क्षेत्रीय आयुर्विज्ञान संस्थान, इंफाल: मणिपुर
REGIONAL INSTITUTE OF MEDICAL SCIENCES, IMPHAL, MANIPUR
(स्वास्थ्य और परिवार कल्याण मंत्रालय,भारत सरकार के अंतर्गत एक स्वायत्त संस्थान)
(An Autonomous Institute under the Ministry of Health & Family Welfare, Govt. of India

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# CIRCULAR

Imphal, the 15th December, 2025

**No. 266/RIMS-MRU/2025:** It is to notify that, the **8th Research Masterclasses 2025**, of the Department of Health Research, Ministry of Health and Family Welfare, Government of India, will be conducted virtually, **on 19th December, 2025 (Friday).**

2. All the faculties (RIMS, Dental College, and College of Nursing), members of EC, LRAC of MRU, Principal Investigators undertaking MRU funding projects (including under process projects) and residents are invited to attend the session at **Banting Hall, RIMS, Imphal**.

**Date**: 19.12.2025 (Friday)
**Time**: 3:00 PM onwards
**Venue: Banting Hall, RIMS, Imphal (DESIGNATED SITE FOR PARTICIPATION FOR RIMS)**
**Event name:** Research Masterclass under DHR-ICMR Research Grand Rounds

3. As per the directives issued by the DHR, **maximum participation** from our institute is highly encouraged. MRU is submitting the attendance sheet to the DHR after the session concludes.

15.12.2025
**Prof. T. Jeetenkumar Singh,**
Nodal Officer,
Multi-Disciplinary Research Unit,
RIMS, Imphal

Copy to:

1. The P.S. to Director, RIMS, for kind information of Director
2. The P.A. to Medical Superintendent, RIMSH, for kind information
3. The Dean (Academic), RIMS, for kind information & permission to utilize the facilities at Banting Hall.
4. The Principal, Dental College, RIMS
5. The Principal, College of Nursing, RIMS — with a request for ensuring maximum participation.
6. The Head of Department, RIMS, Imphal ……………………………………
7. The Chairperson/Co-Chairperson/Member, LRAC, MRU, RIMS……………………………………
8. The Member, EC, MRU, RIMS, Imphal……………………………………
9. The Principal Investigator, RIMS ……………………………………
10. The IT Cell, RIMS – with a request for uploading the notice in the website & technical support on **19.12.25**
11. Asst. Engineer (Elect. /Civil), RIMS - with a request for ensuring uninterrupted power supply & optimum AC functioning.
12. The Care Taker, Banting Hall, RIMS, Imphal- for proper upkeep of the venue & the accompanying facilities.
13. Guard file.

No. R.11016/22/2024-HR
Government of India
Ministry of Health & Family Welfare
Department of Health Research

IRCS Building, 2nd Floor,
Red Cross Road
New Delhi – 110 001
12.12.2025

To
The Dean/ Principal/ Director of Medical Colleges/ Institutes

Subject: Request to attend Research Masterclasses, 2025 for MRU network– reg.

Sir/Madam

DHR-ICMR has initiated a dedicated platform to conduct Research Grand Rounds to strengthen the National research ecosystem through sustained collaboration and knowledge exchange. The objectives of the Research Grand Rounds are as follows:

I. To deliberate on research methodologies, analytical tools, and emerging scientific approaches
II. To strengthen the methodological understanding amongst researchers needed to implement different kinds of research.
III. To foster collaboration and connectivity across research institutions

2. These Research Grand Rounds will be organized as monthly webinars entitled 'Research Masterclass' proposed around the last Friday of each month. The speakers for these Research Masterclasses will be eminent research scientists in the country who will be discussing their original research work in details from methodological point of view.

3. The next Research Masterclass is scheduled for **19.12.2025 (Friday)** at **3:00 PM**. The invited speaker is **Dr. Sitara S.R. Ajjampur,** Professor of Microbiology, Division of Gastrointestinal Sciences, Christian Medical College, Vellore, Tamil Nadu. The research paper to be discussed during the research masterclass is enclosed. The link for the research masterclass will be shared shortly.

4. Accordingly, it is requested to kindly disseminate the information in your institution and ensure maximum participation in Research Masterclass. Also, it is requested from your institute to share at least two questions related to research paper attached on the following email: **dhr-mru@gov.in** latest by 16.12.2025. These questions will be discussed with the speaker during masterclass.

Yours faithfully

(Dharkat R. Luikang)
Deputy Secretary to the Govt. of India

Copy to: The Nodal Officer of Multi-Disciplinary Research Units (MRUs)

# Feasibility of interrupting the transmission of soil-transmitted helminths: the DeWorm3 community cluster-randomised controlled trial in Benin, India, and Malawi

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## Summary

**Background** Soil-transmitted helminths are targeted for elimination as a public health problem. This study assessed whether, with high coverage, community-wide mass drug administration (MDA) could lead to transmission interruption.

**Methods** DeWorm3 is an open-label, community cluster-randomised controlled trial in Benin, India, and Malawi. In each country, a single governmental administrative unit (population ≥80 000 individuals) with soil-transmitted helminth endemicity and participation in at least five rounds of community-wide MDA for lymphatic filariasis, was divided into 40 clusters (population ≥1650 individuals), which were randomly assigned (1:1) to community-wide MDA versus school-based deworming. Laboratory personnel were masked to exposure status and all investigators were masked to post-baseline outcome data until unmasking. In all clusters, preschool-aged and school-aged children received school-based deworming as per national guidelines for 3 years. In intervention clusters, door-to-door community-wide MDA (a single oral dose of 400 mg albendazole) was delivered to all eligible individuals biannually by community drug distributors for 3 years. All individuals aged 12 months and older in India and Benin and aged 24 months and older in Malawi were eligible for treatment, except women in the first trimester of pregnancy, those with adverse reactions to benzimidazoles, those who were acutely ill or intoxicated, or those reporting treatment within the previous 2 weeks. The co-primary outcomes were individual-level prevalence and cluster-level transmission interruption (ie, weighted prevalence of predominant species of ≤2%) of the predominant soil-transmitted helminth species, assessed by quantitative PCR (qPCR) 24 months after the last round of MDA. The analysis set contained a subset of randomly selected participants per cluster who enrolled in the endline assessment, provided a stool sample, and had a qPCR result. All individuals who received treatment were eligible for inclusion in the safety population. This trial is registered with ClinicalTrials.gov (NCT03014167), and is active but not recruiting.

**Findings** Between Oct 10, 2017, and Feb 17, 2023, 120 clusters (40 clusters per country, comprising 357 716 individuals) were randomly assigned, 60 to community-wide MDA and 60 to school-based deworming. 184 030 (51·4%) individuals in the clusters at baseline were female, 173 663 (48·5%) were male, and 23 (<0·1%) were other. The analysis set consisted of 58 827 individuals in the control group and 58 554 in the intervention group 24 months after the cessation of all deworming, *Necator americanus* prevalence (the predominant species at all sites) in the community-wide MDA group was lower than the school-based deworming group in Benin (adjusted prevalence ratio [aPR] 0·44 [95% CI 0·34–0·58]), India (0·41 [0·32–0·52]), and Malawi (0·40 [0·34–0·46]). Transmission interruption was achieved for *N americanus* in 11 (55%) of 20 intervention clusters versus six (30%) of 20 control clusters in Benin (p=0·20), in one (5%) intervention cluster versus no control clusters in India (p=1·00), and in no clusters in either group in Malawi (p=1·00). 984 adverse events were reported among 487 participants over the study, of which 32 among 13 participants resulted in hospitalisation and were classified as serious adverse events (three of which were related to study procedures).

**Interpretation** Soil-transmitted helminth transmission interruption might be possible in focal geographies but does not appear to be programmatically feasible within the evaluated timeframe. Community-wide MDA should be considered as an alternative strategy to school-based deworming programmes to improve equity and outcomes in helminth-endemic areas.

**Funding** The Gates Foundation.



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**This online publication has been corrected. The corrected version first appeared at thelancet.com on September 18, 2025**

See **Comment** page 420

*Members are listed in the appendix 1 (p 1)

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See Online for appendix 1

## Research in context

### Evidence before this study

The current WHO target for soil-transmitted helminths is elimination as a public health problem, delivered through annual and biannual school-based deworming of at-risk groups, including preschool-aged and school-aged children. Previously developed mathematical models indicate that interruption of soil-transmitted helminth transmission might be feasible with intensified deworming. A 2024 systematic review and meta-analysis by Ugwu and colleagues provides further evidence that community-wide deworming interventions lead to greater reductions in soil-transmitted helminth prevalence than school-based deworming. It included studies that overlapped with the timeframe of the DeWorm3 study, but were limited by smaller sample sizes, shorter duration, and restricted geographical representativeness.

### Added value of this study

DeWorm3 was done in three different countries in Africa and Asia to ensure geographical representativeness. The overall population included in the study was large and the sample size of individuals who received the intervention and were evaluated for study outcomes allowed for robust estimation of the study objectives. In addition, the rigorous design of the study—as a cluster-randomised trial—allowed for assessment of the contribution of each intervention while limiting potential bias. Although transmission interruption was not achieved across all clusters, community-wide MDA led to substantially greater reductions in the prevalence of the predominant species of soil-transmitted helminth at all sites compared with school-based deworming.To our knowledge, this is the first large randomised controlled trial to assess the feasibility of interrupting the transmission of soil-transmitted helminths and is by far the largest trial to evaluate the impact of community-wide MDA.

### Implications of all the available evidence

Due to the reductions in soil-transmitted helminth prevalence shown (driven by the predominant species *Necator americanus*) both in our trial and in others, community-wide MDA should be considered as a strategy to achieve the stated WHO goal of elimination as a public health problem, particularly in areas with high baseline prevalence (as lower prevalence reduced morbidity at the population level). However, transmission interruption using community-wide MDA was not achieved at programmatically relevant scale within the 3-year timeframe of the study, which was the first trial to look at this endpoint. The data from this large, rigorous, and geographically representative study adds to the previous evidence, to directly inform policy and programmes in soil-transmitted helminth-endemic areas. The study adds evidence to support policy development as countries transition donor financing and assess the role of community-wide MDA in achieving global targets for addressing neglected tropical diseases. Our data can also be used to reparametrise existing models to better inform prevalence thresholds and treatment duration and frequency to inform future efforts to interrupt soil-transmitted helminths transmission. In addition, our trial showed the feasibility of using high-throughput molecular assays as an alternative to existing coproscopic methods to provide robust estimates of soil-transmitted helminth prevalence and intensity. Given the improved diagnostic performance of quantitative PCR over traditional microscopy, quantitative PCR should be used in research and programmatic settings in which prevalence and intensity are low to improve estimates.

## Introduction

WHO recognises 21 conditions as neglected tropical diseases (NTDs) that disproportionately affect populations living in poverty, half of which are targeted for either elimination or eradication under the existing WHO roadmap for NTDs.[1,2] Among these diseases, soil-transmitted helminths, including roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), and hookworms (*Necator americanus* and *Ancylostoma duodenale*) are not currently targeted for elimination. Instead, the roadmap targets reduced morbidity (elimination as a public health problem, defined as <2% prevalence of moderate and heavy intensity infections) in 96% of endemic countries by 2030.[3]

Soil-transmitted helminths are among the most prevalent human infections, with an estimated 1·15 billion individuals in 87 low-income and middle-income countries considered at risk in 2022.[4] These infections are associated with substantial disease burden, accounting for 1·9 million disability-adjusted life-years annually, largely as a result of anaemia, malnutrition, and micronutrient deficiencies.[5] Soil-transmitted helminths have been associated with reduced school attendance, poor school performance, lower income earning potential, and reduced societal economic development.[6] WHO currently recommends targeting improved access to safe water, sanitation, and hygiene (WASH) facilities, behaviour change, and preventive chemotherapy with anthelmintic agents (largely benzimidazoles) in populations at highest risk of morbidity (preschool-aged [2–4 years] and school-aged [5–15 years] children, women of reproductive age, and groups at high occupational risk [eg, miners and agricultural workers]).[3,7] Our study defined preschool-aged children as ages 1–4 years.

Current national soil-transmitted helminth programmes are supported by a robust drug donation programme, through which more than 337 million doses were provided in 2023 alone.[8] Although reported coverage of preschool-aged and school-aged children often approaches or exceeds national targets, coverage for other populations at risk, including women of reproductive age, is considerably lower and often ineffectively monitored. In addition, there is strong evidence that children are rapidly re-infected after treatment due to exposure to untreated individuals in the community and ongoing environmental contamination (of soil, water, or food with soil-transmitted helminth larvae or eggs).[9] Given the dioecious nature of these parasites and the inability for most soil-transmitted helminth species to complete their lifecycle within the human host, there is an unstable equilibrium of infection intensity and prevalence, below which transmission cannot be sustained. As a result, as infection numbers in a human population fall, the likelihood that transmission can be sustained within the population decreases.[10] Several modelling studies have suggested that biannual (every 6 months) community-wide mass drug administration (MDA), in which all eligible community members are treated, could achieve interruption of transmission of soil-transmitted helminths within 3 years, assuming high coverage of the entire population over each campaign round.[11,12] These models suggest that more than 90% of clusters in which the true prevalence is reduced to less than 2% do not bounce back to the pre-MDA endemic state, suggesting that transmission will be eliminated in most of these settings.[13] In addition, a number of previous studies have shown greater reductions in prevalence using community-wide MDA compared with school-based targeted deworming.[14] High-quality trial data demonstrating the feasibility of soil-transmitted helminth elimination are needed to support any potential change in guidelines or policy.

We tested the feasibility of interrupting soil-transmitted helminth transmission at study sites in Benin, India, and Malawi in a large community-wide, cluster-randomised trial. We identified areas where multiple previous rounds of community-wide MDA using albendazole had already been delivered by previous lymphatic filariasis programmes. We hypothesised that prevalence might already be reduced in these areas and six additional rounds at high coverage and targeting all eligible individuals would be sufficient to reduce infection prevalence below the threshold necessary to sustain transmission. We aimed to compare the prevalence of the predominant soil-transmitted helminth species and to establish whether transmission of the predominant species could be interrupted using community-wide MDA versus school-based deworming.

## Methods

### Study design and participants

DeWorm3 was a community-wide, cluster-randomised controlled trial comparing biannual community-wide MDA to standard-of-care school-based deworming conducted in Benin, India, and Malawi between Oct 10, 2017, and Feb 17, 2023. DeWorm3 was reviewed and approved by the National Ethics Committee for Health Research (002–2017/CNERS-MS) of the Ministry of Health in Benin, the London School of Hygiene & Tropical Medicine (12013), the College of Medicine Research Ethics Committee (P.04/17/2161) in Malawi, and the Christian Medical College Institutional Review Board in Vellore, India (10392). It was also approved by the Human Subjects Division at the University of Washington, WA, USA (STUDY00000180). The trial was registered at ClinicalTrials.gov (NCT03014167). Community advisory boards were established at each study site to guide and inform the appropriate implementation of study procedures and facilitate effective community engagement. A summary of the study methods are provided here and a detailed description of the methods has been published previously (appendix 2).[15] In each country, predefined geographical areas contained within a single governmental administrative unit and comprising at least 80 000 individuals were selected to be the study sites. Criteria for selection were baseline soil-transmitted helminth endemicity, participation in at least five rounds of community-wide MDA for lymphatic filariasis, no active lymphatic filariasis programmes in the area, and existing support from national soil-transmitted helminth programmes. A census of the entire population was conducted at baseline (before randomisation and MDA delivery) and updated in years 2, 3, and 5. During each census, each site was visualised using mapping software available online (Bing, Google Satellite, and OpenStreetMap XYZ raster tiles in qGIS). All potential structures were identified from these maps and cross-referenced with visited structures to ensure the entire population of each study site was enumerated, including individuals considered migratory. The head of household or other adult household member consented to participation in the baseline census and annual updates on behalf of their households. Data collected during the census included demographic information (the head of the household or responding adult provided the sex of all household members, the options for which were male, female, and other), occupation, assets, household construction materials, and access to WASH. Data on ethnicity were collected and reported per standard country reporting guidance. WASH facilities were grouped and categorised according to the 2017 WHO–UNICEF Joint Monitoring Programme criteria.[16] Following the baseline census, each study site was divided into 40 clusters, each with a minimum population of 1650 individuals and adhering to local administrative boundaries where possible.

See Online for appendix 2

For OpenStreetMap see https://www.openstreetmap.org

At baseline, 150 participants per cluster were sampled by age-stratified random sampling to include 30 preschool-aged children, 30 school-aged children, and 90 adults, who were tested for infection by the Kato–Katz technique and enrolled in a cohort to be followed annually. A further 500 individuals were selected by true random sampling from the list of cluster residents with a target of enrolling and collecting stool samples from 20 000 participants per site. To assess baseline prevalence, all participants enrolled in the cohort and were tested by quantitative PCR (qPCR). Due to resource constraints, a further 250 of the 500 cross-sectionally sampled individuals per cluster were selected and tested by qPCR. Participants who provided stool samples provided either written or witnessed oral consent, and children older than 7 years provided assent. Individuals who consented but did not provide a stool sample were excluded from prevalence analyses. All selected individuals who were found to have moderate or heavy-intensity soil-transmitted helminth infection by the Kato–Katz technique were treated with albendazole 400 mg.

### Randomisation and masking

Clusters were randomly assigned in a 1:1 ratio to community-wide MDA or school-based deworming. Laboratory personnel were masked to treatment allocation during assessment. Study investigators were masked to all prevalence and laboratory testing data post-baseline. The unmasked statistical team managed masked study data and conducted the final analysis as per the published statistical analysis plan (appendix 3) until the data lock occurred and unmasking to the study investigators occurred on Jan 18, 2024. The success of masking was not assessed. A lead member of the central DeWorm3 data team (KHÁ) used covariate-based restricted randomisation to ensure the clusters were balanced with regard to baseline soil-transmitted helminth prevalence as measured by the Kato–Katz technique, population size, age distribution, relative socioeconomic status, WASH access, and urban or rural designation of clusters. In addition randomisation in India was restricted to ensure balance of clusters in the Jawadhu Hills and Timiri subsites. Randomisation in Malawi was restricted to ensure the balance of clusters identified as potentially resistant to study activities by the site team. (appendix 1 p 2).[17] A simulation of 100 000 scenarios randomising 40 clusters 1:1 to two groups was done in Stata (version 14.2). For each main study site (one in each country), a scenario was then randomly selected from a list of scenarios that met balancing criteria (127 scenarios in Benin, 485 in India, and 297 in Malawi).

