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## Original Research Article

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# Prevalence of Urinary Schistosomiasis in Umuowele, Agulu Community, Anambra State, Nigeria

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## Abstract

**Purpose:** To apply molecular techniques in producing a proper data on the prevalence of urinary schistosomiasis in Umuowele community located around Agulu Dam, Southwest Nigeria.

**Methods:** Urine samples from 108 pupils were screened for schistosomiasis using haematuria and polymerase chain reaction (PCR) amplification of schistosome Dra1 repeat. Six snails collected from the human-water contact site in the lake were also screened for schistosome infection by PCR amplification of the Dra1 repeat while PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) was used for snail species identification.

**Results:** Haematuria revealed 48.1% prevalence rate among the pupils while PCR showed 58.3%. Three snails were also positive for schistosome infection one of which was identified as *Bulinus truncatus* while the other two belonged to the genus, *Physa*.

**Conclusion:** There was a high prevalence of *S. haematobium* infection among the participants. PCR was able to detect infection in cases otherwise shown to be negative by haematuria, thereby making it possible for all the infected participants to receive treatment. *Bulinus truncatus* is one of the snails which may be responsible for the transmission of urinary schistosomiasis in the community.

**Keywords:** *Bulinus truncatus*, *Schistosoma haematobium*, PCR-RFLP, schistosome infection.

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## Introduction

According to the World Health Organization (WHO), schistosomiasis is a worldwide public health problem affecting 200 million people in the third world. *Schistosoma haematobium*, which causes urinary schistosomiasis in humans, afflicts about 150 million people in 53 countries in Africa and the Middle East [1]. Urinary schistosomiasis caused by *Schistosoma haematobium*, a trematode parasite, has been reported to be endemic throughout all the States of Nigeria [2,3]. Brown [4] also reported that freshwater pulmonate snails of the genus *Bulinus* are the intermediate hosts for *S. haematobium* and occur commonly throughout much of Africa and adjacent regions. The snails are also reported by Akogun and Akogun [5] to be more prevalent in the rural riverine areas and in areas where developments of projects to promote irrigation and provide hydroelectric power are taking place. According to Cowpear, their distributions are focal, aggregated and are usually related to the presence of several man-made impoundments, small seasonal streams, irrigation canals and ponds [6].

Available diagnostic methods of schistosomiasis are search for eggs in stools or urine and detecting eggs or adult worm antigens in urine and sera of infected individuals. Sturrock *et al* [7] observed that surveillance techniques include monitoring schistosome eggs output by humans, human water contact activities, snail infection rates and numbers of cercariae in the water. They also observed that detection of eggs or adult worm antigens in urine and sera of infected individuals could differentiate between past and current infections with specificity close to 100%. However, they stated that the sensitivity could be low in light infections with disadvantages such as high cost, difficult approach and dependence on monoclonal antibodies. Recently a polymerase chain reaction (PCR) was developed by Rabello *et al* [8] for the detection of schistosome DNA in faeces. Other detection methods of

schistosome infections in snails include snail crushing in search of larvae, repeated shedding of cercariae in the laboratory, detection of schistosomal antigens in snail hemolymph and lately, polymerase chain reaction (PCR) assays [9-14]. Unlike other methods that were previously used for identifying snails with pre-patent infection, it was observed by Hamburger *et al* [11] that PCR can enable detection of snail infection from its very earliest stages and can identify the entire population of infected snails, regardless of whether they eventually shed cercariae.

In this study, we aimed at producing an appropriate data on the prevalence of urinary schistosomiasis in Umuowe community located around Agulu Dam, Southwest Nigeria and also at understanding more about the snails that play an indispensable role in its transmission. To achieve these, we applied some current molecular techniques to screen the study participants for *S. haematobium* infection, identify and screen the snails species collected from the water body serving the study community.

## Materials and Methods

### Study site

The study site is Umuowe community of Agulu, which is at the eastern side of Agulu Lake basin in Anaocha Local Government Area of Anambra State. The community lies between latitude 6°12' N and longitude 7°00' E. Agulu Lake is the largest water body in the area and is about 10km away from Awka, the state capital. The residents are mainly artisans but also engage in fishing and farming. With their domestic animals, they depend on the lake for water for all their domestic use. Just like many rural areas in Nigeria, the community lacks some basic infrastructures such as electricity, pipe borne water, safe waste disposal and tarred roads. However, there is a primary school and a

health centre that serve the entire community.

