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Prevalence of *Schistosoma haematobium* Infection in a Neglected Community, South Western Nigeria

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Abstract

Purpose: Schistosomiasis ranks second to malaria among parasitic diseases of socio-economic and public health importance. In Nigeria, urinary schistosomiasis caused by *Schistosoma haematobium* is endemic. This study aimed at producing an accurate data on the prevalence of urinary schistosomiasis in Apojula, a neglected community located around Oyan Dam, southwest Nigeria, using parasitological and molecular techniques.

Methods: Parasitological examinations were carried out on urine samples from 63 participants whose ages ranged between 7 and 63 years. Matched blood and urine samples were also screened for *S. haematobium* infection by polymerase chain reaction (PCR) amplification of the schistosome Dra1 repeat.

Results: Of the 63 participants, 33 (52.4%) were positive for heamaturia while 6 (9.5%) had *S. haematobium* ova in their urine. PCR amplification of *S. haematobium* Dra1 repeat from their urine and blood samples showed that 59 (93.65%) and 62 (98.4%) were infected respectively.

Conclusion: There was a high prevalence of *S. haematobium* infection as detected by PCR amplification of schistosome Dra1 repeat from the urine and blood samples of the study participants. In addition, the PCR was able to detect schistosome infection in cases otherwise shown to be negative by parasitological examinations thereby making them also to receive chemotherapy.

Keywords: *Schistosoma haematobium*, heamaturia, urine, blood, PCR, Dra1.

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Introduction

Schistosomiasis is a worldwide problem afflicting more than 200 million people living in developing countries with at least 600 million people at risk of infection and 1.7 million Disability Adjusted Life Years (DALYs) lost annually [1]. In many African countries, there occur both urinary and intestinal schistosomiases caused by *Schistosoma haematobium* and *Schistosoma mansoni,* respectively. Urinary schistosemiasis is endemic in Nigeria with substantial transmissions occurring in all the states of the federation and a high prevalence rate among school children [2]. The infection is associated with water resource development projects such as dams and irrigation schemes, slow-flowing or stagnant water, where the snail intermediate host of the parasite breeds. The main courses of large rivers are not usually a major source of schistosomiasis, but waters sustained by them through seasonal flooding, impoundment and extraction for irrigation are important transmission sites [3]. The disease is essentially an infection of rural and agricultural communities where the way of life of people promotes the contamination of inland water with human excreta.

The upsurge in dam construction in Nigeria linked to the Sahelian drought of the 1970s has contributed largely to the shift in the disease transmission bionomics, with a shift from flowing rivers, streams and ponds to artificial lakes and dams. Of 325 registered dams in Nigeria, over 246 (77%) were constructed since 1970 [4]. More than 200 (62%) of these dams were built in 10 most endemic states, the rest in the other 26 less endemic states [5]. One of such dams is Oyan Dam in Ogun State, south-west Nigeria, which was constructed in 1984. However, within four years of its construction, an outbreak of urinary schistosomiasis in two resettlement communities (Abule-titun and Ibaro) located around the reservoir was reported [6].

Schistosomiasis control programmes are chiefly based on treatment of infected populations, hence adequate case finding is crucial. Up to now, the 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. Detection of eggs or adult worm antigens in urine and sera of infected individuals can differentiate between past and current infections with specificity close to 100%. However, the disadvantages include low sensitivity in light infections, high cost, difficult approach and dependence on monoclonal antibodies. Also, haematuria and egg counts are the only indicators of morbidity being used presently for surveillance but Poggensee *et al* [7] reported urinary tract morbidity in some infected Tanzanian women with negative haematuria and scanty or no egg output in their urine. This suggests the need for a more sensitive and specific method of detection of the infection in the human definitive host.

Recently, Hamburger *et al* [8] identified a tandemly repeated DNA sequence, termed Dra I, in the genome of *S. haematobium* and demonstrated that a highly sensitive PCR assay amplifying this sequence can easily detect individual cercariae in infected snails. In the present study, we adapted this Dra I assay for the detection of *S. haematobium* infection in the urine and whole blood samples of infected humans. The urine samples were also examined for heamaturia using urine reagent strips (Hemastix; Boehringer Mannheim, Germany). We aimed at producing an accurate data on the prevalence of urinary schistosomiasis in Apojula – a neglected community located around Oyan Dam, southwest Nigeria –by using both parasitological and molecular techniques.

Methods

Study population

The study was conducted in Apojula community situated around Oyan Dam. The

dam is located at latitude 7º14'N and longitude 3º13'E near Abeokuta, Ogun state, southwest Nigeria. It is a multipurpose dam used mostly for water, flood control, fishing and irrigation. The main occupations in this community are fishing and farming and it has a population of about 150 people. Just like many rural areas in the country, it lacks some basic infrastructures such as electricity, pipe borne water and safe waste disposal.

