**STUDY PROTOCOL**

# Mikono Safi Study

**Hand hygiene intervention to optimise helminthic infections control: a cluster-randomised controlled trial in NW Tanzania**

(mikono safi – clean hands)

Principal investigator:

Prof Saidi Kapiga, Mwanza Intervention Trials Unit (MITU) / London School of Hygiene and Tropical Medicine (LSHTM)

Co-Principal investigator:

Dr Safari Kinung'hi (National Institute for Medical Research, Mwanza Centre)

Co-Investigators

Prof Heiner Grosskurth (MITU/LSHTM)
Dr Christian Hansen (MITU/LSHTM)
Dr Robert Dreibelbis (LSHTM)

## Executive summary

The proposed research is a proof of concept trial to establish how effective a high intensity hand hygiene intervention after deworming can be in reducing both intensity and prevalence of selected helminthic infections among primary school students.

This work will be conducted in the Kagera Region of Tanzania, NW-Tanzania. Surveys conducted in this populous area have shown a very high prevalence of infection with *Ascaris lumbricoides* and *Trichuris trichiura,* ranging from18% to more than 50% (Brooker 2009; Siza 2015), particularly among children aged 6 – 12 years, indicating both the urgent need for an effective intervention and also the suitability of this environment for the proposed study.

The proposed behaviour change intervention will be a combination intervention package comprising of the following components: teacher-led health education in primary schools, low-cost structural improvements with respect to water supply and sanitation, nudges to increase students intention to wash hands after defecation, screening of students for current worm infection combined with feedback of results to parents and health information given to students' parents. The intervention will use a health promotion strategy that aims to generate emotional drivers similar to that validated in the SuperAmma study (Biran 2014) which had shown substantial increases in hand-washing behaviour in India. This intervention package will be designed to fit into the cultural context of Tanzania. Implementation will be primarily school-based, but will involve children's parents and guardians. The exact operational details of the above intervention components will be informed through

qualitative (formative) research that is currently being conducted at 3 pilot schools and communities in Kagera Region (under a separate protocol with separate ethical clearance).

The efficacy of the intervention will be tested through an open-label cluster-randomised controlled trial, with primary schools being the unit of randomisation, involving students recruited from intervention and control schools. The primary outcome will be the prevalence of infection with ascaridiasis and trichuriasis following deworming in both intervention and control schools. Secondary outcomes will include self-reported hand-washing behaviour both at home and in schools, the prevalence of worm eggs retrieved from hands in a subgroup of pupils, and the prevalence of hookworm infection.

## Background

Chronic diarrhoea and helminthic infections, especially soil transmitted helminths (STH), are frequent infections in childhood, and are strongly associated with malnutrition, poor child development and cognitive performance. Both diarrhoea and helminthic infections have a strong association with poor access to water supply, sanitation and hygiene. Malnutrition is responsible for 45% of children's deaths in low and middle income countries, and also has long term consequences such as: short adult height and associated complications during delivery, low birth weight, impaired cognitive, motor and social development, and reduced economic productivity.

Control of STH has largely depended on the administration of anthelmintic drugs, though a recent systematic review has shown that the use of school based deworming programmes had little to no impact on child development and cognitive performance (Taylor-Robinson 2012). Reinfection is often rapid, and for Ascaris infection sometimes reaches pre-treatment levels within six months post-treatment (Yap 2013), as the root causes of transmission have not been addressed. Open defecation has been shown to have a strong association with all STH infections (Ziegelbauer 2012). Although the results of recently published randomised controlled trials on the impact of sanitation interventions on STH in India have shown that sanitation programmes can increase the coverage of latrines, actual use of latrines remains low, with especially men and children still practicing open defecation (Patil 2014, Clasen 2014).

STH have a low infective dose, where a single egg can cause an infection, and especially Ascaris eggs can survive and remain infective for several months in the environment (Nordin 2009), and are particularly sticky (Brownell 2007). They are predominantly found in soil, but also on vegetables, especially in areas where human faeces or wastewater are used as fertilizer (Ensink 2008, Do 2007). People pick up Ascaris and trichuris infections when they ingest these eggs after they have matured in the environment by eating raw, unwashed vegetables or by not washing their hands after handling contaminated soil which is thought to be a common transmission route for children (Ziegelbauer 2012).The exact role of hands in the transmission of eggs is unclear, as a systematic review on the impact of hand washing with soap and ascariasis remained inconclusive (Fung and Cairncross 2009). However, results from a more recently completed randomised controlled trial in China found that the promotion of hand washing with soap in schools resulted in a 50% reduction in STH prevalence (Bieri 2013). A pilot study to test a method developed under SHARE phase 1 to quantify eggs on hands found 34% of hands of farmers who used fresh excreta as a fertilizer positive for STH eggs (Gulliver 2014), while a study among school children found a prevalence of about 20% (Cranston 2015).

Deworming campaigns remain a cost-effective way to reduce the burden of STHs (Clarke 2017), but as deworming does not target the root causes of STH and re-infection occurs rapidly, an integrated approach that combines sustainable hygiene behaviour change with deworming could prove an effective way to control STH infections.

The proposed study aims to establish whether the effect of routine deworming campaigns can be enhanced and sustained by combining it with an appropriate Water, Sanitation and Hygiene (WASH) behaviour change intervention.

In preparation of our protocol we took careful note of the findings from the large scale 'SWASH project' intervention programme that has been conducted at schools in Kenya (Freeman 2013; Freeman 2014; Caruso 2014; Saboori 2013; Greene 2012). This programme focused on improving the availability of clean toilets and soap at schools and was combined with some hygiene promotion, and was successful in reducing the prevalence of ascariasis by about 50% and Ascaris egg work load by nearly 70% (Freeman 2013).

