SURVEY PROTOCOL

NATIONAL SURVEY ON NEGLECTED TROPICAL DISEASES IN SOUTHERN SUDAN

REVISED FEBRUARY 2009
BACKGROUND

After more than 20 years of war and limited disease control, Southern Sudan is thought to be among the countries with the highest burden of neglected tropical diseases (NTDs) in the world. In recognition of this, the Ministry of Health (MoH) of the Government of Southern Sudan (GoSS) has included control of NTDs among its health sector priorities and has committed itself to the integrated control of NTDs using preventive chemotherapy (PCT), as recommended by the World Health Organization (WHO). This approach targets schistosomiasis (*Schistosoma haematobium* and *S. mansoni*), soil-transmitted helminths (STH: hookworm, *Ascaris lumbricoides* and *Trichuris trichiura*), lymphatic filariasis, trachoma and onchocerciasis through community-based mass drug administration (MDA).

Appropriate targeting of MDA requires information on the geographical distribution and prevalence of infection in order to identify high-risk areas that might benefit most from integrated control. Targeting by locality not only maximizes the health impact and cost-effectiveness of intervention, but also minimizes the risk of treating uninfected individuals. In Southern Sudan, relevant information on NTD prevalence and distribution is not generally available.

For onchocerciasis, disease mapping is ongoing and expected to be completed by the Southern Sudan Onchocerciasis Task Force (SSOTF) and the African Programme for Onchocerciasis Control (APOC) by the end of 2008. APOC is also aiming to complete mapping of loiasis in onchocerciasis endemic areas during 2008. Some data are available for the other diseases, but surveys to date were generally limited to relatively small areas that, in most cases, are not overlapping. No data is available on *Loa loa* prevalence in areas not targeted under the onchocerciasis control programme. For this reason, the MoH GoSS plans to undertake comprehensive mapping of the geographical distribution of four of the five diseases targeted with PCT: schistosomiasis and soil-transmitted helminth (STH) infection; lymphatic filariasis (LF); and trachoma. Loiasis will also need to be mapped in areas outside those endemic for onchocerciasis, if LF is believed to be prevalent in them. Treatment of LF with ivermectin plus albendazole in areas with high endemicity for loiasis may result in severe adverse events (SAE). Areas co-endemic for LF and loiasis could be excluded from MDA if loiasis microfilarial load is high, and be provided with alternative interventions, such as high coverage with long-lasting insecticidal nets (LLINs) to reduce LF transmission.

Starting-up of NTD control will build on the existing volunteer network for community-directed treatment with ivermectin (CDTI) to control onchocerciasis. Mapping surveys will therefore commence in areas where onchocerciasis endemicity has already been confirmed through mapping, and where CDTI structures for onchocerciasis are currently in place or are planned. Co-Implementation of MDA using albendazole and ivermectin will be conducted during the dry season, from September to March, targeting counties endemic for both onchocerciasis and LF.

Mapping for LF and schistosomiasis will generate data on co-endemicity that will allow use of the CDTI structure for co-implementation of onchocerciasis and schistosomiasis control and LF/trachoma elimination. Distribution of albendazole (in conjunction with ivermectin) for LF elimination will simultaneously reduce STH infection.

This survey protocol describes the design and implementation of field surveys to determine the prevalence of infection with schistosomiasis, STH and LF, as well as loiasis, to guide the development and implementation of control strategies. A separate survey protocol has been developed for trachoma, following WHO operational guidelines, and the rapid assessment of avoidable blindness (RAAB). When possible, sites sampled for trachoma may be studied for schistosomiasis and LF. The present protocol is based on international recommendations and the experiences of national NTD programmes. According to the operational experience gained during the first round of NTD surveys in Southern Sudan, this protocol will be reviewed to ensure that sampling approaches are as efficient as possible, thus minimizing time and costs.
SURVEY PROTOCOL

**Purpose:** To determine the prevalence of schistosomiasis and LF infection in Southern Sudan, to establish which areas qualify for MDA, and contribute to the knowledge on loiasis distribution so that areas that are both endemic for LF and highly endemic for loiasis can be excluded from MDA.

