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# *Schistosoma mansoni* antigen detects *Schistosoma mekongi* infection

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ABSTRACT

Northern Cambodia and Southern Laos are highly endemic for *Schistosoma mekongi*. However, there is currently no immunological assay available that is specific for this form of schistosomiasis. We have validated *Schistosoma mansoni* antigens to detect *S. mekongi*-directed antibodies in human sera collected from a highly *S. mekongi* endemic region in Laos. On two consecutive days stool samples of 234 individuals were analyzed by Kato-Katz for presence of *S. mekongi* eggs and the results were correlated with serology. A sensitivity of 94.5% was calculated for a combination of ELISA and indirect fluorescence assay (IFA) as compared to the detection of *S. mekongi* eggs in stool samples as gold standard. The results demonstrate that *S. mansoni* antigens can be used for the diagnosis of *S. mekongi* infections.



## 1. Introduction

Schistosomiasis is a widely distributed trematode infection in tropical regions with an estimated >200 million people infected. *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma intercalatum* occur in Africa and South America, whereas *Schistosoma japonicum* and *Schistosoma mekongi* are the causative agents of intestinal schistosomiasis in Asia.

*S. mekongi* is endemic in two provinces in northern Cambodian (Kratié and Stung Treng) and in the most southern Champasack Province in Lao People's Democratic Republic (Lao PDR, Laos) (Muth et al., 2010). 150,000 people are estimated at risk of infection (Urbani et al., 2002). Foci of intense transmission are communities of the Mekong island areas of Southern Laos (Sayasone et al., 2011, 2012).

In the recent decade, the Mekong river area has emerged as an attractive destination for travellers. Several case reports of Mekong schistosomiasis in travellers have been published in the last decade, some of them documenting severe sequelae (Clerinx et al., 2013; Houston et al., 2004; Leshem et al., 2009).

In the *S. mekongi* endemic area diagnosis is mainly based on stool examination using standard parasitological techniques like Kato-Katz (Ohmae et al., 2004). This has a high specificity and only detects active infections, but the sensitivity is low and light infections are frequently missed (Utzinger et al., 2011). Examination of multiple stool samples with at least duplicate Kato-Katz thick smears per sample is recommended (Lovis et al., 2012), but cannot fully compensate for the low detection limit of the method. Immunological assays promise a higher sensitivity by detecting antibodies or antigens. For detection of *Schistosoma*-specific antibodies ELISAs employing soluble egg antigens (SEA) of *S. mansoni* (van Gool et al., 2002) or *S. japonicum* (Zhu, 2005), as well as cercarial antigen preparations (cercarial transformation fluid, SmCFT) are being used (Smith et al., 2012).

To date, no specific serological diagnosis is available for *S. mekongi*. Development of a specific assay would imply production of *S. mekongi* antigen, which is hampered by the challenging rearing of *Neotricula aperta*, the intermediate snail host of the parasite.

The use of cross-reacting antigens from other *Schistosoma* species would be an alternative. ELISAs using *S. japonicum* SEA for detection of *S. mekongi* antibodies were successfully used in control programmes in the Mekong River basin (Ohmae et al., 2004) and the dipstick dye immune assay (DDIA) with *S. japonicum* SEA showed a sensitivity of more than 97% for detection of *S. mekongi* infections (Zhu et al., 2005). A serodiagnostic assay using adult worm antigen

*Abbreviations:* AWE, adult worm antigen extract from *S. mansoni*; EPG, eggs per gram Kato-Katz stool examination; SEA, soluble egg raw antigen from *S. mansoni*; STH, soil-transmitted helminth infection; IFA, indirect fluorescence assay; DDIA, dipstick dye immune assay.

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extract from *S. mansoni*; (AWE) and SEA of *S. mansoni* for detection of *Schistosoma*-specific antibodies is routinely performed at the diagnostic centre of the Swiss Tropical and Public Health Institute (Swiss TPH) with a sensitivity for *S. mansoni* of 98% and 80% for AWE and SEA, respectively, and a specificity of 96% and 92% (unpublished data). Positive or equivocal results in one or both tests are always further examined with a confirmatory *Schistosoma* indirect fluorescence assay (IFA). Both antigens show cross-reactivity with antibodies elicited by *S. haematobium* or *S. japonicum* infections.

In our study we tested the *S. mansoni* antigen preparations for the detection of *S. mekongi*-specific antibodies. We validated the *S. mansoni* antigen-based serology for the diagnosis of *S. mekongi* infections in a cross-sectional study in Southern Laos using the results of Kato-Katz stool examinations as gold standard.

## 2. Method and population

### 2.1. Ethics statement

This study was integrated in a larger study on multi-parasitic infections and their control approved by the Lao National Ethics Committee for Health Research (NECHR), Ministry of Health, Lao PDR. Study participants were informed about the study procedures, the benefits and risks as well as their voluntary participation. Before enrolment written informed consent was obtained from each study participant and/or parents or legal guardians of children below the age of 15 years. In addition a written assent was obtained from children and adolescent below age of 18 years. Participants were informed about the examinations. All infections diagnosed were treated according to the Lao National treatment guidelines (MOH, 2004), *i.e. S. mekongi* infection was treated with praziquantel (40 mg/kg BW, single oral dose).

