LLINEUP2 Study Statistical Analysis Plan

LLIN Evaluation in Uganda Project 2 (LLINEUP2)	
Study title	Impact of long-lasting insecticidal nets (LLINs) treated with pyrethroid plus pyriproxyfen vs LLINs treated with pyrethroid plus piperonyl butoxide on malaria incidence in Uganda: a cluster-randomised trial
Trial registration	ClinicalTrials.gov Identifier: NCT04566510
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1 Introduction

1.1 Background

In Uganda, LLINs are the primary tool for malaria prevention, and considerable efforts have been made to achieve universal coverage of LLINs [1]. In 2013-14 Uganda became the first country to deliver LLINs free-of-charge nation-wide, with over 90% of households reporting ownership of at least one LLIN following the mass distribution campaign [2]. In 2017-18, the Ugandan Ministry of Health conducted a 2nd national mass-distribution campaign including LLINs with and without PBO, providing a unique opportunity to rigorously evaluate PBO LLINs across different epidemiological settings. In close collaboration with the Ministry of Health, we embedded a cluster-randomised trial (LLINEUP1) to evaluate the impact of the LLINs delivered in the 2017-18 national campaign at an unprecedented scale in Eastern and Western Uganda. Overall, 104 clusters (health sub-districts) were included, covering 40% of Uganda [3]. Proportionate randomisation was used to assign clusters to one of four arms, including LLINs with PBO (32 PermaNet 3·0, 20 Olyset Plus), and conventional LLINs (37 PermaNet 2·0, 15 Olyset Net). At baseline, 6, 12, 18 and 25 months after LLIN distribution, cross-sectional surveys were conducted in 50 randomly selected households per cluster (5,200 per survey); a sub-set of 10 households per cluster (1,040 per survey) were randomly selected for entomology surveys. The primary outcome was parasite prevalence by microscopy in children aged 2-10 years. Baseline surveys were conducted in 2017 [4-6]. LLINs were delivered from March 2017 to March 2018. In the 'as treated' analysis, three clusters were excluded because no predominant LLIN was received, and four clusters were reassigned, resulting in 49 PBO LLIN (31 PermaNet 3.0, 18 Olyset Plus) and 52 non-PBO LLIN clusters (39 PermaNet 2.0, 13 Olyset Net). At six months, parasite prevalence was 10.7% in the PBO arm vs 14.5% in the non-PBO arm (prevalence ratio [PR] adjusted for baseline values 0.74, 95% CI: 0.62–0.87, p<0.001). Results were similar at 12 months (10.6% vs 13.0%, PR 0.73, 95% CI: 0.63–0.85, p<0.001) and at 18 months (11.8% vs 14.0%, PR 0.84, 95% CI: 0.72–0.98, p=0.03). In the 90 clusters for which follow-up data were available at 25 months (42 PBO vs 48 non-PBO), parasite prevalence remained lower in the PBO arm than the non-PBO arm (17.1% vs 19.8%, PR 0.80, 95% CI: 0.69–0.93, p=0.005). Although overall parasite prevalence at 25 months was trending upward, it remained significantly lower than at baseline (18.6% vs 27.0%, PR 0.71, 95% CI: 0.67–0.77, p<0.001), which was true for both PBO and non-PBO clusters. Thus, in the first LLINEUP trial, we found that PBO LLINs provided superior protection against malaria in the setting of high-level insecticide resistance in Uganda. This innovative trial, embedded within a national LLIN distribution campaign, serves as a paradigm for future assessment of malaria control interventions, including the trial proposed here.

Other next generation LLINs combine a pyrethroid insecticide with a second active ingredient, such as pyriproxyfen [7-9]. Treating LLINs with a combination of insecticides with different modes of action may improve efficacy and help to prevent or delay the spread of insecticide resistance. Pyriproxyfen (PPF) is an insect growth regulator, which has traditionally been used as a larvicide [10, 11], but also acts as a sterilizing agent, reducing the fecundity (egg laying), fertility (production of viable offspring), and longevity of adult mosquitoes [12-16]. PPF has a different mechanism of action than pyrethroids and other commonly used insecticides, is effective at very low concentrations, and has been demonstrated to be safe to humans [10, 17]. In theory, pyrethroid-resistant mosquitoes that survive initial contact with a PPF-treated LLIN would be sterilized by the PPF. Thus, a dual active-ingredient LLIN including PPF is an attractive option.