**Study design:** Population-based cross-sectional survey using a multi-stage cluster sampling design with stratification by county.

Surveys for schistosomiasis and LF will be implemented simultaneously, as the required survey approach is very similar, and due to a lack of infrastructure, access to communities is considered the major cost associated with NTD mapping in Southern Sudan. STH infection will be recorded while stool samples are being examined for intestinal schistosomiasis (*S. mansoni*). Loiasis will only be studied in areas outside those that have already been surveyed for *Onchocerca volvulus* and *L. loa*.

**Study area:** All ten states of Southern Sudan will need to be mapped for above NTDs. Southern Sudan is divided into ten states (the first administrative unit): Central Equatoria, Eastern Equatoria, Jonglei, Lakes, Northern Bahr el Ghazal, Unity, Upper Nile, Warrab, Western Bahr el Ghazal and Western Equatoria (Figure 1). The second administrative unit is the county (n=50) and the third administrative unit is the payam (n=209). The total population is estimated at c. 13 million.

Since NTD control is planned to start in areas where onchocerciasis is endemic, these areas will be surveyed first. This means that mapping will be conducted first in the states with the largest onchocerciasis endemic areas; Western Equatoria and Northern Bahr el Ghazal. Table 1 shows the number of payams in each state and suspected endemicity of different NTDs. STH infections are suspected to be endemic throughout the country.

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1 The number of counties and payams is based on United Nations generated maps, under the realization that the number of administrative units may change.
Table 1. Distribution of population by state and currently known areas endemic for NTDs in Southern Sudan

<table>
<thead>
<tr>
<th>State</th>
<th>Est. pop</th>
<th>Pop as % of total</th>
<th>No. of payams</th>
<th>Oncho</th>
<th>LF</th>
<th>Schisto</th>
<th>Trachoma</th>
<th>Loiasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Equatoria</td>
<td>1,218,340</td>
<td>9%</td>
<td>12</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Eastern Equatoria</td>
<td>1,090,148</td>
<td>8.1%</td>
<td>25</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Jonglei</td>
<td>1,724,404</td>
<td>12.7%</td>
<td>49</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Lakes</td>
<td>1,258,725</td>
<td>9.3%</td>
<td>17</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern Bahr el Ghazal</td>
<td>1,580,695</td>
<td>11.7%</td>
<td>18</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unity</td>
<td>1,469,962</td>
<td>10.8%</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper Nile</td>
<td>1,388,560</td>
<td>10.3%</td>
<td>23</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Warrab</td>
<td>2,364,491</td>
<td>17.5%</td>
<td>19</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western Bahr el Ghazal</td>
<td>573,996</td>
<td>4.2%</td>
<td>6</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western Equatoria</td>
<td>838,625</td>
<td>6.2%</td>
<td>26</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>13,507,946</strong></td>
<td></td>
<td><strong>209</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Study population and sampling unit:** The survey will include a random selection of households in selected villages. Sampling will be carried out at the level of the implementation unit for which MDA will be administered to the population. In Southern Sudan, this is either the county (admin 2, n=50) or the payam (admin 3, n=209).1

Schistosomiasis tends to be focally distributed because its transmission depends on the distribution of the appropriate snail species and suitable environmental conditions. Identifying local areas of transmission is therefore more difficult. To adequately capture the focality of schistosomiasis in Southern Sudan the implementation unit will be at the payam level.

Generally, STH infections are widely geographically distributed, and in Southern Sudan are likely to occur in any area endemic for LF or schistosomiasis.

For LF, the implementation unit is the administrative unit in a country in which anti-filarial drugs are administered to the entire population once the area has been identified as having prevalence ≥1%. In most countries, the second administrative level is identified as the implementation unit. In Southern Sudan the implementation unit for LF elimination will probably be the county level. To determine whether the county or payam is the more appropriate implementation unit, sampling will initially be conducted at the payam level.