### Study design

#### *Ethical consideration*

Ethical clearance was obtained from Anaocha Local Government and Agulu Local School Authorities while informed consent was obtained from the parents/guardians of the participants.

#### *Participants selection*

All the pupils in Umuowe Community Primary School were invited to participate in the study. Included in the study were 108 volunteers made up of 66 (61.1%) males and 42 (38.9%) females. This did not include children under the age of five and any girl menstruating at any point of urine collection.

#### *Sample collection*

For each of the recruited pupil, demographic data including the name, surname, age, sex and weight were recorded and a unique study code of three digits was assigned. About 200 ml of urine was collected into sterile wide mouth bottle (labeled with the corresponding study code) from each of them between 10.00 am and 2.00 pm, on each collection day, for maximum schistosome egg yield as observed by Weber *et al* [15]. Haematuria was detected in the field using commercially prepared reagent strips (Hemastix; Boehringer Mannheim, Germany) and the samples were transported to the laboratory immediately for further analysis.

#### *Snail material*

Snails were collected from identified human-water contact sites in the lake with the aid of a snail identification field guide. A total of six snails with similar morphological characteristics to the genus, *Bulinus*, were collected from the study site and were kept in 70% ethanol.

### Sample Preparation

#### *Urine cell pellets preparation*

Each urine sample was centrifuged at 5,000 *xg* for 10 min, the supernatant decanted and cell pellets washed three times with 25 ml PBS (0.8% NaCl, 2.7 mM KCl, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 8 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.4). The cell pellets were stored immediately at -80 °C until used.

#### *Genomic DNA extraction from urine cell pellet*

Urine cell pellets were digested with 1% SDS and 50 µg/ml proteinase K (Roche Diagnostics, Mannheim, Germany) at 48 °C overnight. Genomic DNA was extracted from the solution by adding an equal volume of chloroform/isoamyl alcohol (24:1) to each tube. The organic and aqueous layers were gently mixed for 5 min and spun at 13,000 rpm for 20 min. The upper aqueous layer was removed into another sterile Eppendorf tube and an equal volume of 100% ethanol was added, mixed and incubated at -20 °C overnight in order to enhance DNA precipitation. Resulting solution was spun at 13,000 rpm for 20 min and the pellets were washed with 70% ethanol and spun for another 20 min. The supernatant was removed and the pellets were dried at room temperature. When completely dry, the pellets were re-suspended in 25 µl of water and stored at 4 °C until used.

#### *Genomic DNA extraction from snails*

Genomic DNA was extracted from each snail using CTAB extraction buffer containing 2-mercaptoethanol, hexadecyltrimethyl-ammonium bromide (CTAB) (solid), *tris*(hydroxymethyl) amino-methane, ethylenediamine-tetraacetic acid, disodium salt solution (EDTA), and sodium chloride. Each snail was removed from the 70% ethanol and soaked in TE (10mM Tris HCl and 1mM EDTA) overnight so as to get rid of the remaining ethanol. Tissue from each of the snails was placed in a sterile 1.5 ml

Eppendorf tube, 500 µl of CTAB solution added and the tissue was ground followed by the addition of 10 µl of proteinase K solution (20 mg/ml) and incubated at 55 °C for 1 hr, with occasional gentle mixing. Genomic DNA was extracted from the CTAB buffer by adding an equal volume of chloroform and isoamyl alcohol (24:1) to each tube. The organic and aqueous layers were gently mixed for 5 min and spun at 13,000 rpm for 20 min. The upper aqueous layer was removed into another sterile Eppendorf tube and an equal volume of 100% ethanol was added, mixed and incubated at -20 °C overnight in order to enhance DNA precipitation. Resulting solution was spun at 13,000 rpm for 20 min and the pellet was washed with 70% ethanol and spun for another 20 min. The supernatant was removed and the pellets were dried at room temperature. When completely dry, the pellet was re-suspended in 25 µl of water and stored at 4 °C until used.

#### *Molecular Screening of Urine Samples and Snails for Schistosomiasis*

Genomic DNA extracted from the urine cell pellets and snails was subjected to PCR amplification of the schistosome *Dra1* repeat using forward primers 5'GATCTCACCTA TCAGACGAAAC3' and reverse primers 5'TCACAACGATACGACCAAC 3' [9]. All the PCR amplifications were performed with the Thermal Cycler (Bio-Rad iCycler) and the amplified products were visualized on 1.5% agarose gel. Photo documentation was performed with Gel Documentation and Analysis System (Clix Science Instruments, USA).

