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Prevalence of Urinary Schistosomiasis in Part of Ogun State, Nigeria

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ABSTRACT
The current study is to apply molecular techniques in producing data on the prevalence of urinary Schistosomiasis in parts of Ogun state, Nigeria. This study evaluated the prevalence of *S. haematobium* in urine samples collected from 250 primary school pupils in 8 communities of 8 local government areas (LGAs). The number of pupils selected are 48 from Ijebu Ogbere ((Ijebu East LGA), 20 from Fidiwo (Obafemi Owode LGA), 40 from Sabo (Shagamu LGA), 22 from Iweke (Yewa South LGA), 20 from Ketu/Adiowe (Ado Odo/Ota LGA), 36 from Abule-titun (Odeda LGA), 24 from Itori (Ewekoro LGA) and 40 from Ijoun (Yewa North LGA) of Ogun State, Nigeria Urine samples from 250 pupils were screened for Schistosomiasis using haematuria and polymerase chain reaction (PCR) amplification of schistosoma Dra1repeat. Heamaturia revealed 28.8% prevalence rate among the pupils while PCR showed 45.6%. Results revealed a cumulative prevalence of 29% and 46% *S. haematobium* infection in the pupils as detected by haematuria and PCR techniques respectively. Ijebu Ogbere recorded the highest prevalence of 83.0% and 64.5% PCR and haematuria respectively. This was followed by Abule titun (66.6%) and (33.3%), Ijoun (55%) and (27.5%), Shagamu (35%) and (25.0%), Iweke (27.2%) and (13.6%), Itori (25%) and (16.6%) and Fidiwo recorded the least prevalence of 10% and 5.0% respectively. The mean prevalence of schistosomiasis for PCR and haematuria dip stick were 37.7% and 23.2% respectively. The proportion of males infected using haematuria were 16% when compared to females 13%, whereas the ratio by PCR was males (22%) and females (23%). There was a high prevalence of *Schistosoma haematobium* infection among the participants. PCR was able to detect infection in cases otherwise shown to be negative by haematuria, thereby making possible for all the infected participants to receive treatment.
Keywords: PCR-RFLP; Urinary schistosomiasis; Prevalence; Pupils; Ijebu east;

INTRODUCTION

Schistosomiasis is one of the most widespread of all human parasitic diseases, ranking second only to malaria in terms of its socioeconomic and public health importance in tropical and subtropical areas (Chitsulo, 2000). Schistosomiasis is a major public health problem affecting over 200 million people worldwide (WHO, 2010). As a mainly rural, often occupational disease, Schistosomiasis principally affects people who are unable to avoid contact with natural water sources, either because of their profession (agriculture, fishing) or because of lack of reliable water for drinking, washing and bathing (Chandiwana, 1987; Emejulu *et al.*, 1994, Ekpo *et al.*, 2010). Increased population movement seems to enhance the spread of the disease, and Schistosomiasis is now occurring increasingly in periurban areas (Oliveira, 2004). The establishment of water resource development projects has beenassociated with outbreaks of schistosomiasis in many parts of tropical Africa (Ofoeze *et al.*, 1991, Sam-Wobo *et al.*, 2009). Although the development of water resources is overall immensely beneficial to a developing country like Nigeria which seeks to boost agricultural production to feed its growing population, evidence abounds to show that the planning, design and execution of such projects are often undertaken without considering their health implications (Oladejo and Ofoeze, 2006; Akinwale *et al.*, 2010). As a consequence water related diseases, such as schistosomiasis, are spreading (Oladejo and Ofoeze *et al.*, 2006). 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., (2001)* 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 (2002)* 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 (Hamburger *et al.*, 1989, Hamburger *et al.*, 2001, Hamburger *et al.*, 2004, Hertel *et al.,* 2004, Driscoll *et al.*, 2005., Ten Hove *et al.*, 2008. Unlike other methods that were previously used for identifying snails with pre-patent infection, it was observed by Hamburger *et al* (2004*)* 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. This study aims at producing an appropriate data on the prevalence of urinary schistosomiasis in Lopo Korede community, in Ijebu East Local Government, Ogun State, Southwest, Nigeria

To achieve these, we applied some current molecular techniques to screen the study participants for *S. haematobium* infection, identified and screened the snails species collected from the water body serving the study community.