There are a number of differences between the work in Kenya and the work we are proposing here. Different from our proposed intervention, the SWASH project did not include an educational behaviour change intervention per se, it did not aim at changing WASH behaviour both *at home and at school*, it did not involve parents and guardians, and there is also evidence from the SWASH publications that water has not been sufficiently available even at those schools that had water 'within reach'. Importantly, the creation of a strong *emotional motivation* to wash hands at key times is the central component of our proposed intervention, and in this respect goes substantially beyond the intervention efforts in the Kenyan studies.

One important observation from the SWASH programme was the total lack of consumables at schools for anal cleansing after defecation, mainly for cost reasons (poverty). We are aware that this is also a problem at primary schools in Tanzania, and may affect our proposed trial. It can be argued that under such circumstances open defecation in an area that at least provides access to plant material may be better than using an insufficiently equipped and dirty latrine. However, we argue that cleansing of the anal area by hands or with inappropriate materials as often used (e.g. stones, old garbage paper) *but followed by careful hand-washing*, whilst less than ideal, may go a long way to reduce the risk of faecal-oral transmission, both with respect to bacteria and helminths.

## Aims and objectives

The overall aim of the proposed cluster-randomised controlled trial is to assess the effectiveness of a behaviour intervention package for the promotion of hand washing with water and soap, among school aged children in NW Tanzania in reducing both the prevalence and intensity of *Ascaris lumbricoides* and *Trichuris trichiura* infections in these children. Through this work, we aim to lay the foundation for a future (cost-)effectiveness study of the intervention to be conducted within the context of the ongoing school-based national deworming programme in Tanzania.

### Specific objectives

1. To introduce a scalable, school-based combination intervention to promote hand-washing with water and soap at key times during the day among school aged children (6-12 years) in NW Tanzania.
2. To assess the effectiveness of this intervention in changing hand-washing behaviour in the study population.
3. To assess the effectiveness of this intervention in reducing the prevalence and intensity of *Ascaris lumbricoides* and *Trichuris trichiura* infections in the study population.
4. To explore the costs and the cost-effectiveness of the intervention in order to inform the Tanzanian government with respect to the possible integration of the intervention into the national STH control efforts.

## Methods

To achieve these objectives we propose to conduct a study in which a high intensity hygiene behaviour change programme will be implemented in order to determine 'the maximum' reduction in prevalence and intensity of infection that hand-washing with soap can achieve, after routine deworming as provided by the ongoing Tanzanian national programme for the control of neglected tropical diseases (NTDs). We propose a cluster-randomised controlled trial in 16 schools in Kagera Region of NW Tanzania.

### Study Area

Kagera Region is the large area situated between the shores of Lake Victoria in NW Tanzania, and the borders of Tanzania to Uganda and Rwanda. The region has a population of about 2.46 million people (NBS 2012). The capital city of the region is Bukoba with about 300,000 inhabitants. The region comprises seven districts, each with sizable district towns, of which Bukoba, Muleba and Karagwe are the largest. The main economic activity in the region is agriculture, with farmers growing a range of food crops, including coffee and tea for export markets. Other activities include fishing and industrial processing of fish for export. The continuing urbanisation ofthe region's towns, coupled with high population growth and often inadequate infrastructure, contributes to the existing high burden of infectious diseases such as malaria, STH, respiratory tract infections, diarrhoea, and HIV/AIDS, and an emerging epidemic of non-communicable diseases.

All major villages of the region have primary schools, and the total number of primary schools in the region is about 800.The schooling rate is high, exceeding 90% in urban areas. The prevalence of ascariasis among school children has been estimated to exceed 25% in most places (Brooker 2009). In a recent still unpublished survey conducted by our team among 2000 students from 34 primary schools in Kagera, the prevalence of ascariasis and trichuriasis ranged from 0 - 71%, exceeding 25% in most schools (Kinung'hi – personal communication).

**Intervention design**

Design of the intervention will draw on experience from India (Biran 2014), Kenya (Freeman 2014, Saboori 2013) and Bangladesh (Dreibelbis 2016) as well as recent successful behaviour change interventions in Zambia and ongoing work in Nigeria (unpublished).

*Underlying principles*

1. The intervention will be mainly school based, but will involve students' parents or guardians to ensure their support.

2. The intervention will be intensive, but affordable and feasible within the public primary school education system allowing it to be replicated and upscaled in other primary schools in Tanzania, if shown to be effective.

3. The behaviour change intervention will combine emotional drivers (in line with current thinking on behaviour change) and structural facilitators ('nudging'), with classical health educational messages.

4. Practical details of this combination intervention will be adapted to the cultural context in Tanzania, and will be designed based on qualitative (formative) research currently conducted at pilot schools in Kagera.

5. Deworming treatment with helminths will be provided in all primary schools involved in this trial, as part of the national deworming programme. All participating schools will have a reliable water supply and hand washing points. Schools in the intervention arm of the trial will, in addition, receive the proposed behaviour change intervention to promote hand washing with water and soap. Deworming will be timed so that it is executed just after the behaviour change intervention has started in intervention schools. Timing it in this way ensures that the intervention is in place in intervention schools at the time of the deworming exercise, and that potential infections would occur in-spite of the intervention (and not because the intervention is not yet up and running).

