINs required). A centralized point in each village will be used to distribute LLINs. After the distribution is complete, all households with enrolled children will be visited to a) ensure LLINs are deployed properly, and b) register each LLIN to the appropriate household, and c) label each LLIN with a vinyl wristband on which is pre-printed a unique LLIN identification number.

3.10 LLIN use verification

Verification exercises will be conducted twice during the study period. Children participating in the cohort will be selected at random and visited at home within two days of a monthly visit. The use or non-use of an LLIN will be verified through direct observation and compared with data self-reported during the monthly visit.

3.11 Passive surveillance of sick visits

Children enrolled in the incidence cohort will have a sticker placed in their health passport identifying them as participating in the LLIN effectiveness study and reminded to show the sticker each time the child visits Machinga District Hospital over the next 12 months: this is the only government health facility within the study area. They will be told that transportation to the district hospital for sick visits will be reimbursed. Clinicians at the hospital will be briefed before the study and will be asked to refer children to the study clinician for examination. The study clinician will perform a full clinical examination and provide appropriate treatment free of charge. Similar to the monthly active surveillance visits described above, a brief questionnaire on symptoms and LLIN use will be administered, and testing for malaria and anemia will be conducted as described above.

3.12 Entomological monitoring

Two primary types of entomologic monitoring will be carried out: PSCs to assess vector species, density and infection rates, and resistance testing, each of which is described below.

Pyrethrum spray catches (PSCs)

PSCs will be carried out twice monthly in each of the nine villages in the study area to calculate entomologic inoculation rates (EIRs; the number of malaria infectious bites received per person per night) and determine the vector species composition. During the first week of the month in each village, two households in each village will be randomly

sampled from the list of households. The four houses closest to each sampled index house will also be included in the PSC collections. During the third week of the month, the process will be repeated, resampling the two index houses in each village. Two entomology field technicians will be trained to carry out the PSCs. In each selected house, all food items will be removed and then white sheets will be laid upon the floor and over the furniture. Two collectors, one inside and one outside the house, will spray around the eaves with cans of insecticide, such as DOOM or Raid. The collector inside the house will then spray the roof and walls. The house will be closed for 10–15 minutes, after which time dead mosquitoes will be collected from the sheets and transferred to the laboratory on filter paper inside petri dishes. In the laboratory, the species of *Anopheline* mosquites collected will be recorded, along the number of each species. The sporozoite rate will be calculated using an ELISA test on a sample of the collected mosquitoes.

Resistance monitoring

Phenotypic resistance monitoring will be carried out in the study area at baseline and 12 months at five geographically dispersed sites within the study area (e.g., North, South, East, West, and central locations). Permethrin and deltamethrin will be tested for local mosquito susceptibility using the WHO tube assay. Live mosquitoes will be collected and their progeny (F-1 generation) will be exposed to the WHO tube assay and the percentage of mosquitoes dead after 24 hours after will be recorded and adjusted based on the mortality rate in a control tube assay.

3.13 Cell phone assessment:

During the second household census, we will also census the cell phones owned by the household and request information on the use of cell phones for text messaging. We will explore ownership and access to cell phones among family members to determine whether it is feasible to pass health communication messages through this channel.

4 Study team composition

A full-time study coordinator employed by the Malaria Alert Centre (MAC) will be based in Machinga to oversee day-to-day activities of the LLIN resistance study. Three teams, each composed of one nurse and one surveyor, will be hired for the duration of the study. Assuming teams will collect cohort surveillance data a total of 18 days per month (four days per week, with the fifth day used to prepare supplies, touch base with the study coordinator, and resolve problems arising during data collection), a team will need to collect data on a minimum of 27 children/day, assuming a total initial sample size of 1,380 children. Three additional surveyors and three additional nurses (baseline) will be hired during the enrolment to expedite data collection.

A full-time study nurse will be hired and based at Machinga District Hospital. This study nurse will conduct sick child visits and collect relevant study data for all children in the cohort study visiting Machinga District Hospital during the study.

Three full-time entomology field assistants will be hired and trained to carry out the PSCs and conduct the bioavailability data. The entomology field assistants will work closely with an expert entomology at MAC, who will oversee their work and analyze samples collected.

The village field worker will assist with the LLIN censuses and the cohort study. Among other things, the field worker will be asked to remind parents to bring their enrolled children to the monthly monitoring visits, to seek care at the health facility if they are ill, and to verify the type of net used by each child who reports LLIN use during the monthly survey. A small monthly incentive will be provided to field workers to compensate them for their time spent assisting with this research study.

5 Laboratory procedures

5.1 Microscopic blood examination

Thick and thin blood smears for parasite counts will be obtained at baseline and at each monthly incidence cohort visit and each sick visit. Specimens will be labeled with the participant's name (for easier treatment of positives), study identification number, and the date. A fresh Giemsa or Field's stain dilution will be prepared at least once per day and possibly more often, depending on the number of slides to be processed. The stained thick and thin blood films will be examined at a magnification of 1,000x to identify the parasite species and to determine the parasite density.