Materials and Methods

Study sites

The study was conducted in some Local Governments in Ogun state: Yewa North Local Government Primary School, Yewa, Obafemi Owode Local Government Primary School; Yewa South Local Government Iweke, Ilupeju Primary School Abule tuntun, Local Government Secondary School Sagamu, Muslim Primary School Ado Odo Ota, Saint John Catholic Primary School Lopo-Korede Ijebu East Local Govt, and St. Michael Primary School, Itori. Ogun State is situated in the South West part of Nigeria and covers a land of 16,370 square kilometers with an average density of 206 person square kilometers. The state lies between longitude 2^0 45^1 E and 3^0 55^E and latitude 7^{0n}.01 and 7^1.18N it share boundary with Oyo and Osun state to the north Lagos and Atlantic Ocean to the South, Benin republic the west and Ondo state to the east.

It has a projected population of about 3.4million people in 2004 with a population growth rate of 2.8% (NPC) 1991). There are 20 local government areas and 4 main geopolitical groups viz: the Egbas, Yewas, Remos and Ijebus in the state.

The vegetable ranges from fresh water swamp with mangrove forest in South East, through diverse forest community to the woody guinea savannah, in the north western tip of the state. Rain forest is the largest ecological zone running the centre of the state from east to west. It had annual rainfall of 1206. 7mm and 1112.7mm in 2009 and 2010 respectively and mean temperature of 23.64^{0c} and 23.18^{oc} in 2009 and 2010 respectively. The comparison analysis was carried out in eight communities across eight local government areas of the state.

Study design

Ethical consideration

Ethical clearance was obtained from Ogun state ministry of health and from the School authorities while informed consent was obtained from the parents/guardians of the participants. All the pupils in St. John Primary School were invited to participate in the study. Included in the study were 250 volunteers made up of 128 (51.2%) males and 122 (48.8%) 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 schistotome egg yield as observed by Weber *et al (*Stothard *et al*., 1996). 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.

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 mMKH2PO4, 8 mM Na2HPO4, pH 7.4).The cell pellets were stored immediately at -80 °C until used.

Collection of Urine samples

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 13,000rpm 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 −20oC 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 resuspended in 25 µl of water and stored at 4^{oC} until used.

Molecular Screening of Urine Samples for Schistosomiasis

Genomic DNA extracted from the urine cell pellets was subjected to PCR amplification of the schistosome *Dra1* repeat using forward primers 5'GATCTCACCTATCAGACGAAAC3'and reverse primers

5'TCACAACGATACGACCAAC 3'. All the PCR amplifications were performed with the Thermal Cycler (Bio-RadiCycler) and the amplified products were visualized on 1.5% agarose gel. Photo documentation was performed with Gel Documentation and Analysis System (Clinx Science Instruments, USA).

Statistical analysis

Descriptive analysis was used to express gender occurrence while the prevalence of infection was expressed in percentage.

RESULT:

4.1 Prevalence of *Schistosoma haematobium* in the study area

The table presents the prevalence of urinary schistosomiasis by both hematuria and PCR technique (table 1). Female pupils (23.2%) showed higher prevalence than the male pupils (22.4%) for the Polymerase Chain Reaction (PCR) method; while male pupils (16.0%) showed higher prevalence than female pupils (12.8%) in the hematuria method. A total number of 72 (28.8%) and 114 (45.6%) were shown to be positive for both hematuria and PCR amplification of Dra1 repeat methods respectively.

The table further showed that PCR was more sensitive than dip stick method in detecting *S. haematobium* infection. The dip stick showed 16 and 26 false negative results for both males and females respectively that were confirmed positive by PCR (Table 1). Also from the table 1, pupils from Korede village in Ijebu East LGA had the highest percentage prevalence of 83.3% and 64.5% for both PCR and hematuria respectively. This was followed by pupils from Abule Titun in Odeda LGA with 66.6% and 33.3% for both PCR and hematuria respectively while the least prevalence was recorded in Ketu/Adio-owe in Ado-Odo LGA with no infection detected in both PCR and hematuria methods.

