# Serodiagnosis of *Schistosoma japonicum* infection: genome-wide identification of a protein marker, and assessment of its diagnostic validity in a field study in China

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## Summary

**Background** Schistosomiasis remains a highly prevalent and serious parasitic disease. A major factor preventing its effective management is the scarcity of effective diagnostic tools. We did a genome-wide identification of diagnostic protein markers for schistosome infection and assessed their diagnostic validity in a field study.

**Methods** We predicted putative secreted proteins of *Schistosoma japonicum* (SjSPs) and expressed them as glutathione S-transferase (GST)-fusion proteins. The fusion proteins were arrayed on glutathione (GSH)-immobilised microplates and screened with serum samples from patients with schistosomiasis diagnosed by the Kato-Katz method. We further assessed an identified protein marker for sensitivity and specificity, first in infected serum samples collected from Jiangxi and Hunan Provinces, China, and then through a field study, done in two villages located in a high schistosomiasis-endemic area of the southeast of China.

**Findings** Of 204 recombinant proteins, 35 yielded seropositive reactions, eight showed strong immunoreactivity, and only one (SjSP-13) reacted to the entire panel of 14 archived samples. The reactivity of SjSP-13 to 476 serum samples showed 90·4% (95% CI 86·5–93·5) sensitivity and 98·9% (95% CI 95·9–99·9) specificity. Of 1371 residents enrolled in a field study from Dec 6, 2010, to June 23, 2011, only 74 individuals were identified as being egg-positive, whereas 465 were diagnosed as positive by the SjSP-13-based ELISA kit (rSP13-ELISA). Of the 394 individuals found egg-negative but rSP13-ELISA-positive, 363 (92·4%) were confirmed to be positive for schistosome infection by PCR detection of *S japonicum* SjR2 retrotransposon.

**Interpretation** The application of this sensitive, specific, and affordable rSP13-ELISA method should help reduce schistosomiasis transmission through targeted treatment of individuals, particularly with low intensity infections, and therefore support schistosomiasis control and elimination strategies.

**Funding** National 973 project in China.

*Lancet Infect Dis* 2014; 14: 489–97

Published Online March 19, 2014 http://dx.doi.org/10.1016/S1473-3099(14)70067-2

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## Introduction

Schistosomiasis is one of the oldest parasitic infestations of man, yet it remains a serious public health concern throughout the world's tropical and subtropical regions, affecting more than 200 million people.[1,2] One factor contributing to high disease prevalence is the scarcity of effective diagnostic methods to detect infections and response to drug treatment and to assess the success of control measures.[3] The traditional gold standard for the diagnosis of schistosomiasis includes parasitological detection, commonly done via the use of Kato-Katz thick smears.[4] The sensitivity of this diagnostic technique depends on the rate of egg excretion,[5] which can be very low or even absent when parasite transmission is low, or when intestinal fibrosis has occurred. Hence, a major disadvantage of parasitological detection as a diagnostic technique is that it exhibits low sensitivity in areas of low endemicity.[6,7] Additionally, since parasitological detection is labour-intensive and time-consuming, this approach is not suitable for large-scale disease surveillance. However, all current schistosomiasis control measures, including targeted treatment of all infected individuals, especially those with low-intensity infections, and large-scale surveillance of disease transmission are strongly dependent on sensitive and accurate diagnostic methods. Therefore, such methods are urgently needed for case detection and surveillance of the disease, which are crucial for the disease control programme.

Immunodiagnostic detection of schistosome antigens or their antibodies is more sensitive and less time-consuming than parasitological diagnosis and has thus become a more attractive option for the diagnosis of schistosomiasis.[8,9] These alternative diagnostic techniques have proven particularly useful for diagnosing atypical forms of schistosomiasis (eg, infections with low parasite burdens) and in disease surveillance.[10,11] However, the immunodiagnostic antibody detection assays that are currently available have low specificity and poor reproducibility because they use whole crude extracts (eg, soluble egg antigens [SEA] or soluble worm antigens [SWA]) consisting of thousands of schistosome proteins, which enable cross-reactivity with the antibodies to other parasitic flukes or soil-transmitted helminths, thereby substantially lowering the test specificity.[12–14] Additionally, as with parasitological diagnosis, the antibody-detection approach based on the use of SEA or SWA cannot

distinguish between current and past infection, or detect the response to drug treatment because the antibodies to the crude extract antigens declined very slowly.[9,15]

The ideal immunodiagnostic assay for schistosome infection should use one protein or a combination of very few proteins as antigens. Therefore, the identification of specific antigens that could be targeted (and thus recognised by host antibodies) in an SEA-ELISA-based approach is essential. The recent availability of the genome sequences of *Schistosoma japonicum* along with the corresponding proteomic and transcriptomic datasets have enabled a comprehensive and unbiased analysis of the antigenicity and immunogenicity of *S japonicum* proteins at the whole-genome scale.[16,17] Since the parasite undergoes development but not multiplication in the definite host, we expected that the most immunologically important proteins could be secreted or transmembrane proteins. Therefore, in the current study, we developed a glutathione *S*-transferase (GST) fusion protein array assay, in which each protein is arrayed in solution and at similar amounts to analyse host antibody responses to pathogen infection. The putative secreted proteins of *S japonicum* (SjSPs) were produced at the whole-genome scale using the GST fusion protein system.

