Immunolocalization of the 29 kDa Schistosoma haematobium species-specific antigen: a potential diagnostic marker for urinary schistosomiasis

Uri S Markakpo, George E Armah, Julius N Fobil, Richard H Asmah, Isaac Anim-Baidoo, Alfred K Dodoo, Parnor Madjitey, Edward E Essuman, Somei Kojima and Kwabena M Bosompem

Abstract

Background: The 29 kDa Schistosoma haematobium species-specific antigen (ShSSA) is of remarkable interest in the diagnosis of urinary schistosomiasis although it had not been fully characterized.

Method: To determine the biological importance of ShSSA in S. haematobium and pathogenesis of the disease, we immunolocalized ShSSA in schistosome eggshells, miracidia and adult worm sections using indirect fluorescent antibody test (IFAT).

Results: ShSSA was strongly immunolocalized in the schistosome eggshells, selective regions of the miracidia body and walls of internal organs such as oviduct, ovary, vitelline duct and gut of the adult worm.

Conclusion: The strong immunolocalization of ShSSA in schistosome eggshells and adult worm internal organs suggests that the antigens involved in the pathogenesis of urinary schistosomiasis could have originated from the eggs and adult worms of the parasite. The findings also indicate that ShSSA may play a mechanical protective role in the survival of the parasite.

Keywords: Schistosomiasis, S. haematobium, Antigen, MAb, Immunolocalization, Fluorescence
Immunolocalization to characterize this antigen at the morphological and ultrastructural levels in *S. haematobium* will provide answers to critical questions about the use of the antigen in estimating infection intensity. Furthermore, immunolocalization of the antigen will provide data on its role in the survival of the parasite and significance in its taxonomy [10]. A major objective of this study, therefore, was to immunolocalize ShSSA in all life-cycle stages of *S. haematobium*. In addition, in order to ascertain the suitability of infected urine samples and parasite eggs for immunolocalization studies on ShSSA, this study was conducted with a view to determine the reactivity of MAb to crude antigens from eggs and urine of study subjects. Finally, to identify urine samples that can provide adequate amount of parasite eggs for generation of *S. haematobium* life-cycle stages and crude antigen extracts, this study was conducted to determine the sensitivity and specificity of microscopy or MAb dipstick test at detecting parasite eggs or antigens from the urine of study subjects.

**Methods**

**Study design and population**

The study was a purposive cross sectional study involving elementary school pupils who answered yes to whether or not they have any of the signs and symptoms of urinary schistosomiasis.

The *S. haematobium* species-specific MAb required for detection of the 29 kDa antigen was purified and the reactivity confirmed. Active MAb fractions were utilized for the urinary schistosomiasis MAb dipstick assay (USDA), microplate enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFAT). Urine samples for the study were collected from a total of 292 elementary school pupils from two villages, Kwashikumahman (*n* = 190) and Kojo Ashong (*n* = 102), hyperendemic for urinary schistosomiasis [11]. Aliquots of urine samples from subjects showing urinary schistosomiasis symptoms, haematuria and dysuria, were tested for *S. haematobium* antigens and eggs using USDA and microscopy respectively. Schistosome eggs were isolated from urine samples with >100 eggs/10 ml of urine for soluble egg antigen preparation, generation of parasite stages and for immunolocalization.

**Study area**

The study was conducted at Kojo Ashong and Kwashikumahman in the Greater Accra Region of Ghana. These villages are located on 5°43’N, 0°23.5’E and 5°43’N, 0°21.5’E, respectively. The vegetation along the banks of the slow flowing Densu River and Dobro stream, running at the outskirts of the villages, comprises mainly grassland and a few trees. The weedy river and stream banks contain decomposing plant leaves and twigs infested with urinary schistosomiasis vector snails, *Bulinus globosus*. The Densu River and the Dobro stream constitute the principal sources of water for domestic use and transmission of urinary schistosomiasis.