#### *Molecular identification of snails*

Snails identification was performed by PCR amplification of the snails' ribosomal internal transcribed spacer (ITS) region using forward primer *Etts1* 5'TGCTTAAGTTCAG CGGGT3' and reverse primer *Etts2* 5'TAACAAGTTTTCCGTAGGTGAA3' [14]. The amplification was confirmed by visualization on 1.5% agarose gel and the

amplified products were digested with *RsaI*, a 6-base cutting restriction enzyme, following the method of Stothard *et al* [14]. The digest was visualized on 1.5% agarose gel followed by photo documentation using Gel Documentation and analysis System (Clix Science Instruments, UA).

#### **Statistical analysis**

The data was analyzed using Epi Info version 2008 (CDC, USA). Proportional data were compared using chi-square test while other data comparison was carried out using analysis of variance (ANOVA). At 95% confidence interval, p values less than 0.05 were considered significant.

## **Results**

### ***S. haematobium* infection**

Both screening methods revealed that more males than females were infected (Table 1). Out of the 108 participants, 52 (48.1%) were positive for haematuria suggesting *S. haematobium* infection, while 63 (58.3%) were shown to be infected by the PCR amplification of schistosome *Dra1* repeat. The gel picture of the infection status of 10 children and three out of the six snails discovered to be infected with schistosomes after PCR amplification are shown in Figures 1 and 2.

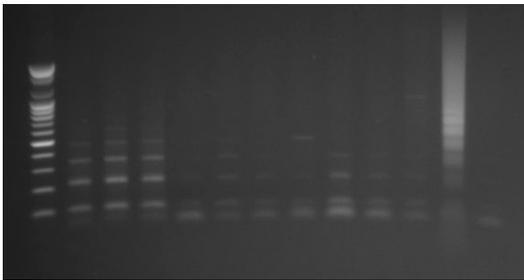
**Table 1:** The prevalence of urinary schistosomiasis using both haematuria and PCR

Sex	Number examined (%)	Number infected haematuria (%)	Number infected PCR, <i>Dra1</i> (%)
Male	66 (61.1)	32 (29.6)	40 (37.0)
Female	42 (38.9)	20 (18.5)	23 (21.3)
Total	108 (100)	52 (48.1)	63 (58.3)

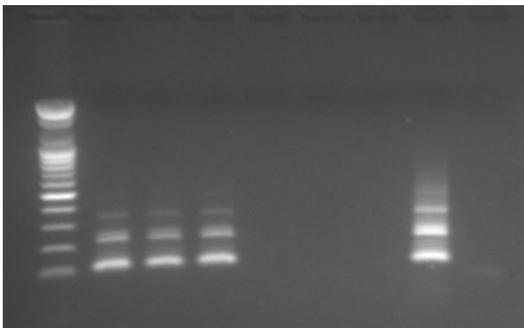
### ***Identification of snails***

The amplification of the ITS region of the 6 snails produced bands of different sizes and the resulting fragments following digestion by restriction enzyme *RsaI* are shown in Figure

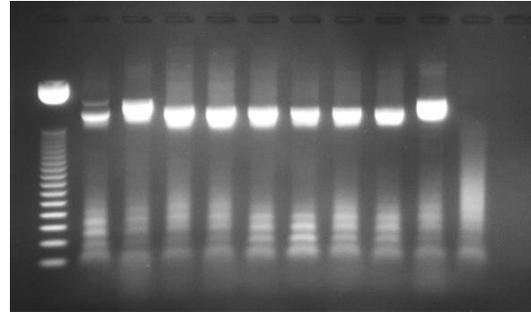
3. One of the 6 snails was identified by restriction fragment length polymorphism (RFLP) as *Bulinus truncatus* while the remaining five were observed to belong to the genus *Physa*. The identification was confirmed by including in the PCR-RFLP, gDNA from *Bulinus globossus*, *Bulinus truncatus* and *Physa acuta*, whose species have been confirmed earlier through sequencing (Akinwale *et al.* unpublished data) and using their banding patterns after RFLP for reference (Figure 4). The *B. truncatus* was infected while 2 of the remaining five snails belonging to the genus *Physa* were also infected.