Parasite density will be assessed by counting the number of asexual parasites in a set number of white blood cells (typically 200) with a hand tally counter. Once a field has been started, it must be counted to completion; the final number of white blood cells will therefore rarely be exactly 200. If more than 5,000 parasites have been counted before 200 white blood cells have been reached, the count will be stopped after the reading of the last field has been completed. Parasite density, expressed as the number of asexual parasites per µl of blood, will be calculated by dividing the number of asexual parasites by the number of white blood cells counted, then multiplying by an assumed white blood cell density (typically 8,000 per µl).

$$\text{Parasite density (per µl)} = \frac{\text{number of parasites counted} \times 8000}{\text{Number of leukocytes counted}}$$

The same technique will be used to establish the parasite count on each subsequent blood film. When the number of asexual parasites is less than 10 per 200 white blood cells in follow-up smears, counting will be done against at least 500 white blood cells (i.e., to completion of the field in which the 500th white blood cell is counted). A blood slide will be considered negative when examination of 500 white blood cells reveals no asexual parasites.

Microscopists will be blinded to LLIN use of the child. Two qualified microscopists will read all the slides independently, and parasite densities will be calculated by averaging the two counts. Blood smears with discordant results (e.g., differences between the two microscopists in species diagnosis, in parasite density of > 50% or in the presence of

parasites) will be re-examined by a third, independent microscopist, and parasite density will be calculated by averaging the two closest counts.

5.2 Measurement of hemoglobin concentration

Hemoglobin will be determined as per Table 2 using Hemocue Hb 201+ Analyzer (Hemocue, Inc, Cypress, CA, USA). About 10 microliters of blood will be collected via venipuncture for hemoglobin testing.

5.3 Measurement of *P. falciparum* by PCR

Filter paper blood samples will be taken at enrollment to detect *P. falciparum* by PCR (to identify subpatent infections). Filter paper samples will be labeled using a unique sample ID printed on a bar code printer and the date. The unique sample ID will be recorded on the questionnaires at the time the samples are taken.

5.4 Specimen handling

All laboratory results forms will be labeled with the participant name and study ID. Blood smears will be labeled with the participant name and study ID for rapid follow-up if they are positive. Filter paper samples will be labeled using a unique sample ID printed on a bar code printer, and the date of collection. The same unique sample ID will be recorded on the questionnaires at the time the samples are taken. After the laboratory results forms are completed, they will be returned to the data management staff, and the filter paper samples will be returned to the laboratory staff. All filter paper samples will be retained at MAC or CDC until molecular analyses have been completed and then they will be destroyed.

6 Data management and analysis

6.1 Data management

Data will be collected using hand-held personal digital assistants (PDAs). Range and logic checks will be built into the data collection programs. Data from each PDA will be backed up onto secure digital (SD) cards each evening. The study coordinator will download and merge data from all study PDAs on a regular basis, and he and CDC staff will conduct interim quality checks on data collected and make any adjustments necessary to data collection programs.

PDAs will be password protected, and downloaded data will be stored on password-protected computers accessible only to study investigators. Names of household members will be collected to identify who slept under LLINs, and names of enrolled children will be written on blood smears during monthly cohort visits and sick visits, but will names be stripped from the final dataset for analysis. Households will be uniquely identified by their GPS points, and cohort children will each be assigned a unique five-digit code that will be pre-printed and affixed to their study card, each collected blood smear, and the child's health passport, in case the study card is lost.

6.2 Data analysis

All data will be analyzed using SAS 9.3. The primary outcome of malaria incidence will be measured using GEE, to account for the correlated nature of the longitudinal data [16], and a Poisson regression model. Each cohort will be analyzed separately. LLIN use for each enrolled child will be allowed to vary by time period. Additional variables will be included in the model if they are related to the outcome and/or are potential confounders, including fixed covariates (e.g., socio-economic status, mother's education) and time-dependent covariates (season, child's age). Person-time in the 14-day period following any antimalarial treatment will be excluded from the analysis, as these children are not considered "at risk" of developing malaria infection. The rate of sick child visits to the health facility, for malaria and for all illnesses, will also be calculated using Poisson regression for each age group. Mosquito numbers will be compared using Poisson regression with the number of people in the house, the number using LLINs the previous night, the use of other household insecticides (e.g. sprays, coils), housing characteristics (open fires for cooking in house, type of roof, type of walls, open/closed eaves), distance from the river and season. The model will be adjusted for clustering at the village level.

7 Ethical considerations

7.1 Informed consent

Verbal consent (see Appendices 1 and 2 for scripts) will be asked from all households in the study area to conduct the initial and repeat household, LLIN and sleeping space census. Verbal consent (see Appendix 3) will be asked to apply a brief screening questionnaire to determine eligibility of children attending the study enrollment activity. Written, informed consent will be obtained from caregivers of children eligible for the cohort study. The study will be explained to each eligible child's caregiver, and the consent form read to the caregiver. The consent form will detail the design of the study, the questionnaires, risks and benefits to participants, and samples collected from children. Study team members will ask caregivers if they have any questions, and will answer any questions or address any concerns that arise. Study team members will emphasize that caregivers are free to withdraw their child at any time from the study, with no penalty whatsoever. After the initial 12-month period of the study, a new cohort will be enrolled and we will consent all participants for an additional nine months of the study.

7.2 Risks to study participants

This study poses minimal risk to participants. Blood samples will be collected by finger or heel sticks at all visits. The amount of blood collected will be very small (0.2-0.5 ml from a finger stick) and participants may experience only a small bruise at the blood draw site. Adhesive bandages will be put on all blood draw sites. Staff will be trained extensively on safe and effective finger sticks. Children will be given comfort measures, such as breastfeeding or sucrose, if appropriate, during finger sticks.

