**Lake Victoria Island Intervention Study on Worms and**

**Allergy-related diseases (LaVIISWA)**

**Statistical Analysis Plan**

18th October 2016

# Overview of study setting and design

LaVIISWA is being conducted on the Lake Victoria islands of Koome sub-county in Mukono district, Uganda. The island communities consist of well-defined, geographically separated villages, each governed by a single administrative committee, and mainly located on the islands’ shores. For this study, fishing villages only (not inland villages) are included.

LaVIISWA is an open cluster-randomised trial of intensive versus standard anthelminthic treatment. Fishing villages are the units of randomisation. Villages receive either intensive or standard anthelminthic treatment over a three-year period. A baseline household survey was conducted immediately prior to the first treatment round; outcomes will be assessed at the end of the three years using a second household survey.

# Objectives

The overall objectives of LaVIISWA are to:

1. Describe the phenotypes, prevalence and risk factors for asthma, eczema and atopy in island communities in Uganda
2. Investigate effects of intervention against helminths on asthma, eczema and atopy, and on other health outcomes, in a cluster-randomised trial among the island communities of Lake Victoria.
3. Determine the effects of exposure to helminths and their treatment on the expression of immunological pathways related to allergy, and to vaccine antigens.
4. Develop a DNA archive. Samples will be used to relate genetic polymorphisms and epigenetic DNA modification to gene expression, asthma, eczema and atopy, and helminth infection; for these studies additional funding will be sought.

This SAP describes the analyses to be conducted for objective 2 and the main trial analyses to be conducted for objective 3. Objective 1 was assessed in cross-sectional analyses of the baseline survey [1]. Further immunological work for objective 3 will be done in selected subgroups of participants and is not described here. Objective 4 does not require statistical analysis.

# Randomisation

Twenty-six clusters were randomised in a 1:1 ratio between the two trial arms. The randomisation was restricted based on village size, distance to Koome health centre and previous helminth treatment coverage. Full details of the restriction criteria are provided in the protocol paper [2].

# Description of the intervention

***Standard intervention*** comprises home-delivered single-dose albendazole (400 mg) given twice-yearly to all community members aged ≥1 year, and annual praziquantel treatment (approximately 40 mg/kg, estimated by height pole) to all individuals measuring ≥94cm (the standard height pole range) [3]. This height range excludes many pre-school children [4]. Individuals who are sick are excluded.

***Intensive intervention*** comprises home-delivered, quarterly, triple-dose albendazole and quarterly single-dose praziquantel to all community members aged ≥1 year. For praziquantel administration to young children the extended height pole (≥60cm) is used, with tablets crushed and given in juice [5] because a paediatric formulation for praziquantel is not yet available. For albendazole, a three-day course 400 mg daily, is given to provide more effective treatment for *Trichuris trichiura* [6, 7]. The first dose is directly observed, and the subsequent two doses provided. Pregnant women are given only single-dose albendazole.

# Outcomes

Primary outcomes, assessed at the three-year survey, together with a description of the group or groups of participants in which they are evaluated and their definitions are listed in Table 1.

**Table 1. LaVIISWA primary outcomes**

|  |  |  |
| --- | --- | --- |
| Outcome | Age group | Definition |
| **Wheeze, last 12 months** | **≥5 years** | **Self-report** |
| <5 years | Self-report |
| **Atopy (SPT)** | **≥1 year** | **SPT positive to any allergen versus SPT negative to all allergens** |
| SPT positive to *Dermatophagoides* mixversus SPT negative to *Dermatophagoides* mix |
| SPT positive to *Blomia tropicalis* versus SPT negative to *Blomia tropicalis* |
| SPT positive to German cockroach versus SPT negative to German cockroach |
| **Atopy (IgE)1** | **≥1 year** | ***Dermatophagoides* asIgE>0.35kUa/L or cockroach asIgE>0.35kUa/L, ImmunoCAP** |
| *Dermatophagoides* asIgE>0.35kUa/L, ImmunoCAP |
| Cockroach asIgE>0.35kUa/L, ImmunoCAP |
| *Dermatophagoides* asIgE (continuous variable), ImmunoCAP |
| Cockroach asIgE (continuous variable), ImmunoCAP |
| In-house ELISA *Dermatophagoides* asIgE (continuous variable), ImmunoCAP |
| In-house ELISA cockroach asIgE (continuous variable), ImmunoCAP |

1ImmunoCAP results are available for a subgroup of approximately 30 participants per village.