Infection with *L. loa* will be studied at payam level, alongside the above surveys. The existing rapid assessment procedure for *L. loa* (RAPLOA) will be followed.

**Survey timetable:** Five counties (Tambura, Ezo and Yambio in Western Equatoria, and Aweil East and Aweil Centre in Northern Bahr el Ghazal) will be mapped before the end of April 2009, provided that all the payams are accessible. From these areas, mapping will be expanded to all counties of the two states, and beyond until all the whole of Southern Sudan has been mapped.

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1 Based on REMO and RAPLOA surveys conducted by APOC and the SSOTF, trachoma surveys conducted by the MoH and The Carter Center, and schistosomiasis and LF surveys conducted by a number of MoH implementing partners.
Sample size: In each implementation unit, the number of individuals to be sampled depends on the prevalence threshold recommended to denote the need for MDA. This varies for schistosomiasis and for LF.

**Schistosomiasis**

The goal of schistosomiasis control is to reduce morbidity and transmission. To assess the need for MDA for schistosome and STH infection, WHO recommends that a sample of 200–250 individuals in each ecologically homogeneous area in terms of climate, topography and ecology is considered to be adequate for evaluation of infection prevalence (Lwanga & Lemeshow, 1991). Owning to the focal nature of schistosomiasis up to four communities will need to be surveyed per payam, with the exact number depending on ecological diversity and payam size. In each study village, 60 children of school age (30 males and 30 females) will be randomly selected. This sample size will adequately capture information needed to determine the prevalence of STH.

Both *S. mansoni* and *S. haematobium* are endemic in Southern Sudan, but it is presently unclear in which areas the two species are coendemic and in which areas they are not. For this reason, both stool and urine samples will need to be analyzed, to ensure that areas endemic for only one schistosome species are not accidentally excluded from control.

**Lymphatic filariasis**

The goal of LF control is the interruption of filariasis transmission and to achieve this goal WHO recommends MDA in areas where prevalence is 1% or above. Therefore, the objective of the LF survey is not to precisely determine infection prevalence, but rather to determine if infection prevalence in each implementation unit is high enough to warrant MDA.

Initially, one community in each implementation unit (payam) will be selected. If this survey indicates that the intervention threshold for LF (1% prevalence) is not exceeded, one more community will be selected before concluding that a payam is not endemic for the disease.

For LF surveys, the WHO recommends a Lot Quality Assurance Sampling (LQAS) approach, whereby a specified number of individuals are randomly selected to provide finger-prick blood samples that are examined until either a predetermined number of infected individuals are identified, or the specified number of individuals sampled without reaching the threshold. It is estimated that at least 250 individuals in each implementation area (initially designated as the payam) need to be examined before it is statistically valid to say that prevalence is less than 1%. If one or more individuals are found to be infected after examining 100 individuals, then no further testing is required; otherwise testing continues in another village until a total of 250 individuals have been examined in the payam.

**Loiasis**

In communities that are highly endemic for loiasis there is a risk of SAEs to the drug ivermectin following its use for treatment of onchocerciasis or LF. The risk of SAEs is related to the intensity of infection with *L. loa*, with the risk becoming high when the microfilarial load exceeds 30,000 mf/ml. Information on loiasis prevalence is therefore required to plan in which communities ivermectin distribution can be safely implemented.

A rapid assessment procedure, referred to as RAPLOA, was therefore developed by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). It employs a simple questionnaire on one of the clinical signs of loiasis: the migration of adult *L. loa* under the conjunctiva (often referred to as a history of ‘eye worm’). It is

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recommended that **80 adults** are interviewed per study village, using only three questions: i) Have you ever noticed worms moving along the white part of your eye? If the answer is 'Yes', the interviewer will show a picture of an adult *L. loa* worm in the eye and ask: ii) Have you ever had the condition in this picture?; and iii) The last time you had this condition, how long did the worm stay before disappearing? Results from the questionnaire-based study are used to predict whether or not loiasis is present in a community at a high level of endemicity (40% or above).

