



World Health
Organization

ASSESSING THE EPIDEMIOLOGY OF

STH

DURING A

TAS



ASSESSING THE EPIDEMIOLOGY OF SOIL-TRANSMITTED HELMINTHS
DURING A TRANSMISSION ASSESSMENT SURVEY IN THE
GLOBAL PROGRAMME FOR THE ELIMINATION OF LYMPHATIC FILARIASIS



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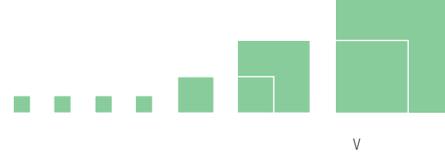
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Abbreviations and glossary

Abbreviations

MDA	mass drug administration
STH	soil-transmitted helminthiasis
TAS	transmission assessment survey

Glossary

The definitions provided here are for the use of terms in this manual and may not be valid in other contexts.

Antigenaemia

Presence of an antigen circulating in the bloodstream

Census

A means of identifying children for testing in the context of a TAS and linked-surveys of STH. In a census, there is no sampling; the entire target population is tested.

Cluster sampling

See Sampling

Critical cut-off

The maximum number of individuals with STH required to classify a community in terms of STH transmission. For example, if 25 children are infected in a cluster sample of 332 children, then the evaluation unit is classified as being in the STH prevalence range 10% to <20% (see *Table 3*).

Drug coverage

Proportion of individuals in a population who are given a drug or a combination of drugs, expressed as a percentage.

Drug combinations used for the control of lymphatic filariasis

One of the following drug combinations should be administered to the entire eligible population of an implementation unit over a period of 5–6 years:

- ivermectin and albendazole in areas where onchocerciasis is present and
- diethylcarbamazine and albendazole in areas where onchocerciasis is not endemic.

Evaluation unit

A geographical area selected for implementation of a TAS for lymphatic filariasis. Evaluation units usually consist of one or more implementation units, but an exceptionally large implementation unit might be divided into more than one evaluation unit.

Global Programme for the Elimination of Lymphatic Filariasis

A programme launched in 2000 by WHO for the elimination of lymphatic filariasis

Immunochromatographic test

Rapid test used for the detection of *Wuchereria bancrofti* antigen

Implementation unit

A geographical area in a country, frequently a district, to which decisions on implementation of MDA apply; must be defined before mapping

Lymphatic filariasis

A parasitic human infection caused by nematodes of the Filarioididea family. *W. bancrofti* causes most (90%) human infections, usually acquired in childhood; *Brugia malayi* and *B. timori* cause the remainder. Lymphatic filariasis parasites are transmitted by mosquitos (vectors) during blood-feeding.

Mass drug administration (MDA)

A modality of preventive chemotherapy in which anthelmintic medicines are administered to the entire population of an implementation unit at regular intervals, irrespective of individual infection status

Microfilariae

Microscopic larval stages of lymphatic filariasis parasites that circulate in the blood and are transmitted by mosquitos

Microfilaraemia

Presence of microfilariae in the blood

Neglected tropical diseases

A group of primarily infectious diseases that thrive in impoverished settings, especially in the heat and humidity of tropical climates. They have been largely eliminated elsewhere and are thus often forgotten (hence neglected). WHO focuses on the prevention and control of 17 neglected tropical diseases: Buruli ulcer, Chagas disease, dengue, dracunculiasis, echinococcosis, foodborne trematode infections, human African trypanosomiasis, leishmaniasis, leprosy, lymphatic filariasis, onchocerciasis, rabies, schistosomiasis, STH, taeniasis or cysticercosis, trachoma and yaws.

Net primary-school enrolment ratio

The number of children enrolled in primary school who are in the age group that officially corresponds to primary schooling, divided by the total population of the same age group

Preschool-age children

Children aged 1–4 years

Population at risk

The definition differs by the disease targeted. In the case of *lymphatic filariasis*, the population at risk is the entire population living in an implementation or an evaluation unit. The entire population should be targeted for treatment because any individual can harbour the parasite and be a source of infection for the rest of the population. In the case of *STH*, when the aim is to control STH-attributable morbidity, the population at risk is represented by preschool-age children, school-age children and women of child-bearing age living in an endemic area. These groups are those in a critical period of growth and development and, for children, also cognitive development, and are therefore at increased risk of adverse health outcomes caused by STH.

Preventive chemotherapy

Use of drugs, either alone or in combination, in public health action against helminth infections and trachoma. Preventive chemotherapy can be applied by *MDA* (see above); by *targeted chemotherapy*, in which specific risk groups, defined by age, sex or a social characteristic such as occupation (e.g. school-age children, fishermen) are given anthelmintic drugs at regular intervals, irrespective of their infection status (i.e. individuals are not screened); or by *selective chemotherapy*, in which, after screening, all individuals found to be (or suspected of being) infected are given anthelmintic drugs.

School-age children

Usually defined as children aged 5–14 years, who may or may not be enrolled in school. The ages of school enrolment vary slightly by country. As the peak prevalence and intensity of STH infection occur primarily in school-age children and because this at-risk population can be accessed readily in schools, this age group is often the first to be targeted with deworming activities.

Schistosomes

Six schistosome species infect humans: *Schistosoma mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum* and *S. guineensis*, all of which cause intestinal schistosomiasis; and *S. haematobium*, which causes urogenital schistosomiasis. Intestinal schistosomiasis is usually diagnosed by analysing faecal specimens, whereas in cases of urinary schistosomiasis, a sample of urine is examined. Preventive chemotherapy for these parasites consists of the administration of praziquantel, frequently with albendazole or mebendazole.

Sampling

Two sampling strategies are used for TAS and are therefore suggested in this manual for TAS-linked STH prevalence surveys:

- Cluster sampling: The primary sampling units (schools or census enumeration areas) are systematically selected and children in the selected schools or in households in census enumeration areas are systematically selected.
- Systematic sampling: Individuals are selected systematically, at a fixed interval, at all sites; e.g. children are lined up at school, each child is given a consecutive number and then children are selected according to a specified sampling interval (e.g. every third or every fourth child), depending on the sample size required.

Soil-transmitted helminths

Four species of nematodes are collectively referred to as soil-transmitted helminths: the roundworm, *Ascaris lumbricoides*; the whipworm, *Trichuris trichiura*; and the hookworms, *Necator americanus* and *Ancylostoma duodenale*. These four species are frequently considered together because infection is diagnosed by the same laboratory method and treated with the same drugs. The nematode *Strongyloides stercoralis* can also be considered a soil-transmitted helminth, but, because special laboratory methods are used for proper diagnosis and a different class of medicine is used in its treatment, it is not included within the STH group.

