

Video Article

Determining Soil-transmitted Helminth Infection Status and Physical Fitness of School-aged Children

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Abstract

Soil-transmitted helminth (STH) infections are common. Indeed, more than 1 billion people are affected, mainly in the developing world where poverty prevails and hygiene behavior, water supply, and sanitation are often deficient^{1,2}. *Ascaris lumbricoides*, *Trichuris trichiura*, and the two hookworm species, *Ancylostoma duodenale* and *Necator americanus*, are the most prevalent STHs³. The estimated global burden due to hookworm disease, ascariasis, and trichuriasis is 22.1, 10.5, and 6.4 million disability-adjusted life years (DALYs), respectively⁴. Furthermore, an estimated 30-100 million people are infected with *Strongyloides stercoralis*, the most neglected STH species of global significance which arguably also causes a considerable public health impact^{5,6}. Multiple-species infections (i.e., different STHs harbored in a single individual) are common, and infections have been linked to lowered productivity and thus economic outlook of developing countries^{1,3}.

For the diagnosis of common STHs, the World Health Organization (WHO) recommends the Kato-Katz technique^{7,8}, which is a relatively straightforward method for determining the prevalence and intensity of such infections. It facilitates the detection of parasite eggs that infected subjects pass in their feces.

With regard to the diagnosis of *S.stercoralis*, there is currently no simple and accurate tool available. The Baermann technique is the most widely employed method for its diagnosis. The principle behind the Baermann technique is that active *S.stercoralis* larvae migrate out of an illuminated fresh fecal sample as the larvae are phototactic⁹. It requires less sophisticated laboratory materials and is less time consuming than culture and immunological methods⁵.

Morbidities associated with STH infections range from acute but common symptoms, such as abdominal pain, diarrhea, and pruritus, to chronic symptoms, such as anemia, under- and malnutrition, and cognitive impairment¹⁰. Since the symptoms are generally unspecific and subtle, they often go unnoticed, are considered a normal condition by affected individuals, or are treated as symptoms of other diseases that might be more common in a given setting. Hence, it is conceivable that the true burden of STH infections is underestimated by assessment tools relying on self-declared signs and symptoms as is usually the case in population-based surveys.

In the late 1980s and early 1990s, Stephenson and colleagues highlighted the possibility of STH infections lowering the physical fitness of boys aged 6-12 years^{11,12}. This line of scientific inquiry gained new momentum recently^{13,14,15}. The 20-meter (m) shuttle run test was developed and validated by Léger *et al.*¹⁶ and is used worldwide to measure the aerobic fitness of children¹⁷. The test is easy to standardize and can be performed wherever a 20-m long and flat running course and an audio source are available, making its use attractive in resource-constrained settings¹³. To facilitate and standardize attempts at assessing whether STH infections have an effect on the physical fitness of school-aged children, we present methodologies that diagnose STH infections or measure physical fitness that are simple to execute and yet, provide accurate and reproducible outcomes. This will help to generate new evidence regarding the health impact of STH infections.

Video Link

The video component of this article can be found at <http://www.jove.com/video/3966/>

Protocol

1. Kato-Katz Technique

1. Place a standard Kato-Katz template with a hole for holding 41.7 milligrams (mg) of stool on a microscope slide.
2. Scoop 2-3 grams (g) of a fresh fecal sample onto a piece of newspaper or aluminum foil, and press a piece of wire or plastic mesh on top to sieve it.
3. Using a small plastic spatula, scrape the sieved material off the mesh and completely fill the hole in the Kato-Katz template with it. To remove excess fecal material, level the content of the hole with the spatula.

- Vertically remove the template without disturbing the fecal material now adhering to the microscope slide. The template and spatula can be cleaned in water with detergent, and reused.
- Place a piece of cellophane, pre-soaked in glycerine-malachite green solution for at least 24 hours (hr), over the fecal sample on the microscope slide.
- To spread the fecal material into a thick smear, gently press a clean microscope slide against the sample slide, evenly distributing the material within a circle of a diameter slightly smaller than the breadth of the microscope slide.
- Allow the slide to clear for 30-60 minutes (min), during which the slides must be kept away from direct sunlight. Then, systematically examine the thick smear under a light microscope (40-100x magnification). Count the number of STH eggs and stratify them by species (i.e., *A.lumbricoides*, *T.trichiura*, and hookworm). Of note, other helminth eggs (e.g. *Schistosoma mansoni*) can also be detected by this method and should be counted and recorded separately.
- To get a standardized estimate of the intensity of infection, conventionally expressed as number of eggs per gram of stool (EPG), multiply the egg count by 24 ($24 \times 41.7 \text{ mg} \approx 1 \text{ g}$).

