

Malawi Re-assessment Survey 2018



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Background to the Re-assessment Survey

Background

The Malawian Ministry of Health (MoH) has an on-going national scale treatment programme for schistosomiasis control. All 28 districts are at varying stages of programme implementation. All districts are endemic for *Schistosoma mansoni* and *S. haematobium* and have received four or more rounds of annual treatment with Praziquantel (PZQ) and Albendazole (ALB) for Soil Transmitted Helminths (STH) since 2009 (MoH report). According to WHO guidelines, after five to six rounds of treatment re-assessment should be carried out (WHO, 2011). The reasons for this re-assessment survey are two-fold: first to re-determine the required frequency of treatments and secondly to re-focus resources and ensure the programme is continuing to have a maximum impact on infection. It has been determined, by the MoH, in collaboration with their partners at the Schistosomiasis Control Initiative (SCI), that 8 districts which have received five or more rounds of treatment should be re-assessed given the number of treatment rounds and evidence from the annual impact surveys which show a decrease in both prevalence and intensity of *Schistosoma* infection over the last five years. The mean prevalence of *S. mansoni* has reduced from 2.23% at baseline to 0.82% at the third follow up. With the prevalence of heavy infection reducing from 0.19% to 0%. The prevalence of *S. haematobium* has also reduced from 9.21% at baseline to 3.64% at follow up three. Prevalence of heavy infection has reduced from 1.60% at baseline to 0.73% at baseline and mean intensity of infection (eggs per cl) has reduced from 3.59 to 1.12.

Previous mapping

Intestinal and urogenital schistosomiasis caused by infection with *S. mansoni* and *S. haematobium* is a widespread public health problem in Malawi. Prior to SCI working with the MoH in 2011 baseline mapping surveys had been conducted in a number of the districts between 2003 and 2010 to enable the national program to commence mass treatment campaigns¹. The first mapping exercise was carried out in Malawi in 2003 to determine the distribution of infection and help guide planned control through treatment. The surveys that were conducted focused on urine filtration to detect *S. haematobium* and found there was a significant burden of disease in all targeted districts. Results indicated both a widespread occurrence of infection, and a marked variability in infection prevalence. However, information relating to *S. mansoni* and STH infection was not gathered. Since the baseline mapping in 2003, 26 out of 28 districts have been mapped. Figure 1 shows a map of Malawi with districts coloured coded into re-assessment year based on when baseline mapping was conducted and prevalence category at baseline based on WHO guideline results.

In 2017, 13 districts that were mapped prior to SCI support were re-assessed for schistosomiasis and STH.

The [Reassessment Survey 2017 Recommendations Report](#) illustrates the findings.

In 2012, 9 districts were mapped for *S. mansoni*, *S. haematobium* and STH's using a protocol developed by SCI and the MoH (Knowles et al 2017). These 9 districts are now due to be re-assessed following 5 rounds of treatment, with the same survey design as the re-assessment conducted in 2017.

Data from the baseline mapping in 2012 will provide added information on the district-wide impact of the intervention over the last six years in these 9 districts.

¹ S:\SCI - post 3 June 2011\Current programmes\DFID\ICOSA\COUNTRIES\Malawi\Mapping\pre-ICOSA mapping\Baseline Prevalence studies from Dr Jemu

Baseline report can be found here:

https://imperiallondon.sharepoint.com/sites/fom/schisto/mer/Forms/AllItems.aspx?web=1&id=%2Fsites%2Ffom%2Fschisto%2Fmer%2F2_Country_M%26E%2FMWI%2FMapping%2FFY_1213%2F3_Reports%2FMWI_FY3_Mapping_Report_CG_JW%2EPDF&parent=%2Fsites%2Ffom%2Fschisto%2Fmer%2F2_Country_M%26E%2FMWI%2FMapping%2FFY_1213%2F3_Reports

Figure 1. Map of Malawi showing year of planned re-assessment surveys

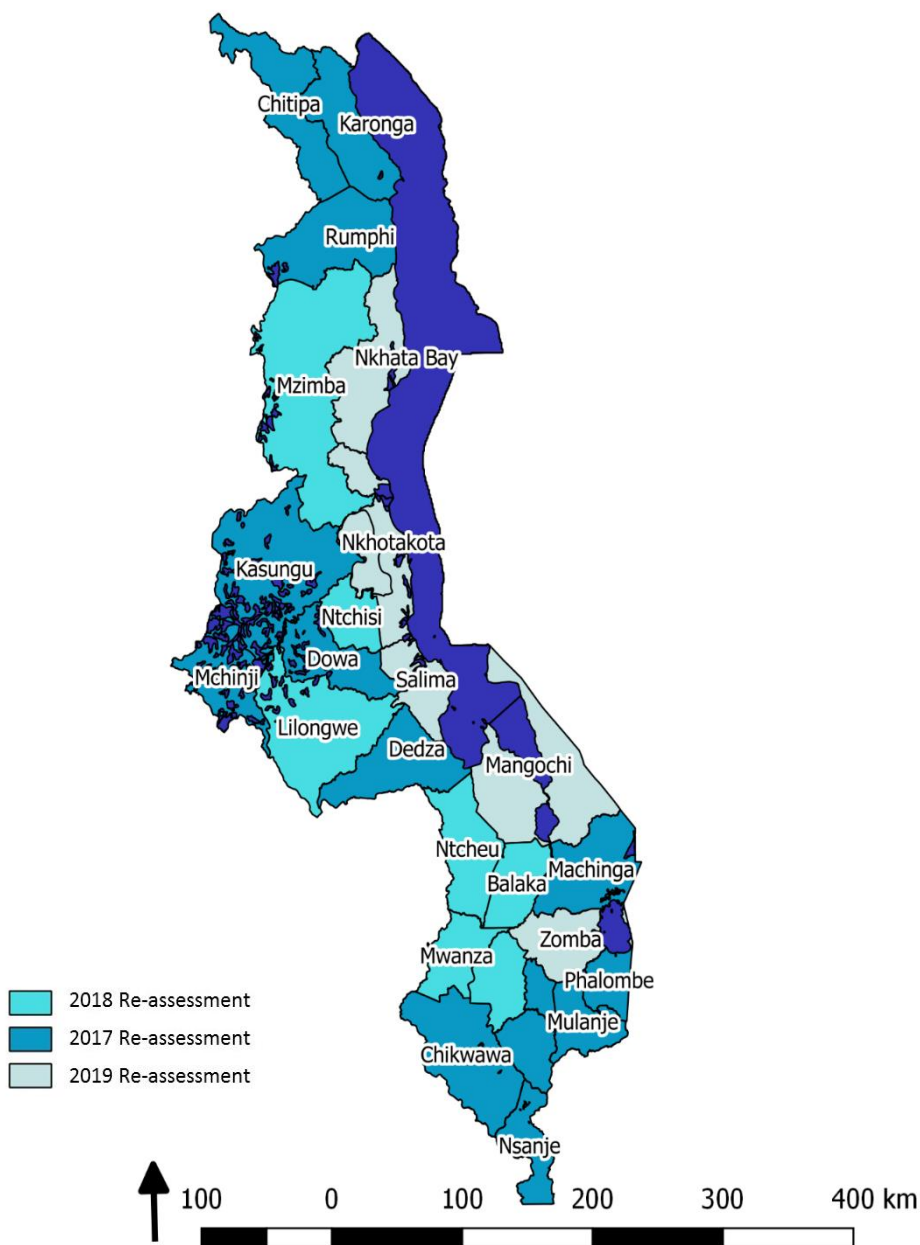


Table 1: WHO guidelines for treatment of schistosomiasis (WHO 1993, 1998)

Category	Prevalence	Action	
High-risk	≥50%	Treat all school-age children once a year	Simultaneously treat all adults once a year
Moderate-risk	≥10% but <50%	Treat all school-age children once a year	Also treat adults considered to be at risk
Low-risk	<10%	Treat all school-age children twice during their primary schooling	Praziquantel should be available in dispensaries and clinics

Treatment History

Due to the strong political and programmatic commitment of the MoH, financial support from SCI and availability of purchased and donated PZQ and ALB, Malawi has conducted national treatment annually since 2012. This followed limited treatments which were distributed between 2009 - 2012 in several parts of the country.

To date the implementation, and therefore mapping units, for all surveys has been the district which encompasses multiple traditional authorities, health zones and ecological zones. The majority of surveys used random selection of schools from within the districts to determine the districts prevalence category and subsequent treatment frequency based on the 1998 WHO guidelines as seen in Table 1 rather than the more recent guidelines published in 2012.