Survey sample builder

A tool used in planning TAS to automate calculation of the appropriate survey design and to facilitate selection of clusters and children or households within clusters (available online at <http://www.ntdsupport.org/resources>)

Systematic sampling

See Sampling

Target population

The population for which preventive chemotherapy is intended: the entire population in the case of lymphatic filariasis, and school-age children, preschool-age children and women of child-bearing age living in an endemic area in the case of STH



Threshold prevalence

In the context of lymphatic filariasis, the threshold infection prevalence below which transmission is assumed to no longer be sustainable, even in the absence of MDA. For lymphatic filariasis elimination, the threshold prevalence is 1% or 2%, depending on the vector. For STH, multiple prevalence thresholds correspond to a different decision about the frequency of drug administration (see section 9).

Vectors of lymphatic filariasis

Anopheles, *Aedes* and *Culex* mosquitoes are the main vectors responsible for transmission. Mosquitos serve as biological hosts that permit both the development and the transmission of the parasite during blood-feeding, which then establishes the infection in humans.

Water, sanitation and health education strategy

A strategy that combines improving water supplies and sanitation standards with the provision of health education. This strategy is essential for maintaining reductions in STH prevalence and intensity of infection.

Women of child-bearing age

Women between puberty and menopause, usually defined as between 15 and 44 years of age

■ *Assessing the epidemiology of STH during a TAS*

1. Introduction and aims of the manual

WHO recommends an integrated approach to the control of neglected tropical diseases in order to avoid duplication of effort and to reduce costs (WHO 2006). When a programme for the elimination of lymphatic filariasis is implemented in an endemic area, the drug combination of albendazole plus ivermectin or diethylcarbamazine also affects other neglected tropical diseases, including soil-transmitted helminthiasis (STH).

After at least 5 years of annual mass drug administration (MDA) with a coverage over 65%, the prevalence of lymphatic filariasis in a population can be expected to be so low that the parasite will be unable to maintain its transmission cycle. Once a transmission assessment survey (TAS) has been conducted to confirm that the prevalence of infection is below a level at which recrudescence is unlikely to occur, MDA can be stopped (WHO, 2011a).

In this context, it is important to have information about the epidemiological situation of STH in the area and, in particular, to determine whether administration of albendazole and mebendazole for the control of STH should be continued in the absence of further MDA for lymphatic filariasis.

The aims of this manual are:

- to propose a standardized approach for collecting data on STH when a TAS is conducted in the context of a programme for the elimination of lymphatic filariasis; and
- to provide guidance, on the basis of the data on STH obtained, on whether drugs for STH should continue to be administered regularly to the at-risk populations living in the area.

These data will be useful for:

- confirming the expected impact of the lymphatic filariasis programme on STH prevalence and intensity;
- determining the frequency of school-based STH treatment required after community-wide MDA for lymphatic filariasis elimination ceases; and
- determining a new baseline for monitoring the impact of school-based preventive chemotherapy for the control of STH infection.

The method for collecting data on STH proposed in this manual is cost-effective when a TAS for collecting data on lymphatic filariasis is organized and the two activities can be coordinated. When only data on STH are to be collected, the standard WHO method (WHO, 2011b) is likely to be more cost-effective.

2. Preventive chemotherapy and the elimination of lymphatic filariasis

Preventive chemotherapy, with wide-scale distribution of anthelmintic drugs to population groups at risk, is the main intervention recommended by WHO for reducing morbidity from and transmission of the four main helminth infections, lymphatic filariasis, onchocerciasis and schistosomiasis (Gabrielli et al., 2011).

In a programme for the elimination of lymphatic filariasis, a drug combination is administered annually to the entire population of an implementation unit in which the prevalence of infection is estimated to be $\geq 1\%$ (WHO, 2010). This mode of administration is known as MDA. Except in areas where *Loa loa* is also endemic, the drug combination used to eliminate lymphatic filariasis is either:

- ivermectin plus albendazole in areas where onchocerciasis is present or
- diethylcarbamazine plus albendazole in areas where onchocerciasis is not endemic.

The strategy for lymphatic filariasis elimination comprises six steps (Figure 1).

Figure 1. Steps in the lymphatic filariasis elimination strategy (WHO, 2010)



The **first step** (mapping) is conducted in an area where lymphatic filariasis is thought to be transmitted in order to determine whether MDA is required. If this is confirmed, the implementation unit is classified as endemic, and the second step of the strategy is taken.

In the **second step**, MDA is conducted annually in the entire population of the implementation unit in order to reduce the number of microfilariae circulating in the blood of the human hosts and to prevent further transmission. When MDA is conducted over 5–6 years and if drug coverage is $\geq 65\%$ for the entire population each year, lymphatic filariasis transmission is expected to be eventually interrupted (WHO, 2010).

MDA for lymphatic filariasis is also expected to result in a significant reduction in the prevalence of STH because:

- The drug combinations administered for the elimination of lymphatic filariasis are also active against STH species (*Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale*).¹
- When ivermectin is part of the drug combination administered, the efficacy against *T. trichiura* against STH is greater than that of albendazole alone.
- As the entire population over 2 years of age is targeted in areas of diethylcarbamazine distribution and over 90 cm in height in areas where ivermectin is distributed, the impact is greater than that of treatment of school-age children only.

The **third step** consists of organizing a TAS to confirm that the prevalence of lymphatic filariasis among 6–7-year-old children is too low to be self-sustaining. If this is the case, MDA is stopped, and a period of surveillance is established (**fourth** and **fifth steps**) in which two additional assessments are organized at 2–3-year intervals to confirm that there has been no recrudescence of lymphatic filariasis.

When a TAS is organized (**step 3, 4 or 5**) and if the resources are available, data on STH could be collected with the method suggested in this manual. The next chapter briefly presents the characteristics of a TAS to show how additional data can be collected.

¹ Both ivermectin and albendazole are effective against STH (Marti et al., 1996).

3. Transmission assessment survey for lymphatic filariasis

A detailed description of the method for conducting a TAS is given in the WHO manual (WHO, 2011a). The main features are summarized below.

The **aim** of a TAS is to provide information that will allow programme managers to determine whether an area has reached a critical threshold of infection prevalence, below which transmission is no longer sustainable. The results of a TAS provide evidence for deciding whether to stop or continue MDA.

The **geographical area** selected for a TAS is an evaluation unit, which may be the same as the implementation unit, comprise multiple implementation units or be part of an implementation unit. Implementation units within an evaluation unit can be geographically contiguous, but they should all have completed at least five rounds of MDA and have similar features, such as the level of MDA coverage and the prevalence of microfilaraemia in sentinel or spot-check sites. In general, evaluation units should have a population of no more than 2 million.

