

Epidemiological Survey

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Scope: questions/ challenges the tool addresses

This tool uses standard biomedical methodologies to determine infection rates for key water-related (or “reservoir-related”) diseases including schistosomiasis and other intestinal parasites, and malaria. These diseases were selected for discussion because they are widespread and important in developing countries, especially Africa. In any particular country, of course, there may be a completely different set of priorities with regard to reservoir-related health issues. In these cases it is wise to check with local health personnel about priorities, and to use local communities’ perceptions with respect to reservoir-related problems. For diseases and infections not described in this tool, local health professionals, literature, and the internet provide good starting points.

For schistosomiasis and other intestinal parasites, urine and stool samples are collected and analyzed. Normally this is done for children under 14 years of age (often between 5 and 10 years) because they can easily be sampled at school. For malaria, blood smears are taken from finger pricks. If anemia is also studied, a few drops of blood can be collected in micro tubes for determination of hemoglobin levels.

Target group of the tool

Planners, designers, builders, managers, and users of small, multi-purpose reservoirs who wish to increase health benefits and reduce health risks from these reservoirs. It may also be of use to health professionals and researchers.

Requirements for tool application

This tool requires participation by health care professionals, typically from government research institutes or universities.

Materials include sampling pots, microscopes, microscope slides, chemicals, filters, and drugs (depending on the diseases selected for detailed study). Since most of the methods are expensive, they are usually applied at the level of selected schools and communities. Under these circumstances, site selection becomes very important.

When blood samples are collected from children, ethical clearance is required. Usually this has to be given by the Ministry of Health, but many health research institutes and universities have umbrella arrangements from which a project may benefit.

In all parasitological surveys it is important to provide treatment for infected people, usually free of charge and according to national or WHO guidelines. For example, in case of urinary or intestinal schistosomiasis, praziquantel should be given at 40 mg/kg body weight. Albendazole is the proper treatment of soil-transmitted helminth infections, with doses dependent on species according to the World Health Organization (WHO Expert Committee 2002). For malaria the most recent local protocols need to be followed because of fast-developing resistance.

Tool: description and application

A number of specific research methods are described below. Methods should be selected in accord with the importance of disease, the expertise of the team, and other preference and practical considerations. In some cases alternative methods are presented for similar purposes. An appropriate selection from the following methods can be compiled into a site-specific protocol for requesting ethical clearance.

Set up

Decisions on sample size should be based on previous, preferably recent, studies of the same infection in the same country. Simple shareware such as Win Episcopy (download from <http://www.clive.ed.ac.uk/winepiscopy/>) can be used to facilitate sample size estimation. Classical approaches such as those proposed by Snedecor and Cochran (1980) can also be used. Normally, confidence levels for statistical tests are set at 95%.

Appropriate sampling frames for epidemiological studies vary over diseases. Often, school children of a certain age range (e.g. between five and ten years) are sampled because of their relatively high morbidity and because surveys are relatively easy to organize and implement. However, there is always a risk that a very important fraction of the population is missed (Cecchi 2007) and often national control programs target the same group so close collaboration is needed to prevent overlap or even interference. Targeted questionnaires can be used to select specific classes. Children can be asked to bring from the village their friends of the same age who normally do not go to school. For ethical reasons, all children who test positive for a disease should be treated according to national protocols.

Example from Burkina Faso: For the sampling campaign in Koubri (Burkina Faso), schools with six classes were selected. All children of the first two classes were tested, approximately 60-120 children per school, mostly between six and eight years old. In addition, 20 children from each of the other classes were tested, as well as all pupils and teachers who volunteered. In principle only those children were selected who lived close (less than 3-4 km) to the school.

Blood sampling

For malaria, pre-school children under five years of age are the best indicator group. However, in a region of low endemicity, the age group of 6-8 year olds shows a similar prevalence. Under these conditions, school children can be tested instead of pre-school children. A thick blood

smear is made from a finger prick. The slides are stained with Giemsa (Hira and Behbehani 1984) and are examined the same day at the school. All positive slides and 10% of the negative slides are taken to a laboratory (usually in a neighboring city) for confirmation. In order to detect cases that did not test positive the first time, and to eliminate infections, all tested children are re-tested after four weeks. All positive children are treated, following either the first or the second round of testing. Ideally sampling is done several times a year depending on major transmission seasons (normally malaria control programs know when this is). For instance in West Africa this would be three times: at the end of the main rainy season, at the end of the dry season and again at the start of the rainy season.

At the same time of sampling for the thick blood smear, some drops are put in a small cup (Eppendorf) to determine the level of hemoglobin and assess anemia.

Serological tests are available as well, to determine the level of antigens against a variety of parasitic diseases (e.g. for schistosomiasis as analyzed by Polman et al. 2000 and Van Lieshout et al. 2000). However, most of these are for financial and practical reasons beyond the capacity of national health research institutes in developing countries and are not discussed here.

Stool sampling

A - For intestinal schistosomiasis and geo-helminthes, children are provided with containers for stool collection one day in advance. The next day, small portions of stool (1-2 g) are placed in small plastic tubes containing a sodium-acetic acid-formalin solution (SAF), and shaken rigorously for 20-30 seconds. Then, single standard 42mg Kato-Katz thick smears are prepared (Katz et al. 1972). The slides are examined on the spot for hookworm eggs (only visible in the first half hour), *Schistosoma mansoni* ova and for eggs of *Ascaris lumbricoides*, *Ancylostoma duodenale* and *Trichuris trichuria*. All parasites are counted and recorded separately for each sample.