**Ethical consideration**

Study subjects were elementary school pupils assigned by the Ghana Health Service, Ministry of Education and Noguchi Memorial Institute for Medical Research for schistosomiasis examination and treatment. Subjects were recruited if they discharged urine with blood and/or pain. Informed consent was obtained from Education Authorities and parents and/or guardians of pupils before recruitment. Infected children were treated with 40mg/kg body weight of praziquantel (Merck KGaA, Darmstadt, Germany) as recommended by WHO [12]. Approval for this study was given by the Noguchi Memorial Institute for Medical Research Institutional Review Board, referenced NMIRM-IRB CPN 042/06-07 rev 2008.

**Collection and analysis of urine specimens**

**Field procedures**

**Collection and handling of urine samples** Twenty to 100ml of fresh clean catch urine was collected from each of 292 school pupils in the period between 11:00 and 14.00hours Greenwich Mean Time (GMT) into a 200ml urine container. The samples were then transported to the laboratory at the Noguchi Memorial Institute for Medical Research within 1hour on ice in ice-chest.

**Laboratory procedures**

**Examination of urine samples for parasite antigens and eggs** Within 3hours after arrival at the laboratory urine samples were tested individually for *S. haematobium* antigen by MAb dipstick as described elsewhere [8,9,11]. Also, 10ml of the urine was filtered through a 25mm Nucleopore filter (12μm pore size) [11] to determine parasite density. The rest of the urine was centrifuged at 1,290 X g to isolate *S. haematobium* eggs.

**Generation of *S. haematobium* parasite life-cycle stages**

*S. haematobium* eggs were isolated by centrifugation and sedimentation as described by Bosompem and others [13] and then cleaned by density centrifugation through ficoll-paque™ (GE Healthcare Life Sciences, Buckinghamshire, UK). They were subsequently hatched into miracidia by exposure to clean aged tap water and light as described by Huyse and others [14]. Some of the miracidia were used to infect *Bulinus* snails (five miracidia/snail) to generate cercariae as described elsewhere [15,16]. Some of the live cercariae were transformed into schostosomula by vortexing (Ikemoto Rikakogyo Co. Ltd., Japan) as described by Ramalho-Pinto and others [17] for 20min. Some cercariae were also used to infect
BALB/c mice to generate adult worms [6,15]. Fractions of the eggs, miracidia, cercariae, schistosomula and adult worms were respectively homogenized by sonication [15] to prepare crude antigens or treated with fixatives for immunolocalization studies.

*S. haematobium* parasite stages and fixatives for immunolocalization

*S. haematobium* parasite stages were fixed for immunolocalization according to the method described elsewhere [18] with modification.

Washed *S. haematobium* eggshells, miracidia, cercariae, schistosomula and adult worms were suspended in PBS containing different concentrations of fixatives namely, paraformaldehyde, glutaraldehyde, Karnovsky’s fixative, methanol, ethanol and acetone, and incubated at 4°C for 5 min, 30 min, 60 min, 90 min and overnight (12 hrs). The concentrations of the various fixatives tested were 0.1, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 10 and 100%. Fixed specimens were washed four times with PBS by centrifugation at 16,000 xg for 10 min at 4°C, stored in PBS at 4°C and analyzed by a preliminary IFAT to determine and select the best fixatives and conditions for immunolocalization studies.

Preparation of *S. haematobium* adult worms for (IFAT)

Adult worms were prepared for IFAT as described elsewhere [19-21] with modification. Fixed *S. haematobium* adult worms were incubated for 5 mins in saturated picric acid to colour the specimen and enhance visualization. The worms were dehydrated stepwise by transferring them into ethanol (85% for 10 min, 95, 100 and 100%, each for 20 min) and then 100% chloroform (3 times, each for 10 min). The worms were transferred into molten paraffin wax at 56°C (2 times for 10 min each) to fill open cavities with wax, embedded in wax and then cut into 4 μm sections with a microtome (Yamato Koki Individuals. Co., Ltd., Tokyo, Japan.). Worm sections on glass microscope slides were deparaffinized by heating at 50°C for 2 hr and then washed twice in xylene (5 min/wash). The worms were then re-hydrated stepwise in 100, 100, 95 and 90% ethanol (5 min/step). Finally, the worm sections were rinsed, 5 min each, under running distilled water and in PBS.