See Online for appendix 3

### Procedures

In intervention clusters, a single oral dose of 400 mg of albendazole was provided biannually for 3 years via community-wide MDA by community drug distributors and volunteer members of the community selected to distribute drugs for diseases targeted by neglected tropical disease programmes. Community drug distributors were accompanied by study data collectors. Community-wide MDA treatment lists were based on the most recent census at that time and targeted all individuals eligible for treatment as per national guidelines (ages 12 months and older in India and Benin and 24 months and older in Malawi), with the exception of women who reported being in the first trimester of pregnancy, those with a history of adverse reactions to benzimidazoles, individuals who were acutely ill or intoxicated at the time of treatment, or those reporting treatment within the previous 2 weeks. Albendazole was provided to all eligible household members present at the visit and ingestion of the drug was directly observed when possible. Study staff made up to three attempts to visit each household and treatment was left at the household at the third visit for any individuals not reached over 1–2 weeks. Mop-up campaigns targeting untreated individuals were conducted in all intervention clusters within 1–2 weeks of each MDA round.

In all study clusters, eligible preschool-aged and school-aged children were treated in schools following country guidelines (ages 1–14 years in Benin, 1–19 years in India, and 2–19 years in Malawi) either annually (Benin and Malawi, coinciding with community-wide MDA rounds two, four, and six) or biannually (India, coinciding with all rounds of community-wide MDA). Non-enrolled preschool-aged and school-aged children were encouraged to go to schools for treatment in both India and Benin, but not in Malawi, as per existing national guidelines. Teachers, community drug distributors, or both recorded all treatments and documented whether treatment was directly observed. Once treated, the children had their finger marked with ink. In intervention clusters, school-based deworming preceded community-wide MDA and finger markings were used to ensure that children were not re-treated during campaigns that were conducted shortly after school-based deworming. As per agreements with local and national agencies, following the sixth round of community-wide MDA, no deworming was delivered in either study group for 24 months until the endline prevalence assessment was completed.

Cross-sectional assessments of soil-transmitted helminth prevalence were conducted at the end of the study (24 months following the sixth MDA round; 24 months after the sixth round of school-based deworming in India and approximately 30 months after the third round in Benin and Malawi). For each cluster, 1000 participants were randomly selected by true random sampling without stratification from the most recent census at that time with a target of enrolling and collecting stool samples from 40 000 participants per site (1000 per study cluster). Up to three attempts were made to reach each sampled individual, after which a replacement was selected from a backup sampling list. Individuals who consented but did not provide a stool sample were excluded from the study and were not part of the analysis set.

Prevalence (at both baseline and endline) was assessed using a multiplex qPCR assay that was optimised and then validated in three laboratory sites.[15] Due to the very high sensitivity of the assay, cycle threshold cutoffs were set for *N americanus* and *A lumbricoides* to differentiate transmissible infections from detection of DNA more likely associated with non-transmissible ova or helminth fragments.[18] A finite mixture model was applied to the observed bimodal distributions of cycle threshold values observed at baseline, with a true positive qPCR result defined as any value with a 5% or greater chance of belonging to the primary peak. Values with a greater than 95% chance of belonging to the secondary peak were defined as indeterminate and samples with no amplification up to a cycle threshold of 40 (the number of cycles run) were considered negative. Given the very low sample numbers for *T trichiura* and *A duodenale*, all samples with a cycle threshold below 40 for these species were considered positive (appendix 1 p 3). All data were recorded electronically using Android phones with SurveyCTO software (Dobility; Cambridge, MA, USA).[19]

## Outcomes

There were two prespecified primary outcomes, individual-level soil-transmitted helminth infection and cluster-level transmission interruption, both measured by qPCR 24 months after the final round of treatment at each study site. Individual-level soil-transmitted helminth infection was defined as an individual's test result for the predominant baseline soil-transmitted helminth species (*A lumbricoides, A duodenale, N americanus,* or *T trichiura*). Cluster-level transmission interruption was defined as age-weighted and sex-weighted prevalence with finite population correction of the predominant baseline soil-transmitted helminth species with a one-sided 95% CI less than or equal to 2%, based on previously published studies.[12,13] The predominant soil-transmitted helminth species was established using the results of the baseline prevalence survey.

Prespecified secondary outcomes were to compare individual-level soil-transmitted helminth infection with any of the four predominant species and cluster-level transmission interruption for all four predominant species, defined as any prevalence less than or equal to 2% with 95% CIs following the methods as above. In intervention clusters, the prespecified outcome of treatment coverage was defined as the proportion of censused and eligible individuals who received albendazole at each round of community-wide MDA; individuals were reached by home visits, confirmed to be eligible, provided with treatment, directly observed taking treatment (doses were left for absent members), and were assessed using electronic MDA treatment registers, which were completed by study data collectors during house-to-house delivery of treatment.[19] In the control clusters, aggregate coverage of children in schools was assessed using routine treatment records collated by teachers, community drug distributors, or both, according to national programme requirements.[20]

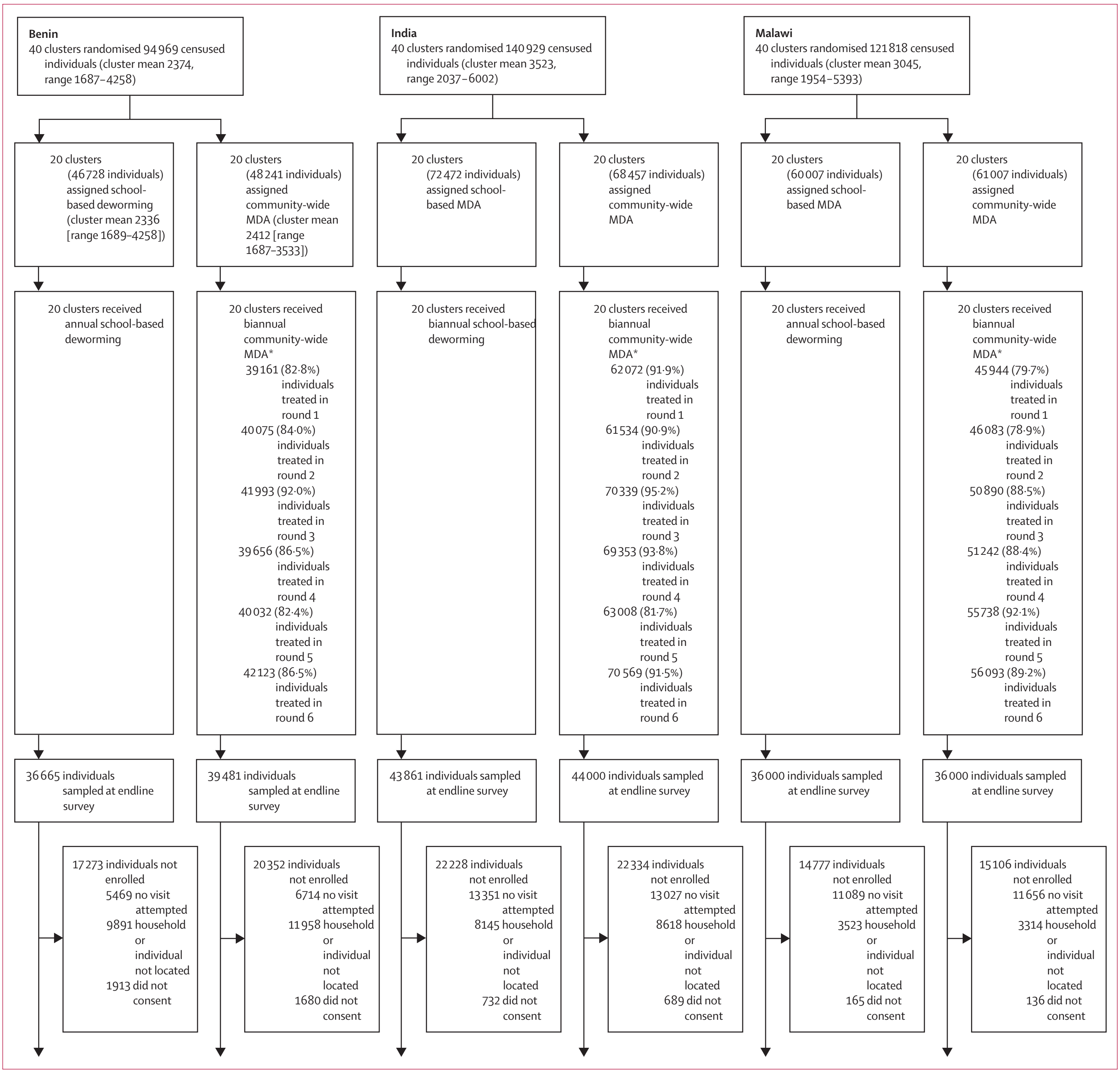
Teachers and health workers were instructed to observe all treated individuals for adverse events following treatment. Adverse events were recorded by the study teams during and following MDA in the intervention group in India and Benin and in both groups in Malawi. In addition, all participants in both study groups were encouraged to report any adverse events that occurred after receipt of MDA to their local health facility or equivalent, where authorities were given contact information for reporting to the trial investigators. Adverse events were only classified as adverse events or serious adverse events, defined as hospitalisation, death, or both.

## Statistical analysis

All analyses and power calculations to detect differences in prevalence by group and in transmission interruption at the cluster level were conducted in R (version 4.4.2). A data safety and monitoring committee provided oversight. All details of the study protocol and statistical analysis plan were made publicly available before unmasking of data for analysis.[15] Simulations estimated power to detect a difference in the proportion of clusters in which transmission was interrupted by each group for each study site. Simulations assumed 20 clusters per group, 500 or 1000 individuals per cluster, a binomial distribution of soil-transmitted helminth prevalence with a mean prevalence of 7% across clusters in the control group and ranging from 0·1% to 4% ($\pi_1$) in the intervention group, an intraclass correlation coefficient (ICC) range of 0·003–0·05, and an α value of 0·05; 10 000 repetitions were run for each scenario (appendix 1 p 4). Measuring prevalence of soil-transmitted helminth infection among 1000 people per cluster at endline would provide adequate power for the transmission interruption objective in most scenarios given $\pi_1 \leq 2\%$. For the objective comparing endline prevalence by group, power was greater than or equal to 80% to detect a difference in endline prevalence up to a prevalence of 4% in the intervention group, given an ICC of ≤0·02 (appendix 1 p 5).

All analyses were conducted separately for each site unless otherwise specified. The effect of biannual community-wide MDA on prevalence of soil-transmitted helminths was analysed according to the group that the individual's cluster was assigned to, with a two-sided type I error rate of 0·05. Participants were included in the analysis set if, 24 months after the last round of community-wide MDA, they enrolled in the endline assessment and provided a stool sample that was correctly processed by the laboratory and obtained a qPCR result. For ease of interpretation, we estimated the effect as the prevalence ratio for each group using modified Poisson regression with robust variance estimation and exchangeable correlation matrix using generalised estimating equations, which adjusts SEs of the Poisson model coefficients for binomial outcome[21] and accounts for the clustered study design.[22] Models were adjusted for age, sex, migration status, household size, population density within 0·5 km of the household, socioeconomic status (asset index quintile), WASH access, and cluster-specific age-weighted and sex-weighted baseline prevalence of soil-transmitted helminths. The effect of the intervention on the secondary outcome of prevalence of any soil-transmitted helminth was analysed in the same manner. Secondary analyses pooled data across all sites and tested for effect modification using an interaction term between study site and randomisation group.

The effect of biannual community-wide MDA on transmission interruption was tested using a Fisher's exact test comparing the proportion of clusters in each group achieving transmission interruption. The effect on the secondary outcome of transmission interruption of




(Figure 1 continues on next page)

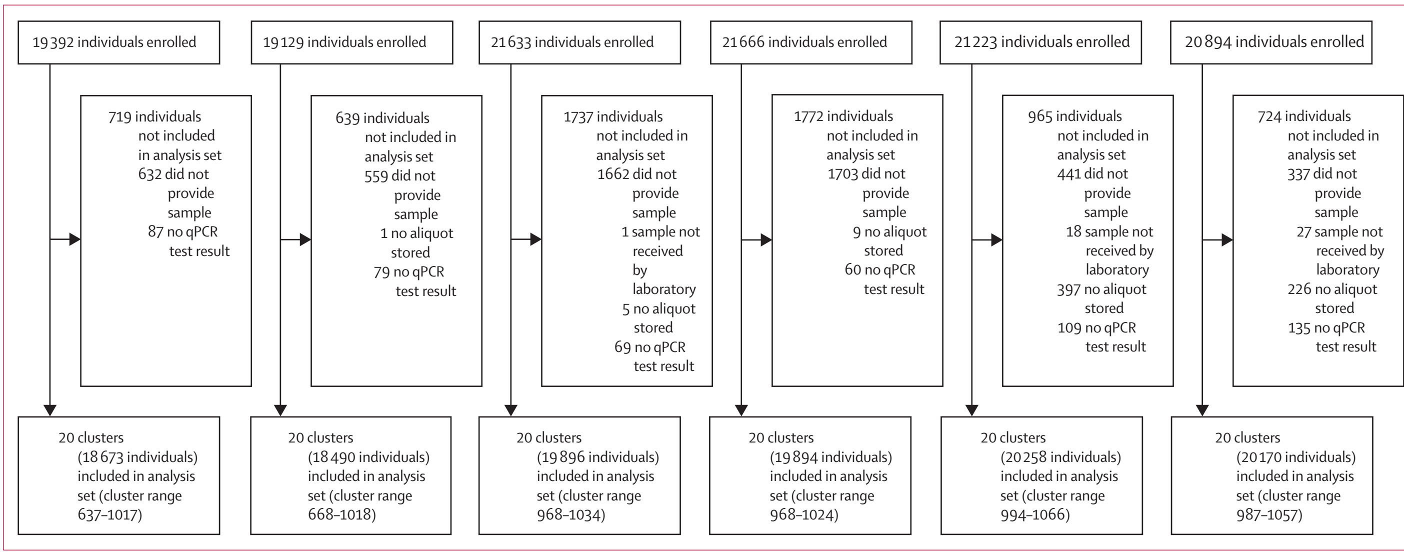



***Figure 1:*** **Trial profile**

MDA=mass drug administration. qPCR=quantitative PCR. *Denominators vary because MDA depends on the national guidelines in each country, with exclusions of anyone younger than 1 year, anyone in their first trimester of pregnancy, and anyone who died between the time of the census and treatment delivery.

any soil-transmitted helminth was analysed in the same manner. A prespecified exploratory analysis assessed cluster-level correlates of transmission interruption in the intervention group using modified Poisson regression.

Post-hoc descriptive subgroup analyses compared the unweighted prevalence of each soil-transmitted helminth species by timepoint, study randomisation group, and participant age.

### Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

## Results

Data collection took place between Oct 10, 2017, and Feb 17, 2023. 357 716 individuals were enumerated in the baseline census and formed the population from which 40 clusters per site were identified and randomly assigned for the trial (figure 1). The sites ranged in geographical size from 148 km² (Benin) to 477 km² (India) and in baseline population from 94 969 to 140 929 individuals. Baseline demographics were similar between treatment groups (table 1). No clusters declined to participate or were lost to follow-up after randomisation. Age-weighted, sex-weighted, and cluster-weighted baseline prevalence was calculated from participants with a valid stool qPCR result and age and sex data available (10 045 in Benin, 9995 in India, and 9940 in Malawi), and was 10·6% (95% CI 10·1–11·2) in Benin, 29·9% (29·0–30·8) in India, and 13·7% (13·1–14·4) in Malawi, driven primarily by *N americanus* infections at all sites. 157 individuals (71 in the control group and 86 in the intervention group) were identified with moderate or heavy-intensity infection by the Kato–Katz technique at baseline and were treated with a single dose of albendazole in schools or by the study team.

In the intervention group, community-wide MDA coverage was high at all sites across the six rounds, ranging from 40 032 (82·4%) of 48 564 to 41 993 (92·0%) of 45 637 in Benin, 46 083 (78·9%) of 58 042 to 55 738 (92·1%) of 60 518 in Malawi, and 63 0088 (1·7%) of 77 096 to 70 339 (95·2%) of 73 924 in India (figure 1; appendix 1 p 6). Coverage of directly observed therapy ranged from 35 296 (75·1%) of 47 005 to 40 873 (91·1%) of 44 890 in Benin, 32 538 (68·6%) of 47 412 to 55 198 (91·6%) of 60 235 in Malawi, and 61 422 (80·1%) of 76 704 to 53 861 (89·8%) of 59 963 in India. Some rounds had higher denominators because those already treated in school were excluded and no school-based deworming was delivered in some cases (rounds 1, 3, and 5 in Malawi and Benin due to national policies and rounds 5 and 6 in India due to the COVID-19 pandemic). Treatment uptake among individuals reached during door-to-door MDA exceeded 90% at all sites and rounds (data not shown).