**Sampling method:** Two stage clustering convenient sampling will be used. The first stage is to sample communities in each payam, and the second stage is to sample households within chosen communities. All persons aged 5 years and above living in the selected households will be invited to participate. To capture the peak prevalence of schistosomiasis the focus will be on individuals aged 5–15 years (see detailed description below), while individuals age 16 years and above will be tested for LF. If no LF is found in the first study village, then the age-range will be extended to include school-age children in the second village, to facilitate the recruitment of the required 250 individuals per payam.

**Primary sampling unit**
The primary sampling unit for the survey is accessible communities. At the payam level, communities suspected to be endemic for either schistosomiasis or LF will be deliberately selected for sampling. Communities that cannot be reached by car or are potentially insecure will be regarded as inaccessible.

For schistosomiasis infection, communities will be selected based on environmental data (presence of water bodies, land surface temperature, coverage with vegetation, rainfall data, etc.). The number of communities to be tested for schistosomiasis in a payam will be calculated based on the population size of each payam. For this calculation, the population of large towns will be subtracted from the overall population of the payam. If a large town also equates to a payam, one administrative unit within the town will be randomly selected and sampled. In payams suspected to have no or low transmission of schistosomiasis and where more than two study communities have been selected, sampling will stop after the first two study communities if neither had any cases of schistosomiasis and the county/payam health staff indicate that the environmental conditions in these communities are representative of those in the payam as a whole.

Schistosomiasis and STH testing will be conducted in communities already selected for LF. The selection of communities for LF testing will be based on anecdotal reports of the occurrence of disease, particularly lymphoedema and hydrocele, rather than randomly. Up to three communities will be tested for LF per payam.

**Secondary sampling unit**
The secondary sampling unit will be households randomly selected within each village using the random walk approach. The procedure will be as follows:

1. **Choosing the first household.**
   - From the approximate centre of the village, spin a bottle to randomly select a direction.
   - If there are no households in that direction, move the bottle clockwise until the first house is encountered. This becomes the new direction.
   - Consider only occupied households in the sampling.

2. **Choosing the next household.**
   - Each time you leave a house, you go left and do the interview in the next house you meet.
• If you pass a small road that leads to a house, take it. The next house is the house at the left. If you can’t go anywhere to the left of that house, return to the road you were walking on before you turned left and continue the same direction previously taken.
• If you meet a crossing, turn left.
• If you reach the boundary of the village, you go back to the centre of the village, spin the bottle and start all over again.

Note: Other methods of selecting the secondary sampling unit may be explored during surveys, should the random walk prove too labour intensive to conduct. Selection of individuals at a central point, such as the house of the village chief or a school, are alternatives that have been successfully used for NTD surveys elsewhere.

3. Inclusion/exclusion criteria.
In each sampling unit (village), all eligible individuals in selected households are to be included. The following age groups are eligible:
• Schistosomiasis: Children age 5-15
• LF: School children (older age-groups are preferable) and adults
• Loiasis: Adults

To ensure an adequate sample size for the study of schistosomiasis, recruitment of households will be continued in each study community until 70 children between the ages of 5-15 years have been recruited. Meanwhile, all individuals age 16 and above will be recruited to provide a blood sample for analysis of LF antigen until 110 individuals have been enrolled. *L. loa* questionnaires will be administered to all individuals age 16 and above attending for LF testing.

Individuals will be excluded from the study if s/he:
• Has not lived in the same payam for at least six months
• Does not provide informed consent

Field team: A single field team will be comprised of the following persons:

• One team leader: in charge of coordinating survey activities and ensuring compliance with the mapping protocol
• Two interviewers/ auxiliary workers: to identify households using the random walk approach, distribute faecal and urine sample containers, collect household information and refer individuals to a central location to deliver their sample containers for parasitological examination
• Three laboratory technicians: one to analyze stool samples, one to analyze urine samples, and one to collect finger-prick blood samples and conduct ICT tests
• One driver: in charge of vehicle and team safety and security. While survey teams conduct field work the driver will guard the vehicle and man the HF radio

Two field teams will simultaneously conduct the survey in a payam, covering nearby villages.