Wheeze will be assessed separately in two age groups (≥5 years, <5 years): the principle age group of interest will be ≥5 years.

Atopy (SPT) is defined as both positive SPT response to any allergen and as positive SPT responses to individual allergens. During the LaVIISWA planning stage, we anticipated that the trial intervention would have similar effects on SPT responses for all allergens. However, findings from our baseline survey and unpublished data comparing allergen-specific SPT responses between a rural environment (with heavy helminth burden) and an urban environment (with light helminth burden) indicate that associations between worms and atopy may vary depending on the allergen. To address this, results for each allergen will be presented, alongside the principal atopy (SPT) outcome of “any positive SPT versus none”.

For atopy (IgE), in-house ELISAs were done on all available blood samples from participants aged ≥1 year. However, data from the baseline survey indicated that results from the in-house ELISAs did not correlate well with ImmunoCap results. For purposes of international, inter-study comparability, we decided to conduct ImmunoCap assays as well as in-house ELISAs in the outcome survey. For reasons of cost, ImmunoCAP assays were done on a randomly selected subgroup of approximately 30 participants from each village. For the same reasons as described above for the atopy (SPT) outcome, we will present principle results using the definition “ImmunoCAP Dermatophagoides asIgE>0.35kUa/L or cockroach asIgE>0.35kUa/L”, but results from other definitions will be reported alongside.

Secondary and exploratory outcomes, assessed at the three-year survey, together with description of the group or groups of participants in which they are evaluated and their definitions are listed in Table 2.

**Table 2. LaVIISWA secondary and exploratory outcomes**

|  |  |  |
| --- | --- | --- |
| Outcome | Age group | Definition |
| **Secondary outcomes** |  |  |
| Visible flexural dermatitis | all | Clinical examination |
| Helminth infections | all | *Schistosoma mansoni* positive versus negative, stool Kato Katz |
| *Schistosoma mansoni* positive versus negative, urine CCA1 |
| Hookworm positive versus negative, stool PCR |
| *Strongyloides stercoralis* positive versus negative, stool PCR |
| *Trichuris trichiura* positive versus negative, stool Kato Katz |
| *Ascaris lumbricoides* positive versus negative, stool Kato Katz |
| Haemoglobin | ≥1 year | Hemocue |
| Growth | ≤19 years | Height-for-age z-score |
| Weight-for-age z-score |
| Hepatosplenomegaly, clinical examination | all | Hepatomegaly, palpation |
| Splenomegaly, palpation |
| Hepatosplenomegaly, palpation |
| Hepatosplenomegaly, ultrasound2 | <18 years, selected adults2 | Liver size left lobe (normal, enlarged, much enlarged), ultrasound |
| Spleen size (no splenomegaly, moderate splenomegaly, marked splenomegaly), ultrasound |
| Portal vein score (normal, dilation, marked dilation), ultrasound |
| Degree of hepatic fibrosis (normal, peri-portal, other), ultrasound |
| Vaccine responses | [1,4) years | Measles antibody responses, ELISA |
| Tetanus toxoid antibody responses, ELISA |
| Mycobacterial antigen cytokine responses, whole blood assay |
| Tetanus toxoid antigen cytokine responses, whole blood assay |
| Immune response profile | [1,18) years | Responses to schistosome worm antigen, whole blood assay  |
| Responses to schistosome egg antigen, whole blood assay |
| Responses to *Dermatophagoides pteronyssinus* antigen, whole blood assay |
| Responses to *Dermatophagoides farinae* antigen, whole blood assay |
| Responses to cockroach antigen, whole blood assay |
| **Exploratory outcomes** |  |  |
| Vaccine responses | [4,18) years | As listed above for the [1,4) years age group |
| Wheeze, last 12 months | All | Self-report |
| Rhinitis, last 12 months | all | Self-report |

1CCA, circulating cathodic antigen; 2Ultrasound results are available for all children <18 years, all hepatitis B positive adults, and a randomly selected subgroup of 45 hepatitis B negative adults per village, and will be analysed separately in these three subgroups