Impact surveys conducted prior to each round of treatment are reporting a decline in the prevalence and intensity of all parasites. A copy of the most recent impact showing this decline in prevalence and intensity over four rounds of treatment can be found here:

https://imperiallondon.sharepoint.com/:w:/r/sites/fom/schisto/_layouts/15/WopiFrame.aspx?sourcedoc=%7B4C1D0BC7-5FE1-457A-86CA-DBC71086E5D8%7D&file=MWI_FU3ImpactSurveyRecommendationsReport_EN.docx&action=default&lsList=1&ListId=%7B825796A2-CB19-4B65-97EB-1957505FED4A%7D&ListItemId=2316

All districts in Malawi have now received at least five rounds of treatment. From 2012 to date the target population within each district has expanded from targeting SAC through school treatments, to treating SAC through school and communities and some adults, to now conducting both school and community treatments targeting all SAC as well as adults in known high risk areas (which may sometimes be whole districts) based on previous mapping results. In 2014 districts were asked to begin categorising health centres, schools and villages into areas of high risk or not. This was based on either previous mapping results, abundance of water bodies or high-risk agricultural activities i.e. rice farming or fishing. Categorisation of a high-risk areas is based on local knowledge and practices of the areas and population.

Aim

In order to efficiently allocate PZQ and measure the impact of the program across multiple districts after multiple years of treatment, a re-assessment survey will be conducted to re-categorise districts into prevalence categories to allow a bespoke treatment frequency and determine how the program should move forward.

Objectives

Primary Objective

The primary aim of this re-assessment exercise is to re-categorise endemic districts in Malawi into WHO prevalence categories (Table 1) for *S. mansoni*, *S. haematobium* and STH in order that funds and donated drugs are utilised appropriately.

It is hypothesized that the district level prevalence of schistosomiasis has reduced since baseline mapping. Impact surveys conducted in these nine districts have provided this evidence and results from the 2017 re-assessment show that 12 of the recently re-assessed 13 districts have reduced to lower WHO prevalence categories.

This re-assessment survey will re-visit 9 districts which were mapped in 2012.² Data is available to allow the impact of district level prevalence's to be evaluated after 5 rounds of treatment.

Secondary Objectives

- To enhance knowledge of the spatial distribution of STH in Malawi after years of treatment through the schistosomiasis and Lymphatic Filariasis (LF) treatment program;
- To assess the appropriateness of the current high-risk categorisation given to areas or districts based on local knowledge and practices.
- To determine the impact of 5 rounds of treatment on prevalence in specific schools that will be re-visited from baseline.

The Lymphatic Filariasis program has carried out a very successful campaign and have now stopped national treatment campaigns. The majority of the districts in Malawi have benefitted from bi-annual treatment with albendazole through the LF and SCH programmes. In order allow the programme to monitor STH levels once treatment for LF ceases it is important to have a cross section of the prevalence one year after the last round of bi-annual treatment which will allow the country to monitor STH levels when only annual treatment is delivered.

Pre-survey data collection

All schools in the district to be surveyed were classified into whether they were situated within a high-risk area or not based on local knowledge, topography, historical mapping results and agricultural practices within the districts. This is the same categorisation used by the national programme to determine which communities should include treatment for adults during MDAs. This information enabled a stratified sampling method to be carried out within each district, which is simple variation on the WHO grouping sample method (WHO, 2014). This database can be found here:

² Note these 9 districts are broken down into 14 educational zones.

Materials and Methods

Study Design

The surveys will use a cross-sectional design whereby a random selection of children aged 10-14 will be sampled to determine the overall prevalence of the schools.

Study Outcomes

The following outcomes will be measured:

- *S. haematobium*: eggs per 10ml of urine
- *S. mansoni*: eggs per gram of faeces
- *Ancylostoma duodenale*, *Necator americanus*³: eggs per gram of faeces
- *Ascaris lumbricoides*: eggs per gram of faeces
- *Trichuris trichiura*: eggs per gram of faeces
- Age, how long lived in the area, and sex
- WASH indicators

Study Setting

The survey will be conducted in 9 districts (see figure 1) that were previously mapped in 2012 and categorised as moderate prevalence according to WHO definitions. These districts have received multiple treatment rounds. WHO guidelines state that moderate-risk communities should receive treatment every two years. The frequency of treatment will be governed by the highest level of risk from any schistosomiasis.

Sampling schools within each district

Per the sample size calculations between 15 and 22 schools will be sampled per district, depending on the number of high-risk schools within each district. As we will also be revisiting schools sampled in the 2012 mapping survey the total schools sampled per district ranges from 15 to 27. Survey teams will not know whether or not a school is in a high-risk area.

The sampling frame was reviewed, and schools sampled during the 2012 mapping survey identified. 156 such schools were identified. These were allocated into the risk strata (high risk and low risk). If the number of schools allocated per strata was less than the required sample size, additional schools were selected randomly from the sampling frame with no reference to size of the school. The list of all schools was developed during the pre-survey data collection exercise. See Appendix C for full details of sample size calculations and sampling.

Type of schools

The survey will be conducted in primary schools for a number of reasons, including:

³ Hookworm need not be monitored where it presents logistical demands if mapping results have shown it to be prevalent at very low frequency.

1. Higher primary school enrolment in Malawi ensures that the majority of children of the desired age group will be included in the sampling frame, minimising selection bias
2. Primary schools present a convenient platform for conducting surveys and delivering treatment to the greatest at-risk individuals

Sampling children within each school

A total of 30 students will be sampled per school. We will follow WHO guidelines in to sampling children aged 10-14, equally split between boys and girls.

Study Participant Recruitment

For re-assessment activities, schools will be contacted as the site of the study. The director/head teacher of the school will be informed fully about the study and requested to provide overall informed consent, allowing the study to collect samples from children within the school.

Head teachers will obtain informed consent from parents of children at the school in advance. Parents will be informed of the study through school meetings and be requested to provide informed consent for their children to participate within the study. Prior to consent they will be provided with detailed information as to why the study is taking place and questions will be answered by technical staff that are providing the information for the meeting. Random selection will be undertaken by the health workers from those children whose parents have provided informed consent.

Exclusion criteria: Any child who is unwell (e.g. fever) should not take part in the study and be referred instead to the health workers.

Data collection and analysis

Phones installed with survey CTO will be used to collect the data. Two phones will be used per team and will be uploaded with the forms necessary to capture the information regularly collected on paper forms. This will avoid issues with data entry which has occurred in the past.

2018 RE-ASSESSMENT DATA COLLECTION PROTOCOL

Arriving at the school

The school information form (Appendix B) should be completed by the team leader.

The GPS coordinates of the school will be recorded as part of the questionnaire on the phones. The interviewer will not be able to continue with the survey until this has been done.

Selecting the grades

Students with the desired age groups for the study will most probably be found at the last four grades in the primary schools. Students with appropriate ages (10-14 years) should be selected from grades 3, 4 and 5 (15 girls and 15 boys from the selected age group). If there are less than 30 pupils in the desired age groups within the sampled school, it will be necessary to top up the sample from younger children from the same school.

Selecting the students

All students within a school that meet the required ages should be assembled in separate lines – one line of boys and one line of girls for each age.

1. If number of children in the age group are much more than the required number, 30 students will be picked randomly.
2. To select the children randomly, calculate the sampling interval (SI) for each grade/gender group (i.e., the number of positions in the line after which a child is selected).
3. $SI = \frac{\text{total number of students in the line}}{\text{number of students to be surveyed in that age/gender group}}$, rounded to the nearest whole number.

Example *There are 105 boys aged 10 years old, 52 girls aged 11 years old. The SIs are:*

Boy aged 11: $105/15 = 7$

Girls aged 11: $52/15 = 3$

4. Select an arbitrary “start” number between 1 and the SI, which corresponds to the position of the first student to be selected.
5. Subsequent students are selected by adding SI to the position of the previously selected child (in other words, if $SI = 5$, every 5th child is selected). Continue to the end of the line. This may result in not enough students being selected (e.g. in the aged 11 girls example above). If this is the case, top up the sample to the required number by taking students from the very end of the line. In other situations, 20 students will be obtained before reaching the end of the line.
6. A list of the students selected to be in the survey should also be given to the school for their records.

Collecting the samples

1. Each student is asked for consent to provide stool and urine samples.
2. The student is given empty stool containers (if appropriate) and is instructed on how to collect sufficient amounts of urine and stool for testing.
3. The team leader registers the student, labels the specimens with an identification number and enters the child’s personal details on the Pupil Case Record Form (Appendix B).

