MossieGo Trial (AFR1577) Statistical Analysis Plan

Version 1.3

Statistical analysis plan for a randomised control trial to evaluate the protective efficacy of a spatial repellent to reduce malaria prevalence in Uganda.

V1.3 updated to reflect revised study timelines and associated updates to the planned statistical analysis.

1. Introduction

Malaria remains one of the greatest healthcare problem facing the African continent, with 330 million people contracting the disease annually. In 2020 alone, 627,000 deaths were attributed to malaria and ninety five percent of these infections occurred in Sub-Saharan Africa (SSA). SSA bears the highest burden of morbidity and mortality worldwide, despite improvements with diagnostics, treatment, vaccines and large-scale deployment of vector control measures. Children and pregnant women remain the highest risk groups, with a five percent mortality rate for children under five due to malaria. The burden of malaria also greatly impacts GDP and economic growth due to lower productivity, missed school, missed work and inaccessible price of healthcare.

In 2020, Uganda had the third highest global burden of malaria cases and deaths (5.4%) and the fifth highest levels of death.[1] The entire population is considered to be at risk of contracting malaria.[2] The primary malaria vectors in Uganda are *Anopheles gambiae s.l.* and *An. funestus s.l.*[3] The progress towards elimination and eventual eradication of the disease is threatened by challenges such as the rise in insecticide resistance and low coverage of existing vector control tools. New vector control tools are urgently needed to address the growing threat of insecticide resistance and target outdoor biting vectors.

Spatial repellents offer a promising solution to complement existing vector control tools and help to resolve issues being faced in deployment of current strategies. Spatial repellents are intended to be used in combination with existing World Health Organization (WHO) recommended vector control methods and the WHO has recently recommended methods to evaluate the efficacy of new spatial repellent products. Spatial repellents reduce human-vector contact by repelling the vector from the chemical stimuli, interfering with host detection, attraction inhibition and / or reduced feeding response.[4],[5] This provides protection against daytime and early evening biting and protection in enclosed/semi enclosed and peri-domestic spaces. Several active ingredients have been shown to provide spatial repellent effects and provide protection from mosquito bites. Although few studies have investigated effects on malaria transmission, two studies of limited power suggest that the volatile pyrethroids transfluthrin and metofluthrin, when used as coils inside people's houses, have potential uses as part of malaria control programmes.[6],[7] Placing hessian ribbons, treated with transfluthrin, around the eaves of experimental huts, has been shown to significantly reduce human landing rates by *Anopheles* mosquitoes.[8] Importantly, these results were seen regardless of the insecticide-resistance status of the mosquitoes, leading the authors of the study to conclude that transfluthrin-treated eave-positioned ribbons could be useful in areas with pyrethroid-resistant mosquitoes.

Noted limitations with spatial repellents include the limited residual effect of the repellent and the need for external power or heat for diffusion of the volatile active ingredients if they are to be actively dispersed. However, seventy percent of households in SSA are off-grid. The intervention proposed in this study (Mossie-GO) allows for active emanation of transfluthrin into the air, powered by off-grid solar power technology. Although similar types of devices are widely available in Europe, they have not been investigated for domestic use in developing countries, presumably because of the perceived costs of the device, of the repellent refill units, and of the batteries or requirement for mains power to operate them. The main benefit is that the devices can be charged throughout the day and used to provide protection to members of households when they are not under a bed net. A further advantage is the ease of installation; both the solar devices and the repellent units can be readily installed. A powered device is expected to provide greater protection than a passive device because the powered fan allows for controlled and stronger distribution of the active ingredient.

This study will serve as a proof-of-principle of the efficacy of an active transfluthrin-based spatial repellent device (Mossie-GO) against malaria in Uganda. Trial results will be submitted to the WHO Vector Control Advisory Group (VCAG) to inform recommendations for the use of spatial repellents in malaria control.

