DeWorm3
MASTER TRIAL PROTOCOL

June 15, 2017

1. INTRODUCTION

Neglected tropical diseases (NTDs) are caused by a group of parasitic, viral and bacterial infections that affect over 1 billion people globally, particularly socio-economically disadvantaged populations living in low-resource settings [1]. The clinical and economic consequences of NTD infections are significant, as these diseases result in disabling chronic conditions, delayed growth and cognitive development, severe social stigma, and lost economic productivity [2]. In an effort to respond to the NTD's as a major public health priority, the World Health Organization (WHO) and other global partner institutions endorsed the London Declaration, a commitment to pursuing control or elimination of select NTDs by 2020.

The soil-transmitted helminths (STH) are group of intestinal parasites targeted by the London Declaration. These parasites infect an estimated 1.45 billion people globally, resulting in the loss of almost 5 million disability adjusted life years (DALYs) annually [3, 4]. High to moderate intensity STH infections are associated with increased risk of malnutrition, iron-deficiency anaemia and other adverse physical and cognitive morbidities, particularly in children. The current WHO strategy for controlling STH is based on mass drug administration (MDA) of deworming medications to pre-school age children (pre-SAC) and school age children (SAC) with the goal of controlling morbidity [5].

While the current strategy of MDA for STH control has proven successful in controlling morbidity, targeting of pre-SAC and SAC alone with MDA is unlikely to break transmission of STH in many settings, in large part due to adult reservoirs of disease [6, 7]. In addition, despite the large commitment from pharmaceutical industries to donate large quantities of the medications (albendazole and mebendazole) needed to treat these infections, current estimates suggest that the global geographic coverage of MDA for STH is below WHO targets [8]. However, even if coverage of all pre-SAC and SAC was optimized, the current STH strategy of targeting children would likely need to be continued indefinitely, or at least until significant economic and social developments are able to interrupt transmission. As a result, interest in alternative strategies has led to several studies suggesting that it may be possible to interrupt STH transmission using broadly administered preventative chemotherapy strategies targeting all age groups [9].

Other NTD programmes have been successful in achieving high treatment coverage of MDA to entire communities, including adults. For example, programmes targeting lymphatic filariasis (LF) elimination have achieved considerable community acceptability and high MDA coverage in many LF-endemic countries worldwide. Of the 73 countries endemic for LF in 2014, 18 have discontinued MDA and are in a state of post-MDA surveillance, pending confirmation that LF transmission has been interrupted [10]. Given that LF programmes provide five to seven rounds of MDA to entire communities with albendazole (in combination with ivermectin or diethylcarbamazine (DEC)), there is a unique opportunity to leverage the LF programme platform to attempt to break the transmission of STH.

The Natural History Museum, in partnership with the Bill & Melinda Gates Foundation, has established the DeWorm3 Project to demonstrate the feasibility of interrupting the transmission of STH (*Ascaris lumbricoides*, *Ancylostoma duodenale*, *Necator americanus* and *Trichuris trichiura)* by building upon the success of the LF elimination platform. The project will conduct a series of community-based cluster randomised trials in Asia and Africa (India, Malawi and Benin) in order to determine whether continuing community-wide MDA with albendazole following cessation of LF programmes can interrupt STH transmission in focal geographic areas. These findings and accompanying implementation science research will provide the evidence necessary to inform relevant guidelines, policies, and operational plans.

2. OBJECTIVES

2.0 General objective

To determine the feasibility of interrupting the transmission of STH (*A. lumbricoides*, *A. duodenale*, *N. americanus* and *T. trichiura)* in focal geographic areas in Africa and Asia by expanding the population targeted and the frequency of delivery of MDA with albendazole.

2.1 Primary objectives

In focal geographic areas in India, Malawi and Benin, where LF programmes have delivered at least five rounds of MDA of albendazole (plus ivermectin or DEC);

2.1.1 To compare the prevalence of the predominant STH species driving transmission at a particular study site (*most frequently A. duodenale* or *N. americanus)* measured by quantitative PCR 24 months after stopping MDA between clusters randomised to receive twice-yearly community-wide MDA versus clusters randomised to receive standard of care pre-SAC and SAC targeted MDA.
Hypothesis: In settings where the prevalence and intensity of STH infections have been reduced through annual MDA of albendazole delivered by LF elimination programs, an additional three years of twice-yearly community-wide MDA with albendazole will result in a lower final cross-sectional prevalence of the predominant STH species driving local transmission as compared to a strategy of pre-SAC and SAC targeted MDA.

2.1.2 To determine whether the transmission of the predominant STH species driving transmission at a particular study site (*most frequently A. duodenale* or *N. americanus)* can be interrupted using MDA with albendazole, defined as reaching a combined point prevalence of ≤2% of that species measured by quantitative PCR 24 months after stopping MDA in clusters receiving either twice-yearly community-wide MDA or standard of care pre-SAC and SAC targeted MDA.
Hypothesis: In settings where the prevalence and intensity of STH infections have been reduced through annual MDA of albendazole delivered by LF elimination programs, an additional three years of twice-yearly community-wide MDA with albendazole will be sufficient to interrupt transmission of a the predominant STH species driving transmission at a particular study site, whereas MDA targeting pre-SAC and SAC will not achieve interruption of STH transmission.

2.2 Secondary objectives:

2.2.1 To determine whether the transmission of each STH species *(A. lumbricoides*, *A. duodenale*, *N. americanus* and *T. trichiura)*) can be interrupted using MDA with albendazole, defined as reaching a cluster point prevalence of ≤2% 24 months after stopping MDA in clusters receiving either twice-yearly community-wide MDA or standard of care pre-SAC and SAC targeted MDA.
Hypothesis: In settings where the prevalence and intensity of STH infections have been reduced through annual MDA of albendazole delivered by LF elimination programs an additional three years of community-wide MDA of albendazole will be sufficient to interrupt transmission of each individual species, whereas MDA targeting pre-SAC and SAC will not achieve interruption of species-specific transmission.

2.2.2 To compare the proportion of clusters in each of the randomisation arms that interrupt transmission, defined as reaching a cluster prevalence of ≤2% for each STH species (*A. lumbricoides*, *A. duodenale*, *N. americanus* and *T. trichiura)* by quantitative PCR measured 24 months after stopping MDA.
Hypothesis: In settings where the prevalence and intensity of STH infections have been reduced through annual MDA of albendazole delivered by LF elimination programs, an additional three years of twice-yearly community-wide MDA with albendazole will result in a greater proportion of clusters achieving elimination of all STH species as compared to a strategy of pre-SAC and SAC targeted MDA measure by qPCR 24 months after stopping MDA with albendazole. Reductions in prevalence will be driven primarily by reductions in the predominant species of STH in each geographic area (usually hookworm species), as documented at the baseline assessment.

2.2.3 To compare the combined prevalence of all STH species detected by quantitative PCR 24 months following the last round of MDA in children under 5 years of age residing in clusters receiving twice-yearly community-wide MDA versus those residing in clusters receiving standard of care pre-SAC and SAC targeted MDA.
Hypothesis: Detection of infection in children under 5 years of age 24 months following the last round of MDA is a proxy marker of continued transmission in the community. In settings where the prevalence and intensity of STH infections have been reduced through annual MDA of albendazole delivered by LF elimination programmes, an additional three years of twice-yearly community-wide MDA will result in significantly fewer STH infections occurring in young children, as compared to the number of infections in young children exposed to pre-SAC and SAC targeted MDA.

2.2.4 To compare the proportion of individuals excreting fertilized *Ascaris* eggs 24 months following the last round of MDA in clusters receiving twice-yearly community-wide MDA versus those residing in clusters receiving standard of care pre-SAC and SAC targeted MDA.
Hypothesis: In settings where the prevalence and intensity of STH have been reduced through annual MDA of albendazole delivered through LF elimination programmes, an additional three years of community wide MDA of albendazole will result in significantly fewer individuals excreting fertilized Ascaris eggs 24 months after discontinuing MDA in clusters randomised to twice-yearly community-wide MDA, as compared to individuals in clusters that receive MDA targeting pre-SAC and SAC only.

