Statistical Analysis Plan

TRIAL FULL TITLE	Eliminating human rabies: impact of enhanced vaccination coverage
SAP VERSION	FINAL v1.0
SAP VERSION DATE	24/04/2024
ISRCTN NUMBER / LINK	ISRCTN14813279 / http://www.isrctn.com/ISRCTN14813279
TRIAL STATISTICIAN	Dr Paul Johnson, University of Glasgow, UK (paul.johnson@glasgow.ac.uk)
TRIAL CHIEF INVESTIGATOR	Prof Felix Lankester, Washington State University (felix.lankester@wsu.edu)
SAP AUTHOR	Paul Johnson, Felix Lankester

1 SAP Signatures

I give my approval for the attached SAP entitled "Eliminating human rabies: impact of enhanced vaccination coverage" dated 24/04/2024.

Chief Investigator

Name: Felix Lankester

Signature: 

Date: April 24, 2024

Co-Principal Investigator

Name: Katie Hampson

Signature: 

Date: 24 April 2024

Statistician

Name: Paul Johnson

Signature: 

Date: 24 April 2024

2 Table of Contents

1 SAP Signatures 1
2 Table of Contents 2
3 Abbreviations and Definitions 3
4 Introduction 4
4.1 Preface 4
4.2 Purpose of the analyses 5
5 Study Aims and Endpoints 5
5.1 Study Aims 5
5.2 Outcome measures 6
6 Study Methods 7
6.1 General Study Design and Plan 7
6.2 Inclusion-Exclusion Criteria and General Study Population 8
6.3 Randomisation and Blinding 8
6.4 Study Variables 9
7 Sample Size 9
8 General Considerations 10
8.1 Timing of Analyses 10
8.2 Analysis Populations 10
8.3 Covariates and Subgroups 10
8.4 Missing Data 11
9 Summary of Study Data 11
10 Efficacy Analyses 12
11 Figures 14
12 Reporting Conventions 14
13 Technical Details 15

3 Abbreviations and Definitions

AE	Adverse Event
CRF	Case Report Form
MDV	Mass Dog Vaccination
GLMM	Generalized Linear Mixed-effects Model
SAP	Statistical Analysis Plan

4 Introduction

4.1 Preface

Rabies has the highest case fatality rate of any known human infectious disease and kills around 59,000 people annually. The vast majority (99%) of these fatalities occur in Africa and Asia due to canine rabies. While human rabies can be prevented with post-exposure prophylaxis (PEP), the intervention is expensive and often not available in the remote communities where it is most needed. Targeting control efforts at the reservoir host through mass dog vaccination (MDV) is a socially equitable and effective approach to eliminating human rabies. However, implementing MDV across the rural landscapes where rabies remains endemic is logistically challenging and, consequently, expensive. Moreover, there has only been limited empirical evidence to demonstrate the cost-effectiveness of MDV in achieving public health outcomes. As a result many countries spend substantial resources on provision of PEP with only limited investment in MDV and, without eliminating the transmission source, human rabies deaths continue. The standard method of delivering MDV across Africa is a centralized team-led strategy. Based in central locations where power supplies allow vaccine storage in cold-chain conditions (4°C), teams drive to rural villages and set up temporary MDV clinics. To eliminate rabies on a regional scale, these pulsed once-per-year campaigns must vaccinate at least 70% of each community's dog population in order to maintain the minimum coverage above 20-45% (critical threshold - Pcrit) throughout the year. Otherwise natural turnover in the dog population leads to drops in coverage that allow sustained rabies transmission. Achieving this coverage level consistently across remote landscapes with pulsed team-led delivery, which is expensive and often results in a heterogeneous coverage, is challenging. Novel, cost-effective MDV delivery strategies that enable consistently high vaccination coverage to be achieved at the scale required for regional elimination are urgently needed. Decentralized community-led delivery strategies are a promising way of improving access to health interventions and have been used in Africa for the control of neglected tropical diseases such as onchocerciasis. In the case of rabies control, it has been hypothesized that moving towards continuous vaccination throughout the year via a community-led model will improve consistency of coverage and reduce delivery costs relative to annual pulsed

MDV. A key barrier to implementation of community-led interventions has been the inability to store rabies vaccines under cold-chain conditions in resource-limited rural communities. However, the availability of a thermotolerant rabies vaccine, storable without loss of potency for extended periods at temperatures exceeding cold-chain conditions, would allow community-led delivery options to be explored. Our recent study investigating immunogenicity of a widely used canine vaccine (Nobivac™ Rabies) shows that immunogenicity to a protective level is not diminished following storage at 30°C for 3 months. This important outcome now enables implementation and testing of novel decentralized delivery strategies.