In initial experimental hut trials conducted in Benin and Cote d'Ivoire, LLINs treated with the pyrethroid permethrin and PPF (Olyset Duo, Sumitomo Chemical) were associated with higher mosquito mortality and reduced blood-feeding rates, compared to standard LLINs treated with permethrin only (Olyset Net) [9, 18]. Moreover, surviving mosquitoes exposed to PPF-treated nets had substantially lower fecundity and fertility rates [7, 18]. In Kenya, a field trial comparing permethrin + PPF nets (Olyset Duo) to permethrin-only LLINs (Olyset Net) and a PPF-only treated net showed similar sterilizing effects against wild pyrethroid-resistant *An gambiae s.s.* [8]. In a step-wedge, cluster-randomised trial conducted in Burkina Faso, permethrin + PPF LLINs (Olyset Duo) were associated with lower clinical incidence in children aged 6-59 months than permethrin-only LLINs (Olyset Net) (1.5 vs 2.0 episodes per child-year, incidence rate ratio 0.88, 95% CI 0.77-0.99, p=0.04) [19]. The entomologic inoculation rate was also lower in the permethrin + PPF LLIN arm compared to permethrin-only LLINs (42 vs 85 infective bites per transmission season, rate ratio 0.49, 95% CI 0.32-0.66, p<0.0001). The PPF-treated LLINs appeared to work by reducing the vector population density and lifespan of adult mosquitoes, thus reducing the number of infective bites [19]. Another study from Burkina Faso found that the bio-efficacy and durability of PPF-treated LLINs (Olyset Duo) was superior to permethrin-only LLINs (Olyset Net) but that net survivorship for both net types was poor at 36 months [20]. The World Health Organization has pre-qualified one dual active-ingredient LLIN, which is treated with both a pyrethroid (alpha-cypermethrin) + PPF (Royal Guard LLIN, which produced by Disease Control Technologies) [21]. PPF-treated dual active-ingredient LLINs are promising, but additional epidemiologic studies in different settings are needed.

1.2 Rationale

LLINs provide the foundation for vector control in Uganda, and elsewhere in Africa. However, the effectiveness of LLINs is threatened by widespread pyrethroid resistance. In the first LLINEUP trial, we found that PBO LLINs were superior to conventional LLINs [22]. However, PBO LLINs have several potential limitations. PBO is a synergist, not an insecticide, and can only restore sensitivity of pyrethroid insecticides if resistance is due to specific metabolic mechanisms. Moreover, PBO cannot fully restore susceptibility in all resistant mosquito populations. Newer dual active-ingredient LLINs treated with a combination of insecticides using different modes of action are attractive alternatives; these LLINs may provide greater protection and delay the spread of insecticide resistance, but like PBO LLINs, they are more expensive than conventional nets. Further evidence of the effectiveness and cost-effectiveness of PPF-treated LLINs is urgently needed. Royal Guard LLINs, treated with alphacypermethrin and PPF, are one of only two dual active-ingredient LLINs prequalified by the WHO [21], which are available for widescale distribution.

In Uganda, the National Malaria Control Division (NMCD) and implementing partners are delivering LLINs nationwide in 2020-21, through a mass distribution campaign supported by generous contributions from international donors. LLINs will be distributed free-of-charge to all Ugandan households, aiming to achieve universal coverage. The Against Malaria Foundation has agreed to provide LLINs treated with a pyrethroid insecticide plus PPF (Royal Guard, Disease Control Technology) and LLINs treated with a pyrethroid insecticide plus PBO (PermaNet 3.0, Vestergaard), presenting an opportunity to rigorously evaluate and compare these two LLINs at scale across Uganda. In collaboration with the MOH, we propose to embed a cluster-randomised trial to compare the impact of LLINs with PPF to LLINs with PBO into Uganda's 2020-21 LLIN distribution campaign, as we did successfully for the last LLIN distribution campaign conducted in 2017-18.