After enrollment in the cohort study, all children will be given a full weight-appropriate dose of the first-line antimalarial treatment in Malawi (AL) to clear all parasitemias. AL is considered to be a very safe drug with minimal side effects that is safe for use in young

children. Children less than 5 kg at enrolment will not be enrolled in the cohort study, as AL has not been approved for use in children less than 5 kg, due to lack of safety data. During monthly visits, cohort study participants found to be parasitemic according to microscopy will be given AL if they have not taken it within 28 days. If a child has had a full course of AL in the last 28 days, they will be treated with a weight appropriate dose of the second line antimalarial, artesunate-amodiaquine. Children will also be given a weight-appropriate two-week supply of iron syrup if monthly hemoglobin testing reveals anemia (Hb <10.5 g/dl). Anemic children ≥ 12 months who have not received antihelminthic treatment in the last six months will be given an appropriate deworming agent, and their parents will be instructed to take them to the health facility for re-assessment after two weeks. Children found to be seriously ill during the monthly visits will be transported to Machinga District Hospital by the study team. Only hospitalization costs related to malaria will be paid by the study.

PSCs are a standard entomologic technique to collect indoor mosquitoes; it has been used by CDC for more than 20 years without any adverse events. Care will be taken during PSCs to cover or remove from the house any food or drinking water. Household members will be instructed not to re-enter the house for at least 30 minutes after spraying.

We will take steps to ensure the protection of each study participant's personal information. Each household captured in the LLIN census will be identified using GPS coordinates and the name of the head of household. Children enrolled in the cohort will be assigned a unique five-digit study identification number that will be tied to the household information. The unique ID code, along with the date, will be placed on all samples taken from the child. Blood smears will also be labeled with child's name for rapid follow-up if they are positive. All names will be removed from the dataset before analysis.

7.3 Benefits to study participants

All children enrolled in the cohort study will receive a full course of antimalarial treatment at the beginning of the study, which will clear any parasite infections, including sub-patent parasitemia. They will also receive treatment for anemia if found to be anemic. In addition, children will be tested for anemia and parasitemia monthly, and will be given treatment accordingly (i.e. a full course of AL if parasitemic and iron syrup (plus mebendazole for those over two years), if anemic). Although malaria testing at government facilities is free during sick visits in Malawi, the monthly monitoring visits will detect asymptomatic cases of parasitemia, which can still have negative health consequences, and will treat them accordingly. In addition, testing will take place close to participants' homes, reducing the burden on mothers to bring their children to the district hospital for treatment.

7.4 Confidentiality

All information will remain confidential and will only be accessible to study investigators and the study team. Data will be stored on password-protected PDAs and computers, and all names will be stripped out during data analysis.

7.5 Compensation

Participants in the cohort study will be reimbursed for their transport for sick visits to Machinga District Hospital. Actual transport reimbursement will be individualized for distance traveled, but maximum reimbursement per visit will be approximately 500 Malawi kwacha or ~$3 US. However, given the continuing problems with fuel shortages in Malawi, we may need to increase this amount should the cost of transport significantly increase. The reimbursement amount will be calculated to only cover reasonable transport costs. No other gifts or payments will be provided.

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8 Appendices

7.6 Appendix 1: Verbal consent to participate in the first household, LLIN and sleeping spaces registration – English

Hello, my name is _____. I work for the Malaria Alert Centre of the Malawi College of Medicine. We work together with the National Malaria Control Programme and the US Centers for Disease Control and Prevention. We are doing a study in this area on how well insecticide-treated bednets are working. During this study, we will be following children 6 months to 5 years old to see who uses bednets and who gets malaria. As part of this study, we need to know:

1. How many bednets are in the house and who slept under them.
2. The type of bednets you have.
3. How old the bednets are.
4. The number of people usually sleeping in the house
5. Number of usual sleeping spaces in the house.

We will do this again in about one year's time. So, as part of the study, we will tag every house in this area so we can identify it in future.

If you have a child between 6 months and 5 years old, we would like to invite you and your children to be part of this study. We will give you an appointment card for a date in about 1 month's time when you can come to _____ and we will explain the study in more detail and ask whether you want to participate.

Do you agree for us to come into your house to see all of your bednets, sleeping places and to ask you about who usually sleeps in this house?

7.7 Appendix 2: Verbal consent to participate in household, LLIN and sleeping spaces– Chichewa

Mulibwanji, dzina langa ndi…… Ndimagwira ntchito ku Malaria Alert Centre imene ili nthambi ya sukulu ya ukachenjede ya Malawi College of Medicine. Tikugwira ntchito limodzi ndi a Unduna wa za Umoyo komanso US Centre for Disease Control and Prevention. Tikupanga kafukufuku okhudzana ndi mmene masikito onyikidwa mmakhwala akugwirira ntchito. Mukafukufuku wathuyu tizitsatira ana oyambira miyezi 6 mpaka zaka zisanu (5) kuti tione ana amene akugona mmasikito onyikidwa mmankhwala komabe akumadwala malungo. Mukafukufuku ameneyu tiyenda nyumba ndi nyumba mdera lino kuti tidziwe ;

1. Chiwerengero cha masikito amene ali mnyumbayi komanso anthu amene akugwiritsa ntchito masikotowo
2. Dzina la masikito amane mukugwiritsa ntchito
3. Nthawi yomwe mwakhala mukugwiritsa ntchito masikitowa
4. Chiwerengero cha anthu amene amagona mnyumbayi

5. Chiwerengero cha malo omwe amagwiritsiridwa nthcito kugona mnyumbayi.

Kalemberayu tidzapanganso pakatha chaka chimodzi pachifukwa ichi tidziyika chizindikiro pa nyumba yanu kuti tidzathe kuizindikira panthawiyo.