# Sample size justification

For the three-year survey we aim to sample 70 households comprising 125 individuals per cluster, with ImmunoCAP results available for approximately 30 individuals per cluster. Based on data collected in the baseline survey, prevalence of wheeze in individuals aged 5+ years was estimated to be 5%, the proportion with a positive SPT response was estimated to be 20% and the proportion with ImmunoCAP IgE>0.35kUa/L was estimated to be 40%. Table 3 indicates the power that the trial will have to detect risk ratios (RR) of 1.4-1.8 for intensive versus standard anthelminthic treatment at 5% significance level, assuming coefficient of variation 0.3 for wheeze and SPT responses and coefficient of variation 0.2 for IgE (conservative estimates, based on the baseline survey) [8].

**Table 3. LaVIISWA sample size calculations**

|  |  |  |  |
| --- | --- | --- | --- |
|  |  |  | Power to detect difference between intervention arms at p<0.05 |
| Outcome | Sample size per arm | Expected prevalence in standard arm | 1.4-fold difference | 1.5-fold difference | 1.6-fold difference | 1.8-fold difference |
| Wheeze, ≥5 years | 1450 | 5% | 39% | 53% | 66% | 85% |
| Positive SPT response, ≥1 year | 1540 | 20% | 65% | 81% | 90% | 98% |
| IgE>0.35kUa/L, ≥1 year | 390 | 40% | 81% | 95% | 98% | 99% |

# Analysis populations

The primary analysis will be an intention-to-treat analysis. The population for this will consist of all individuals surveyed in the villages at the three-year survey, regardless of how long they have lived in the village and whether they have received the trial interventions.

A secondary analysis will be done on a per protocol basis. The population for this will consist of individuals who report that they have lived in the same village throughout the three-year intervention period (or for children aged <3 years, were born in the village). We will not use data on treatment compliance to define the per protocol population since it is often not possible to match the treatment register data to the outcome survey data. Instead, alongside the results from the per protocol analysis, we shall report data on treatment compliance for each village (using study treatment registers), and on treatment compliance at the individual level, where available.

# Subgroup analysis

Subgroup analysis by age will be conducted for wheeze in last 12 months and for vaccine responses.

The subgroup analysis for wheeze will assess whether the effect of the intervention on wheeze in the last 12 months varies for the two age groups (<5 years, ≥5 years) because it is recognised that wheeze below age five years may not be indicative of asthma.

The subgroup analysis for vaccine responses will assess whether the effect of the intervention on vaccine responses varies for the two age groups (<4 years, ≥4 years to <18 years) because children under four years of age are likely to have received their routine immunisations during the trial intervention.

# Recruitment and trial profile

A trial profile figure will be produced illustrating the following, by trial arm:

* Number of clusters in each randomisation group
* Total number of individuals selected for final outcome survey
* Total number of individuals who participated in final outcome survey (i.e. for whom we have UMPC data)
* Mean (range) number of individuals per cluster who participated in final outcome survey
* Number of households/individuals that were selected but who did not take part in final outcome survey, with reasons
* Number of individuals who participated in final outcome survey who provided stool, blood and SPT responses
* Number of individuals analysed for each primary outcome

#  Baseline characteristics and descriptive analyses

Characteristics of clusters, households and individuals who participated in the final outcome survey will be tabulated by trial arm. We shall also refer to the tables of baseline (pre-intervention) characteristics in the Trials protocol paper [2], to address whether characteristics of clusters were similar between trial arms at baseline.

Characteristics tabulated for the final outcome survey will include:

Cluster-level characteristics

* Total number of households per village (mean, range)
* Number of households per village who are participating in the survey (mean, range)
* Number of individuals per village who are participating in the survey (mean, range)
* Proportion of villages with any public toilets
* Median (range) of number of public toilets
* Median (range) of number of private toilets
* Proportion with water supply other than lake, with breakdown of other source

Household-level characteristics

* Total number of households participating in the survey
* Household size (median, IQR)

Individual-level characteristics

* Age, median (IQR) and in 5-year age bands
* Sex
* Birth order
* Proportion resident (or born into) in the village throughout the intervention period
* Where born (this fishing village, other fishing village, other rural village, town, city)
* Maternal tribe
* Paternal tribe
* Any maternal history of eczema, asthma or allergy
* Any paternal history of eczema, asthma or allergy
* Occupation
* Frequency of lake contact
* Malaria treatment with Coartem in last 12 months
* Reported worm treatments in last 12 months
* Immunisations
* HIV status, overall and stratified by ART status
* Malaria infection status

We will report the proportion of cases of wheeze (separately for both age groups) that have atopy based on any positive SPT. We do not anticipate doing any further analysis stratifying by atopy, unless the prevalence of wheeze is higher than currently expected.