A TAS is **conducted** when all implementation units in the evaluation unit have completed at least five rounds of annual MDA with a coverage rate $\geq 65\%$ of the entire population of the implementation unit. After the fifth MDA, the prevalence of microfilariae at sentinel and spot-check sites should be $< 1\%$, or the prevalence of antigen should be $< 2\%$.

The **target population** for a TAS is children aged 6–7 years. In community-based household surveys, all children aged 6–7 years in selected households are eligible for inclusion. In school-based surveys, grades (which vary by country) can be used to approximate the target age population. When grades are used as a proxy for age, some children may be younger or older than 6–7 years, but all should be considered eligible for inclusion in the sample for the survey.

The **diagnostic tools** recommended for a TAS are mainly rapid point-of-care tests: in areas where *W. bancrofti* is endemic, immunochromatographic card tests (ICT) or filariasis test strips are used; in areas where *Brugia* spp. are endemic, the *Brugia* Rapid™ test should be used.

The TAS **design** is flexible and can be adapted to fit different local situations, depending on the attributes of the evaluation unit, such as:

- the net primary school enrolment rate,
- the main vector,
- the number of children aged 6–7 years and
- the number of schools or enumeration areas.

An algorithm shown in the WHO manual on monitoring and epidemiological assessment of MDA in the elimination of lymphatic filariasis (WHO, 2011a) helps in selecting the appropriate survey design according to the combination of the four attributes of the evaluation unit.

There are three possible designs for a TAS:

- **A cluster survey of children in the target age group** is used when there are ≥ 1000 children in an evaluation unit in an area endemic for *Anopheles* and *Culex* vectors or ≥ 1800 children in an area endemic for *Aedes*, and when there are at least 40 schools or enumeration areas in the evaluation unit. (This is the approach used for most assessments.)
- **Systematic sampling of children in the target age group** is recommended when the number of children in the evaluation unit is between 400 and 999 in areas endemic for *Anopheles* and *Culex*, between 1000 and 1799 in areas endemic for *Aedes*, and remains an option for larger populations in areas with either vector. Every k^{th} child in the target population is included systematically.
- **A census**, with testing of all children in the target age group, is used when there are < 400 children in areas endemic for *Anopheles* and *Culex* and < 1000 children in areas endemic for *Aedes* in the evaluation unit. Samples are collected from all children aged 6–7 in a sample size consisting of < 400 or < 1000 .

Each of these approaches can be used in school- or community-based surveys, depending on the evaluation unit population and the number of primary sampling units. The cluster survey is the approach used in most cases. The survey design and sample size can be calculated with Survey Sample Builder; the sample size can also be determined from tables in the WHO manual (WHO, 2011a). Appropriate critical cut-off values are defined from the tables or the survey sample builder.

The next section gives suggestions on collecting data on STH in evaluation units in which a TAS is conducted by each of the survey designs.

4. Collection of data on soil-transmitted helminths during a transmission assessment survey

This section suggests ways of collecting data on the prevalence and intensity of STH in order to decide whether preventive chemotherapy for STH will be required in the evaluation unit. The suggestions are coherent with the STH control programme (see *Annex 1*) and will reduce logistic complications and the number of extra personnel in the field. The characteristics of TAS and STH survey are summarized in *Table 1*.

Aim: The aim of collecting data on STH during a TAS is to help programme managers to decide whether an intervention is required to control STH in areas covered by MDA for lymphatic filariasis for 5–6 years, when treatment for lymphatic filariasis may stop.

Timing of data collection: Data on STH should be collected at an interval between 6 months and 1 year after drug administration. Thus, the interval recommended for TAS (at least 6 months after the last MDA) is ideal for collecting STH data.

Geographical area: The evaluation unit defined for a TAS will be the area for collection of data on STH to which the decision about continuing STH control will be applied.

Survey design: For logistic reasons, the survey design for STH will generally be the same as that for the TAS. The sampling strategy most often used for TAS is school-based cluster sampling.

Scheduling of the STH evaluation: The STH evaluation should be conducted with the TAS for lymphatic filariasis, usually after 5–6 years of MDA. Information of the prevalence of STH at this time allows an informed decision on preventive chemotherapy for STH, such as suspension or a change in frequency. The TAS is repeated twice at 2–3-year intervals after MDA is stopped, and these assessments are appropriate for monitoring the prevalence of STH to detect recrudescence. Therefore, data on STH should be collected whenever a TAS is done, if the resources are available.

Target population for collection of stool specimens:

- For school surveys, the results of pilot studies suggest that selection of children aged 8–10 years is the most efficient logistically. Once a field team has reached a school, it can separate into two, with one group responsible for conducting rapid tests for lymphatic filariasis and the second for collecting stool specimens for STH testing from a different group of children.
- For community surveys, the results of pilot studies suggest that selection of the same age group as that for TAS is the most efficient logistically because it reduces the number of houses to be investigated. Once the field team has reached the village and the purpose of the visit clearly communicated it can separate into three, with two groups responsible for explaining the reason for the survey, obtaining the informed consent from the parents, and providing the container for the faecal specimen to a subsample of the children (two groups conduct this exercise because it is the more time-consuming) and the third group responsible for filling the child form, conducting the rapid test and collecting the containers of faecal specimen.

Table 1. TAS and STH survey characteristics in a coordinated approach

Characteristic	TAS	STH survey
Aim	Stop MDA, interrupt transmission	Determine frequency of MDA required for control
Geographical area	Evaluation unit	Same as TAS
Timing	After five or more rounds of effective MDA repeated twice at 2–3-year intervals after MDA is stopped	Same as TAS
Primary sampling unit	Schools if primary enrolment is $\geq 75\%$ Enumeration areas if primary enrolment is $< 75\%$	Same as TAS
Target population	6–7-year-olds for both school-based and household surveys	8–10-year-olds for school-based surveys 6–7-year-olds for household surveys
Survey design	Cluster or systematic survey or census	Same as TAS
Target sample size	Cluster, 759–1692; systematic, 284–895	Cluster, 332; systematic, 166 ^a
Child selection ^a	Fixed sampling interval from TAS lists in survey sample builder	Fixed sampling interval from STH lists in survey sample builder
Diagnostic specimen	Blood	Stool
Diagnostic tool	Immunochromatographic test (ICT), filariasis test strip, <i>Brugia</i> Rapid	Kato-Katz or Mini-FLOTAC

^a See Table 3

5. Sample size

The sample size depends on the design chosen for both the TAS and the STH survey, although the sample size required for STH assessment is smaller than that for lymphatic filariasis. The survey sample builder (available at <http://www.ntdsupport.org/resources>) is a program used to create sampling lists for TAS. It can also be used to produce sampling lists for selecting children to be examined for STH; alternatively any other method to randomly selecting children can be used.