Mabanja amene ali ndi mwana woyambira miyezi 6 mpaka zaka zisanu, timafuna kukupemphani inuyo ndi mwana wanu kuti mutengeko mbali mukafukufuku wathuyu.

Tikupatsani tsiku loti tidzakumane pakatha mwezi kuti muzabwere ku ……. kuti tidzakufokotozereni mwa tsatanetsatane zokhudzana ndikafukufuku wathuyi ndinso kukufunsani ngati mukufuna kutengako mbali.

Mungatilore kuti tilowe mnyumba mwanu ndikuona masikito onyikidwa mankhwala amene muli nawo komaso kukufunsani za anthu amene anagona mnyumbamu usiku wathawu?

7.8 Appendix 3: Verbal consent to screen children for eligibility to participate in the cohort study - English

Hello, my name is _____. I work for the Malaria Alert Centre of the Malawi College of Medicine. We work together with the National Malaria Control Programme and the US Centers for Disease Control and Prevention. We are doing a study in this area on how well insecticide-treated bednets are working. During this study, we will be following all children 6 months to 5 years old in part of TA Sitola to see who uses bednets and who gets malaria. I would like to ask you a few questions to see whether or not your child is eligible to take part in this study. If your child is eligible, I will explain in more detail about what we will ask you to do in the study. Do you agree for me to ask you these questions?

7.9 Appendix 4: Verbal consent to screen children for eligibility to participate in the cohort study - Chichewa

Mulibwanji, dzina langa ndi…… Ndimagwira ntchito ku Malaria Alert Centre imene ili nthambi ya sukulu ya ukachenjede ya Malawi College of Medicine. Tikugwira ntchito limodzi ndi National Malaria Control Programme komanso US Centre for Disease Control and Prevention. Tikupanga kafukufuku okhuzana ndi mmene masikito onyikidwa mmakhwala akugwilira ntchito. Mukafukufuku wathuyu tizitsatira ana amene ali osachepera miyezi isanu ndi umodzi(6) mpaka zaka zisanu(5) mu dera la a T/A Sitola kuti tione ana amene akugona mmasiskito onyikidwa mmankhwala ndi omwe amadwala malungo.

Ndimati ndikufunsenikoni mafunso kuti tione ngati mwana wanu ayenera kutengako mbali mukafukufuku ameneyu. Ngati mwana wanu ali oyenera, ndikufotokozerani mwa tsatanetsatane zinthu zimene ndikufunseni mu kafukufuku ameneyu.

Mukuvomera kuti ndikufunseni mafunso amenewa?

7.10 Appendix 5: Verbal consent to participate in the subsequent household censuses

Hello, my name is _____. I work for the Malaria Alert Centre of the Malawi College of Medicine. We work together with the National Malaria Control Programme and the US Centers for Disease Control and Prevention. We are doing a study in this area on how well insecticide-treated bednets are working. We are also interested in understanding how households use cell phones. As part of this study, we need to know:

1. How many bednets are in the house and who slept under them.
2. The type of bednets you have.
3. How old the bednets are.
4. The number of people usually sleeping in the house
5. Number of usual sleeping spaces in the house.
6. The number of cell phones and who uses them.

We will do this again in about six month's time.

Do you agree for us to come into your house to see all of your bednets, cell phones, sleeping places and to ask you about who usually sleeps in this house?

7.11 Appendix 6: Written consent form for household participation in the cohort study —English

Flesch-Kincaid Reading Level=8.5

Title: Insecticide-treated mosquito net effectiveness in a setting of high pyrethroid resistance: an observational cohort study of malaria incidence in children in Malawi

1. PURPOSE OF RESEARCH:

The Malaria Alert Centre, the US Centers for Disease Control and Prevention and the Ministry of Health are doing a research study. We want to learn whether insecticide-treated mosquito nets are protecting your family against malaria. We are asking all parents and children whose ages are between 6 months and 5 years old and who live in selected villages of the Sitola traditional authority to participate.

2. WHAT WE WILL DO:

Malaria is a disease that is spread by mosquitoes. When your child sleeps under a treated mosquito net, he or she gets some protection from mosquito bites and malaria. Using a mosquito net all the time lowers your child's chance of getting malaria. We want to see whether mosquito nets are still working to reduce your child's chance of getting malaria. To know whether mosquito nets are working, we would like to see your child every month for 12 months. We will come to a place close to your home and ask you to bring your child to this place. At each visit, we will ask you some questions about your child's health. We will ask you who in your family is sleeping under a mosquito net. At each visit, we will test your child for malaria and anemia. Your child will receive free treatment if he or she has malaria or anemia. Each monthly visit should take about 30 minutes of your time.