Note: During the initial month of surveys, teams will be comprised of four technicians, two of whom will be staff of the Vector Control Division of the Ugandan Ministry of Health or WHO technicians. This composition will be required to train Southern Sudanese staff in the diagnostic and survey procedures.
**Survey preparations:** Health and administrative authorities at state, county, and payam levels, and community leaders will be contacted, for permission to conduct the survey and to obtain their support and collaboration in planning and undertaking the survey. In areas where a questionnaire for loiasis will be included in the survey, preliminary community visits will be conducted to administer a community questionnaire to identify the local names for ‘eye-worm’.

**Household survey:** Household encountered by means of the random walk method will be selected and the household head will be invited to participate in the study. If written consent is provided, all individuals above the age of 5 years will be invited to participate in the study. Eligible study participants will be asked to provide a stool, urine and/or finger-prick blood sample. If some inhabitants are absent at the time of the visit, sample containers for them will be left with a household member. Household visits will be repeated until the required number of study participants for a community has been recruited.

Should the above method prove too labour intensive to allow completion of one village per day, modifications will be made as follows: Drawing on the assistance of the village chief and/or other community leaders, village inhabitants will be requested to gather at a central location. Individuals will then be invited to participate in the survey and be requested to provide verbal consent. Sample containers will be provided to all individuals that provide consent and finger-prick blood samples will be taken for analysis of LF infection. Stool and urine samples will be analysed on site.

**Collection of biological samples:** Each individual aged 5 – 15 years selected will be provided with a container for a urine sample plus a piece of polyethylene sheeting for collection of a stool sample, with an indication of the amount of stool needed. The containers and pieces of polyethylene will be distributed to individuals either on the day of collection or during the previous day. Parasitological examination of stool and urine samples will be conducted in the field.

A finger-prick blood sample will also be collected from consenting adults aged over 16 years, on the day of the survey and used for the detection of *Wuchereria bancrofti* antigens using immunochromatographic card tests (ICT).

**Data collection:** Survey data will be entered and stored on handheld personal digital assistants (PDAs) linked to a GPS device. Data entry templates will be prepared and uploaded onto each PDA in advance, to facilitate data-entry and minimize mistakes. Survey data will be downloaded onto a laptop computer at the end of each survey day and backed up externally once a week.

**Data analysis:** The information relevant for guiding the implementation of MDA is:

- The percentage of individuals in each community infected with each parasite species.
- For schistosomiasis and STH, individuals can also be classified according to their intensity of infection and categories into light, moderate and heavy infection using the following classes:

<table>
<thead>
<tr>
<th>Species</th>
<th>Light</th>
<th>Moderate</th>
<th>Heavy</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. lumbricoides</em></td>
<td>1–4999 epg</td>
<td>5000–49999 epg</td>
<td>≥50000 epg</td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>1–999 epg</td>
<td>1000–99999 epg</td>
<td>≥100000 epg</td>
</tr>
<tr>
<td>Hookworms</td>
<td>1–1999 epg</td>
<td>2000–39999 epg</td>
<td>≥40000 epg</td>
</tr>
<tr>
<td><em>S. mansoni</em></td>
<td>1–99 epg</td>
<td>100–39999 epg</td>
<td>≥400 epg</td>
</tr>
<tr>
<td><em>S. haematobium</em></td>
<td>1–50 eggs/10ml urine</td>
<td>≥50 eggs/10ml urine</td>
<td></td>
</tr>
</tbody>
</table>

- For schistosomiasis and STH, the mean intensity of infection and the prevalence of heavy infection can be calculated for each community. This information is especially useful in determining the future frequency of MDA.
Parasitological methods: The following information has been adapted from relevant WHO manuals:

- WHO (1994). Bench Aids for the diagnosis of intestinal parasites
- WHO (2002). Helminth control in school-age children

**Schistosomiasis**

Parasitological diagnosis of schistosomiasis is made by either examining (i) urine (for *S. haematobium*) using the reagent strip for haematuria or (ii) stool samples (for *S. mansoni*) using the Kato-Katz method. Because both species are present in Southern Sudan but are not necessarily co-endemic, both techniques will need to be used to confirm whether a payam qualifies for intervention.