Baseline and endline weighted prevalence of *N americanus* in each cluster are displayed in figure 2. After 3 years (six rounds) of intervention followed by 24 months during which no deworming was provided, mean cluster-specific age-weighted and sex-weighted prevalence of *N americanus* decreased in all sites in both groups (in Benin from 7·2% [SD 7·1] to 4·0% [4·1] in the control group and 7·2% [7·7] to 1·8% [1·9] in the intervention group; in India from 29·3% [14·9] to 22·0% [15·0] in the control group and 28·6% [17·4] to 9·8% [11·3] in the intervention group; and in Malawi

| | Benin | | India | | Malawi | |
|---|---|---|---|---|---|---|
| | School-based deworming | Community-wide MDA | School-based deworming | Community-wide MDA | School-based deworming | Community-wide MDA |
| **Study site characteristics** | | | | | | |
| Geographical area of study site, km² | 84·5 | 63·8 | 285·2 | 289·5 | 140·3 | 148·3 |
| Urbanicity in study clusters* | | | | | | |
| Rural | 9 (45·0%) | 9 (45·0%) | 17 (85·0%) | 16 (80·0%) | 16 (80·0%) | 15 (75·0%) |
| Peri-urban | 4 (20·0%) | 4 (20·0%) | 3 (15·0%) | 4 (20·0%) | 4 (20·0%) | 5 (25·0%) |
| Urban | 7 (35·0%) | 7 (35·0%) | 0 | 0 | 0 | 0 |
| **Household characteristics** | | | | | | |
| Households enumerated | 11 905 | 12 473 | 18 716 | 17 819 | 13 762 | 13 988 |
| Household residents† | 4 (2–5) | 4 (2–5) | 4 (3–5) | 4 (3–5) | 4 (3–6) | 4 (3–6) |
| Population density within 0·5 km of the household | | | | | | |
| <1000 people per km² | 2098 (17·6%) | 2650 (21·2%) | 9729 (52·0%) | 8694 (48·8%) | 5848 (42·5%) | 4098 (29·3%) |
| 1000–4999 people per km² | 6733 (56·6%) | 5003 (40·1%) | 8084 (43·2%) | 8715 (48·9%) | 7914 (57·5%) | 9890 (70·7%) |
| ≥5000 people per km² | 3074 (25·8%) | 4820 (38·6%) | 903 (4·8%) | 410 (2·3%) | 0 | 0 |
| Owner-occupied dwelling | 7369 (61·9%) | 7720 (61·9%) | 16 703 (89·2%) | 15 928 (89·4%) | 11 769 (85·5%) | 12 195 (87·2%) |
| Flooring material | | | | | | |
| Natural | 2086 (17·5%) | 2213 (17·7%) | 2413 (12·9%) | 2151 (12·1%) | 10 847 (78·8%) | 11 229 (80·3%) |
| Manmade | 9784 (82·2%) | 10 192 (81·7%) | 16 284 (87·0%) | 15 647 (87·8%) | 2905 (21·1%) | 2751 (19·7%) |
| Other or unknown | 35 (0·3%) | 68 (0·5%) | 19 (0·1%) | 21 (0·1%) | 10 (0·1%) | 8 (0·1%) |
| Sanitation‡ | | | | | | |
| Basic facilities | 2835 (23·8%) | 2838 (22·8%) | 6200 (33·1%) | 5473 (30·7%) | 9367 (68·1%) | 9515 (68·0%) |
| Limited facilities | 3297 (27·7%) | 3430 (27·5%) | 359 (1·9%) | 294 (1·6%) | 3492 (25·4%) | 3478 (24·9%) |
| Unimproved facilities | 1142 (9·6%) | 1300 (10·4%) | 186 (1·0%) | 254 (1·4%) | 534 (3·9%) | 659 (4·7%) |
| No facilities (open defecation) | 4388 (36·9%) | 4636 (37·2%) | 11 954 (63·9%) | 11 792 (66·2%) | 352 (2·6%) | 305 (2·2%) |
| Other or unknown | 243 (2·0%) | 269 (2·2%) | 17 (0·1%) | 6 (<0·1%) | 17 (0·1%) | 31 (0·2%) |
| Drinking water source‡ | | | | | | |
| Basic | 9967 (83·7%) | 10 197 (81·8%) | 17 328 (92·6%) | 16 553 (92·9%) | 10 524 (76·5%) | 10 271 (73·4%) |
| Limited | 688 (5·8%) | 865 (6·9%) | 631 (3·4%) | 512 (2·9%) | 2970 (21·6%) | 3513 (25·1%) |
| Unimproved | 1196 (10·0%) | 1357 (10·9%) | 616 (3·3%) | 634 (3·6%) | 241 (1·8%) | 170 (1·2%) |
| Surface water | 20 (0·2%) | 17 (0·1%) | 49 (0·3%) | 30 (0·2%) | 26 (0·2%) | 27 (0·2%) |
| Other or unknown | 34 (0·3%) | 37 (0·3%) | 92 (0·5%) | 90 (0·5%) | 1 (<0·1%) | 7 (0·1%) |
| Household has electricity | 5253 (44·1%) | 5493 (44·0%) | 17 581 (93·9%) | 16 615 (93·2%) | 786 (5·7%) | 541 (3·9%) |
| Household has livestock | 1687 (14·2%) | 1488 (11·9%) | 7580 (40·5%) | 6872 (38·6%) | 4871 (35·4%) | 4858 (34·7%) |
| Household has a mobile phone | 8889 (74·7%) | 9012 (72·3%) | 15 688 (83·8%) | 15 004 (84·2%) | 6032 (43·8%) | 5965 (42·6%) |
| **Study population** | | | | | | |
| Enumerated | 46 728 | 48 241 | 72 472 | 68 457 | 60 811 | 61 007 |
| Sex | | | | | | |
| Male | 22 700 (48·6%) | 23 188 (48·1%) | 36 141 (49·9%) | 34 153 (49·9%) | 28 589 (47·0%) | 28 892 (47·4%) |
| Female | 24 028 (51·4%) | 25 052 (51·9%) | 36 318 (50·1%) | 34 300 (50·1%) | 32 218 (53·0%) | 32 114 (52·6%) |
| Other | 0 | 1 (<0·1%) | 13 (<0·1%) | 4 (<0·1%) | 4 (<0·1%) | 1 (<0·1%) |
| Age distribution | | | | | | |
| Infants (<1 year) | 1297 (2·8%) | 1319 (2·7%) | 900 (1·2%) | 850 (1·2%) | 2199 (3·6%) | 2168 (3·6%) |
| Preschool-age children (1–4 years) | 5413 (11·6%) | 5775 (12·0%) | 4453 (6·1%) | 4029 (5·9%) | 8768 (14·4%) | 8687 (14·2%) |
| School-age children (5–14 years) | 12 897 (27·6%) | 13 146 (27·3%) | 11 260 (15·5%) | 10 578 (15·5%) | 18 905 (31·1%) | 18 747 (30·7%) |
| Adults (≥15 years) | 26 919 (57·6%) | 27 963 (58·0%) | 55 859 (77·1%) | 53 000 (77·4%) | 30 840 (50·7%) | 31 321 (51·3%) |
| Women of reproductive age (15–49 years) | 10 388 (22·2%) | 10 876 (22·5%) | 20 180 (27·8%) | 19 098 (27·9%) | 13 720 (22·6%) | 13 702 (22·5%) |
| Age unknown | 202 (0·4%) | 38 (0·1%) | 0 | 0 | 99 (0·2%) | 84 (0·1%) |

(Table 1 continues on next page)

| | Benin | | India | | Malawi | |
|---|---|---|---|---|---|---|
| | School-based deworming | Community-wide MDA | School-based deworming | Community-wide MDA | School-based deworming | Community-wide MDA |
| (Continued from previous page) | | | | | | |
| School attendance among school-age children | | | | | | |
| Attending school | 10 070/12 897 (78·1%) | 9961/13 146 (75·8%) | 10 560/11 260 (93·8%) | 10 033/10 578 (94·8%) | 16 953/18 905 (89·7%) | 16 819/18 747 (89·7%) |
| Not attending school | 1705/12 897 (13·2%) | 1805/13 146 (13·7%) | 697/11 260 (6·2%) | 545/10 578 (5·2%) | 1934/18 905 (10·2%) | 1908/18 747 (10·2%) |
| Unknown | 1122/12 897 (8·7%) | 1380/13 146 (10·5%) | 3/11 260 (<0·1%) | 0 | 18/18 905 (0·1%) | 20/18 747 (0·1%) |
| Highest level of education among adults aged ≥20 years | | | | | | |
| No education or less than primary school | 8122/22 554 (36·0%) | 8124/23 430 (34·7%) | 16 669/49 579 (33·6%) | 15 576/47 031 (33·1%) | 9927/24 300 (40·9%) | 10 352/24 790 (41·8%) |
| Primary school incomplete or complete | 4094/22 554 (18·2%) | 4344/23 430 (18·5%) | 14 988/49 579 (30·2%) | 14 215/47 031 (30·2%) | 10 887/24 300 (44·8%) | 11 283/24 790 (45·5%) |
| Secondary school incomplete or complete | 3589/22 554 (15·9%) | 3691/23 430 (15·8%) | 8572/49 579 (17·3%) | 8180/47 031 (17·4%) | 2440/24 300 (10·0%) | 2149/24 790 (8·7%) |
| Above secondary school | 4119/22 554 (18·3%) | 4081/23 430 (17·4%) | 9104/49 579 (18·4%) | 8812/47 031 (18·7%) | 66/24 300 (0·3%) | 42/24 790 (0·2%) |
| Other or unknown | 2630/22 554 (11·7%) | 3190/23 430 (13·6%) | 246/49 579 (0·5%) | 248/47 031 (0·5%) | 980/24 300 (4·0%) | 964/24 790 (3·9%) |
| Ethnicity§ | | | | | | |
| Majority language | 41 669 (89·2%) | 43 104 (89·4%) | 69 974 (96·6%) | 66 263 (96·8%) | 57 953 (95·3%) | 58 663 (96·1%) |
| Minority language | 5031 (10·8%) | 5122 (10·6%) | 2487 (3·4%) | 2194 (3·2%) | 2854 (4·7%) | 2371 (3·9%) |
| Unknown | 28 (0·1%) | 15 (<0·1%) | 11 (<0·1%) | 0 | 4 (<0·1%) | 3 (<0·1%) |
| Migration¶ | | | | | | |
| Lived outside the household most of the past year | 701/22 554 (1·5%) | 715/23 430 (1·5%) | 1812/49 579 (2·5%) | 1976/47 031 (2·9%) | 2287/24 300 (3·8%) | 2342/24 790 (3·8%) |
| Slept elsewhere the night before the census | 1318/22 554 (2·8%) | 1232/23 430 (2·6%) | 3963/49 579 (5·5%) | 3816/47 031 (5·6%) | 3354/24 300 (5·5%) | 3387/24 790 (5·6%) |
| **Baseline quantitative PCR prevalence estimates\|\|** | | | | | | |
| Unweighted | | | | | | |
| Any soil-transmitted helminth | 477/5010 (9·5%) | 488/5035 (9·7%) | 1302/5013 (26·0%) | 1300/4982 (26·1%) | 589/4838 (12·2%) | 656/5102 (12·9%) |
| *N americanus* | 347/5010 (6·9%) | 349/5035 (6·9%) | 1291/5013 (25·8%) | 1287/4982 (25·8%) | 576/4838 (11·9%) | 652/5102 (12·8%) |
| *A duodenale* | 0/5010 | 2/5035 (<0·1%) | 8/5013 (0·2%) | 9/4982 (0·2%) | 15/4838 (0·3%) | 6/5102 (0·1%) |
| *A lumbricoides* | 142/5010 (2·8%) | 136/5035 (2·7%) | 4/5013 (0·1%) | 4/4982 (0·1%) | 2/4838 (<0·1%) | 1/5102 (<0·1%) |
| *T trichiura* | 10/5010 (0·2%) | 9/5035 (0·2%) | 9/5013 (0·2%) | 8/4982 (0·2%) | 4/4838 (0·1%) | 2/5102 (<0·1%) |
| Weighted** | | | | | | |
| Any soil-transmitted helminth | 10·7% (9·9–11·6) | 10·5% (9·7–11·4) | 29·5% (28·3–30·8) | 30·3% (29·0–31·6) | 13·2% (12·2–14·3) | 14·3% (13·3–15·4) |
| *N americanus* | 8·2% (7·5–9·1) | 7·8% (7·1–8·5) | 29·3% (28·0–30·6) | 30·0% (28·7–31·3) | 12·9% (11·9–14·0) | 14·2% (13·2–15·3) |
| *A duodenale* | 0 | 0·1% (0·0–0·2) | 0·2% (0·1–0·3) | 0·2% (0·1–0·4) | 0·4% (0·2–0·7) | 0·1% (0·0–0·2) |
| *A lumbricoides* | 2·8% (2·4–3·3) | 2·7% (2·4–3·3) | 0·1% (0·0–0·2) | 0·1% (0·0–0·1) | <0·1% (0·0–0·1) | <0·1% (0·0–0·1) |
| *T trichiura* | 0·2% (0·1–0·5) | 0·2% (0·1–0·3) | 0·2% (0·1–0·4) | 0·1% (0·1–0·3) | 0·1% (0·0–0·3) | <0·1% (0·0–0·2) |

Data are n, n (%), or n/N (%) unless otherwise specified. *A duodenale=Ancylostoma duodenale*. *A lumbricoides=Ascaris lumbricoides*. MDA=mass drug administration. *N americanus=Necator americanus*. *T Trichiura=Trichuris trichuria*. *As defined by each study team. 20 clusters per intervention group. †Median (IQR). ‡ As defined by the WHO–UNICEF Joint Monitoring Programme for Water Supply, Sanitation, and Hygiene 2018.[16] § Majority language is defined as Pedah, Sahouè, Watchi, Mina, Adja, and Xwla in Benin, Tamil in India, and Chiyao in Malawi. Minority language is defined as Fon or other language in Benin, Hindi, Telegu, Urdu, or other language in India, and Chichewa or other language in Malawi. ¶Column does not sum to total as respondents could report living outside of the household, sleeping elsewhere, or neither. ||Prevalence as measured by quantitative PCR from the baseline prevalence survey. Soil-transmitted helminth positivity defined as a cycle threshold <34·43980 for *N americanus*, <28·57587 for *A lumbricoides,* and <40·00 for *A duodenale* and *T trichiura*. **Prevalence estimates (95% CIs) weighted to match the age, sex, and cluster distribution of the census.

***Table 1:*** **Characteristics of participants at baseline**

from 13·6% [5·6] to 10·0% [3·6] in the control group and 14·5% [5·2] to 4·1% [1·4] in the intervention group; appendix 1 p 13). After adjustment, compared with the control group, the prevalence of *N americanus* in the intervention group at endline was 56% lower (prevalence ratio 0·44 [95% CI 0·34–0·58]; p<0·0001) in Benin, 59% lower (0·41 [0·32–0·52]; p<0·0001) in India, and 60% lower (0·40 [0·34–0·46]; p<0·0001) in Malawi. In the analysis that pooled all sites, prevalence was 59% lower in the intervention group than the control group (0·41 [0·36–0·48]; p<0·0001) with no effect modification observed by site (p=0·51). The prevalence of any soil-transmitted helminth species in the intervention group at endline was 48% lower in Benin, 59% lower in India, and 60% lower in Malawi than in the control group (p<0·0001 for all comparisons) with a pooled reduction of 58% (p<0·0001) and no effect modification by site (p=0·24; table 2). Greater reductions in prevalence

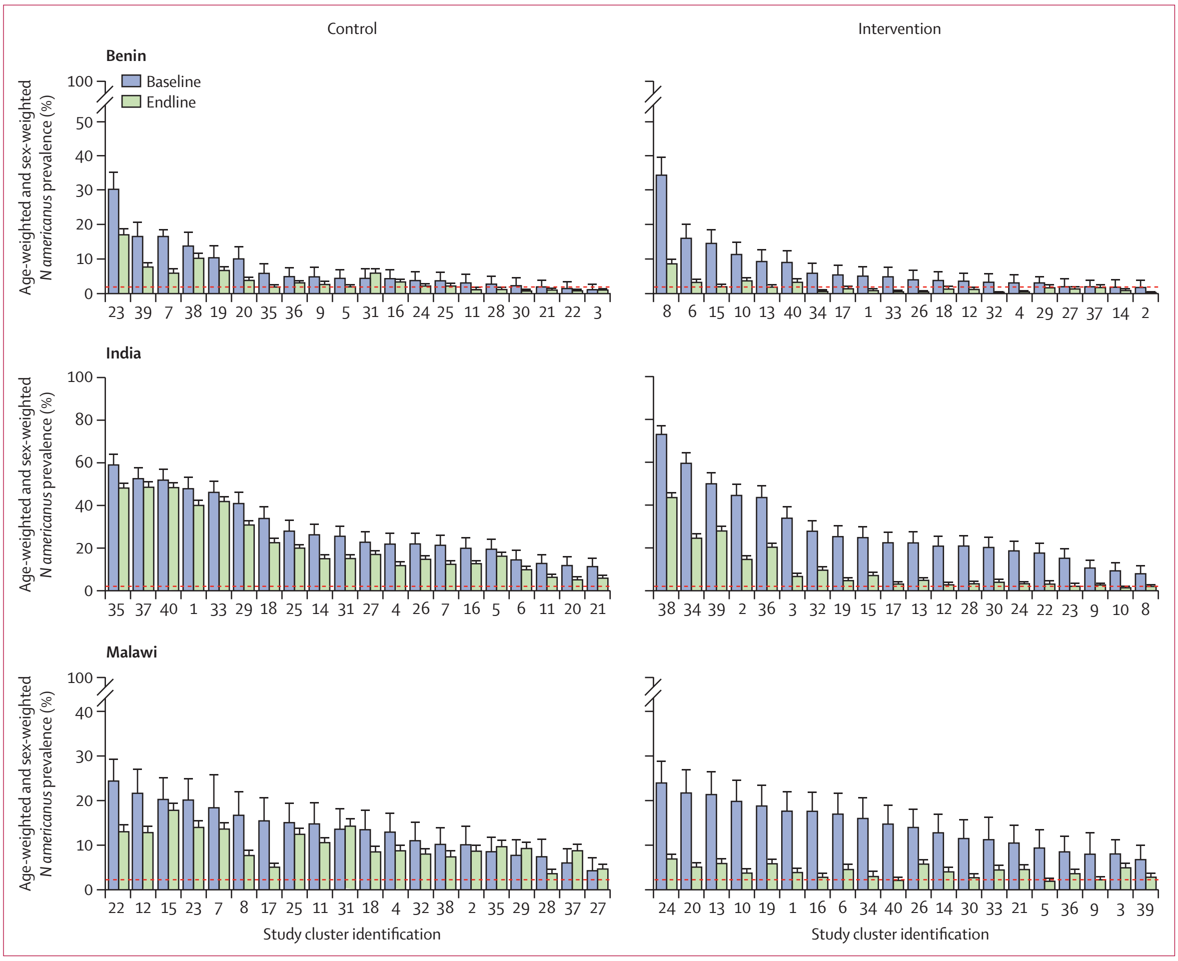



***Figure 2:*** **Baseline and endline individual-level *N americanus* qPCR prevalence for each cluster, by treatment group and country**
*N americanus* qPCR prevalence was computed for each study cluster at baseline (mean 403 [SD 34] samples per cluster) and endline (978 [80] samples per cluster) weighted to the age distribution and sex distribution of the most recent census at the time of survey. Clusters have been ordered by prevalence at baseline (highest to lowest). Error bars represent one-sided binomial 95% CIs and prevalences are displayed separated by site and study treatment group with an overlaid transmission interruption threshold (≤2%). The y axes are presented to different scales per country to enable better visualisation of the data. *N americanus*=*Necator americanus*. qPCR=quantitative PCR.

of all soil-transmitted helminth species combined were observed in the community-wide MDA group than the school-based deworming group at all three sites; however, no significant differences in the prevalence of specific species other than *N americanus* (ie, *A duodenale, A lumbricoides,* and *T trichiura*) between groups at any of the sites were found. In subgroup analyses of preschool-aged children, school-aged children, women of reproductive age, and all adults, greater reductions were also observed in the community-wide MDA group than the school-based deworming group in all descriptive analyses across all sites, with the exception of preschool-aged children in Malawi (appendix 1 p 14).