If you agree for you and your child to take part in this study, we would like to ask you some questions. We would like to take a sample of blood from your child's finger. Some of this blood will go on a glass slide to test your child for malaria. One drop of blood will go into a machine to test your child for anemia. After today, one drop of blood will be used in a rapid test for malaria. Today only, a few drops of blood will be put on a paper that we will test in the laboratory to see if your child has malaria that we could not see under the microscope.

We would like for you and your child to come back to this place every month for the next year. We will repeat the blood sample and most of the questions.

New mosquito nets will soon be given out free to your household. After you get your new mosquito net, we would like to come to your house to put a tag on the net. We will use this tag to identify which mosquito net your child sleeps under.

After each monthly visit, a member of the study team from your village will come to your house to identify the mosquito net your child is sleeping under. At this visit, we will just check the tag on the mosquito net and record the tag number. We will not ask you any questions.

Today we would like to give your child treatment for malaria, whether or not they have the disease. This will make sure that all children start the study free of malaria. If your child has anemia today or at any monthly visit, we will give you iron to treat the anemia. If your child has anemia and is 12 months or older, we will also give a deworming medicine to get rid of any worms your child might have.

If your child gets sick at any time while you are enrolled in the study, we will ask you to bring your child to see a study clinician at Machinga District Hospital. The study nurse will take a blood sample from your child's finger to see whether your child has malaria or anemia. If your child has malaria or anemia, the study clinician will give your child treatment free of charge. The study clinician will do a complete health check, and will treat any other common illness that can be managed at the outpatient department free of charge. You will be reimbursed for transportation for you and your child to get to the hospital.

3. **POTENTIAL BENEFITS:**

Your child will get a test for anemia right now. Your child will receive treatment for malaria and if she or he has anemia, she or he will be treated for that, too. Secondly, while your child is enrolled in the study, your child can see a study clinician at Machinga District Hospital free of charge. We will provide free treatment for malaria, anemia and treatment for common illnesses that do not require hospitalization. The study will not be able to pay for any cost of hospitalization of your child unless your child is suffering from malaria.

4. **POTENTIAL RISKS:**

Today, your child will receive the government's recommended treatment for malaria. However, we will not know if your child has malaria. Anytime a drug is given there can be side effects. Common side effects for antimalarial drugs are cough, vomiting, loss of appetite and headache. Rarely, a child can have an allergic response or an abnormal heartbeat.

There may be a small bruise or temporary mild pain on your child's arm or finger where the blood is taken. There is also a small chance of infection when blood is drawn. We will use clean techniques to prevent any infection from occurring.

5. PRIVACY AND CONFIDENTIALITY:

Information about your child will be kept confidential to the maximum extent allowable by law. The data we collect will be stored on computers at the Malaria Alert Centre. Only the staff researchers will have access to the data. Your child will (each) be given a unique code which will be used on all data and samples collected each month. Your child's name and house number will be stored with the data but will not appear on any reports. At the end of the study, we will remove your child's name and the house number from the data so that no one can identify your information.

6. YOUR RIGHTS TO PARTICIPATE, SAY NO, OR WITHDRAW

You are free to choose to be part of this study. You have the right to refuse. If you do not want your child to receive LA treatment today, you can choose not to be part of the study. If anyone does not want to go on with this study they can stop at any time. If you do not want to participate in this study, your child can still be checked and treated for malaria or anemia at a hospital or health facility.

7. COSTS AND COMPENSATION FOR BEING IN THE STUDY:

There are no costs to participate in this study. If you have a sick child and want to bring them to Machinga District Hospital, we will reimburse you for the costs of your travel.

8. CONTACT INFORMATION FOR QUESTIONS AND CONCERNS

If you have any questions about this study or you feel that your child has been harmed by taking part in this study, you can bring your child to Machinga District Hospital at any time and ask to see Mr. Dyson Mwandama or you can contact Dr. Don Mathanga at the Malaria Alert Centre on telephone number 01 870 145.

This proposal has been reviewed and approved by the ethics committees of the Malawi College of Medicine Research and Ethics Committee and the US Centers for Disease Control and Prevention. These committees make sure that study participants are protected from harm. If you have questions about your rights as a study participant or you wish to find out more about the institutional review board, you may contact the Chairman of the College of Medicine Ethical Review Committee, Prof. Joseph-Mfutso Bengo, at telephone number 01 871 911.

If you are sick, do not call these numbers. Please go to the nearest health facility.

Your signatures or thumb prints below means that you voluntarily agree for you and your child to participate in this research study:

Today's study: The above has been explained to me and I agree that I and my child will take part in the study. I understand that I am free to choose whether I and my child will be in this study and that saying "NO" will have no negative effects for me or my child. I agree for my child's blood to be tested for malaria and anemia.	If you agree, circle "YES," if you do not agree, circle 'NO'.	
	YES	NO

Child 1	**Name:**		
Child 2	**Name:**		
Child 3	**Name:**		
Parent/ guardian or adult providing consent	**Name:**	**Signature/ thumbprint:**	**Date**
Witness*	**Name:**	**Signature:**	**Date**
Name of person obtaining consent	**Name:**	**Signature:**	**Date:**

*A parent or caregiver can sign, or place a thumbprint and verbally state his/her consent in the presence of a wLLINess who will then sign.