4. The student submits the stool specimen to the “Kato-Katz” table and proceeds to the “urine” table where the urine sample is submitted.
5. The following Standard Operating Procedures (SOPs) (Appendix A) must be followed without deviation. If questions or clarifications are needed please SMS, skype or call the SCI Programme Manager *Jane Whitton*

Treatment in schools involved in monitoring process

Schools/communities selected for re-assessment surveys **must** be dealt with in exactly the same way as those not included in the survey, to ensure the results represent the whole treatment programme, which will not be true if conditions are different for those groups of people involved in the survey.

- **Drug treatments to schools/communities involved in the monitoring survey should be administered at the same time as the national programme**
- Drug treatments to schools/communities involved in the monitoring survey should not be given at the time of the survey
- Drug treatments to schools/communities involved in the monitoring survey should be delivered not more than 2 months after survey

Although individuals will be identified as being infected with schistosomes and/or soil-transmitted helminths during the baseline data collection and follow-up surveys, it is essential that these individuals do not receive treatment during data collection to allow extrapolation of the results for this sentinel group to the whole treatment programme. Therefore, it is important to arrange that the data collection in the selected schools and communities be carried out no more than 2 months before the national drug administration takes place. This will ensure that all individuals identified as infected during the survey can receive treatment within 8 weeks of diagnosis.

No special care or treatment should be given to those schools/communities involved in monitoring surveys. In particular, the following should be **avoided**:

- Extra drug treatments
- Extra training
- Extra education / IEC

A list of any children testing positive should be kept by the school and the district health officers, such that treatment can take place if there is any unexpected delay to the MDA.

APPENDIX A: Standardised Operating Procedures

Kato Katz SOP

Diagnosis of: Schistosoma mansoni, Trichuris trichiura, Ascaris lumbricoides, Ancylostoma duodenale and Necator americanus

General Principle: people infected with STH or intestinal schistosomes pass the eggs of the worms through their faeces. By examining a stool specimen under a microscope it is possible to count the number and the type of eggs that are present.

Safety precautions

- The stool should be considered potentially infectious.
- Wear gloves and lab coats whenever handling stool samples.
- Benches, instruments and equipment should be routinely decontaminated with disinfectants after use.
- Materials contaminated with infectious waste should be disinfected before disposal.
- Drinking or eating during laboratory procedures is prohibited.
- Appropriate disinfectant(s) should be used for disposal of contaminated materials, wooden spatulas and specimen containers and for cleaning of workbenches.
- Used specimen containers must be disinfected before washing.

Equipment for Kato Katz

Kato-Katz:

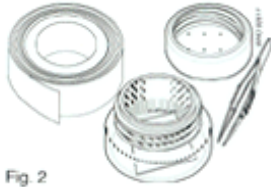
- Stool sample in container (polythene squares tied with grass or plastic pot)
- Microscopic glass slides
- Cellophane sheets (hydrophilic, 30 - 50µm thick)
- Malachite green (or methylene blue)
- Glycerol
- Metal sieve (Endecott Sieve) with 200 - 250µm mesh size
- Slide boxes
- Newspapers
- Wooden or plastic applicators
- Forceps
- Kato-Katz plastic template with a hole of 6mm on a 1.5mm thick template (delivering 41.7mg of faeces)

Microscopic examination:

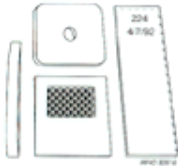

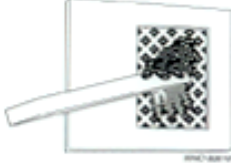

- Microscope
- Hand tally counter
- Laboratory forms


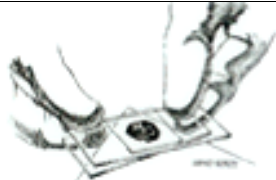
Disinfectants and waste disposal:

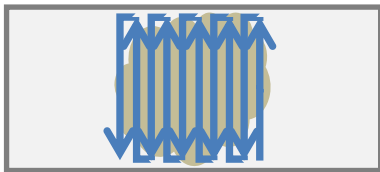
- Disinfectant wipes
- Medicated soap
- Methylated spirit
- Waste container (containing disinfectant)

Preparation of Kato Katz Reagents	Images
Step 1: Weigh out 3g of Malachite green powder (or methylene blue).	
Step 2: Dilute it in 100ml of distilled water (this is the “stock solution”).	
Step 3: Dilute 60ml of glycerine in 40ml of distilled water*.	
Step 4: Take 1 ml of Malachite green (or methylene blue) stock solution and add it to 100ml of the 60% glycerol solution (this is the “working solution”).	
Step 5: Cut cellophane into 25mm x 30mm pieces and soak them overnight in the working solution .	

*In reference books the ratio is 50% or greater glycerol solution (50ml glycerine and 50ml distilled water). In Uganda they have found this makes too light a solution and thus makes it difficult to read slides after some time has passed.

Kato-Katz Steps	Images
Step 1: Place two glass slides alongside each other and label both slides with the sample number and then place a plastic template on top of each.	
Step 2: Place a small amount of the faecal specimen on a newspaper and press through the metal sieve. Using a spatula, scrape the sieved faecal material through the sieve so that only the debris remains on the top.	
Step 3: Scrape up some of the sieved faeces from the underside to fill the hole in the templates, avoiding air bubbles and levelling the faeces off to remove any excess.	
Step 4: Carefully lift off the templates and place it in a bucket of water mixed with concentrated detergent so that they can be reused.	

<p>Step 5: Place one piece of the cellophane, which has been soaked overnight in the malachite green (or methylene blue) working solution, over the faecal specimen.</p>	
<p>Step 6: Place a clean slide over the top and press it evenly downwards to spread the faeces in a circle (this can be done by inverting the slide onto clean newspaper and pressing firmly). If done well, it should be possible to read newspaper print through the stool smear.</p>	
<p>Step 7: If hookworm is present in the area, the slide should be read within 60 minutes of processing. After that time, the hookworm eggs disappear.</p> <p>The ideal time for observing <i>S. mansoni</i> eggs is 24 hours after preparation, however, in bright sunlight the slides clear rapidly and a 24hr delay is not necessary.</p>	

Microscopic Examination for <i>S. mansoni</i> and STH	Images
<p>Step 1: After 10 minutes place a little amount of eosin on the slide and place it under microscope using x10 / x40 objective.</p>	
<p>Step 2: Count ALL eggs present using a hand tally counter; start in one corner of the sample and systematically scan the whole sample in a 'zig zag' scheme</p>	
<p>Step 3: Record the number and the type of each egg on a recording form alongside the sample number. If no eggs are seen, record "0".</p>	
<p>Step 4: Remove the faeces and cellophane using a tissue into the waste container and place all slides used when conducting Kato-Katz into the disinfectant. These slides should be cleaned and used again for the survey.</p>	

Note:

The quality control when reading the Kato-Katz slides is important. The supervising or senior technician will need to be cross check 10% of samples. Sample ID to be cross checked will be randomly selected at the end of the SurveyCTO forms.

Hemastix SOP

Diagnosis of: Schistosoma haematobium.

All manufactured kits come with instructions on how to use them. It is very important to follow the instructions to ensure the quality of the results.

Equipment for Hemastix test




- Case record form
- Hemastix test strip and Hemastix pot with scale
- Scissors
- Gloves
- Disinfectants and waste disposal

•



haemastix.MPG

[Video demonstration: click on the icon](#)

Steps for Reagent Strips	Images
Step 1: Collect a fresh urine specimen in a clean plastic container. Ensure that the urine is tested in the field within 2 hours of collection. If there is a delay, refrigerate the specimen if possible.	
Step 3: Remove one strip from its bottle (you can cut the strip in two to save resources) and label the strips with the patient identification.	
Step 4: Completely immerse the reagent areas of the strip into the urine specimen for a few seconds.	
Step 5: When removing the strip, run its edge against the rim of the container to remove any excess urine.	
Step 6: Put the strip horizontally on the table so that the chemicals do not mix together.	
Step 7: Read the strip between 1 and 2 minutes after it has been dipped in the urine specimen.	
<p>Step 8: Match the colour of the strip with the colour chart on the bottle label and record the results on the monitoring form. Record "0" if the result is negative.</p> <p>1= trace non-haemolysed</p> <p>2 = trace haemolysed</p> <p>3 = +</p> <p>4 = ++</p> <p>5 = +++</p>	
<p>Important Note:</p> <ul style="list-style-type: none"> • DO NOT LAY THE STRIP ON THE COLOUR CHART AS THIS WILL SOIL THE CHART • It is extremely important to read the strip 1-2mins after it has been dipped in the urine sample. Any colour changes that occur after 2 minutes are of no diagnostic value and should be ignored. 	