4.2 Purpose of the analyses

These analyses will assess the efficacy of community-led *continuous* MDV in comparison with standard team-led *pulsed* MDV.

5 Study Aims and Endpoints

5.1 Study Aims

Aim 1: Test the effectiveness of a decentralized community-led continuous delivery strategy against the standard centralized team-led pulsed delivery via a randomized controlled trial (RCT). We will carry out a RCT to compare metrics of vaccination coverage under these two intervention strategies. We hypothesize that community-led continuous MDV delivery will result in:

- higher mean coverage across each year;
- higher coverage at 11 months (because puppies should be vaccinated earlier in their lives than under pulsed MDV);
- lower likelihood (proportion of year) with coverage below the critical threshold of 40%;
- more consistent coverage over time;

- more consistent coverage over space (between villages).

Aim 2: Compare the cost-effectiveness of the two delivery strategies.

We hypothesize that a community-led continuous mass dog rabies vaccination strategy will enable higher levels of vaccination coverage to be achieved when compared to a team-led pulsed mass dog rabies vaccination strategy and at lower cost. The data generated will allow estimation and comparison of vaccination cost-effectiveness and the net benefits of public health outcomes under the two MDV delivery strategies.

Aim 3: Assess the impact of the introduction of MDV across the Mara region on human health

We hypothesize that the introduction of MDV over a 3-year period to a region with little prior exposure to MDV will result in a reduction in numbers of dog bites and cases of human rabies. Note that the resolution of human health data does not allow these outcome measures to be compared between trial arms. This aim does not therefore assess the efficacy of the continuous MDV intervention.

5.2 Outcome measures

Primary efficacy outcome measure

Mean vaccination coverage across each year of the trial, which is equivalent to predicted coverage at 6 months. Coverage is defined as the proportion of dogs in each community (village) that have been vaccinated. A dog will be identified as having been vaccinated based on the possession by the owner of a vaccination certificate.

Secondary efficacy outcome measures

1. Vaccination coverage at 11 months.
2. Proportion of a year when coverage is predicted to be below the critical threshold of 40%.

3. Variability in coverage over time.
4. Variability in coverage between villages.

Cost-effectiveness outcome measures

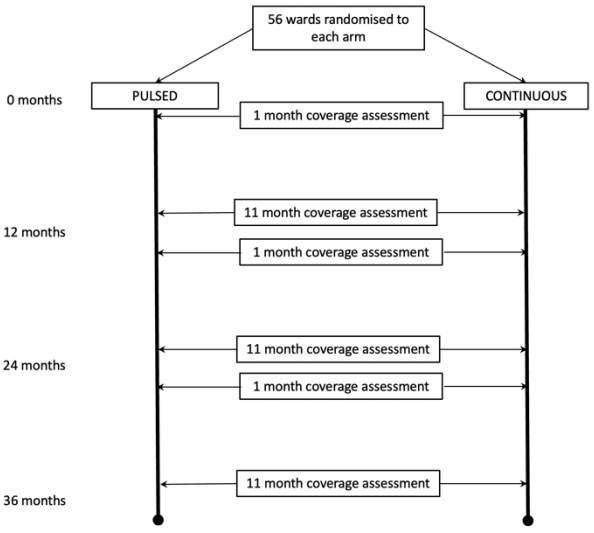
Cost-effectiveness outcomes will be added in a future version of the SAP.

Human health outcome measures

1. Dog bite injuries assessed using hospital record data collected at district hospitals in the Mara region of Tanzania from October 2019 to August 2023.
2. Number of human rabies cases assessed using hospital record data collected at district hospitals in the Mara region of Tanzania from October 2019 to August 2023.

6 Study Methods

6.1 General Study Design and Plan



The study design will be a cluster randomised controlled trial in which the villages within 112 administrative wards will be randomly assigned to receive MDV through either of the two delivery strategies. The ward is the cluster. Randomisation will be stratified by district. All wards will be randomised before the start of the trial. Due to the nature of the intervention, the allocation of wards to treatment arms will be unblinded to the participants (the ward population) and the research

team. However, the trial statistician will be blinded to the allocations until after the trial has been completed and the SAP has been approved.