7.12 Appendix 7: Written consent form for household participation in the cohort study —Chichewa

Chilolezo Chovomereza Kulowa Kafukufuku Wofuna Kuona Mphamvu Ya Masikito Onyikidwa M'mankhwala opha Udzuzu

1. **Cholinga cha Kafukufuku**

Bungwe la Malaria Alert Centre, unduna wa zakafukufuku wa matenda ndi kuteteza matenda ku US ndi unduna wa za umoyo ku Malawi, akuchita kafukufuku. Akufuna ku phunzirapo mmene masikito a udzudzu onyikidwa mmankhwala angatetezere maanja ku malungo. Masikito onyikidwa mmankwhala ali ndi mphamvu yopha udzudzu. Tifunsa makolo and ana amene amakhala mwa mfumu yaikulu Sitola kuti atengo mbale pakafufukukuyu.

2. **Ndondomeko ya kafukufuku:**

Matenda a malungu amatengedwa kupyolera pa kulumidwa ndi tidzirombo tomwe timanyamulidwa ndi udzudzu.Mwana wanu amatetedzedwa ku matenda a malungo pamene akugona mu masikito onyikidwa mu mankhwala a malungo.Kafukufukuyu tikupanga ndi ana oyambira miyezi isanu ndi umodzi (6) mpaka zaka zisanu (5). Nyumba yanu yasankhidwa chifukwa muli ndi mwana (ana) yemwe zaka zake ndi zokwanira kulowa nawo kafukufukuyu. Ngati munga vomereze kutenga nawo mbali pa kafukufukuyu, tidzafuna kudziwa za masikito a udzudzu omwe muli nawo ndipo mukuwagwiritsa ntchito tidzakufunsaninso mafuso okhuza kafukufukyu komanso titenga magazi pang'ono pa msempha.Magazi amenewa akayezedwa malungo, akayezedwanso kuchuluka kwa magazi ndiposo akayikanso pa pepala ndikuwasunga ndipo amazayeza kuti aone ngati mwana wanu anali ndi malungo omwe sanaoneke ndi makina owunikira malungo.

Magazi omwe atatengedwewo, akayezedwa ku chipatala kukaona ngati mwana (ana) wanu ali ndi malungo kapena ai, koma kuchuluka kwa magazi mudziwiratu pompano. Lero tiperekeratu mankhwala a malungo a LA, kaya mwana akapezeka ndi malungo kapena ai. Tikufuna tionesetse kuti poyamba kafukufukuyu mwana (ana) alibe malungo ,akakayeza ndipo mwana akapezeka ndi malungo, tidzabweranso pakatha masabata awiri kuti tidzaone ngati mankhwala omwe anamwa aja agwira ntchito, ngatinso mwana wanu wapezeka kuti magazi ake ndi ochepa, lero timpatsa mankhwala amagazi otchedwa Iron, omwe amathandiza kuchulukisa magazi.

Mwana wanu akalowa nawo mu kafukufukuyu, tidzakhala tikumamuona kamodzi pa mwezi, ndipo pakhazikitsidwa malo pafupi ndi kwanu kuno komwe ana adzikapimidwa za umoyo wawo ndikutengedwa magazi kukayeza malungo, kuchuluka kwa magazi komanso kuyika pa pepala kuti adzayeze malungo omwe sanathe kuoneka pa makina owunikira malungo. Kupima mwana wanu ndikutenga magazi zizidzatenga mphindi makumi atatu (30) ulendo uli wonse.

Ngati mwana wapezeka ndi malungo kepena kuchepa kwa magazi, tidzampasa chithandizo chomwecho amapeleka ku chipatala cha boma. Ngati mwana walowa kafukufukyu ndipo wadwala tsiku lodzamuona lisanakwane, muyenera kubwera

naye ku chipitala cha Machinga, kumeneko a dokotala a zakafukufuku adzamupima ndipo adzayedzedwa malungo ndi kuchuluka kwa magazi. Ngati mwana wapezeka ndi malungo kapena kuchepa kwa magazi, a dokotola adzamupatsa chithandizo cha mankhwala molingana ndi ndondomeko ya Malawi.

3. **Ubwino Olowa Kafukufuku**

Pamene mwana(ana)wanu wayezedwa magazi, mudzadziwiratu kuchuluka kwa magazi ake komanso ngati ali ndi malungo, adzathandizidwa nthawi yomweyo molingana ndi vuto lake. Mwana adzathandizi dwa ndi a dokotala a zakafukufuku ku chipatala cha Machinga nthawi ili yonse wadwala mwa changu, koma ngati mwana wagonekedwa mchipatala, akafukufuku salipirapo chilichonse.

4. **Zobetchera**

Mwana (ana)wolowa kafukufuku amalandira mankhwala a malungo a LA, omwe amaperekedwa ndi boma, ndiye nthawi zina mankhwala amapezeka kulibe. Mankhwala aliwonse amakhala ndizovuta zake, ngati LA, ena amasokomola, kusanza ndiponso ena chilakolako cha chokudya chimachoka ndiponso kupwetaka mutu. Nthawi zochepa ena amatha kutuluka zironda, ena ngakhale kugunda kwa mtima kumasokonekera.

Pobayidwa, mwana amamva kupweteka pang'ono ndiponso pa malo pobayidwapo pa makhala kabala kakang'ono .Timagwiritsa ntchito njira yaukhondo kuopesa kulowa tizirombo toyambitsa matenda pa malo pobayidwapo.