A major strength of the proposed trial is the use of malaria incidence as the primary outcome measure. Incidence of malaria, defined as the number of symptomatic cases of malaria occurring in a population at risk over time, is the gold standard for assessing malaria burden. However, cluster-randomised trials using malaria incidence as the primary outcome are very expensive and logistically challenging. A novel approach for measuring malaria incidence, which we have proposed here, is to utilize data collected routinely at health facilities. By defining target areas around health facilities and collecting data on the location of residence of patients diagnosed with malaria, we will be able to generate longitudinal measures of malaria incidence at an unprecedented scale across Uganda.

1.3 Study site

The NMCD and supporting partners will distribute LLINs nationwide across Uganda, including the 32 districts included in this study. Districts were selected to participate in the study based on the following criteria: (1) Not receiving IRS, (2) Selected by the NMCD to receive PBO LLINs, based on available insecticide resistance data and guided by Uganda's insecticide resistance management plan [23], (3) high malaria transmission intensity. Once the districts were identified, we then selected MRCs to bring the total to 64.

The selection criteria for the LLINEUP2 MRC sites, included: (1) Level III/IV high-volume, public health facility (HC III or HC IV), (2) Total OPD attendance between 1000-2000 patients per month, (3) Evidence of weekly and monthly reporting in DHIS2, (4) Presence of a functional laboratory at the facility. In addition, we aimed to ensure that MRCs within the same district were comparable in terms of level-of-care and were located in different sub-counties to avoid contamination.

1.4 Purpose of this document

This document details the planned analyses for the randomised comparison of the study arms (PBO vs Royal Guard LLINs) for the LLINEUP2 trial, including the 12 and 24-month follow-up surveys. The CONSORT checklist item number for reporting a cluster randomised trial is included in Appendix B.

2 Study objectives and endpoints

2.1 Study objectives

We propose to address the following research question: Are LLINs treated with a pyrethroid insecticide plus pyriproxyfen (PPF LLINs) more effective than LLINs treated with a pyrethroid plus piperonyl butoxide (PBO LLINs) for malaria control in Uganda, particularly in high-burden areas?

The primary objective of the study is: To evaluate the impact of LLINs treated with a pyrethroid insecticide plus pyriproxyfen (PPF LLINs), as compared to LLINs treated with a pyrethroid plus piperonyl butoxide (PBO LLINs), on malaria incidence in Uganda. We will test the hypothesis that malaria incidence will be lower in intervention clusters (randomised to receive PPF LLINs) than in control clusters (randomised to receive PBO LLINs).

In addition, the following secondary objectives will be addressed:

1 To evaluate the impact of PPF LLINs, as compared to PBO LLINs, on parasite prevalence and prevalence of anaemia. We will test the hypothesis that parasite prevalence and prevalence of anaemia will be lower in intervention clusters (PPF LLINs), than in control clusters (PBO LLINs).

2 To estimate the cost-effectiveness of delivering PPF LLINs, as compared to PBO LLINs. We will estimate incremental cost-effectiveness ratios.

2.2 Endpoints

2.2.1 Primary outcome

The primary outcome of the study will be malaria incidence (defined as the number of cases of laboratory-confirmed malaria diagnosed at the MRC among patients residing in the target area per unit time/the population of the target area) in patients of all ages.

2.2.2 Secondary outcomes

- Community surveys: Prevalence of parasitemia (in children 2-10 years), anemia (in children 2-4 years), and LLIN ownership, coverage, and use.
- Economic evaluation: Incremental cost-effectiveness ratios (USD per disability-adjusted life year averted and per malaria case averted).

3 Study methods

3.1 General study design and plan

We propose to conduct a rigorous, cluster-randomised trial to evaluate the impact of LLINs distributed in Uganda through the 2020-21 national universal coverage campaign. A cluster has been defined as the target area of an MRC. A total of 64 clusters have been included in the study, covering 32 high malaria burden districts in Uganda where IRS is not being implemented. Clusters have been randomised in a 1:1 ratio in blocks of two by district to receive one of two types of LLINs: (1) PPF LLINs (Royal Guard [n=32] and (2) PBO LLINs (PermaNet 3.0) [n=32] (Appendix A).

The intervention, including delivery of the LLINs and social and behaviour change communication (SBCC), will be led by the Ugandan NMCD and other stakeholders. Currently, LLINs are scheduled to be delivered in the study areas from November 2020 to March 2021. The evaluation will include health facility surveillance at the MRCs to generate continuous estimates of malaria incidence for each MRC target area and cross-sectional community surveys at 12- and 24-months after LLIN distribution to gather information on net ownership and use, parasite prevalence in children 2-10 years of age, and anemia in children 2-4 years of age.