The reagent strip is a quick and easy method that is also highly sensitive and specific in endemic areas. The reagent strip is dipped into the urine sample and then, after about one minute, compared with a colour scale supplied with the strips. Intensity of infection can be estimated according to the quantity of blood detected by the strip. Since haematuria tends to be more consistent than excretion of eggs, the strips can be used at any time of the day.

As an alternative, the urine filtration technique involves the microscopic examination of a filter used to collect the eggs of *S. haematobium* from a urine sample. 10 ml of a urine specimen collected from each child is filtered through a 13mm diameter polycarbonate membrane with a 12µm pore size (Millipore Ltd) and the concentration of the eggs of *S. haematobium*, expressed as eggs/10ml of urine.

*The urinary excretion of S. haematobium eggs follows a daily rhythm, with a peak around noon. Urine specimens for filtration are therefore best collected between 10:00 and 14:00 (10 am and 2 pm).*

The Kato-Katz technique involves microscopic examination of a fixed amount of faecal material to detect and count *S. mansoni* eggs and is estimated in eggs per gram of faeces (epg). Epg values are obtained by multiplying the number of eggs counted on the slide by a multiplication factor that varies according to the size of the template used. WHO recommends the use of a template holding 41.7mg of faeces, which corresponds to a multiplication factor of 24. Ideally, all samples should be collected in the morning and processed and examined in the afternoon of the same day. This simplifies the daily routine and reduces the number of containers and slides needed since they can be cleaned at the end of each day and reused.

**Soil-transmitted helminths**

STH will be studied while faecal samples are being analyzed for intestinal schistosomiasis. The Kato-Katz method will be used to detect the presence of STH eggs, and the concentration of eggs of STH species will be estimated in eggs per gram of faeces (epg).

*Whenever possible, eggs will be counted within one hour of the preparation of slides; hookworm eggs tend to become transparent over time and may be overlooked. If the egg count is completed after one hour, a note will be made that hookworm may be underestimated.*
**Lymphatic filariasis**  
Two standardized diagnostic tools are currently available for detecting LF infection in the field: (i) night-blood films for microfilaraemia; and (ii) ICT tests that detect *Wuchereria bancrofti* antigen in a human blood sample and can take place at any time of the day.

Because the filarial infection generally shows nocturnal periodicity, slides are made from blood obtained from finger-pricks between 22:00 and 02:00 to detect microfilariae. This makes blood films more difficult to collect and they require specially trained technical staff. The ICT measures the presence of the adult worm antigen and is a useful tool to detect the infection in the community during the initial assessment phase when determining which implementation units require MDA.

Thus, for LF mapping, a small drop of blood will be collected and used for the diagnosis of lymphatic filariasis using the NOW® Filariasis ICT. Results will show the prevalence of LF infection.

**Laboratory safety:** Team members will be trained on laboratory safety including wearing of latex gloves during the collection and microscopic examination of faecal and urinary specimens and collection of blood samples. Any material contaminated with stool, urine or blood will be soaked in a suitable disinfectant, such as sodium hypochlorite solution, before disposal or cleaning for reuse. Sharps, such as lancets used to collect finger-prick blood samples, will be collected in safety containers and disposed according to best-practice.

**Quality control:** The consistency of microscopic results during the survey should be verified by quality control; this is particularly important for the Kato-Katz technique. Before the survey is undertaken, a day will be spent evaluating the consistency of egg counting among laboratory technicians. Each day during the survey, the team leader will read 10% of the slides handled by each microscopist without prior knowledge of the results. In the case of a discrepancy larger than 10%, the slide will be discussed by the two readers and further slides will be examined to avoid repeated errors.

