

Study Protocol

Efficacy and safety of a single-dose regimen and a multi-dose regimen of mebendazole against hookworm infections in children: a randomized controlled trial


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Study site	Public Health Laboratory Ivo de Carneri, Pemba, Tanzania		
Funding Agency	PATH, Seattle, USA		

1. General Information

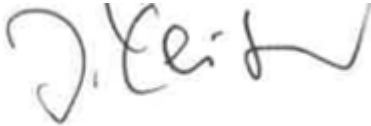
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I have read this protocol and agree that it contains all necessary details for carrying out this trial. I will conduct the trial as outlined herein and will complete the trial within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this trial. I will discuss this material with them to ensure they are fully informed regarding the drug and the conduct of the trial.

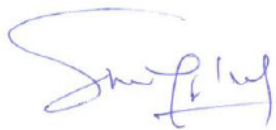
I will use only the informed consent forms approved by the Sponsor or its representative and will fulfil all responsibilities for submitting pertinent information to the Independent Ethics Committees responsible for this trial.

I agree that the Sponsor or its representatives shall have access to any source documents from which Case Report Form information may have been generated.


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Abbreviations

AE: adverse event

CI: confidence interval

CR: cure rate

CRF: case report form

DALYs: disability-adjusted life years

EMA: European Medicines Agency

EPG: egg per gram

ERR: egg reduction rate

GCP: Good Clinical Practice

HB: haemoglobin

ICH: International Council for Harmonisation

IEC: independent ethics committee

MDA: mass drug administration

PCR: polymerase chain reaction

PHL: Public Health Laboratory

PI: Principal Investigator

SAE: serious adverse event

SSL: Secure Sockets Layer

STH: soil-transmitted helminths

WHO: World Health Organization

Synopsis

Study Title	Efficacy and safety of a single-dose regimen and a multi-dose regimen of mebendazole against hookworm infections in children: a randomized controlled trial
Study Type	Phase 4 trial
Indication	Hookworm infection (eggs in stool)
Sample size	180
Investigational Product and Reference Treatment	Mebendazole 100 mg solid tablets 500 mg solid tablets
Protocol Number, Date and Version	14 July 2017, V2.01
Trial registration	Will be registered on (http://www.controlled-trials.com/)
Study Rationale	To provide evidence on the efficacy of a single dose regimen and a multiple dose regimen of mebendazole in children.
Study Objectives	To assess the efficacy and safety of two dose regimens of mebendazole: i) 100 mg solid tablets twice a day for 3 days, and ii) one dose of 500 mg solid tablets of mebendazole in participants aged 6-12, inclusive, infected with hookworm. The primary objective of the trial is to assess the cure rate (CR) of a 3-day regimen of mebendazole against hookworm and a single dose mebendazole treatment. The secondary objectives are to determine if the multi-dose regimen is superior to the single dose regimen, evaluate the efficacy against concomitant soil-transmitted helminth infections, and assess the safety of both mebendazole regimens.
Study -design	Double blind, randomized trial (both participants and outcome assessors are blinded)
Study product intervention	Mebendazole
Comparator(s)	None
Key inclusion / Exclusion criteria	Inclusion: individuals aged between 6 and 12 with a hookworm infection, absence of major systemic illnesses, written informed consent signed by parents/caregivers; and oral assent by the participants. Exclusion: Menarche, any abnormal medical conditions or chronic disease, negative diagnostic result for hookworm, no written informed consent

Primary Endpoints	CR against hookworm
Secondary Endpoints	Egg reduction rate (ERR, based on geometric means) against hookworm, CR/ERR against <i>A. lumbricoides</i> and <i>T. trichiura</i> , safety
Exploratory Endpoints	<ul style="list-style-type: none"> • Sensitivity of Kato Katz compared to quantitative polymerase chain reaction assays • Prevalence of mebendazole genetic resistance markers • Prevalence of STH species
Interim Analyses	None
Study Duration	12 weeks total; up to 8 weeks per participant
Schedule	07/2017 of first-participant in (planned) 10/2017 of last-participant out (planned)
Measurements & procedures	<p>Two stool samples will be collected if possible on two consecutive days or otherwise within a maximum of 5 days apart. The medical history of the participating individuals will be assessed with a standardized questionnaire, in addition to a clinical examination carried out by the study physician on the treatment day.</p> <p>Randomization of participants into the two treatment arms will be stratified according to intensity of infection. Participants will be interviewed before treatment for clinical symptoms and 3 hours after every morning treatment and 24 hours after every morning treatment about the occurrence of adverse events. The efficacy of the treatment will be determined 14-21 days post-treatment by collecting another two stool samples. All stool samples will be examined with duplicated Kato-Katz thick smears.</p>
Statistical Analyses	<p>The primary analysis will include all participants with primary end point data (available case analysis). Supplementary, two sensitivity analyses will be conducted imputing all missing endpoint data as treatment failures or all as treatment success. CRs will be calculated as the percentage of egg-positive participants at baseline who become egg-negative after treatment. CRs will be compared by using unadjusted logistic regression. To assess model robustness with respect to covariates, adjusted logistic regressions (adjustment for age, sex, school, weight and strata) will be performed.</p> <p>Geometric and arithmetic mean egg counts will be calculated for the different treatment arms before and after treatment to assess the corresponding ERRs. Bootstrap resampling method with 5,000 replicates will be used to calculate 95% confidence intervals (CIs) for ERRs and the difference of the ERRs.</p>
GCP statement	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, ICH-GCP E6 as well as all national legal and regulatory requirements.
Key explanation for the inclusion of	This study will be carried out in individuals aged 6-12 years old, since an infection with hookworm and other soil-transmitted

participants	helminths occurs most often in this age group.
Recruitment procedure	The study will be carried out in primary schools in Pemba Island, Tanzania.
Coverage of damages	Winterthur Police Nr. 4746321, National Insurance Cooperation of Tanzania LTD, GTA No: 00062.
Storage of data and samples for future research aims	After the study has been completed all samples will be destroyed and the trial master file will be kept for a minimum of 15 years.
Conflict of interest in relation to the investigated drugs	We declare no conflict of interest in relation to the investigated drugs.
Study site	Public Health Laboratory Ivo de Carneri, Pemba, Tanzania

2. Background information

The most recent estimates suggest that between 600 and 800 million people are infected with one or several of the common soil-transmitted helminths (STHs), which are *A. lumbricoides*, *T. trichiura*, and hookworm [1]. Recently, the global burden due to STHs has been estimated at 3.3 million disability-adjusted life years (DALYs) [2]. The symptoms of STH infections are non-specific and may only be apparent in heavily infected individuals. Children make up the age group which is most at risk of infection with helminths and infections in children are typically the most intense and debilitating [3]. Chronically infected children might suffer from malnutrition, physical and cognitive retardation, and reduced work performance [4].

Currently there are five drugs on the World Health Organization (WHO) model list of essential drugs against STH infections and these drugs have been widely and effectively used [5, 6]. Against *A. lumbricoides*, *T. trichiura* and hookworm infection, the two benzimidazoles (albendazole and mebendazole) are the treatment of choice; the other drugs used against these infections are levamisole and pyrantel pamoate [5-7]. Ivermectin is used to treat *Strongyloides stercoralis* infection, but it is also effective against *A. lumbricoides* infections and moderately effective against *T. trichiura* infection [8, 9]. Both albendazole and mebendazole are used at large scale, within the frame of preventive chemotherapy, which is the regular administration of anthelmintic drugs to at-risk populations (e.g. school-aged children). Preventive chemotherapy is advocated by WHO, because this strategy has a rapid impact on morbidity, and its ease of implementation; the drugs can be administered by personnel outside the health sector (e.g. teacher) independent of recipients body weight, e.g. albendazole or mebendazole are administered at a single oral dose [5].

Mebendazole displays a broad spectrum of activity [6, 10, 11] and it interferes with cellular tubulin formation in the worms' intestine leading to its death [12]. This drug has been extensively used worldwide for more than 30 years both alone and, more recently, in combination with drugs such as praziquantel and ivermectin [13-16]. Mebendazole has shown excellent cure rates (CR) against *A. lumbricoides*. In addition, it reveals low to moderate efficacy against *T. trichiura* and hookworm at single oral doses [6, 17].

Through mass drug administration (MDA) campaigns for STH treatment, hundreds of millions of doses of mebendazole have been distributed worldwide, including to many pregnant women. Multiple studies have failed to find a statistically significant increase in congenital abnormalities among infants born to women exposed to mebendazole during pregnancy as compared to those born to untreated women [55-58]. Over 6,000 women treated with mebendazole are included in these studies, providing more data than for other anthelmintic drugs. Of particular relevance to the proposed trial, a study in Israel found no teratogenic

effects when either a single-dose or a three-day regimen of mebendazole was administered to women in pregnancy, including during the first trimester [57].

The WHO notes in its guidelines on MDA, Preventative chemotherapy in human helminthiasis, that “despite excellent empirical safety profiles, none of the anthelmintic drugs used in MDA campaigns, including mebendazole, is licensed for administration during pregnancy [59]. Nonetheless, risk-benefit analyses show that the advantages of treating women of reproductive age, including those who are pregnant, far outweigh the risks to their health and that of their unborn child.” [60].