We will investigate the agreement between KK and CCA for assessing *S. mansoni* infection status. We will calculate the sensitivity and specificity of KK using CCA as the gold standard.

We will visualise the impact of the interventions on the relationship between age and intensity of *S. mansoni* infection status by plotting this separately for the two intervention arms, using the three-year survey data. Formal statistical analysis will not be done.

We will investigate the impact of the interventions on helminth prevalence over the course of the three-year intervention period, using data from the baseline survey and from two smaller interim surveys that were conducted at the ends of years one and two of the intervention. For each village, point estimates of helminth prevalence together with 95% confidence intervals will be tabulated by time point, and graphed.

We will describe the pattern of compliance to the intervention during the three-year intervention period, by trial arm.

#  Statistical analysis of outcomes

## *Overview of analysis approach*

All analyses will employ statistical methods that allow for within-cluster correlations. The primary analysis will be done at the cluster-level since this is most robust when the number of clusters is relatively small. Exploratory, secondary analyses will be done at the individual-level. These will be used to support and interrogate findings from the primary cluster-level based analyses.

For each outcome, and for both cluster-level and individual-level analyses, results from three models will be presented: (1) a crude analysis with no adjustment, (2) a minimally adjusted analysis, adjusting for pre-intervention baseline survey data on the outcome (if available), (3) a fully adjusted analysis, adjusting for pre-intervention baseline survey data on the outcome (if available) and for key prognostic factors that showed imbalance between treatment arms in the final survey participants.

If interpretation of findings deviates between models (1)-(3) then the reasons for this will be investigated (i.e. which adjusting factors have the biggest impact) and results from approach (3) will be used as the basis for interpretation.

## *Analysis of binary outcomes – cluster-level analysis*

For binary outcomes, cluster-specific proportions will be calculated as follows:

$$\frac{number of participants in cluster who experience the outcome}{number of participants in cluster who have data on the outcome}$$

The distribution of these cluster-specific proportions will be examined graphically by trial arm and a logarithmic transformation of the cluster-specific proportions applied prior to further analysis if the distributions are positively skewed (and amenable to correction to normality by log transformation).

The arithmetic mean of the cluster-specific proportions within each intervention arm will be used as a summary measure of the proportion experiencing the outcome in that arm. The overall proportion (i.e. the number of participants in the arm who experience the outcome divided by the number of people in the arm providing data for the outcome) will also be calculated, but the cluster-based summary measure described above will be the primary point estimate presented.

If cluster-level summaries do not require logarithmic transformation, then crude risk ratios for the effect of the intervention on binary outcomes will be calculated directly from the cluster-based summary measures by dividing the mean proportion in the intensive intervention arm by the mean proportion in the standard intervention arm. P-values for the effect of the intervention will be calculated using t-tests comparing cluster-level mean proportions between the trial arms. A 95% confidence interval for the risk ratio will be calculated using a Taylor series approximation approach to estimate the standard error.

If cluster-level summaries require logarithmic transformation, crude risk ratios for the effect of the intervention will be calculated by taking the exponential of the difference in the mean of the cluster-specific log proportions between the two arms. T-tests will be used to calculate p-values for the effect of the intervention on the log proportions, and 95% confidence intervals for the risk ratios will be obtained directly using the t-distribution and standard formulae for the standard error of the difference between two means, and then taking exponentials.

As described above in the “Overview of analysis approach” section, adjusted analyses for the effect of the intervention will also be conducted. For each outcome the cluster-specific prevalence (or cluster-specific mean for quantitative outcome) of the outcome as assessed at the baseline survey will be adjusted for, *a priori*, to improve precision of intervention effect estimates. We will also investigate adjustment for prognostic factors that showed imbalance between treatment arms in the baseline survey, despite randomisation. See the section below for a list of prognostic factors that will be considered.