**Training:** To undertake the field survey, certain expertise on diagnostic methods and data collection is required. Currently this expertise is rare or not available in Southern Sudan, particularly at state level. A team of experienced Uganda laboratory technicians, previously involved in the mapping of NTDs in their own country, will therefore be used for training of trainers in Southern Sudan, both at central level and in a number of states. Initial mapping will then be carried out jointly by Ugandan and Southern Sudanese technicians until teams of national trainers are experienced in the methods and practicalities of field surveys. These national trainers will be used to expand the capacity for rapid mapping to all states and, with additional manpower from each state, to map the target diseases.

**Ethical considerations:** Study participants will have the purpose of the study explained to them in their local language at the time of recruitment. Written consent will then be requested from heads of household. No payment or other form of compensation will be provided to participants. However, all individuals found to be infected with one or more of the diseases under investigation will be treated with the appropriate anthelminthic drug. If participants want to stop being examined after giving full consent, they are free to do so, and will be excluded from the study.
Appendix 1. Selection of communities for schistosomiasis, STH and LF surveys

The purpose of the survey is to try and establish:

1. The current prevalence of schistosomiasis/STH in selected payams
2. Whether at least one community in each payam has a LF prevalence of >1%

This information will be used to determine whether mass drug administration is required according to WHO guidelines.

There are several parameters that need to be taken into consideration for the selection of survey communities:

- Population figures will be used to calculate the number of survey communities per payam proportional to its population size relative to the overall population size of the study area.
- In payams with a large town, the population of the town will be subtracted from the overall population size of the payam, due to the expected difference between urban and rural areas. For the rural area, the number of survey sites will be calculated using the adjusted population figures. For the large town, only one survey site in the town will be selected.
- Anecdotal reports of LF will be taken into consideration where possible so that suspected LF endemic village are surveyed.
- All villages selected for LF will be surveyed for schistosomiasis and STH.
- At least two community in each payam will be surveyed for schistosomiasis and STH. The exception to this are large towns that are also a payams. In this case only one survey site will be chosen at random from within the towns’ administrative structure.
- In payams suspected to have no or low transmission of schistosomiasis and where more than two study communities have been selected, sampling will stop after the first two study communities, if both had no case of schistosomiasis and if the county/payam health staff indicate that these environmental conditions in these communities are representative of those in the payam as a whole.
Appendix 2. Standard Operating Procedures - Village level

Day 1

1. Once a village has been selected, it will be visited by the supervisor and two staff during the day before the survey. The purpose of the visit will be to:
   a. Sensitize the community and identify a respected member of the village to accompany the survey team during recruitment
   b. Carry out selection of households via random walk
   c. Recruit individuals from each of the selected households

2. State, county, payam and village information will be filled in (on PDA). This form also includes GPS coordinates and the diseases to be surveyed. This will act as a check list for parasitological data sheets.

3. Once a household has been identified, the study will be explained to the head of household and s/he will be asked to sign the consent form.

4. If informed consent is provided, eligible individuals in the household will be identified. Urine containers and black polyethylene sheeting for faecal samples will distributed to each eligible individual. If the individual is present, s/he will be given a urine sample container and a piece of polyethylene sheeting for faecal sample collection. Both will be labelled with the individuals name and unique identifying number. For those individuals that are not present, the team will leave containers and pieces of polyethylene sheeting labelled with the household information with the head of household and explain that samples should be returned by the designated individual. Individuals will be asked to return the samples to a central location in the village the next morning. At this point the age and sex of individuals that were not present when sample containers were distributed will be recorded.

5. Individuals eligible for LF testing will be requested to come to a central location the next morning to provide a blood sample.