Following approval by the Institutional Review Board of the Nigerian Institute of Medical Research, and permission to carry out the study in the village was taken from Ogun State Ministry of Health and Abeokuta North Local Government Authority, all inhabitants of the community were invited to participate in the study. However, children under the age of five, the elderly and debilitated people, pregnant women, lactating mothers, and any female menstruating at any time of urine sample collection were excluded from the study. This led to the final recruitment of 63 volunteers (35 males and 28 females; age: 7-63 years; mean age: 21 years) (Table 1).

Table 1: Demographic characteristics of the study participants

Variable	Number	%
Sex		
Male	35	55.6
Female	28	44.4
Total	63	100.0
Age (years)		
7-19	36	57.1
20-30	12	19.0
30+	15	23.8
Total	63	100.0
Weight (kg)		
19-30	21	33.3
31-50	13	20.6
51-70	25	39.7
>71	4	6.3
Total	63	100.0

Informed consent was obtained from each participant or parents/guardians of children aged below 18 years prior to the investigation.

Sample collections

The study participants were registered on household forms and the name, surname, age, sex and weight of all participants were recorded while every participant was allocated a unique code of six digits representing village, household and individual numbers. About 200 ml of voided mid-stream urine was collected between 10.00 am and 2.00 pm on each collection day into sterile containers labeled with the study number of each participant and taken immediately to the laboratory in icebox at 4 ºC. Also 2 ml of blood was collected from each of the participants into labeled EDTA bottles and stored at 4 ºC.

Parasitological examinations

The participants were examined for hematuria using commercially prepared reagent strips (Hemastix; Boehringer Mannheim, Germany) while presence of *S. haematobium* eggs was detected by centrifugation of about 100 ml of the urine samples and examination of the sediment under the microscope.

Molecular examination

Genomic DNA (gDNA) extraction

About 100 ml of each urine sample was centrifuged at 5,000 *x g* for 10 min and the supernatant decanted and cell pellets washed three times with 25 ml PBS (0.8% NaCl, 2.7mM KCl, 1.8mM KH2PO4, 8mM Na2HPO4, pH 7.4). The cell pellets were stored immediately at -80ºC until use. Genomic DNA was extracted from the urine pellet using Qiagen DNeasy® blood and tissue kit (Qiagen Group) as well as from whole blood collected from each participant using GenSpin™ Genomic DNA purification kit (Whatman, UK) according to the manufacturers' instructions. The extracted DNA was then stored at 4 ºC until use.

PCR screening of the participants for schistosome infection

The gDNA extracted from the urine cell pellets and whole blood was subjected to PCR amplification of the schistosome *Dra1* repeat using forward primers 5'GATCTCACCTATCAGACGAAAC3' and reverse primers, 5'TCACAACGATACGACC AAC 3' following the method of [8]. All the PCR amplifications were performed with the Thermal Cycler (BioRad iCycler) and the amplified products were visualized on 1.5% agarose gel. Photo documentation was performed with Gel Documentation and analysis System (Clinx Science Instruments).

Data analysis

The differences in prevalence of infection between the gender groups (as shown by haematuria) were determined using Chi-square test. At 95% confidence interval, p-values less than 0.05 were considered to be significant.

Results

More than half of the participants (33, 52.4%) were positive for haematuria while 30 (47.6%) were negative. This was made up of 17 (27%) males and 16 (25.4%) females and there was no significant difference ($p > 0.05$) in the prevalence between the gender groups. Eggs were seen in the urine samples of 6 (9.52%) participants, comprising of 3 (4.76%) males and 3 (4.76%) females. The PCR amplification of Dra1 gene from the urine pellets of the 63 participants showed that 59 (93.65%) of them were positive while 4 (6.35%) were negative for the infection. However, the PCR amplification of Dra1 gene from the whole blood of the 63 participants confirmed that 62 (98.4%) of them were infected while 1 (1.6%) was not. The only participant that was shown as negative by the blood PCR was also negative for haematuria and urine PCR (Figures 1 and 2; lane 20).

The PCR assays were repeated on the urine and blood samples of the 4 participants shown earlier as uninfected by the PCR performed on their urine samples. The repeated assay was performed alongside 16