2.2.5 To compare the economic and financial costs and incremental cost-effectiveness of providing six twice-yearly rounds of community-wide MDA versus three annual pre-SAC and SAC targeted MDA for the purpose of interrupting the transmission of all STH species.
Hypothesis: Three years of twice-yearly community wide MDA with albendazole will be a more cost-effective method to (i) reduce STH prevalence and (ii) interrupt transmission than MDA targeting pre-SAC and SAC only. This observed cost effectiveness will be maintained when evaluating cost to the participant, the health system or the funder and in predictive models assessing impact over 5 years, 10 years and 20 years.

2.2.6 To develop and evaluate a community-wide STH MDA model that is sustainable and scalable in STH endemic areas.

This objective is addressed through a package of observational activities described in the supplemental proposal attached with this protocol (Supplemental Implementation Science Proposal).

3. SIGNIFICANCE AND IMPLICATIONS

The current momentum for achieving NTD elimination provides a unique opportunity to test the feasibility of using innovative strategies to reduce and interrupt the transmission of STH. We propose to demonstrate the feasibility of interrupting STH transmission using a strategy of twice-yearly community-wide MDA following the discontinuation of community-wide MDA targeting LF elimination. This Project will provide evidence necessary for optimizing a delivery model based on LF elimination efforts that can inform future guidelines, policies, and operational plans in STH endemic countries.

4. METHODS

4.1 Trial sites

The DeWorm3 trial sites include focal geographic areas in Benin, India, and Malawi (Figure 1).

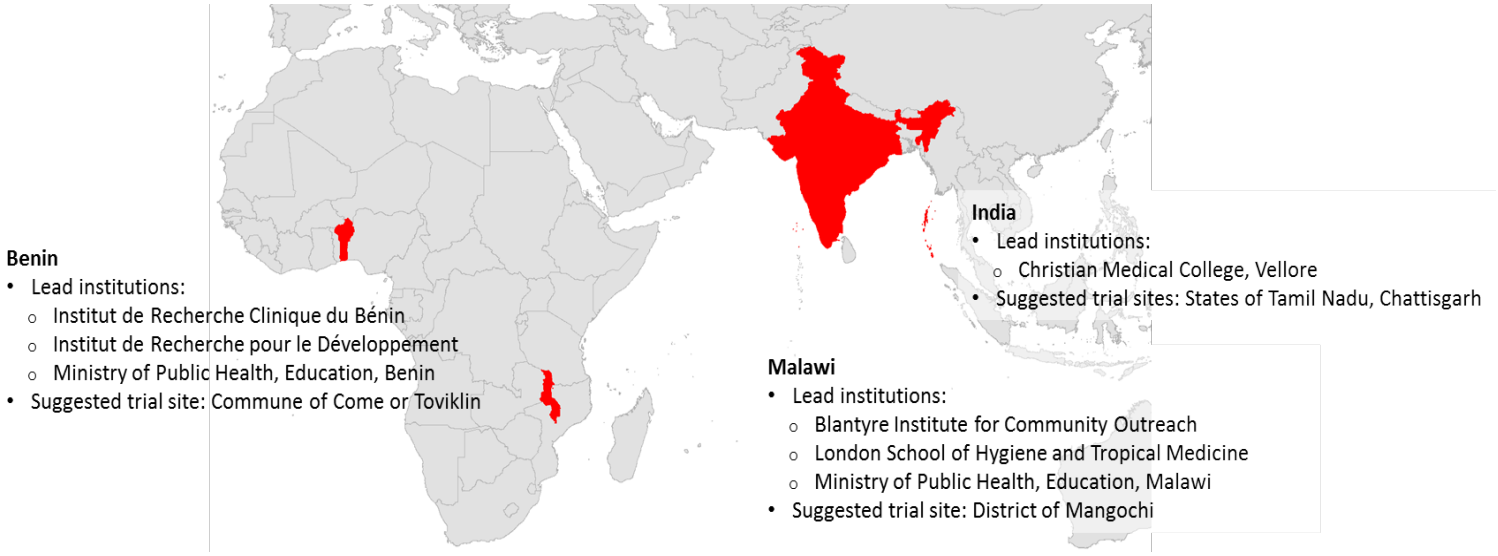


Figure 1: Main DeWorm3 trial sites and partners

In each site, clusters will consist of villages and/or groups of villages, depending on village population size.

4.2 Trial Description

DeWorm3 is a series of community cluster randomised trials comparing twice-yearly community-wide and standard of care targeted (pre-SAC and SAC only) MDA for interruption of STH transmission.

Study Design:	Community cluster randomised controlled clinical trial
Intervention:	Bi-annual (twice-yearly) community-wide MDA of albendazole for 3 years delivered to all individuals over 24 months of age

Control:	Targeted MDA of pre-SAC and SAC with albendazole for 3 years delivered in accordance with national Ministry of Health (MOH) guidelines
Primary Outcome:	STH transmission interruption (prevalence ≤2% 24 months following the final round of MDA)
MDA eligibility criteria:	Resident in the cluster and eligible for treatment with albendazole according to WHO and national guidelines
MDA exclusion criteria:	Children under two years of age Pregnant women in their first trimester History of adverse reaction to benzimidazoles
Sampling eligibility criteria:	Individuals considered residents of the study cluster based on questionnaire responses Provide consent and/or assent (as applicable)
Sampling exclusion criteria:	Individuals who do not reside in the study cluster Refusal to consent/assent
Sampling schedule:	Cross-sectional sampling of randomly selected eligible participants will take place at baseline (pre-MDA), post-treatment (six months following the third year of MDA), and at endline (24 months following the final round of MDA) Longitudinal monitoring cohorts will be sampled annually

Table 1. Study summary

Primary Outcome

Transmission interruption is defined as a cluster-level point prevalence ≤2% of any detectable STH species. The prevalence threshold of 2% was selected based on the threshold's diagnostic performance in accurately predicting transmission interruption of any STH species 24 months after stopping MDA (Figure 2).

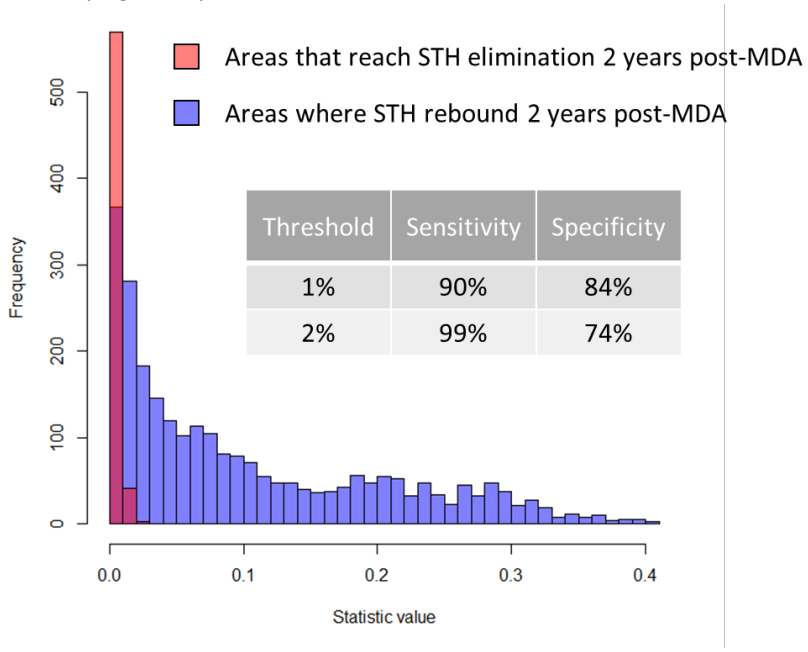


Figure 2: Optimal transmission interruption thresholds identified by STH transmission models (Anderson et. al. 2016, *publication pending*)

Trial Design

Each study site will include a total population of approximately 80,000 to 100,000 individuals. Clusters of villages that have completed at least five rounds of annual LF MDA with albendazole (plus ivermectin or DEC) will be randomised to three consecutive years of either;

A. Twice-yearly community-wide MDA (intervention arm), or
B. Targeted MDA of pre-SAC and SAC (control, standard-of-care arm) delivered in accordance with national Ministry of Health (MOH) guidelines.