` In the age distribution of Urinary Schistosomiasis in all the communities studied (table 2) Korede community had the highest percentage of infection of 87% 100% for PCR among pupil of ages 5-9years old and 10-14years old while hematuria recorded 60% and 66.7% respectively. This was followed by Abule titun community with 64% and 54.5% in PCR and hematuria for pupils of aged 5-9years old and 10-14years old. There was no significant difference between the ages (Table 2).

Table 1: Sex prevalence of urinary Schistosomiasis among pupil in the study areas using both haematuria and PCR

Communities/LGA	Male			Female			Total		
	No Exam	PCR (%)	% Hem	No Exam	PRC%	% Hem	No Exam	PCR (%)	s% Hem
Korede/Ijebu East	20	90	90	28	79	46.4	48	83.3	64.5
Fidiwo/Obafemi - Owode	08	0.0	12.5	12	17	0.0	20	10	5.0
Sabo/Sagamu	22	36	18.1	18	33	33.3	40	35.0	25.0
Iweke/Yewa South	12	17	16.6	10	40	10	22	27.3	13.6
Ketuadio/owe/Ado-Odo-Ota	12	0.0	0.0	08	0.0	0.0	20	0.0	0.0
Abule titun/Odeda	20	60	40	16	75	25	36	66.6	33.3
Itori/Ewekoro	10	30	30	14	21	7.1	24	25.0	16.6
Ijoun/Yewa North	24	54	16.6	16	56	43.75	40	55	27.5
Total	128	44	31.25	122	48	26.22	250	45.6	28.8

PCR -- Polymerase Chain Reaction
Hem -- Hematuria
LGA – Local Government Area

Table 2: Age Prevalence of urinary schistosomiasis among the study population using haematuria and PCR

COMM/ LGA.	5 - 9 YEARS NUMBEX	PCR %	HEM %	10 – 14 YEARS NUMBEX	PCR %	HEM %	15 – 19 YEARS NUMBEX	PCR %	HEM%
Korede/ Ijebu East	15	87	60	21	100	66.7	12	50	50
Fidiwo/ Obafemi –Owode	16	0.0	0.0	11	18	0.0	3	67	67
Sabo/Sagamu	14	7	0.0	20	65	40	6	0.0	0.0
Iweke/ Yewa south	6	0.0	0.0	12	42	25	4	25	25
Ketu/Ado/Odo-Ota	4	0.0	0.0	10	0.0	0.0	6	0.0	00
Abule-Titun/Odeda	11	64	54.5	16	87.5	37.5	9	33.3	33.3
Itori/Ewekoro	8	25	12.5	10	40	0.0	6	0.0	0.0
Ijoun/Yewa North	14	21.4	14.2	14	93	28.6	12	50	50
TOTAL	**78**	**33.3**	**23.07**	**105**	**68.57**	**32.3**	**58**	**34.48**	**34.48**

INFECTION STATUS OF PUPILS IN THE STUDIED AREA

The prevalence of Schistosomiasis in the studied area using PCR is shown in Plate 1. For the bottom; Agarose gel stained with ethidium bromide showing the infection status of 10 children lane – 1 and 14 size marker (Bioneer ladder 50-2500bp); lanes 2-4 were uninfected; lane 5-6 and 10 showed that the children were infected, lanes 7, 8, 9 and 11were also uninfected children. The Lane 12 served as a positive control and lane 13 a negative control.

The infection status of pupils in the studied area is as shown in Plate 2. The lane at the top that shows bands signifies pupils infected with schistosomes as indicated in lanes 1-5 and lanes 7,8,10,11,17,18,19,20,21 and 22. Those without band as indicated in lanes 6, 12, 13, 14, 15, 16, 23-26 and 28-30 were not infected with schistosomes