At endline, transmission interruption was achieved for the predominant soil-transmitted helminth species (*N americanus)* in 17 (43%) of 40 clusters in Benin (11 [55%] of 20 clusters in the intervention group *vs* six [15%] of 20 clusters in the control group, p=0·20), in one (5%) of 20 intervention clusters versus none of 20 clusters in the control group in India (p=1·00), and in no clusters in either group in Malawi (p=1·00; appendix 1 p 15). Transmission interruption of all soil-transmitted helminths species was achieved in 14 (35%) of 40 clusters in Benin (nine [45%] of 20 clusters in the intervention group *vs* five [25%] of 20 clusters in the control group, p=0·32) and in no clusters in India or Malawi. Clusters that achieved transmission interruption had lower baseline prevalence than those that did not in both control and intervention groups; in Benin, the 11 clusters achieving interruption of *N americanus* in the intervention group had a higher baseline point prevalence of *N americanus* (median 3·6% [IQR 2·5–4·4], mean 3·5% [SD 1·4]) compared with the six clusters achieving *N americanus* interruption in the control group (median 2·1% [1·5–2·6], mean 2·1% [0·8]; appendix 1 p 16).

| | Benin | | | India | | | Malawi | | | Pooled | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control (n=18 673) | Intervention (n=18 490) | p value* | Control (n=19 896) | Intervention (n=19 894) | p value* | Control (n=20 258) | Intervention (n=20 170) | p value* | Control (n=58 827) | Intervention (n=58 554) | p value* |
| ***N americanus*** | | | | | | | | | | | | |
| Endline prevalence† | 748 (4·0%) | 322 (1·7%) | ·· | 4310 (21·7%) | 1906 (9·8%) | ·· | 1989 (9·8%) | 827 (4·1%) | ·· | 7047 (12·0%) | 3055 (5·2%) | ·· |
| Treatment effect | 1 (ref) | 0·43 (0·23–0·83) | 0·011 | 1 (ref) | 0·44 (0·25–0·79) | 0·0061 | 1 (ref) | 0·42 (0·34–0·52) | <0·001 | 1 (ref) | 0·43 (0·30–0·63) | <0·001 |
| Adjusted treatment effect‡ | 1 (ref) | 0·44 (0·34–0·58) | <0·001 | 1 (ref) | 0·41 (0·32–0·52) | <0·001 | 1 (ref) | 0·40 (0·34–0·46) | <0·001 | 1 (ref) | 0·41 (0·36–0·48) | <0·001 |
| ***A duodenale*** | | | | | | | | | | | | |
| Endline prevalence† | 11 (0·1%) | 11 (0·1%) | ·· | 3 (<0·1%) | 3 (<0·1%) | ·· | 15 (0·1%) | 6 (<0·1%) | ·· | 29 (0·1%) | 20 (<0·1%) | ·· |
| Treatment effect | 1 (ref) | 1·02 (0·37–2·83) | 0·97 | 1 (ref) | 1·00 (0·23–4·39) | >0·99 | 1 (ref) | 0·41 (0·09–1·75) | 0·23 | 1 (ref) | 0·70 (0·34–1·45) | 0·34 |
| Adjusted treatment effect‡ | 1 (ref) | 0·83 (0·27–2·57) | 0·75 | 1 (ref) | 0·92 (0·17–4·94) | 0·92 | 1 (ref) | 0·31 (0·07–1·40) | 0·13 | 1 (ref) | 0·70 (0·33–1·51) | 0·37 |
| ***A lumbricoides*** | | | | | | | | | | | | |
| Endline prevalence† | 59 (0·3%) | 123 (0·7%) | ·· | 7 (<0·1%) | 17 (0·1%) | ·· | 15 (0·1%) | 12 (0·1%) | ·· | 81 (0·1%) | 152 (0·3%) | ·· |
| Treatment effect | 1 (ref) | 2·08 (0·49–8·87) | 0·32 | 1 (ref) | 2·44 (0·83–7·14) | 0·10 | 1 (ref) | 0·80 (0·39–1·67) | 0·56 | 1 (ref) | 1·88 (0·59–6·00) | 0·28 |
| Adjusted treatment effect‡ | 1 (ref) | 1·50 (0·83–2·72) | 0·18 | 1 (ref) | 2·07 (0·81–5·29) | 0·13 | 1 (ref) | 0·71 (0·37–1·38) | 0·31 | 1 (ref) | 1·40 (0·89–2·18) | 0·14 |
| ***T trichiura*** | | | | | | | | | | | | |
| Endline prevalence† | 6 (<0·1%) | 13 (0·1%) | ·· | 5 (<0·%1) | 7 (<0·1%) | ·· | 4 (<0·1%) | 2 (<0·1%) | ·· | 15 (<0·1%) | 22 (<0·1%) | ·· |
| Treatment effect | 1 (ref) | 2·20 (0·88–5·51) | 0·093 | 1 (ref) | 1·40 (0·38–5·18) | 0·61 | 1 (ref) | 0·50 (0·09–2·81) | 0·43 | 1 (ref) | 1·48 (0·75–2·91) | 0·26 |
| Adjusted treatment effect‡ | 1 (ref) | 2·26 (0·85–6·02) | 0·10 | 1 (ref) | 0·95 (0·34–2·67) | 0·93 | 1 (ref) | 0·28 (0·03–2·63) | 0·27 | 1 (ref) | 1·54 (0·76–3·12) | 0·23 |
| **Any soil-transmitted helminth species** | | | | | | | | | | | | |
| Endline prevalence† | 819 (4·4%) | 467 (2·5%) | ·· | 4318 (21·7%) | 1930 (9·7%) | ·· | 2016 (10·0%) | 846 (4·2%) | ·· | 7153 (12·2%) | 3243 (5·5%) | ·· |
| Treatment effect | 1 (ref) | 0·57 (0·31–1·05) | 0·073 | 1 (ref) | 0·45 (0·25–0·80) | 0·0061 | 1 (ref) | 0·42 (0·34–0·52) | <0·001 | 1 (ref) | 0·46 (0·32–0·65) | <0·001 |
| Adjusted treatment effect‡ | 1 (ref) | 0·52 (0·38–0·71) | <0·001 | 1 (ref) | 0·41 (0·33–0·52) | <0·001 | 1 (ref) | 0·40 (0·35–0·47) | <0·001 | 1 (ref) | 0·42 (0·37–0·49) | <0·001 |

Data are prevalence ratio (95% CI) or n (%) unless otherwise specified. For Benin, sample sizes are 37 163 (unadjusted) and 37 058 (adjusted). For India, sample sizes are 39 790 (unadjusted) and 39 598 (adjusted). For Malawi, sample sizes are 40 428 (unadjusted) and 40 294 (adjusted). For pooled countries, sample sizes are 117 381 (unadjusted) and 116 950 (adjusted). *A duodenal=Ancylostoma duodenale*. *A lumbricoides=Ascaris lumbricoides*. *N americanus=Necator americanus*. *T Trichiura=Trichuris trichuria*. *Assessed by estimating prevalence ratios using modified Poisson regression with robust SEs. The primary analysis is the adjusted analysis. †Unweighted; soil-transmitted helminth positivity by quantitative PCR defined as a cycle threshold <34·43980 for *N americanus*, <28·57587 for *A lumbricoides*, and <40·00 for *A duodenale* and *T trichiura* . ‡Adjusted for baseline study cluster-specific age-weighted and sex-weighted prevalence, age distribution, sex, individual migration status, household size, population density per 1000 individuals within 0·5 km of the household, socioeconomic status as measured by an asset index divided into quintiles, and water and sanitation access as measured by WHO–UNICEF Joint Monitoring Programme for Water Supply, Sanitation, and Hygiene indicators.

***Table 2:*** **Comparison of the individual-level soil-transmitted helminth species-specific endline quantitative PCR prevalence**

| | Transmission interrupted (N=11 clusters) | Transmission not interrupted (N=9 clusters) | RR (95% CI)* | p value |
|---|---|---|---|---|
| Baseline prevalence of *N americanus*† | 3·5% (1·4) | 11·6% (9·8) | 0·80 (0·71–0·90) | 0·0003 |
| Population density‡ | 4761·4 (1652·3) | 2493·4 (2021·9) | 1·27 (1·10–1·47) | 0·0011 |
| Households with open defecation§ | 24·0% (32·5) | 49·4% (32·4) | 0·92 (0·83–1·03) | 0·17 |
| Individual MDA treatment coverage in rounds 1–6 | 84·4% (4·2) | 86·5% (5·0) | 0·95 (0·86–1·05) | 0·30 |
| Clusters that achieved mean 90% coverage in rounds 1–6 | 1/11 (9·1%) | 2/9 (22·2%) | 0·57 (0·11–2·95) | 0·50 |
| Individual MDA treatment acceptance¶ | 57·6% (9·8) | 63·7% (10·6) | 0·97 (0·92–1·02) | 0·24 |
| Migration of individuals‖ | 5·0% (2·8) | 4·1% (1·9) | 1·07 (0·94–1·22) | 0·32 |
| Households with earthen household floor materials** | 9·9% (7·1) | 23·4% (10·0) | 0·70 (0·57–0·86) | 0·0007 |

Data are mean (SD) or n/N (%) unless specified. *N americanus*=*Necator americanus*. RR=risk ratio. *All models were modified Poisson regression with robust SEs. †Study cluster-specific *N americanus* prevalence was weighted to the age and sex distribution of the baseline census population. *N americanus* positivity by quantitative PCR was defined as a cycle threshold <34·43980. RR is expressed per 1% increase. ‡The number of study residents living within 0·5 km of each household expressed per km². RR is expressed per 1000 individuals. §RR is expressed per 5% change in open defecation. ¶The percentage of cluster residents who were treated at all MDA rounds in which they were eligible. RR is expressed per 1% increase. ‖Defined as the percentage of cluster residents who reported living in the household less than 6 months in the previous year during the endline census. RR is expressed per 1% increase. **Defined as the percentage of cluster residents who lived in a household with floor materials made from earth, sand, mud, clay, or dung during the endline census. RR is expressed per 5% change in floor material.

***Table 3:* Cluster-level factors associated with transmission interruption of *N americanus* in the intervention group in Benin**

Factors associated with transmission interruption of *N americanus* in the intervention group were only assessed for Benin and included baseline age-weighted and sex-weighted cluster prevalence (risk ratio 0·80 [95% CI 0·71–0·90] per 1% increase; p=0·0003), cluster mean population density (1·27 [1·10–1·47] per 1000 individuals per km² within 0·5 km of households; p=0·0011), and percentage of households with earthen flooring material (0·70 [0·57–0·86] per 5% increase; p=0·0007; table 3).

Nearly all adverse events recorded were in the intervention group as they were only passively reported during MDA (the intervention), with the exception of in Malawi, where the Government requested the study team deliver the standard of care during the study. In India and Benin, the Government delivered the standard of care.

Adverse events were not collected disaggregated by group and were not a study outcome that was compared by randomisation group to assess if there were differences between groups (given the large body of evidence showing albendazole is safe). To be conservative and ensure the maximal protection of research subjects, we instructed participants to passively report adverse events and followed up all reports to ensure resolution.

Over the course of the study, 984 adverse events were reported among 487 participants, of which 32 adverse events among 13 participants resulted in hospitalisation and were classified as serious adverse events. There were no deaths and all participants reporting serious adverse events recovered. All but three serious adverse events were considered not related to study procedures.

One serious adverse event in Benin was classified as probably related to study procedures when a woman aged 50 years developed diarrhoea and vomiting leading to fainting and hospitalisation following receipt of MDA. In Malawi, two girls aged 12 years had fainting leading to hospitalisation following school-based deworming administration, which was classified as possibly related to study procedures. Study protocol deviations were tracked and monitored by the data safety and monitoring committee and are presented in the appendix 1 (pp 7–12).

## Discussion

We have shown that achieving transmission interruption of soil-transmitted helminths is not feasible at a programmatically relevant scale using MDA delivered over a 3-year period (based on a prevalence of ≤2% by qPCR measured 2 years after cessation of deworming). Transmission interruption was only achieved in focal geographical areas (the transmission of infection involving individuals residing in close spatial and temporal proximity) and was observed almost exclusively in Benin. Although transmission interruption was achieved in more intervention clusters than control clusters in Benin (11 [55%] of 20 *vs* six [15%] of 20), this difference was not statistically significant. In the intervention group, features associated with urban environments, including improved flooring and high population density, were associated with greater likelihood of achieving *N americanus* transmission interruption. Baseline prevalence was strongly negatively associated with *N americanus* transmission interruption in the intervention group. In the control group, prevalence in clusters that achieved interruption approached the transmission interruption threshold (≤2%) before the start of the trial (appendix 1 p 16). These findings suggest that although it might be biologically feasible to break transmission over six rounds of MDA, it is unlikely to be attained across broad geographical settings within a timeframe of 3 years, even when coverage is exceptionally high. The substantial reductions in prevalence in the community-wide MDA group observed across all sites suggest that a longer period of deworming than that studied in this trial could potentially achieve transmission interruption in more clusters. These results support the current WHO targets of eliminating soil-transmitted helminth infection to a prevalence level where it is no longer a public health problem (defined as <2% prevalence of moderate or heavy-intensity infection) and do not appear to support a shift towards a global soil-transmitted helminth policy targeting transmission interruption.

Importantly, we observed substantially greater reductions in the overall prevalence of all soil-transmitted helminth species (driven by the predominant species *N americanus*) using community-wide MDA as opposed to school-based deworming 24 months following cessation of all deworming, a finding that adds greatly to findings from smaller trials.[14,23,24] These greater reductions in prevalence were observed in all age groups, including among preschool-aged children (other than in Malawi), school-aged children, and women of reproductive age, which are populations that are targeted by most current soil-transmitted helminth guidelines and programmes. However, controversy exists regarding the health benefits of deworming, particularly in populations with low levels of moderate or heavy-intensity infection, and these data do not necessarily imply that community-wide MDA would lead to greater reductions in overall morbidity due to helminth infection.[25]

Strengths of this study include the recruitment of large populations across multiple geographies, thereby increasing the generalisability of these results. In addition, the study achieved exceptionally high validated treatment coverage, suggesting that the failure to achieve transmission interruption in all clusters was not due to inadequate coverage.[26] We also showed the feasibility of establishing a high-throughput qPCR platform for soil-transmitted helminth surveillance that can be used to effectively monitor areas with low prevalence in which performance of microscopy-based diagnostics is suboptimal.[27] However, the study did have some limitations. Prevalence was only assessed 24 months following cessation of MDA and samples collected immediately following the final round of MDA have not yet been tested due to a shortage of resources. As such, the trajectory of prevalence following treatment cannot be accurately established. *N americanus* was the predominant species in all three sites and the effects of community-wide MDA might differ in areas where other species predominate. Additional modelling calculations are needed with improved epidemiological data (including with data from DeWorm3) to establish the veracity of a true prevalence of less than 2% as the threshold for transmission interruption across a wide range of transmission settings for the three major soil-transmitted helminth species.[14,23,28] Such simulation-based studies should also establish a range of confidence bounds on qPCR diagnostic tests using positive predictive values for elimination. Finally, prevalence was markedly reduced in both intervention and control clusters, suggesting potential Hawthorne effects, cross-cluster contamination of treatment, or indirect benefits due to reduced exposure to adults with a soil-transmitted helminth infection or environmental contamination with helminth eggs. As clusters were geographically contiguous, reducing the number of individuals shedding soil-transmitted helminth eggs could reduce the reservoir of eggs in the soil and thereby reduce the incidence of new infection.