2. Baermann Technique

- Set up a stand that can hold a glass funnel and attach a rubber tube, closed with a clip, to the bottom of the funnel.
- Place a sieve or a piece of wire screen shaped like a cone, in the funnel and layer it with a piece of medical gauze.
- Fill up the funnel with tap water and ensure that the whole set-up does not leak.
- Scoop 20-30 g of fresh stool onto the middle of the medical gauze and ensure that it is completely submerged in water. Add more water if necessary. Fold the medical gauze over the sample.
- From below, shine artificial light at the funnel for 2 hr.
- Drain 50 milliliters (ml) of the lower portion of the water in the funnel into a centrifuge tube by gently releasing the clip on the rubber tube.
- Centrifuge the collected water at 600 x g (relative centrifugal force) for 5 min and carefully pour out the supernatant without disturbing the sediment. Retain the last few drops in the tube.
- Re-suspend the sediment and pipette 2 drops of the solution onto a microscope slide and examine the slide under a light microscope (40x magnification for detection and 100-400x magnification for species confirmation). First-stage larvae (L1) of *S. stercoralis* are alive and actively move under the microscope.

3. 20-m Shuttle Run Test

- Measure a 20-m flat and straight running course and mark it with cones.
- Help the test participant wear a heart rate monitor on the wrist and the corresponding transmitter around the chest¹⁸. This equipment allows the heart rate of the participant to be measured before and after the test and thus monitors whether the participant has exerted maximal effort.
- Instruct the participant to run back and forth on the course by following the pace of pre-recorded sound signals delimiting 20-m laps. Starting with a running speed of 8.5 kilometers per hour (km/hr), the frequency of the signals indicating 20-m intervals increases by an equivalent of 0.5 km/hr every min.
- Ensure that the participant starts a new 20-m interval each time a signal is sounded and finishes it before the next signal arrives.
- Stop the participant if he/she fails to follow the pace for two consecutive 20-m intervals.
- Record the highest running speed indicated for the last interval which the participant completed fully, along with the age and sex of the participant.
- Verify that the heart rate achieved at the end of the test is the child's maximal heart rate¹⁹. If this heart rate is not reached, the child might not have exerted maximal effort, and hence the test should be repeated.
- Estimate the maximum aerobic capacity ($\text{VO}_2 \text{ max}$) of the participant, using an equation put forth by Léger and co-workers: $\text{VO}_2 \text{ max} = 31.025 + 3.238 * \text{speed (in km/hr)} - 3.248 * \text{age (in years)} + 0.1536 * \text{speed (in km/hr)} * \text{age (in years)}$.

4. Representative Results

In terms of STH diagnosis with the Kato-Katz technique, unique characteristics of the different helminth eggs allow for the identification of most species²⁰. The eggs of *A.lumbricoides* (approximately 45-75 micrometer (μm) long and 35-50 μm wide, **Figure 1**) have thicker shell walls than the eggs of *T.trichiura* (**Figure 2**) and hookworm (**Figure 3**). A fertilized egg of *A.lumbricoides* (**Figure 1**) has an outer albuminous coat, which makes it look golden brown, while a non-fertilized egg lacks this outer coat and is slightly elongated and larger overall. The eggs of *T.trichiura* (approximately 50 μm long and 22 μm wide, **Figure 2**) are elongated and have distinct polar plugs. Hookworm eggs (approximately 64-76 μm long and 36-40 μm wide, **Figure 3**), on the other hand, have a very thin wall, followed by a clear ring around a dense cluster of cells in the center of the egg.

S.stercoralis L₁ larvae (**Figure 4**) are approximately 180-380 μm long and have a very short buccal cavity. A distinct genital primordium (highlighted with a blue arrow in **Figure 4**) can be observed and the tail of the larvae (highlighted with a red arrow in **Figure 4**) has a sharp end.