Monoclonal antibody (MAb)

The IgG1 MAb used in this study was generated by immunizing BALB/c mice with antigens extracted from *S. haematobium* infected human urine [6,7]. This antibody did not cross-react with *Necator americanus* (hookworm) egg antigens in micro-plate ELISA, and could bind ShSSA, from the eggs of both Ghanaian and Egyptian strains of *S. haematobium* and infected human urine [6-8]. The antibody in culture supernatant was concentrated ten-fold by Amicon filtration or by precipitation with 50% (v/v) ammonium sulphate [(NH$_4$)$_2$SO$_4$] and then purified by ion-exchange chromatography. Microplate ELISA was used to determine the reactivity of this MAb to ShSSA in infected human urine and homogenates of parasite stages as described earlier [6].

### Table 1 Reactivity of purified MAb.F fractions with crude antigens from *S. haematobium* parasite stages and infected human urine

| Antibody fraction            | Crude antigens | Egg lysate | P$_2$J$^*$ | Miracidium | Cercaria | Schistosomulum | Adult worm |
|------------------------------|----------------|------------|------------|------------|----------|----------------|------------|
| Amicon concentrated          | 2+             | 3+         | >+         | +          | <+       | >+             | >+         |
| Ion-exchange purified         | 2+             | 2+         | +          | >+         | <+       | +              | +          |
| Precipitated by 50% (NH$_4$)$_2$SO$_4$ solution | 3+             | 2+         | >+         | +          | <+       | >+             | >+         |
| Immuneonized mouse serum$^a$  | 3+             | 3+         | >+         | +          | <+       | >+             | >+         |
| Normal mouse serum$^b$        | -              | -          | -          | -          | -        | -              | -          |
| Antibody-free culture medium$^c$ | -              | -          | -          | -          | -        | -              | -          |

$^a$Crude antigens extracted from *S. haematobium* infected human urine.

$^b$Negative control sample.

$^c$Background blank sample.

+ Positive reaction.

- Negative reaction.

> + Stronger than positive, but weaker than 2+.

↔ Weak reaction or trace.
Table 2 Prevalence of urinary schistosomiasis in the study communities as determined by microscopy and dipstick assay

| Community | Number tested | Microscopy+ve (%) | Dipstick+ve (%) | *Sensitivity(%) | *Specificity(%) |
|-----------|---------------|-------------------|----------------|----------------|----------------|
| K A       | 102           | 30 (29.41)        | 42 (41.18)     | 100            | 75.00          |
| K M       | 190           | 98 (51.58)        | 121 (63.68)    | 100            | 83.33          |
| Total     | 292           | 128 (43.84)       | 163 (55.82)    | 100            | 78.66          |

K A Kojo Ashong.
K M Kwashikumahman.
*Relative sensitivity and specificity based on microscopy as gold standard test.

Table 3a & b: evaluation of different fixatives for processing *S. haematobium* (Sh) parasite stages for immunolocalization (IL)

Table 3a

| Type of fixative       | Duration of treatment of Sh parasite stages | Concentration of fixative | 0.10 | 0.25 | 0.50 | 1.00 | 1.50 | 2.00 |
|------------------------|-------------------------------------------|---------------------------|------|------|------|------|------|------|
| Karnovsky’s fixative   | 90 min                                    |                           |      |      |      |      |      |      |
|                        |                                           |                           | + (+) |      |      |      |      |      |
|                        | 12 hr                                     |                           | + (+) |      |      |      |      |      |
| Paraform-aldehyde      | 90 min                                    |                           |      |      |      |      |      |      |
|                        | 12 hr                                     |                           |      |      |      |      |      |      |
| Glutar-aldehyde        | 90 min                                    |                           |      |      |      |      |      |      |
|                        | 12 hr                                     |                           |      |      |      |      |      |      |
| Methanol               | 90 min                                    |                           |      |      |      |      |      |      |
|                        | 12 hr                                     |                           | + (±) | ± (±) |      |      |      |      |
| Ethanol                | 90 min                                    |                           | ± (±) | + (±) |      |      |      |      |
|                        | 12 hr                                     |                           | + (±) | ± (±) |      |      |      |      |
| Acetone                | 5 min                                     |                           |      |      |      |      |      |      |
|                        | 90 min                                    |                           |      |      |      |      |      |      |
|                        | 12 hr                                     |                           |      |      |      |      |      |      |