WHO notes that using LMP is appropriate for identification of women who are pregnant and to determine the stage of pregnancy. However, among school-age children, defined as those aged 6 to 15 years, the WHO guidance has no requirements for pregnancy testing or screening. The mean age of menarche in Tanzania has been described as 14.3 ± 1.1 years [61], but especially on Zanzibar, the location for this study, there is often a lengthy delay between menarche and sexual activity. The median age of first birth in Zanzibar is 21.7 years and it is clear from demographic surveys that pregnancy is rare among adolescents [62]. The population of Zanzibar is almost entirely Muslim and quite conservative culturally. Sexual activity before marriage is stigmatized and highly discouraged, so testing for pregnancy among 6 to 12 year old girls would be socially inappropriate. As a result, this study proposes to exclude all girls who have started their menses to avoid the possibility of exposure to mebendazole during the first trimester.

Mebendazole can be administered in different doses. The present study will explore the efficacy of the single-dose (500 mg) and the triple-dose (six doses of 100 mg over three consecutive days twice per day) against hookworm. Like albendazole, both regimens of mebendazole (the single- and triple-dose) are included in the National Essential Medicine List for mainland Tanzania [18].

A summary of randomized controlled trials using a single dose of mebendazole (500 mg solid tablets) against hookworm is given in Table 1. The recorded CRs of single-dose mebendazole are very conflicting ranging from 7.6 to 91.1% [19, 20]. Of note, CRs and ERRs of mebendazole observed on Pemba are very low.

Table 1. Randomized-controlled studies which assessed the efficacy of mebendazole (500 mg) against hookworm.

Study	Country	Diagnostic approach	Treatment evaluation	Study design	Age	N	CR	ERR
Abadi 1985 [19]	Indonesia	Kato-Katz and Harada Mori	1-4 wk after treatment	Double-blinded	2-70	45	91.1%	98.3 %

Albonico <i>et al.</i> , 2002 [21]	Tanzania	Kato-Katz	21 d after treatment	Not blinded	9.5 (mean)	424	13.2%	67%
Albonico <i>et al.</i> , 2003 [20]	Tanzania	Kato-Katz	21 d after treatment	Not blinded	7-18	224	7.6%	52.1 %
De Clercq <i>et al.</i> , 1997 [22]	Mali	Kato-Katz	4 wk after treatment	Single-blinded	5-54	35	22.9%	0%
Knopp <i>et al.</i> , 2010 [23]	Tanzania	Kato-Katz	3 wk after treatment	Single-blinded	≥ 5	34	35.3%	78.7 %
Sacko <i>et al.</i> , 1999 [24]	Mali	Kato-Katz	10 d after treatment	Single-blinded	3-70	35	51.4%	68.5 %
Soukhathammavong <i>et al.</i> , 2012 [25]	Laos	Kato-Katz	21-23 d after treatment	Not blinded	6-12	82	17.6%	76.3 %
Speich <i>et al.</i> , 2014 [17]	Tanzania	Kato-Katz	18-23 d after treatment	Double-blinded	6-14	109	17.4%	58.7 %
Steinmann <i>et al.</i> , 2011 [26]	China	Kato-Katz	3-5 wk after treatment	Not blinded	≥ 5	81	31%	83.6 %

We identified 22 studies exploring the efficacy of the triple-dose regimen of mebendazole [27-48]. These studies were conducted between 1973 and 2007 in Asia (N=14), North America (N=1), South America (N=3), Africa (N=2), Europe (N=1) and in the Pacific (N=1) using relatively small samples (average of 40 participants). CRs varied considerably ranging from 30.8% to 100% [36, 37]. Still, the lowest CR of this regimen was considerably higher than the lowest CR of the single-dose regimen CR=7.6%) (Table 1).

The trial presented in this protocol would be, to our knowledge, the first randomized clinical trial comparing the single-dose (500 mg) to the triple-dose (100 mg twice a day for three days) of mebendazole. The fact that the efficacy of the different mebendazole regimens is still highly variable and, therefore, not entirely clear, points to the pressing need to perform further trials to increase evidence. Furthermore, it is important to note that our study will take place in an African setting where the prevalence of hookworm is particularly worrisome and where, up to date, few (2/22) trials testing the efficacy of the multiple dose regimen have taken place. On Pemba, where this trial will take place, the most recent studies have found a prevalence of hookworm ranging from 36 to 97% and the efficacy of a single dose mebendazole is low [17, 49, 50]. Thus, there is a pressing need to develop treatment alternatives. Albendazole is the current standard of care in Pemba for STH treatment; however mebendazole is used in many other settings because of its greater efficacy against infections such as pinworm and Trichinosis. There could be a great public health benefit if an effective mebendazole regimen was identified for the treatment of hookworm in Pemba as the drug would also be effective against numerous other concomitant STH infections.

3. Trial objective and purpose

We hypothesize that both regimens of mebendazole will be effective against hookworm and concomitant soil-transmitted helminths.

The primary objective of the study is to assess the CR (*i.e.* conversion from being egg positive pre-treatment to egg negative post-treatment) of two different mebendazole regimens against hookworm in children (6 - 12 years old): (i) 100 mg of mebendazole twice a day for 3 days and (ii) 500 mg of mebendazole single dose. Thus, the **primary endpoint** of the study is the CR of these two regimens of mebendazole against hookworm.

Additional **secondary endpoints** are (1) the ERR of these two regimens against hookworm, (2) the CR and ERRs of both mebendazole regimens against concomitant soil-transmitted helminth infections, and (3) the safety of both mebendazole regimens. **Exploratory endpoints** include comparison of the sensitivity of Kato Katz to quantitative polymerase chain reaction assays, prevalence of genetic resistance markers among participants, and distribution of hookworm species among participants.

4. Methodology

4.1 Type of trial

Double blind randomized trial.

4.2 Trial design

4.2.1 Baseline survey and screening

This trial will take place in the two primary schools (Bagamoyo School and Piki School) in Pemba, Tanzania, which have been found to have a high prevalence of hookworm in previous studies. Among those children for whom informed consent is obtained, each participant will receive an empty container at school, which they return the following day with a stool sample. Stool samples will be collected and transported by car to the central laboratory. Two stool samples will be collected from each participating individual on different days, to adequately cover the egg shedding cycle of 24-48 hours, until a total of 180 hookworm-infected participants have been identified, preferable with *A. lumbricoides* and/or *T. trichiura* co-infections (Fig. 1). The prevalence for hookworm infected individuals is expected to be between 20% and 40% [17, 49, 50], hence we anticipate screening around 500-1,000 individuals to reach the total number of participants. The number of screened individuals will depend on the prevalence of the parasite.

The Kato-Katz technique will be used for the quantitative assessment of STH infections. Two stool samples will be collected from each participant before treatment and from each sample

duplicate Kato-Katz thick smears (41.7 mg each) will be prepared [51]. The slides will be analysed under a microscope by experienced technicians and a subsequent independent quality control of sample results (10%) will be conducted. Results are considered correct if the following tolerance margin is not exceeded: (i) No difference in presence/absence of hookworm, *A. lumbricoides* and *T. trichiura*, (ii) Egg counts are ± 10 eggs for counts ≤ 100 eggs or $\pm 20\%$ for counts > 100 eggs (for each species separately) [52]. In case discrepancies above the tolerance margin are noted in one or more slides, all slides are re-read by the local technicians. The new results are discussed, so that in case of discordant results, slides can be re-evaluated to reach consensus. Infection intensity expressed as the arithmetic and geometric mean egg count per gram of stool (EPG) will be calculated for each treatment arm.