As the true impact of STH infections on the physical fitness of children is still being debated^{13,14,15}, there is no representative result for their impact at this point. Interpretation of 20-m shuttle run test results can be performed in various ways. The physical fitness of children can be compared between infected and non-infected subjects, who are similar in terms of age and sex, other potentially relevant health conditions, socioeconomic status, and cultural habits. This can be done in a cross-sectional survey^{13,14,15} where the STH infection status and physical fitness of infected and non-infected children are assessed at a particular time point. The strength of the evidence will be higher when following a randomized controlled trial design where the physical fitness of school-aged children is assessed before and after anthelmintic/placebo treatment, allowing for both short- and long-term evaluation. One can further try to identify a possible dose-response relationship between the intensity of STH infections and physical fitness and compare different levels of multiparasitism¹ with physical fitness. In the work presented

by Yap and co-workers¹⁵, physical fitness, as depicted by VO_2 max, was found to be significantly reduced in children infected with *T.trichiura* compared to children who were not infected with *T.trichiura* (Figure 5).

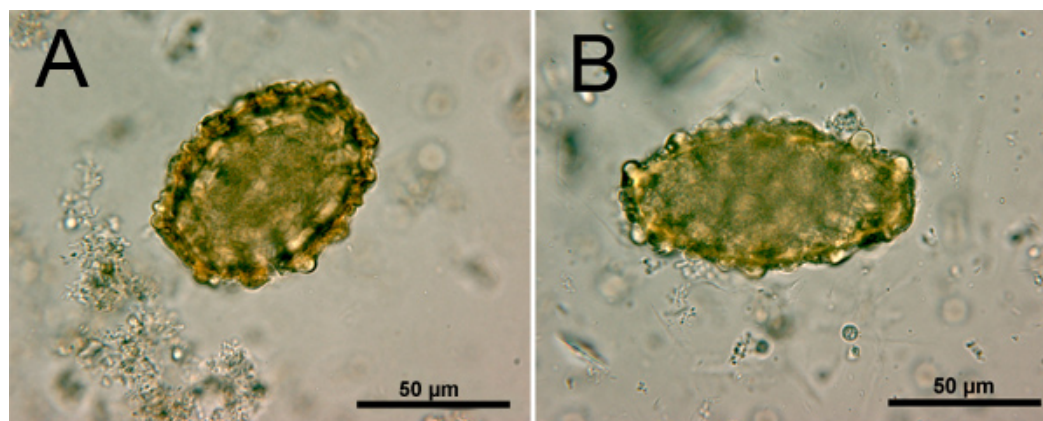


Figure 1. Microscopic images of *A.lumbricoides* eggs: fertilized (A) and non-fertilized (B) under 500x magnification. Scale bar = 50 μ m.



Figure 2. Microscopic image of a *T.trichiura* egg under 500x magnification. Scale bar = 50 μ m.

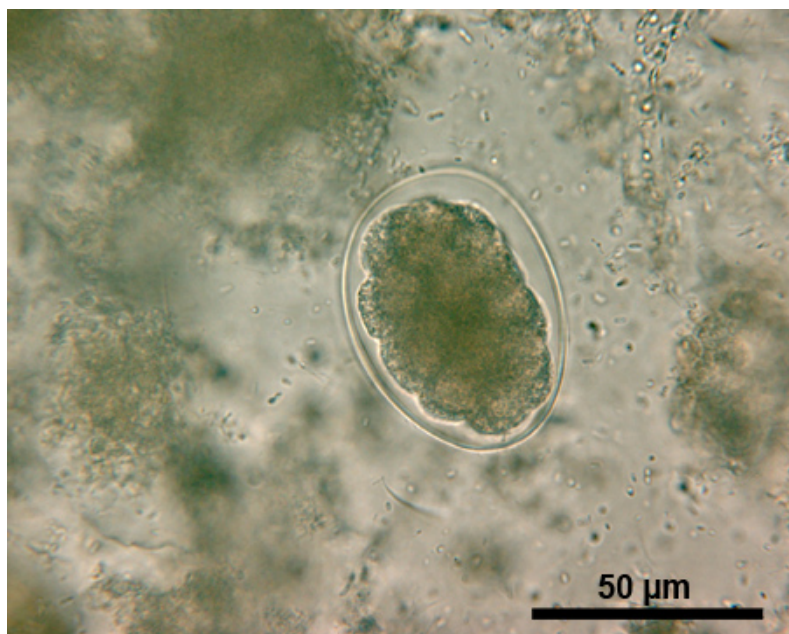


Figure 3. Microscopic image of a hookworm egg under 500x magnification. Scale bar = 50 μ m.