5. **Chinsinsi**

China chili chonse cholembedwa chokhuza mwana wanu chidzasungidwa mwa chisinsi monga mwa malamulo. Zonse zidzasungidwa mu ma computer ku bungwe la Malaria Alert Centre(MAC). Okhawo omwe akugwira ntchito yakafukufuku ndiomwe angathe kuona mbiri ya mwana wanu. Mwana wanu adzapatsidwa nambala imene izidzalembedwa pali ponse pamene pakulembedwa za iye,dzina la mwana ndi nambala ya nyumba yanu zidzasungidwa. Pamapeto pa zonse, dzina la mwana ndi nambala ya nyumba yanu, zidzachotsedwa paliponse pomwe panalembedwa,ndipo palibe anagazindire kuti mbiriyo ndi ya mwana(ana) wanu.

6. **Ufulu wotenga nawo mbali**

Muli ndi ufulu onse utenga nawo mbali mukafukufukuyu, kapena osatenga nawo mbali kapena kusiya mutayamba kale kutenga nawo mbali. Muli ndi ufulu kukana mwana wanu kuti alandire mankhwala a LA, ndipo ngati salandira sangalowe nawo kafukufuku. Ngati simunafune kuti mwana wanu alowe nawo mukafukufuku, sizipangisa kuti musamabwere naye mwana kuchipatala ngati mwanayo asakupeza bwino ,thandizo la kuchipitala adzalandira monga mwa nthawi zonse.

7. **Kulipira Kapena Malipiro Okhala Mu Kafukufuku**

Chithandizo chili chonse ndi cha ulere pa nthawi yomwe mwana ali mukafukufuku. Palibe malipiro ena aliwonse amene amaperekedwa chifukwa cholowa kafukufuku. Mwana

akadwala mukabwera naye kuchipitala, timapereka ndalama yosaposera K500 kuti mulipire mayendedwe.

8. Mafunso Kapena Madandalo

Ngati muli ndi mafunso okhuza kafukufuku kapena mwaona kuti mwana wanu wapwetekedwa mwa njira inailiyonse potenga nawo mbali mukafukufuku, bwera nayeni mwanayo ku chipatala cha Machinga ndipo mukafunse kuti muonane ndi Mr. Dyson Mwandama kapena mutha kuimba foni kwa Dr. Don Mathanga ku bungwe la Malaria Alert Centre(MAC) pa nambala iyi 01870145.
Ndondomekoyi yavomelezedwa ndi kuunikidwa ndi a College of Medicine Research and Ethics committee ndi a Centers for Disease Control and Prevention. Makomiti amenewa amaonetsetsa kuti otenga mbali mukafukufuku ali otetezeka ku zoopsa zili zones. Ngati mungakhale ndi mafunso okhuza ufulu wanu pa kafukufuku kapena za mbiri za makomiti amenewa, mutha kuimba foni kwa wa pampando wa College of Medicine Ethical Review committee, Prof. Joseph Mfutso Bengo pa nambala iyi ;01871911.

Ngati mwana wadwala musayimbe manambala amanewa mungobwera naye ku chipatala.

Mukalemba dzina kapena kudinda ndi chala mumizere ili mmusiyi, ndiye kuti mwamvetsetsa zakafukufuku ndipo mwavomereza kulowa nawo osakakamizidwa.

Lero andifotokozera ndondomeko yonse yakafukufuku ndipo ndavomereza mwana wanga kutenga nawo mbali mu kafukufuku. Ndamvetsetsa kuti ndili womasuka kulowanao mukafukufukyu ndipo kukana sikunga sinthe mwana wanga kulandira chantitdzo choyenelera. Ndavomera zakuti magazi a mwana wanga aziyezedwa malungo kapena kuchuluka kwa magazi.	Ngati mwavomereza zunguzani mau oti 'Inde', Ngati simukuvomereza zunguzani mau oti 'Ayi'	
	Inde	Ayi

.

Mwana Woyamba	Dzina		
Mwana Wachiwiri	Dzina		
Mwana Wachitatu	Dzina		
Kholo kapena woyang'anira Mwana	Dzina	Saini/ chidindo cha chala	Tsiku
Mboni	Dzina	Saini	Tsiku
Wopempha chilolezo	Dzina	Saini	Tsiku

Appendix 7: Written consent form for household participation in the (second) cohort study —English

Flesch-Kincaid Reading Level=8.5

Title: Insecticide-treated mosquito net effectiveness in a setting of high pyrethroid resistance: an observational cohort study of malaria incidence in children in Malawi

9. PURPOSE OF RESEARCH:

The Malaria Alert Centre, the US Centers for Disease Control and Prevention and the Ministry of Health are doing a research study. We want to learn whether insecticide-treated mosquito nets are protecting your family against malaria. We are asking all parents and children whose ages are between 6 months and 5 years old and who live in selected villages of the Sitola traditional authority to participate.