The study site will be divided into 40 total clusters, 20 in the intervention arm (biannual community-wide MDA) and 20 in the control arm (targeted MDA). The population of each cluster will range between 1,650 and 4,000 individuals. Cluster randomisation will be performed within each site. Restricted randomisation may be performed to ensure similarity in baseline characteristics across clusters.

In each cluster, 500 randomly selected individuals aged 12 months and up will be sampled via cross-sectional surveys at study baseline and 6 months following the final round of MDA. Fecal samples will be collected from the sampled population and will be stored for subsequent qPCR analysis. A final STH prevalence survey of 500-1000 individuals per cluster will be conducted 24 months following the last round of MDA to determine whether transmission has been successfully interrupted in each cluster.

In addition to the sampled population, 150 individuals will be included in longitudinal monitoring cohorts. Participants in the longitudinal monitoring cohorts will be randomly selected and age-stratified.

The trial design is summarized below in Figure 4.

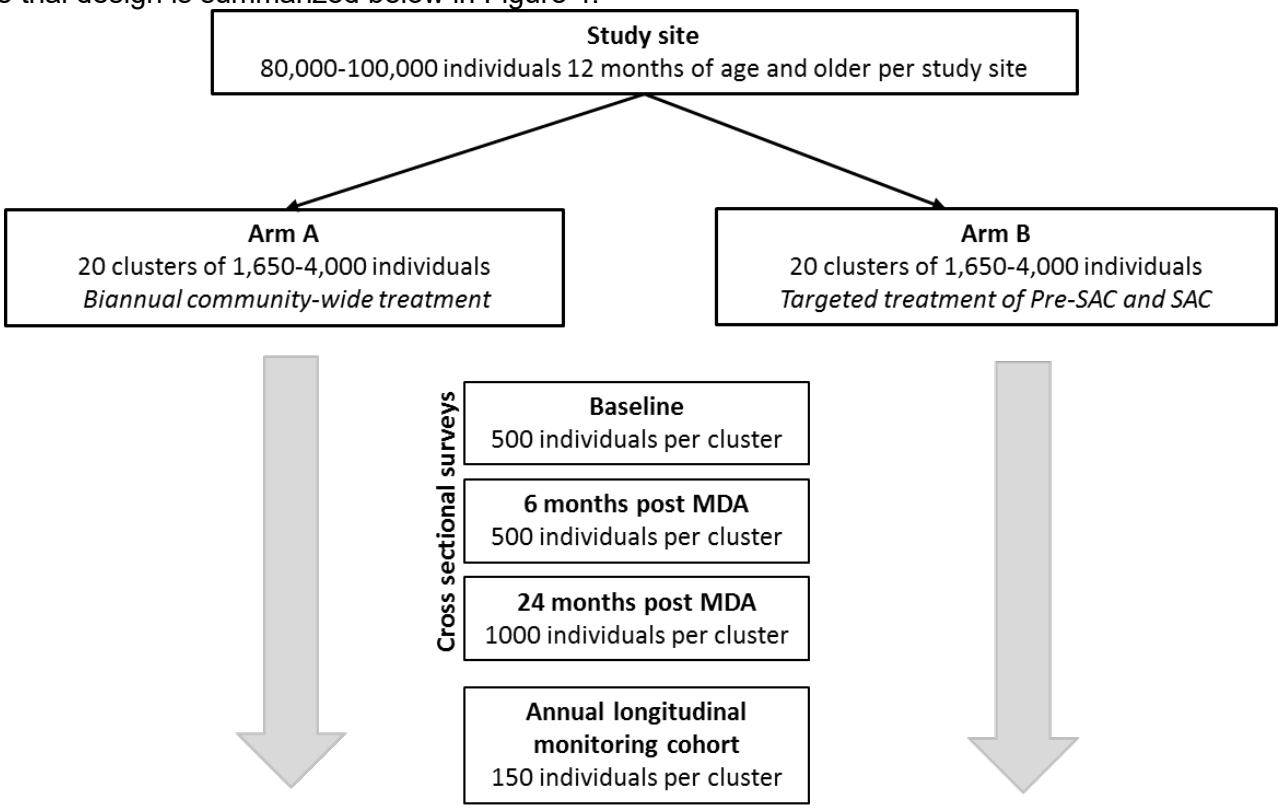


Figure 3: Summary study flowchart

4.3 Intervention

In the intervention arm, all eligible individuals above the age of 24 months will receive a single dose of albendazole during twice-yearly time-restricted campaigns[11].