There is increasing pressure for national governments to finance and deliver national neglected tropical disease programmes.[29] In addition, there is global interest in further optimising the impact of the global drug donation programme and other philanthropic support. As such, the prospect of soil-transmitted helminth elimination is of interest as a potentially cost-effective means of reducing the overall need for resources to support these programmes over time. Our results, from areas with a predominance of hookworm, suggest that transmission interruption is only feasible in focal geographical areas and is unlikely to be achievable at large scale over a period of 3 years, even with high MDA treatment coverage. As a result, elimination of soil-transmitted helminths in most low-income settings does not appear feasible in the timeline tested in this trial. Resources will need to be allocated to allow national programmes to ensure continued delivery of anthelmintics, maintain surveillance, improve coverage, and more effectively integrate MDA with broader health, WASH, and education services. The substantially greater reductions in population prevalence observed with community-wide MDA compared with school-based deworming, deserves careful consideration by neglected tropical disease policy makers given the continued burden of soil-transmitted helminths globally.[30]

### Contributors

JLW, KHÁ, RB, SSRA, KA, SRG, MI, KK, DTJL, AJFL, ARM, WEO, RP, and KKT conceptualised this Article SRG, SSRA, KHA, RB, GC, EA, KEH, PH, MI, GJI, KK, HL, AJFL, MM, ARM, WEO, NP, RP, KKT, and SAW contributed to data curation. KHÁ, SRG, PH, EA, GJI, MM, ARM, NP, RP, KKT, and JLW did formal analyses of the data. SRG and KKT double coded and verified the reported data. SSRA, KHÁ, RB, MI, KK, DTJL, AJFL, AM, ARM, RP, SAW, and JLW contributed to supervision of the overall trial conduct and analyses. All authors contributed to writing the original draft and contributed equally to investigation, methodology, review, and editing. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. All authors have seen and approved the final manuscript.

### Declaration of interests

We declare no competing interests.

### Data sharing

The DeWorm3 datasets, data dictionaries, statistical analysis plan, and study protocol were made publicly available on the Vivli repository in November, 2024. The datasets were anonymised by a third-party vendor. Access to the data and supporting documents is available on request at vivli.org and requires the execution of a Data Use Agreement.

For the **data and supporting documents** see https://doi.org/10·25934/PR00010754

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RESEARCH ARTICLE

# Epidemiology of soil transmitted helminths and risk analysis of hookworm infections in the community: Results from the DeWorm3 Trial in southern India

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## Abstract

Since 2015, India has coordinated the largest school-based deworming program globally, targeting soil-transmitted helminths (STH) in ~250 million children aged 1 to 19 years twice yearly. Despite substantial progress in reduction of morbidity associated with STH, reinfection rates in endemic communities remain high. We conducted a community based parasitological survey in Tamil Nadu as part of the DeWorm3 Project—a cluster-randomised trial evaluating the feasibility of interrupting STH transmission at three geographically distinct sites in Africa and Asia—allowing the estimation of STH prevalence and analysis of associated factors. In India, following a comprehensive census, enumerating 140,932 individuals in 36,536 households along with geospatial mapping of households, an age-stratified sample of individuals was recruited into a longitudinal monitoring cohort (December 2017-February 2018) to be followed for five years. At enrolment, a total of 6089 consenting individuals across 40 study clusters provided a single adequate stool sample for analysis using the Kato-Katz method, as well as answering a questionnaire covering individual and household level factors. The unweighted STH prevalence was 17.0% (95% confidence interval [95% CI]: 16.0–17.9%), increasing to 21.4% when weighted by age and cluster size. Hookworm was the predominant species, with a weighted infection prevalence of 21.0%, the majority of which (92.9%) were light intensity infections. Factors associated with hookworm infection were modelled using mixed-effects multilevel logistic regression for presence of infection

(dw3data@uw.edu) for researchers who meet the criteria for access to these data.

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and mixed-effects negative binomial regression for intensity. The prevalence of both *Ascaris lumbricoides* and *Trichuris trichiura* infections were rare (<1%) and risk factors were therefore not assessed. Increasing age (multivariable odds ratio [mOR] 21.4, 95%CI: 12.3–37.2, p<0.001 for adult age-groups versus pre-school children) and higher vegetation were associated with an increased odds of hookworm infection, whereas recent deworming (mOR 0.3, 95%CI: 0.2–0.5, p<0.001) and belonging to households with higher socioeconomic status (mOR 0.3, 95%CI: 0.2–0.5, p<0.001) and higher education level of the household head (mOR 0.4, 95%CI: 0.3–0.6, p<0.001) were associated with lower odds of hookworm infection in the multilevel model. The same factors were associated with intensity of infection, with the use of improved sanitation facilities also correlated to lower infection intensities (multivariable infection intensity ratio [mIIR] 0.6, 95%CI: 0.4–0.9, p<0.016). Our findings suggest that a community-based approach is required to address the high hookworm burden in adults in this setting. Socioeconomic, education and sanitation improvements alongside mass drug administration would likely accelerate the drive to elimination in these communities.

**Trial Registration:** NCT03014167.

## Author summary

Approximately 1 in 5 people in India are infected with soil transmitted helminths (STH), leading to anaemia and malnutrition. To tackle this large burden of infection, the government of India launched one of the world's largest school-based deworming programs in 2015 aiming to deworm all pre-school and school-aged children between 1 to 19 years of age twice yearly on the National Deworming Days. Deworming programs, including those in India, are focused on pre-school aged children, school aged children and women of reproductive age group. However, prevailing environmental and socioeconomic conditions, including poor sanitation, can contribute to high rates of reinfection from untreated adults and children. The DeWorm3 Project is a cluster-randomised trial evaluating the feasibility of interrupting STH transmission with community wide deworming of all individuals aged one to 99 years of age or older. As part of the study, we conducted a parasitological survey in the Deworm3 trial site in rural Tamil Nadu. Here we present the factors associated with STH infection and burden in these communities.

## Introduction

Soil-transmitted helminths (STH)—*Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*) and *Trichuris trichiura*—are among the most common infections globally, with India estimated to have the highest number of cases (375 million) according to the Global Burden of Disease estimates, 2013 [1]. Significant worldwide reductions in prevalence of *Ascaris* (-25.5% since 1990) have been estimated, but these reductions have been modest for *Trichuris* (-11.6%) and even smaller for hookworm (-5.1%) [2]. In more recent estimates (2015), 258 million (or 1 in 5) individuals in India are estimated to be infected with STH, with 148 million *Ascaris*, 109 million hookworm and 41 million *Trichuris* infections, indicating a lower prevalence of *Ascaris* and *Trichuris*, but a higher prevalence of hookworm than previous reports [3]. Moderate- and heavy-intensity (MHI) hookworm infections are associated with lower haemoglobin levels and anaemia particularly affecting pregnant women and young children who often have low baseline iron stores [4–6]. While a recent Cochrane review indicated that regular deworming of children in public health programmes does not seem to improve outcomes [7], a study using data from Demographic and Health Surveys (DHS) of 45 STH endemic countries found that there was a consistent association between deworming and reduced stunting in pre-school-age children (PSAC) [8]. This is especially relevant in India, where more than half the children under 5 years are stunted [9]. Deworming has also been shown to improve nutritional status, cognition and school performance in school-age children (SAC) [10–12].

The WHO-recommended strategy is focused on controlling morbidity through mass drug administration (MDA) of anthelmintic drugs, albendazole or mebendazole, targeted to PSAC, SAC, women of reproductive age (WRA) and other at-risk populations, aiming for 75% coverage in these populations by 2020 [13,14]. Although the lymphatic filariasis (LF) control programme has delivered albendazole alongside diethylcarbamazine (DEC) through community-wide treatment in over 250 endemic districts in India since 2004 [15], STH burden has remained high [3,16]. The Ministry of Health and Family Welfare (MOHFW) in India has since introduced the world's largest school-based deworming program, targeting ~240 million children aged 1 to 19 years twice yearly (biannual) during the 'National Deworming Days' (NDD) conducted in February and August since 2015 [17]. Eleven states/union territories participated at the launch (including Tamil Nadu) and this program expanded to 33 states/union territories in 2019. With primary school enrolment exceeding 99% in India [18] and the involvement of anganwadi centres (a government run centre in each village providing care for pregnant women and children under 6 years of age under the Integrated Child Development Services Scheme), this is a highly effective way of reaching out to PSAC and SAC to carry out a targeted deworming program.

Reinfection rates in endemic communities with ongoing targeted deworming programs are often high due to poor sanitation, high rates of open defecation, migration and persistent reservoirs of infection in untreated adults [19]. While India has initiated large-scale programs to provide toilet access and reduce open defecation, [20] in the absence of significant structural improvements in sanitation, targeted deworming programs would likely need to be continued indefinitely [21]. Furthermore, meta-analyses, mathematical models and empirical field studies suggest that a community-wide deworming strategy including individuals of all ages may be effective in interrupting transmission of STH infections [22–24]. The recent launch of the WHO 2030 targets for STH control programmes has also emphasised the goal of achieving and maintaining elimination of STH morbidity in pre-SAC and SAC as well as the need to establish an efficient STH control programme for WRA. This has highlighted the need for robust epidemiological data to inform the strengthening of future efforts to control or interrupt transmission of STH [25].

The vast majority of empirical data monitoring the progress of the NDD and demonstrating STH burden reductions in children in India have been collected through school surveys [16,26]. To fully understand the current STH epidemiology, especially for hookworm infections known to increase and plateau in adulthood, community-wide data are also required [27]. We present the results of an age-stratified community STH survey conducted at baseline with participants enrolled into a longitudinal monitoring cohort as part of the DeWorm3 trials, evaluating the feasibility of interrupting STH transmission by comparing community-wide MDA to school-age-targeted deworming [28]. We estimate age-stratified community species-specific STH prevalence and describe individual, household and environmental factors associated with infection in this study population in Tamil Nadu.

## Methods

Reporting of this study has been verified in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist [29] (S1 STROBE Checklist).

### Ethical considerations

The DeWorm3 study was reviewed and approved by the Institutional Review Board at Christian Medical College, Vellore, India as well as the Institut de Recherche Clinique au Benin (IRCB) through the National Ethics Committee for Health Research, Ministry of Health in Benin, The London School of Hygiene and Tropical Medicine, The College of Medicine Research Ethics Committee in Malawi and the Human Subjects Division at the University of Washington. The trial is registered at ClinicalTrials.gov (NCT03014167). Prior to the initiation of the study in India, a technical review meeting was convened with government authorities at the national level and subsequently sensitization meetings were held with officials at the state, district and block levels. Meetings were held with local community leaders to explain the purpose of the study and procedures. Information sheets in Tamil, the local language, were provided to participants before each study activity. Written informed consent was sought from the household head for households' participation in the census. All LMC participants $\geq$18 years provided written informed consent while parental consent for participants $<$18 years was obtained along with verbal assent for children aged 7–11 years and written assent for children aged 12–17 years.

### Study setting

This study was carried out in two sub-sites in Tamil Nadu: the Timiri block in the Vellore Health Unit District (HUD) and selected villages in the Jawadhu Hills block of the Thiruvannamalai HUD (**Fig 1**). The last round of LF MDA was carried out in Vellore district in 2013 and Thiruvanamalai in 2015 [30]. The Timiri block is located in the plains of Vellore district and comprises four primary health centres (PHCs), each in turn divided into 25 health subcentres (HSCs). Each PHC serves a population of ~30,000 and each HSC ~5,000 respectively. This area has an average annual rainfall of 971 mm and has the following soil types; sandy and sandy loam 19%, red loam soil 20.8%, clay and clay loam 57.9% and black cotton soil 4.27% [31]. Although the most common occupation in this rural block is agriculture (20%), individuals also work in nearby industries as skilled and semi-skilled labourers. The Jawadhu Hills block comprises of three PHCs and is further subdivided into 13 HSCs with each PHC serving a mostly tribal population of ~20,000 and each HSC ~3,000 respectively. This block is located 762 metres above mean sea level in a reserve forest area. The tribal or indigenous people that populate this area are the Malayali and are classified as a scheduled tribe (disadvantaged communities or group of people listed in a schedule of the Indian constitution under article 342) by the government. The mean annual rainfall in Jawadhu Hills is 1100 mm, the mean maximum temperature is 36.6˚C and about 50% of the soil is red loamy clay and sandy soil. The main occupation is subsistence farming with more than 90% of the population involved in agricultural activities. Seasonal migration to nearby districts and states is common when residents work as semi-skilled labourers [32].

### Baseline census

The protocol and aims of the DeWorm3 trial have been previously published [28,33]. In the India site, 115 trained field workers conducted a baseline household census between October and December 2017 across 219 villages in Timiri and 154 villages in Jawadhu Hills. At each

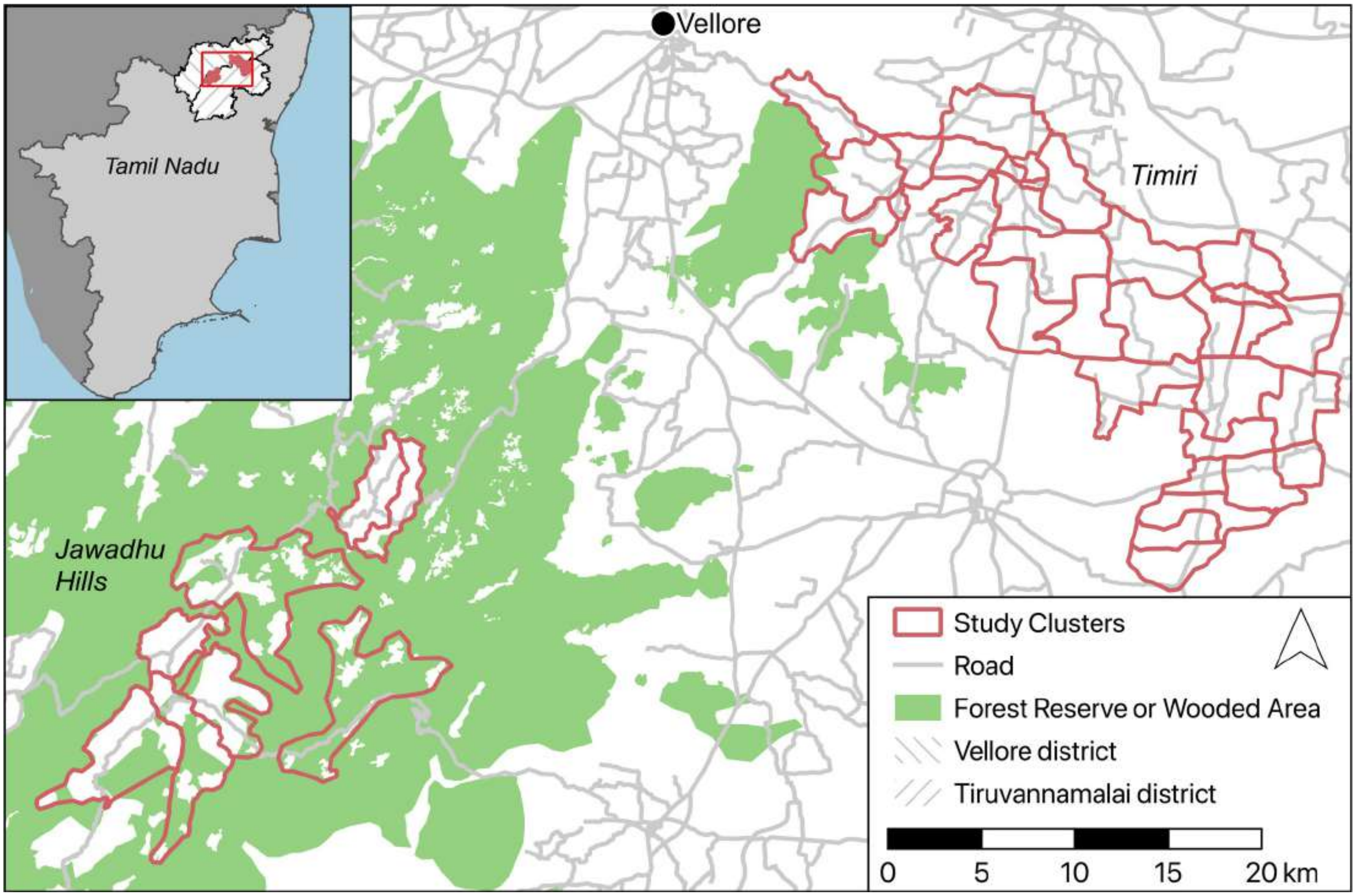


**Fig 1. Map of the Deworm3 India trial sub-sites at Timiri (32 clusters) and Jawadhu Hills (8 clusters) in Vellore and Thiruvanamalai districts* of Tamil Nadu (inset).** *District administrative boundaries obtained from gadm.org (https://gadm.org/license.html) and map data obtained from OpenStreetMap (https://www.openstreetmap.org/copyright).

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household, following informed written consent provided by the household head or equivalent adult, household- and individual-level data were collected with a questionnaire programmed using SurveyCTO software (Dobility, Inc; Cambridge, MA and Ahmedabad, India) on an Android smartphone [34]. Demographic details such as age and sex were collected for all household members and were verified using state or central Government of India (GOI) issued identification cards (Aadhar card, Electoral Identity Card, Driving Licence or Birth Certificate). Household-level information including number of persons in the family, assets, sources of income and access to water and sanitation facilities were collected. Housing characteristics such as flooring, roof and walls were observed by field workers. All households were provided a study ID card that contained the household ID linked QR code sticker, name of the head of household and the address. Global Positioning System (GPS) coordinates were collected for all households censused, as well as for all structures at which no household members were found on three separate visits—these were classified as vacant or non-residential structures.

## Cluster demarcation

Following the census, all households were allocated to one of 40 study clusters. Contiguous cluster boundaries were confirmed on the basis of administrative and geographical boundaries. Clusters were, on the whole, demarcated in line with HSCs, being divided along village boundaries where necessary, based on the requirement for clusters to comprise populations between 1,650 and 4,000. All villages within the study site boundaries were included. A total of 692 consented households from different health blocks that were located on the periphery of the cluster boundaries had been censused but were excluded during the demarcation process. A total of 32 clusters were defined in Timiri and 8 in Jawadhu Hills. In Jawadhu Hills, each HSC equated to a single cluster. While a majority of HSCs could be equated to single cluster in a Timiri, six HSCs were split into multiple clusters due to high population density.