*Deworming*

Deworming campaigns are usually conducted at regular intervals of 12 months under the auspices of the national neglected tropical diseases (NTD) control programme, Ministry of Health, Community Development, Gender, Elderly, and Children of the United Republic of Tanzania. In this trial, deworming will be performed in both intervention and control schools by the national NTD control programme if timing permits, or by research staff according to guidelines of the Ministry of Health to ensure that deworming and the hand-washing intervention are synchronised (see Figure 2). Treatment will be with Albendazole according to guidelines (400 mg, single dose).

*Intervention components*

Classes 1 to 6 of the participating primary schools will receive the intervention; and the majority of the children in these classes are expected to fall into the age-band of 6 – 12 years. The intervention will consist of the following components:

1. *Initial worm infection survey in intervention schools:* To generate maximum parental support for the intervention among students from schools in the intervention arm, we will conduct an initial screening for STH in these students, using the Kato-Katz methods.

2. *Parents' and guardians' meeting:* Following the survey, parents and guardians of students from the same class will be invited for a meeting at the school. On this occasion, the results about the infection of their individual children will be given to the respective parents, provided in individual (confidential) envelopes. Dedicated teachers, supported by project staff, will explain the meaning of the results to the audience. They will provide information on the potential consequences of worm infections for the health and development of children, briefly explain routes of infection and how to reduce risk of infection through hand-washing behaviour at key times. The purpose of this intervention component will be to raise personal emotional concern among parents and guardians, establishing the desire to improve hygiene behaviour in the home and to provide parental support to the proposed class–room based behaviour-change education described under point 3 below. The aim is to improve hygiene behaviour in the family in general, and among children both at home and at school. Messages will focus on hand-washing behaviours at key times, but the need for clean food and water will also be discussed.

   This strategy has been employed with high and statistically significant effect in the Ukerewe District of Mwanza Region from 1993 – 1996, in order to promote hygiene and latrine building (personal communication, Dr Mwasha, HESAWA SIDA Project). Unfortunately this experience has not been published as the PI died soon after the end of the intervention project.

3. *Class-room based health education:* In each school we will train two interested teachers who will over time provide up to 3 teacher-led sessions of up to 2 x 1 hour to each class about personal hygiene, during the course of one school year. In this effort they will be supported by project staff. All classes 1 to 6 of the participating primary schools will receive the intervention; and the majority of the children in these classes are expected to fall into the age-band of 6 – 12 years. The sessions will (i) focus on basic essential health information on STH, adapted to the mean age level of each respective class; (ii) use educational

material to generate behaviour change intentions using emotional drivers(feelings of disgust about faecal hand and food contamination), and (iii) offer solutions through demonstration of hand-washing which will be embedded in hygiene messages.

Sessions will be delivered using health information and the education materials described below under point 5. The sessions will be delivered according to the schedule shown in Figure 2: the first intervention session will be delivered about 4 weeks after the initial worm infection survey described above and about 2 weeks after the parents' meeting, and will be timed to occur about 2 weeks prior to the deworming campaign. A re-enforcement session will be provided within 2 weeks after the deworming campaign. A second re-enforcement session will be conducted after about a further 6 months (Figure 2). During these intervention sessions, correct hand-washing behaviour with water and soap will be demonstrated and practiced.

4. Hand washing areas will be equipped with soap bars or with soap water solutions (Saboori 2013), depending on findings from the formative phase of the project (see below).

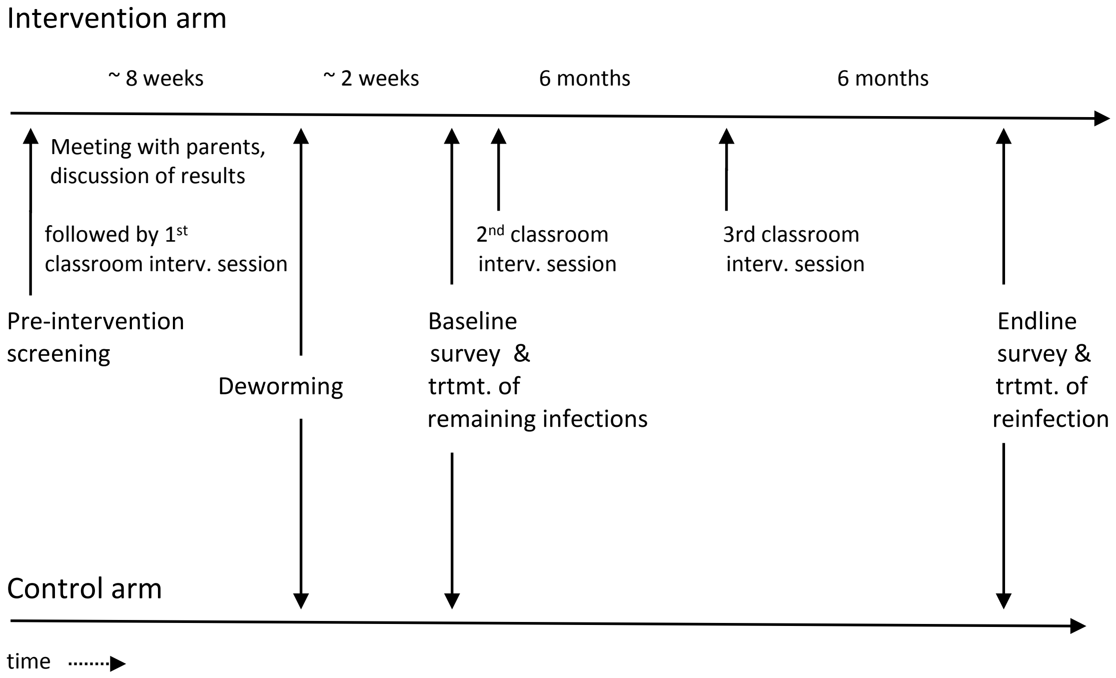
5. *Educational materials for use during school sessions:* These will comprise pictorial displays. The material will aim to both provide information and raise the feeling of disgust among children with respect to the possible contamination of hands and food by faecal materials as described under point 3. The materials may be enhanced through storytelling and role-plays, depending on the results from the formative research phase (see below).