2. Study Design

This study is a blinded cluster-randomised placebo-controlled trial with two arms. A total of 56 clusters will be randomly assigned in equal quantities to receive the intervention or placebo-control. Primary endpoint sampling was conducted at baseline, 6 months and 12 months. Participants will be sampled independently at each timepoint, representing repeated cross-sectional surveys rather than longitudinal follow-up of individual participants. A subset of households within each cluster will be selected for secondary endpoint sampling. All households will be encouraged to continue use of other malaria control tools.

3. Study Endpoints

Primary outcome(s)

1. **Malaria Prevalence in children aged ≤5 years:** The primary endpoint for analysis will be malaria infection status among sampled children aged ≤5 years. Malaria prevalence will be summarised by study arm and cluster. The primary comparison will assess malaria infection prevalence between intervention and control arms at 12 months, with an additional comparison at 6 months
   Blood samples will be collected from one child aged ≤5 years per selected household. The target will be up to 100 children per cluster at baseline and

approximately 60 children per cluster at the 6- and 12-month follow-up timepoints. Participant selection will be conducted independently at each timepoint
Malaria infection status will be defined using a composite of RDT and microscopy results. Participants will be classified as malaria-positive where either diagnostic test is positive and as negative where both tests are negative. Where only one diagnostic test result is available, classification will be based on the available result. Participants with neither RDT nor microscopy results available will be excluded from the primary analysis.

Secondary outcomes

1. **Mosquito vector surveillance and biology**: Mosquito species composition, population density and sporozoite rates in mosquitoes caught using indoor CDC-light traps and human-landing catch (HLC).
    - CDC light trapping will be conducted in seven households across all 56 clusters at baseline, 6 months and 12 months. Households will be selected independently at each sampling timepoint.
    - HLC will be conducted in three households across 10 clusters at baseline, 6 months and 12 months. Households will be selected independently at each sampling timepoint. HLC sampling will be conducted indoors and outdoors for three consecutive nights. Sampling will be conducted from 18:00 to 00:00 at baseline and 6 months and from 18:00 to 06:00 at 12 months.

    For both entomological protocols, households will be randomly selected with replacement at each of the four six-monthly timepoints meaning that households can be sampled multiple times. Sample selection is nested within the households selected for the primary endpoint per timepoint, per cluster. Mosquitoes will be collected and subject to the same analysis. For each trap sample, we will report:

    a. **Species composition**: Will be reported as the total count of mosquito species present.
    b. **Population and subgroup densities** Will be reported as the total counts of mosquitoes per house per night by species, sex, and feeding status for *Anopheles* mosquitoes, including the number blood fed/unfed, half gravid, and fully gravid females.
    c. **Sporozoite rates:** Will be reported as the proportion of mosquitoes carrying sporozoites.

2. **Mosquito vector pyrethroid resistance**: Prevalence of resistance to pyrethroids in Anopheles mosquitoes using WHO tube test bioassays. This will be reported as the proportion of mosquitoes susceptible and grade as below:
    a) **Susceptible**: 98-100% mortality suggests that the mosquito population is susceptible to the insecticide.
    b) **Possible Resistance**: 90-97% mortality indicates potential resistance, warranting further investigation.
    c) **Confirmed Resistance**: Less than 90% mortality confirms resistance to the pyrethroid.

3. **Household survey**: Household-level information and information on participants living within each selected household will be collected alongside primary endpoint sampling at baseline, 6 months and 12 months. Household selection will be conducted independently at each timepoint. Where information is not expected to change over time, previously collected information may not be collected again.
   The data collected through this survey we be:
    a. Participant information, including demographics information on all members of the household.
    b. Household information, including construction features that could affect the clinical and entomological endpoints and socio-economic factors. This information will include qualitative and quantitative data which will be summarised as below:
        i. **Quantitative Data**: Descriptive statistics will summarise the responses, and inferential statistics will be used to examine differences in acceptability over time and between subgroups.
        ii. **Qualitative Data**: Responses will be analysed for recurring themes and patterns, providing contextual depth to the quantitative findings.