3.2 Cluster definition

The unit of randomisation (cluster) was the target area around each MRC; 64 MRCs from 32 districts were included. Target areas around the MRCs were identified before LLINs were distributed and included the village where the MRC is located and adjacent villages that meet all of the following criteria: 1) do not contain another health facility, 2) are within the same sub-county as the village where the MRC is located, 3)

have a similar incidence of malaria as the village where the MRC is located, and 4) provide an estimated total target area population of at least 1200 persons.

3.3 Cluster randomisation

Given the open-label study design and the need to generate estimates of the targeted number of LLINs for distribution in advance, randomisation was completed at the time of the protocol development. The randomisation was carried out by a member of the study team who is not based in Uganda and not directly involved in the field work. The unit of randomisation (cluster) was at the level of the MRC and the surrounding sub-county targeted for LLIN distribution. Randomization was done in blocks of 2, with each block representing a district containing 2 clusters with one cluster assigned the letter "A" and one cluster assigned the letter "B". For each block, a random number between 0 and 1 was generated using the 'runiform' command in STATA (StataCorp, Texas, USA). If the random number was <0.5, cluster "A" was assigned to PBO LLINs and cluster "B" was assigned PPF LLINs. If the random number was $\geq$0.5, cluster "A" was assigned to PPF LLINs and cluster "B" was assigned PBO LLINs. The final treatment allocations are summarized in Table 1, and the full intervention allocation list is provided in Appendix A.

Table 1. Allocation of LLINs

Type of LLIN	Targeted total number of LLINs for distribution	Number of clusters allocated
PPF LLIN	632,359	32
PBO LLIN	696,914	32
Total	**1,329,273**	64

4 Delivery of the intervention

LLINs were distributed according to detailed national guidelines, which built on prior experience from a similar net distribution campaigns carried out in 2013-2014 and 2017-18, and incorporated guidance for LLIN distribution in the context of COVID-19, as well as lessons from food distribution during COVID-19. The overall goal of the 2020-21 LLIN distribution campaign was to reduce malaria morbidity and mortality in Uganda by achieving universal coverage with LLINs, aiming to ensure that: (1) 85% of the targeted population has access to a LLIN, and (2) 85% of LLINs distributed are utilised. Members of the research team engaged with Uganda's national committees that coordinated the LLIN universal coverage campaign, including the National Coordination Committee (NCC), which was responsible for overall coordination and oversight of campaign planning, implementation, and engagement with political and traditional authorities, the operations sub-committee, the logistics sub-committee and the advocacy, communication and social mobilisation sub-committee. All LLINs procured for the campaign were stored centrally in at the National Medical Stores warehouses in Entebbe and were distributed across the country in waves. The 32 districts selected for this study were included in Waves 3-5 and were scheduled to receive nets from November 2020 to March 2021. The research team worked closely with the NCC and other stakeholders to ensure that the nets were allocated per the randomisation scheme.

For each cluster we used a 'fried egg' approach for delivering the intervention ('egg white') and measuring our outcomes ('egg yolk'). The 'white' of the egg included one sub-county per cluster, where the MRC is located. PPF LLINs and PBO LLINs were distributed to the designated sub-county, as allocated in the

randomisation. The 'yolk' of the egg will be the target area directly surrounding each MRC, where care-seeking at the MRC is expected to be high (i.e. if someone within the target area develops malaria, they are likely to seek care at the MRC). To determine the population of the MRC target areas and to generate a sampling frame for the community surveys, the following were done: (1) define the target area of each MRC before the onset of the trial using data on village of residence from patients attending the MRCs, (2) map and enumerate all households within the MRC target areas before the 12-month community survey, (3) conduct a census survey within each MRC target area to generate an accurate estimate of the study population in which study outcomes will be measured concurrently with the 12-month community survey.