Table 3b

| Type of fixative       | Duration of treatment of Sh parasite stages | Concentration of fixative | 2.50 | 3.00 | 4.00 | 5.00 | 10.00 | 100.00 |
|------------------------|-------------------------------------------|---------------------------|------|------|------|------|-------|--------|
| Karnovsky’s fixative   | 90 min                                    |                           |      |      |      |      |       |        |
|                        |                                           |                           | + (+) |      |      |      |       |        |
|                        | 12 hr                                     |                           | + (+) |      |      |      |       |        |
| Paraform-aldehyde      | 90 min                                    |                           | ± (–) | ± (–) |      |      |       |        |
|                        | 12 hr                                     |                           | + (–) | + (–) |      |      |       |        |
| Glutar-aldehyde        | 90 min                                    |                           |      |      |      |      |       |        |
|                        | 12 hr                                     |                           |      |      |      |      |       |        |
| Methanol               | 90 min                                    |                           | ± (±) | ± (±) |      |      |       |        |
|                        | 12 hr                                     |                           | ± (±) | ± (±) |      |      |       |        |
| Ethanol                | 90 min                                    |                           |      |      |      |      |       |        |
|                        | 12 hr                                     |                           |      |      |      |      |       |        |
| Acetone                | 5 min                                     |                           |      |      |      |      |       |        |
|                        | 90 min                                    |                           |      |      |      |      |       |        |
|                        | 12 hr                                     |                           |      |      |      |      |       |        |

Results in parenthesis, ( ), indicate the intensity of background staining.
+ Indicates weak fluorescence.
Blank spaces on the table represent negative fluorescence and background staining results. Except for 100% acetone which gave positive fluorescence at 5 min of fixation, all the reagents at all the concentrations gave negative fluorescence and background staining at 5, 30 and 60 min of fixation, and were not represented on the table.
the wells were incubated with Sh2/15.F, positive control (immune) sera or negative control (free medium) at 20 μl/well for 1 hr at room temperature. Primary antibodies, Sh2/15.F and immune sera were used at 1:40 and 1:50 dilution, respectively. The slides were washed twice (5mins per wash) with PBS and then incubated for 30 min (20 μl/well) with secondary antibody reagent [goat-anti-mouse IgG conjugated with fluorescein isothiocyanate (FITC) (Sigma Immuno Chemicals, St. Louis, MO) and 0.01% trypan blue (counter stain), all in PBS]. After incubation the slides were washed four times in PBS, blotted and then mounted in 50% glycerol (Sigma-Aldrich Co. Ltd.-Gillingham-Dorset, UK) in PBS. The specimens were observed using a fluorescent microscope (Olympus Optical Co. Ltd., Japan) at x120 magnification.

Results
Reactivity of purified MAb fractions
Table 1 summarizes the reactivity of different fractions of MAb to crude antigens from S. haematobium parasite stages and infected human urine as determined by micro-plate ELISA. The results show that MAb was reactive to crude antigens from all life-cycle stages of the parasite and infected human urine.