Urine Filtration SOP

Diagnosis of: Schistosoma haematobium

All manufactured kits come with instructions on how to use them. It is very important to follow the instructions to ensure the quality of the results.

Safety precautions

- The urine should be considered potentially infectious.
- Wear gloves and lab coats whenever handling urine samples.
- Benches, instruments and equipment should be routinely decontaminated with disinfectants after use.
- Materials contaminated with infectious waste should be disinfected before disposal.
- Drinking or eating during laboratory procedures is prohibited.
- Appropriate disinfectant(s) should be used for disposal of contaminated specimen containers and for cleaning of workbenches.
- Used specimen containers must be disinfected before washing

Equipment

General use:

- Gloves
- Laboratory Forms

Urine Filtration:

- Urine pots (250ml)
- Swinnex Filter Holder
- Tweezers/Forceps
- Syringe, plastic, 10ml
- Nucleopore Membrane Filter, 13mm diameter and pore size 12µm

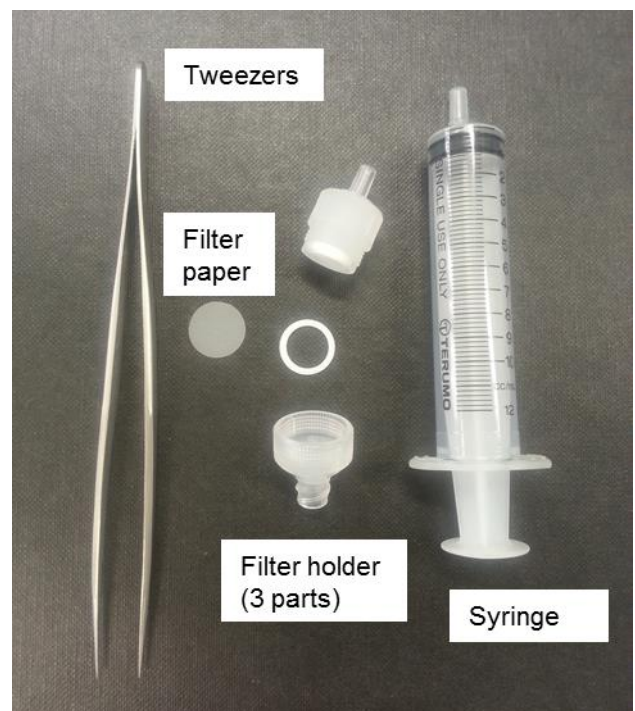
- Microscope glass slides
- Lugol's Iodine (5% solution)

Microscopic examination:

- Microscope
- Hand tally counter

Disinfectants and waste disposal:

- Bucket (to discard urine)
- 1% hypochlorite solution (domestic bleach)
- Methylated Spirit
- Medicated soap
- Rubber washing gloves
- Disinfectant wipes
- Waste container (containing disinfectant)






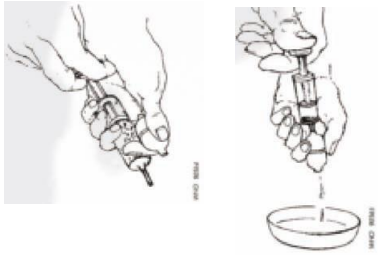


Sample collection:

The number of ova in the urine varies throughout the day, with the highest between 10am and 2pm. The specimen should be taken between these times and consist of a single urine sample. Since eggs are more often

found at the end of a urine flow, at least 10ml should be collected at the end of urination (the terminal urine). The easiest way to ensure a terminal urine sample is to ask individuals to ‘try to fill’ a large pot, e.g. 250ml. Note that some children, particularly those who are heavily infected with schistosomiasis, may not be able to provide 10ml of urine. Do not discard these smaller samples, but note the volume (ml) of urine provided. Specimens should be examined as soon as possible after collection as the eggs may hatch and then become invisible, or crystals may form, making a correct diagnosis more difficult.

IMPORTANT NOTE: To increase the volume of urine provided during sample collection, it would be advisable to promote fluid intake and physical exercise prior to micturition (e.g. provide the children with 2 glasses of water, one hour before urine collection, and request the children to participate in 10 minutes of exercise) (Doehring et al. 1983).

Steps for Urine Filtration	Images
<p>Step 1: Unscrew the filter holder and insert a nucleopore filter between the two parts of the filter holder. Make sure it is correctly held in place before screwing the unit together again.</p>	
<p>Step 2: Thoroughly shake and mix the urine specimen before drawing a 10ml specimen into the syringe. Then attach the filter unit.</p> <p>If less than 10ml urine sample is available, withdraw all urine in the sample pot and note the quantity of urine (ml) on the laboratory form next to the ID number. Do not discard the urine sample if it is less than 10ml.</p>	
<p>Step 3: Keeping the syringe and the unit in a vertical position, press the plunger down to push all the urine through the filter and out into a bucket.</p>	

<p>Step 4: Carefully detach the syringe from the filter unit. Draw air into the syringe, reattach the syringe to the filter unit holder and expel the air again. This is important as it removes any excess urine and ensures that the eggs are firmly attached to the filter.</p>	
<p>Step 5: Unscrew the filter holder and use a pair of tweezers to remove the filter and place it inverted, onto the glass microscope slide labeled with a unique identification number. The top side of the filter, where the eggs were captured, should be face-up on the slide.</p> <p>DO NOT DISCARD THE FILTER HOLDER OR SYRINGE.</p>	
<p>Step 6: Add one drop of Lugol's iodine and wait 15 seconds for the stain to penetrate the eggs. This makes the eggs more easily visible.</p>	
<p>Step 7: Immediately examine the whole filter under a microscope at a low power (x40). Schistosome eggs can be seen clearly because they stain orange. Record the <i>total number of eggs on the filter</i>.</p>	
<p>Step 8: At the end of the day, wash all reusable equipment (forceps, filter holders, syringes, urine containers, glass slides) in 1% hypochlorite solution (domestic bleach) for use next day, discard used filters and clean the workbench.</p>	
<p>IMPORTANT: Read the slide within an hour of the urine sample being taken otherwise the eggs may be non-viable and become translucent. Do not leave the samples exposed to the sun.</p>	

Where two urine samples are required: Repeat Steps 1-7 to prepare a second duplicate filter from the same urine sample, and place it on the glass slide next to the first filter, or on another slide labeled with the same ID code. The syringe can be re-used for this second filtration on the same urine sample. However, ensure that a clean syringe is used for each different urine sample (i.e. from two individuals). Two filters from the urine sample should be read by two independent laboratory technicians.

The quality control when reading the urine filtration slides is important. The supervising or senior technician will need to be cross check 10% of samples. Sample ID to be cross checked will be randomly selected at the end of the SurveyCTO forms

APPENDIX B: Data Collection Forms

Form 1: School Record Form

The School Form is critical for the programme to succeed; it will allow us to gather background information required for the programme. This form must be filled out upon arrival at each of the schools that are participating in the activities of the Schistosomiasis and Soil Transmitted Helminth programme.

Section A: Site Details

Site Details should be filled out on arrival at the location as outlined on the forms.

1. Date of Visit: To be filled on the day of Mapping activities following:
Day (DD) – Month (MMM) – Year (YYYY)
Example: (DD-MMM-YYYY): |2|7|-|F|E|B|-|2|0|1|1|
2. Team Leader Initials: The data collector will record his/her initials in the allocated spot on the form:
Team Leader Initials |_|_|_|_|
Example: John Jones Smith |J|J|S|
3. District Name: Record the name of the District here in **BLOCK Capitals** to ensure it is easy to read
4. Educational District Name: Record the name of the Educational District here in **BLOCK Capitals** to ensure it is easy to read. This is our sampling area to be consistent with mapping.
5. Educational District Code: Fill in the district code (DDD) in accordance with the assigned codes (see Schools list) – these are constant with codes used for mapping for consistency, and is a 3 digit number: 001 – 013.

Section B: GPS

The GPS device is likely to arrive with the default setting of degrees and minutes. At first use, you will need to change this to decimal degrees (instructions for the GARMIN eTrex® H):

1. Press PAGE and switch to the menu page
2. Select SETUP and press ENTER
3. Select UNITS and press ENTER
4. Select POSITION FRMT and press ENTER
5. Select the decimal degrees format hddd.ddddd and press ENTER.