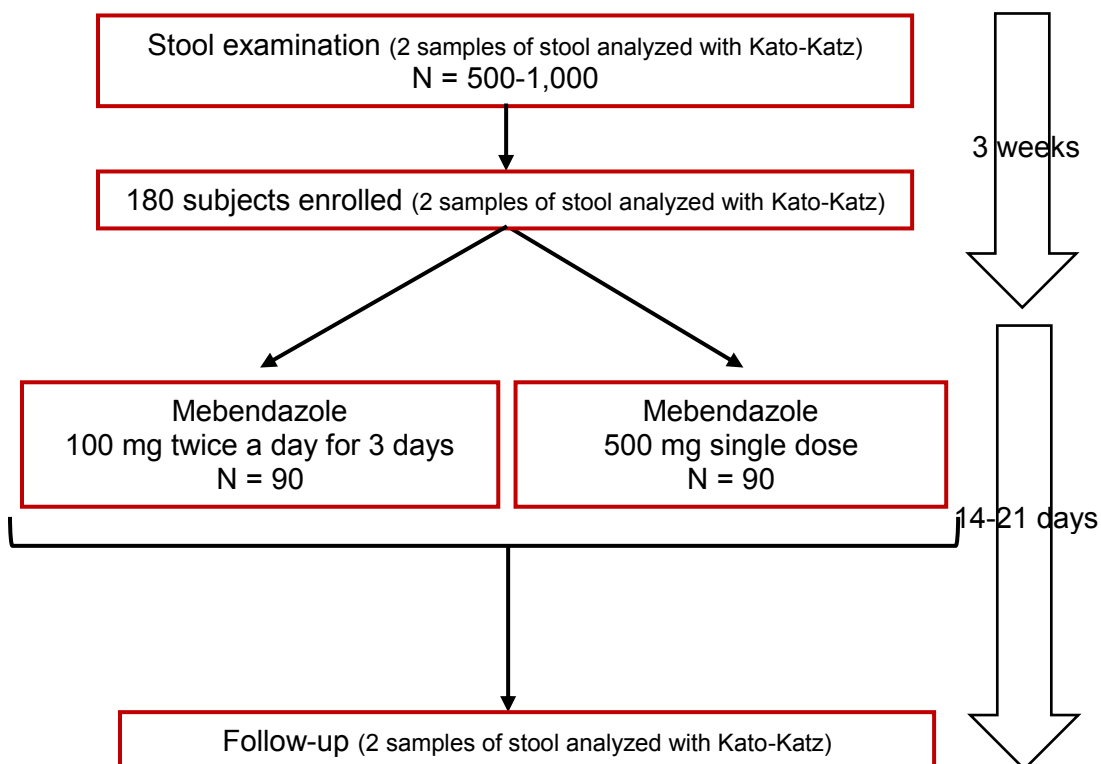


Figure 1: Mebendazole efficacy study-flow.

All stool samples will be prepared and analysed in the Public Health Laboratory Ivo de Carneri on Pemba, United Republic of Tanzania. All quadruplicate Kato-Katz thick smears will be microscopically analysed within the first 60 minutes and will then be destroyed within a few days (after passing the quality control). Also, two aliquots (about 1 g of stool each) of positive samples will be stored in ethanol. One aliquot will remain in Pemba as a “back-up”

sample and the other aliquot will be transported to the Swiss Tropical Public Health Institute for subsequent DNA extraction, diagnostic and assessment of drug resistance-associated single-nucleotide polymorphisms. Upon successful PCR analysis, the aliquot which was stored in Pemba will be destroyed. Finally, a Harada Mori culture will be prepared from one of the stool samples of each child at baseline and at follow-up to extract hatched larvae. Larvae will be stored in ethanol and will subsequently undergo the same process as the previously mentioned stool aliquots.

Among those participants who tested positive for hookworm, the second stage of screening involves a physical examination and will occur one to three days before treatment is scheduled to begin. At baseline the medical history of STH-infected participants will be assessed at school during clinical examination carried out by the study clinician. Additionally, during this clinical examination, haemoglobin levels will be detected using HemoCue conducted by nurses to exclude severely anaemic participants (below 80 g/l Hb according to WHO definition). In all girls, the clinician will assess the possibility of pregnancy by asking if menarche has occurred. If menarche has occurred and thus pregnancy cannot be ruled out, the participant will be excluded from the trial, but will still be given the option of receiving only placebo to avoid any stigmatization.

4.2.2 Assessment of efficacy after treatment

The efficacy of the treatment in participants will be determined 14-21 days post-treatment by collecting another two stool samples and microscopically examined for STH using duplicate Kato-Katz thick smears, creating a total of four slides for this time point assessment. Kato-Katz is the standard method used to diagnose STH and is the reference standard for much of the literature reporting anti-helminthic treatment efficacy. Kato-Katz thick smears are not as sensitive as more recently developed methodologies, such as quantitative PCR, and slides have to be read within a strict window for accurate results. Appendix 1 includes instructions that will be followed for Kato-Katz preparation and analysis.

Individuals will be considered hookworm negative if EPG < 100 (total of the four slides) and/or if only one Kato-Katz thick smear slide with more than one hookworm egg. EPG will be assessed by adding up the egg counts from the quadruplicate Kato-Katz thick smears and multiplying this number by a factor of six. Geometric and arithmetic mean egg counts will be calculated for the two treatment arms before and after treatment to assess the corresponding ERRs.

Additionally, an aliquot from each participant stool sample positive for any of the soil-transmitted helminths (*A. lumbricoides*, *T. trichiura* or hookworms) at either baseline or follow-up survey, will be stored at Public Health Laboratory Ivo de Carneri in absolute ethanol. Aliquots will be shipped to the Swiss Tropical Public Health Institute (Basel,

Switzerland) to undergo DNA extraction and quantitative polymerase chain reaction (PCR) analysis to perform molecular genotyping for drug resistance-associated single-nucleotide polymorphisms studies. At the end of the study all participants in whose post-treatment samples we still find hookworm eggs will be contacted at school with their test results and treated with albendazole (400 mg single dose), the current local standard drug against hookworm.

4.3. Measure to minimize bias

Study participants eligible for treatment will be randomly assigned to one of the two treatment arms using a computer-generated stratified block randomization code. The random allocation sequence with varying random blocks of four or eight and stratified by 2 levels of baseline infection intensity (light: 1-1999 EPG, and moderate plus heavy: ≥ 2000 EPG hookworm infections) will be provided by a statistician. Both treatment arms will have an equal number of participants with light infection intensity, although the number of light versus moderate/heavy infections are not expected to be equal in each arm, depending on the distribution of infection intensity in the recruited cohort. The codes will be held in a locked cabinet at the Swiss Tropical and Public Health Institute. A copy of this code will be kept in a sealed envelope by one of the co-investigators (will only be unblinded in case there is a serious adverse event, determined by the principal investigator upon consultation with the co-investigators). The blinding will be maintained throughout the trial until data entry and processing are complete and the data have been verified. Following release of the final data, the randomization codes will be released.

4.4. Study duration and duration of subject participation

The trial will last twelve weeks with each participant's involvement lasting up to eight weeks. The screening for the baseline will start three weeks prior to the treatment. Follow up screening will take place 14-21 days post-treatment and last for about three weeks. Schedules of visits are summarized below.

4.5. Schedule of visits

Table 2 briefly presents the distribution of tasks along the trial. Further detail is available in Appendix 2.

Table 2. Schedule of visits during treatment period.

Screening							Follow-up
- 21 to -1	-3 to -1	D1	D2	D3	D4	D5	14 to 21 days
Hours							

	days	days	0	3	10	0	3	10	0	3	10	0	0
Diagnosis (stool examination)	X												X
Informed consent	X												
Demographics	X												
Medical History		X											
Administration of treatment/placebo			X		X	X		X	X		X		
Physical Examination, including menarche and anemia assessments		X											
Capturing AEs/ SAE				X		X	X		X	X		X	X
Administration of albendazole (still infected at follow-up)													X

5. Selection of the trial subjects

5.1 Recruitment

The study will be carried out in children (age: 6-12 years) in one primary school on Pemba, United Republic of Tanzania in areas endemic for hookworm [17, 21, 49, 50, 53].

The parents/caregivers of participants will be invited to participate in an information meeting to explain the purpose and procedures of the study, including potential benefits and risks. Parents/caregivers will be encouraged to ask questions in an open discussion forum. During this session, parents will be informed of preventive actions they can take to help protect their children from acquiring hookworm and other STH infections in the future (e.g., wearing shoes, hand washing procedures).

Those parents/caregivers and their children who are interested in the study will be invited to complete the process of informed consent. See section 9.3 for details on obtaining informed consent. Only those participants who have written informed consent will be assessed for study eligibility criteria during screening procedures.

5.2 Inclusion criteria

1. Written informed consent signed by parents and/or caregiver; and oral assent by participant.
2. Able and willing to be examined by a study physician at the beginning of the study.
3. Able and willing to provide two stool samples at the beginning (baseline) and approximately three weeks after treatment (follow-up).

4. Positive for hookworm eggs in the stool (≥ 100 EPG and at least two Kato-Katz thick smears slides with more than one hookworm egg).
5. Absence of major systemic illnesses, e.g. diabetes, severe anemia (HB <8.0 g/l) as assessed by a medical doctor at school, upon initial clinical assessment.
6. No known or reported history of chronic illness as cancer, diabetes, chronic heart, liver or renal disease.
7. No recent anthelmintic treatment (within past 4 weeks).
8. No known allergy to study medications (mebendazole and albendazole).

5.3. Exclusion criteria

1. No written informed consent by parents and/or caregiver; no oral assent by participant.
2. Menarche, based on self-report
3. Presence of major systemic illnesses, e.g. diabetes, severe anemia (HB <8.0 g/l) as assessed by a medical doctor, upon initial clinical assessment.
4. History of acute or severe chronic disease.
5. Recent use of anthelmintic drug (within past 4 weeks).
6. Attending other clinical trials during the study.
7. Negative diagnostic result for hookworm eggs in the stool (< 100 EPG (total of the four slides) and/or only one Kato-Katz thick smear slide with more than one hookworm egg).

Participants who were diagnosed with a STH infection, but who were excluded from the study due to one or several of the above-mentioned exclusion criteria, including withdrawals, will be offered standard anthelmintic treatment (albendazole).