Prevalence of Urinary Schistosomiasis
Table 2 summarizes urinary schistosomiasis prevalence both at Kojo Ashong and Kwashikumaman as determined by microscopy and MAb-dipstick, USDA. Of the 292 individuals interviewed verbally for the presence or absence of urinary schistosomiasis, 58.56% (171/292) answered yes. Also, USDA detected S. haematobium antigens in more (55.82%) of the 292 subjects than microscopy (p < 0.01). The analysis showed that the urinary schistosomiasis prevalence estimated by microscopy in Kwashikumaman (51.58%) (98/190) was higher (p < 0.01) than that of Kojo Ashong (29.41%) (30/102). A similar trend was found using the USDA (p < 0.01). All individuals who were S. haematobium egg positive also tested positive for parasite antigens by the USDA.

Evaluation and selection of fixatives for immunolocalization
Table 3 summarizes the results of the experiment to determine the suitability of six fixatives (paraformaldehyde, glutaraldehyde, Karnovsky's fixative, acetone, methanol and ethanol) for processing S. haematobium parasite stages for immunolocalization. As shown, 2.0% Karnovsky's fixative (2% paraformaldehyde plus 2% glutaraldehyde) applied for 90 min at 4°C, produced the highest positive fluorescence with a weak background staining as compared to the other fixatives. Consequently, this fixative at the application conditions was used to process the specimens for immunolocalization of the diagnostic antigen.

Immunolocalization of the 29 kDa S. haematobium antigen using IFAT
The 29 kDa S. haematobium diagnostic antigen, ShSSA, was immunolocalized to the scleroproteic eggshell (Figure 1). However, the inner wall of the eggshell (vitelline membrane) showed more intense fluorescence in some specimens. As shown in Figure 2, the antigen was also immunolocalized mostly to the cilia and patches on the dorsal surface of the tegument of the miracidia. In the adult worm, ShSSA was immunolocalized in the entire section. However, the antigen appeared to be concentrated in the inner lining of internal organs such as the ovary and gut (Figure 3).

Discussion
The aim of this study was to immunolocalize the 29 kDa Schistosoma haematobium species-specific antigen (ShSSA) in all the life-cycle stages of the parasite. Even though a monoclonal antibody (Sh2/15.F) against this antigen has enabled it to be identified in a field-applicable dipstick test.
as a potential diagnostic marker for the disease [8,9,11], ShSSA has not been fully studied. Immunolocalization of ShSSA at the morphological and ultrastructural levels in the parasite, therefore, is of paramount importance to provide answers to critical questions about the relative abundance of the antigen in the parasite stages and its applicability in estimating infection intensity. Also, immunolocalization studies on this antigen will provide data on the possible role of ShSSA in the parasite stages and its applicability in estimating infection intensity. Also, immunolocalization studies on this antigen will provide data on the possible role of ShSSA in the parasite stages and its applicability in estimating infection intensity. Also, immunolocalization studies on this antigen will provide data on the possible role of ShSSA in the parasite stages and its applicability in estimating infection intensity. Also, immunolocalization studies on this antigen will provide data on the possible role of ShSSA in the parasite stages and its applicability in estimating infection intensity. Also, immunolocalization studies on this antigen will provide data on the possible role of ShSSA in the parasite stages and its applicability in estimating infection intensity.

From this study, the 29 kDa antigen (ShSSA) was detected in all the life-cycle stages of *S. haematobium* and in the urine of infected individuals. To our knowledge, this is the first study to immunolocalize a species-specific potential diagnostic marker antigen in the various life-cycle stages of the *S. haematobium* parasite.

Immunolocalization of proteins provides answers to critical questions concerning the roles of specific antigens in classification and survival of parasites [10]. For example, immunolocalization studies confirmed paramyosin as a muscle protein and contributed to the understanding of its multiple functions within the schistosome [10]. In this study, the ShSSA present in both Ghanaian and Egyptian strains of *S. haematobium* [7] was immunolocalized in the eggshells, miracidia and adult worm. This observation coupled with the detection of ShSSA in homogenates of *S. haematobium* adult worm, eggs, miracidia, cercariae and schistosomula by microplate ELISA and USDA, indicated that ShSSA may be distributed in all the life-cycle stages of the parasite.