6. *Information leaflets for parents and guardians:* These will be designed to reinforce messages provided at the parents' and guardians meeting (see point 2 above), and to reach parents who did not attend this initial meeting. The leaflets will be written in Kiswahili. Children will be asked to return the lower part of the leaflet with a signature by their parent confirming that the leaflet has been seen.

7. *Nudging:* Nudges are environmental cues engaging unconscious decision-making processes to prompt behaviour change (Dreibelbis 2016). Typical nudges comprise footprints on footpaths painted in bright colours that aim to guide students to handwashing stations (Figure 1). They can also include handprints painted at stations. The potential use of these nudges and their exact design will depend on the outcome of the formative research (see below).

*Figure 1*

**Figure 2 Relative timing of intervention events, surveys, and deworming campaign**




*Development and piloting of the intervention*

The trial is preceded by a preparatory phase to conduct qualitative (formative) research in three schools of Kagera region with the aim to pilot details of the intervention package described above as components 1 – 7. These schools are different from those to be recruited for the trial. This preparatory work is being conducted under a separate protocol (with separate ethical clearance) and is being performed by two social scientists under the supervision of a senior WASH experienced anthropologist and of the PI of the main trial, in close collaboration with local authorities of the Ministry of Health and Social Welfare, and the Ministry of Education in Kagera region.

**Activities in the control arm**

There will be no WASH promotion related intervention activities in the control arm. The deworming campaign will be conducted in both arms of the trial as depicted in Figure 2 above. However, students from schools in the control arm will have access to water and devices to wash hands like those in the intervention arm. They will not receive (additional) soap through the intervention project (as this would be an intervention in itself that may change behaviour and therefore possibly dilute the measurable intervention effect between arms).

**Evaluation of the intervention**

*Overview*

The intervention will be evaluated through a cluster randomised controlled trial. Sixteen primary schools will be randomised to receiving the above intervention package (N=8) or to be in the control arm (N=8).

Whilst the intervention will be delivered to all classes 1 – 6 of the schools randomised to the intervention arm, a random selection of classes will be recruited for the evaluation. For the evaluation, we will select an equal number of classes in both arms, and the random selection will be restricted so that selected classes represent comparable age bands (to avoid that by chance there would be more 'younger' classes in one of the trial arms than in the other).The target group will be children aged 6 – 12 years, but all children in the participating classes will be enrolled, including those that may fall outside of this age band.

The evaluation will involve two cross-sectional surveys: a baseline survey (conducted just after the deworming campaign in both arms of the trial, and just after the intervention has been started in the intervention arm); and an endline survey to be conducted about 12 months after the baseline survey in each respective school (Figure 2).

*Baseline and endline surveys*

The baseline survey has three objectives: (i) to confirm whether the deworming campaign was successful, (ii) to document any baseline differences in STH prevalence between arms in order to facilitate the analysis and interpretation of trial results, and (iii) to identify specific students in schools from both arms who are still infected. These students will be treated again. The aim is to ideally eradicate STH infections in both arms so that for the purpose of the trial, infections found at the endline survey are more likely to be reinfections, and differences between infection prevalences in the two arms may be attributed to the intervention in the intervention arm. For this reason, the initial introduction of the intervention in intervention schools will be timed to occur just before the deworming campaign (so that the risk of spontaneous reinfection is reduced – see Figure 2).

*Outcomes*

The primary outcome will be the combined prevalence of ascariasis and trichuriasis in students' stool samples in both intervention and control schools determined at about 12 months after initial deworming in both intervention and control schools.

Secondary outcomes will include

(i) hand-washing behaviour in schools (reported and observed) and at home (reported only)
(ii) intensity of ascariasis and trichuriasis infections at baseline and 12 months after deworming
(iii) levels of hand contamination with worm eggs and E. coli bacteria at 12 months after deworming

(iv) prevalence and intensity of hookworm infection at baseline and at 12 months after deworming

**Sample size considerations**

With respect to the primary outcome, the expected combined prevalence for Trichuris and Ascaris infection in school children in study schools will be about 30%. In the absence of the hand-washing intervention, the prevalence is assumed to reach original levels within one year after deworming. The intervention is expected to reduce this by at least 40%. Table 1 shows the different numbers of clusters (schools) required per arm for different scenarios. The scenarios differ with respect to the number of students per school, the size of the intervention effects, and the inter-cluster coefficient of variation (k). We will aim to enrol a total of 3200 pupils from a total of 16 primary schools (8 to be allocated randomly to each trial arm). This sample size will provide at least 80% power to detect an intervention effect of at least 40%, assuming a between-cluster co-efficient of variation of 0.3 (Table 1). If the baseline prevalence is indeed about 30% and the reduction of prevalence during the endline survey about 50%, this sample will provide about 95% power to detect this effect size (Hayes and Bennett).