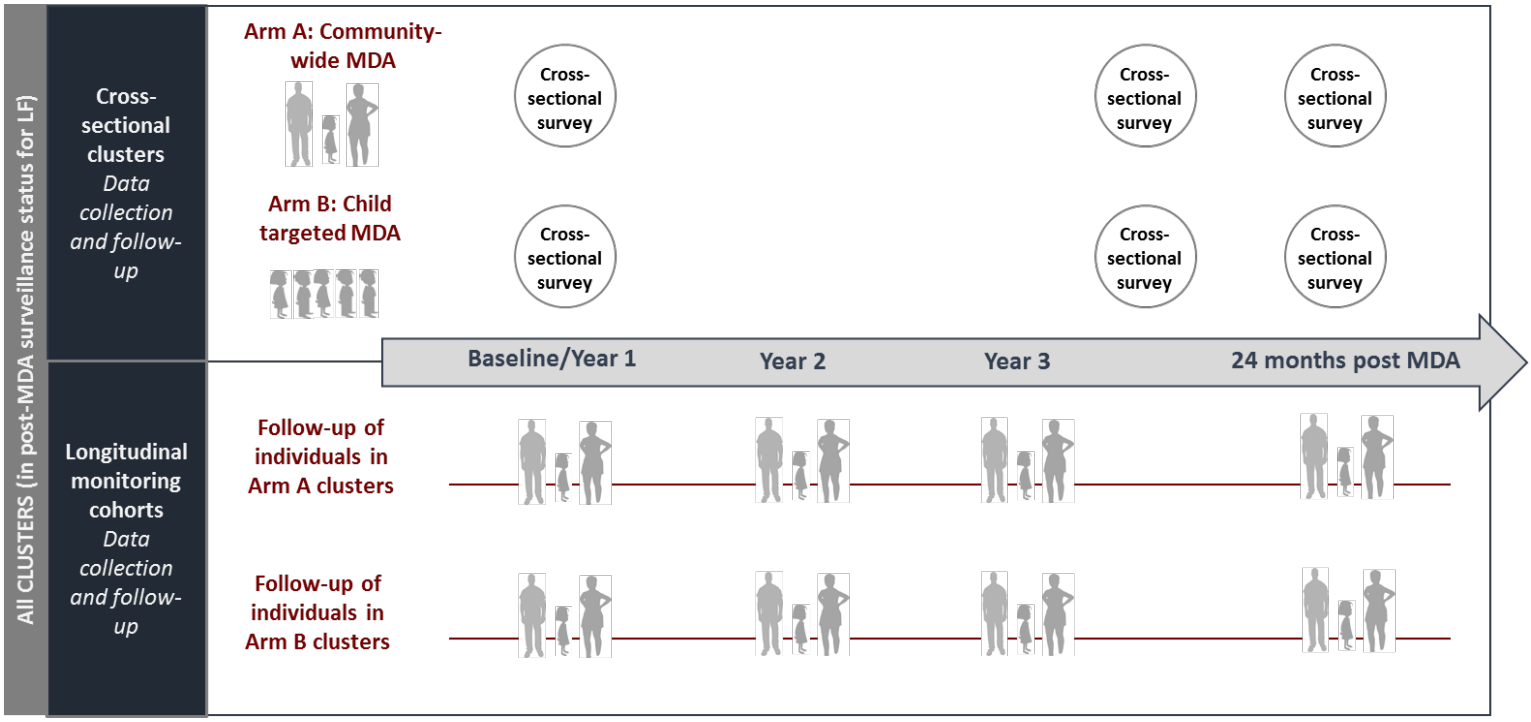
Delivery of MDA

In the intervention clusters (population 40,000-50,000), house-to-house MDA will be conducted and treatment will be delivered to all adults and children. Study staff or village volunteers will directly observe MDA compliance and record when each tablet is ingested by all individuals meeting treatment inclusion criteria. Mop-up campaigns targeting untreated individuals will be conducted in all clusters. Social mobilisation for community-wide MDA of albendazole will be ensured through pre-MDA sensitization and health education.

In control clusters, MDA will be provided to all pre-SAC and SAC through the existing National STH Programme or a similar distribution structure. Eligible children aged 2-14 years will receive a single dose of albendazole in school or during alternative MDA campaigns, as detailed in site-specific national guidelines. Non-enrolled school-age children and pre-SAC will be encouraged to come to school for treatment on deworming days, or will be reached by strategies appropriate to the local context in collaboration with local ministries and authorities. MDA will be administered by teachers, health workers or volunteers, according to national practice. All doses of MDA provided to pre-SAC and SAC through the National Programme will be directly observed and recorded in programme logbooks. Summary data from the National Programme logbooks will be extracted into the study database (not individually identifiable).

Figure 5: Outline of the DeWorm3 trial design

4.4 Data collection



An **annual census** will be performed of the entire study population, identifying and describing the inhabitants of each household along with the GPS coordinates of their dwellings. The baseline census will be vital for initial cluster randomisation, as well as to understand STH risk factors at a household level. Households may receive a DeWorm3 ID card during the baseline census, which they will be asked to keep for the remainder of the trial. This card has their household ID on it, to facilitate identification of the household during future DeWorm3 activities. All censuses following the baseline census will be formulated as census updates, where the births, deaths, and migration status of household members will be verified. Censuses will be used to quantify in- and out-migration and describe migration patterns influencing overall coverage of MDA and potential importation of infection from outside of the study area. Migration-specific questions on the census will also be used to determine when mop-up MDA activities will occur in a given cluster. Geolocation data will be de-associated from patient identifying information at the time of data de-identification.

DeWorm3 will use two primary definitions of cluster population. The total population is comprised of all members of households within the cluster boundaries, where a household is defined as "A person or group of persons, related or unrelated, who consider the house their permanent residence and are affected by the decisions of the head of household. A household member may travel for work or education, but regularly returns to the household as their primary permanent residence." The non-migratory population excludes all members of eligible households who consider the house their permanent residence but have spent the majority of the six months prior to the most recent census living elsewhere for work or education.

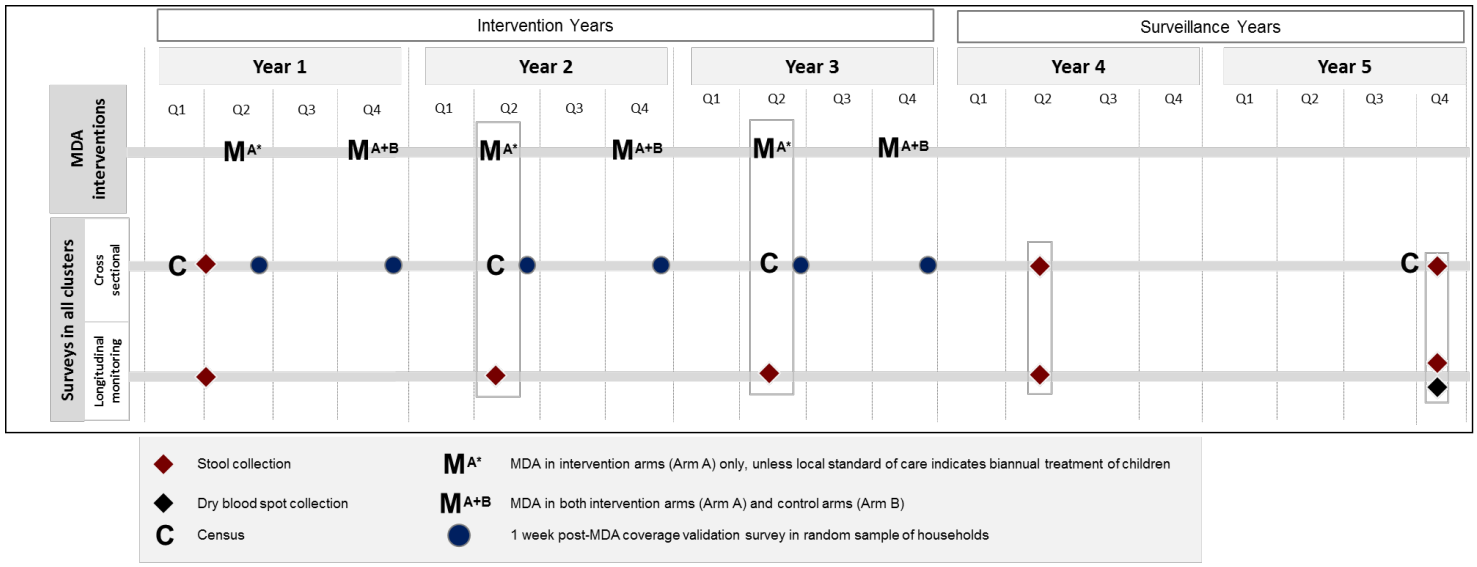


Figure 6: Study schematic of data and sample collection time points

Baseline (pre-MDA), post-MDA (6 months following the third round of MDA), and endline (24 months following the third round of MDA) **cross-sectional assessments** of STH prevalence and intensity will take place in all clusters via random sampling of pre-SAC, SAC, and adults. Cross-sectional assessment data will be used to inform the study's primary aims, measuring endline STH prevalence and determining whether or not transmission interruption has been achieved at a cluster-level. During cross-sectional surveys stool samples or rectal swabs (where stool samples cannot be provided) will be collected for qPCR analysis. Microscopic examination using Kato-Katz technique will be performed on a subset of whole stool samples during the second cross-sectional survey (6 months post-MDA), and during the final cross-sectional survey (24

months following the final round of MDA). Samples will be stored for subsequent analysis; including molecular identification of parasites, drug resistance, and enteric function. Each sample collected will be labeled with unique patient ID barcodes. Individuals cannot volunteer or electively choose to participate in the survey; samples will only be collected from the individuals who have been randomly selected. Study staff will make at least three attempts to reach each randomly selected individual in the cross-sectional survey.

All samples collected during the baseline cross-sectional survey will be stored for subsequent qPCR analysis. Samples collected 6 months following the final round of MDA will be analysed by qPCR and a random subset of 50-100 samples per cluster will be analysed using Kato-Katz. Samples collected at endline will be analysed by qPCR and a random subset of 75-100 samples per cluster collected at endline (24 months following the final round of MDA) will be analysed using Kato-Katz.