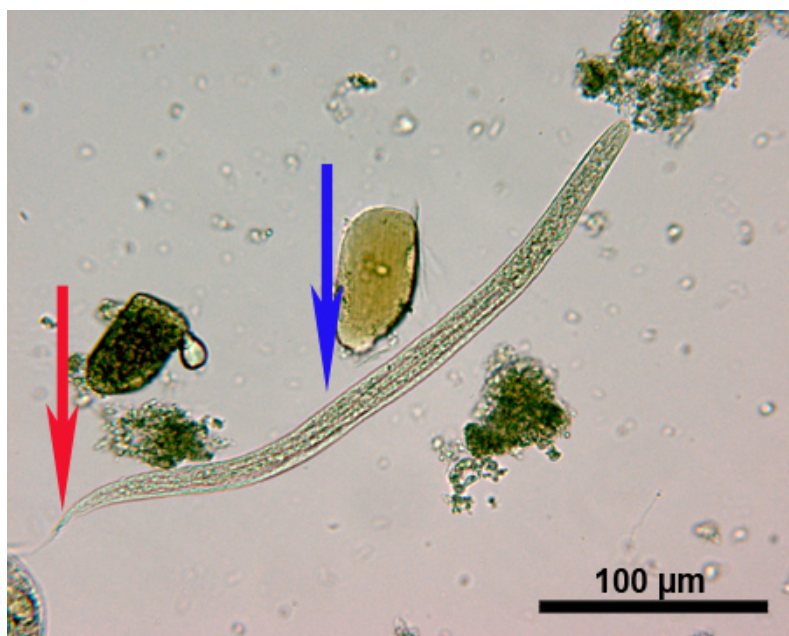


Figure 4. Microscopic image of a *S. stercoralis* L₁ larva under 250x magnification. Arrows indicate tail (red) and genital primordium (blue). Scale bar = 100 μ m.

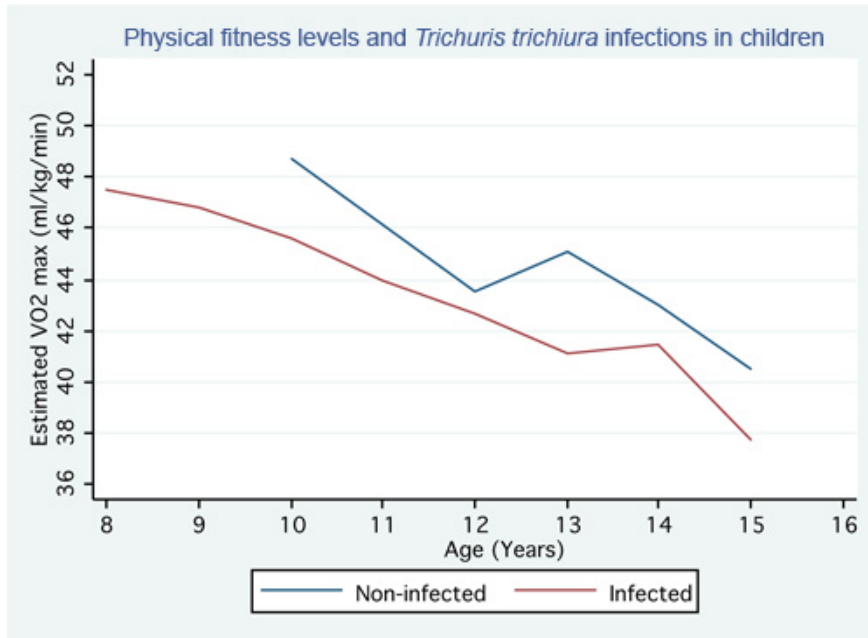


Figure 5. Graph showing the physical fitness level of children infected with *T.trichiura* compared to the physical fitness level of children not infected with *T. trichiura*.

Discussion

The three protocols described in this paper have been tested and executed in the south-western part of Yunnan province, People's Republic of China among members of the Bulang ethnic minority²¹ and in different parts of Africa^{6,13,14}.