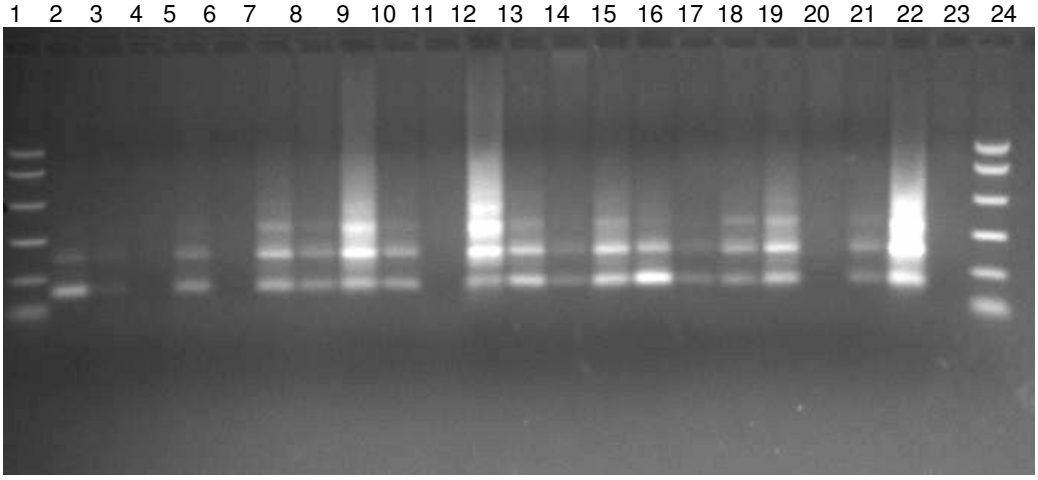

Figure 1: PCR amplification of the Dra1 from the urine samples of 20 participants as shown by agarose gel stained with ethidium bromide. Lanes – 1 and 24: size marker (Promega BenchTop PCR Markers 50 - 1000bp); 2, 3, 5, 7 – 10, 12 – 19 and 21: infected; 4, 6, 11 and 20: uninfected; 22: DNA from an adult *S. haematobium* as positive control; 23: negative control.

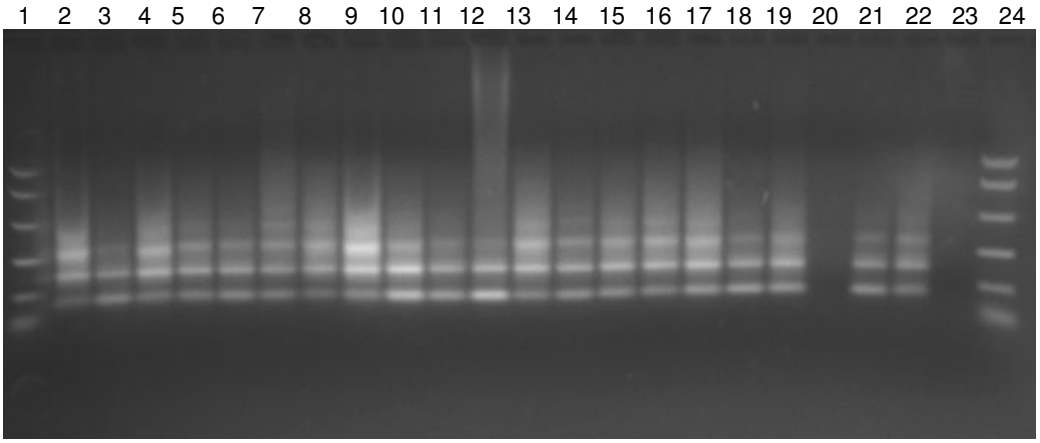

Figure 2: PCR amplification of the Dra1 from matched blood samples of the same 20 participants in figure 1 as shown by agarose gel stained with ethidium bromide. Lanes – 1 and 24: size marker (Promega BenchTop PCR Markers 50 - 1000bp); 2 – 19 and 21: infected; 20: uninfected; 22: DNA from an adult *S. haematobium* as positive control; 23: negative control.

urine and matched blood samples, which were also confirmed earlier as positive by both assays (Figures 1 and 2). The urine PCR was still negative for the 4 participants while the blood PCR still showed that they were positive for the infection. However, the difference between the sensitivity of the PCR amplifications of the Dra1 gene from urine pellet and the whole blood for the detection of *S. haematobium* infection was examined. The difference was not statistically significant ($p > 0.05$).

Figure 1 also demonstrated the ethidium bromide stained agarose gel of the amplified Dra1 from the urine samples of 20 participants (lanes 2 – 21). Sixteen of them were infected as shown on the gel, while 4 were uninfected. Figure 2 also revealed the infection status of the same 20 participants (lanes 2 – 21) as seen on the ethidium bromide stained agarose gel after the PCR amplification of the Dra1 from their whole blood samples. Nineteen of them were infected as shown on the gel, while one participant was not infected.

Discussion

In this study, PCR amplification of schistosome, Dra1 gene, as well as parasitological examinations were carried out on urine and blood samples of participants drawn from Apojula community situated around Oyan Dam. The result of the blood PCR showed that all but one of the participants were infected with *S. haematobium*. This study has therefore demonstrated that *S. haematobium* infection is endemic in Apojula, a neglected rural community situated around Oyan Dam. All the study participants were treated with a single dose of praziquantel at 40 mg/kg body weight at the end of the investigation.