5 Evaluation procedures

5.1 Enumeration surveys

To estimate the population of the MRC target areas, and to generate a sampling frame for the cross-sectional community surveys, we will enumerate and map all households within each target area prior to the evaluation. In advance of the enumeration surveys, investigators will meet with local officials and community representatives to discuss the study and plans for the household enumeration. Using a map of the boundaries of the MRC target areas, project personnel will systematically cover the entire area within the boundaries to identify and enumerate all households. A household will be defined as any single permanent or semi-permanent dwelling structure acting as the primary residence for a person or group of people that generally cook and eat together. Some households may include members who sleep in other dwelling structures within the same compound, if the members are still dependent on the head of household in the main household. All households identified will be assigned sequential unique IDs. Household locations will be mapped using GPS receivers. Readings will be taken from the door of the household, if possible, or from a point that is most representative of the household. At each household, a reading will be taken every five seconds for 2 minutes, and the average values from these readings will be recorded (Easting, Northing, and Altitude) in UTM units. Only GPS coordinates will be picked from the households. No additional data will be collected during the enumeration survey.

5.2 Health facility-based surveillance

At each MRC (Appendix A), individual-level data from standardised registers for all patients presenting to the outpatient departments are entered into an Access database by on-site data entry officers. Primary data captured comes from the HMIS 002 standardised form (Appendix C) and includes location of residence (parish and village), age, gender, body temperature, history of subjective fever, type of malaria test done (rapid diagnostic test or microscopy), malaria diagnostic test results, any diagnoses given, and any treatments prescribed. Data from each MRC will be submitted to the team in Kampala on a monthly basis using a secure on-line system. Standardised data checks will be applied to assess for missing data and data errors. Data queries will be submitted back to the sites and corrected whenever possible. Interval data submitted from the MRCs will be merged into an existing master database. Each time the master database is updated, existing programmes will be run to perform variable transformations and generate standardised indicator variables.

5.3 Community surveys

Cross-sectional community surveys will be conducted 12 and 24 months after nets are distributed. The surveys will include two components: (1) a household survey targeting heads of households, and (2) a clinical survey of children aged 2-10 years.

5.3.1 Sampling frame

The sampling frame for the community surveys will be generated from the enumeration of the MRC target areas (which will be conducted prior to the onset of the evaluation). Households will be randomly selected from each of the 64 clusters and screened until 50 households with at least one child aged 2-10 years are enrolled (at least 3,200 households). If an enrolled household has no children of appropriate age, they will be included in the household survey only, and will not take part in the clinical survey.

5.3.2 Definitions

Household: A household will be defined as any single permanent or semi-permanent dwelling structure acting as the primary residence for a person or group of people that generally cook and eat together. Some households may include members who sleep in other dwelling structures within the same compound, if the members are still dependent on the head of household in the main household.

Head of household: The head of household is an adult person or persons who primarily make decisions for the general household (e.g. decisions on healthcare, income, etc.), including emancipated minors.

Household resident: A resident within each household will be defined as a person who intends to have a sleeping place primarily at that location for a period of the next 6 months. This may include people who sleep in a separate house within the same compound, if they are still dependent on the head of household for decisions on finances and health care.

5.3.3 Household selection criteria

The inclusion criteria for household participation in the community surveys are: (1) at least one adult aged 18 years or older present; (2) adult is a usual resident who slept in the sampled household on the night before the survey; (3) agreement of the adult resident to provide informed consent for the household survey. The exclusion criteria are: (1) dwelling destroyed or not found; (2) household vacant; (3) no adult resident home on more than 3 occasions.

5.3.4 Household questionnaire

The household questionnaire will be administered to the head of the household (or their designate), after obtaining their consent using a hand-held tablet computer. Information will be gathered on the characteristics of households and residents, proxy indicators of wealth including ownership of assets, and ownership and use of LLINs in the households, specifically focusing the nets distributed in the LLIN campaign. The household survey questionnaire has been adapted from prior cross-sectional community surveys conducted in Uganda, including the national Malaria Indicator Survey [24-27].

5.3.5 Clinical survey recruitment

All children aged 2-10 years from enrolled households who are present will be eligible for participation in the clinical survey. Children will be identified from the household survey questionnaires.

5.3.6 Clinical survey selection criteria

The inclusion criteria are: (1) child aged 2-10 years; (2) usual resident who was present in the sampled household on the night before the survey; (3) agreement of parent/guardian to provide informed consent; (4) agreement of child aged 8 years or older to provide assent. The exclusion criterion is: (1) child not home on day of survey.