There are several templates available for the Kato-Katz technique. Each distinct hole-size will deliver a distinct amount of fecal material. For the calculation of EPGs, it is therefore necessary to know the unit of the template, so as to multiply the counted number of eggs with the correct factor to arrive at the EPG estimate. Furthermore, when hookworm is present in the community under investigation, it is essential to read the slides shortly after preparation (after a clearing time of 30 min but within a maximum clearing time of 60 min). The main reason is that at high temperatures, hookworm eggs rapidly disintegrate²². The time between fecal production by the study participant and the examination of the samples is also important as an interval of 3 hr or more can lead to a 50% decrease in the sensitivity of the Kato-Katz technique for hookworm diagnosis²³. Lastly, to improve the diagnostic sensitivity of the Kato-Katz technique, especially in areas with low-intensity STH infections, multiple fecal samples should be obtained from a participant, and double or triplicate Kato-Katz thick smears should be prepared from each stool sample^{7,24}.

With regard to the Baermann technique, caution must be taken when performing the experiment as infective larvae might be present and thus, gloves must be worn. Although the Baermann technique shows a considerably higher sensitivity than other concentration techniques and is less laborious than culture methods, there are important shortcomings. For example, a large amount of fresh fecal material is required, which is a problem when patients submit small fecal samples. On the other hand, a large fecal sample might increase the chances of larval detection in low-intensity infections⁹. In addition, there is a risk that the larvae of other nematodes, such as hookworm or *Trichostrongylus spp.*, might be present, thus careful microscopic examination is warranted.

There are direct and indirect ways of measuring VO₂ max for aerobic fitness assessments. Direct protocols require treadmills, cycle ergometers, and other specialized and bulky equipment, which can measure the volume and gas concentrations of the exhaled air. Such an approach is not feasible in a resource-constrained rural setting in the developing world. For such settings, indirect ways, such as the 20-m shuttle run test, are the preferred methods. Although indirect methods are never as accurate as direct methods, the 20-m shuttle run test established by Léger and co-workers has demonstrated high reproducibility ($r=0.89$) and validity against maximal oxygen uptake on a treadmill¹⁶. Furthermore, the investigator and participant ratio can be lower and several participants can perform the test at the same time, saving human resources and time. Heart rate monitors are not typically used in such tests^{13,16}. However, their use allows the investigator to obtain additional information on whether the participant has exerted maximal effort in the test. Finally, it is advisable to let the participants do a trial run of the first few intervals so that they can familiarize themselves with the running speed and course.

In conclusion, we believe that a combination of the above-mentioned methodologies, following standard protocols, allows for the assessment of the prevalence and intensity of STH infections and physical fitness of school-aged children in a scientifically sound, yet manageable and affordable manner.

Disclosures

No conflicts of interest declared.