At baseline, a subset of 150 individuals will be invited to participate in **longitudinal monitoring cohorts** in each cluster for the duration of the trial**.** The longitudinal cohort will be selected at random from an age-stratified census (30 PSAC, 30 SAC, 90 adults). These individuals will provide stool samples annually for immediate analysis using Kato-Katz and whole stool samples (or rectal swabs where stool samples cannot be provided) will be stored for subsequent testing via qPCR. A series of at least three blood spots will also be taken from participants in these cohorts at the final round of follow-up (Year 5) for evaluation of antigen detection of infection and measurement of immune response to STH infection and clearance. And, from a subset of cohort members, saliva and urine will be collected for evaluation of novel saliva and urine based STH diagnostics detecting DNA, antigen or immunologic responses. Individuals who are part of the longitudinal monitoring cohorts will not contribute outcome data to the cross-sectional analysis. Data collected at interim timepoints in these cohorts will serve to assess rates of change in STH prevalence, quantify reinfection after a negative test where applicable, and to validate and adapt mathematical models. Study staff will make at least three attempts to reach each randomly selected individual in the longitudinal monitoring survey at each timepoint.

Between the cross-sectional survey and longitudinal monitoring, 650 samples will be collected per cluster at study baseline. These surveys will be administered at the same time in communities, in order to minimize the number of times that households are re-approached throughout the study. During cross-sectional and longitudinal surveys, all individuals in the intervention arm with STH infections identified using Kato-Katz will be treated during community-wide MDA. All pre-SAC and SAC in the control arm with identified infections will be treated during targeted MDA. WHO guidelines do not recommend preventive chemotherapy for adults; however, any adult (≥15 years of age) found to have moderate to heavy intensity infections by Kato-Katz will be provided treatment by study staff. Figure 6 outlines study data and sample collection timepoints.

To ensure high coverage and compliance, a random sample of 50 households in all clusters will be invited to participate in a **post-MDA coverage survey** within one week of receiving MDA, prior to any cluster-wide mop-up activities. All individuals in the selected household will be invited to participate in the coverage survey. This survey will be conducted in all clusters, in both study arms. The purpose of the survey is to collect critical information regarding validated treatment coverage. Treatment coverage is defined as the proportion of eligible individuals who ingested albendazole during MDA. The number of eligible individuals will be ascertained during each annual census. This will provide important information regarding the benefits of different delivery systems in maximizing treatment coverage. Individuals in randomly selected households will be asked if they participated in the MDA event and, if not, their reason for not participating. Questions in the survey will align with WHO-endorsed coverage survey for use by national programmes. In accordance with the same NTD coverage survey guidelines, the individuals who conduct the coverage survey in a given cluster will not be the same individuals who delivered the drugs [12].

Additionally, up to 250 urine samples per site will be collected from a subset of individuals in the post-MDA sentinel survey and stored for future measurement of albendazole metabolites to confirm compliance with MDA.

An annual **school facility survey** will also be conducted to assess the presence of water and sanitation facilities at schools in all clusters and to validate coverage of MDA delivered to SAC. At baseline, a comprehensive survey will be administered with confirmation of the facilities listed. Subsequent surveys will be designed as short updates to confirm the status of water and sanitation facilities, record any changes, and review and abstract data from school records regarding treatment coverage as an opportunity to validate post-MDA sentinel survey data.

4.5. Recruitment, eligibility, exclusion criteria, consent, and follow-up procedures

4.5.1 Treatment recruitment

In order to encourage high treatment coverage in the community-wide intervention arm, community sensitization activities will take place within one month of scheduled MDA events. Sensitization activities will be designed in close consultation and collaboration with the National NTD programme and in accordance with site norms and past MDA experiences. Where possible, MDA activities will be scheduled to accommodate seasonal and cultural activities that might influence MDA participation, such as holidays, migratory events, or crop harvesting.

4.5.2 Treatment eligibility

In intervention clusters, all individuals 24 months of age and older and not in their first trimester of pregnancy will be eligible for treatment with albendazole, in accordance with WHO guidelines. In targeted MDA clusters, all pre-SAC and SAC 24 months of age and older will be eligible for treatment.

4.5.3 Study eligibility and enrollment

The population eligible for sampling in the intervention and control arms include all individuals 12 months of age or older who are confirmed by study staff as residing within the study clusters.

During the cross-sectional surveys, participants will be approached and asked to provide fecal samples. If participants are unable or unwilling to produce whole stool, a self-administered rectal swab will be offered and instructions for fecal collection provided. Fecal collection kits will be collected within 24 hours of the sample being produced.

In the longitudinal monitoring cohort, participants will be enrolled in conjunction with the baseline cross-sectional surveys, prior to the first round of MDA. Fecal collection kits will be dispersed to each participant at the time of enrollment and annually thereafter, coinciding with planned MDA activities.

4.5.4 Treatment exclusion criteria

Populations excluded from treatment include children under 24 months of age and pregnant women in their first trimester.

4.5.5 Study exclusion criteria

Survey (cross-sectional and longitudinal) exclusion criteria include: individuals who cannot be confirmed as living in the study clusters, who do not provide informed consent, or for children 7 years and above who refuse assent. Additionally, participants will be excluded from the longitudinal monitoring cohorts if they report that they plan to move out of the study area within the study period.

4.5.6 Consent
The head of household or other adult member of the household will give written informed consent for participation in the census, or will provide a thumbprint if unable to write. Participants in each round of cross-sectional sampling will give written informed consent for their participation or will provide a thumbprint if unable to write. Participants in the longitudinal monitoring cohorts will provide consent for repeated sample collection and repeated rounds of data collection at enrollment. If a child is selected, parents or guardians will give written informed consent on behalf of the child and children aged 7 and older will provide assent.

4.5.7 Follow-up
Up to three attempts will be made to reach each household or individual for the purposes of the censuses and the cross-sectional prevalence assessments, respectively. Individuals participating in the longitudinal cohort will be asked to provide contact information and every effort will be made to ensure high rates of retention in the cohort. Contact will be attempted by phone or household visits if individuals are missed during routine follow up.

4.6 Adverse event monitoring
Adverse events will be passively monitored for all participants in the study. Reports of severe adverse events, including all deaths and any events classified as “definitely”, “probably” or “possibly” related to the study drug according to WHO criteria will be reported to the Data Safety Monitoring Committee (DSMC) and to all appropriate regulatory bodies.

4.7 Costs data collection
The DeWorm3 project will conduct cost-effectiveness evaluations following three years of MDA and then again following two years of surveillance. Financial and non-financial economic costs will be collected using standardized cost collection forms over the duration of the study. The perspectives used will be individual (i.e. participant), societal, and health systems-level (i.e. government). We will utilize WHO guidelines [15] and an ingredients-based approach to quantify the resources and associated unit costs required to deliver twice-yearly community-wide MDA and annual school-based MDA. We will organize the costs according to standard expenditure categories, including: personnel (salaries), volunteer community drug distributor time, supplies including drugs, equipment, services, space and overhead. We will also attempt to measure the time demanded by community-members to participate in different aspects of the intervention. Full incremental costs will be derived, measured in the local currency, and converted to US dollars.

4.8 Data management
The DeWorm3 coordinating team will ensure harmonization of data collection and data management processes across all sites. All sites will collect information on a core set of variables with standard definitions using digital data collection methods or, when necessary, paper-based methods. Each site will be responsible for data entry and initial cleaning of the data, including running range and consistency checks as well as periodic reviews of distributions and identification of outliers. Each study site will resolve any inconsistencies within their database in consultation with their field data collection team and with field verification if needed. DeWorm3 will provide a set of range and consistency checks that must be applied to these variables.

De-identified data will be shared with the DeWorm3 coordinating center at the Natural History Museum, London, UK and with the clinical trials support unit at the University of Washington, Seattle, USA and a harmonised central database will be maintained. The DeWorm3 data manager will run another set of range and consistency checks including checking of consistency of data quality across sites. Any inconsistencies or queries will be communicated to the study site, which

will be expected to check and address the list of queries and resubmit data. Cleaned de-identified data from all sites will be pooled and stored in a secure database at the Natural History Museum.