B - Samples are preserved in 10% formalin and taken to the laboratory where two thin slides from each sample are prepared. For the identification of *Schistosoma* eggs, the slides can be examined as they are. For the estimation of schistosomiasis prevalence in a population, this is just as reliable and much simpler than the traditional Kato-Katz method (De Vlas et al. 1997; Kongs et al. 2001).

C - For the detection of *Cryptosporidium* oocysts, the slides are stained according to international protocol¹ or as follows, based on Ayalew et al. (2008). After noting the consistency of each stool sample, a portion of stool is preserved with SAF (15g sodium acetate, 20ml glacial acetic acid, 40ml formalin and 925ml distilled water) in a proportion of 1 g of stool in 3 ml of SAF. The remaining part is processed using the direct wet mount method with saline to observe motile intestinal parasites and trophozoites under a light microscope at 10x and 40x magnification. The preserved stool samples are transported to the laboratory and a portion of the samples processed by the formalin-ether concentration method as described by

¹ E.g. by using the modified acid-fast staining procedure according to CDC, as described on their website www.dpd.cdc.gov/dpdx/ under diagnostic procedures, stool specimens, staining.

Ritchie (1948), if necessary with modification. The samples are observed under a light microscope at 100x and 400x magnifications for the presence of cysts and ova of the parasites.

D - For the detection of *C. parvum* oocysts, a thin smear is prepared directly from fresh as well as from sediments of concentrated stool and processed by the Ziehl-Neelsen method (Ayalew et al. 2008). These slides are observed under a light microscope with 1000x magnification (Garcia et al. 1993; Endeshaw et al. 2004). Each slide is observed for 10 minutes to decide whether it is negative or positive.

Urine sampling

For urinary schistosomiasis, school children are tested twice during the main transmission season. If time and resources allow, a combination of different diagnostic methods is used to increase precision, to allow for an evaluation of prevalence, and to estimate parasite load (intensity of infection). If resources are limited, the various methods and their results can be compared in space and time to select the most efficient.

First children are provided with a drink and then asked to produce a urine sample between 10:00 and 14:00 hours. Three techniques are practical:

- Macroscopic evaluation of the urine sample for transparency, bloodiness (hematuria);
- Testing the samples immediately after that for micro-hematuria using reagent strips;
- Mixing and filtering of 10 ml of urine through a paper filter according to Plouvier et al. (1975), left to dry, stained with ninhydrin and examined for *S. haematobium* ova after drying. As far as possible this is to be done the same day at the school. This method allows even the detection of low infections (Tiemersma et al. 1997).

It is also possible to assess *Schistosoma* infections by measuring the level of soluble circulating antigen in urine (see e.g. Van Etten et al. 1996, 1997; Kahama et al. 1999). However, this technique is not available to most national health researchers in developing countries and is not further discussed here.

All school children, whether sampled or not, positive or not, receive the standard treatment (in the case of Burkina Faso this is a single dose of praziquantel at 40 mg/kg body weight). The treatment is done as soon as possible after testing, but in any case no more than two weeks later. To verify that all treated children really became negative (indicator of praziquantel effectiveness, level of infection, as well as rapid re-infection: Gryseels *et al.* 2001), children who had tested positive are re-tested four weeks after treatment. Those who are still positive are treated again to eliminate all schistosomes and other helminths from their bodies.

Eye examination

This technique can only be applied by trained personnel, so collaboration with staff working in National Control Programs is necessary. A simple diagnosis is carried out based on clearly visible signs with binocular loupes (2.5x) of all children with acute eye infections. Symptoms are classified according to the WHO grading system for trachoma, as described on their website www.who.int/pbd/trachoma/gradcard/grading.htm. All children positive for trachoma are

treated with tetracycline. Depending on baseline health data, a selection of adults (especially women since they tend to be up to three times more affected than men) may also be examined for signs of advanced or past eye infections. In case of serious symptoms, the nurses can refer for surgery.

Other indicators

As all school children are measured or weighed at the time of sampling to determine the correct dosage of medication against schistosomiasis, this presents an opportunity to calculate additional indicators. The data are noted with the name of the child and date of birth to determine weight for length or “stunting” and weight for age or “wasting”.

Lessons learned

The value of these epidemiological methods has long been established and is recognized by the health sector. They provide detailed information and primary data that may not be available for the areas being studied. At the same time they contribute to the broader participatory health assessment (described in a separate tool).

Recommendations

If national control programs exist for water-related disease in the country, it is important to collaborate with them. This also helps embed environmental disease mitigating measures for small reservoirs into regular control efforts.

Limitations of the tool

Because these studies are expensive and cumbersome, questionnaires and rapid appraisals should be used to target them at the most suitable sites. Users of this tool should be aware that in its current form it only covers a few of the techniques for only a few water-related diseases. While the methods described above are “state of the art” and are recognized as such by health professionals, the tool is by no means complete for all reservoir-related health issues in all countries. Hence it has to be used critically and with an open mind for other locally important health issues.

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Regional Water Demand Initiative/Initiative Regionale de la Demande en Eau International
Development Research Centre (IDRC) Middle East/North Africa Regional Office, PO Box
14 Orman, Giza, Cairo, Egypt

Selected websites on water-related diseases

Index World Health organization <http://www.who.int/topics/en/>

UNICEF - UNDP - World Bank - WHO Special Programme for Research and Training in
Tropical Diseases <http://www.who.int/tdr/>

Further reading on methodologies for water-related diseases and hygiene

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