Also, the identification of ShSSA in strains of the parasite from Egypt and different regions of Ghana [7-9] suggested that this antigen has a wide geographic distribution. In addition the wide distribution of this antigen in *S. haematobium* both stage-wise and geographically *vis a vis* its absence in *S. mansoni* and *N. americanus* [7-9], emphasizes its possible specificity to *S. haematobium* and potential usefulness in taxonomic classification of the parasite and diagnosis of urinary schistosomiasis.

Labelling of ShSSA in the eggshells and miracidia confirmed the presence of the antigen in the *S. haematobium* eggs. The *S. haematobium* eggshell is scleroproteic and has sub-microscopic pores through which antigens are excreted [23-27]. It is therefore, possible that the antigen immunolocalized is a structural component of the eggshell, had diffused from the miracidia into the shell, or both.

In addition, the immunolocalization of ShSSA in the eggshell, tegument and cilia of miracidia and walls of
internal organs such as ovary, vitelline duct and gut of the adult worm, suggests that, possibly, this protein has a muscular function in the parasite. Further studies are however required to ascertain this observation. Furthermore, the localization of ShSSA in both eggs and adult worms of *S. haematobium* suggests that the antigen detected in infected persons may originate from both stages of the parasite, since these are the stages found in humans. Hence, it may be difficult but interesting to use measured antigen levels to estimate egg or worm burden.

High relative sensitivity (99.1%) and specificity (98.3%) were reported for USDA in earlier studies [11], however, in this work, the specificity (78.66%) of the MAb-dipstick compared to microscopy as a gold standard test was significantly lower (*p* < 0.01) whilst the sensitivities were the same (100%). The rather low relative specificity recorded by USDA in this study could be attributable to either a low sensitivity of the microscopic technique or high false positivity rate of the USDA. The first explanation is more likely to be correct because, Bosompem *et al.* [11] re-examined urine samples with low egg counts, which readily tested positive by USDA, seven times more before confirming their positivity by microscopy. The higher (*p* < 0.01) prevalence of urinary schistosomiasis obtained by USDA compared to microscopy was therefore not surprising. One limitation of this study is the inability to repeat testing of the samples to ascertain parasite infectivity rates.

**Conclusions**

In conclusion, this study has confirmed the suitability of the USDA for detecting *S. haematobium* infections in humans. The 29 kDa antigen was found in all the life-cycle stages of *S. haematobium* and in the urine of infected individuals. The 2% Karnovsky’s fixative was shown to be suitable for immunolocalization studies on ShSSA. The presence of ShSSA in both eggs and adult worms of *S. haematobium* suggests that the antigen detected in infected persons may originate from both stages of the parasite.

**Recommendations**

It is recommended that further immunolocalization studies be conducted on the 29 kDa *S. haematobium* species-specific antigen at the ultrastructural level by electron microscopy to elucidate the function(s) of this diagnostic antigen in the various stages of *S. haematobium*.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contribution**

USM conceived the idea, conducted most of the studies reported in this paper and drafted the manuscript; KS was a supervisor and contributor to the formulation of the study. KMB contributed to the development of concept and supervision of the research activities as well as review of the manuscript. GEA contributed to supervision of the studies and review of the manuscript. JNF, IAB, RHA, AKD, PM, and EEE assisted in the conduct of some of the experiments and proof reading of the manuscript. All the authors have read the manuscript.