5.4. Criteria for discontinuation of trial

A subject can be discontinued from the study for the following reasons:

1. Withdraws from the study (this can happen anytime as participation is voluntary and there are no further obligations once a participant withdraws).
2. At the discretion of the Principal Investigator (PI) or co-PI, if the participant is not compliant to the requirements of the protocol.

Discontinued subjects will not be replaced. If, for any reason, a subject is discontinued from the study before the end of treatment evaluations, the safety procedures planned (adverse events monitoring) will be conducted.

5.5. Treatment of subjects

Mebendazole tablets (100 and 500 mg) will be commercially purchased from Johnson & Johnson (Zug, Switzerland) (expiration date 2019). Matching placebo tablets will be produced by our collaborators at the Pharmacenter of the University of Basel. Tablets will be put into sealed and labelled plastic bags by an independent pharmacist according to the randomisation list and will always be stored at room temperature together with silica gel (desiccant) against humidity. Because we do not have independent pharmacists in the study site, all plastic bags will be prepared prior to departure at the Swiss Tropical Public Health Institute (Basel, Switzerland) and labelled with their treatment ID. There will be six treatment time points: three mornings and three evenings (evening treatments will take place about 10 hours after morning treatments), thus, a total of 1080 bags will be prepared (180 participants x 6 time points): 90 contain 500mg mebendazole and 100mg placebo, 90 contain 100mg mebendazole and 500mg placebo, 450 contain 100mg mebendazole, and 450 contain 100mg placebo (see Table 3). At this point, all bags will be labelled with the treatment ID of each participant, e.g. participant number 13 will have six bags corresponding to the six time points (13.1, 13.2, 13.3, 13.4, 13.5, and 13.6). In the study site, the tablet bags will be kept in air conditioned rooms (up to 20°C). Drugs will be administered up to one month prior to the preparation of the envelopes.

Participants will stay all day at school for the morning and evening treatments. All drugs will be administered orally with a small amount of water in the presence of the investigator(s), and ingestion confirmed. This will be recorded with the time and date of dosing. On the first morning, depending on which treatment arm they were randomly allocated to, all participants will receive two tablets: one 100mg tablet (mebendazole or placebo) and one 500mg tablet (mebendazole or placebo). On the remaining five treatment points, also depending on which treatment arm they were allocated to, each participant will receive either one 100mg mebendazole tablet or one 100mg placebo tablet (Tables 2 and 3). Participants will be all day at school where the administration of mebendazole and capturing of AEs will take place. The morning dosing will occur when participants first arrive at school and the evening dosing will occur right before participants leave the school, approximately 10 hours later.

Table 3. Distribution of the administration of mebendazole and placebo in both groups.

	Day 1		Day 2		Day 3	
	Morning	Evening	Morning	Evening	Morning	Evening
Arm A	MEB 100 + plac 500	MEB 100	MEB 100	MEB 100	MEB 100	MEB 100

Arm B	MEB 500 + plac 100	plac 100	plac 100	plac 100	plac 100	plac 100
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Note: MEB 100, 100 mg of mebendazole; MEB 500, 500 mg of mebendazole; plac 100, placebo matching 100 mg mebendazole; plac 500, placebo matching 500 mg of mebendazole.

Subjects will be asked not to take any drugs other than those prescribed by the study medical team. After ingestion of the medication, which will be done at school, the subjects will be observed for 3 hours to ensure retention of the drug. Vomiting within 1-hour post-dosing will require re-dosing. The subjects will not be allowed more than one repeated dose. No re-administration will be needed for subjects vomiting after one hour. The site co-PI is responsible for drug accountability at the study site. Maintaining drug accountability includes careful and systematic study drug storage, handling, dispensing and documentation of administration. Any study product that is unused at the conclusion of the study will be destroyed.

5.6 Concomitant therapy

All medications taken one month before and during the study period until the last stool examination between day 14 and 21 (follow-up) must be recorded with indication, dose regimen, date and time of administration.

Medication(s)/treatment(s) permitted during the trial:

- Analgesics and antipyretics are allowed to be given to the subjects in case of fever, antiemetics to prevent nausea and vomiting and/or antibiotics to prevent or treat bacterial superinfection.

Medication(s)/treatment(s) NOT permitted during the trial:

- No other active drugs against helminths are permitted during the trial.

6. Safety assessments

Few adverse events have been reported following mebendazole administration. The most common reported adverse events are abdominal cramps, headache, diarrhoea, fever and fatigue [17, 23, 26]. The safety profile of mebendazole will be assessed through the recording, reporting and analysis of baseline medical conditions, adverse events and a physical examination. Participants will be encouraged to notify study staff of any adverse events that may occur outside of scheduled safety assessments. The medical doctor will be responsible for this part of the trial.

6.1. Adverse event definitions

The term “adverse event” could include any of the following events which develop or increase in severity during the course of the study, after administration of the study product:

- a) Any unfavourable and unintended signs, symptoms or disease temporally associated with the use of a medicinal product, whether or not considered related to the condition under study and the study product;
- b) Any abnormality detected during physical examination.

The medical conditions present at the initial trial visit that do not worsen in severity or frequency during the trial will not be defined as adverse events but as be considered baseline medical conditions. For the purpose of this trial, disease progression and relapse will be considered as treatment failure, not as an Adverse Event.

The observation time for adverse events starts when the treatment is initiated until day 5 (48 hours after last drug administration).

These data will be recorded on the appropriate CRF sections, regardless of whether they are thought to be associated with the study or the drug under investigation. Associated with the use of the drug means that there is a reasonable possibility that the event may have been caused by the drug (see also relatedness definitions below).

6.1.1. Severity grading

Adverse signs or symptoms will be graded by the Investigator as mild, moderate, severe or life threatening according to the following definitions:

Grade	Definition
1	<u>Mild</u> : the subject is aware of the event or symptom, but the event or symptom is easily tolerated.
2	<u>Moderate</u> : the subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity.
3	<u>Severe</u> : significant impairment of functioning: the subject is unable to carry out his or her usual activities.
4	Life threatening or disabling
5	Death related to adverse events

6.1.2. Relatedness

Relatedness will be assessed as defined below based on the temporal relationship between the adverse event and the treatment, known side effects of treatment, medical history, concomitant medication, course of the underlying disease and trial procedures.

Possibly related: an adverse event which can medically (pharmacologically/clinically) be attributed to the study treatment.

Unrelated: an adverse even which is not reasonably related to the study treatment. A reasonable alternative explanation must be available.

An adverse event that is determined to be related to the administration of a study product is referred to as an “adverse drug reaction.”

6.1.3. Expectedness

Expected adverse drug reaction: Any adverse event possibly related to the administration of mebendazole reported in the literature or on the drug package leaflet and listed in the consent form. These adverse events are detailed in the drug package leaflet (Appendix 3).

Unexpected adverse drug reaction: Any adverse event possibly related to the study product administration, the nature, frequency, specificity or severity of which is unanticipated and not consistent with the available risk information described for these drugs.

6.1.4. Serious adverse events

According to the ICH “Clinical Safety Data Management: Definitions and standards for expedited Reporting E2A” [54], a serious adverse event includes any event (experience) or reaction in any untoward medical occurrence that at any dose:

1. results in death;
2. is life threatening, meaning, the subject was, in the view of the Investigator, at immediate risk of death from the reaction as it occurred, *i.e.* it does not include a reaction that, had it occurred in a more serious form, might have caused death;
3. results in persistent or significant disability/incapacity, *i.e.* the event causes a substantial disruption of a person’s ability to conduct normal life functions;
4. requires in patient hospitalisation or prolongation of existing hospitalisation;
5. creates a congenital anomaly or birth defect (not relevant for this study);
6. is an important medical event, based upon appropriate medical judgment, that may jeopardize the patient or subject or may require medical or surgical intervention to prevent one of the other outcomes defining serious.

A “severe” adverse event does not necessarily meet the criteria for a “serious” adverse event. Serious adverse events are reported from consent to 48 hours post-treatment (Day 5).

Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome.

The causality of any serious adverse event that occurs after the study period and its possible relatedness to the study treatment or study participation will also be assessed by investigators as described in section 6.1.2.

6.1.5. Suspected unexpected serious adverse reactions

A suspected unexpected serious adverse reaction (SUSAR) is an unexpected adverse drug reaction which also meets the definition of serious adverse events.

6.2. Methods of recording and assessing adverse events

Patients will be kept for observation for at least 3 hours following each morning treatment for any acute adverse events. During the reporting period, any unfavorable changes in the subject’s condition will be recorded as adverse events, whether reported by the subject or observed by the Investigator. In case of any abnormal finding, the local study physician will

perform a full clinical examination and findings will be recorded. An emergency kit will be available on site to treat any medical conditions that warrant urgent medical intervention.

In addition, patients will be also interviewed by a nurse and/or a physician about the occurrence of adverse events 24 hours after every morning treatment (before the next treatment) and 48 hours after the last treatment (day 5) (Table 2).