In summary

If TAS survey design is	Then the STH survey design is	STH target sample size
Cluster sampling	Cluster sampling	332 (398 including 20% non-response rate)
Systematic sampling	Systematic sampling	166 (199 including 20% non-response rate)
Census, N° of children targeted for STH data collection* <300	Census	All children in the target age group
Census, N° of children targeted for STH data collection* ≥300	Census or systematic sampling (survey manager can choose)	<ul style="list-style-type: none"> •If census: All children in the target age group •If systematic sampling: 166 (199 including 20% non-response rate)

* School-based surveys: 8–10 years old; community based survey: 6–10 years old.

Note: Regardless of the survey type, additional children should be added to the target sample, as it is difficult to predict the number of faecal specimens that will be returned. In addition, children may be absent on the survey date, refuse to participate or lack parental consent. The sampling method should factor the non-response rate into the calculations, which is suggested to be 10%–20% on the basis of pilot TAS–STH surveys.

6. Collection of faecal specimens

School-based surveys

Separate teams should be responsible for collecting blood for screening for lymphatic filariasis and stool specimens for monitoring STH. In each school, while the lymphatic filariasis team is selecting children aged 6–7 years, the STH team should select children aged 8–10 years according to the list provided by the sample builder. Even if the data collection is not dangerous for the health of the children, the children and their parents should be informed about the purpose of the specimen collection and their consent should be obtained.

For each survey separately, all eligible children should be lined up and a number assigned to each. The survey team then selects children to be sampled by matching the numbers provided by the sampling list. Note that the Sample Survey Builder produces two lists, A and B, and one should be selected randomly before arrival at each school.

For the STH survey, each selected child receives a container for one stool specimen. The plastic container should have a cover and hold approximately 100 mL. When the stool containers are distributed, the amount of stool required should be indicated, with a demonstration of putting it into the container with a wooden stick. The containers can be distributed to the children either on the day of collection (preferable because reduces the number of visit to each school) or the previous day.

The number of specimens returned is usually independent of the timing of container distribution, but the first option simplifies the operation by requiring only one visit to each school. The cultural appropriateness of this approach must be checked before the start of the survey. Each container should be marked with an identification number corresponding to the child's name and age, which is recorded on a form to enable identification of the child if necessary.

Community-based surveys

In community-based surveys, children of the same age should be sampled for STH and for TAS, and the STH sample should be a sub-sample of the TAS sample. Houses chosen for STH testing will be designated by the sample builder, and all target-age children in the selected houses will be sampled for both lymphatic filariasis and STH testing.

The distribution and collection of stool containers follow the same protocol as in school surveys. Ideally, the containers are distributed at the time of the survey, but they might have to be sent in advance. Collection and processing of the specimens depends on the diagnostic test used; however, whereas Kato-Katz tests can be prepared and examined on site, it is not practical to do this in each house, and either a central field laboratory or a dedicated space in the village or enumeration area is required.

Safety and waste disposal

Team members are advised to wear latex gloves during the collection of faecal specimens and the preparation and reading of microscope slides. Any material contaminated with stools should be cleaned with water and soap and then soaked in sodium hypochlorite solution (or another suitable disinfectant). The containers and slides can then be rinsed and dried for reuse or disposed of by incineration.

7. Diagnostic tools

The two laboratory techniques recommended for collecting data on STH during a TAS are the Kato-Katz and mini-FLOTAC tests.¹ Details of the two techniques are given in *Annex 2*.

The operational steps for stool examination depend on the laboratory method selected:

- If the Kato-Katz technique (see *Annex 2*) is used, the specimen should be processed within a few hours. In tropical climates, slides should be prepared and read within 4–6 h of collection of the specimen.
- If mini-FLOTAC (see *Annex 2*) is used, the specimen should be transferred to an apparatus for measuring an exact quantity, which is mixed with a standard quantity of 5% formalin. The specimen can then be maintained at room temperature and processed within 2 weeks (Barda 2015).

¹ The laboratory material required to examine faecal specimens collected during a TAS can be obtained from the Department of Neglected Tropical Diseases at WHO (wormcontrol@who.int).

Kato-Katz is the method usually used in STH surveys. It is based on analysis of 41.7 mg of faeces, while the newer mini-FLOTAC technique requires 2 mL of a solution of 2 g of faeces. Both techniques can be used to identify STH eggs and to quantify the intensity of infection and have acceptable sensitivity and specificity for a single stool specimen. The choice of technique depends on the available laboratory expertise, the availability of laboratory supplies and logistic arrangements. The Kato-Katz method requires laboratory analysis of unfixed specimens shortly after collection at the field site, while the mini-FLOTAC method permits collection and fixing of the specimen in the field for laboratory analysis later. Stool specimens to be examined by the Kato-Katz method can be transported to the next school or a central laboratory for preparation and examination, which must be completed within 4–6 h. For the mini-FLOTAC, specially designed faecal containers are used for collecting stool specimens at the site, which can then be transported to a central laboratory for processing.

If Kato-Katz is the method selected for examination of stool specimens, two to three field workers are required to distribute containers to the selected children, collect the faecal specimens, prepare slides and examine the slides microscopically. Personnel in central laboratory are not required because specimens are processed and analysed at the collection site, unless the team can return to a central laboratory every day. As the Kato-Katz method is well known, normally minimal training of laboratory technicians is required. It is advisable to carry extra supplies to the field each day; any supplies to be re-used should be cleaned and prepared before arrival at the field site.

If mini-FLOTAC is the laboratory method selected for examination of stool specimens, one field worker is needed at the collection site to collect faecal specimens, transfer the faecal material into the containers and fix the specimens. In central laboratory two technicians will be sufficient to process and analyse the specimens collected each day, and one auxiliary worker is necessary to dispose of faeces after the examination and to clean the containers so that enough are available for field work. Although the mini-FLOTAC is a relatively simple technique, adequate training is necessary in advance of a survey. Space should be assured to store stool specimens if they are not processed and analysed immediately. If the specimens are not processed immediately, they might have to be transferred to storage containers so that the collection containers can be re-used. The two methods are compared in *Table 2*.

For each specimen analysed, the presence of STH parasites should be recorded separately and the number of eggs per gram of faeces should be calculated for each species and recorded (for examples of forms, see *Annex 3*). A report should be written at the end of the survey (for an example of a survey report, see *Annex 4*).