6.2 Inclusion-Exclusion Criteria and General Study Population

The trial will take place in nine local government authority districts within the Mara Region of northern Tanzania. Each district is comprised of administrative wards, with each ward comprising three to four villages. The RCT will cover 112 wards and 428 villages in total. All wards within this study area will be included in the trial, therefore there are no inclusion or exclusion criteria at the cluster level beyond inclusion in the study area.

6.3 Randomisation and Blinding

Fifty-six wards will be randomly allocated to each of pulsed or continuous MDV (n=112) by permuted block randomisation with a block size of six wards, stratified by district. Randomisation will be carried out by running a program (a script for the R statistical software), written by the trial statistician, that will generate a spreadsheet assigning each ward to a treatment arm. To maintain blinding of the trial statistician to the treatment allocations, a statistician with no connection to the study (Dr Theo Pepler, University of Glasgow) will run the program using a different random seed chosen by and known only to him. He will send the spreadsheet of treatment allocations directly to the Chief Investigator. An additional 19 wards (n=131) might be included in the trial if time and costs allow. In order to preserve the integrity of the randomisation, the spreadsheet will include a randomisation order within each district indicating the order in which wards should be enrolled in the study.

6.4 Study Variables

	Baseline	Year 1-3 Month 2	Year 1-3 Month 11	Year 1-3 Monthly
Coverage in pulsed arm		X	X	
Coverage in continuous arm		X	X	
Human dog bite injuries	X			X
Human rabies cases	X			X
Dog rabies cases	X			X
Cost/dog of delivery (pulsed)				X
Cost/dog of delivery (continuous)				X

7 Sample Size

We estimate that randomizing 56 wards to each arm of the trial will give 87% power at the 5% significance level to detect coverage superiority of continuous to pulsed MDV delivery, assuming a mean coverage (the mean of coverage at 2 and 11 months) of 50% with pulsed and 58% with continuous delivery (equivalent to an odds ratio of 1.34). Power was estimated by analysing 10,000 simulated data sets, assuming sampling of one village per ward, three sub-villages per village, 10 households per sub-village and an average of 2.5 dogs per dog-owning household (a total of 9,000

dogs per assessment) at 2 and 11 months for three years. Logit-normal variances between households (11.5), sub-villages (4.4), villages (0.55) and wards (0.023) and the distribution of dogs over households were based on survey data from previous studies, and have an effect on required sample size equivalent to a design effect of 23. On the basis of following up vaccinated dogs in 112 study villages corresponding to 112 wards, we expect to record coverage in approximately 43,700 dogs in the first phase of the trial and a further 16,100 in each of the second and third years (assuming half of the dogs selected for follow-up each year will have died and been replaced with new dogs by the next). Mean coverage at 2 months in both arms was assumed to be 60% following static-point vaccination clinics. Mean coverage is assumed to decline in Arm 1 to 41% at 11 months due to deaths and births (assuming an exponentially distributed lifespan with a mean of 26 months), while declining less sharply to 55% in Arm 2 due to ongoing vaccination of puppies. If time and costs allow, the more villages in addition to the central village could be included.

8 General Considerations

8.1 Timing of Analyses

The final statistical analysis will be performed after the study has closed and all of the outcome data has been delivered to the trial statistician. To maintain blinding of the trial statistician, these data will not include the allocations of the wards to the study arms. The final primary analysis will be developed and approved using dummy allocations, before the final analysis is performed using the true allocations.

8.2 Analysis Populations

It is not expected that any wards will drop out during the trial, therefore the analysis population will be the full population of 112 wards, which is the intention to treat (ITT) population.

8.3 Covariates and Subgroups

The final analysis model will allow include random effects to allow for clustering of the outcome measures at the level of districts and wards, sub-villages and households. Where possible, two types of random effect will be fitted at each level, allowing clustering that is both consistent over the six survey visits, and specific to each survey visit. The efficacy analyses will not be adjusted for any ward characteristics. Subgroup analyses will be performed by tribal group and by prior exposure to MDV.

8.4 Missing Data

Missing data on a large scale (e.g. loss-to-follow-up of entire wards) is not expected. Missing data on a small scale (e.g. the trial team cannot collect outcome data at a particular time point in a particular village) is possible. Sparsely missing outcome data is effectively naturally imputed in random effects models.