5. STUDY MANAGEMENT

5.1 Coordination

The Principal Investigator (PI) of the Project is assisted by two Deputy Chief Operating Officers - a Director of Finance and Operations and a Director of Science and Policy and one or more Project Managers based at the Natural History Museum in London. These Project leads will work closely with a support team internal to the Project, and are responsible for the conduct and output of the project, and for communication with the Bill & Melinda Gates Foundation. Each site will appoint a PI responsible for ensuring the agreed protocol is comprehensively and precisely implemented at the respective site according to the agreed time frame. The site PI will represent the interests of the respective site partners in communication with the Project leads and support team of the Project. The Project leads will work closely with the site PIs throughout the Project to ensure the interests, views and priorities of all sites are taken into consideration. The DeWorm3 coordinating team at the Natural History Museum is supported by three units;

- Clinical Trial and Implementation Science Support Unit, University of Washington, USA
- Modeling and Trial Simulation Support Unit, Imperial College London
- Economic Analysis Support Unit, Swiss Tropical and Public Health Institute

These support units may receive copies of data from the trial sites to conduct associated modeling and data quality assurance activities.

5.2 Advisory Groups

A **community advisory board (CAB)** will be established at each study site and designed in accordance with site preferences in order to guide and inform appropriate implementation of all study procedures and engagement with community members.

A Programme Partnership and Advisory Committee (PPAC) consisting of funding partners and leadership at the Natural History Museum as the DeWorm3 primary grantee institution will provide support to Project leadership on major Project decisions. A Strategic Advisory Committee will provide external advice on overall strategic decisions and will support advocacy for the Project internationally. Subject matter experts will form a Technical Advisory Board to provide technical expertise on specific aspects of the Project. The Project leadership will be responsible for inviting the members of the respective committees, and site PIs will suggest relevant local experts for consideration as Advisory Group members.

5.3. Data Safety Monitoring Board Review

Per standard practice, MDA programs distributing albendazole conduct passive adverse event (AE) monitoring and reporting. In addition, DeWorm3 will form a Data Safety and Monitoring Committee (DSMC) prior to study initiation to monitor severe adverse events (SAEs) and to evaluate the statistical analysis plan. The DSMC membership will include expertise in clinical trials, statistics, STH infections, and ethics in resource limited settings. SAEs will be reported to the DSMC and all relevant IRBs within 48 hours of the study team becoming aware of such events and will be summarized and reported to study investigators and relevant institutional review boards on a twice-yearly basis.

5.4 Quality Assurance

Field supervisors will check all forms completed by field workers before data entry or upon data upload. Data cleaning quality assurance will be performed using standard operating procedures, and consistency and range checks both at the study sites and centrally at the Natural History

Museum and/or the University of Washington. Data quality checks will also be applied on a quarterly basis and feedback will be provided to the PIs and study managers in all sites. PIs will provide brief monthly progress reports during the entire study period and will participate in regular telephone conferences with DeWorm3 staff. The monthly progress reports will include details regarding preparations for future MDA, MDA treatment coverage, drug supply chain management, workforce training and management, and interim laboratory findings.

6. LABORATORY PROCEDURES AND SPECIMEN COLLECTION AND STORAGE

6.1 Specimen collection

Consenting participants will be provided plastic containers for collection of a fresh morning/first stool sample, to be submitted within 24 hours of the time it is collected. Stool will be collected from households or, if necessary, delivered to a central location, depending on participant preference. Rectal swabs may be collected in circumstances where individuals are not able to provide stool samples. Instructions for rectal swab self-collection (or caregiver collection for children) will be provided at the time of stool collection.

Participants in the longitudinal cohort will have finger prick blood samples taken at the final round of follow-up to contribute whole blood, stored as dried blood spots, and a subsample will provide urine and saliva samples as well.

6.2 Specimen analysis

Stool samples will be divided into aliquots for microscopic examination, DNA extraction, and storage. Each unit will be labeled with barcoded patient ID codes.

Aliquots containing a minimum of 2g of stool and/or rectal swabs collected during the cross sectional surveys and longitudinal monitoring surveys will be stored for parasite DNA extraction, enteric pathogen detection, enteric function and qPCR analysis, in order to understand factors associated with helminth clearance or persistance. Stool samples or extracted DNA may be pooled to improve efficiency of testing.

All sites will ship duplicates of 10% of all study samples to the central laboratory facility at Christian Medical College (CMC) in Vellore, India for quality assurance and control. CMC will ship 10% of their samples collected to Smith College, Massachusetts, USA for quality assurance and control. Where standardization of assays is required or where technical limitations affect the ability to complete testing in a timely manner using methods described by Williams et al. [13]), stool samples or extracted DNA from stool will be shipped to central laboratory facilities at the Natural History Museum, the National Institutes of Health, CMC, the University of Washington or Smith College.

A subset of samples will be analysed in duplicate by microscopy (Kato-Katz) to facilitate rapid assessment of cluster-level prevalence, to identify prevalence trajectories, and to provide treatment to those still in need post-MDA. Aliquots of stool will be analysed within 24 hours of collection and within 30 minutes of sample preparation, to prevent the degradation of hookworm eggs. Two slides will be prepared and analysed per specimen. Ten percent of slides will be submitted for quality control checks to be performed by laboratory supervisors. Laboratory personnel will be blinded to treatment allocation during assessment.

At least three dried blood spots will be collected on filter paper and stored using appropriate application of stacking, desiccant packets, and humidity cards [16].

Urine will be collected and frozen from a subset of participants during coverage validation surveys. These specimens will be sent to Laboratorio de Farmacología CIVETAN (Argentina) or the Natural History Museum, London and analysed using HPLC or rapid chromatographic immunoassays for the detection of albendazole metabolites.

6.3 Specimen storage

Stool samples intended for DNA extraction and stored for subsequent analyses will be cryopreserved at -80°C. Parasite material identified within stool samples will be isolated and stored at each respective site for future molecular testing for drug resistance and parasite diversity analysis. Parasite material will be shipped to the central parasite repository at the Natural History Museum, London.

Dry blood spot cards will be transported to refrigerators within 7–10 days of collection and stored at -20ºC [16]. Where feasible, haemoglobin measurements made be conducted using whole blood on site. Along with stored stool and rectal swabs, one of the three cards will be held in DeWorm3 processing areas at the Natural History Museum in London. Future applications to relevant ethical review committees may be made for the purposes of other unspecified analyses.

7. STATISTICAL ANALYSIS PLAN

All analyses will be conducted separately for each site, unless otherwise specified.

Primary objectives

The effect of the primary exposure on endline infection with the predominant STH species driving transmission in each site (Objective 2.1.1) will be analysed using generalized estimating equations with binomial family and exchangable correlation matrix. The primary exposure variable will be randomisation arm, while the primary outcome will be individual-level STH infection status (positive or negative for the dominant species driving transmission species by qPCR). Models may be adjusted for baseline cluster-level STH prevalence and urban/rural designation, socioeconomic status (SES), water sanitation and hygiene (WASH) access, age, gender of the participant, household size, or other relevant variables as appropriate. In the primary analysis, these data will be analysed separately by site, while a secondary analysis will pool all data and test for effect modification using an interaction term between study site and randomisation arm.

Transmission interruption in a cluster (Objective 2.1.2) will be defined as achieving a cluster prevalence $\leq$2% of the dominant STH species driving transmission at each site by qPCR 24 months after the final round of MDA. The effect of the primary exposure on the proportion of clusters in which transmission is successfully interrupted will be calculated by comparing the proportion of clusters in each arm in which transmission is successfully interrupted using a chi-squared test.