Information on all adverse events (onset, duration, intensity, seriousness and causality) will be immediately entered in the appropriate adverse event module of the case report form (CRF) which is considered as a source document. For all adverse events, sufficient information will be pursued and/or obtained so as to permit i) an adequate determination of the seriousness of the event (i.e. whether the event should be classified as a serious adverse event); ii) an assessment of the casual relationship between the adverse event and the study treatments (i.e. whether the event should be classified as an adverse drug reaction); and iii) an assessment of intensity of adverse events by the study physician.

All serious adverse events, unexpected adverse drug reactions, or SUSARs must be reported as described in Section 6.3.

6.3. Reporting of serious adverse events

Any study-related unanticipated problem posing risk of harm to subjects or others (including all unexpected adverse drug reactions), and any type of serious adverse event will be immediately (within a maximum of 24 hours after becoming aware of the event) notified to the study sponsor-investigator and co-PI:

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Within the following 48 hours, the local co-investigator must provide to study sponsor-investigator further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of a completed SAE form, and any other diagnostic information that will assist the understanding of the event. In exceptional circumstances, a serious adverse event may be reported by telephone. In these cases, a written report must be sent immediately thereafter by fax or e-mail. Names, addresses and telephone for serious adverse event reporting will be included in the trial-specific SAE form. Relevant pages from the CRF may be provided in parallel (e.g., medical history, concomitant medications).

6.5. Safety reporting to Health Authorities and Ethics Committees

The sponsor-investigator will send appropriate safety notifications to Health Authorities in accordance with applicable laws and regulations. Additionally, this information will be provided to the 'Ethik Kommission Nordwest- und Zentralschweiz' (EKNZ, Switzerland) and 'Zanzibar Medical Research and Ethical Committee' (ZAMREC, Tanzania) according to national rules. Fatal or life-threatening serious adverse events or SUSARs will be reported within 24 hours followed by a complete report within 7 additional calendar days. Other serious adverse events and SUSARs that are not fatal or life-threatening will be filed as soon as possible but no later than 14 days after first knowledge by the sponsor.

7. Statistics

7.1. Definition of primary endpoint

Cure rate of mebendazole against hookworm is the primary endpoint in our study. Since this might be influenced by infection intensity, treatment groups will be equally balanced in terms of infection intensity by 2 levels of baseline infection intensity (light infections and moderate/heavy infections).

7.2. Justification of number of trial subjects

Sample size calculations for this study are based on the secondary objective comparing the efficacy of the two mebendazole regimens. Based on the published literature with special attention to studies conducted on Pemba Island, we assume that the cure rate of single dose mebendazole against hookworm infections is 20% compared to 40% in the triple treatment regimen [17, 33, 45, 46, 49]. To detect the difference with 80% power at a two-sided 5% significance level we require 79 participants per study arm. To account for potential loss to follow up (which was low in our previous studies in this setting) we aim to recruit in total 180 participants.

7.3. Description of statistical methods

The primary available case analysis will include all participants with primary end point data. In addition, an intention-to-treat analysis will be conducted considering all participants with missing endpoint data as treatment failure or all as treatment success to ensure that the results are not sensitive to potential loss to follow-up bias. CRs will be calculated as the percentage of egg-positive children at baseline who become egg-negative after treatment. EPG will be assessed by adding up the egg counts from the quadruplicate Kato-Katz thick smears and multiplying this number by a factor of six. The ERR will be calculated as:

$$ERR = 1 - \frac{\frac{1}{n} e^{\sum \log(EPG_{follow-up} + 1)} - 1}{\frac{1}{n} e^{\sum \log(EPG_{baseline} + 1)} - 1}$$

In the primary model we estimate the difference among CRs by using unadjusted logistic regressions. In a subsequent analysis an adjusted logistic regression (adjustment for age, sex, school, weight and strata) will be performed.

Geometric mean egg counts will be calculated for the different treatment arms before and after treatment to assess the corresponding ERRs. Bootstrap resampling method with 5,000 replicates will be used to calculate 95% confidence intervals (CIs) for ERRs and the difference between the ERRs.

Adverse events will be evaluated descriptively as the difference of proportion reporting adverse events before and after treatment.

7.4. Description of data management

The investigators are responsible for an adequate data quality. Prior to the initiation of the study, a short investigator's meeting will be held with the investigators and their study

coordinators and a member from Swiss TPH. This meeting will include a detailed discussion of the protocol, performance of study procedures (SOPs from previous studies available on site), CRF completion, and specimen collection and diagnostic methods.

Screened patients will be listed in a confidential “subject screening log”. Enrolled patients will be listed in a confidential “subject enrolment log” and attributed a unique study number; this document will constitute the only source to decode the pseudonymised data and will only be accessible to the local principal investigator. All data that have been hand-entered in the database will be verified by a double-key entry procedure in a validated electronic data base system and error, range and consistency checks will be programmed. Any discrepancies will be reviewed against the hard copy CRF and corrected. Electronic data files will be stored on secured network drives with restricted access for study personnel only. Data analysis will be conducted with pseudonymised data and reporting of findings will be fully anonymised.

Essential infrastructure such as lockable cabinets for safe storage of hardcopy data will be made available. Network drives with restricted access for authorised personnel only and appropriate analysis software are available.

8. Duties of the investigator

8.1. Investigator’s confirmation

This trial will be conducted in accordance with the protocol, International Conference on Harmonisation Good Clinical Practice E6 (R2) (ICH-GCP) and the current version of the Helsinki Declaration.

All protocol modifications must be documented in writing. A protocol amendment can be initiated by either the Sponsor/PI or any Investigator. The Investigator will provide the reasons for the proposed amendment in writing and will discuss with the Sponsor/PI and Co-PIs. Any protocol amendment must be approved and signed by the Sponsor/PI and must be submitted to the appropriate Independent Ethics Committee (IEC) for information and approval, in accordance with local requirements, and to regulatory agencies if required. Approval by IEC must be received before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the change involves only logistical or administrative aspects of the trial, e.g. change of telephone number(s).

8.2. Damage coverage

A general liability insurance of the Swiss TPH is in place (Winterthur Police Nr. 4746321) and a patient liability insurance will be issued by the National Insurance Cooperation of Tanzania LTD, which will cover any eventual study related injuries or deaths.

8.3. Project management

The trial team will include the PI (Prof. Jennifer Keiser), a trial and data manager (Marta Palmeirim), a trial statistician (Dr. Jan Hattendorf), as well as a physician (Dr. Sauda Kassim Omar), nurses and laboratory technicians. Prof. Jennifer Keiser and Marta Palmeirim will be responsible for staff management, communication with the collaborative group, recruitment monitoring, data management, safety reporting, analysis, report writing and dissemination of the trial results. Marta Palmeirim will monitor all field activities at the study site. Dr. Sauda Kassim will be responsible for clinical examinations and adverse events monitoring. Shaali Ame and Said Ali (Co-PI in Tanzania) are responsible for supervision of the lab- and field technicians, staff management, recruitment monitoring, supply of the material, contact to the local authorities and participating schools to obtain necessary permissions before beginning recruitment.

The investigator team is responsible for ensuring that the protocol is strictly followed. The investigator should not make any changes without the agreement of the Principal Investigator and the Co-Investigators, except when necessary to eliminate an apparent immediate hazard or danger to a study participant. The investigator will work according to the protocol and GCP. The investigator may take any steps judged necessary to protect the safety of the participants, whether specified in the protocol or not. Any such steps must be documented. During the treatment the records are maintained by the responsible medical doctor. All entries have to be made clearly readable with a pen. The investigator must be thoroughly familiar with the properties, effects and safety of the investigational pharmaceutical product.

9. Ethical considerations

9.1. Independent Ethics Committee (IEC)

The study will be submitted for approval by the institutional research commission of the Swiss TPH and the ethics committees of Switzerland (EKNZ: Ethical Commission of northwest/central Switzerland) and Zanzibar (ZAMREC: Zanzibar Medical Research and Ethics Committee). The study will be undertaken in accordance with the Declaration of Helsinki and good clinical practice (GCP).

9.2. Evaluation of the risk-benefit ratio

Few adverse events have been reported for mebendazole (package insert Appendix 3). The most common reported adverse events are abdominal cramps, headache, diarrhoea, fever and fatigue [17, 23, 26].

All participants will benefit from a clinical examination and a treatment against STHs. All participants in whose stool we still find hookworm eggs will be treated with albendazole (according to WHO recommendations).

9.3. Subject information and consent

Community meetings will be conducted to explain to caregivers the purpose and procedures of the study. Parents or caregivers attending this meeting will receive a small provision to cover their costs for transportation (~US\$ 2). Their level of comprehension of the trial's purpose and procedures will then be assessed using a short multiple-choice questionnaire. Finally, one the parents/caregiver of an eligible individual will be asked to sign a written informed consent form (translated into the local language, *i.e.* Kiswahili) after having had sufficient time for reflection of their child's participation. Even if the participant gives oral assent, the parent/caregiver has to sign the consent. In case the parent/caregiver is illiterate, an impartial witness that can read and write has to sign the consent and the parent/caregiver to give a thumb print. Participation is voluntary and individuals have the right to withdraw from the study at any given point in time with no further obligations. Participation itself will not be awarded with compensation. Only after the informed consent form is signed will participants undergo any screening procedures.