4. **The concentration of transfluthrin** present in indoor air will be quantified to assess variation in airborne concentration following activation of the spatial emanator. Air sampling will be conducted at 9 and 12 months in two households from each of seven selected clusters. Samples will be collected approximately one hour and four hours after activation of the spatial emanator. Transfluthrin concentration will be reported as nanograms per litre of air sampled.

5. **Safety of intervention** through monitoring of adverse events (AEs) and serious adverse events (SAEs). Adverse events reported will include a combination of qualitative and quantitative information.

6. **Acceptability of intervention** will be collected in households completing the household survey, described above, for the 6- and 12-month timepoints. This information includes qualitative and quantitative questions.
    - **Quantitative Data**: Descriptive statistics will summarise the responses, and inferential statistics will be used to examine differences in acceptability over time and between subgroups.
    - **Qualitative Data**: Responses will be analysed for recurring themes and patterns, providing contextual depth to the quantitative findings.

4. Sample Size

The sample size calculations are based on the assumption that the prevalence of malaria infection, detected by rapid diagnostic test (RDT) in children under 5 years of age, is approximately 20% in the study region. A conservative coefficient of variation (CV) of 0.5 has been assumed to account for variability in prevalence between clusters.

To detect a 40% reduction in malaria prevalence with 90% power, the required sample size is 28 clusters per study arm, with 100 children sampled per cluster. The original target will be to sample up to 100 children per cluster at baseline. Due to funding and resource constraints, the follow-up sampling target will be reduced to approximately 60 children per cluster at the 6- and 12-month timepoints.

5. Randomization and Blinding

Random selection

All randomisation procedures will be handled electronically, using R and functions from the dplyr package (R Core Team, 2024; Wickham et al., 2023).

Eligible clusters will be identified according to the predefined population and demographic eligibility criteria, from which 56 clusters will be selected at random for inclusion in the trial. Villages located within the two study regions, Buikwe and Jinja, will be identified and selected as eligible clusters based on restricting criteria. Clusters will only be eligible based on population size and demographic stability criteria. The eligible population size range for clusters was a minimum of 150 households and maximum of approximately 250 households. The lower limit was defined by the need to ensure that there are at least 100 households with children under 5 where a local census revealed that around 70% of households would have children aged 5 and under. The upper limit was defined by logistics depending on the availability of the Mossie-GO device. The populations in some clusters were identified as having a higher risk of not meeting sampling requirements as their populations were more likely to change between sample collection time points. This was mostly explained by seasonality in local fishing productivity, meaning that the population was only present at certain times of the year.

For this reason, villages locally characterised by seasonal fishing activity will be excluded from the list of eligible clusters.

Random allocation

The 56 clusters will be randomly assigned to intervention or control in a 1:1 ratio by an independent statistician using unrestricted randomisation. Allocation will be conducted prior to baseline sampling and intervention distribution.

Baseline malaria prevalence will not be available prior to allocation and will therefore not be used to balance clusters between study arms. Other cluster characteristics, including study district, land type and environmental or socioeconomic factors, will not be used to constrain randomisation. Where relevant, these factors may instead be considered in descriptive or exploratory analyses.

The treatment allocation will remain blinded, with clusters assigned to coded treatment arms. Access to the allocation code will be restricted to staff who require this information for intervention manufacture, distribution or study oversight.

Blinding

This trial is conducted as a double-blinded study to ensure the integrity and impartiality of the results. Observer bias was reduced as much as possible where all staff are considered blinded to study, with exceptions made on a needs basis which will be monitored and regulated by the trial manager.