GPS coordinates must be recorded on site at arrival and departure (stand in the same place for each recording).

Section C: School Details

School information will be gathered on site through conversations with the Headteacher who will assist you in the mapping activities.

1. School Name: Record the name of the school here in **BLOCK Capitals** to ensure it is easy to read
2. School Code: Fill in the school code (SSS) in accordance with the assigned codes (see Schools list) (this is a 3 digit code: 021 – 023. Schools are numbered (arbitrarily) 21 to 23 within each Educational District (can be assigned by field team on arrival in 'Mapping Area'/District, or via Schools list).
3. Name of Headteacher: Record the name of the Headteacher here in **BLOCK Capitals** to ensure it is easy to read
4. Have pupils in the school received deworming treatment in the last year?: Write the corresponding number in available space.

1=Yes

0=No

2=Don't know

5. Lowest Standard Taught: Write the corresponding number to the lowest Standard taught in the school in the available space.
6. Highest Standard Taught: Write the corresponding number to the highest grade taught in available space

Section D: Enrolment Numbers

Record the enrolment in the available space. The headteacher will be able to assist you with this section. The total refers to the total school enrolment.

Example:

D. Enrolment numbers		
	Boys Enrolled	Girls Enrolled
Total	1. __ 1 8 4	2. __ 1 6 3
Grade 3 (Age 9)	3. __ 9 3	4. __ 9 6
Grade 4 (Age 10)	5. __ 5 0	6. __ 4 5
Grade 5 (Age 11)	7. __ 7 5	8. __ 6 3
Grade 6 (Age 12)	9. __ 5 9	10. __ 5 5

Form 2: Participant Identification Form

The completion of this form allows each survey participant to be given a unique identification (ID) number comprised of DD – district code, SS – school code and NN – ID number (00-99). This ID allows the individuals' names to be absent from the F3_Individual Form where the diagnostic results are recorded. This form also collects individual level indicators for WASH.

Please complete the information at the top of the page:

Date of survey: To be filled on the day of survey following:

Day (DD) – Month (MMM) – Year (YYYY)

Example: (DD-MMM-YYYY): |2|7|-|F|E|B|-|2|0|1|1|

Registers Initials: The data collector will record his/her initials in the allocated spot on the form:

Registers Initials |_|_|_|_|

Example: John Jones Smith |J|J|S|

Implementation Unit Name: Record the name of the District here in **BLOCK Capitals** to ensure it is easy to read.

Implementation Unit Code: Fill in the District code (DD) in accordance with the assigned codes decided pre-survey this should be a 3 digit number: 01 – XX.

School Name: Record the name of the School here in **BLOCK Capitals** to ensure it is easy to read.

School Code: Fill in the School code (SS) in accordance with the assigned codes decided pre-survey this should be a 3 digit number: 01 –XX.

Form 2. Participant Identification Form

Date of Survey (DD-MMM-YYYY)	_ _ . _ _ . _ _ . _ _	Registers Initials	
District (Admin 2) Name			
District (Admin 2) Code (DD)	_ _	Survey Type	
School Name			
School Code (SS)	_ _	Page Number	

	Name	Identification Number (DDD.SSS.NN)	Used a basic sanitation facility last time they defecated ✓ or X	Hygiene is taught in schools ✓ or X	Photo of urine sample taken ✓ or X
1.		_ _ . _ _ . _ _			
2.		_ _ . _ _ . _ _			
3.		_ _ . _ _ . _ _			
4.		_ _ . _ _ . _ _			
5.		_ _ . _ _ . _ _			
6.		_ _ . _ _ . _ _			
7.		_ _ . _ _ . _ _			
8.		_ _ . _ _ . _ _			
9.		_ _ . _ _ . _ _			
10.		_ _ . _ _ . _ _			
11.		_ _ . _ _ . _ _			
12.		_ _ . _ _ . _ _			
13.		_ _ . _ _ . _ _			
14.		_ _ . _ _ . _ _			
15.		_ _ . _ _ . _ _			

Form 3: Pupil Case Record Form

The Mapping Pupil Form is critical for the mapping programme to succeed; it will allow us to gather background information required for the programme. This form must be filled out as samples are collected from students. The pots that these samples are collected in must be recorded with the ID number so that the analysis looking for eggs, haematuria and the dipstick results can be recorded next to the correct individual.

At the School

- Fill out the top of the form
 - Date of Survey
 - School Code SSS (from 'ICOSA Mapping School Form')
 - Page Number
- ID Number: This will be recorded using the district code (DD), the school code (SS) and the individual number (NN) which numbers 1-99; where the first student will be have an NN = 01
 - Label slides or take the corresponding sticker and put in the ID Number location.
 - E.g. The following would be the ID Number for the district with code 001, the school with code 001 and first student (01):
 $(DD.SS.NN) |0|1|. |0|1|. |0|1|$
- Identical ID stickers should also be stuck on the child's sample containers.
- Sex: Record the gender of the student as either **M** for Male and **F** for Female
- Age: Record the age of the student in years.
 - E.g. |1|1|
- Take the sample containers and label with ID number (written or sticker)

At the school/In the lab

- Microscopist Initials: The lab technician who is handling the samples must record his/her initials in the provided location.
 - Lab Technicians Initials |__|__|__|
 - Example: John Jones Smith |J|J|S|
- Egg Detection (stool): Slides will be read for hookworm on the day of collection. Slides will be read for the follow species of parasitic helminths at least 24 hours later: *Schistosoma mansoni*, *Ascaris lumbricoides* and *Trichuris trichiura*.
 - A simple presence/absence test is being used for the mapping activities. However this re-assessment will measure intensity also and therefore will count and record the total number of eggs found for each species.

The Pupil CRF will be filled out throughout the examination of each of the children taking part in the impact survey. There is one form per child.

Form 4: Water and Sanitation Form

These questions must be filled in when the individual presents their samples on Day 1.

- *Do you have access to an improved water source at school?:* Record ✓ for **YES** or **X** for **NO**
- *Did you use a basic sanitation facility last time you defecated at school?:* Record ✓ for **YES** or **X** for **NO**
- *Are you taught about good hygiene in school?:* Record ✓ for **YES** or **X** for **NO**

Mapping and Impact Survey - WASH Form

Date of survey (DD-MM-YYYY)	_ _ _ - _ _ - _ _ _ _ _ _	Interviewer initials	_ _ _ _ _
District Name		District Code	_ _ _ _ _
School Name		School code	_ _ _ _ _
*DDD – implementation unit code, SSS – school code			

A. Observation by Interviewer

	Yes	No	Observations on....
School Water			type/location
An improved water source is located on site			
An improved water source is accessible to all children at school			
School Hygiene			height of station/ease of use
A handwashing station with water and soap is present near the latrines			
A handwashing station with water and soap is present near to kitchen/food preparation area			
A handwashing station with water and soap is accessible to all children at school			
School Sanitation			Physical structure/cleanliness/access for disability students+staff
Latrines are functioning and accessible to all children at school			
Latrine floors (internal and external) are free from excreta			
There is ≥1 latrine per 25 girls			
There is ≥1 latrine per 50 boys			
There is ≥1 latrine per female teacher/staff			
There is ≥1 latrine per male teacher/staff			

B. Answers from Headteacher or teacher

	Yes	No
School Water		
Do all staff have access to an improved water source at school?		
Do all children have access to an improved water source at school?		
School Hygiene		
Is good hygiene taught at this school?		
School Sanitation		
Did you use a basic sanitation facility last time you defecated at school?		

APPENDIX C: Detailed survey methodology & sample size estimation

Sample size details

Pre-survey data collection

SCI received a file detailing all schools in the districts to be surveyed, along with information on whether each school was considered to be in a 'high risk' area, based on whether or not adults in the community surrounding the school are also treated. The decision on whether adults in the community are also treated is believed to be taken in each district based on expert knowledge of the local area. Health Surveillance Assistants who are based within communities inform District Health Offices (DHO's) on the topography of the area that they work, populations reporting symptoms that could relate to SCH or STH and agricultural practices of the communities. DHO's then use this information and available historical mapping results to determine if an area should be classed as high risk and therefore all those aged five and above should receive treatment.