9.4. Subject Confidentiality

The obtained data will be handled strictly confidentially. Only members of the study team will have access to the data. Personal data will be coded for data analysis. The codes will be filled with the participant's identity on a separate file (subject identification list) and filled in a secured place at the Public Health Laboratory Ivo de Carneri and will only be accessible to investigators. No names will be published at any time, and published reports will not allow for identification of single subjects. Confidentiality and anonymity will be ensured throughout the entire research project.

The investigators have all been trained in GCP. None of the investigators declare to have any conflicts of interest.

9.5. Subjects requiring particular protection

This study will be carried out in children, since an infection with hookworm often occurs in this age group and they are at high risk of infection. Our trial will produce more evidence to support the search of safer and more effective treatment of STH infections in children.

10. Quality control and quality assurance

10.1. Monitoring and auditing

We will work with a locally based monitor. He/she will conduct site visits to the investigational facilities for the purpose of monitoring the study. Details will be described in a separate monitoring plan. The investigator will permit them access to study documentation and the clinical supplies dispensing and storage area. Monitoring observations and findings will be documented and communicated to appropriate study personnel and management. A corrective and preventative action plan will be requested and documented in response to any audit observations. No sponsor initiated audits are foreseen, but audits and inspections may be conducted by the local regulatory authorities or ethics committees. The Investigator agrees to allow inspectors from regulatory agencies to review records and is encouraged to assist the inspectors in their duties, if requested.

10.2. Access to data, handling of data and samples (data protection), archiving (place, duration) and destruction

Information about study subjects will be kept confidential and managed accordingly. A CRF will be completed for each subject enrolled into the clinical study. The investigators will review, and approve each completed CRF. The study CRF is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked "N/D" will be entered. If the item is not applicable to the individual case "N/A" will be written. All entries will be printed in blue ink. All corrections must be initialled and dated.

All data on parasitology and questionnaires about adverse events and self-reported clinical signs and symptoms will be doubled entered into a database by two independent persons and cross-checked. Discrepancies between data entries will be corrected by consulting the hard copy.

The collected data together with the hard copy CRFs, ICFs and other study documents will be stored at server of the Public Health Laboratory (PHL) in Pemba and are encrypted with Secure Sockets Layer (SSL).

The results of the research study will be published, but subjects' names or identities will not be revealed. Records will remain confidential. To maintain confidentiality, the Sponsor-Investigator will keep records in locked cabinets and the results of tests will be coded to prevent association with participant's names. Data entered into the ACCESS data entry mask will be accessible only by authorized personnel directly involved with the study and will

be encoded. Subject-specific information may be provided to other appropriate medical personnel only with the subject's permission.

After the study has been completed all samples will be destroyed and research data and related material will be kept for a minimum of 15 years to enable understanding of what was done, how and why, which allow the work to be assessed retrospectively and repeated if necessary.

10.3. Data entered directly in the Case Report Form (CRF) – definition of source data

Source Data are the clinical findings and observations, laboratory data maintained at the study site. Source data are contained in source documents. Local authorities are allowed to access the source data. Data will be entered directly onto the case report forms. The case report form is considered as a **source document**. All CRFs will be kept for at least 15 years. The study site will retain a copy of the CRF to ensure that local collaborators can provide access to the source documents to a monitor, auditor, or regulatory agency.

10.4. Data and safety monitoring board (WHO)/ data monitoring committee (EU/FDA)

In our study no data and safety monitoring board will be established, since we work in a small sample size and treatment will be completed after 3 days. This study is anticipated to be no greater than minimal risk to participants.

11. Dissemination of results and publication

The final results of this study will be published in a scientific journal and presented at scientific conferences. PATH will be acknowledged as study funder. All results from this investigation are considered confidential and shall not be made available to any third party by any member of the investigating team before publication. A summary of study conclusions will be shared with the local ethics committee, ZAMREC.

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Appendix 1: Kato-Katz manual from the World Health Organization (assessed: http://www.who.int/neglected_diseases/preventive_chemotherapy/pctnewsletter11.pdf;

Step 1: Label a glass slide⁴ with the sample number and then place a plastic template on top of it.

Step 2: Place a small amount of the faecal sample on a newspaper and press a piece of nylon screen on top. Using a spatula, scrape the sieved faecal material through the screen so that only the debris remains.

Step 3: Scrape up some of the sieved faeces to fill the hole in the template, avoiding air bubbles and levelling the faeces off to remove any excess.

Step 4: Carefully lift off the template and place it in a bucket of water mixed with concentrated detergent so that it can be reused.

Steps 5 and 6 of the Kato-Katz technique

Step 5: Place one piece of the cellophane, which has been soaked overnight in methylene blue glycerol solution, over the faecal sample.

Step 6: Place a clean slide over the top and press it evenly downwards to spread the faeces in a circle. If done well, it should be possible to read newspaper print through the stool smear.

Steps 5 and 6 of the Sandwich technique


Step 5: Turn the slide containing the small amount of stool upside down and place it on a clean slide to make a "sandwich".

Step 6: Using a circular motion, press the top slide firmly onto the bottom slide to spread the stool in an even circular layer. If done well, it should be possible to read newspaper print through the stool smear.

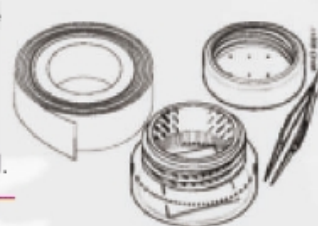
IMPORTANT: A slide prepared using the Sandwich technique must be read within 1 hour.

Step 7: If hookworm is present in the area the slide should be read within 30–60 minutes, irrespective of the technique used. After that time, the hookworm eggs disappear.


Place the slide under a microscope and examine the whole area in a systematic zigzag pattern. If the sample created by the Sandwich technique is too thick, it may be necessary to use a x400 magnification objective. Record the number and the type of each egg on a recording form alongside the sample number. Finally, multiply the number of eggs by 24⁵ to give the number of eggs per gram (epg) – the standard measurement to assess the intensity of infection.



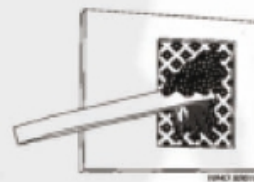
Step 1




Step 2




Step 3




Step 4




Step 5



Step 6



Step 5



Step 6

⁴ The Kato-Katz technique uses a 25 x 75 mm slide. The Sandwich technique uses a 58 x 75 mm slide.

⁵ This assumes that the standard 41.7 mg template is used. If a template of another size is used, multiply the number of eggs by the correct multiplier to give the epg.

05.03.2013).

SHORTAGES OF KATO-KATZ KITS Sandwich or Teesdale technique

Kato-Katz kits contain a roll of cellophane that is cut into small pieces and soaked in methylene blue glycerol solution (not included in the kit) the night before the field work. The cellophane is then placed directly on the faeces sample, making the eggs more easily visible and allowing long-term storage of the slides.

Unfortunately, the company that supplied the bulk of the cellophane rolls recently discontinued production, resulting in a shortage of kits. WHO is therefore field-testing cellophane produced by alternative manufacturers to identify a high-quality product. Until the situation is resolved, the Sandwich or Teesdale technique, which is very similar to the Kato-Katz technique, is the most appropriate alternative. There is one important difference: the slides prepared with the Sandwich technique must be read within one hour of preparation.

² *Preventive chemotherapy in human helminthiasis: coordinated use of anthelmintic drugs in control interventions: a manual for health professionals and programme managers*. Geneva, World Health Organization, 2006 (available at: http://whqlibdoc.who.int/publications/2006/9241547103_eng.pdf).

³ For example, the modified Ritchie technique.

Appendix 2: Schedule of the mebendazole study procedures in Tanzania.

	Mo	Tu	We	Th	Fr	Sa	Su	Mo	Tu	We	Th	Fr	Sa	Su		
	Week 1 --							Week 2 --								
Training Interviewers	X	X	X													
Panflet distribution		X														
Invite for Consenting				50	50			50	50	50	50	50				
Shows Info/Consenting				20	20			20	20	20	20	20				
Consents/Questionnaires				20	40			60	80	100	120	140				
	Week 3 --							Week 4 --								
Invite for Consenting	50	50	50	200	200			200	200	200	200	200				
Shows Info/Consenting	20	20	20	120	120			120	120	120	120	120				
Consents/Questionnaires	160	180	200	120	240			360	480	600	720	840				
	Week 5 --							Week 6 --								
Cup distribution	120	120	120	120	120			120		120	120					
Baseline stool 1		100	100	100	100			100		100	100	100				
Stool 1 positives		20	20	20	20			20		20	20	20				
Baseline stool 2				20	20			20 + 20		20		20				
Stool 2 positives					16			16		16 + 16	16					
Sum of positives					16			32		64	80					
	Week 7 --							Week 8 --								
Cup distribution	120	120	120	120	120											
Baseline stool 1		100	100	100	100			100								
Stool 1 positives		20	20	20	20			20								
Baseline stool 2	20 + 20			20	20			20 + 20		20						
Stool 2 positives	16	16 + 16			16			16	16 + 16		16					
Sum of positives	96	112			128			144	176		192					

	Week 9 --							Week 10 --						
Treatment		 School holidays											
	Week 11 --							Week 12 --						
Treatment *	180	180	180											
AE's recording	X	X	X	X	X									
	Week 13 --							Week 14 --						
Cup distribution								96	96	96	96			
Follow up stool 1								90	90	90	90	90		
Follow up stool 2								90	90	90	90			

Appendix 3: package leaflet mebendazole

PACKAGE LEAFLET: INFORMATION FOR THE USER

Vermox[®] 100 mg tablets

Mebendazole

Vermox is a registered trademark

Read all of this leaflet carefully before you start using this medicine.