5.3.7 Clinical survey procedures

The clinical surveys will include measurement of temperature, subjective fever and a finger-prick blood sample for measurement of thick blood smear and haemoglobin (in children < 5 years), and filter paper blood sample.

5.4 Economic evaluation

An economic evaluation will be conducted to compare the incremental costs and cost-effectiveness of LLIN strategies using either Royal Guard LLINs or PBO LLINs in accordance with the reference case for economic evaluations in low- and middle-income countries [28]. The analysis will combine primary data on costs and effectiveness from the trial with additional secondary data to inform policy choices regarding the choice of LLIN. Efficiency will be measured in incremental cost-effectiveness ratios. A decision tree will be used to calculate the incremental cost-effectiveness of the Royal Guard LLIN compared to PBO LLINs [29, 30].

6 Sample size calculations

6.1 Primary outcome

Our sample size of 32 clusters per arm was calculated to detect a 26% decrease (incidence rate ratio (IRR) = 0.74) in the incidence of malaria over the 24 month period following the intervention (the primary endpoint of the study) between the two study arms, given a power of 80% and a two-sided significance level of 0.05. This sample size calculation assumes an incidence of 332 malaria cases per 1000 person-years in the control arm and a coefficient of variation (CV) of 0.42 calculated from the 14 MRCs where estimates of malaria incidence are available over the last 6 month at the time of protocol development.

6.2 Secondary outcomes

We will sample all eligible children aged 2-10 years from 50 households in the 64 clusters in each round of surveys, aiming to maximise the potential prevalence ratio detectable in the intervention arm, as well as the cost/value of the trial. Assuming an average of 1.8 children aged 2-10 years per household, we estimate that we will survey 5,760 children from 3,200 households. Assuming a coefficient of variation of 0.4, across a wide range of prevalence measures in the control arm (10-70%) our sample size will allow us to detect a 25-28% decrease in the prevalence measure of interest (prevalence ratio (PR) = 0.72-0.75), given a power of 80% and a two-sided significance level of 0.05.

7 Summary of study data

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, and intra-quartile range. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. All summary tables will be structured with a column for each study arm and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

7.1 Trial Profiles

7.1.1 Intervention

The overall study profile for the intervention will be presented as a figure following CONSORT guidelines. This will include the number of clusters allocated to the 2 treatment arms, the estimated number of household targeted for the intervention in the sub-county surrounding each MRC, the estimated number of households in the target area where the outcomes will be assessed, and the estimated population of the target area where the outcomes will be assessed.

7.1.2 Community surveys

For the community surveys additional flow diagrams will detail participation in the survey, including the number of households recruited and screened, reasons for exclusion, and number of households enrolled. The figure will include details of the number of children aged 2-10 years recruited and screened for the clinical survey, reasons for exclusion, number of children included, and the number with complete data included in the analysis.

7.2 Characteristics of clusters, households, and residents

The baseline characteristics of the clusters from the enumeration surveys and characteristics of the households and residents assessed at the time of the 12 and 24 month cross-sectional surveys will be presented by study arm. For continuous variables the mean and standard deviation will be presented. For skewed continuous variables, either geometric mean or the median and inter-quartile range will be presented, and for categorical variables the number and percentage in each category.

8 Efficacy analyses

8.1 Primary outcome

The primary outcome of the study will be the cumulative incidence of malaria over the 24 month follow-up period. The total number of cases of malaria for each MRC target area will be the number of laboratory confirmed cases of malaria from patients who report residing in the target area over the 24 month follow-up period, with two correction factors: 1) for patients who reside within the target area with suspected malaria who do not undergo laboratory testing, and 2) for patients with laboratory confirmed malaria whose village of residence is missing. The population of the MRC target area will be used for incidence calculations.