Adjustment will be performed using a two-stage approach. First, a logistic regression model, including terms for the covariates to be adjusted for, but not a term for intervention arm, will be fitted to individual-level data from all clusters. From this the predicted prevalence of the outcome for each cluster and the ratio between the observed and predicted prevalence will be calculated (risk ratio-residuals). These risk ratio-residuals (one for each cluster) will then be used as the data points for the cluster-level analysis, using the same methods as described above for the unadjusted analysis. When the above two-stage procedures are used to adjust for cluster-level covariates (for example, the cluster-specific proportion with the outcome variable as measured at baseline), an adjustment will be made to the degrees of freedom of the *t*-distribution used for statistical inference. Because information on between-cluster variability is used to fit regression parameters for these cluster-level covariates, as well as to make inferences about the intervention effect, we will reduce the degrees of freedom by the number of parameters corresponding to the cluster-level covariates in the regression model. No such adjustment is needed for individual-level covariates.

## *Analysis of quantitative outcomes – cluster-level analysis*

Analysis of quantitative outcomes will follow a similar approach, with the effect of the intervention quantified as the difference in mean outcome between the two arms, and a 95% confidence interval for this calculated directly using the t-distribution and standard formulae for the standard error of the difference between two means. For the adjusted analysis following the two-stage approach, linear regression will be used in the first stage and mean difference-residuals will be used as the data point for the cluster-level analysis.

## *Analysis of ordered categorical outcomes – cluster-level analysis*

For the analysis of ordered categorical outcomes (which each have three categories), a proportional odds model will be used. For random outcome variable Y, the average of the cumulative odds for each cluster: pr(Y>k)/pr(Y≤k), k=0, 1, 2 will be calculated. These cluster-specific summary measures will then be log transformed and compared between intervention arms using a t-test. For the adjusted analysis following the two-stage approach, ordinal logistic regression will be used in the first stage and predicted probabilities for each level will be used as the data points for the cluster-level analysis.

## *Analysis of nominal categorical outcomes – cluster-level analysis*

For the analysis of this outcome (liver fibrosis, which has three categories), we shall conduct two separate analyses comparing the risk ratios for each of peri-portal and other abnormalities with “normal” as the baseline, using the methods outlined above for binary outcomes.

*Analysis of all outcomes – individual-level analysis*

Exploratory, secondary analyses will be done at the individual-level and used to support and interrogate findings from the primary cluster-level based analyses.

Random-effects models, including a random intercept for villages (clusters) will be used to investigate the impact of the intervention on primary, secondary and exploratory outcomes.

For binary outcomes, random-effects logistic regression will be used. For these analyses, we will assess the reliability of the model fit using a quadrature check. If there is more than a 1% relative difference in the likelihood or any of the parameter estimates, then we will consider an alternative approach using robust standard errors or generalised estimating equations (GEE).

For continuous outcomes, random-effects linear regression (mixed effects linear regression) will be used. For ordered categorical outcomes, random-effects ordered logistic regression will be used. For this, we will again assess the reliability of the model fit using a quadrature check. If there is more than a 1% relative difference in the likelihood or any of the parameter estimates, then we will consider an alternative approach using robust standard errors or generalised estimating equations (GEE). For nominal categorical outcomes, random-effects multinomial regression will be used.

As for the cluster-level analysis, we shall conduct (1) a crude analysis with no adjustment, (2) a minimally adjusted analysis, adjusting for pre-intervention baseline survey data on the outcome (if available), (3) a fully adjusted analysis, adjusting for pre-intervention baseline survey data on the outcome (if available) and for key prognostic factors that showed imbalance between treatment arms in the final survey participants. Adjustment will be done by including covariates in individual-level regression models.

## *Additional variables to be considered for adjusted analyses*

Analyses may be adjusted for potential confounders, based on information from characteristics of clusters and how these differ by trial arm. Variables pre-specified as potential confounders are indicated below. These were selected due to cross-sectional associations with the primary outcomes when assessed in the baseline survey [1].