Both tests provide information on the intensity of infection and on the occurrence of the different STH species, which is important for selecting the deworming drug to be distributed. For example, if hookworms are the most prevalent STH species, albendazole is the preferred drug; if *T. trichiura* is the most prevalent species, mebendazole is preferred.

Future strategies

Additional approaches to STH diagnosis will depend on diagnostic tools that are not yet fully developed or standardized. Molecular-based techniques are promising because they will reduce the time between specimen collection and diagnosis, the requirement for laboratory personnel and they will improve sensitivity in light-intensity infections. Studies are still required, however, to compare and validate the results of these new methods with those used presently.

Table 2. Characteristics of the two methods for analysing stool samples for STH

Characteristic	Kato-Katz	Mini-FLOTAC
Standard	Used regularly in endemic countries	Relatively new
Specimen	Fresh faeces	Fresh or fixed faeces
Sensitivity	Good sensitivity for moderate- or heavy-intensity infection Poor sensitivity for very light-intensity infection	Good sensitivity for moderate- or heavy-intensity infection Poor sensitivity for very light-intensity infection
Equipment	Requires equipment that is simple to use, low cost and recyclable; readily available in endemic countries	Requires equipment that is simple to use, low cost and recyclable; not readily available in endemic countries
Ease of implementation	Very easy	Easy
Requirement for fixative	No fixative required	2 ml of 5% solution of formalin required to fix each faecal sample
Personnel	Should be trained	Should be trained
Timing	Examination of faecal specimens within 4–6 h of collection	Faecal specimens can be fixed after collection and examined within 1–2 weeks.
Logistic implications	If the site at which specimens are collected is more than 3–4 h from a laboratory, processing and examination should be done on site.	Specimens can be collected on site, fixed and transported to a central laboratory for examination.
Personnel required	If the specimens are analysed on site, one to three people are required to expedite examination so as not to delay the TAS team.	One staff should join the TAS team to collect and fix specimens while blood samples for lymphatic filariasis testing are collected.

8. STH survey design and critical cut-off ranges

In the STH survey, it is suggested that the same survey design be used as in the TAS. The one exception is if the TAS is conducted as a census and there are at least 300 children in the STH target population. In this case, the STH survey can be conducted as either a census or with systematic sampling.

Table 3 presents sample sizes and critical cut-off values for systematic and cluster sampling. These are the survey designs most often used in the TAS (cluster-sample surveys are more common than ones using systematic sampling). If the STH survey is conducted as a census, see Annex 5 for classification of the evaluation unit by STH prevalence range.

The sample size and cut-off numbers presented in table 3 are selected so that there is no more than about a 5% chance that the true STH prevalence range is higher than the range identified using the cut-off numbers.

Table 3. Sample sizes and STH cut-off values for classifying evaluation units by STH prevalence range, STH prevalence surveys conducted as part of the TAS.

TAS survey sampling design		Critical cut-off values (number of STH-positive children in sample) for classifying an evaluation unit as being in one of the following STH prevalence ranges				
Sampling design	STH sample size	< 2% ^a	2% to < 10% ^b	10% to < 20% ^c	20% to < 50% ^d	≥ 50%
Systematic sampling	166	0	1–10	11–24	25–72	≥73
Cluster sampling ^e	332	0	1–20	21–48	49–144	≥145

^a The level of power for demonstrating that STH infection lower than 2.0% is less than for other prevalence ranges.

^{b,c,d} The power to conclude with 95% confidence that STH prevalence is in the indicated prevalence range is 75% or higher when STH prevalence is b) 5%, c) 13%, d) 41%.

^e Assumed design effect = 2.0.

Example of the use of the critical cut-offs

A school-based cluster-sample survey is conducted with a sample size of 332 children:

- if 14 STH-positive children are found, the evaluation unit is classified as being in the STH prevalence range 2 to <10%.
- If no STH-infected child is found in the survey, the evaluation unit is classified as being in the STH prevalence range < 2%.

Note that the STH prevalence range identified by the cut-off numbers may be higher than the prevalence of STH infection among children in the survey sample. For example, the survey may conclude that STH prevalence is in the 10% to <20% range even though only 8% of children in the survey sample are found to have an

STH infection. This is because the estimate of prevalence from the survey is not far enough below 10% to conclude with 95% confidence that STH prevalence range in the evaluation unit is <10%.

As it is difficult to predict in advance the exact number of faecal specimens that will be returned by children, it is suggested that 20% more containers be distributed at each site. This may result in a larger number of specimens than the target sample size. When oversampling is minimal (less than 10% more children than the requested sample size), the critical cut-offs presented in Table 3 can be used. If the actual sample size exceeds the target sample size by 10% or more, the critical cut-off values in Annex 6 should be used.

Finally, the SSB analysis tool can be used for any STH prevalence survey conducted by systematic sampling or cluster sampling, regardless of the sample size. By taking the actual sample size, the actual design effect and the size of the of the target population size into account, the result is likely to be more accurate than use of the cut-off values.

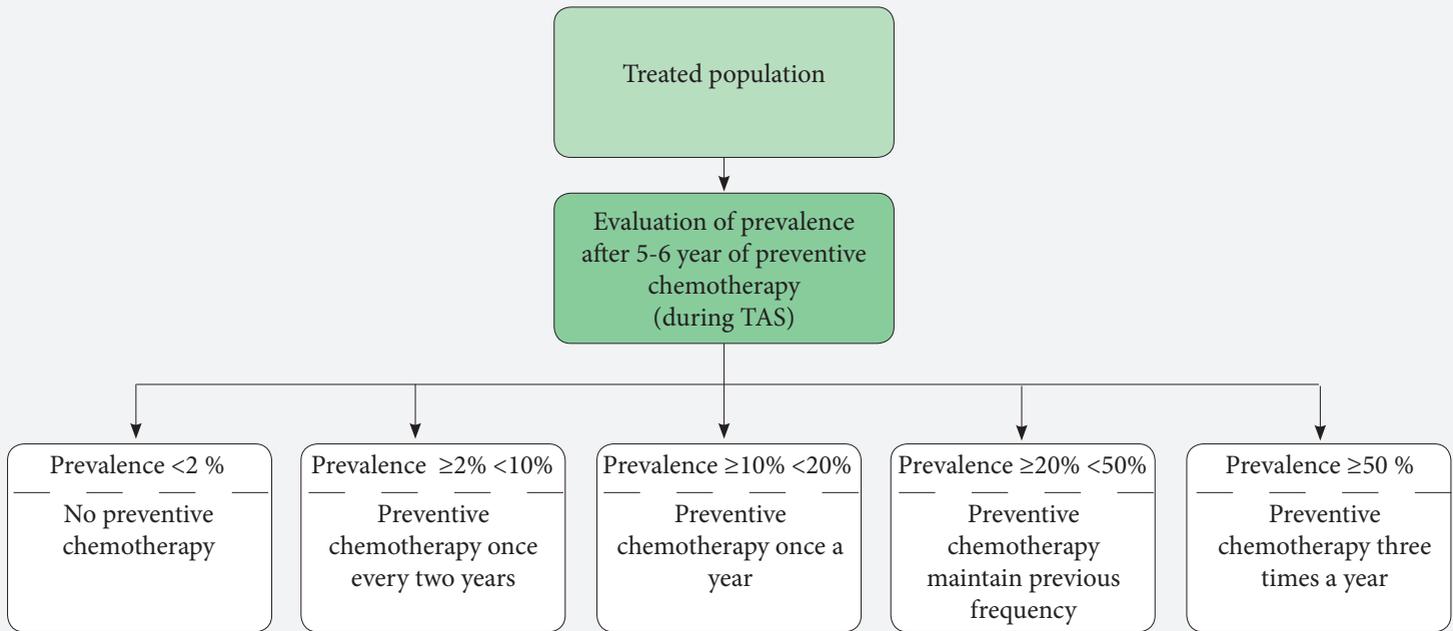
9. Treatment options for soil-transmitted helminthiasis