- **Trial Participants:** All participants remain unaware of whether they are receiving the intervention or control device.
- **Study Staff:** No staff collecting primary data should be aware of whether clusters are assigned to different study arms.
- **Study management**: Staff managing device distribution and producing outputs where the differences between trial arms is required are granted permission to information on the trial arms as “A” or “B” where the identity of the trial arm cannot be detected. For example, the data manager who is handling the random allocation process.
- **Unblinded Staff**: Very few staff are fully unblinded to the trial arms and these staff are minimised as much as possible. These include two staff members who are part of the core study team, one staff member each from the study sponsor and study field team, to handle the distribution of the intervention devices to control or treatment households. Furthermore, five members of the DSMB, including a DSMB chair, will be unblinded to review data related to participant safety between intervention arms. This is an independent body who have no input on the study

design ahead of the commencement of the trial and will act as independent advisory board.

Upon completion of data collection and final cleaning, the database will be locked and transferred to the statistician for analysis.

6. Statistical Analysis

Preparation of data for analysis

Regular quality assurance will take place after periods of data collection to ensure that appropriate data is captured to meet the sample size requirements for study and to ensure that data available is of a good, reliable quality. Efforts to enhance data quality and accuracy include both proactive training of staff and reactive measures to control data entry, aiming to minimise errors such as mis-entries. Each data collection protocol requires a mandatory training session, which is documented with signatures from the relevant personnel. We prioritise the use of electronic data capture through ODK Collect on tablets and smartphones, where data entry fields are specifically designed to limit errors. This includes restrictions on sensitive variables, such as household or participant IDs. Routine quality assurance checks are performed on these critical fields, with feedback provided directly to the data collection teams for any necessary corrections. Additionally, metadata and equipment quality queries are embedded within the forms to help trace sources of errors and facilitate corrective follow-ups when discrepancies are detected.

Missing data will be handled using an available-case approach and no formal imputation will be undertaken. For the primary malaria endpoint, where only one of RDT or microscopy is available, malaria infection status will be based on the available diagnostic result. Participants with neither RDT nor microscopy results available will be excluded from the primary analysis

Analysis of primary outcomes

The primary endpoint for analysis will be malaria infection status among sampled children aged ≤5 years. Because participants will be sampled independently at baseline, 6 months and 12 months, analyses will be conducted as repeated cross-sectional comparisons rather than longitudinal within-participant analyses. Malaria infection status at each follow-up timepoint will be compared between intervention and control using mixed-effects logistic regression models with a logit link and a random intercept for study cluster to account for the non-independence of observations within clusters.

Models will be fitted separately for the 6-month and 12-month follow-up timepoints. Unadjusted models will include trial arm as the only fixed effect. The primary adjusted models will include trial arm and baseline cluster-level malaria prevalence as fixed effects. Baseline cluster-level malaria prevalence will be calculated as the mean malaria infection

status among sampled children within each cluster at baseline. Models will be fitted using participant-level records, with clustering accounted for using the random intercept for study cluster. No formal weighting will be applied. Baseline-adjusted estimates will be treated as the primary inferential estimates.

Analysis of secondary outcomes

Entomological data

- **Mosquito species composition** will be reported as total abundance and species-specific proportions per sample, household and night. Differences between treatment arms will be assessed using regression models incorporating a random effect for study cluster where appropriate.
- **Mosquito density** will be reported as the total abundance of mosquitoes collected stratified by species. Additionally, for female *Anopheline* mosquitoes, the abundances will be broken down blood feeding status; blood fed/unfed, half gravid, and fully gravid. The outcomes will be compared across treatment arms. To address potential confounding factors and account for non-independence of data within clusters, a regression model will be employed with a negative-binomial log-link function to account for overdispersion typical of count data. These models will incorporate a random effect for each study cluster to adjust for intra-cluster correlation. Mosquito abundance will be analysed using mixed-effects negative binomial regression models with a log-link function and a random effect for study cluster. Unadjusted models will include treatment arm as the primary fixed effect. Where possible, adjusted models will be fitted to account for baseline variation in mosquito density. Where species-specific analyses contain sparse observations or substantial baseline imbalance between study arms, adjusted models may be considered unreliable or non-estimable and will not be interpreted.
- **Mosquito sporozoite rate** will be reported as the proportion of mosquitoes (%) with sporozoites present per sample. The outcomes will be compared across treatment using the same linear regressions models employed for mosquito species composition.
- The **prevalence of resistance to pyrethroids** in Anopheles mosquitoes will be reported as the proportion of samples that are susceptible (98% - 100% resistance), possibly resistant (90% - 97% resistance) or confirmed resistant (less than 90% resistance) per sample. The outcomes will be compared across treatment arms. To address potential confounding factors and account for non-independence of data within clusters, logistic regression models will be employed, as described for the primary endpoint