**Table 1**

| Type-1 error | Power | Prevalence in comparison arm | Prevalence in intervention arm | Nu. of students per school | Coefficient of variation k | Nu. of schools per arm | Total nu. of participants |
|---|---|---|---|---|---|---|---|
| 0,95 | 0,8 | 40.00% | 30.00% | 300 | 0.25 | 15 | 6000 |
| 0,95 | 0,8 | 40.00% | 25.00% | 300 | 0.3 | 8 | 3200 |
| 0,95 | 0,8 | 40.00% | 20.00% | 200 | 0.3 | 5 | 2000 |
| 0,95 | 0,8 | 30.00% | 20.00% | 300 | 0.25 | 9 | 3600 |
| **0,95** | **0,8** | **30.00%** | **18.00%** | **200** | **0.3** | **8** | **3200** |
| 0,95 | 0,8 | 30.00% | 15.00% | 200 | 0.3 | 6 | 2400 |
| 0,95 | 0,8 | 20.00% | 15.00% | 300 | 0.3 | 22 | 8800 |
| 0,95 | 0,8 | 20.00% | 13.00% | 200 | 0.3 | 12 | 4800 |
| 0,95 | 0,8 | 20.00% | 10.00% | 200 | 0.3 | 5 | 2000 |
| **0,95** | **0,95** | **30.00%** | **15.00%** | **200** | **0.3** | **8** | **3200** |

When selecting participants within schools, we will aim for a gender balance of 1:1 overall. In each school we expect to recruit about 3 - 5 classes of pupils in whom the majority of pupils is aged between 8 and 12 years, aiming for 200 students per school, and an even distribution of age groups within the target age band. All assenting students in a selected class will be enrolled, even if their number exceeds the target, and if some students would fall outside of the target age.

**Measuring outcome indicators**

***Primary outcome:* Helminth infection at 12 months after deworming**

This will be determined through a cross-sectional survey conducted in intervention and control arms at 12 months after the baseline survey (Figure 2). (The baseline survey is required to identify and treat students who were still infected in spite of the initial deworming, and to generate data about potential imbalances between trial arms at the beginning of the follow-up period, in order to facilitate adjustments in the data analysis). A questionnaire will be administered to collect information on age, sex, school grade, number of family members, personal risk factors potentially associated with STH, including last time hands were washed with/without soap or soil-eating behaviours, materials used for anal cleaning after defecation (Freeman 2013), and information about potential confounding variables related to the environment, including availability of water at home, hand-washing behaviours at home, source of food eaten etc. Study ID-numbers will be assigned and stool samples will be collected and processed as described below.

***Stool sample collection:***
Pre-labelled 50 ml sample bottles will be distributed in the school and the fresh stool samples collected on the next day. Each student participating in the study will receive an individual container labelled with the unique ID number and the individual's name. On distribution of the sample containers the interviewer will explain how the containers should be used. Using a leaflet, the purpose of the stool examination will be explained and parents/ guardians will be asked to help their children with sample collection. In the schools, when the filled sample containers are returned, the stool samples will be stored on ice in a cool box and brought to the laboratory for analysis as soon as possible.

The prevalence and intensity of intestinal nematode infections (in particular; *Ascaris lumbricoides,* and *Trichuristrichuria,* but also hookworm) and the prevalence of protozoa infections (in particular; *Giardia intestinalis*) will be determined.

All study participants who have a positive stool sample (in spite of the general deworming treatment just days before the baseline survey), will be provided with anthelmintic or anti-protozoal medication according to the lab results obtained. The same will apply to participants with positive samples at the endlinesurvey.

*Laboratory methods: helminth and protozoa analysis*
We will use the formol-ether concentration method to identify helminth ova and protozoa cysts, and to determine infection intensity. On the day of collection, stool samples will be fixed in 10% formalin in the field laboratory. On arrival at the parasitology laboratory of NIMR Mwanza centre, the samples will be classified based on their consistency as either F(ormed), S(oft), L(oose) or W(atery).

The formal-ether concentration technique will be used for analysis, as it has consistently shown higher recovery, especially in light infections, in comparison to other techniques (Young 1979). For safety reasons, ether will be replaced by ether-acetate, which has been shown not to affect the results (Ridley 1956).

Intensity (as eggs per gram) of intestinal nematode infections will be quantified by taking a pre-weighted stool sample of approximately 2 grams, which will be dissolved in 6 ml of formalin (30% formaldehyde). After proper mixing, this will be transferred to a glass tube in which 2 ml of ether-acetate will be added. The tubes will be centrifuged at 2400 rpm for two minutes, after which fat and formal water will be drained off. All residues in the tube will be analyzed in a saline solution under a 10 by 10 magnification for helminth eggs. A sample will be declared negative if 3 slides yield no ova. Intensity of infection will be determined by both egg counts and infection intensity categories (i.e. light, moderate, heavy).

***Secondary outcome:* hand-washing behaviours**

Hand-washing behaviours will be assessed by direct, structured observation in schools, and by self-reports asked of students in schools regarding both home and school-based behaviours. Observations will be conducted by trained monitors who will anonymously record whether students wash their hands and make use of soap, after using a toilet. The percentage of students washing hands with / without soap will be determined.

Hand washing behaviours at home will be assessed based on students' self-reports. For these self-reports simple questionnaires will be administered to students themselves (whilst in school).

Self-reported hand-washing behaviour is subject to recall bias, but also desirability bias. We will therefore collect this information using techniques that minimises desirability bias (e.g. we will not ask whether a participant washed hands, but how often s/he could not do so because of time pressure or other external factors).

***Secondary outcome:* intensity of worm infections**

The intensity of worm infections will be graded, and will be recorded during the microscopic assessment of all stool samples as described above in the section on laboratory methods.