Sample-size calculations

Per the protocol for reassessment mapping conducted in 2017:

It was decided to stratify sampling by high risk or not within each district for the following reasons:

- We would like to test in what way the high risk classification is related to prevalence of schistosomiasis
- If high risks do indeed have more schistosomiasis, then the stratification will have been justified and treatment strategy may be different between the groups
- If high risks do not have more schistosomiasis, then combining the results into one district level prevalence estimate should not introduce any large biases

The budget was for 20 schools per district to be surveyed, giving a total of 240 schools. We performed sample size calculations for each district separately, incorporating fpcs to account for the proportion of schools sampled in each district, to look at estimated 50%, 80% and 95% confidence intervals at expected true prevalence of 10% and 20%, and different sampling strategies of how to split the 20 schools per district. An example for Dedza is shown below in figure C1. We then examined confidence intervals by eye for each of the districts to determine the most appropriate sampling strategy to give fairly equal confidence intervals for each grouping of high risk or not: in Dedza, sampling 8 schools in high risk areas and 12 not in high risk areas was deemed to be adequate.

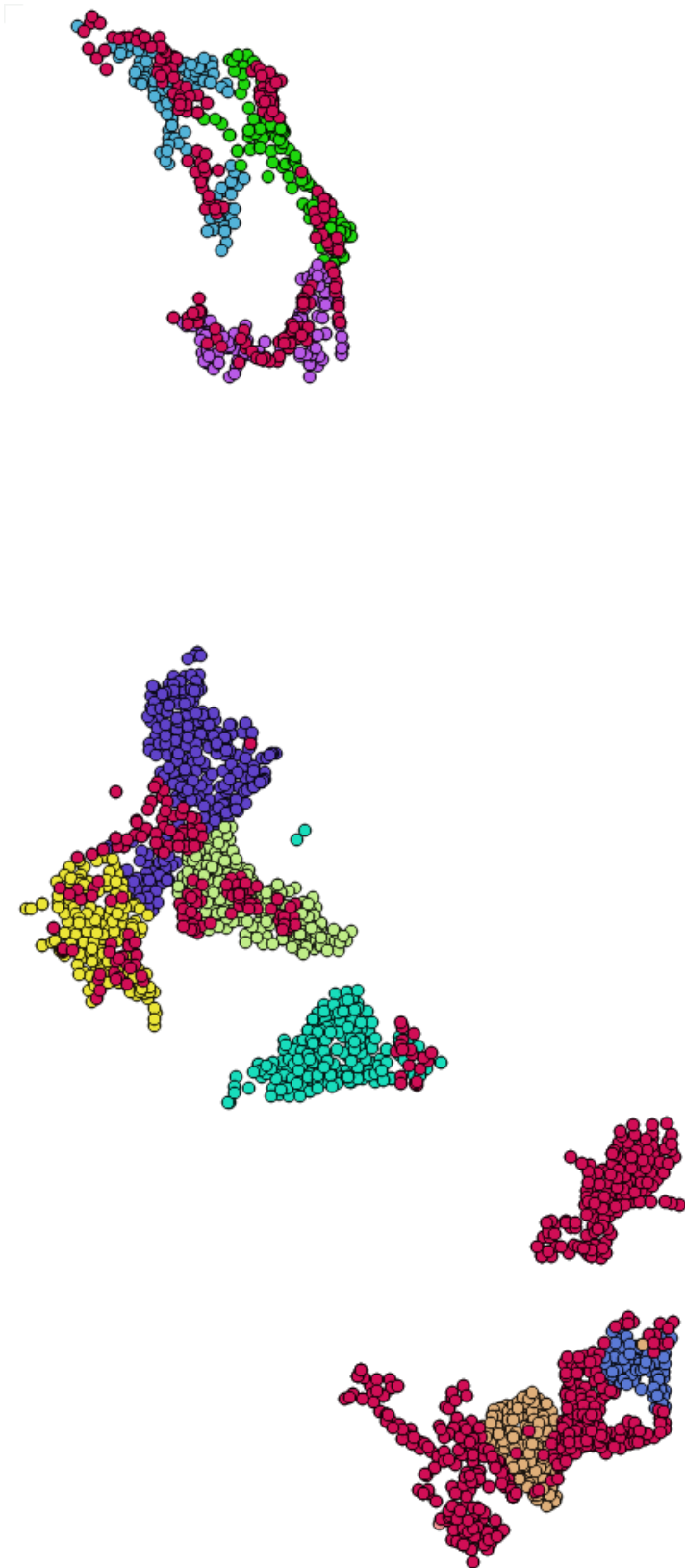


Figure C1: 'high risk' villages (red) and non-high-risk villages (other colours) in districts to be surveyed, excluding Nsanje as data was not available at the time the map was made. Although there seems to be some clustering of the red high-risk schools, the pattern is not always clear and consistent.

Expected precision in Dedza: 19 hotspot schools and 225 not hotspot

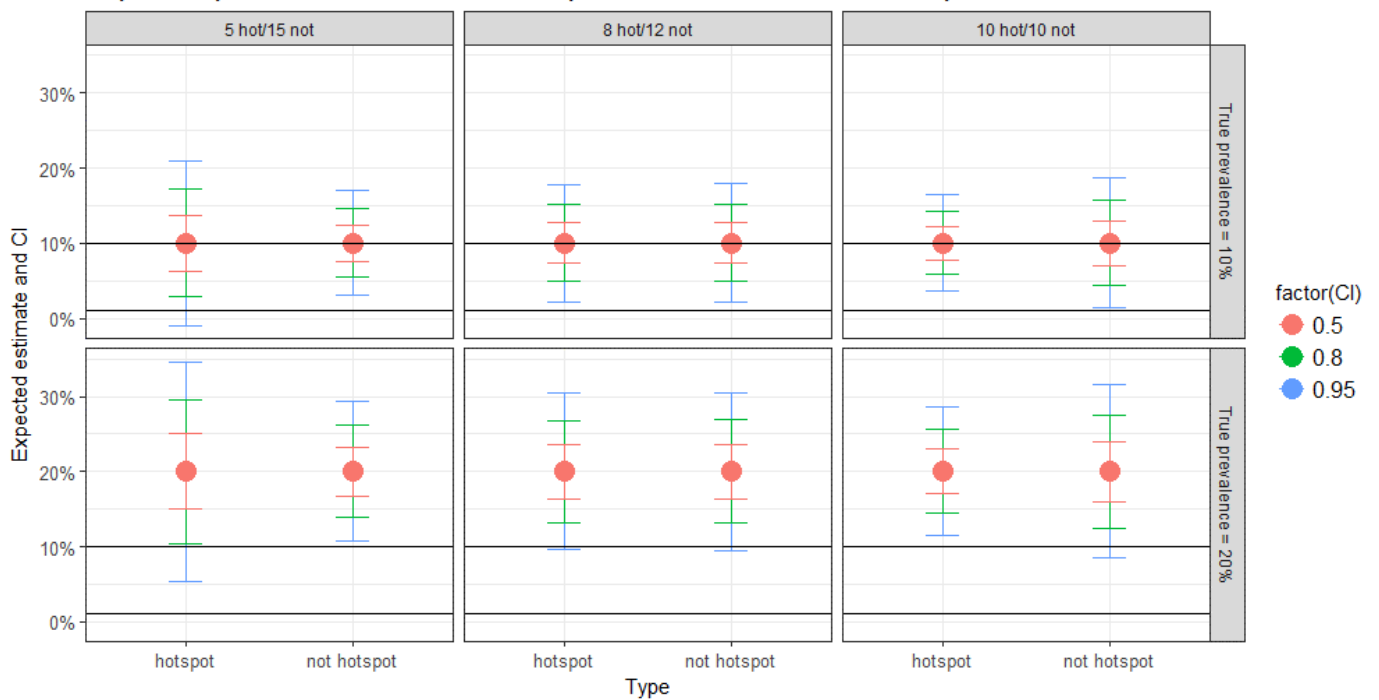
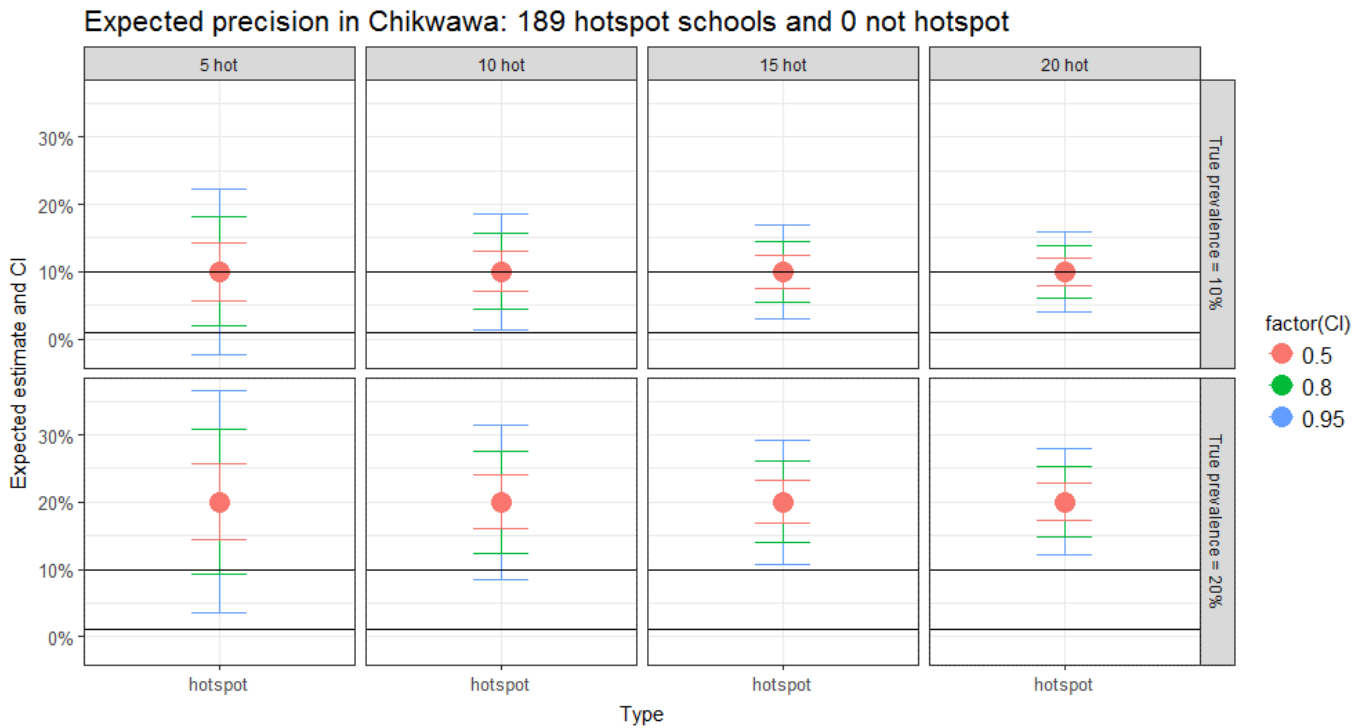