The treatment options listed in Figure 2 provide guidance on the control of STH after 5–6 years of MDA for lymphatic filariasis (see last row of the diagram). Five categories are listed, according to the prevalence of STH:

- **prevalence < 2%:** indicates that transmission is very low; no preventive chemotherapy is necessary.
- **prevalence 2–10%:** indicates that transmission is low; preventive chemotherapy can be administered once every 2 years.
- **prevalence 10–20%:** indicates that transmission is not intense; preventive chemotherapy can be administered once a year.
- **prevalence 20–50%:** indicates that transmission is still intense and that reducing the frequency of preventive chemotherapy could result in a rebound in prevalence; maintain the existing frequency of preventive chemotherapy. (i.e. if only MDA for LF was administered to the population, then once year administration of albendazole or mebendazole should be organized for school age children, if also an additional distribution of albendazole or mebendazole was administered to school-aged children, two distributions at year with albendazole or mebendazole should be organized).
- **prevalence ≥ 50%:** indicates that the strategy used was not successful in controlling STH; increase the frequency of preventive chemotherapy to three times a year.

Any change in the frequency of preventive chemotherapy should be accompanied by regular monitoring at sentinel sites (WHO, 2011b) to detect, as early as possible, any recrudescence of infection.

Figure 2. Treatment options for STH, according to prevalence assessed during TAS



Additional information collected on STH during a TAS can help programme managers to improve the effectiveness of STH control (*Annex 7*). For example, data on the relative predominance of the remaining STH species guides decisions on which drug to use, while information on heavy-intensity STH infection would indicate the effectiveness of the intervention. For example, when a large number of children still have heavy-intensity infections after 6 years of mass distribution of anthelmintics, further investigation should be carried out to verify the coverage of drug administration, compliance of the target population and the efficacy of the drug.

Improvements in water, sanitation and health education once a low prevalence and low intensity of STH have been achieved by preventive chemotherapy facilitate maintenance of these low levels for a long time, delaying and even preventing re-infection.

10. Key messages

- Programmes for the elimination of lymphatic filariasis distribute albendazole, a medicine that is effective against soil-transmitted helminthiases (STH).
- After a number of years of implementation, all programmes for the elimination of lymphatic filariasis conduct a Transmission Assessment Survey to evaluate if the programme's objective has been reached.
- This is the ideal time to evaluate STH epidemiology and to decide whether interventions for STH control are needed.
- Most TAS evaluations involve school-based cluster sampling and can easily collect STH epidemiological data.
- The sample required for STH testing is smaller than that required for lymphatic filariasis testing.
- Children will be selected for STH testing at the same sites as testing for lymphatic filariasis is conducted.
- The team conducting a TAS for lymphatic filariasis usually conducts rapid diagnostic tests on site (immunochromatographic test, filariasis test strip, Brugia Rapid) and therefore, in principle, spends a relatively short time collecting and analysing blood specimens in the field.
- An STH survey can be conducted using two diagnostic methods:
 - The Kato-Katz technique requires time-sensitive specimen processing and microscope reading, which may increase the overall time at each site.
 - The mini-FLOTAC technique allows the specimen to be fixed and read in the following week or two, but requires special equipment.
- Critical cut offs provided in the manual help to classify the evaluation unit and decide the need of intervention for STH.



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Annexes

Annex 1. Characteristics of a soil-transmitted helminthiasis control programme

Some characteristics of STH control programmes, in comparison with lymphatic filariasis elimination programmes, should be taken into account in conducting a survey for the collection of data on STH during a TAS.

The aims of the two types of programme are different. While that of a lymphatic filariasis elimination programme is to reduce the prevalence of the parasite to a level that is incompatible with transmission, the aim of a STH control programme is to eliminate morbidity due to STH infection. The differences in the aims of the two programmes are linked to the transmission efficiency of the parasite. Transmission of lymphatic filariasis is not efficient because passage of filarial larvae from the mosquito to a human host is not always successful (because of the relatively large size of the larvae); therefore, it is possible, by chemotherapy alone, to reduce the number of parasites in the host to a number so low that the few remaining parasites will not be able to maintain the parasite life cycle. In contrast, STH transmission does not involve vectors, and humans can acquire STH infections directly from infective stages in the environment. It is therefore difficult to reach a number of parasites in the environment that is so low that transmission is interrupted. Elimination can be attained only if improvements in sanitation accompany preventive chemotherapy (Gabrielli et al., 2011).

Eliminating morbidity due to STH infection is achieved by eliminating infections of moderate to heavy intensity, which are the main drivers of STH-associated morbidity. Information on the prevalence of STH infection is sufficient to decide to change the frequency of preventive chemotherapy (*Figure 2*). Information on the intensity of infection (i.e. light, moderate or heavy) is important for assessing the effectiveness of the programme in reaching its objectives.

The consequence of ceasing or halting drug administration is different. In lymphatic filariasis elimination programmes, cessation of drug administration is, in principle, a terminal step; while for STH control programmes, stopping drug administration is only temporary if sanitation is not adequate. Therefore, in the case of STH, drug administration is expected to recommence once monitoring shows a tendency for the prevalence of STH to return to the original levels.

For these reasons, the degree of confidence in the results of assessments of the two infections is different. In the case of lymphatic filariasis, the entire TAS method is constructed to ensure with 95% confidence that MDA is not phased out until the prevalence is < 2%; whereas, in the case of STH, a very low prevalence is often only a temporary indication of the effectiveness of preventive chemotherapy and of the possibility of interrupting the intervention for a few years in order to contain costs and reduce drug pressure (Gabielli et al., 2011).