6. As it is expected that not all containers will be returned and that some individuals will not report for LF examination, an excess of individuals will be recruited according to the following village categories:
   a. The first village in a payam to be surveyed for LF, schistosomiasis and STH: 70 individuals age 5-15 and 110 individuals age 15+ will be recruited for schistosomiasis/STH and LF examination, respectively.
   b. If LF tests are negative in the first village, a second one will be surveyed for LF, schistosomiasis and STH: 70 individuals age 5-15 and 110 individuals age 15+ will be recruited for schistosomiasis/STH and LF examination, respectively. All individuals age 5-15 will also be tested for LF at the time they return their stool/urine sample.
   c. Villages that are only surveyed for schistosomiasis/STH: 70 individuals age 5-15

7. The supervisor records household number plus the initials, age and sex of each eligible individual on the PDA.
Day 2

1. On morning of the following day a laboratory will be set up at the agreed central point.

2. Three technicians will examine samples using paper data entry sheets.

3. The technicians have to ensure that the details (age, sex) are recorded for individuals that were not present during household registration and for which sample containers were only labelled with the household identifier

4. The consent form should be used to identify households that have not provided samples

5. The driver, together with a village guide, should follow up these households to ensure that all enlisted individuals provide a sample
Appendix 3. Standard operating procedures - Faecal egg count

1. Take microscope slide and write ID number of stool sample at one end of slide.
2. Put white plastic template over slide
3. Thoroughly mix sample by stirring with wooden applicator stick
4. Remove corn seed size sample with stick and place on small piece of mesh
5. Place mesh on clean newspaper, sample downwards and press mesh over sample with clean wooden applicator
6. Scrape sieved stool sample off mesh and place into hole in plastic template
7. Make sure hole is filled and surface of sample is level with sides
8. Remove plastic cover without disturbing the sample and leaving sample in a ring on the slide
9. Place glycerine-soaked cellophane cover slip over sample
10. Invert microscope slide onto clean newspaper and press firmly to spread sample to edges of cover slip
11. After 10 minutes place microscope slide onto microscope and count all eggs present
12. Record numbers of eggs present under each heading

Note: Repeat twice for each child’s stool sample to provide two estimates of faecal egg counts

Appendix 4. Standard operating procedures - Urine egg count

1. Mix sample thoroughly by vigorous inversion
2. Withdraw 10ml by syringe and attach syringe to pre-prepared filter holder
3. Take sample ID number and write on microscope slide
4. Push whole of sample through filter holder
5. Disengage syringe, fill with 2 ml air and push air through filter holder
6. Unscrew filter holder and with a pair of forceps, pick up and place filter membrane carefully onto centre of microscope slide
7. Add 100ul water to cover sample and count using x100 magnification and with microscope condenser closed down to give maximum contrast. Count all eggs on slide, recording egg numbers for each species of parasite separately.
8. Record total numbers of each type of egg present on data sheet provided
Appendix 5. Standard operating procedures - Hemastix

1. Remove the reagent strip from the bottle. Replace the cap immediately and tightly. Do not touch the test pad or the reagent strip.
2. Dip the test end of the reagent strip into a fresh urine sample and remove it immediately drawing the edge of the reagent strip against the rim of the urine container to remove excess urine.
3. Immediately begin timing.
4. At 60 seconds, match the test pad to the colour chart on the bottle label.
5. Record the result.

Appendix 6. Standard operating procedures - Immunochromatographic card tests

1. Remove the test card from the pouch just prior to use. Open the card and lay it flat on the work surface.
2. Fill the capillary tube to the 100µl mark using capillary action, with blood from a finger puncture.
3. Add the 100µl of sample **slowly** from the capillary tube onto the top of the pink and white pad. **Note:** See arrow on test device showing correct placement of sample at **top** of pad. Should there be a sample remaining in the capillary tube that will not flow out freely, the tip may be gently pressed against the pad. **Important:** **Wait for each drop to soak in before adding the next drop. Incorrect addition of sample may result in device failure.**
4. Wait until the sample has flowed into the pink area and it is completely wet (this should take about 30 seconds to 1 minute.
5. Remove and discard the adhesive liner. Ensure that the adhesive on the right hand side of the test card is exposed.
6. Close the card. To ensure good test flow, press very firmly along the entire area to the right of the window. Start timing.
7. Read the result through the viewing window at 10 minutes. Refer to the details printed on the card for test interpretation. Two clearly visible lines indicate a positive result.