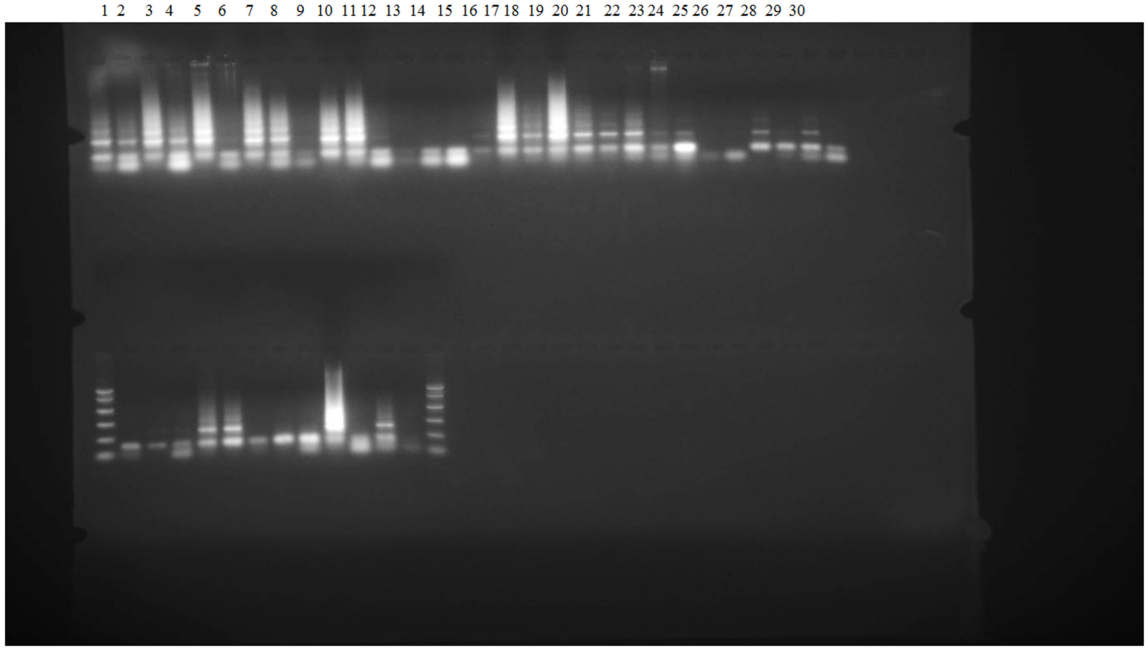

Figure: 1 Agarose gel stained with ethidium bromide showing the infection status of pupils.
Top: Lanes 1-5 indicate infected pupils, lane 7, 8, 10, 11, 17, 18, 19. 20, 21 and 22 infected children. Lane 6, 12, 13, 14, 15, 16, 23-26 and 28-30 uninfected children.

Bottom: Agarose gel stained with ethidium bromide showing the infection status of 10 children lane – 1 and 14 size marker (Bioneer ladder 50-2500bp); lane 2-4 uninfected lane 5-6, and 10 infected children, lane 7, 8, 9 and 11 uninfected children. Lane 12 positive control lane 13 negative control.

DISCUSSION

The prevalence of infection is almost half of the participant (45.6%) as indicated by the PCR amplification of schistosome Dra1 repeat showed that most of the study areas were endemic with urinary schistosomiasis. The high prevalence may possibly be a reflection of intense water contact activities in the areas. The relatively low sensitivity of haematuria and detecting schistosome infection as compared with the PCR technique confirmed earlier observations that the PCR technique is more sensitive than any other method in diagnosing schistosome infection (Hamburger *et al*, 1998; Ten Hove *et al*, 2008), as methods of determining the prevalence of schistosomiasis could be misleading and give inaccurate data on the occurence of the infection in such localities. The non significant differences observed in the prevalence of infection in both sexes possibly indicate that both school age males and females are equally exposed to water activities. Researchers has posited that the school-age children are the most vulnerable group to urinary schistosomiasis because of frequent engagement with water activities such as swimming, fishing, and washing of clothes and plates. (Bello *et al*, 2003) Thus, the estimation of the prevalence of the infection in school age children can be used as an index for assessing community prevalence (Guyatt *et al, 1999)*. The study revealed that 45.6% of the study participants were infected with S. *haematobium*. Therefore, as stated by WHO (1985) , that prevalence rate above 40% is considered high, while those below 40% and 25% are considered moderate and low respectively, one can say that the studies area is endemic with urinary schistosomiasis. This result confirmed the observation made by Emejulu *et al* (1994). Similar prevalence rate were also recorded by other researchers Bello *et al* (2003) recorded 52% prevalence rate in some villages around the Guronyo dam in sokoto state. Ngele and Onyeukwu, (2008) recorded 45% prevalence rate in Afipko, Ebonyi state. 47.9% prevalence rate was measured by Olofitoye and Oninya (2008) in Ekiti state. Similar but fairly higher prevalence rates were measured in other areas. These include Anigbo Nwarogu (1990) in Amagunze (73%) Mafiana *et a*l (2003) in Ogun State (71.8%) Basompem *et a*l (2004) in Ghana (78%), Brouwer *et al* (2004) in Zimbabwe (60%), Nmorsi *et al (*2005) in Edo state (65%), and Sam-wobo *et al (*2008) 86% and 88% in two communities in Ogun State.