## Survey design

Following the census and cluster demarcation, 6,000 individuals from the 40 clusters were recruited into a longitudinal monitoring cohort (LMC) to be followed up for five years. Age-stratified random sampling of 150 enumerated individuals with PSAC aged 1–4 years, SAC aged 5–14 years and adults aged 15 years and above in a ration of 1:1:3 was used to recruit 150 individuals in each cluster. All censused individuals above one year of age who were permanent residents, i.e. residing in the study area for more than six months and not planning to move out of the study area for the study duration, willing to provide informed consent (and assent where applicable) and provide samples, were eligible for recruitment. The survey was conducted between December 2017 to February 2018 in the local language spoken by all participants (Tamil) during which individual-level data including school enrolment, highest education level achieved, deworming history and shoe-wearing at the time of the survey were collected. Additionally, household water and sanitation facilities were observed, where possible, with indicators on construction materials and usage recorded. Data pertaining to household drinking water, sanitation facilities, and hygiene were collected according to the WHO UNICEF Joint Monitoring Program classification [35].

## Laboratory methods

A clean wide mouth container along with instructions on sample collection in Tamil, a wooden spatula and paper were provided to the participants. The containers had QR code stickers displaying the 9-digit participant ID, which was scanned upon receipt. The stool samples were transported on ice to the laboratory daily. All samples were read in duplicate by the Kato-Katz method by trained technicians who screened each slide (also labelled with the QR code sticker) for a minimum of six to eight minutes and within 30 minutes of preparation. The number of eggs in each slide was recorded on smartphones also using SurveyCTO software-based forms. The presence of other helminth ova and larvae was recorded but not quantified. With each batch, 10% of the slides were randomly checked by a supervisor for quality control. Presence of infection was determined if either one of the slides had at least one egg. Intensity of infection was calculated as the arithmetic mean of eggs per gram of faeces (EPG) by multiplying the eggs counted with a factor of 24 since the template used delivered 41.7 mg of stool. The WHO classification for intensity of infection for each of the species was used to categorise light, moderate and heavy intensity infections [36].

## Environmental data

Environmental and topographic data were explored as potential risk factors for infection [37]. Raster datasets on elevation and aridity at one km$^2$ resolution were obtained from the Consortium for Spatial Information [38]. Normalised Difference Vegetation Index (NDVI), Enhanced Vegetation Index (EVI), Middle Infrared (MIR) [39] and Land Surface Temperature (LST) [40] were produced by processing satellite images provided by the Moderate Resolution

Imaging Spectroradiometer (MODIS) instrument operating in the Terra spacecraft (NASA) at a resolution of 250m (NASA LP DAAC). Estimates of soil properties, such as sand fraction and soil acidity, were extracted from soilgrids.org at a resolution of 250m [41]. Environmental and topographic data were extracted using point-based extraction for each household using ArcGIS 10.3 (Environmental Systems Research Institute Inc. Redlands, CA, US).

## Statistical methods

Descriptive statistics of the baseline LMC characteristics were generated and prevalence was calculated in the three age categories (PSAC, SAC and adult). Age- and cluster-population-weighted estimates were calculated using the proportion of the censused population living in the cluster. Population density per $km^2$ was estimated through totalling the number of censused individuals falling within a one $km^2$ buffer placed around each household in ArcGIS. For households near study area boundaries, the number of censused individuals was divided by the buffer area falling within the boundary. Principal component analysis (PCA) in line with Filmer and Pritchett's widely used method [42] was used to arrive at a composite wealth index using various assets that were available to the households that included ownership of cooking fuel, electricity, radio, stove, DVD, television, computer, refrigerator, sofa set, mattress, solar lamp, ceiling fan, watch, mobile phone bicycle, motorcycle, autorickshaw, cart, car, livestock, house ownership, and housing materials. Cronbach's alpha assessed the dimensionality of the items included in the composite wealth index, and an item-rest correlation of 0.1 was set as a minimum threshold for including the item in the PCA. Variables with item-rest correlation less than 1 were removed and Cronbach's alpha value was computed again to see if all the variables were pointing to a similar direction with overall alpha set at 0.7. The wealth indices were divided into five SES quintiles, 1 being low and 5 being high. Household water source and sanitation facilities were categorised as improved and unimproved facilities for analyses according to JMP guidelines (JMP).

Univariable and multivariable mixed-effects multilevel logistic regression analysis was performed to build a model to assess the association between exposure factors and presence of infection, accounting for clustering at the household, village, and cluster level. Since STHs are highly aggregated and distributed in a negative binomial manner, the association between the intensity (EPG) of infection and associated factors was analysed using mixed-effects negative binomial regression method of the egg counts offsetting the actual quantity of stool used per sample and accounting for the clustering at all the levels. To fit the models, all significant ($p<0.05$) variables in the mixed-effects multilevel univariable analyses, were included in the multivariable model and a backwards stepwise approach was used to arrive at the most parsimonious models. Data with more than 500 missing values were excluded from multivariable analysis. Data management and analyses were performed using STATA version 16.0 (STATA Corporation, College Station, TX, USA).

# Results

The baseline census at the two sub-sites in India, Timiri and Jawadhu hills, enumerated 36,536 households comprising 140,932 individuals across an area of 477 $km^2$ between October and December 2017 (**Fig 2**). The demographic spread of the censused population was found to be skewed to the older ages, with 77.2% aged 15 years or above, the sex ratio was 1:1 and 30% of household heads reported having some secondary education or above. Only 34.6% of the households reported having access to improved sanitation, whereas 95.8% had access to an improved water source. Based on the census, the study area was then demarcated into 40 clusters. Final cluster sizes ranged from 494 to 1509 households and 2037 to 6002 individuals with

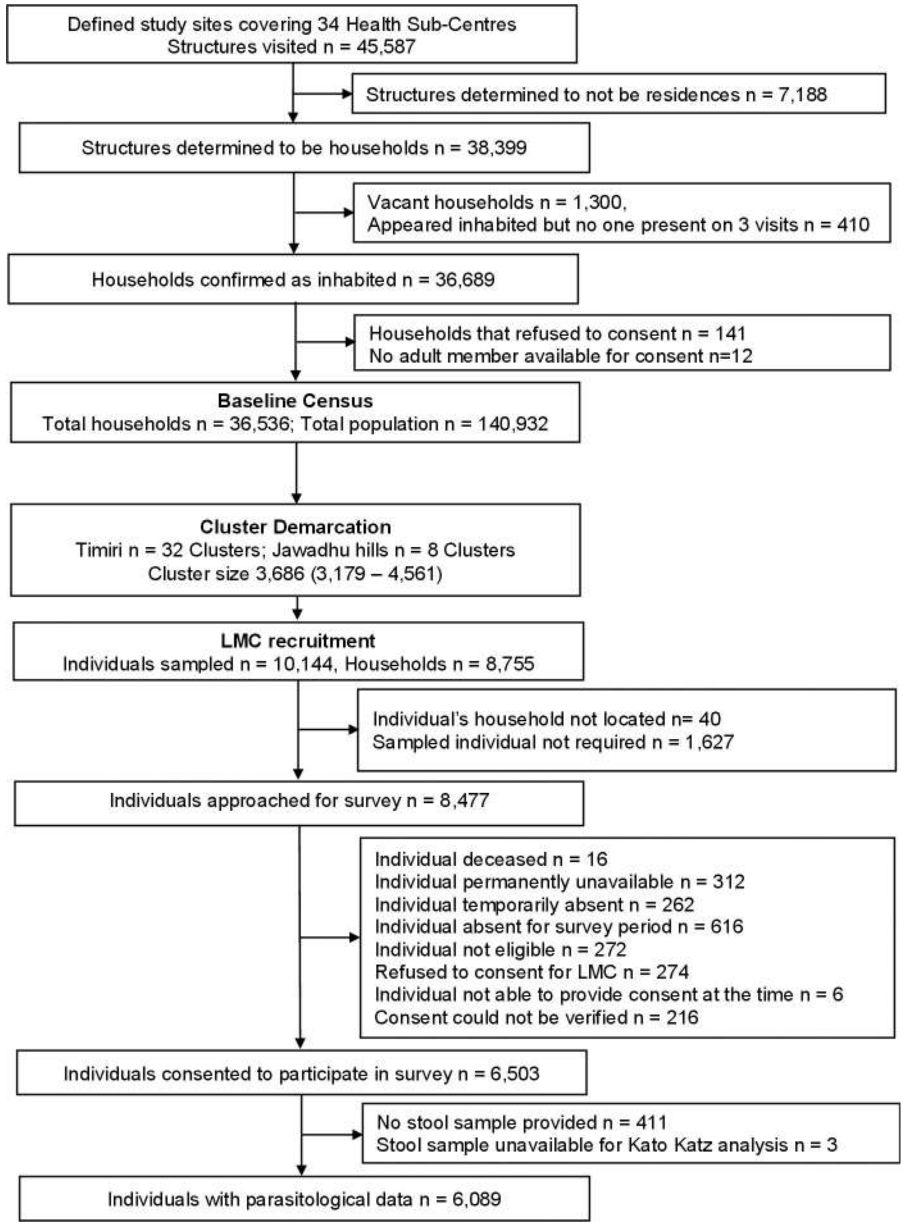


**Fig 2. Flow chart of activities during the baseline census and recruitment of the longitudinal monitoring cohort (October 2017- February 2018) Footnote: "Not required" refers to the number of individuals in the ranked, age-stratified, cluster-specific reserve/replacement lists generated who were not approached as the cohort sample size was achieved in the cluster.**

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a mean of 989 (SD 273) and 3820 (SD 1072) households and individuals respectively. The median cluster area was 11.4 (interquartile range [IQR]: 7.5–15.7) $km^2$.

## Longitudinal monitoring cohort enrolment

From the age-stratified list of 10,144 individuals sampled for recruitment to the LMC, 8517 were approached between December 2017 and February 2018. Of these, 6998 (82.2%) were present and 6503 (92.9%) individuals consented to participate in the study. Among those who consented, 6370 (98.0%) completed the survey questionnaire and 6089 (93.6%) provided an adequate stool sample for examination (**Fig 2**). Of the 6089 participants recruited from 5474 households in 368 villages, 1179 (19.4%), 1305 (21.4%), and 3605 (59.2%) were PSAC, SAC, and adults respectively. Recruitment of the target of 150 individuals was achieved in all the 40 clusters. When those who refused to participate in the LMC (n = 274) were compared to those who consented (n = 6503), the populations were broadly similar across characteristics, although the proportions of adults were higher in the group who refused (79.2% vs 59.9%), and in this group there was a higher proportion belonging to households in the high SES quintile (38.0% vs 22.6%), and where the head of the household had higher secondary or college education (18.3% vs 10.4%) (S1 Table). Among those who consented to participate but did not provide stool during the survey (n = 411), again, a higher proportion were adults (70.3%) than those who did provide a sample (59.2%).

## Characteristics of the LMC participants and their households at enrolment

The median age of the recruited participants in each category was 3.1 years (IQR: 2.2–4.0) among PSAC, 10.0 years (IQR: 7.3–12.6) among SAC and 40.0 years (IQR: 27.7–53.1) among adults. Participation among females (3252, 53.4%) was slightly higher compared to males (2836, 46.6%) (ratio 1.15). The LMC population was very closely representative of the census population, with the exception of age group, due to the age-stratified sampling. A third of participants came from households where the household head had no education. Among enrolled participants 3370 (55.4%) lived in houses made of man-made materials (improved) and 1510 (24.8%) lived in houses made of natural, non-durable materials (unimproved) with 10.4% and 9.4% living in houses made of mixed materials and government provided housing respectively. When flooring alone was categorized, 5370 (88.2%) had flooring of man-made materials as opposed to natural flooring. The majority of households used improved water source facilities (5824, 95.7%). However, 3968 or 65.2% of participants used unimproved sanitation facilities (including pit latrine without platform or open pit or open defaecation). Nearly half the individuals lived in households where livestock were owned (2745, 45.1%) and farming was carried out (2590, 44.5%).

## Prevalence and intensity of STH

The unweighted prevalence of any STH in the LMC at enrolment was 17% (95% CI: 16.0–17.9%) (**Table 1**). When weighted by age and cluster size the prevalence was 21.4% (95% CI: 20.4–22.4). Hookworm was the most common STH species detected with an unweighted prevalence of 16.6% (95% CI: 15.7–17.6) and weighted prevalence of 21% (95% CI: 20.0–22.2). Six individuals in the LMC had *Ascaris lumbricoides* and 17 had *Trichuris trichiura* infections, respectively, while two individuals with a dual infection were detected (*Ascaris* and hookworm). The mean intensity of hookworm infections was 634 EPG (SD 1493.6, median 198, IQR: 72–552) with EPG counts ranging from 12 to 18,756 EPG. Among the 6 individuals infected with *Ascaris*, the mean intensity was 1116 EPG (SD 1847.4, median 360, IQR: 216–1188) and ranged from 24 to 4848 EPG. Similarly, among the 17 individuals infected with *Trichuris*, the mean intensity was 209.6 EPG (SD 518.5, median 96, IQR 48–96) and ranged from 12 to 2196 EPG. Among the 1033 individuals who were positive for any STH, a very small proportion were found to have moderate to heavy intensity (MHI) infections for any STH species (n = 73, unweighted estimate 1.2%, 95% CI: 0.9–1.5%) and this was seen across all age categories (**Table 1**). Nearly all MHI were due to hookworm except for one *Trichuris* infection. Six other helminth species were also identified in 132 (2.2%) LMC participants during the survey and the more common species were *Enterobius vermicularis* (96, 1.6%) and *Hymenolepis nana* (27, 0.4%) (S2 Table).

**Table 1. Prevalence and intensity of soil transmitted helminths in the surveyed participants by age categories.**

| n = 6089 | Any STH | Hookworm | *Ascaris* | *Trichuris* |
|---|---|---|---|---|
| ***Unweighted prevalence n (%)*** | 1033 (17.0) | 1012 (16.6) | 6 (0.1) | 17 (0.3) |
| PSAC (1179) | 35 (3.0) | 31 (2.6) | 2 (0.2) | 2 (0.2) |
| SAC (1305) | 92 (7.0) | 87 (6.7) | 1 (0.1) | 5 (0.4) |
| Adult (3605) | 906 (25.1) | 894 (24.8) | 3 (0.1) | 10 (0.3) |
| ***Intensity of infections among positives n (%)*** | | | | |
| ***PSAC (n = 35)*** | | | | |
| Light-intensity | 32 (91.4) | 28 (90.3) | 2 (100) | 2 (100) |
| Moderate-intensity | 3 (8.6) | 3 (9.7) | 0 (0.0) | 0 (0.0) |
| Heavy-intensity | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| ***SAC (n = 92)*** | | | | |
| Light-intensity | 87 (94.6) | 83 (95.4) | 1 (100) | 4 (80.0) |
| Moderate-intensity | 4 (4.3) | 3 (3.4) | 0 (0.0) | 1 (20) |
| Heavy-intensity | 1 (1.1) | 1 (1.1) | 0 (0.0) | 0 (0.0) |
| ***Adult (n = 906)*** | | | | |
| Light-intensity | 841 (92.8) | 829 (92.7) | 3 (100) | 10 (100) |
| Moderate-intensity | 43 (4.7) | 43 (4.8) | 0 (0.0) | 0 (0.0) |
| Heavy-intensity | 22 (2.4) | 22 (2.5) | 0 (0.0) | 0 (0.0) |

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## Individual and household characteristics associated with hookworm infection

As infections with *Ascaris* and *Trichuris* were very low, further analysis was carried out only for hookworm infections. The results of the univariable and multivariable mixed-effects logistic regression analysis for hookworm infection are presented in **Table 2**. In the univariable analysis, individual and household factors were associated with hookworm infection and nearly half of these variables remained significant in the multivariable mixed-effects logistic regression analysis. PSAC, SAC and adults had a hookworm prevalence of 2.6% (95% CI: 1.8–3.7), 6.7% (95% CI: 5.4–8.2), and 24.8% (95% CI: 23.4–26.2) respectively. In the multivariable regression, SAC (multivariable odds ratio [mOR] 3.8, 95% CI: 2.3–6.3) and adults (mOR 21.4, 95% CI: 12.3–37.2) were more likely to be infected ($p<0.001$) compared to PSAC (**Table 2**, **Fig 3**). Sex was not found to be associated with hookworm infection at the univariable or multivariable level. Among the other individual characteristics analysed, those who had a history of deworming in the past 12 months were less likely to be infected than those who did not (mOR 0.3, 95%CI: 0.2–0.5, $p<0.001$) but wearing shoes (based on observations during the survey) was not associated with reduced hookworm infection risk. After accounting for other variables, migratory status was no longer associated with hookworm infection and neither was livestock ownership or family size.