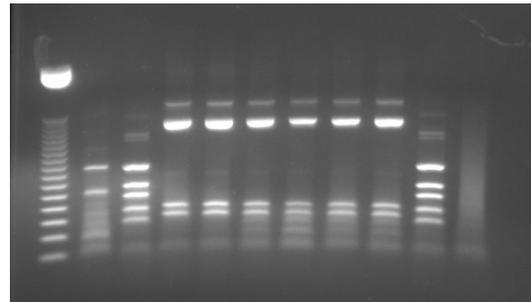
**Figure 1:** Agarose gel stained with ethidium bromide showing the infection status of 10 children. Lanes - 1: size marker (Bioneer ladder 100bp); 2 - 11 infected children; 12: DNA from an adult *S. haematobium* as positive control; 13: negative control.



**Figure 2:** Agarose gel stained with ethidium bromide showing the infection status of 6 snails. Lanes - 1: size marker (Bioneer ladder 100bp); 2 - 4, infected snails; 5 - 7, uninfected snails; 8: DNA from a previously confirmed infected *Bulinus globossus* as positive control; 9: negative control.



**Figure 3:** Agarose gel stained with ethidium bromide showing the different bands obtained from amplification of the ITS region of the 6 snails. Lanes - 1: size marker (Promega ladder 50 - 1500bp); 2 - 4: (previously confirmed snail species through sequencing) *Bulinus globossus*, *Bulinus truncatus* and *Physa acuta* respectively; 5 - 9 (snails under study): *Physa acuta*; 10: *Bulinus truncatus*; 11: negative control.



**Figure 4:** Gel showing species-specific banding patterns obtained from digestion of the 1.3kbp PCR product containing the ITS region using *RsaI*. Lanes - 1 : size marker (Promega ladder 50 - 1500bp); 2 - 4: (previously confirmed snail species through sequencing) *Bulinus globossus*, *Bulinus truncatus* and *Physa acuta* respectively; (Snails under study) 5 - 9: *Physa acuta*; 10: *Bulinus truncatus*; 11: negative control.

## Discussion

The results showed that about half of the study participants (58.3%) were infected with *S. haematobium* while 3 out of the 6 snails were infected with schistosomes. All the infected pupils were treated with a single oral dose of praziquantel tablets (Bayer) at 40 mg/kg body weight, at the end of the investigation. Our results confirmed the observations made earlier by Emejulu *et al*

[16] about prevalence of urinary schistosomiasis around Agulu Lake. They had earlier observed that the communities surrounding the lake were endemic for *S. haematobium* infection. We observed that the snail species that may be responsible for the transmission of *S. haematobium* in Umuowele, Agulu community is *Bulinus truncatus* as confirmed by the infection status revealed by PCR amplification of the *Dra1* as well as species identification by PCR-RFLP.

Emejulu *et al* [17] reported that snails belonging to the genus *Physa* could be infected with *S. haematobium* but the snails could not carry the infection to patency. We confirmed this in our laboratory from our previous work by keeping some snails belonging to the genus *Physa* over a period of 21 days, observing them weekly for cercariae shedding after exposing them to artificial light.

We observed that the PCR assay applied in this study could serve as an alternative to parasitological diagnostic techniques for the detection of *S. haematobium* infection. However, it demands a more sophisticated laboratory and more complex operational efforts than parasitological techniques. Earlier reports made by some researchers [9-13] confirmed that parasitological techniques surpass both molecular and serological techniques in terms of low costs and ease of operation. However we observed that PCR may particularly represent a tool for diagnosis of the infection when high sensitivity and specificity are required and infrastructure is available. We also observed that the restriction fragment analysis of the ribosomal ITS applied in this study could be a cheaper and more rapid method compared to sequencing and could be a promising technique for differentiating between *B. globosus* and *B. truncatus* species (Figure 4).

## Conclusion

It is evident from our study that *S. haematobium* infection is endemic in Umuowele community. PCR was able to detect infection in cases otherwise shown to be negative by haematuria, thereby making it possible for all the infected participants to receive treatment. *Bulinus truncatus* is one of the snail species which may be responsible for the transmission of urinary schistosomiasis in the community. We therefore urge the relevant health authorities to embark on an urgent intervention so as to save the inhabitants from the socio-economic effects of the burden of the disease. Pipe borne water and safe waste disposal system should be provided, not only to Umuowele community, but to all the communities around the lake. All these should be accompanied with appropriate health education in order to improve the hygiene of the people.

## Acknowledgements

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## Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. EAU and CNU developed the concept. OPA designed the experiments. EAU and CNU carried out sample collections. MBA, DOA and AAD prepared the samples for molecular assays. OPA, EAU, PVG and MAA performed molecular assays and analysis.

EAU, OPA and CNU prepared the manuscript.

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