Secondary objectives

The effect of randomisation arm on endline infection with each individual species of STH (2.2.1), on the proportion of clusters interrupting transmission of each individual species (2.2.2), on endline infection with any STH species among children (2.2.2) and on shedding of fertilized *Ascaris* eggs (2.2.3) will be analysed using generalized estimating equations with binomial family and exchangable correlation matrix or chi-squared tests, as detailed above. Models may be adjusted for baseline cluster-level STH prevalence and urban/rural designation, SES, WASH access, age, gender of the participant, household size, or other relevant variables as appropriate.

Secondary analyses

The influence of MDA strategy on treatment coverage of Pre-SAC and SAC will be analysed using self-reported coverage (defined as receiving and taking albendazole during the last round of MDA) from coverage validation surveys in each arm. The effect of randomisation arm on self-reported coverage will be analysed using generalized estimating equations with binomial family and exchangable correlation matrix and may be adjusted for child's age, gender, current school attendance, and study year or other relevant variables as appropriate.

Cluster-level correlates of STH transmission interruption (as defined by reaching a cluster prevalence of any STH ≤2% 24 months after stopping MDA) will be assessed using logistic regression. Key correlates of breaking the transmission of STH will include baseline prevalence and species-specific distribution of STH infection, age distribution, population migration levels, treatment coverage and compliance, rates of open defecation and population density.

The incremental cost-effectiveness ratios (ICERs) of community-wide MDA compared to the standard of care (targeted MDA) will be measured using outcome metrics such as the cost per person treated, the cost per percent reduction from baseline to endline prevalence, and the cost per incident case avoided as determined through mathematical models informed by cluster-level prevalence and intensity data. We will utilize both a short-term time horizon of the study duration as well as longer-term time horizons with extrapolations of 5, 10, and 15 years. Costs will be discounted at 3% per year and sources of uncertainty will be explored in univariate and probabilistic sensitivity analyses [14, 15].

8. SAMPLE SIZE ESTIMATES

In each country, study areas will correspond to an approximate minimum population of 80,000 to 100,000 people. The study site will be divided into 40 total clusters, 20 in the intervention arm (biannual community-wide MDA) and 20 in the control arm (targeted MDA). Cluster sizes will vary according to administrative and / or geographic barriers but should range between a minimum size of 1,650 individuals and a maximum of 4,000 individuals. The minimum cluster size was selected to reduce the probability of repeatedly sampling the same individuals at all three time points to <10%, allowing for 10% refusal rate and the exclusion of the longitudinal monitoring cohort from the sampled population. Randomization of clusters to community-wide vs. school-age targeted MDA will be performed within each site. Restricted randomisation may be performed to ensure balance in baseline characteristics across clusters.

Cross-sectional prevalence surveys at baseline and 6 months post-MDA, and the primary outcome assessment of prevalence at 24 months post-MDA, will be conducted on a random sample of 500 individuals per cluster while the endline cross-sectional survey will sample 1,000 people per cluster in order to precisely assess prevalence on a cluster-by-cluster basis.

8.1 Sample size calculations for Primary Objectives

Primary Objective 2.1.1

To compare the prevalence of the predominant STH species driving transmission at a particular study site (*most frequently A. duodenale* or *N. americanus)* measured by quantitative PCR 24 months after stopping MDA between clusters randomised to receive twice-yearly community-wide MDA versus clusters randomised to receive standard of care pre-SAC and SAC targeted MDA.

Power calculations for a range of scenarios were conducted. The following formula outlined by Hayes and Moulton [16]:

$$c = 1 + (Z_{\propto/2} + Z_{\beta})^2 \frac{[\pi_0(1-\pi_0) + \pi_1(1-\pi_1)][1+(m-1)\rho]}{m(\pi_0 - \pi_1)^2}$$

was used to calculate the prevalence in the control clusters (π_0) that would enable detection of a difference between arms with 80% power, given an endline prevalence in the intervention clusters (π_1) of 2% and a range of assumptions of intracluster correlation coefficient ($\rho = 0.003$ to $\rho = 0.005$), number of clusters per arm ($c = 15$ to $c = 20$) and number of people sampled per cluster ($m = 500$ or $m = 1{,}000$), assuming α=0.05. The detectable alternative π_0 ranged from 3% to 3.5%.

Power to detect differences in final prevalence between arms given plausible values for π_0, ranging from 7% to 10% (comparable to pre-intervention prevalences in each of the study sites), was then estimated using simulations conducted in R. Each simulation assumed 1,000 individuals per cluster and a binomial distribution of STH prevalence with a mean of 2% in the intervention clusters and π_0 in the targeted arm, a range of ICC and α=0.05; 10,000 repetitions were run for each scenario. Power was ≥98% for all scenarios simulated.

Primary objective 2.1.2

To determine whether the transmission of the predominant STH species driving transmission at a particular study site (*most frequently A. duodenale* or *N. americanus)* can be interrupted using MDA with albendazole, defined as reaching a combined point prevalence of ≤2% of that species measured by quantitative PCR 24 months after stopping MDA in clusters receiving either twice-yearly community-wide MDA or standard of care pre-SAC and SAC targeted MDA.

Power to detect transmission interruption and differences by arm was also estimated using simulation in R. The threshold for transmission interruption was set at 2.0% prevalence, and a cluster was considered to have interrupted transmission if prevalence could be declared to be below the threshold with 95% confidence, using a one-sided binomial test. Simulations estimated power to a) declare elimination in 50% of intervention clusters, which was selected as the minimum proportion of intervention clusters that would need to achieve elimination in order for the intervention to be recommended for scaleup, and b) detect a difference in the proportion of clusters in which transmission was interrupted by arm.

Simulations assumed 20 clusters per arm, 500 or 1,000 individuals per cluster (m), and a binomial distribution of STH prevalence with a mean of 7% in the targeted arm (π_0) and π_1 in the intervention arm, a range of ICC ($\rho = 0.003$ or $\rho = 0.005$), and α=0.05; 10,000 repetitions were run for each scenario.

a) The power to declare elimination in 50% of the intervention clusters varies with the mean endline prevalence, the ICC, and the number sampled per cluster as shown in Figure 7.

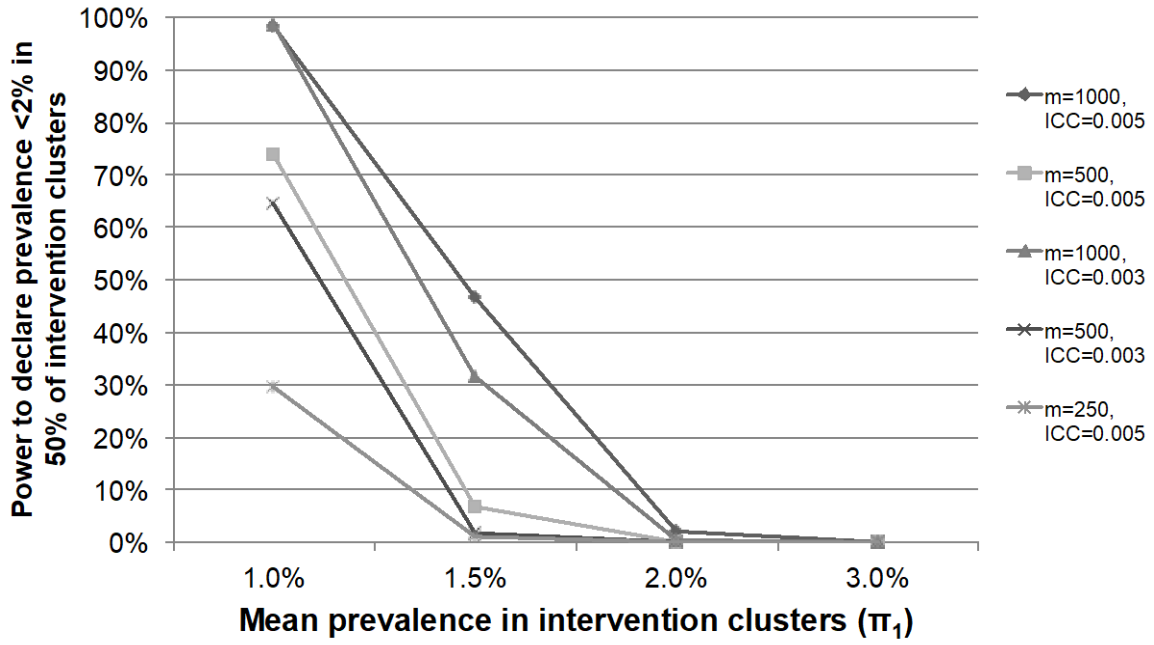


Figure 7: Power to declare elimination in 50% of intervention clusters (Primary Objective 2.1.2) by intracluster correlation (ICC) and number of people estimated per cluster(m) estimated by simulation.

b) For a fixed mean endline prevalence of 7% in the targeted arm (π_0), power to detect a difference in the proportion of clusters eliminated varies by number of individuals sampled per cluster, ICC and endline prevalence in the intervention arm (π_1), as shown in Figure 8.