At the household level, a decreasing prevalence of hookworm was seen as education of the head of household increased, with a prevalence of 27.3% among those belonging to a household where the head had no education and 7.7% among those with household heads having higher secondary or college education. In the multivariable model the odds of infection were significantly lower among those with household heads having higher secondary or college education than in individuals from a household where the head had no education ($p<0.001$). Female literacy in the family also showed a similar correlation but was not included in the multivariable analysis. A decrease in odds of hookworm infection with increase in socioeconomic status of the household was also seen (mOR 0.3 per quintile, 95% CI: 0.2–0.5, $p<0.001$) (**Fig 4**). Although belonging to a household with flooring made of man-made materials was found

**Table 2. Univariable and multivariable analysis of factors associated with hookworm infection.**

| | Census (n = 140932) | LMC (n = 6089) | Hookworm infected (n = 1012) | Univariable | | Multivariable | |
|---|---|---|---|---|---|---|---|
| | n (%) | n (%) | n (%) | OR (95%CI) | p | mOR (95% CI) | p |
| **Individual factors** | | | | | | | |
| **Age** | | | | | | | |
| Infants (<1 year) | 1750 (1.2) | 0 (0) | 0 (0) | | | | |
| PSAC (1–4 years) | 8482 (6.0) | 1179 (19.4) | 31 (2.6) | 1 | <0.001 | 1 | **<0.001** |
| SAC (5–14 years) | 21839 (15.5) | 1305 (21.4) | 87 (6.7) | 3.3 (2.0, 5.4) | | 3.8 (2.3,6.3) | |
| Adult (15+ years) | 108861 (77.2) | 3605 (59.2) | 894 (24.8) | 27.2 (15.3, 48.3) | | 21.4 (12.3, 37.2) | |
| **Sex*** | | | | | | | |
| Male | 70295 (49.9) | 2836 (46.6) | 460 (16.2) | 1 | 0.215 | | |
| Female | 70620 (50.1) | 3252 (53.4) | 552 (17.0) | 1.1 (0.9, 1.3) | | | |
| **Lived there most of past 6 months** | | | | | | | |
| Yes | 137144 (97.3) | 6041 (99.2) | 1006 (16.7) | 1 | 0.037 | | |
| No | 3788 (2.7) | 48 (0.8) | 6 (12.5) | 0.3 (0.1, 0.9) | | | |
| **Slept there last night** | | | | | | | |
| Yes | 133153 (94.5) | 5975 (98.1) | 992 (16.6) | 1 | 0.225 | | |
| No | 7779 (5.5) | 114 (1.9) | 20 (17.5) | 0.7 (0.4, 1.3) | | | |
| **Wearing shoes during survey†** | | | | | | | |
| No | - | 3273 (54.8) | 609 (18.6) | 1 | 0.899 | | |
| Yes | - | 2698 (45.2) | 390 (14.5) | 1.0 (0.8, 1.2) | | | |
| **Dewormed in last 12 months†** | | | | | | | |
| No | - | 4963 (83.1) | 942 (19.0) | 1 | <0.001 | 1 | **<0.001** |
| Yes | - | 1008 (16.9) | 57 (5.7) | 0.2 (0.1, 0.2) | | 0.3 (0.2,0.5) | |
| **Household factors** | | | | | | | |
| **Population density ‡** | | | | | | | |
| <50 | 6750 (4.8) | 262 (4.3) | 79 (30.1) | 1 | 0.416 | | |
| 50–249 | 70424 (50.0) | 3091 (50.8) | 648 (21.0) | 0.9 (0.6, 1.3) | | | |
| 250–999 | 55013 (39.1) | 2386 (39.2) | 268 (11.2) | 0.9 (0.6, 1.4) | | | |
| > = 1000 | 8956 (6.1) | 344 (5.7) | 16 (4.7) | 0.5 (0.2, 1.2) | | | |
| **Family size** | | | | | | | |
| < = 4 members | 71670 (50.9) | 2899 (47.6) | 556 (19.5) | 1 | <0.001 | | |
| > = 5 members | 69262 (49.2) | 3190 (52.4) | 446 (14.0) | 0.6 (0.5, 0.7) | | | |
| **Farming household§** | | | | | | | |
| No | 75470 (56.1) | 3235 (55.5) | 571 (17.7) | 1 | 0.082 | | |
| Yes | 59027 (43.9) | 2590 (44.5) | 414 (16.0) | 1.2 (1.0, 1.4) | | | |
| **Livestock possession** | | | | | | | |
| No | 78179 (55.5) | 3344 (54.9) | 446 (13.3) | 1 | 0.197 | | |
| Yes | 62753 (44.5) | 2745 (45.1) | 566 (20.6) | 1.1 (0.9, 1.3) | | | |
| **Highest education level of any female family member¶** | | | | | | | |
| No education | 38426 (27.5) | 1509 (25.0) | 463 (30.7) | 1 | <0.001 | | |
| Some primary | 18894 (13.5) | 812 (13.5) | 143 (17.6) | 0.7 (0.6, 1.0) | | | |
| Some middle | 24089 (17.3) | 1091 (18.1) | 139 (12.7) | 0.5 (0.3, 0.6) | | | |
| Some secondary | 27731 (19.9) | 1318 (21.9) | 141 (10.7) | 0.4 (0.3, 0.5) | | | |
| Some higher secondary / college | 30451 (21.8) | 1301 (21.6) | 112 (8.6) | 0.3 (0.2, 0.4) | | | |

(*Continued*)

Table 2. (Continued)

| | Census (n = 140932) | LMC (n = 6089) | Hookworm infected (n = 1012) | Univariable | | Multivariable | |
|---|---|---|---|---|---|---|---|
| | n (%) | n (%) | n (%) | OR (95%CI) | p | mOR (95% CI) | p |
| **Highest education level of household head#** | | | | | | | |
| No education | 46198 (33.0) | 1835 (30.4) | 500 (27.3) | 1 | <0.001 | 1 | **<0.001** |
| Some primary | 25705 (18.4) | 1121 (18.6) | 167 (14.9) | 0.6 (0.5, 0.8) | | 0.7 (0.6, 1.0) | |
| Some middle | 26124 (18.7) | 1186 (19.6) | 158 (13.3) | 0.5 (0.4, 0.7) | | 0.7 (0.5, 0.9) | |
| Some secondary | 27900 (19.9) | 1267 (21.0) | 129 (10.2) | 0.4 (0.3, 0.5) | | 0.6 (0.5, 0.8) | |
| Some higher secondary / college | 14112 (10.1) | 634 (10.5) | 49 (7.7) | 0.3 (0.2, 0.4) | | 0.4 (0.3, 0.6) | |
| **Household floor**** | | | | | | | |
| Natural materials | 16334 (11.6) | 708 (11.7) | 220 (31.1) | 1 | 0.004 | | |
| Manmade materials | 124437 (88.4) | 5370 (88.4) | 791 (14.7) | 0.7 (0.5, 0.9) | | | |
| **SES (1 = Low, 5 = High)** | | | | | | | |
| 1 | 23884 (16.9) | 937 (15.4) | 364 (38.9) | 1 | <0.001 | 1 | **<0.001** |
| 2 | 26674 (18.9) | 1100 (18.1) | 228 (20.7) | 0.5 (0.4, 0.7) | | 0.6 (0.4, 0.8) | |
| 3 | 27545 (19.5) | 1251 (20.6) | 165 (13.2) | 0.4 (0.3, 0.6) | | 0.6 (0.4, 0.8) | |
| 4 | 30689 (21.8) | 1424 (23.4) | 147 (10.3) | 0.3 (0.2, 0.5) | | 0.4 (0.3, 0.6) | |
| 5 | 32140 (22.8) | 1377 (22.6) | 108 (7.8) | 0.2 (0.2, 0.3) | | 0.3 (0.2, 0.5) | |
| **Water source** | | | | | | | |
| Unimproved facilities | 5937 (4.2) | 265 (4.4) | 116 (43.8) | 1 | 0.213 | | |
| Improved facilities | 134995 (95.8) | 5824 (95.7) | 896 (15.4) | 0.8 (0.5, 1.2) | | | |
| **Sanitation facilities** | | | | | | | |
| Unimproved facilities | 92216 (65.4) | 3968 (65.2) | 821 (20.7) | 1 | <0.001 | | |
| Improved facilities | 48716 (34.6) | 2121 (34.8) | 191 (9.0) | 0.6 (0.5, 0.8) | | | |
| **Handwashing facility§§** | | | | | | | |
| No | - | 3687 (66.3) | 734 (19.9) | 1 | <0.001 | | |
| Yes | - | 1873 (33.7) | 195 (10.4) | 0.6 (0.5, 0.7) | | | |
| **Environmental factors** | | | | | | | |
| **Mean normalised difference vegetation index (NDVI)‡** | | | | | | | |
| First tertile | 47073 (33.4) | 1959 (32.2) | 268 (13.7) | 1 | 0.031 | 1 | **0.007** |
| Second tertile | 46850 (33.3) | 2059 (33.9) | 332 (16.1) | 1.4 (1.1, 1.7) | | 1.5 (1.2, 2.0) | |
| Third tertile | 46860 (33.3) | 2065 (34.0) | 411 (19.9) | 1.4 (1.1, 1.8) | | 1.4 (1.1, 1.9) | |
| **Mean of middle infrared (MIR)‡** | | | | | | | |
| First tertile | 47136 (33.5) | 2056 (33.8) | 424 (20.6) | 1 | 0.175 | | |
| Second tertile | 46761 (33.2) | 2071 (34.1) | 328 (15.8) | 0.8 (0.6, 1.1) | | | |
| Third tertile | 46886 (33.3) | 1956 (32.2) | 259 (13.2) | 0.8 (0.6, 1.0) | | | |
| **Elevation‡** | | | | | | | |
| First tertile | 47036 (33.4) | 2053 (33.8) | 245 (11.9) | 1 | <0.001 | 1 | **<0.001** |
| Second tertile | 47455 (33.7) | 2014 (33.1) | 151 (7.5) | 1.0 (0.6, 1.6) | | 0.9 (0.6, 1.5) | |
| Third tertile | 46292 (32.9) | 2016 (33.1) | 615 (30.5) | 4.2 (2.4, 7.2) | | 3.9 (2.3, 6.8) | |
| **Sand fraction‡** | | | | | | | |
| First tertile | 50457 (35.8) | 2436 (40.1) | 313 (12.9) | 1 | 0.177 | | |
| Second tertile | 50588 (35.9) | 1643 (27.0) | 193 (11.8) | 0.8 (0.7, 1.1) | | | |
| Third tertile | 39738 (28.2) | 2004 (32.9) | 505 (25.2) | 1.1 (0.8, 1.5) | | | |
| **Soil acidity (pH KCL)‡** | | | | | | | |
| First tertile | 66905 (47.5) | 2656 (43.7) | 605 (22.8) | 1 | 0.289 | | |
| Second tertile | 28233 (20.1) | 1596 (26.2) | 167 (10.5) | 0.8 (0.6, 1.1) | | | |

(*Continued*)

**Table 2.** (Continued)

| | Census (n = 140932) | LMC (n = 6089) | Hookworm infected (n = 1012) | Univariable | | Multivariable | |
|---|---|---|---|---|---|---|---|
| | n (%) | n (%) | n (%) | OR (95%CI) | p | mOR (95% CI) | p |
| Third tertile | 45645 (32.4) | 1831 (30.1) | 239 (13.1) | 0.8 (0.5, 1.2) | | | |

*1 missing value

†118 missing values

‡ 6 missing values

§ 264 missing values

¶ 58 missing values and not included in the multivariable model as education of the head of the household was included

# 46 missing values

**11 missing values

§§529 missing values, not included in the multivariable model due to high number of missing values

Natural flooring includes earth, dung, palm or bamboo and stone; Man-made flooring includes wood, brick, vinyl or asphalt strips, tiles, cement, carpet and polished stone like marble or granite

Improved water source refers to limited and basic facilities, Unimproved refers to surface water and unimproved facilities

Improved sanitation refers to limited and basic facilities, Unimproved refers to open defaecation and unimproved facilities

Acronyms: LMC- Longitudinal Monitoring Cohort, OR–odds ratio, mOR–multivariable OR, 95% CI—95% confidence interval

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to be associated with decreased odds initially (OR 0.7, 95%CI: 0.5–0.9, p = 0.004), after accounting for other variables, this did not remain significant in the multivariable analysis. When household WASH factors were analysed in the univariable analysis, those belonging to households with improved sanitation and having handwashing facilities had a reduced odds of infection, (OR 0.6, 95% CI: 0.5–0.8, p<0.001) and (OR 0.6, 95% CI: 0.5–0.7 p<0.001) respectively. Sanitation did not remain significant in the multivariable analysis and handwashing was not analysed further due to missing data.

## Factors associated with intensity of hookworm infection

The mean (SD) EPG in PSAC, SAC and adults was 538.8 (847.7), 389.7 (746.3), 661.1 (1562.3) respectively with a trend of increased EPG in adults seen in both sexes (Fig 3). In the multivariable analysis (Table 3), an increased infection intensity ratio (IIR) was seen for both SAC (multivariable IIR [mIIR] 9.2, 95% CI: 4.3–19.7) and adults (mIIR 332.5, 95% CI: 166.4–664.5, p<0.001) compared to PSAC. Although female sex appeared associated with higher intensity infections, after accounting for other associated factors, this relationship was no longer significant. Those who had a history of deworming in the past 12 months had a lower EPG (mIIR 0.1, 95% CI: 0.1–0.2, p<0.001). No other factors at the individual level were associated with intensity of infection.

At the household level, decreasing intensity of hookworm as education of the head of household increased was seen (mIIR 0.2, 95%CI: 0.1–0.5, p<0.001). Female education also showed a similar correlation with intensity of infection. The association with SES was also similar to that seen with presence of infection (Fig 4) with a decreasing intensity of hookworm infection with increase in socioeconomic status of the household (mIIR 0.2, 95% CI: 0.1–0.4, p<0.001). When household WASH factors were analysed, those belonging to households with improved sanitation had a lower infection intensity than those residing in households with unimproved facilities (mIIR 0.6, 95%CI: 0.4–0.9). Belonging to households with a handwashing facility was also associated with lower intensity compared to households that did not have facilities in the univariable analysis (IIR 0.3, 95% CI: 0.2–0.5).

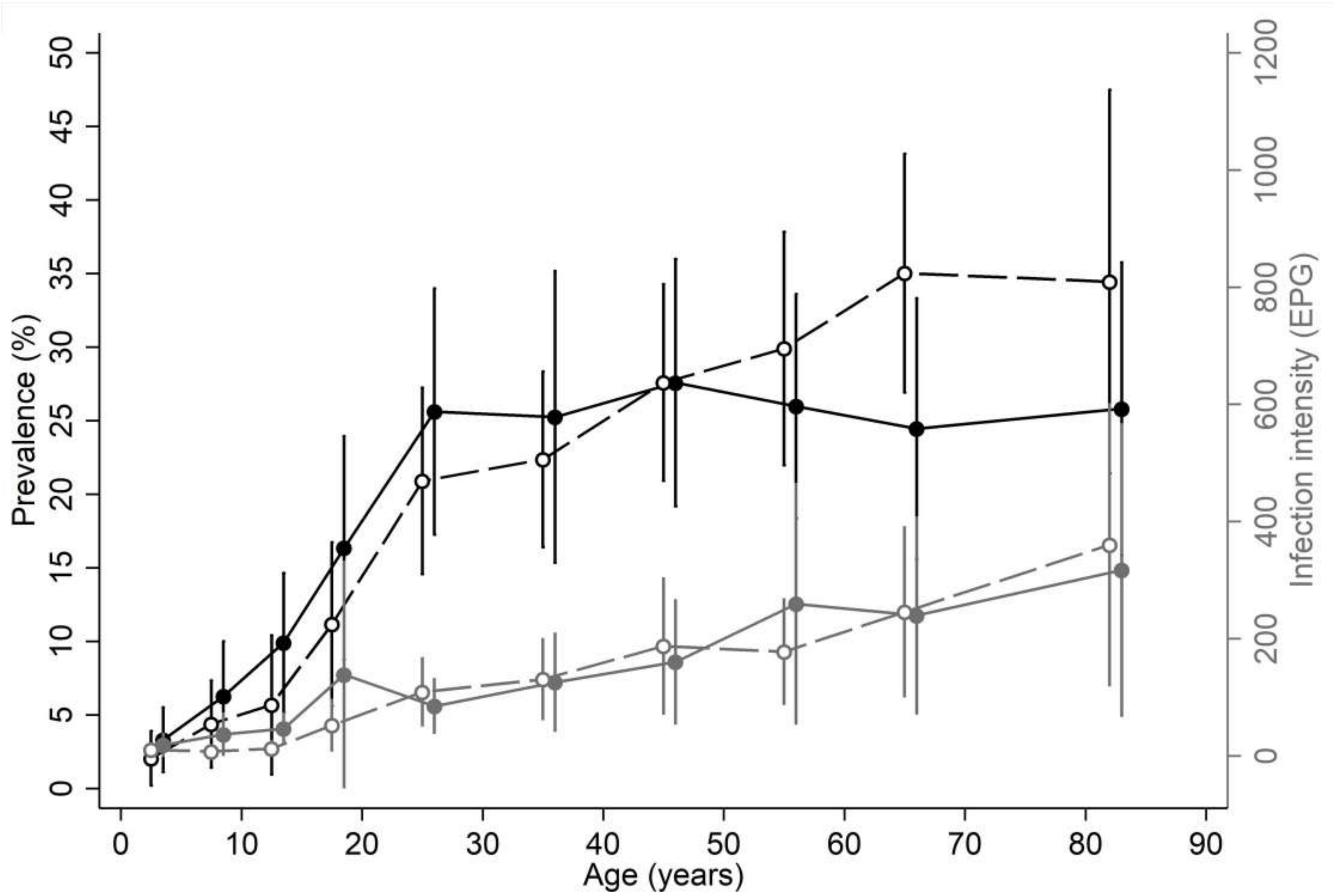


**Fig 3.** Age-infection profiles of hookworm among surveyed participants at enrolment—prevalence (black lines) and intensity (grey lines) of hookworm infection for males (solid line and circles) and females (dashed lines and empty circles).

https://doi.org/10.1371/journal.pntd.0009338.g003

### Environmental risk factors associated with presence and intensity of hookworm infection

Assessment of environmental factors in the multivariable analysis indicated that higher vegetation coverage (Normalized Difference Vegetation Index or NDVI) (mOR 1.4, 95% CI: 1.1–1.9) and higher elevation (mOR 3.9; 95% CI: 2.3–6.8) were associated with the increased odds of hookworm infection. Upon assessing the environmental factors associated with intensity of infection the same parameters were associated with increased egg counts (mIIR for NDVI 2.4, 95%CI: 1.4–3.9, $p<0.001$ and for elevation, mIIR 14.1, 95%CI: 6.5–30.7, $p<0.001$). Among environmental parameters, the aridity index was not included in the analysis as all households in the study site were in the same sub-humid category (range among households 0.54–0.62). Enhanced vegetation index (EVI) and land surface temperature (LST) were also excluded as they were highly correlated with NDVI and elevation respectively.