An additional characteristic of an STH control programme is that STH-attributable morbidity is directly related to the intensity of infection. It is therefore important to obtain data on the intensity of STH infection to evaluate morbidity, which best reflects the true health impact of the programme. The laboratory method used to assess STH should include an evaluation of the number of eggs per gram of faeces.

Annex 2. Laboratory techniques for the analysis of faecal specimens

Kato–Katz technique

Materials and reagents

- applicator sticks
- screen, stainless-steel, nylon or plastic: 60–105 mesh size
- template, stainless-steel, plastic or cardboard. Templates of different sizes have been produced in different countries. A hole of 9 mm on a 1-mm thick template will contain 50 mg of faeces; a hole of 6 mm on a 1.5-mm thick template, 41.7 mg; and a hole of 6.5 mm on a 0.5-mm thick template, 20 mg. The templates should be standardized, and the same size template should always be used, to ensure the repeatability and comparability of prevalence and intensity data.
- spatula, plastic
- microscope slides (75 x 25 mm)
- hydrophilic cellophane, 40–50 g, strips 25 x 30 or 25 x 35 mm
- flat-bottom jar with lid
- forceps
- toilet paper or absorbent tissue
- newspaper
- glycerol–malachite green or glycerol–methylene blue solution (1 ml of 3% aqueous malachite green or 3% methylene blue added to 100 ml of glycerol and 100 ml of distilled water and mixed well). This solution is poured onto the cellophane strips in a jar and left for at least 24 h before use.

Procedure

- Place a small mound of faecal material on newspaper or scrap paper, and press the small screen on top so that some of the faeces are sieved through the screen and accumulate on top.
- Scrape the flat-sided spatula across the upper surface of the screen to collect the sieved faeces.
- Place the template with a hole on the centre of a microscope slide, and add faeces from the spatula so that the hole is completely filled. Pass the side of the spatula over the template to remove excess faeces from the edge of the hole.
- Remove the template carefully so that the cylinder of faeces is left on the slide.
- Cover the faecal material with the pre-soaked cellophane strip. The strip must be very wet if the faeces are dry and less so if the faeces are soft. If excess glycerol solution is present on the upper surface of cellophane, wipe with toilet paper.

- Invert the microscope slide and firmly press the faecal sample against the hydrophilic cellophane strip on another microscope slide or on a smooth hard surface. The faecal material will be spread evenly between the microscope slide and the cellophane strip. It should be possible to read newspaper print through the smear after clarification.
- Carefully remove the slide by gently sliding it sideways to avoid separating the cellophane strip or lifting it off. Place the slide on the counter with the cellophane upwards. Water evaporates while glycerol clears the faeces.
- Read the slide after 30–60 min at ambient temperature. (The exact time should be determined in each setting to avoid over-clearing of hookworm eggs, which become transparent with time and are thus impossible to see.)
- Examine the smear systematically, and record the number of eggs of each species.
- The multiplication factors used to obtain the number of eggs per gram from the number of eggs per slide are: 20 if using a 50-mg template, 50 if using a 20-mg template and 24 if using a 41.7-mg template.

Link to Youtube: https://www.youtube.com/watch?v=WpcZeJHa_jM

Mini-FLOTAC technique

Materials and reagents

- formalin 5%
- flotation solution
- spatula
- Fill-FLOTAC
- Mini-FLOTAC
- timer
- microscope adaptor for Mini-FLOTAC

Fill-FLOTAC



Mini-FLOTAC apparatus



Technique

Fixation (can be done in the field)

- Add 2 ml of 5% formalin to the Fill-FLOTAC container using, for example, a squeeze bottle. (The Fill-FLOTAC stool container has a graduated scale.)
- Fill the conical collector of the Fill-FLOTAC with faeces using the spatula, and level the surface. (The conical collector contains 2g of faeces).
- Close the Fill-FLOTAC and homogenize the specimen (by pumping the conical applicator in the container up and down).

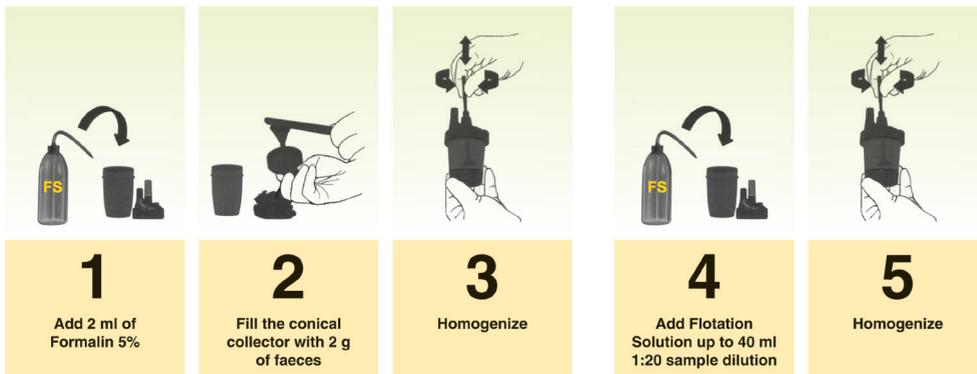
Analysis (can be done in the laboratory within 2 weeks of specimen collection)

- Add 38 mL of a salt flotation solution (sodium chloride, NaCl, specific gravity 1200) to the Fill-FLOTAC container using, for example, a squeeze bottle. (The container should now have 40 mL of liquid.)
- Close the Fill-FLOTAC and homogenize the specimen (as described below).
- Filter and fill the two chambers of the Mini-FLOTAC through the filling holes until a small meniscus is formed. In order to avoid formation of air bubbles, the chambers should be filled while the apparatus is held at a slope.
- After 10 min, rotate the reading disc and put the Mini-FLOTAC under the microscope using the microscope adaptor. The Mini-FLOTAC permits a maximum magnification of x 400.
- Read both chambers of the mini-FLOTAC. The multiplication factor used to obtain the number of eggs, larvae, oocysts and cysts per gram of faeces is 10.

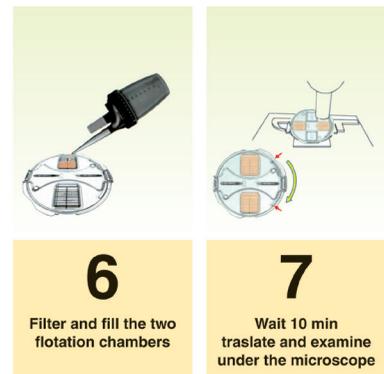
The quantity of formalin is minimal and is sufficient to preserve the faeces for 1–2 weeks before analysis.