9 Summary of Study Data

All continuous variables will be summarised using the following descriptive statistics: n (non-missing sample size), mean and standard deviation. Categorical variables will be summarised with frequency and percentages (based on the non-missing sample size) of observed levels. All summary tables will be structured with a column for each treatment in the order (pulsed, continiuous) and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

10 Efficacy Analyses

Primary efficacy analysis

Mean vaccination coverage will be compared between the two arms using a generalized linear mixed-effects regression model (GLMM) fitted using maximum likelihood. Logit-normal random effects will be fitted to account for variation in coverage between districts, wards, subvillages and households. For each of these levels, except household, additional round-specific random effects will be included to allow for unexplained variation in coverage that is inconsistent between rounds. Intervention arm, assessment visit (2 or 11 months) and year will be modelled as fixed effects. All three two-way interactions and a three-way interaction between year, visit, and arm will be fitted. The effect of fitting these interactions will be to allow coverage to differ between the six rounds (year $\times$ visit), and to allow the intervention effect to differ between the six rounds (arm $\times$ year, arm $\times$ visit, arm $\times$ year $\times$ visit). The primary analysis will be a test of the null hypothesis of equal coverage (Arm One (pulsed) : Arm Two (continuous) odds ratio $= 1$) between continuous and pulsed delivery against the two-sided alternative hypothesis of unequal coverage. The full model, including the main effect of trial arm and the arm $\times$ year, arm $\times$ visit, and arm $\times$ year $\times$ visit interactions, will be compared with a null model not including trial arm or its interactions with round or year. In effect, the null model allows coverage to vary over the six time points (the six household surveys), while the full model includes an additional six parameters that allow coverage to vary independently between trial arms at each of the six time points. Under the null hypothesis, therefore, there is no intervention effect at any of the six time points, while the alternative hypothesis is that there is an intervention effect at one or more time points.

The intervention effect will be estimated as an odds ratio with 95% confidence interval and the null hypothesis will be tested using a likelihood ratio test and rejected at the 5% significance level if $p < 0.05$. If the null hypothesis is rejected and the continuous:pulsed odds ratio > 1 then community-led delivery will be considered superior.

Secondary efficacy analyses

1. Vaccination coverage at the eleven-month time point within each annual vaccination cycle will be compared between arms in order to test the hypothesis that eleven-month vaccination coverage in the community-led delivery arm is higher than the coverage at the same time-point in the team-led delivery arm. A model including the fixed effects of year, trial arm, and their interaction will be compared with a model with only year as a fixed effect. Both models will be fitted to data from the second (11-month) visit only, excluding data from the first (2-month) visit.
2. The full model will be used to compare the likelihood of coverage dipping below the threshold of 40% in each arm by implementing the following plan:
    a. Use parametric bootstrapping from the fitted full model to generate at least 1000 samples from the sampling distribution of the model parameters, capturing uncertainty in the model parameter estimates (fixed effects and random effect variances).
    b. For each of the 1000 sets of parameters, simulate a new set of wards with ward-level coverage, including random effect variation, giving the distribution of predicted coverage in each arm for a random ward, taking account of parameter estimation uncertainty and random effect variation.
    c. Calculate the proportion of wards with coverage below 40% for each arm, using the 1000 parametric bootstrap samples to calculate 95% confidence intervals.
3. The hypothesis that variation in vaccination coverage over the three-year time frame of the study was lower in the community-led arm than in the team-led arm will be tested. The fixed effects of assessment visit and year will be replaced by a variance parameter to capture variation in coverage over time. A model representing the null hypothesis, with the temporal variance constrained to be equal between continuous and pulsed delivery, will be compared to a model where these variances are allowed to differ. These models may be fitted using Markov Chain Monte Carlo (MCMC) because variance component estimation can be more reliable than using maximum likelihood. The null hypothesis of equal variances will be rejected if the 95% CI for the ratio of variances (continuous:pulsed) does not include 1, and

continuous delivery will be considered more consistent than pulsed if the upper bound of the 95% CI is below 1.

4. Using the same methods (except that the target of inference will be the continuous:pulsed ratio in inter-village variance) the null hypothesis of equal inter-village variation in vaccination coverage will be tested.

Cost-effectiveness analyses

Cost-effectiveness analyses will be added in a subsequent version of the SAP.

Human health outcome analyses

Human health outcome analyses will be added in a subsequent version of the SAP.

11 Figures

Analyses comparing efficacy outcomes (coverage and consistency) between the study arms will be illustrated by figures showing for each arm the mean outcome with 95% confidence limits over the three years of the trial.

12 Reporting Conventions

P-values $\geq$0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

13 Technical Details

All analyses will be performed in the most recent version of the R statistical software and its packages. Maximum likelihood GLMMs will be fitted using the lme4 package. Bayesian models will be fitted using MCMC in the runjags and/or MCMCglmm packages. Quality measures will include comparison of source and analysis data sets and code-review.