**Authors’ information**

USM, MPH, PhD candidate, School of Public Health, College of Health Sciences, University of Ghana, P. O. Box LG13, Legon Accra, Ghana. Cell Phone: 233 246 373898; E-mail: umckakpo@noguchi.mimcom.org. SK, Asian Centre for International Parasite Control, Mahidol University, 120–126 Rajayothi Road, 10400 Bankok, Thailand. kojima@ims.tsukuba.ac.jp. KMB, PhD, Prof. George E. Armaah, PhD, Mr. Alfred K. Dodo, M Sc; Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, P.O. Box LG581, bloosompem@noguchi.mimcom.org, gammah@noguchi.mimcom.org, adodoo@noguchi.mimcom.org. JNF, School of Public Health, College of Health Sciences, University of Ghana, P. O. Box LG13, Legon Accra, Ghana. E-mail: jfobi@ug.edu.gh, PM, M Sc; Principal Research Assistant, School Of Public Health, College of Health Sciences, University of Ghana, P. O. Box LG13, Legon Accra, Ghana. pmardjitey@gmail.com. EEE, M Sc, Ph D candidate, School of Public Health, College of Health Sciences, University of Ghana, P. O. Box LG13, Legon Accra, Ghana. IAB, Ph D, School of Allied Health Sciences, College of Health Sciences, University of Ghana, Accra. RHA, M Phil, School of Allied Health Sciences, College of Health Sciences, University of Ghana, Accra. rhasmah@chs.edu.gh.

**Acknowledgements**

We wish to express our sincere gratitude and deep appreciation to Drs. J. K. Kpikpi, D. Wilson, I. Ayi, W. K. Aryan, S. Y. Amelor, D. Boamah, Mr. I. Odoi, Ms. C. O. Ampomah and Ms. S. Damankah for their technical support. Also, we are particularly grateful to Prof. F. K. Mrumah of the Noguchi Memorial Institute for Medical Research. The authors particularly acknowledge the immense contribution of the study participants to the success of this study. This work was part of a thesis research undertaken by the first author in partial fulfilment for the award of an M Phil degree at the University of Ghana, Legon. The research was conducted at the Noguchi Memorial Institute for Medical Research with joint financial assistance from the Government of Ghana and the Japan International Cooperation Agency (JICA).

**Author details**

1. Department of Biological, Environmental and Occupational Health Sciences, School of Public Health, College of Health Sciences, University of Ghana, P. O. Box LG13, Legon, Accra, Ghana. 2. Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, P. O. Box LG581, Legon, Accra, Ghana. 3. School of Allied Health Sciences, College of Health Sciences, University of Ghana, Accra, Ghana. 4. Asian Centre for International Parasite Control, Mahidol University, Bankok, Thailand.

**Received: 7 November 2014 Accepted: 15 April 2015**

**Published online: 26 April 2015**

**References**

1. van der Werf MJ, de Vlas SJ, Brooker S, Looman CNW, Nagelkerke N, Habbema JD, et al. Quantification of clinical morbidity associated with schistosomiasis infection in sub-Saharan Africa. Acta Trop. 2003;86:125–39.
2. Bonnard P, Remoué F, Schacht A-M, Deuffic-Burban S, Dompnier J-P, Elguero E, et al. Specific isotype immune response in the diagnosis of human schistosomiasis pathology? Am J Trop Med Hyg. 2004;71(2):202–5.
3. Bergquist NR, Colley DG. Schistosome vaccines: research to development. Parasitology Today. 1998;14(3):99–104.
4. El Dridi R, Tallima H. Vaccine-induced protection against murine schistosomiasis mansoni with larval excretory-secretory antigens and papain or type-2 cytokines. J Parasitol. 2013;99(2):194–202. doi:10.1645/GE-3186.1.
5. McManus DP, Loukas A. Current status of vaccines for schistosomiasis. Clin Microbiol Rev. 2008;21(1):225–42. doi:10.1128/CMR.00046.
6. Bosompem KM, Arishima T, Yamashita A, Ayi I, Aryan WK, Kojima S. Extraction of Schistosoma haematobium antigens from infected human urine and generation of potential diagnostic monoclonal antibodies to urinary antigens. Acta Trop. 1996;62(2):91–103.
7. Armanor JD, Bosompem KM, Arishima T, Assouk RKG, Kojima S. Characterization of monoclonal antibodies reactive with *Schistosoma*...
8. Bosompem KM, Ayi I, Anyan WK, Nkrumah FK, Kojima S. Limited field evaluation of a rapid monoclonal antibody-based dipstick assay for urinary schistosomiasis. Hybridoma. 1996;15(3):219–24.