Link to Youtube: <http://www.youtube.com/watch?v=Cz24DVQi90g>

ON THE FIELD



ON THE LABORATORY





Annex 3. Standard form for recording the results of faecal examinations

PARASITOLOGICAL FORM						
Personal data						Date ___/___/___
ID number _____			School (or village) _____			
Name _____		Age _____ (years)		Sex M__F__		
Stool examination						
	Eggs/slide	Eggs/gram	Moderate-intensity infection		Heavy-intensity infection	
			Yes	No	Yes	No
<i>Ascaris lumbricoides</i>						
<i>Trichuris trichiura</i>						
Hookworms						

Other parasites identified:

Annex 4. Examples of report forms

Name of evaluation unit	_____
No. of clusters (schools) targeted	65
No. of clusters (schools) investigated	65
No. of individuals targeted	332
No. of individuals investigated	340
No. of positive cases	48
Cut-off corresponding to category	> 20-< 50
WHO recommendation for STH	Maintain previous frequency

Parasite				
	<i>A. lumbricoides</i>	<i>T. trichiura</i>	Hookworms	Any STH
No. investigated	340	340	340	340
No. infected at light intensity (%)	10 (2.9%)	14 (4.1%)	9 (2.6%)	30 (8.8%)
No. infected at moderate intensity (%)	2 (0.6%)	5 (1.4%)	10 (2.9%)	17 (5%)
No. infected at heavy intensity (%)	0	1 (0.2%)	0	1 (0.2%)
Total number of positives	12 (3.5%)	20 (5.8%)	19 (5.5%)	48 (14.1%)

Annex 5. Classification of the evaluation unit by prevalence range based on a census (when the entire target population is tested)

If a stool sample has been requested of all children in the STH target population, then STH prevalence is equal to the number of STH-positive children divided by the number of children tested, expressed as a percentage. Classify the evaluation unit according to the prevalence range in which the prevalence falls: <2%, 2% to <10%, 10% to <20%, 20% to <50%, or ≥50%.

Examples:

- 15 children are STH positive out of 211 are tested.
Prevalence: $15/211=7.1\%$. Prevalence range: 2% to < 10%.
- 4 children are STH positive out of 200 are tested.
Prevalence: $4/200=2.0\%$. Prevalence is not less than 2.0%.
Therefore, the prevalence range is 2% to < 10%.
- 61 children are STH positive out of 233 are tested.
Prevalence: $61/233=26.2\%$. Prevalence range: 20% to <50%. 22 children are STH positive out of 220 are tested. Prevalence: $22/220=10.0\%$. Prevalence is not less than 10%. Therefore, the prevalence range is 10% to < 20%.

Annex 6. Cut-off values for when the actual sample size exceeds the target sample size by 10% or more

When the actual sample size is 10% or more than the target sample size shown in *Table 3*, use the following cut-off values for classifying evaluation units by STH prevalence range, STH prevalence survey conducted as part of the TAS.

Sampling design			Cut-off values (number of STH-positive children) for concluding the STH prevalence range is				
STH sampling design	Target sample size	If actual sample size is*	< 2%	2% to < 10%	10% to < 20%	20% to < 50%	≥ 50%
Systematic sampling	166	≥183	0	1 – 11	12 – 27	28 – 80	≥81
Cluster sampling	332	≥366	0	1 – 22	23 – 54	55 – 160	≥161

* The more the actual sample size exceeds 183 or 366, the less power there is with the cut-off values shown to conclude that the true prevalence range has been reached (there would be an increasing tendency to conclude that the prevalence range is higher than it truly is). In surveys where the actual sample size is 20% or more higher than the target sample size, the survey manager should make every effort for the survey to be analyzed using SSB.

Annex 7. Additional information that can be obtained during a transmission assessment survey

A TAS–STH survey is a good opportunity to collect additional data on the population sample or additional biological specimens or to conduct rapid physical examinations.

Once **stool specimens** have been collected, the prevalence and intensity of infection not only of STH but also of other parasitological aspects can be determined. These include the prevalence of intestinal schistosomiasis (*Schistosoma mansoni*, *S. japonicum*, *S. mekongi*), as eggs of these parasites may be present in stool specimens in endemic countries. When the Kato-Katz technique is used, *Schistosoma* eggs can be identified at the same time as those of STH; when the mini-FLOTAC is used, a different flotation solution will be necessary. The prevalence of *Strongyloides stercoralis* can also be determined. Data on this parasite are rarely collected because special laboratory techniques are required, which include Harada-Mori culture, the Baermann technique, concentration and, if blood is collected, serology. Knowing the prevalence of these infections may help programme managers to establish control programmes for these parasites and integrate them with existing ones.

Urine specimens can be collected at the same time. The prevalence of urinary schistosomiasis (*S. haematobium*) can be determined, as eggs of this parasite might be present in urine specimens in endemic countries. Reporting their presence can help programme managers to take a decision about establishing control programmes for this parasite.

Conducting a brief **physical examination** of the children in the survey could provide data on nutritional status, as recording age, height and weight would allow estimates of stunting, wasting and underweight in the area. It could also indicate the presence of scabies. *Sarcoptes scabiei* is also sensitive to ivermectin, and it might be interesting to determine whether wide-scale deworming has an impact on scabies.

Visits to villages during the survey could provide an opportunity to inspect and to collect information on WASH at the school (presence of water and soap, presence and condition of latrines or toilets). This can facilitate preparations for the maintenance of low levels of STH achieved through PC.



Soil-transmitted helminths infect more than 2 billion people in more than 100 countries, adversely affecting nutritional status and impairing cognitive processes. Their geographical distribution overlaps with at least one of the other parasitic neglected tropical diseases, including lymphatic filariasis.

When a programme for the elimination of lymphatic filariasis is implemented in an endemic area, the distribution of albendazole plus ivermectin or diethylcarbamazine also affects soil-transmitted helminthiases. After at least 5 or 6 years of implementation of this intervention, normally a transmission assessment survey is conducted to confirm that the prevalence of lymphatic filariasis is below a level at which recrudescence is unlikely to occur and the intervention can be stopped.

At this stage, it is important to have information about the epidemiological situation of soil-transmitted helminthiases in the area and, in particular, to determine whether administration of albendazole and mebendazole for the control of these infections should be continued in the absence of further mass drug administration for lymphatic filariasis.

This manual provides support to programme managers in collecting soil-transmitted helminths, including sample size and critical cut-offs, to take decisions on the treatment option in the event that the programme for the elimination of lymphatic filariasis is interrupted.