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References

- Steinmann, P., Utzinger, J., Du, Z.W., & Zhou, X.N. Multiparasitism: a neglected reality on global, regional and local scale. *Advances in Parasitology*. **73**, 21-50 (2010).
- Bethony, J., Brooker, S., Albonico, M., Geiger, S., Loukas, A., Diemert, D., & Hotez, P.J. The soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet*. **367**, 1521-1532 (2006).
- Hotez, P.J., Molyneux, D.H., Fenwick, A., Kumaresan, J., Ehrlich Sachs, S., Sachs, J.D., & Savioli, L. Control of neglected tropical diseases. *New England Journal of Medicine*. **357**, 1018-1027 (2007).
- Chan, M.S. The global burden of intestinal nematode infections-fifty years on. *Parasitology Today*. **13**, 438-443 (1997).
- Olsen, A., van Lieshout, L., Marti, H., Polderman, T., Polman, K., Steinmann, P., Stothard, R., Thybo, S., Verweij, J.J., & Magnussen, P. Strongyloidiasis - the most neglected of the neglected tropical diseases? *Transactions of the Royal Society of Tropical Medicine and Hygiene* **103**, 967-972 (2009).
- Becker, S.L., Sieto, B., Silue, K.D., Adjossan, L., Kone, S., Hatz, C., Kern, W.V., N'Goran, E.K., & Utzinger, J. Diagnosis, clinical features, and self-reported morbidity of *Strongyloides stercoralis* and hookworm infection in a co-endemic setting. *PLoS Neglected Tropical Diseases*. **5**, e1292 (2011).
- World Health Organization. Action against worms. In *WHO Newsletter*, Geneva, 2008, Issue 11 (2008).
- Montresor, A., Crompton, D.W.T., Hall, A., Bundy, D.A.P., & Savioli, L. Guidelines for the evaluation of soil-transmitted helminthiasis and schistosomiasis at community level. *World Health Organization*, WHO/CTD/SIP/98.1 (1998).
- Garcia, L.S. & Bruckner, D.A. *Diagnostic medical parasitology*, American Society for Microbiology, Washington D.C., (2001).
- Brooker, S. Estimating the global distribution and disease burden of intestinal nematode infection: adding up the numbers - a review. *International Journal for Parasitology*. **40**, 1137-1144 (2010).
- Stephenson, L.S., Latham, M.C., Kinoti, S.N., Kurz, K.M., & Brigham, H. Improvements in physical fitness of Kenyan schoolboys infected with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* following a single dose of albendazole. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **84**, 277-282 (1990).
- Stephenson, L.S., Latham, M.C., Adams, E.J., Kinoti, S.N., & Pertet, A. Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *Journal of Nutrition*. **123**, 1036-1046 (1993).
- Bustinduy, A.L., Thomas, C.L., Fiutem, J.J., Parraga, I.M., Mungai, P.L., Muchiri, E.M., Mutuku, F., Kitron, U., & King, C.H. Measuring fitness of Kenyan children with polyparasitic infections using the 20-meter shuttle run test as a morbidity metric. *PLoS Neglected Tropical Diseases*. **5**, e1213 (2011).
- Müller, I., Coulibaly, J.T., Fürst, T., Knopp, S., Hattendorf, J., Krauth, S.J., Stete, K., Righetti, A.A., Glinz, D., Yao, A.K., Pühse, U., N'Goran, E.K., & Utzinger, J. Effect of schistosomiasis and soil-transmitted helminth infections on physical fitness of school children in Côte d'Ivoire. *PLoS Neglected Tropical Diseases*. **5**, e1239 (2011).
- Yap, P., Du, Z.W., Chen, R., Zhang, L.P., Wu, F.W., Wang, J., Wang, X.Z., Zhou, H., & Zhou, X.N. Utzinger J, Steinmann P, Soil-transmitted helminth infections and physical fitness in school-aged Bulang children of southwest China: results from a cross-sectional survey. *Parasites and Vectors*. **5**, 50 (2012).
- Léger, L.A., Mercier, D., Gadoury, C., & Lambert, J. The multistage 20 metre shuttle run test for aerobic fitness. *Journal of Sports Sciences* **6**, 93-101 (1988).
- Tomkinson, G.R., Léger, L.A., Olds, T.S., & Cazorla, G. Secular trends in the performance of children and adolescents (1980-2000): an analysis of 55 studies of the 20 m shuttle run test in 11 countries. *Sports Medicine*. **33**, 285-300 (2003).
- POLAR Electro, User Manual for POLAR FT1/FT2 in English, Available at http://www.polar.fi/en/support/manuals/User_Manual_for_Polar_FT1_FT2_in_English?product_id=26180&category=manuals, Accessed: 3rd Jan, (2011).
- Mahon, A.D., Marjerrison, A.D., Lee, J.D., Woodruff, M.E., & Hanna, L.E. Evaluating the prediction of maximal heart rate in children and adolescents. *Research Quarterly for Exercise and Sport*. **81**, 466-471 (2010).
- Bogitsh, B.J., Carter, C.E., & Oeltmann, T.N. *Human Parasitology - third edition*, Elsevier Academic Press, (2011).
- Steinmann, P., Zhou, X.N., Du, Z.W., Jiang, J.Y., Wang, L.B., Wang, X.Z., Li, L.H., Marti, H., & Utzinger J. Occurrence of *Strongyloides stercoralis* in Yunnan province, China, and comparison of diagnostic methods. *PLoS Neglected Tropical Diseases* **1**, e75 (2007).
- Martin, L.K. & Beaver, P.C. Evaluation of Kato thick-smear technique for quantitative diagnosis of helminth infections. *American Journal of Tropical Medicine and Hygiene*. **17**, 382-391 (1968).
- Dacombe, R.J., Crampin, A.C., Floyd, S., Randall, A., Ndhlovu, R., Bickle, Q., & Fine, P.E. Time delays between patient and laboratory selectively affect accuracy of helminth diagnosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **101**, 140-145 (2007).
- Knopp, S., Mgeni, A.F., Khamis, I.S., Steinmann, P., Stothard, J.R., Rollinson, D., Marti H, & Utzinger, J. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Neglected Tropical Diseases*. **2**, e331 (2008).