***Secondary outcome:* Levels of hand contamination**

Data on hand contamination is scarce, especially for the presence and concentration of STH ova, and no data on variations between individuals, or for particular risk factors has been found in the literature. A required sample size is therefore difficult to estimate. We plan to sample hand-rinse water from 20% of the study population of the endline survey (about 640 children in total), and will analyse hand-rinse samples for the presence and concentration of STH ova (and if funds permit also for the presence of *Escherichia coli bacteria*. Additional funding is currently being sought for this purpose).

Again a short questionnaire will be administered to collect information on age, sex, last time the student went to the toilet to defecate, last time hands were washed with/without soap, and also on activities performed during the day that may have led to the contamination of hands with faecal material.

The number of ova on hands will be assessed through a concentration technique using McMaster slides and developed under the first phase of SHARE (Jeandron 2014). A 100 ml sample from the hand rinse wash water will be used for the detection of STH ova and (funds permitting) of *Escherichia coli bacteria*. Results from hand rinses will be compared between schools across the trial arms, and also with the individual infection status determined from the stool analysis conducted at about the same time.

**Cost and cost-effectiveness study**

For the costing study, the expenditures made for the design and implementation of the intervention will be documented. Investment costs (development of educational materials; training of teachers) and intervention running costs (pre-intervention worm infection survey, feedback meetings with parents, distribution of leaflets, class-room sessions, provision of hardware such as soap and soap-dispensers, monitoring of intervention in schools, etc.) will be itemised separately. For the costing exercise we will use the ingredients approach. Costs will be documented in US dollars at 2016 prices.

Care will be taken to not include research costs (e.g. baseline and FU evaluation surveys). Costs of the deworming campaign in all schools and worm treatments provided in all schools to infected students following the baseline and endline surveys will also be documented but are not a component of the hand-washing intervention and will not be included in the cost-effectiveness analysis.

For the cost-effectiveness study we will relate the expenditures incurred for the intervention (incremental costs of the intervention over and above that of deworming campaign) to the average number of additional infections prevented due to the intervention over and above those cured due to the deworming campaign (incremental intervention effects). The cost-effectiveness will be expressed as costs per infection prevented.

This work will be conducted by MITU's health economist in collaboration with the Ifakara Health Institute (IHI).

## STUDY LIMITATIONS

The main outcome indicator (prevalence of STHs at the endline survey in the two arms of the trial) is subject not only to the desired intervention (hand-washing behaviour) but also to infections from other sources, and may be modified by environmental and other confounding factors as described further above. Such factors will therefore be carefully documented und used for adjustment in the analysis, but residual confounding may occur. Should the intervention trials how a null-result it will be important that the study can disentangle the reasons: whether the hand-washing intervention has been unsuccessful, or whether other sources of infection may have played a role that may have overridden any intervention effects. Also for this reason, we will conduct the sub-study on hand contamination with helminth eggs described above.

We are aware that even after correct repeat deworming with Albendazole, some children may still be infected, particularly with trichuriasis; and that not all infections will be detected by the stool microscopy-based laboratory technique to be used, particularly lighter infections. However this fact does not invalidate the annual deworming campaigns or jeopardise the validity of the intervention trial: incomplete eradication of helminth infection or misclassification of light infections would occur in both arms of the trial; and their consequence would be a gradual dilution of the measurable intervention effect.

The proposed stool microscopy method for the detection of helminth infections has a lower sensitivity than a polymerase chain reaction (PCR) method. However, the proposed methodology will be sufficient for the purpose of this randomised trial for which the outcome is helminth infection *prevalence* rather than an accurate diagnosis of infection at individual level.

**Timelines and sequence of events**

The overall project will take 2 years and 9 months, which includes the preparation phase (not part of this protocol) and the controlled trial (subject of this protocol).

With regards to the trial, the time from the introduction of the intervention in an intervention school to the end of the endline worm infection survey in the same school will take about 1 year and 3 months (Figure 2).

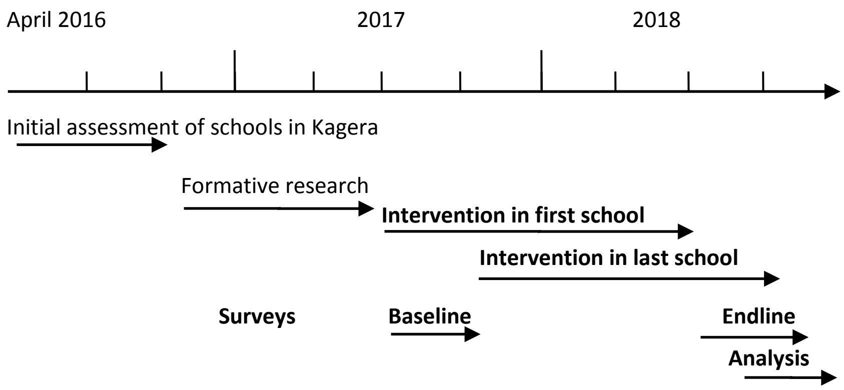
Because the project cannot be delivered simultaneously in all schools it will be phased in over a period of 3 - 4 months (Figure 3).

Data sets from the evaluation surveys will become available over time, and will be complete for the last schools enrolled about 1 year and 4 months after beginning of the trial.

Analysis of data will take about 4 months.

This protocol covers the main trial (i.e. the work to be done after completion of the formative work. The trial is expected to begin in July 2017, and data analysis to be completed by December 2018 (Figure 3).

**Figure 3 Phased project implementation**



**ETHICAL CONSIDERATIONS**

*Ethical clearance and supervision:* The study protocol has been approved by the SHARE Programme Executive Group. The study protocol, questionnaires, information sheets and consent forms for survey participants will be submitted to the ethical review committees at NIMR Tanzania and LSHTM for clearance. The study will be supervised by a Study Steering Committee (TSC).