Village-level characteristics

* Binary variable indicating whether village has any public toilets

Household-level characteristics

* Household size

Individual-level characteristics

* Age
* Sex
* Birth order
* Where born (this fishing village, other fishing village, other rural village, town, city)
* Occupation
* Maternal tribe
* Family history of eczema, asthma, allergy (any versus none)
* HIV status
* Frequency of lake contact

## *Subgroup analysis*

Subgroup analysis will be conducted following the approach outlined by Cheung et al. [9] as follows. For continuous outcomes, the difference in outcome will be calculated between the two subgroups being compared, separately for each cluster. The mean difference in the outcome will then be compared between the intensive and standard treatment arms, to give the point estimate of the interaction effect. A p-value and 95% confidence interval for this interaction will then be obtained by conducting an unpaired t-test on these cluster-specific differences. For binary outcomes, the cluster-specific log proportions will be used in place of the cluster-specific means, in order to calculate risk ratios.

#  Adverse events and safety reporting

For each village, any adverse events or serious adverse events were recorded at the end of each treatment round. Serious adverse events include deaths, miscarriages and stillbirths, life-threatening events, events resulting in disability or permanent damage, congenital abnormalities, hospitalisations, or events that required intervention to prevent one of these outcomes. With the exception of congenital abnormalities, only events that occurred within 48 hours of the study interventions, and that affect individuals who actually took medication, were regarded as serious adverse events for the purposes of this study. For congenital abnormalities, Village Health Team staff reported any events that occur in their village during the trial to the research team.

Higher rates of adverse events and serious adverse events are expected in the intensive arm because these villages were treated more often, and information was requested more often. In addition, adverse events due to praziquantel are expected to be more common for individuals with heavier *S. mansoni* infections (who we expect to be more prevalent in the standard intervention arm than in the intensive intervention arm over the course of the trial intervention period). To allow for these points, we will report numbers of adverse events from the annual treatment rounds, where individuals in both trial arms receive both albendazole and praziquantel treatments. We will compare the numbers at each annual round between the trial arms, and also assess whether there is in interaction between treatment and time, i.e. whether the number of adverse events changes over time in one arm compared to the other.

Serious adverse events will be listed by treatment arm.

#  Sources of bias and contamination

Potential sources of bias which will be examined include:

* Selection bias in one arm over another. This will be assessed by comparison of village-, household-, and individual-level characteristics between the two arms for baseline survey participants, and also for final survey participants.
* Contamination between clusters. We will attempt to document the proportion of people in intensive intervention villages who have previously (during the intervention period) lived in standard intervention villages, and vice versa.
* Selection bias between those participating and not participating in the final survey will be examined in relation to data available (e.g. age, sex), by comparing characteristics of individuals listed in the household with characteristics of those who contribute data to the analysis.

# References

1. Webb, E.L., et al., *Helminths are positively associated with atopy and wheeze in Ugandan fishing communities: results from a cross-sectional survey.* Allergy, 2016. **71**(8): p. 1156-69.

2. Nampijja, M., et al., *The Lake Victoria island intervention study on worms and allergy-related diseases (LaVIISWA): study protocol for a randomised controlled trial.* Trials, 2015. **16**(1): p. 187.

3. Montresor, A., et al., *Development and validation of a 'tablet pole' for the administration of praziquantel in sub-Saharan Africa.* Trans R Soc Trop Med Hyg, 2001. **95**(5): p. 542-544.

4. Sousa-Figueiredo, J.C., et al., *An inclusive dose pole for treatment of schistosomiasis in infants and preschool children with praziquantel.* Trans R Soc Trop Med Hyg, 2010. **104**(11): p. 740-742.

5. Stothard, J.R., et al., *Closing the praziquantel treatment gap: new steps in epidemiological monitoring and control of schistosomiasis in African infants and preschool-aged children.* Parasitology, 2011. **138**(12): p. 1593-606.

6. Speich, B., et al., *Efficacy and safety of nitazoxanide, albendazole, and nitazoxanide-albendazole against Trichuris trichiura infection: a randomized controlled trial.* PLoS Negl Trop Dis, 2012. **6**(6): p. e1685.

7. Steinmann, P., et al., *Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and Taenia spp.: a randomized controlled trial.* PLoS One, 2011. **6**(9): p. e25003.

8. Hayes, R.J. and S. Bennett, *Simple sample size calculation for cluster-randomized trials.* Int J Epidemiol, 1999. **28**(2): p. 319-26.

9. Cheung, Y.B., et al., *A simple approach to test for interaction between intervention and an individual-level variable in community randomized trials.* Trop Med Int Health, 2008. **13**(2): p. 247-55.