8.1.1 Analytical approach

All analyses will be conducted using an intention-to-treat approach according to the treatment allocations the clusters were randomized to. We will compare cluster level estimates of the incidence of malaria

between the intervention (PPF LLINs) and control arm (PBO LLINs) using a mixed effects Poisson regression model with a random effect at the district level (each district includes two clusters, randomized to one of the two study arms) and adjustment for log-transformed estimates of the baseline malaria incidence (3 months prior to the intervention) for each cluster. The effect of the intervention will be expressed as an incidence rate ratio (incidence in the intervention arm/incidence in the control arm). Our primary analysis will evaluate malaria incidence over 24 months following the intervention. We will also perform secondary analyses of malaria incidence stratified by time following the intervention (year 1 vs. year 2) and after adjusting for additional cluster-level covariates from the cross-sectional surveys including treatment seeking behaviour (to account for cases of malaria not captured at the MRCs) and the diagnostic accuracy of RDTs vs microscopy (to account for differences in the use of these diagnostic tests between clusters). These models will include random effects at the cluster level and we will assess the need for an additional random effect at the District level.

8.2 Secondary outcomes from cross-sectional surveys

Secondary outcomes from the 12 and 24 month cross-sectional surveys will include the following:

1. Prevalence of parasitemia in children 2-10 years of age - defined as the proportion of children with a positive thick blood smear by microscopy.
2. Prevalence of anemia in children 2-4 years of age - defined as the proportion of children with a hemoglobin level < 11 g/dL.
3. Measures of LLIN ownership, coverage and use defined as follows:
    a. LLIN ownership - the proportion of households that owned at least one LLIN),
    b. Adequate LLIN coverage - the proportion of households that owned at least one LLIN for every two residents.
    c. LLIN access - proportion of residents who could sleep under a LLIN, if each LLIN in the household were used by up to two residents
    d. LLIN use - the proportion of household residents who reported sleeping under an LLIN the previous night.

8.2.1 Analytical approach

All analyses will be conducted using an intention-to-treat approach according to the treatment allocations the clusters were randomized to. Separate analyses will be conducted for the 12 and 24 month cross-sectional surveys. We will compare individual level estimates of the prevalence of parasitemia and anemia between the intervention (PPF LLINs) and control arm (PBO LLINs) using a mixed effects logistic regression model with random effects at the level of the cluster and household. We will compare household level estimates of LLIN ownership, coverage, and use using a mixed effects logistic regression model with a random effect at the level of the cluster. Effects of the intervention will be expressed as odds ratios (odds in the intervention arm/odds in the control arm).

8.3 Secondary outcomes from economic evaluation

The measure of effectiveness will be number of malaria case averted will be derived from incidence data, which will be calculated by dividing the number of laboratory-confirmed malaria cases diagnosed at each MRC (among patients residing in the target area per unit time) by the total population of the MRC target area [31-33]. All data analyses are addressed in the Health Economics Analysis Plan (HEAP).

9 General Considerations

All data will be analysed using STATA version 14.0 (Stata corporation, College Station, Texas).

9.1 Analysis Populations

All analyses will use an intention-to-treat approach where clusters will be analyzed according to the randomization scheme (as per Appendix A).

9.2 Covariates

In the primary analysis of the primary outcome (malaria incidence) we will adjust for cluster level baseline estimates of malaria incidence (3 months prior to the intervention). In secondary analyses of the primary outcome we will adjust for additional cluster-level covariates from the cross-sectional surveys including treatment seeking behaviour (to account for cases of malaria not captured at the MRCs) and the diagnostic accuracy of RDTs vs microscopy (to account for differences in the use of these diagnostic tests between clusters).

9.3 Subgroups and exploratory analyses

9.3.1 Subgroup analyses

The following subgroup analyses have been planned *a priori:*

1. Primary outcome (malaria incidence) will be stratified according to the following 3 age strata: < 15 years, 5-15 years, and over 15 years.
2. Secondary outcomes for LLIN ownership, coverage and use will be stratified according to the following 3 age strata: < 15 years, 5-15 years, and over 15 years.

9.3.2 Exploratory analysis

No exploratory analyses are planned.

9.4 Missing data

For estimates of malaria incidence, adjustments will be made when calculating the number of cases of laboratory-confirmed malaria by adding estimates for the following categories of patients: 1) Malaria cases due to missing data on diagnostic testing = patients residing in the target area with suspected malaria but no laboratory test done multiplied by the test positivity rate among patients residing in the target area with suspected malaria and tested for malaria. 2) Malaria case due to missing data on location of residence = patients with laboratory confirmed malaria with missing data on village of residence multiplied by the proportion of patients with laboratory confirmed malaria who reside in the target area.

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