Household & acceptability surveys

Household survey and acceptability survey data will be summarised using basic descriptive statistics. Mixed methods will be applied for the quantitative and qualitative data that have been obtained from the entire study. Data will be entered into excel and thereafter exported into a software for analysing qualitative data i.e. NVivo/MAXQDA. We will employ thematic analysis on qualitative data to group sentiments and concepts as patterns collected in the data. These findings will be reported as synthesised narratives that illustrate the most primary perceptions, experiences, and acceptability of the intervention, as well as to provide additional context through description of quantitative data when appropriate.

Air sampling

Transfluthrin concentration in air samples will be reported as nanograms per litre of air sampled. Concentrations will be summarised by treatment arm and sampling timepoint using descriptive statistics and graphical summaries. The proportion of samples above the analytical limit of detection will also be summarised.

Interim analysis

There is no formal interim statistical analysis planned for this study.

Adverse event data

At each data collection timepoint preliminary analysis of safety data and early indications of efficacy will be summarised using descriptive statistics and compared between study arms. This review will include a detailed evaluation of both the frequency and nature of these events, as well as their potential relationship to the intervention.

The DSMB will report an overview of adverse event data, and subgroups of data including:

1. Total AEs,
2. Total SAEs
3. The severity of the event
4. The relation of the event to the intervention
5. Events with respiratory symptoms
6. Events with dermatological symptoms:
7. Mortality

Data on adverse events presented to the DSMB for review will be summarised as:

- **Total counts** reported in each cluster and across all clusters, disaggregated by study arms.
- **Proportion of households** in each cluster and across all clusters, disaggregated by study arms.
- **Incidence rates** (per 1,000 participants), in each cluster and across all clusters, disaggregated by study arms.

For the final analysis, adverse events will be summarised at cluster level as events per 1,000 population. Cluster-level adverse event rates will be compared between trial arms using an independent-samples t-test, with 95% confidence intervals calculated for the difference in mean cluster rates.
Based on their review of the data, the DSMB may recommend actions such as:

1. Modification of trial protocols, such as adjusting the duration or intensity of device use if specific adverse trends are identified.
2. Terminations or temporary suspension of the trial if significant safety concerns arise, particularly if certain clusters or subgroups appear disproportionately affected by AEs or SAEs.
3. Additional exploratory analyses to better understand the nature and causes of adverse events, especially if new patterns or unexpected outcomes are observed.

Subgroup analysis

No formal subgroup analyses will form part of the pre-specified primary analysis. Exploratory post hoc subgroup and restricted analyses may be conducted by socioeconomic status, district and land type. These analyses will be treated as exploratory and hypothesis-generating and will not form part of the primary adjusted analysis.

For exploratory socioeconomic analyses, socioeconomic status will be derived using an asset-based index constructed from electricity access, household transport and durable goods. These variables will be converted into binary asset indicators and combined using principal components analysis, with the first principal component used as the SES score. Households will be grouped into high and low SES categories based on the distribution of this index.

7. Dissemination

Study findings will be disseminated through joint scientific publications between the research consortium, conference attendance and a final project dissemination meeting in Uganda for all stakeholders including government leads and policy makers. Additionally, findings will be disseminated at the community level through community meetings.

8. References

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