See Online for appendix

## Methods

### Cloning, expression, and refolding of GST-SjSP fusion proteins

We analysed all putative open reading frames (ORFs) of *S japonicum* to identify genes potentially encoding signal peptides by using SignalP software (Center for Biological Sequence Analysis at the Technical University of Denmark, Denmark).[18] We amplified the selected ORFs by PCR from a mixture of cDNAs of schistosomula, adult worms, and eggs. The amplified DNA fragments were cloned into the pGEX vector for expression in *Escherichia coli* Rosetta (DE3; Novagen, Germany) by induction with isopropyl-β-D-thiogalactoside (IPTG; 1 mmol/L). We analysed the recombinant GST-SjSP proteins in supernatant or precipitate by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot.

We used the iFOLD Protein Refolding System 1 (Novagen, Germany), which contains a 96-well plate-based protein refolding buffer matrix, to identify an optimum refolding condition for GST-SjSP proteins. We estimated the efficacy of different refolding buffers by analysing the interaction of GST fusion proteins and immobilised glutathione. Briefly, we added the refolded poteins to Immobilizer Glutathione MicroWell plates (Nunc, Denmark) and detected the amount of bound GST fusion proteins by chemiluminescence with SuperSignal ELISA Femto Maximum Sensitivity Substrate (Pierce, USA), and measured the relative light units (RLUs) by luminometer.

### Preparation of GST-SjSP microplate array

We measured the concentration of the bound GST-SjSP proteins in every well using sandwich ELISA with anti-GST-tag mouse monoclonal antibodies and biotinylated anti-GST-tag rabbit polyclonal antibodies (Genscript, China), and by calculating from the standard curve prepared previously using the serial dilutions of the GST protein. We used the recombinant GST protein to test the saturation binding of the protein to glutathione (GSH)-immobilised plates. The recombinant GST protein was produced in *E coli* cultures using pGEX-4T-1 vector and purified by affinity chromatography using glutathione sepharose 4B (GE Healthcare, USA). The saturation assay of GST binding to the plate was done with serial dilutions of GST protein at 0·016 μg/mL to 5 μg/mL. Further, the concentration of GST-SjSP proteins were adjusted to the saturating concentration with phosphate buffered saline before being arrayed onto GSH-immobilised microplates. The bound GST-SjSP proteins in the immobiliser glutathione plate was detected by ELISA with anti-GST antibody.

### Screening of the GST-SjSP proteins using serum samples

To remove antibodies for *E coli* antigens and GST protein before screening, the serum samples of patients with schistosomiasis were incubated with *E coli* extracts and the GST protein. The appendix shows details of methods used for the screening. For the microplate array assay, we incubated diluted serum samples in every well of the microplate containing the bound fusion proteins (appendix). We quantified the bound antibodies by measuring the RLUs, and for each of the GST-SjSP proteins identified, we fused a His-tag to the C-terminus by PCR. We produced these proteins at a large scale via purification by affinity chromatography on nickel-nitrilotriacetic acid column. We compared the diagnostic efficacy of these proteins by chemiluminescent ELISA (appendix).

We collected 302 infected serum samples that were diagnosed by the Kato-Katz method as egg-positive from villagers living in two different areas endemic for schistosomiasis in China—ie, Donting Lake in Hunan Province (144 samples) and Poyang Lake in Jiangxi Province (158). Of them, 184 samples were analysed for eggs per g of faeces (EPG) and used for evaluation of sensitivities among different infection intensities. 14 infected serum samples with various infection intensities were selected for screening of the GST-SjSP proteins. We also collected 174 uninfected serum samples from people living in the Pudong New District in Shanghai, where schistomiasis is not endemic. Of these, we randomly selected ten samples for screening of the GST-SjSP proteins as negative control. To evaluate the cross reactivity of the diagnostic protein marker to other fluke infection, we collected 51 serum samples diagnosed by parasitological detection (fecal smears) as clonorchis infection from the Guangdong Province, where the disease is highly prevalent.

We further selected the antigen SjSP-13 that showed the best diagnostic efficacy and expressed it using a