- Keep this leaflet. You may need to read it again
- If you have any further questions, ask your doctor or pharmacist
- This medicine has been prescribed for you. Do not pass it on to others. It may harm them, even if their symptoms are the same as yours
- If you get side effects and they become serious or if you notice any side effects not listed in this leaflet, please tell your doctor or pharmacist

In this leaflet

1. What Vermox tablets are and what they are used for
2. Before you use Vermox tablets
3. How to use Vermox tablets
4. Possible side effects
5. How to store Vermox tablets
6. Further information
7. Further advice regarding worms

1. What Vermox tablets are and what they are used for

The name of your medicine is Vermox 100 mg tablets (referred to as Vermox tablets in this leaflet). Vermox tablets contain a medicine called mebendazole. It is one of a group of medicines called 'anthelmintics'.

Vermox tablets are used to treat worm infections of the gut such as:

- threadworms (pinworms)
- other common worm infections (such as whipworm, roundworm, hookworm)

You or your child has been advised to take Vermox tablets because you have a worm infection. Worms can infect anyone. It does not necessarily mean that your hygiene is poor.

2. Before you use Vermox tablets

Do not use Vermox tablets if:

- You are allergic to anything in Vermox tablets (listed in section 6 below)
- Your child is under 2 years old

Do not use this medicine if any of the above applies to you. If you are not sure, talk to your doctor or pharmacist before using Vermox tablets.

Taking other medicines

Please tell your doctor or pharmacist if you are taking or have recently taken any other medicines. This includes medicines that you buy without a prescription, herbal medicines, dietary supplements or vitamins.

In particular tell your doctor or pharmacist if you are taking:

- Metronidazole - for certain infections
- Cimetidine - for excess stomach acidity

Talk to your doctor before using Vermox tablets if you are taking any of these medicines.

Pregnancy and breast-feeding

- Do not take Vermox tablets if you are pregnant, think you may be pregnant or might become pregnant
- Ask your doctor or pharmacist for advice if you are breast-feeding

Ask your doctor or pharmacist for advice before taking any medicine if you are pregnant or breast-feeding.

Driving and using machines

This medicine is not likely to affect you being able to drive or use any tools or machines.

Important information about some of the ingredients of Vermox

This medicine contains 0.06 mg of sunset yellow (E110). This ingredient may cause allergic reactions.

3. How to use Vermox tablets

Always use Vermox tablets exactly as your doctor has told you. You should check with your doctor or pharmacist if you are not sure.

Taking this medicine

- Take this medicine by mouth
- The dose will depend on which type of worm you have
- Crush the tablet before giving it to your child. Always supervise a child if they are taking this medicine
- Chew the tablets or swallow them whole
- You do not need to use a laxative or change your diet

How much you should take

Adults and children over 2 years old

For threadworms (pinworms):

- one tablet
A single Vermox tablet will kill threadworms. Your doctor may tell you to take a second tablet after two weeks in case of re-infection.

For other common worm infections:

- one tablet two times a day (morning and evening) for three consecutive days or as directed by your doctor.

If you take more Vermox tablets than you should

If you take more Vermox tablets than you should, talk to a doctor or go to the nearest hospital casualty department straight away.

If you forget to take Vermox tablets

- Do not take the missed dose
- Take your next dose at the usual time, and then keep taking your medicine as your doctor has told you
- Do not take a double dose to make up for a forgotten dose.

If you have any further questions on the use of this product, ask your doctor or pharmacist.

4. Possible side effects

Like all medicines, Vermox tablets can cause side effects, although not everybody gets them.

Stop using Vermox tablets and tell your doctor straight away if you notice or suspect the following serious side effects. You may need urgent medical treatment.

- Sudden swelling of your face or throat. Hives (also known as nettle rash or urticaria), severe irritation, reddening or blistering of your skin. These may be signs of a severe allergic reaction
- Blistering of your skin, mouth, eyes and genitals
- Fits (convulsions)

Tell your doctor or pharmacist if you notice any of the following side effects:

Common (affects less than 1 in 10 people)

- Stomach pain

Uncommon (affects less than 1 in 100 people)

- Stomach discomfort
- Diarrhoea
- Wind

Rare (affects less than 1 in 1,000 people)

- Rash
- Inflammation of the liver
- Changes in liver enzymes (shown in blood tests)
- Reduction in white blood cells (shown in blood tests). You may get more infections
- Unusual hair loss
- Dizziness

Reporting of side effects

If you get any side effects, talk to your doctor, pharmacist or nurse. This includes any possible side effects not listed in this leaflet. You can also report side effects directly via the Yellow Card Scheme at: www.mhra.gov.uk/yellowcard

By reporting side effects you can help provide more information on the safety of this medicine.

5. How to store Vermox tablets

- Keep out of the reach and sight of children
- Do not use Vermox tablets after the expiry date which is stated on the label. The expiry date refers to the last day of that month
- There are no special storage conditions
- Medicines should not be disposed of via wastewater or household waste. These measures will help protect the environment. Return any leftover Vermox tablets to your pharmacist

6. Further information

The active substance in Vermox tablets is mebendazole. The tablets contain 100 mg of mebendazole.

The other ingredients are microcrystalline cellulose, sodium starch glycolate, talc, maize starch, sodium saccharin, magnesium stearate, cottonseed oil hydrogenated, orange flavour, colloidal anhydrous silica, sodium laurilsulfate, sunset yellow (E110).

What Vermox tablets look like and contents of the pack

Vermox tablets are flat, circular, pale orange tablets with "Me/100" on one side and "JANSSEN" on the other.

Vermox tablets are available in blister packs containing 6 tablets.

The product licence is held by:

JANSSEN-CILAG LTD, 50-100 Holmers Farm Way, High Wycombe, Buckinghamshire HP12 4EG, UK

Vermox tablets are made by:

Janssen-Cilag SpA, Via C Janssen, 04100 Borgo San Michele, Latina, Italy
OR

Lusomedicamenta Sociedade Técnica, Farmacêutica, S.A. Estrada Consiglieri Pedroso 69-B, Queluz, 2730-055 Barcarena, Portugal

OR

McGregor Cory Limited, Middleton Close, Banbury, Oxfordshire, OX16 4RS, UK

For information in large print, tape, CD or Braille, telephone 0800 7318450.

This leaflet was last revised in May 2016.

7. Further advice regarding worms

Threadworms (pinworms) produce large numbers of tiny eggs. They may be present in house dust and can stick to clothing, carpets, towels and bed linen. They can also be picked up by contact with someone who already has worms. Because the eggs are so small, it is very easy for them to be swallowed. Then they pass into the bowel where they grow into worms. The female lays her

eggs at night around the bottom. It is this that causes the "itchy bottom" and leads to scratching. The eggs are then transferred to the fingers and finger nails, and can easily get into the mouth by finger sucking or nail biting. The life cycle of an adult worm can be as long as six weeks.

To stop you and your family infecting others or re-infecting yourselves, follow the advice below for at least 6 weeks:

- Keep nails short
- Discourage nail biting or finger sucking
- Wear pyjamas or underclothes in bed
- Each morning, wash your bottom thoroughly
- Use a separate towel for each person in the house
- Change clothes regularly
- Wash and iron bed linen regularly
- Wash hands and nails well after using the toilet and before meals.

Appendix 4: English ICF prepared by funder

Efficacy and safety of a single-dose regimen and a multi-dose regimen of mebendazole against hookworm infections in children: a randomized-controlled trial

The Ministry of Health and Social Welfare (Zanzibar, Tanzania) has permitted this study after careful verification. The Zanzibar Medical Research and Ethical Committee has also reviewed and approved this study.

Introduction

You are being asked to take part in this study because your child is between the ages of 6 and 12 years. This study is sponsored by the Swiss Tropical and Public Health Institute. The person in charge of this study at this site is Mr. Said Ali. The enrollment process includes interview questions, stool sample collection, a finger prick blood test, and a physical exam.

This is an enrollment consent form. It gives you information about the study product, study questions and exams, and what your child will need to do to be in the study. The study staff will explain the exams and tests to you and what is expected of you and your child. You are free to ask questions about the study at any time. If you agree for your child to take part in this study, you will be asked to sign this consent form or make your mark in front of a witness. You will be given a copy of this form to keep.

Why Is This Study Being Done?