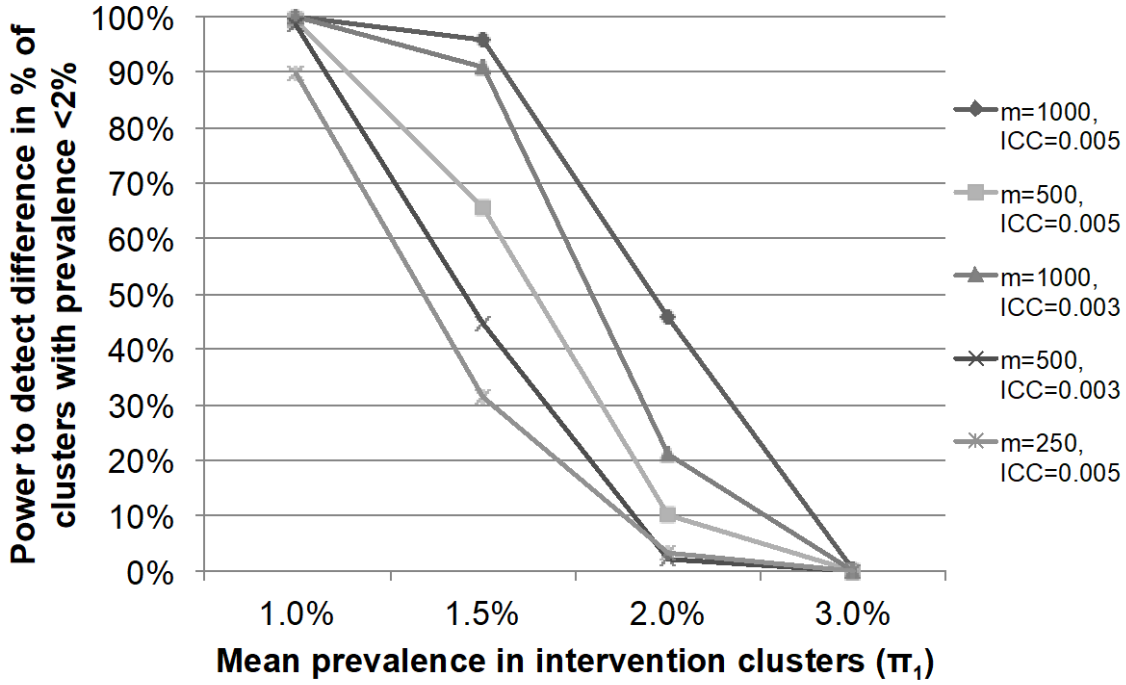


Figure 8: Power to detect a difference in the proportion of clusters achieving transmission interruption (Primary Objective 2.1.2) assuming 7% mean prevalence in the targeted

clusters (π_0), by mean endline prevalence in the intervention clusters (π_1), intracluster correlation (ICC) and number of people measured per cluster (m), estimated by simulation.

These power calculations demonstrate that with 20 clusters per arm, measuring STH infection among 500-1,000 people per cluster at endline will provide adequate power for the primary objectives in most scenarios. Sample size estimates may be refined using data on intra-cluster correlation and baseline prevalence at each site collected during the study.

Prevalence estimates by Kato-Katz

Stool samples collected from longitudinal monitoring cohorts annually will be analysed with Kato-Katz techniques as well as being stored for analysis by qPCR.

Assuming 150 individuals sampled per cluster, 20 clusters per arm, and using Kato-Katz sensitivity of 0.65 and specificity of 0.94 [17], to estimate true prevalence; using Blaker's CI for imperfect diagnostic tests [18] to estimate 95% CI by cluster; and adjusting for design effect with ICC=0.003 to estimate 95% CI by arm, the precision of prevalence estimates by true prevalence are summarized in Table 1.

Table 2. Precision of prevalence estimates by Kato-Katz.

Observed prevalence by Kato-Katz	True prevalence	95% CI by cluster	95% CI by arm (across 20 clusters)
14.9%	**15%**	6.1-25.7%	12.5-17.6%
11.9%	**10%**	2.0-19.4%	7.7-12.5%
9.0%	**5%**	0.0-13.6%	3.0-7.2%
7.2%	**2%**	0.0-9.8%	0.2-3.9%

The prevalence estimate from the first assessment of the longitudinal monitoring cohort will provide baseline prevalence information for clusters prior to randomization. At endline, however, the longitudinal monitoring cohort's testing and treatment history may differ from that of the general population of the clusters. For this reason, a subset of samples collected as part of the cross-sectional sample after the final round of MDA will also be analysed using Kato-Katz.

Coverage estimation by urine albendazole

The primary assessment of coverage will be by self-report. However, a small number of participants in the coverage survey approached between 24-72 hours after MDA will be asked to provide a urine sample to be assessed for albendazole metabolites. Results of the urine analysis will be used to validate self-reported true coverage of MDA, i.e. the proportion of individuals who both received and swallowed pills during MDA. The assay is still being validated and sensitivity / specificity information to be determined. Precision of estimates of true coverage across a range of assay sensitivities, with an assumed specificity of 99%, are shown in Table 3.

Table 3. Precision of estimates of true MDA coverage by urine albendazole.

		95% CI of coverage assessment	
True coverage	**Sensitivity**	**n=100**	**n=150**
95%	95%	87-100%	89-99%

	85%	84-100%	86-100%
	75%	82-100%	84-100%
90%	95%	81-96%	83-95%
	85%	79-99%	81-97%
	75%	76-100%	79-99%
80%	95%	70-88%	72-87%
	85%	68-90%	70-88%
	75%	66-93%	69-90%

Assuming true coverage of 90% or higher and a minimum sensitivity of 85%, assessment of 150 individuals per site will provide adequate power to rule out coverage <80%.

9. POTENTIAL RISKS AND CHALLENGES

A major risk to the DeWorm3 Project is low treatment coverage and/or adherence. Low coverage could be a result of failure to access sufficient proportions of the population, large rates of population migration, or suboptimal adherence (i.e. participants do not consume the drugs they are given). Other potential challenges include political instability, the presence of transmission hotspots in areas with low coverage, and unprogrammed MDA activities in either trial arm. In addition, while STH resistance to benzimidazole anthelmintics has not been documented in humans, it is known to occur in animals and could pose a risk to the success of transmission interruption.

10. TIMELINE

	July-Dec 2016	Jan–June 2017	July-Dec 2017	Jan–June 2018	July-Dec 2018	Jan–June 2019	July-Dec 2019	Jan–June 2020	July-Dec 2020	Jan–June 2021	July-Dec 2021	Jan–June 2022	July-Dec 2022	Jan–June 2023	July-Dec 2023
IRB approvals															
CRF development															
Database development															
SOP development															
Baseline census															
Cluster randomisation															
MDA															
Interim Analysis to feed models															
Surveillance															
Data cleaning															
Data analysis															
Manuscript prep and publications															

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