The sex related prevalence of the infection in the study community was determined. It was found that in haematuria technique male recorded higher prevalence rate than the females. This is in accordance with the works of Abubakar and Adamu (2001), Sam-wobo *et al* (2008), Mafiana *et al* (2003) and Okoli and Iwuala (2004). In the PCR technique the female are more than male. This disparity in the related prevalence, suggest that in every sampled area, prevalence rate is higher among whatever folk that visit the source of infection more often. This could be as a result of some social attitudinal, cultural, religious or occupational differences. The female prevalence rate is higher than male because of occupational differences (Anosike *et al* (2006), Okon *et al*, (2007) and Mbinka *et al* (2008).

Further analysis carried out on age related prevalence showed that children between the ages of 10-15 years had higher prevalence rate than children between ages 5-9years old. Here the haematuria and PCR recorded (7.2% and 10.4%) and (13.6%66and6628.8%) prevalence for children between the ages of 5-9years old and 10-14years old respectively. Similar observation was made by Bello *et al* (2003).

The high prevalence rate among children between ages of 10-14 years can be attributed to the fact that they visit the infected water bodies move often to swim, play, wash or even fish. The children between 5-9 years old are tender and it would be considered that parental restrict must have contributed to the reasons why they less often visit fresh water bodies which lead to lower prevalence rate.

It is important to note that in the analysis of community prevalence (that is both in prevalenece rate among sex and age there was conspicuous disparity in identifying the infection by haematuria and PCR technique. PCR technique recorded a higher prevalence rate for both sex and age. (See table 5 & 6). Generally PCR recorded 114 positive individuals while haematuria recorded 72 positive out of 250 examined pupils which means that, there were 44 false negative records accounted to haematuria technique.This result confirmed early observations by other reseachers (Hamburger *et al*., 1998, 2004; Driscoll *et al*; 2005; Ten Hove *et al*., 2008), the higher sensitivity of PCR technique over other methods for the detection of schistosomes infection.

This study has shown that urinary schistosomiasis is endemic in the studied area though of an average prevalence. This prevalence is of public health significance and could be a treat to important social economic activities in this area if not quickly checked. There is urgent need therefore, for the local govt authority as well as that of the state govt to formalize and establish feasible control programme in the area. Infection can be furthered controlled through the provision of portable water supply, eradication of snail intermediate host and health education on the relationship between water contact and infection.

Conclusion

It is evident from our study that *S. haematobium* infection is endemic in Lopo korede community. PCR was able to detect infection in cases otherwise shown to be negative by haematuria, thereby making3. One of the 23 snails was identified by restriction fragment length polymorphism (RFLP) as *Bulinus globosus and four were Bulinus truncatus* while the remaining eighteen 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. The *B.truncatus* was infected while 2 of the remaining five snails belonging to the genus *Physa* were also infected.

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. globossus* and *B. truncates* species (Figure 4).

It is evident from our study that *S. haematobium* infection is endemic in Lopo korede 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 socioeconomic effects of the burden of the disease. Pipe borne water and safe waste disposal system should be provided, not only to Lopo Korede community, but to all the communities around fresh water. All these should be accompanied with appropriate health education in order to improve the hygiene of the people.

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