*Study implementation:* The intervention will be offered to all students in the intervention arm. Because hygiene education and hand-washing after defecation and before meals are no invasive procedures, consent or assent will not be required for the intervention. In order to enrol students for the *evaluation surveys*, students will be asked for informed assent, and parents or guardians will be asked to provide informed consent based on a circulated information leaflet, and using an opt-out strategy for those who would not wish their children to participate.

Data collected from or about study participants will be treated with strict confidence. Names of study participants will be recorded so that they can be traced for follow up if required, but will be detached from clinical and other personal data. Students infected with STHs will be treated on the spot, students with other apparent ailments will be referred to local health units where they will be managed to the standard of care in accordance with current national guidelines.

The deworming campaign will be conducted in all participating classes according to standard guidelines of Tanzania. The campaign will conducted through the project but will imitate the deworming campaigns usually delivered by the MoHSW at regular intervals. After the baseline and endline surveys, any students with a current worm infection will receive treatment according to national guidelines.

**Project management and governance**

The Principal Investigators will be Dr Saidi Kapiga (MITU/NIMR) and Dr Safari Kinung'hi (NIMR) in Tanzania. Co-investigators will include Dr Christian Holm Hansen (MITU/NIMR), Prof Heiner Grosskurth (LSHTM), Dr Robert Dreibelbis (LSHTM) and Dr.Upendo Mwingira (MoH, Tanzania). Day-to-day implementation of the study will be led by a full-time Mikono Safi project study coordinator (Dr Kenneth Makata). Data management will be supervised by Mr Ramadhan Hashim. Data analysis will be performed under the guidance of Dr Christian Holm Hansen, based on an agreed protocol to be developed before commencing the analysis. The cost and cost-effectiveness studies will be conducted by a MITU health economist in collaboration with the Ifakara Health Institute, Tanzania. The work will be conducted according to the time-lines described in Figures2 and 3.

The study will be supervised by a Trial Steering Committee (TSC). External members will include independent experts: a parasitologist, an epidemiologist, and an educationist. Other members will include the RMO Kagera, the PIs, the senior anthropologist who supervised the formative research prior to the trial, and the study coordinator. The TSC will convene by electronic means, in the beginning of the study, after 6 months, at the end of the study and in addition in case of need.

A trial safety and monitoring board (TSMB) will not be established because (i) an interim follow-up survey (e.g. after 6 months) is not planned, (ii) the intervention is educational and non-invasive, (iii) overall follow-up time per participant does not exceed one year, and (iv) all participants with (re)infection will be treated.

**BUDGET**

The total budget will amount to GBP 388,640. The budget summary is shown below:

| S/N | Item | Amount (GBP) |
|---|---|---|
| 1 | Salaries | 224,028.00 |
| 2 | Consumables | 25,140.00 |
| 3 | Field travel and transport | 20,104.00 |
| 4 | Equipment | 9,750.00 |
| 5 | Field allowances | 12,040.00 |
| 6 | Laboratory testing and related costs | 11,250.00 |
| 7 | Other direct costs | 21,555.00 |
| 8 | Indirect costs | 64,773.00 |
| | **Total (GBP)** | **388,640.00** |

**REFERENCES**

Bieri FA, Gray DJ, Williams GM, Raso G, Li YS, Yuan L, He Y, Li RS, Guo FY, Li SM, McManus DP.Health-education package to prevent worm infections in Chinese schoolchildren. N Engl J Med, 2013. 368(17): p. 1603-12.

Biran A, Schmidt WP, Varadharajan KS, Rajaraman D, Kumar R, Greenland K, Gopalan B, Aunger R, Curtis V. Effect of a behaviour-change intervention on handwashing with soap in India (SuperAmma): a cluster-randomised trial. The Lancet Global Health, 2014. 2(3): p. e145-e154

Brooker S, Narcis B Kabatereine, Jennifer L Smith,Denise Mupfasoni, Mariam T Mwanje, Onésime Ndayishimiye,Nicholas JS Lwambo, Deborah Mbotha, Peris Karanja,Charles Mwandawiro, Eric Muchiri, Archie CA Clements,Donald AP Bundy, Robert W Snow.An updated atlas of human helminth infections: the example of East Africa-International Journal of Health Geographics 2009, 8:42 doi:10.1186/1476-072X-8-42

Brownell, SA,Nelson KL. Inactivation of Single-Celled Ascaris suum Eggs by Low-Pressure UV Radiation. Applied and Environmental Microbiology, 2006. 72(3): p. 2178-2184

Caruso BA, Freeman MC, Garn JV, Dreibelbis R, Saboori S, Muga R, Rheingans R. Assessing the impact of a school-based latrine cleaning and handwashing program on pupil absence in Nyanza Province, Kenya: a cluster-randomized trial. Trop Med Int Health. 2014 Oct;19(10):1185-97. doi: 10.1111/tmi.12360. Epub 2014 Jul 24

Clarke NE, Clements AC, Doi SA, Wang D, Campbell SJ, Gray D, Nery SV. Differential effect of mass deworming and targeted deworming for soil-transmitted helminth control in children: a systematic review and meta-analysis. Lancet. 2017 Jan 21;389(10066):287-297.