10. WHAT WE WILL DO:

Malaria is a disease that is spread by mosquitoes. When your child sleeps under a treated mosquito net, he or she gets some protection from mosquito bites and malaria. Using a mosquito net all the time lowers your child's chance of getting malaria. We want to see whether mosquito nets are still working to reduce your child's chance of getting malaria. To know whether mosquito nets are working, we would like to see your child every month until December 2013. We will come to a place close to your home and ask you to bring your child to this place. At each visit, we will ask you some questions about your child's health. We will ask you who in your family is sleeping under a mosquito net. At each visit, we will test your child for malaria and anemia. Your child will receive free treatment if he or she has malaria or anemia. Each monthly visit should take about 30 minutes of your time.

If you agree for you and your child to take part in this study, we would like to ask you some questions. We would like to take a sample of blood from your child's finger. Some of this blood will go on a glass slide to test your child for malaria. One drop of blood will go into a machine to test your child for anemia. After today, one drop of blood will be used in a rapid test for malaria. Today only, a few drops of blood will be put on a paper that we will test in the laboratory to see if your child has malaria that we could not see under the microscope.

We would like for you and your child to come back to this place every month through December 2013. We will repeat the blood sample and most of the questions.

Some households will be visited in a few months by a member of the study team. These households will be randomly selected. The study team member will ask a few questions about whether your child uses a mosquito net when sleeping.

Today we would like to give your child treatment for malaria, whether or not they have the disease. This will make sure that all children start the study free of malaria. If your child has anemia today or at any monthly visit, we will give you iron to treat the anemia. If your child has anemia and is 12 months or older, we will also give a deworming medicine to get rid of any worms your child might have.

If your child gets sick at any time while you are enrolled in the study, we will ask you to bring your child to see a study clinician at Machinga District Hospital. The study nurse will take a blood sample from your child's finger to see whether your child has malaria or anemia. If your child has malaria or anemia, the study clinician will give your child treatment free of charge. The study clinician will do a complete health check, and will treat any other common illness that can be managed at the outpatient department free of charge. You will be reimbursed for transportation for you and your child to get to the hospital.

11. **POTENTIAL BENEFITS:**

Your child will get a test for anemia right now. Your child will receive treatment for malaria and if she or he has anemia, she or he will be treated for that, too. Secondly, while your child is enrolled in the study, your child can see a study clinician at Machinga District Hospital free of charge. We will provide free treatment for malaria, anemia and treatment for common illnesses that do not require hospitalization. The study will not be able to pay for any cost of hospitalization of your child unless your child is suffering from malaria.

12. **POTENTIAL RISKS:**

Today, your child will receive the government's recommended treatment for malaria. However, we will not know if your child has malaria. Anytime a drug is given there can be side effects. Common side effects for antimalarial drugs are cough, vomiting, loss of appetite and headache. Rarely, a child can have an allergic response or an abnormal heartbeat.

There may be a small bruise or temporary mild pain on your child's arm or finger where the blood is taken. There is also a small chance of infection when blood is drawn. We will use clean techniques to prevent any infection from occurring.

13. PRIVACY AND CONFIDENTIALITY:

Information about your child will be kept confidential to the maximum extent allowable by law. The data we collect will be stored on computers at the Malaria Alert Centre. Only the staff researchers will have access to the data. Your child will (each) be given a unique code which will be used on all data and samples collected each month. Your child's name and house number will be stored with the data but will not appear on any reports. At the end of the study, we will remove your child's name and the house number from the data so that no one can identify your information.

14. YOUR RIGHTS TO PARTICIPATE, SAY NO, OR WITHDRAW

You are free to choose to be part of this study. You have the right to refuse. If you do not want your child to receive LA treatment today, you can choose not to be part of the study. If anyone does not want to go on with this study they can stop at any time. If you do not want to participate in this study, your child can still be checked and treated for malaria or anemia at a hospital or health facility.

15. COSTS AND COMPENSATION FOR BEING IN THE STUDY:

There are no costs to participate in this study. If you have a sick child and want to bring them to Machinga District Hospital, we will reimburse you for the costs of your travel.

16. CONTACT INFORMATION FOR QUESTIONS AND CONCERNS

If you have any questions about this study or you feel that your child has been harmed by taking part in this study, you can bring your child to Machinga District Hospital at any time and ask to see Mr. Dyson Mwandama or you can contact Dr. Don Mathanga at the Malaria Alert Centre on telephone number 01 870 145.

This proposal has been reviewed and approved by the ethics committees of the Malawi College of Medicine Research and Ethics Committee and the US Centers for Disease Control and Prevention. These committees make sure that study participants are protected from harm. If you have questions about your rights as a study participant or you wish to find out more about the institutional review board, you may contact the Chairman of the College of Medicine Ethical Review Committee, Prof. Joseph-Mfutso Bengo, at telephone number 01 871 911.

If you are sick, do not call these numbers. Please go to the nearest health facility.

Your signatures or thumb prints below means that you voluntarily agree for you and your child to participate in this research study:

Today's study: The above has been explained to me and I agree that I and my child will take part in the study. I understand that I am free to choose whether I and my child will be in this study and that saying "NO" will have no negative effects for me or my child. I agree for my child's blood to be tested for malaria and anemia.	If you agree, circle "YES," if you do not agree, circle 'NO'.	
	YES	NO

Child 1	**Name:**		
Child 2	**Name:**		
Child 3	**Name:**		
Parent/ guardian or adult providing consent	**Name:**	**Signature/ thumbprint:**	**Date**
Witness*	**Name:**	**Signature:**	**Date**
Name of person obtaining consent	**Name:**	**Signature:**	**Date:**

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