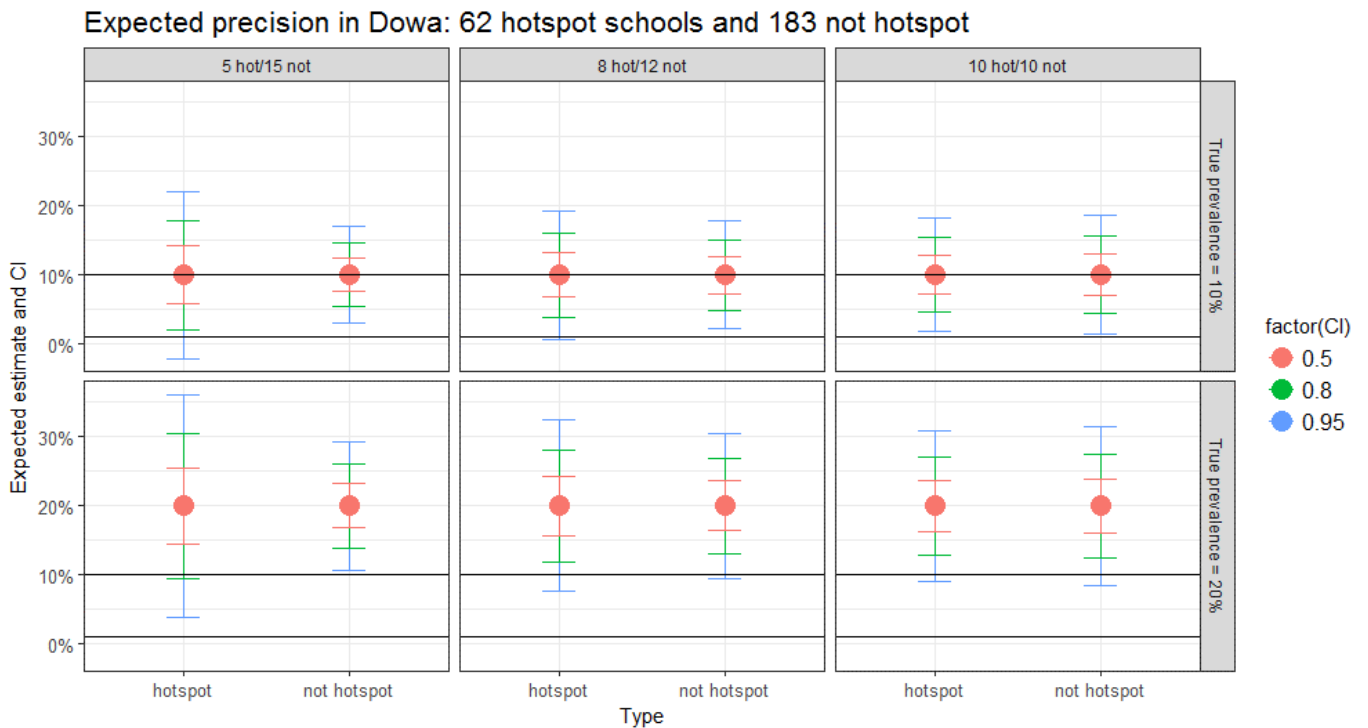
Figure C2: Expected 50% (red), 80% (green) and 95% (blue) confidence intervals for three different sampling strategies of 20 schools per district: 5 schools in hotspot areas, and 15 in not-high risk areas (left); 8 schools in high risk areas and 12 in not-high risk (middle); and 10 schools in each of hot and not (right). The top panel of graphs shows results for expected true prevalence of 10% and the bottom for expected true prevalence of 20%. The black lines show the treatment strategy boundaries at 1% and 10% prevalence, as per WHO recommendations.

Graphs for the four districts in all ‘high risk’ areas showed no large benefit to sampling more than 15 schools per district (graph C3). It was therefore decided to sample 15 schools in the four districts that are in all high risk areas.

For districts with both high risk and not areas, and with more than 20 schools in a high risk area (see graph C4 for an example), it was decided that 10 schools in the high risk area and 12 schools in the not-high risk area would give the best balance of precision between the groups. For areas with less than 20 schools in the high risk group and the rest in the low risk group, 8 high risk schools should be selected and 12 low risk schools giving 20 schools for these districts. This resulted in a total sample size of 256. A further complication was that all schools from the 2012 mapping survey, that still existed in the current sampling frame should be revisited. As the distribution of 2012 schools among the high and low risk groups did not match the sample size requirement, in some districts we will sample more than the minimum required. The final, total sample size is 272 schools.



Graph C3: Expected confidence intervals for sampling in Chikwawa where all schools are classified as being in high risk areas.



Graph C4: Expected confidence intervals for sampling in Dowa where there are both high risk and not areas, and where more than 20 schools are classified as being in a high risk area

District	Group	No. High risk schools to sample	No. not-high risk schools to sample	High risk schools from 2012 survey	Not-high risk schools from 2012 survey	Total schools to sample
Neno	Less than 20 high risk schools	8	12		10	20
Mzimba South	Less than 20 high risk schools	10	12	2	15	25
Ntcheu		10	12			22
Mzimba North		10	12	8	9	22
Lilongwe City		10	12	15	2	27
Ntchisi		10	12			22
Balaka		10	12	7	12	22
Lilongwe Rural West		10	12	12	1	24
Lilongwe Rural East		10	12	6		22
Mzuzu city	More than 20 high risk schools	15	0	20		20
Chiradzulu	All high risk schools	15	0	13		15
Blantyre City		15	0	8		15
Blantyre Rural		15	0	16		16
Total			148	108	107	49

Table C2: Number of schools sampled within each district and high risk or not zone.

Selection of schools

Schools that were part of the 2012 mapping survey were allocated to the risk groups. Where this was less than the required sample size, additional schools were selected randomly to make up the sample for each district and risk zone with no reference to school size. We elected not to sample proportional to school size as SCH and STH may be more prevalent in rural areas with small number of pupils per school.

Three reserves were selected for each district and zone.

APPENDIX D: Field team logistics

Survey team composition

There will be 5 mapping teams in total, each made up of the following members:

- 1 supervising technician
- 3 technicians
- 1 driver

Survey team training

Training has been completed. The training took place over 5 days in the week commencing 26th February 2018.

Timetable of activities

The survey will start on 16th April 2018. Each team will visit 2 schools per day, and data collection will take 6 weeks. The draft schedule is copied below.