Clasen T, Boisson S, Routray P, Torondel B, Bell M, Cumming O, Ensink J, Freeman M, Jenkins M, Odagiri M, Ray S, Sinha A, Suar M, Schmidt WP.Effectiveness of a rural sanitation programme on diarrhoea, soil-transmitted helminth infection, and child malnutrition in Odisha, India: a cluster-randomised trial. Lancet Global Health, 2014. 2(11): p. e645 - e653

Cranston I, Potgieter N, Mathebula S, Ensink JH. Transmission of Enterobius vermicularis eggs through hands of school children in rural South Africa. Acta Trop, 2015. 150: p. 94-96.

Do TT, Mølbak K, Phung DC, Dalsgaard A. Helminth infections among people using wastewater and human excreta in peri-urban agriculture and aquaculture in Hanoi, Vietnam. Tropical Medicine & International Health, 2007. 12: p. 82-90

Dreibelbis R, Kroeger A, Hossain K, Venkatesh M, Ram PK. Behavior Change without Behavior Change Communication: Nudging Handwashing among Primary School Students in Bangladesh. Int J Environ Res Public Health. 2016, 14.13(1)

Ensink JH, Blumenthal U, Brooker S. Wastewater Quality and the Risk of Intestinal Nematode Infection in Sewage Farming Families in Hyderabad, India. The American Journal of Tropical Medicine and Hygiene, 2008. 79(4): p. 561-567

Freeman M,Clasen T, Brooker S, Akoko D, Rheingans R. The Impact of a School-Based Hygiene, Water Quality and Sanitation Intervention on Soil-Transmitted Helminth Reinfection: A Cluster-Randomized Trial. Am. J. Trop. Med. Hyg., 89(5), 2013, pp. 875–883

Freeman M, Clasen T, Dreibelbis R, Saboori S, Greene L, Brumback B, Muga R, Rheingans R. The impact of a school-based water supply and treatment, hygiene, and sanitation programme on pupil diarrhoea: a cluster-randomized trial. Epidemiol Infect.2014 Feb;142(2):340-51

Fung IC, Cairncross S. Ascariasis and handwashing. Trans R Soc Trop Med Hyg, 2009. 103(3): p. 215-22

Greene L,Matthew C. Freeman, Daniel Akoko, Shadi Saboori, Christine Moe, and Richard Rheingans. Impact of a School-Based Hygiene Promotion and Sanitation Intervention on Pupil Hand Contamination in Western Kenya: A Cluster Randomized Trial. Am. J. Trop. Med. Hyg., 87(3), 2012, pp. 385–393

Gulliver F, Jeandron A, Nguyen VA, Do HA, Ensink JH. Transmission of helminth eggs through hands in a high-risk community. Trans R Soc Trop Med Hyg, 2014. 108(10): p. 670-2

Hayes RJ, Bennett S. 1999, "Simple sample size calculation for cluster-randomized trials", International Journal of Epidemiology, vol. 28, pp. 319-326

Jeandron A, Ensink JH, Thamsborg SM, Dalsgaard A, Sengupta ME. A quantitative assessment method for Ascaris eggs on hands. PLoS One, 2014. 9(5):e96731.

NBS (National Bureau of Statistics (2012). Tanzania Population and Housing Census, United Republic of Tanzania.

Nordin A, Nyberg N, Vinnerås B. Inactivation of Ascaris Eggs in Source-Separated Urine and Feces by Ammonia at Ambient Temperatures. Applied and Environmental Microbiology, 2009. 75(3): p. 662-667

Patil SR, Arnold BF, Salvatore AL, Briceno B, Ganguly S, Colford JM Jr, Gertler PJ. The Effect of India's Total Sanitation Campaign on Defecation Behaviors and Child Health in Rural Madhya Pradesh: A Cluster Randomized Controlled Trial. PLoS Med, 2014. 11(8): p. e1001709

Ridley, D.S. and B.C. Hawgood, The value of formol-ether concentration of faecal cysts and ova. J Clin Pathol, 1956. 9(1): p. 74-6

Saboori S,Greene L, Moe C, Freeman M, Caruso B, Akoko D, Rheingans R. Impact of Regular Soap Provision to Primary Schools on Hand Washing and E. coli Hand Contamination among Pupils in Nyanza Province, Kenya: A Cluster-Randomized Trial. Am. J. Trop. Med. Hyg., 89(4), 2013, pp. 698–708

Siza J, Kaatano G, Chai J, Eom K, Rim H, Yong T, Min D, ChangS, Ko Y,Changalucha J. Prevalence of Schistosomes and Soil-Transmitted Helminths among Schoolchildren in Lake Victoria Basin, Tanzania. Korean J Parasitol. 2015 Oct; 53(5): 515–524

Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin and school performance. Cochrane Database Syst Rev, 2012. 7: p. CD000371

Yap P, Du ZW, Wu FW, Jiang JY, Chen R, Zhou XN, Hattendorf J, Utzinger J, Steinmann P. Rapid re-infection with soil-transmitted helminths after triple-dose albendazole treatment of school-aged children in Yunnan, People's Republic of China. Am J Trop Med Hyg, 2013. 89(1): p. 23-31

Young KH, Bullock SL, Melvin DM, Spruill CL. Ethyl acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. J Clin Microbiol, 1979. 10(6): p. 852-3

Ziegelbauer K, Speich B, Mäusezahl D, Bos R, Keiser J, Utzinger R. Effect of sanitation on soil-transmitted helminth infection: systematic review and meta-analysis. PlOS Med. PLoS Med 2012; 9(1): e1001162

**APPENDICES:**

1. Questionnaires (for main trial and sub-study on contamination of hands)
2. Consent forms and information sheets (for school authorities, parents, students)
3. Curriculum vitae of investigators

A sponsor letter has been requested from LSHTM and will be submitted as a further appendix as soon as it has been issued.