## Discussion

The results of this parasitological survey conducted in an age-stratified cohort of 6089 individuals nested within the censused Deworm3 trial population of 140,932 individuals in southern

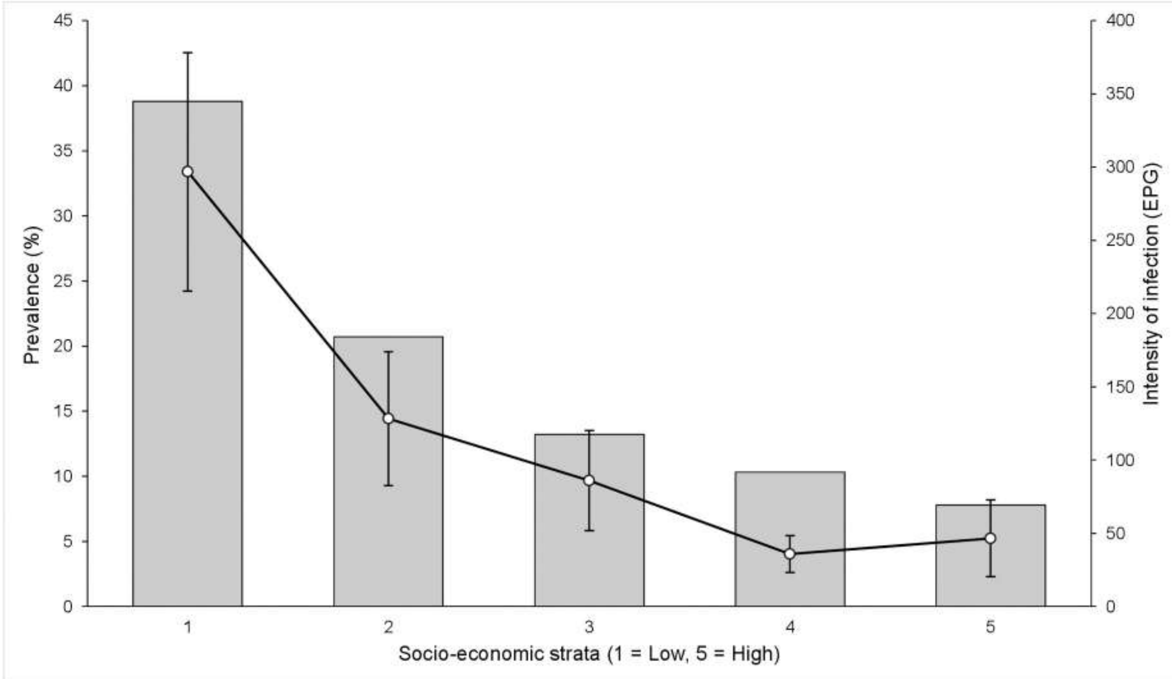


**Fig 4.** Prevalence and intensity of hookworm among surveyed participants stratified by categories of socio-economic status—prevalence (bars) and intensity (lines).

https://doi.org/10.1371/journal.pntd.0009338.g004

India showed that hookworm was the most common STH infection in this region. The prevalence of hookworm was high and consequently remains a significant public health problem. Our study site is unique as it incorporates two subsites—a rural plain area of 32 clusters in Timiri and a difficult-to-reach, hilly area of 8 clusters in Jawadhu Hills with a mostly tribal population. The censused population comprehensively described the communities and age demographic profile that is typical of the region and the LMC surveyed was representative of the population enumerated. Our study indicated that a range of individual, household and environmental factors in these communities including age, SES, education, sanitation and vegetation influence both the odds of infection and the intensity of infection.

As reported in the pooled analysis [43], the age-weighted prevalence of STH in the India site was substantially higher (21.4%) than both the Malawi and Benin study sites, with the vast majority of infections attributable to hookworm. Only a small proportion of infections were of MHI (1.5%). Due to the low number of *Ascaris* and *Trichuris* infections detected, further analysis was limited to factors associated with hookworm prevalence and intensity in this population. While no previous data are available from the Timiri area, previous studies at Jawadhu Hills have shown a high prevalence of hookworm—38% in 2011–12 and 18.5% in 2013–14—despite multiple rounds of treatment with albendazole in the district as part of the LF control program [44,45]. Data collected from the neighbouring district, Villupuram, in Tamil Nadu in 2000, prior to the commencement of the LF programme involving combined administration of Albendazole, found an STH prevalence of 60% among children aged 9–10 years, with particularly high *Ascaris* prevalence. A 70% decrease in STH prevalence after three rounds of MDA with DEC and Albendazole was recorded among SAC [46]. These reductions in prevalence over time and the very low proportion of MHI infections observed in this study would suggest that, despite the ongoing transmission of hookworm, the LF programme and the school-based

**Table 3. Univariable and multivariable analysis of factors associated with intensity of hookworm infection.**

| | EPG (N = 1012)* | Univariable | | Multivariable | |
|---|---|---|---|---|---|
| | Arithmetic mean (SD) | IIR (95%CI) | p | mIIR (95%CI) | p |
| **Individual factors** | | | | | |
| **Age** | | | | | |
| PSAC (1–4 years) | 538.8 (847.7) | 1 | <0.001 | 1 | **<0.001** |
| SAC (5–14 years) | 389.7 (746.3) | 7.4 (3.6, 15.5) | | 9.2 (4.3, 19.7) | |
| Adult (15+ years) | 661.1 (1562.3) | 525.2 (259.3, 1063.8) | | 332.5 (166.4, 664.5) | |
| **Sex†** | | | | | |
| Male | 664.7 (1682.5) | 1 | 0.017 | | |
| Female | 608.4 (1316.6) | 1.5 (1.1, 2.0) | | | |
| **Lived there most of past 6 months** | | | | | |
| Yes | 530.0 (978.0) | 1 | 0.058 | | |
| No | 634.6 (1496.4) | 0.2 (0.0, 1.1) | | | |
| **Slept there the previous night** | | | | | |
| Yes | 480.6 (742.9) | 1 | 0.213 | | |
| No | 637.1 (1504.9) | 0.5 (0.1, 1.5) | | | |
| **Wearing shoes during survey‡** | | | | | |
| No | 638.9 (1509.1) | 1 | 0.304 | | |
| Yes | 618.9 (1473.6) | 0.8 (0.5, 1.2) | | | |
| **Dewormed in last 12 months‡** | | | | | |
| No | 636.8 (1503.3) | 1 | <0.001 | 1 | **<0.001** |
| Yes | 536.4 (1352.1) | 0.0 (0.0, 0.0) | | 0.1 (0.1, 0.2) | |
| **Household factors** | | | | | |
| **Population density§** | | | | | |
| <50 | 593.5 (792.2) | 1 | 0.015 | | |
| 50–249 | 659.2 (1632.9) | 1.0 (0.5, 2.3) | | | |
| 250–999 | 610.9 (1336.7) | 0.8 (0.3, 1.9) | | | |
| > = 1000 | 219.8 (213.8) | 0.2 (0.1, 0.7) | | | |
| **Family size** | | | | | |
| < = 4 members | 652.9 (1374.5) | 1 | <0.001 | | |
| > = 5 members | 610.1 (1633.5) | 0.4 (0.3, 0.6) | | | |
| **Farming household¶** | | | | | |
| No | 722.4 (1691.4) | 1 | 0.483 | | |
| Yes | 534.1 (1210.2) | 1.1 (0.8, 1.6) | | | |
| **Livestock possession** | | | | | |
| No | 627.6 (1214.9) | 1 | 0.178 | | |
| Yes | 639.0 (1682.0) | 1.3 (0.9, 1.7) | | | |
| **Highest education level of any female family member#** | | | | | |
| No education | 718.6 (1682.3) | 1 | <0.001 | | |
| Some primary | 460.0 (962.4) | 0.5 (0.3, 0.9) | | | |
| Some middle | 652.6 (1361.2) | 0.4 (0.2, 0.6) | | | |
| Some secondary | 624.9 (1736.1) | 0.2 (0.1, 0.4) | | | |
| Some higher secondary / college | 475.2 (1012.7) | 0.1 (0.1, 0.2) | | | |
| **Highest education level of household head****| | | | | |
| No education | 670.8 (1563.8) | 1 | <0.001 | 1 | **<0.001** |
| Some primary | 777.1 (1897.2) | 0.5 (0.3, 0.8) | | 0.6 (0.4, 1.0) | |
| Some middle | 592.3 (1132.1) | 0.4 (0.2, 0.6) | | 0.5 (0.3, 0.8) | |
| Some secondary | 385.5 (1111.8) | 0.2 (0.1, 0.3) | | 0.4 (0.2, 0.6) | |

(*Continued*)

**Table 3.** (Continued)

| | EPG (N = 1012)* | Univariable | | Multivariable | |
|---|---|---|---|---|---|
| | **Arithmetic mean (SD)** | **IIR (95%CI)** | **p** | **mIIR (95%CI)** | **p** |
| Some higher secondary / college | 640.4 (1150.9) | 0.1 (0.1, 0.2) | | 0.2 (0.1, 0.5) | |
| **Household floor‡‡** | | | | | |
| Natural materials | 669.1 (1588.3) | 1 | 0.013 | | |
| Manmade materials | 624.6 (1468.0) | 0.5 (0.3, 0.9) | | | |
| **SES (1 = Low, 5 = High)** | | | | | |
| 1 | 764.0 (1786.7) | 1 | <0.001 | 1 | **<0.001** |
| 2 | 618.6 (1555.6) | 0.5 (0.3, 0.9) | | 0.5 (0.3, 0.8) | |
| 3 | 651.7 (1327.8) | 0.4 (0.2, 0.7) | | 0.5 (0.3, 0.9) | |
| 4 | 346.2 (672.9) | 0.2 (0.1, 0.3) | | 0.3 (0.1, 0.5) | |
| 5 | 593.1 (1272.9) | 0.1 (0.1, 0.2) | | 0.2 (0.1, 0.4) | |
| **Water source‡‡** | | | | | |
| Unimproved facilities | 805.2 (2128.6) | 1 | 0.877 | | |
| Improved facilities | 611.8 (1390.5) | 1.1 (0.4, 2.6) | | | |
| **Sanitation facilities§§** | | | | | |
| Unimproved facilities | 655.0 (1564.5) | 1 | <0.001 | 1 | **0.016** |
| Improved facilities | 543.6 (1138.5) | 0.4 (0.3, 0.6) | | 0.6 (0.4, 0.9) | |
| **Handwashing facility¶¶** | | | | | |
| No | 683.2 (1633.7) | 1 | <0.001 | | |
| Yes | 465.7 (858.6) | 0.3 (0.2, 0.5) | | | |
| **Environmental factors** | | | | | |
| **Mean normalised difference vegetation index (NDVI)§** | | | | | |
| First tertile | 511.8 (1069.3) | 1 | 0.018 | 1 | **<0.001** |
| Second tertile | 553.4 (1392.1) | 1.8 (1.2, 2.9) | | 2.4 (1.5, 3.8) | |
| Third tertile | 779.6 (1776.3) | 1.8 (1.1, 2.9) | | 2.4 (1.4, 3.9) | |
| **Mean of middle infrared (MIR)§** | | | | | |
| First tertile | 786.0 (1878.8) | 1 | 0.515 | | |
| Second tertile | 574.5 (1190.3) | 0.8 (0.5, 1.4) | | | |
| Third tertile | 461.8 (1042.0) | 0.7 (0.4, 1.3) | | | |
| **Elevation (N = 6,083)§** | | | | | |
| First tertile | 476.4 (941.3) | 1 | <0.001 | 1 | **<0.001** |
| Second tertile | 458.2 (1193.4) | 0.9 (0.4, 1.8) | | 1.0 (0.5, 2.0) | |
| Third tertile | 740.5 (1715.8) | 8.1 (3.4, 19.3) | | 14.1 (6.5, 30.7) | |
| **Sand fraction (N = 6,083)§** | | | | | |
| First tertile | 610.1 (1433.8) | 1 | 0.348 | | |
| Second tertile | 639.9 (1575.4) | 1.0 (0.7, 1.6) | | | |
| Third tertile | 647.3 (1502.0) | 1.5 (0.8, 2.6) | | | |
| **Soil acidity (pH KCL)§** | | | | | |
| First tertile | 680.7 (1679.0) | 1 | 0.169 | | |
| Second tertile | 576.6 (1300.1) | 0.6 (0.4, 1.1) | | | |

*(Continued)*

**Table 3.** (Continued)

| | EPG (N = 1012)* | Univariable | | Multivariable | |
|---|---|---|---|---|---|
| | Arithmetic mean (SD) | IIR (95%CI) | p | mIIR (95%CI) | p |
| Third tertile | 557.2 (1062.5) | 0.6 (0.3, 1.2) | | | |

*EPG is presented for the infected individuals only, but the negative binomial models include all individuals regardless of infection status

† 1 missing value

‡ 118 missing values

§ 6 missing values

¶ 264 missing values

# 58 missing values and data not included in the multivariable model as education of the head of the household was included

** 46 missing values

†† 11 missing values

¶¶529 missing values, not included in the multivariable model due to high number of missing values

Natural flooring refers to earth, dung, palm or bamboo and stone

Man-made flooring includes wood, brick, vinyl or asphalt strips, tiles, cement, carpet and polished stone like marble or granite

Improved water source refers to limited and basic facilities, Unimproved refers to surface water and unimproved facilities

Improved sanitation refers to limited and basic facilities, Unimproved refers to open defaecation and unimproved facilities

Acronyms: EPG—egg per gram, IIR–Infection intensity ratio, mIIR–multivariable IIR, 95% CI—95% confidence interval

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deworming programmes have been effective in reducing heavy intensity hookworm infections and possibly the prevalence of *Ascaris* in SAC.

The analyses highlighted several important correlates of hookworm infection in this setting. One of the most prominent of these was age, which was associated both with increased odds of infection as well as higher intensity of infection. This age intensity profile associated with hookworm has been described previously in several studies [37,47,48]. In our analyses, sex was not associated with hookworm infection. While adult males are sometimes found to be at higher risk for hookworm infection [49], previous studies conducted in this region have similarly found no association between sex and hookworm infection [44,50]. Although not significant, the prevalence of infection by sex was similar until the 4$^{th}$ decade after which women had a higher prevalence than men. An increase in intensity of infection but not prevalence in older women has been noted in previous studies [47,51]. As expected, a history of deworming was associated with substantially (70%) reduced infection prevalence and intensity. This result is in line with the majority of community surveys conducted within the context of an ongoing school deworming programme [37,50] and highlights the successes of the strategy in the target group, which in the India NDD extends further than in many endemic countries (1 to 19 year olds included).

A higher level of education of the head of household and high SES were both independently strongly associated with decreased odds of infection as well as decreased intensity of infection. These findings are similar to many other community STH studies [47,52]. SES is closely related to several other household-level factors measured in the survey, one such example is flooring. Although a manmade floor was found to be associated with lower odds of infection in univariate analysis, floor type was not significantly associated after accounting for SES. This is likely because flooring was highly correlated with other housing construction variables included in the SES composite variable.

With India currently implementing the world's largest sanitation programme, the Swachh Bharat Mission, there has been an unprecedented scale of toilet building but functionality and uptake remain challenges [20]. In a study in rural Odisha, India increased community sanitation coverage did not reduce diarrheal disease or acute respiratory infections but a reduction in prevalence of helminth infections was seen along with a reduction in stunting in children under 5 [53]. In our study, although unimproved sanitation (including open defecation) was not associated with an increased odds of hookworm infection, residing in a household with access to improved sanitation was associated with decreased intensity of infection. Soil samples from a smaller proportion of households in the study site have been collected to quantify environmental STH contamination. These results will be presented in a future paper and may be useful in elucidating the relationships between sanitation access, peri-domestic risk and intensity of infection. Access to a facility to wash hands with soap and water in the household was also associated with decreased risk of infection as well as lower intensity of infection but was not included in the final multivariable model as data were not available for 590 households. In a meta-analysis of studies that applied JMP definitions to categorize WASH facilities, both access to sanitation as well as access to water and hygiene facilities were associated with reduced odds of infection [54]. While the effect of WASH interventions are not easily evaluated especially in the context of other interventions [55], the importance of integrating comprehensive behavioural and structural WASH interventions and access to potentially sustain the gains made from deworming in the longer term has been highlighted in a recent modelling study [56].

Environmental factors that affect temperature, soil moisture and atmospheric humidity influence the rate of survival and development of hookworm larvae thereby affecting transmission [57]. In this study, both increased vegetation (NDVI) and elevation were associated with increased odds of infection as well as an increase in intensity of infection. In another study using remotely sensed data at a fine resolution in Jawadhu Hills, topographical parameters of elevation and slope were negatively and positively associated with hookworm infections at the village level [58]. Riess *et al*. have shown that ecological variables are associated with hookworm infection but have differing effects within a geographical region, are scale-dependent and urge caution against prediction at smaller scales using large-scale data [52]. Moreover, the effect of elevation in the current study is likely to be associated with the topographical differences between the Timiri and Jawadhu subsites and needs to be explored further using a finer resolution approach.

A robust study design was used to assess the burden of STH among community members in the study site. However, these results presented here are based on a parasitological survey conducted using the Kato Katz technique on a single stool sample, which has been shown to be sub-optimal in estimating prevalence, especially in low intensity settings [59,60]. This limitation will be addressed by future analyses on these samples using field-validated high throughput species specific qPCR [61]. Tribal communities have previously been shown to have higher STH transmission than plains populations, especially urban populations [62]. Further analyses by sub-site and using spatial analyses would be useful to tease out these additional correlates of risk in these different settings and highlight heterogeneity in infection risk. These analyses are not possible at this stage of the trial due to blinding restrictions.

The findings presented here highlight that despite several years of community-based deworming through the LF programme and multiple rounds of school-based deworming, community transmission of hookworm is still persisting in both rural and tribal areas of Tamil Nadu, especially in adults. This study provides important, robust data that will be useful to the research community as well as the Ministry of Health and Family Welfare in planning future potential expansion of the deworming program with synergy across other initiatives including the anemia free India targeting WRA, MDA for lymphatic filariasis in endemic districts and the recently launched Poshan Abhiyaan program that also provide albendazole with a view towards interrupting transmission.

## Supporting information

**S1 STROBE Checklist. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist.**
(DOCX)

**S1 Table. Comparison of characteristics of those consenting and refusing participation in the longitudinal monitoring cohort.**
(DOCX)

**S2 Table. Prevalence of other helminths that were detected by Kato Katz (n = 6089).**
(DOCX)

## Acknowledgments

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## Author Contributions

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