Hookworm infection is an infection by a parasitic worm. These worms, and others like it, live in the small intestine of infected persons. The worms can cause malnutrition, poor growth and development, and reduced work performance later in life.

On Pemba, most children are infected with one or even two or three different types of parasitic worms. This is the reason why children are regularly given drugs to kill these worms. However, the worms may acquire resistance to the drug when it is used for many years, meaning that the medicine loses its capacity to kill the worms. We would like to try two different doses of the same drug (mebendazole) to understand which one is best at killing the worms that your child suffers from. Mebendazole works by killing worms in the body, which are then passed through the stool. Mebendazole is an approved drug for parasitic worms and has been used for many years in Tanzania and other parts of the world for treatment. Mebendazole can be given at a low dose twice a day for 3 days or it can be given once at a high dose.

We would like your child to take part in a research study. The aim of this study is to look at how well two different amounts of mebendazole kill the worms in the children's bodies. Approximately 450 children will be screened for parasites by stool sample and 180 subjects with hookworm will join this treatment study. Your child will be tested to confirm that they are infected with hookworm before being treated. If your child is still infected with hookworm at the end of the study, your child will receive one dose of another anti-parasitic drug called albendazole. Albendazole is also licensed in Tanzania and may cure hookworm infection that is not cured by mebendazole.

An international health non-profit organization, PATH, is providing funds for this study to take place. Each subject will be in the study for a total of about 8 weeks: one month for screening, three days of treatment, and follow up 14 to 21 days after treatment. If your child is in the study, your child will have to attend 11 study visits at your child's school.

What Is The Study Design?

This study will compare the two forms of oral mebendazole. Mebendazole tablets (100 and 500 mg) are from Johnson & Johnson (Zug, Switzerland). Both of these tablet forms of mebendazole are licensed in Tanzania.

Neither you, your child, nor the study staff will know if your child is getting the low dose or high dose drug. This is called keeping the study “blinded.” Not even the study doctor will know, although he can find out if necessary.

The study drugs will be given by mouth. The low dose medication will be given twice a day for 3 days and the high dose will be given once. To keep the study blinded, all subjects will receive a total of 6 small tablets (containing either drug or no medicine, called a “placebo”) and one large tablet (containing either drug or placebo).

Your child will be assigned by chance (like flipping a coin) to be in one of 2 groups. There will be 90 subjects in the low dose group and 90 in the high dose group. So there is an equal chance of being in either group.

What Does My Child Have To Do If My Child Takes Part in the Study?

If you agree for your child to be in the study, your child will have these study visits here at school:

- Enrolment Visit
- Day before treatment
- Treatment days 1-3
- Follow up visit for safety: to look at any signs and symptoms your child may suffer on days 4 and 5
- Follow up visit for efficacy: stool exam between days 14 and 21 to check if your child is still infected.

While your child is in the study, we will ask your child to come in for all study visits. We will also ask you and your child to follow the instructions of the study staff for taking the medications.

Enrolment Visit

Your child’s Enrolment Visit will continue today, after you read, discuss, and sign or make your mark on this form. No study activities will be started before they have been fully explained to you, you have let us know that you understand the enrollment process and you have signed or made your mark on this form.

The Enrollment visit will take about one to two hours. You will be asked to do these things for the Enrollment Visit if you decide you want your child to be in the study:

- Sign this form or make your mark on it after you have read it, understand it, and had the chance to ask questions about the study.
- Tell the study staff how they can stay in contact with you.
- Tell the study staff about any medical problems of your child.
- Tell the study staff about any medicines your child is taking now.
- For all girls, tell the study staff if they have ever had a menstrual period.
- Have your child have a physical exam.
- Have your child provide two stool samples for detection of parasites.
- Have your child provide a finger stick blood sample for anemia testing.

If your child is diagnosed positive for the hookworm parasite and passes the other screening questions and tests, your child can participate in the study and will be assigned by chance to one of the two different treatment groups.

Treatment Days 1, 2 and 3

Your child will visit the study staff twice at school a day so he/she will spend all day at school during the three treatment days. Each day's contact with study staff will take about 30 minutes. At these treatment visits, your child will:

- Tell the study staff how he or she feels and if there are any changes in his or her health since the last visit.
- Take one large pill and one small pill on the first morning. Each pill will be taken by mouth with water.
- Take one small pill in the morning and another one in the afternoon approximately 10 hours later for the remaining days.
- Tell the study staff about any medical problems 3 hours after treatment.

Follow-up Safety Visits on Days 4 and 5

These visits will take about 10 minutes each. At these visits, we will ask your child to:

- Tell the study staff about any medical problems.

Contact the study doctor right away if your child has symptoms or you feel concerned about your child's symptoms and health.

Follow-up Stool Exam between Days 14 and 21

During school hours, two stool cups and instructions on how to collect stool specimens will be provided to your child before the visit. Your child will then return the specimen cup with stool in it to the study team during school hours.

Follow-up Albendazole Treatment

If your child's stool specimen is still positive for hookworm after the mebendazole treatment, you will be notified and your child will be given one tablet of albendazole at the end of the study (approximately 2 months from now). Albendazole is an approved medication in Tanzania for the treatment of hookworm.

Can My Child Be Removed From The Study?

The study doctor may need to take your child out of the study early without your permission if:

- The investigators recommend that the study be stopped early for safety concerns.
- Your child is not able to keep appointments.
- Other reasons that may prevent your child from completing the study successfully.

What Are The Risks of Being in the Study?

Mebendazole and albendazole are usually well tolerated. The most common side effects are stomach/abdominal pain, vomiting, diarrhea, fever, headache, dizziness, or drowsiness. In any case, if anything were to happen your child is insured during the trial. Mebendazole is not recommended for use in the first trimester of pregnancy and anyone who might be pregnant should not participate in the study.

Risk of Blood Draws:

Your child may feel discomfort or pain when your child's blood is pricked from his or her finger.

Are There Benefits To Taking Part In This Study?

Your child will receive a hemoglobin test for anemia, stool examinations and treatment for intestinal parasites free of charge.

Alternatives To Participation

Your child does not have to join this study. Your child's alternative is not to join this study. Your child will receive regularly scheduled treatment for intestinal parasites at school.

What About Confidentiality?

Efforts will be made to keep your child's personal information private. If this study is published, your or your child's names will not be used and your child will not be personally identified. Your child's information will be labeled with an identification code for all data analysis. Your child's records may be reviewed by the following institutions:

- Swiss Tropical Public Health Institute
- National Institute of Public Health or other health authorities
- Study staff
- Study monitors
- Ethics committees
- PATH

However, your child's personal files will always remain in the Public Health Laboratory Ivo de Carneri, in Pemba.

What Are The Costs To Me?

The study procedures will be provided at no cost to you or your child. Ask the study doctor to discuss the costs that will or will not be covered by the sponsor.

Will I Receive Any Payment?

The parents attending this meeting will receive a small provision to cover their costs for transportation (~US\$ 2). Participation is voluntary and therefore itself will not be awarded with compensation.

What Happens If My Child Is Injured?

If your child suffers an injury directly related to participation in this project, a general liability insurance of the Swiss Tropical Public Health Institute (Swiss TPH) and National Insurance Cooperation of Tanzania LTD will help you obtain medical treatment for your child's specific injury and provide referrals to other health care facilities, as appropriate. Swiss TPH, the sponsor of the study, will provide reimbursement of reasonable medical expenses incurred as a result of your participation in this study. The sponsor will not pay for the treatment of other injuries including those caused by non-compliance with the protocol and study staff instruction, and any underlying diseases or conditions that your child may have. No other compensation such as lost wages or pain and suffering will be provided. The study staff can give you more information about this if your child has a study injury. You are not precluded from seeking to collect compensation for injury related to malpractice, fault, or blame on the part of those involved in the research, including the hospital.

Right To Withdraw

Your child doesn't have to be in this study. Your child's participation in this study is voluntary. You or your child may decide not to have your child participate, or your child may leave the study at any time without further obligations. You or your child's decision will not result in any penalty or loss of benefits to which your child is entitled.

- If you want your child to stop being in the study, or your child wants to stop being in the study, please tell us right away.
- Leaving this study early will not stop your child from getting regular medical care.
- If your child leaves the study early, information collected up to the date your child leaves the study can be used only as outlined in the confidentiality section of this consent form.
- If your child leaves the study after taking the study medication, you and your child will be given contact information to reach the study doctor or the study coordinator if your child develops any illness that may be related to the study.

What Do I Do If I have Problems or Questions?

For questions about the screening exams and tests or if your child has a research-related injury, you should contact:

Mr. Said Ali or Mr. Shaali Ame
Public Health Laboratory Ivo de Carneri
P.O. Box 122 Wawi, Chake Chake
Pemba, Zanzibar (Tanzania)
Phone: +255 24 245-2003

For questions about your rights as a research participant, contact:

Zanzibar Medical Research and Ethical Committee (ZAMREC, Tanzania)
Dr. Msafiri L. Marijani, Administrator
Mnazi Mmoja Hospital, P.O. Box 672
Zanzibar (Tanzania)
Phone: +255 77 666-3303