The study showed that transmission of urinary schistosomiasis has continued in the dam, 20 years after the outbreak was first reported by Ofoezie *et al*. (1991). This is typical of many other communities situated around water reservoirs, lakes and dams in Nigeria where there are uneven distribution of natural water resources, poverty, ignorance and low-sanitation standards. Although they carried out a study on the prevalence of urinary schistosomiasis in the only two communities (Abule-titun and Ibaro) that existed then around the dam, this study has shown that the risk of transmission in the dam has also spread to new settlements that

were created thereafter, one of which is Apojula community.

The PCR Dra1 amplification assay used in this study may serve as an alternative to the parasitological diagnostic techniques for the detection of *S. haematobium* infection. However, it requires a very standard laboratory and more complex operational effort than parasitological diagnostic techniques, which surpass both molecular and serological techniques in terms of low costs and ease of operation. Nevertheless, the PCR may particularly represent a tool for the diagnosis of the infection in special situations, when high sensitivity and specificity are required and infrastructure is available.

Conclusion

We concluded that *S. haematobium* infection is endemic in Apojula community and urgent intervention is necessary in order to save the people from the socio-economic effects of the burden of the disease. There should be simultaneous implementation of control measures in all the endemic communities around the dam. The state and local authorities should provide pipe borne water and safe waste disposal system to the communities and this should also be accompanied with appropriate health education. It is hoped that health education together with the basic amenities will help to improve the hygiene of the people and impact on their general well being.

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References

1. Chitsulo L, Engels D, Montresor A, Savioli L. The global status of schistosomiasis and its control. Acta Trop. 2000; 77: 41–51.
2. Mafe MA, Stamm Von T, Utizinger J, N'Goran EK. Control of urinary schistosomiasis: an investigation into the effective use of questionnaires to identify high-risk communities and individuals in Niger State, Nigeria. Trop Med Int Health. 2000; 5(1): 53–63.
3. Ogbeide HE, Uyigue E. Access to safe drinking water and schistosomiasis in Nigeria: survey on Ipogun Community, Ondo State of Nigeria. Submitted to the Society for Water and Public Health Protection (SWAPHEP). 2004. http://swaphep.virtualactivism.net
4. Imevbore AMA, Ofoezie IE, Obot EA. Vector-borne disease problems of small scale water resources development projects in Kano State: Snail vectors of schistosomiasis. The Afrancet. 1988; 1: 17–23.
5. Ofoezie IE. Human health and sustainable water resources development in Nigeria: schistosomiasis in artificial lakes. Nat Res Forum. 2002; 26: 150–160.
6. Ofoezie IE, Imevbore AMA, Balogun MO, Ogunkoya OO, Asaolu SO. A study of an outbreak of schistosomiasis in two resettlement communities near Abeokuta, Ogun State, Nigeria. J Helminth. 1991; 65: 95–105.
7. Poggensee G, Kiwelu I, Saria M, Richter J, Krantz I, Feldmeir H. Schistosomiasis of the lower reproductive tract without egg excretion in urine Am J Trop Med Hyg. 1998; 59, 782–783.
8. Hamburger J, He N, Abbasi I, Ramzy RM, Jourdane J, Ruppel A. Polymerase chain reaction assay based on a highly repeated sequence of *Schistosoma haematobium*: a potential tool for monitoring schistosome-infested water. American Journal of Tropical Medicine and Hygiene. 2001; 65: 907–911.
9. Hamburger J, He-Na, Xin XY, Ramzy RM, Jourdane J, Ruppel A. A polymerase chain reaction assay for detecting snails infected with bilharzia parasites (*Schistosoma mansoni*) from very early prepatency. Am J Trop Med Hyg. 1998; 59(6):872–6.
10. Hamburger J, Hoffman O, Kariuki HC, Muchiri EM, Ouma JH, Koech DK, Sturrock RF, King CH. Large-scale, polymerase chain reaction-based surveillance of *Schistosoma haematobium* DNA in snails from transmission sites in coastal Kenya: a new tool for studying the dynamics of snail infection. Am J Trop Med Hyg. 2004; 71: 765–773.
11. Driscoll AJ, Kyle JL, Remais J. Development of a novel PCR assay capable of detecting a single *Schistosoma japonicum* cercaria recovered from

Oncomelania hupensis. Parasitol. 2005; 131: 497–500.

12. Ten Hove RJ, Verweij JJ, Vereecken K, Polman K, Dieye L, Van Lieshout L. Multiplex real-time PCR for the detection and quantification of Schistosoma msnsoni and S. haematobium infection in stool samples collected in northern Senegal. Trans Roy Soc Trop Med Hyg. 2008; 102: 179-185.