9. Bosompem KM, Ayi I, Anyan WK, Nkrumah FK, Kojima S. A monoclonal antibody-based dipstick assay for diagnosis of urinary schistosomiasis. Trans R Soc Trop Med Hyg. 1997;91:554–6.

10. Gobert GN. The role of microscopy in the investigation of pararnyxos as a vaccine candidate against Schistosoma japonicum. Parasitol Today. 1998;14:3115–8.

11. Bosompem KM, Asigbee J, Otchere J, Abdul A, Kpo KH, Kojima S. Accuracy of diagnosis of urinary schistosomiasis: comparison of parasitological and a monoclonal antibody-based dipstick method. Parasitol Int. 1998;47:211–7.

12. World Health Organization. Preventive chemotherapy in human helminthiasis: coordinated Use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers. Geneva: WHO; 2006.

13. Bosompem KM, Bentum IA, Otchere J, Anyan WK, Brown CA, Osada Y, et al. Infant schistosomiasis in Ghana: a survey in an irrigation community. Trop Med Int Health. 2004;9(8):917–22.

14. Huyse T, Webster BL, Geldof S, Stothard JR, Diaw OT, et al. Bidirectional introgressive hybridization between a cattle and human schistosome species. PLoS Pathog. 2009;5(9), e1000571. doi:10.1371/journal.ppat.1000571.

15. Suzuki T, Osada Y, Kumagai T, Hamada A, Okuzawa E, Kanazawa T. Early detection of Schistosoma mansoni infection by touchdown PCR in a mouse model. Parasitol Int. 2006;55:213–8.

16. Chu KY, Sabbaghian H, Massoud J. Host-parasite relationship of bulinus truncatus and Schistosoma haematobium in Iran. 2. Effect of exposure dosage of miracidia on the biology of the snail host and the development of the parasites. Bulletin of World Health Organization. 1996;34(1):121–30.

17. Ramalho-Pinto FJ, Gazinzinelli G, Howells RE, Mota-Santos TA, Figueiredo EA, Pellegrino J. Schistosoma mansoni: defined system for stepwise transformation of cercaria to schistosomule in vitro. Exp Parasitol. 1974;36:105–22.

18. Li Y, Auliff A, Jones MK, Yi X, Mcmanus DP. Immunogenicity and immunolocalization of the 22´6 kDa Antigen of Schistosoma japonicum. Parasite Immunol. 2000;22:415–24.

19. Dillon GP, Illesh JC, Isaaci HV, Wilton RA. Patterns of gene expression in schistosomes: localization by whole mount in situ hybridization. Parasitology. 2007;134:1589–97. doi:10.1017/S0031182000026395.

20. Liu F, Lu J, Hu W, Wang SY, Cui SJ, Chi M, et al. New perspectives on host–parasite interplay by comparative transcriptomic and proteomic analyses of Schistosoma japonicum. PLoS Pathog. 2006;2:e29.

21. Yang J, Feng X, Fu Z, Yuan C, Hong Y, et al. Ultrastructural observation and gene expression profiling of Schistosoma japonicum derived from two natural reservoir hosts, water buffalo and yellow cattle. PLoS One. 2012;7(10):e47660. doi:10.1371/journal.pone.0047660.

22. Rinaldi G, Okatcha TI, Popratiloff A, Ayuk MA, Suttiratpapa S, et al. Genetic Manipulation of Schistosoma haematobium, the Neglected Schistosome. PLoS Negl Trop Dis. 2011;5(10):e1000348. doi:10.1371/journal.pntd.0000348.

23. de Walick S, Bexkens ML, van Balkom BWM, Wud Y-P, Smit CH, Hokke CH, et al. The proteome of the insoluble Schistosoma mansoni eggshell skeleton. Int J Parasitol. 2011;41(5):523–32.

24. Buergelt D, Greiner C. Fibrosing granulomas in the equine liver and peritoneum: a retrospective morphologic study. J Vet Diagn Invest. 1995;7:102–7.

25. Sabatini DD, Bensch K, Barnett RI. Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J Cell Biol. 1963;17:19–58.