Week 1 beginning				Week 1 beginning				Week 1 beginning				Week 1 beginning				Week 1 beginning			
Haem	District	School 1	School 2	Haem	District	School 1	School 2	Haem	District	School 1	School 2	Haem	District	School 1	School 2	Haem	District	School 1	School 2
Monday	Balaka			Monday	Balaka	Travel	Travel	Monday	Balaka	Travel	Travel	Monday	Chiradzulu	Travel	Travel	Monday	Chiradzulu	Travel	Travel
Tuesday	Balaka	1	2	Tuesday	Balaka	9	10	Tuesday	Balaka	17	18	Tuesday	Chiradzulu	1	2	Tuesday	Chiradzulu	9	10
Wednesday	Balaka	3	4	Wednesday	Balaka	11	12	Wednesday	Balaka	19	20	Wednesday	Chiradzulu	3	4	Wednesday	Chiradzulu	11	12
Thursday	Balaka	5	6	Thursday	Balaka	13	14	Thursday	Balaka	21	22	Thursday	Chiradzulu	5	6	Thursday	Chiradzulu	13	14
Friday	Balaka	7	8	Friday	Balaka	15	16	Friday	Ntcheu	1	2	Friday	Chiradzulu	7	8	Friday	Chiradzulu	15	16
Saturday				Saturday				Saturday				Saturday				Saturday			
Sunday				Sunday				Sunday				Sunday				Sunday			
Week 2 beginning				Week 2 beginning				Week 2 beginning				Week 2 beginning				Week 2 beginning			
Haem	District	School	School 2	Haem	District	School	School 2	Haem	District	School	School 2	Haem	District	School	School 2	Haem	District	School	School 2
Monday	Neno	1	2	Monday	Neno	11	12	Monday	Ntcheu	3	4	Monday	Ntcheu	13	14	Monday	Mwanza	1	2
Tuesday	Neno	3	4	Tuesday	Neno	13	14	Tuesday	Ntcheu	5	6	Tuesday	Ntcheu	15	16	Tuesday	Mwanza	3	4
Wednesday	Neno	5	6	Wednesday	Neno	15	16	Wednesday	Ntcheu	7	8	Wednesday	Ntcheu	17	18	Wednesday	Mwanza	5	6
Thursday	Neno	7	8	Thursday	Neno	17	18	Thursday	Ntcheu	9	10	Thursday	Ntcheu	19	20	Thursday	Mwanza	7	8
Friday	Neno	9	10	Friday	Neno	19	20	Friday	Ntcheu	11	12	Friday	Ntcheu	21	22	Friday	Mwanza	9	10
Saturday				Saturday				Saturday				Saturday				Saturday			
Sunday				Sunday				Sunday				Sunday				Sunday			
Week 3 beginning				Week 3 beginning				Week 3 beginning				Week 3 beginning				Week 3 beginning			
Haem	District	School	School 2	Haem	District	School	School 2	Haem	District	School	School 2	Haem	District	School	School 2	Haem	District	School	School 2
Monday	Blantyre City	1	2	Monday		11	12	Monday	Blantyre Rural	1	2	Monday	Blantyre Rural	11	12	Monday	Mwanza	11	12
Tuesday	Blantyre City	3	4	Tuesday		13	14	Tuesday	Blantyre Rural	3	4	Tuesday	Blantyre Rural	13	14	Tuesday	Mwanza	13	14
Wednesday	Blantyre City	5	6	Wednesday		15	16	Wednesday	Blantyre Rural	5	6	Wednesday	Blantyre Rural	15	16	Wednesday	Mwanza	15	16
Thursday	Blantyre City	7	8	Thursday		17	18	Thursday	Blantyre Rural	7	8	Thursday	Blantyre Rural	17	18	Thursday	Mwanza	17	18
Friday	Blantyre City	9	10	Friday		19	20	Friday	Blantyre Rural	9	10	Friday	Blantyre Rural	19	20	Friday	Mwanza	19	20
Saturday				Saturday				Saturday				Saturday				Saturday			
Sunday				Sunday				Sunday				Sunday				Sunday			
Week 4 beginning				Week 4 beginning				Week 4 beginning				Week 4 beginning				Week 4 beginning			
Haem	District	School	School 2	Haem	District	School	School 2	Haem	District	School	School 2	Haem	District	School	School 2	Haem	District	School	School 2
Monday	Lilongwe RE	1	2	Monday	Lilongwe RE	11	12	Monday	Lilongwe RE	21	22	Monday	Lilongwe RW	1	2	Monday	Lilongwe RW	11	12
Tuesday	Lilongwe RE	3	4	Tuesday	Lilongwe RE	13	14	Tuesday	Lilongwe RW	21	22	Tuesday	Lilongwe RW	3	4	Tuesday	Lilongwe RW	13	14
Wednesday	Lilongwe RE	5	6	Wednesday	Lilongwe RE	15	16	Wednesday	Lilongwe City	1	2	Wednesday	Lilongwe RW	5	6	Wednesday	Lilongwe RW	15	16
Thursday	Lilongwe RE	7	8	Thursday	Lilongwe RE	17	18	Thursday	Lilongwe City	3	4	Thursday	Lilongwe RW	7	8	Thursday	Lilongwe RW	17	18
Friday	Lilongwe RE	9	10	Friday	Lilongwe RE	19	20	Friday	Lilongwe City	5	6	Friday	Lilongwe RW	9	10	Friday	Lilongwe RW	19	20
Saturday				Saturday				Saturday				Saturday				Saturday			
Sunday				Sunday				Sunday				Sunday				Sunday			
Week 5 beginning				Week 5 beginning				Week 5 beginning				Week 5 beginning				Week 5 beginning			
Mansoni	District	School 1	School 2	Mansoni	District	School 1	School 2	Mansoni	District	School 1	School 2	Mansoni	District	School 1	School 2	Mansoni	District	School 1	School 2
Monday	Ntchisi	1	2	Monday	Ntchisi	11	12	Monday	Lilongwe City	7	8	Monday	Lilongwe City	17	18	Monday	Mtuzu City	1	2
Tuesday	Ntchisi	3	4	Tuesday	Ntchisi	13	14	Tuesday	Lilongwe City	9	10	Tuesday	Lilongwe City	19	20	Tuesday	Mtuzu City	3	4
Wednesday	Ntchisi	5	6	Wednesday	Ntchisi	15	16	Wednesday	Lilongwe City	11	12	Wednesday	Lilongwe City	21	22	Wednesday	Mtuzu City	5	6
Thursday	Ntchisi	7	8	Thursday	Ntchisi	17	18	Thursday	Lilongwe City	13	14	Thursday	Ntchisi	21	22	Thursday	Mtuzu City	7	8
Friday	Ntchisi	9	10	Friday	Ntchisi	19	20	Friday	Lilongwe City	15	16	Friday	Ntchisi	21	22	Friday	Mtuzu City	9	10
Saturday				Saturday				Saturday				Saturday				Saturday			
Sunday				Sunday				Sunday				Sunday				Sunday			
Week 6 beginning				Week 6 beginning				Week 6 beginning				Week 6 beginning				Week 6 beginning			
Mansoni	District	School 1	School 2	Mansoni	District	School 1	School 2	Mansoni	District	School 1	School 2	Mansoni	District	School 1	School 2	Mansoni	District	School 1	School 2
Monday	Mzimba Nc	1	2	Monday	Mzimba Nc	11	12	Monday	Mzimba South	1	2	Monday	Mzimba South	11	12	Monday	Mtuzu City	11	12
Tuesday	Mzimba Nc	3	4	Tuesday	Mzimba Nc	13	14	Tuesday	Mzimba South	3	4	Tuesday	Mzimba South	13	14	Tuesday	Mtuzu City	13	14
Wednesday	Mzimba Nc	5	6	Wednesday	Mzimba Nc	15	16	Wednesday	Mzimba South	5	6	Wednesday	Mzimba South	15	16	Wednesday	Mtuzu City	15	16
Thursday	Mzimba Nc	7	8	Thursday	Mzimba Nc	17	18	Thursday	Mzimba South	7	8	Thursday	Mzimba South	17	18	Thursday	Mtuzu City	17	18
Friday	Mzimba Nc	9	10	Friday	Mzimba Nc	19	20	Friday	Mzimba South	9	10	Friday	Mzimba South	19	20	Friday	Mtuzu City	19	20
Saturday				Saturday				Saturday				Saturday				Saturday			
Sunday				Sunday				Sunday				Sunday				Sunday			

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