### 2.2. Study design and area

Between October 2011 and August 2012 a cross-sectional study was conducted in four villages of two Mekong islands, Don Khon (Ban Khon and Ban Hang Khon village) and Don Som (Som Ven Ook and Ban Yai Veun Som village) in Southern Khong district, Champasack Province, Laos. Twenty-five households were randomly selected from each village. All household members older than 2 years were eligible to participate in the study.

For the current study, samples of 234 individuals of the cross-sectional study were selected for detection of *S. mekongi* antibodies using *S. mansoni* antigens.

The Khong district is highly endemic for helminth infections. Recent studies have shown that in certain villages *S. mekongi* infection may reach 50% or more (Sayasone et al., 2011, 2012). *Opisthorchis viverrini* and minute intestinal flukes (MIF) such as *Haplorchis taichui* are most frequent (Chai et al., 2013). Soil-transmitted helminth (STH) infection such as hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* may reach high infection rates (Eom et al., 2014). Recently it was demonstrated that *Strongyloides stercoralis* infection prevalence may reach 40% and higher (Vonghachack et al., 2014). *Taenia* spp. (Jeon et al., 2013) and *Gnathostoma* spp. (Vonghachack et al., 2010) infections were also reported. The district is endemic for *Plasmodium falciparum* malaria.

### 2.3. Field procedures and stool examinations

From each study participant two stool samples were collected and examined using Kato-Katz thick smears technique (Katz et al., 1972). Pre-labelled plastic stool containers were handed out to each participant. The following morning, filled containers were collected and replaced by empty ones for collection on the following day. In the Health Centre of Done Khon, two Kato-Katz thick smears were carried out for each stool sample by experienced technicians. Thirty minutes clearance time was given between smear preparation and reading of the slides. Eggs were counted and noted for each parasite species separately.

### 2.4. Serum collection and examination

From each study participant a 5 ml blood sample was drawn. At the health centre of Don Khon the coagulated blood was centrifuged and separated serum was frozen in a −20 °C freezer. Aliquots of the serum samples were sent frozen to the Diagnostic Centre of the Swiss TPH in Basel, Switzerland, where they were stored at −80 °C until assayed. Samples were defrosted in maximum twice for performing the assay.

### 2.5. Preparation of antigen

Adult worm antigen extract (AWE): Adult *S. mansoni* worms were homogenized in PBS (pH 7.2), 2 mM PMSF for 1 h at 4 °C and the extract was centrifuged at 80,000 × *g* for 3 h at 4 °C. The pellet was re-suspended and homogenized in PBS (pH7.2) containing 1% NP-40, 2.5 mM EDTA, 0.2 mM TPCK, 0.2 mM TLCK, 1 mM o-phenantroline, 2 mM PMSF, 0.05 mg/ml SAM chloride dihydrochloride, 0.05 mg/ml leupeptin and 0.05 mg/ml chymostatin. After overnight incubation on a stirrer at 4 °C the suspension was centrifuged at 80,000 × *g* for 3 h at 4 °C and the resulting supernatant was further concentrated in an Amicon stirred cell (model 402, Millipore corporation) using an Ultracel disc membrane (YM-30). Concentrated antigen was centrifuged at 15,300 × *g* for 5 min at 4 °C, and the supernatant was stored in aliquots at −80 °C until use.

Soluble egg antigen (SEA): Frozen *S. mansoni* eggs were homogenized in PBS (pH 7.2) on ice, subsequently extracted for 3 h on a stirrer at 4 °C and the extract then centrifuged at 100,000 × *g* for 2 h at 4 °C. Supernatant was stored in aliquots at −80 °C until use.

### 2.6. Screening ELISA

For serodiagnosis two in-house ELISAs were carried out, one using *S. mansoni* AWE and the other one using SEA as described (Junghanss and Weiss, 1992).

In brief, antigens were coated in 0.05 M sodium carbonate buffer, pH 9.6, to Immulon 2HB plates (Thermo Labsystems, 735-0462). After washing, diluted sera were added to the plates and incubated for 15 min at 37 °C. After additional washing steps, horseradish peroxidase conjugated goat-anti-human-IgG (KPL, 474-1006) was added. Plates were incubated for 15 min at 37 °C, subsequently washed and o-Phenylendiamine Dihydrochloride (OPD, Sigma) was added. Reaction was stopped with 8 M $H_2SO_4$ and absorption was read with a Multiscan FC reader (Thermo Scientific) at 492 nm. All sera giving positive or equivocal results were additionally tested with an in-house confirmatory *Schistosoma* IFA.

### 2.7. Schistosoma IFA

Adult male *Schistosoma* worms were washed in 0.9% NaCl, packed closely and shock-frozen in petroleum ether with dry ice. Thin sections were cut with a cryomicrotome and mounted on glass microscope slides. Slides were stored at −80 °C until further use. For IFA analysis slides were quickly air-dried and fixed with acetone. Diluted sera were then applied to the slide-slots. Three control sera were present on each slide, one positive, one equivocal and one negative control. After 25 min of incubation at 37 °C, slides were washed with PBS and air-dried. FITC conjugated F(ab)'2 anti-IgG/A/M (BioRad, #30244) diluted in 0.01% Evansblue in PBS was added, slides were incubated for 25 min at 37 °C, washed, dried and a cover glass was mounted with buffered glycerol. Slides were