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Molecular Detection of Schistosoma haematobium DRA1 DNA in Urine and Serum Samples

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Abstract
Urinary schistosomiasis, caused by *Schistosoma haematobium*, remains a significant public health challenge, particularly in rural communities where access to healthcare and preventive measures is limited. Nigeria has the greatest number of cases of schistosomiasis worldwide. The aim of this study was to detect *Schistosoma haematobium* Dra1 DNA fragments in urine and serum Samples. Urine and blood samples from 335 school children were collected and examined using reagent strip urinalysis, urine microscopy, PCR amplification of *S. haematobium* Dra1 DNA (in urine and serum samples). The overall prevalence of haematuria among the study participants was 21.5%. Haematuria was observed to be higher in males (23.4%) than females (18.8%). Age group specific distribution indicated children aged 5-7years significantly ranked higher in haematuria (28.1%; *p = 0.046*). According to the intensity of infection, children with heavy infection significantly recorded the highest percentage of hematuria (100%). All the 335 urine samples were evaluated for *S. haematobium* by microscopic and PCR techniques, of which 66 (19.7%) were positive for microscopy, 73 (21.8%) positives for dra1PCR in urine and 71 (21.2%) for dra1PCR in serum. The prevalence of urinary schistosomiasis in this study was not associated with gender (p = 0.374). The highest prevalence was observed among younger children aged 5-7years using all the four techniques employed in this study. The diagnostic performance recorded for microscopy were Sn = 90.4%, Sp = 100%, PPV = 100% and NPV = 97.4%, dra1PCR in urine (Sn = 100%, Sp = 100, PPV = 100% and NPV = 100%) and dra1PCR in serum (Sn = 97.3%, Sp = 100%, PPV = 10%0 and NPV = 99.2%). Strip test is a good alternative in poor areas where PCR is not available. Polymerase chain reaction amplification is a promising test for diagnosing *S. haematobium* and may serve as a criterion standard in determining the accurate disease prevalence. Our findings suggest that the dra1PCR in serum is suitable for diagnosis of urinary schistosomiasis and might be of value in diagnosing patients during the acute phase of infection before eggs excretion and seroconversion, and in light infections.

Keywords: PCR, Schistosoma haematobium, DRA1, Urine, Serum

Introduction

Schistosomiasis is one of the most common and widely distributed parasitic infections. Today, schistosomiasis causes greater mortality and morbidity than all other parasitic worm infestations. The disease is increasing in prevalence affecting about 10% of the world□s population and ranking second to malaria as a cause of disabling disease and death. Sub-Saharan Africa accounts for 93% of all people suffering from schistosomiasis and is home to the causative agents, including *S. haematobium*; *S. mansoni* and *S. intercalatum*. In Africa alone, Nigeria is the most schistosomiasis-endemic country (Steinmann *et al.,* 2019).

Inadequate diagnosis of schistosomiasis is potentially a serious problem, particularly in cases of chronic infection with *S. haematobium* that has been associated with damage to the urinary tract and eventual development of squamous cell carcinoma of the bladder (ten

Hove *et al.,* 2015). Different diagnostic procedures to detect schistosomiasis have been identified and compared. These procedures include tests for circulating antigens, specific antibody, egg detection, hematuria, and ultrasound scans of the urinary tract. However, the diagnostic performance of these methods varies, and it is hard to set anything like a "gold" standard in areas with different *S. haematobium* prevalence (Koukounari *et al.,* 2016).

Without highly sensitive, specific tests, it will be difficult to ensure the objective control of schistosomiasis. There is strong evidence that detection of parasite specific DNA in urine and DNA is more sensitive and specific than processing urine by standard methods (Lodh *et al.,* 2013). To overcome some of the drawbacks of other diagnostic procedures, attempts have been made to detect Schistosoma CFPD in human serum/plasma and other body fluids (Pontes *et al.,* 2012). Wichmann *et al.* (2019) used the term □cell-free parasite DNA□ (CFPD) to comprise the DNA that was detected in serum. Indeed, due to the high parasite turnover, diverse stadia of the parasite might be present in the blood circulation and are detectable depending on the phase of the infection. CFPD is uniformly distributed in serum, unlike schistosome eggs in urine, so one of the major limitations of egg DNA amplification, i.e., sampling, can be avoided with CFPD detection. Moreover, detection of CFPD can play an important role in situations where diagnostic dilemmas occur, such as in neuro-schistosomiasis cases, which normally present false-negative results by conventional techniques (Härter *et al.,* 2014; Xu *et al.,* 2021).

Materials and Methods

Study Area

The study focused on two villages in Sokoto State, Nigeria, known for their histories of *Schistosoma haematobium* infection: Hamma Ali (Kware Local Government) and Kwalkwalawa (Wamakko Local Government). Both communities lack safe waste disposal, clean water supply, and essential health centers. Residents rely on nearby rivers for their daily needs, including drinking water, washing clothes, and bathing, with fishermen and traders making up the majority of the population.

Hamma Ali, a rural district in Kware Local Government, is home to fishermen and farmers. The River Sokoto serves as the boundary between the two districts. The settlements are mostly situated on low-lying terrains with various freshwater bodies, such as marshes, rivers, and ponds. The vegetation primarily consists of grassland with scattered trees (Mamman and Kudu, 2001).

Kwalkwalawa is a rural settlement in the Wamakko Local Government Area of Sokoto State. Covering about 889 square kilometers, Wamakko has an estimated population of around 181,999 people. The village is located near the Sokoto River Rima valley at latitude 13° 5"N and longitude 5° 12"E, to the northwest of Sokoto city, near the Usman Danfodiyo University main campus (Mamman and Kudu, 2001). The area's climate is influenced by two opposing air masses: the tropical continental and tropical maritime. The dry season lasts from October to April, while the rainy season starts around April-May and ends around October-November, with an average annual rainfall of about 1300 mm. The residents are primarily subsistence farmers involved in arable crop farming, fishing, cattle rearing, and small-scale trading (Taofiq *et al.,* 2017).

Study Design

This cross-sectional study involved the recruitment of school children aged 5 to 13 years, without regard to their infection status.

Sample Size Determination

Using a schistosomiasis prevalence rate of 32.09% (Shuaibu *et al.,* 2017), the minimum sample size was calculated based on the following statistical formula:

$$n = \frac{Z^2 PQ}{d^2}$$

Where;

n = Minimum sample size required

Z = Confidence level at 95% (standard error from the mean 1.96)

P = 32.09% is the prevalence of *S. haematobium* in school children (Shuaibu *et al.,* 2017).

Q = 1-P, 1- 0.3209 = 0.6791

d = 5% margin of error (0.05)

$$n = \frac{(1 \cdot 96)^2 x (0 \cdot 3209) x (1 - 0 \cdot 3209)}{(0.05)^2} = 335$$

Inclusion and Exclusion Criteria
The study included male and female school children aged 5 to 13 years, who were lifelong residents of the study area, had not received treatment for schistosomiasis in the past six months, and whose parents or guardians provided consent. Children who did not meet these inclusion criteria were excluded from the study.

Ethical Considerations
Ethical approval for this study was obtained from the Sokoto State Ministry of Health (Ref: No. SMH/1580/V.IV) and the Universal Basic Education Board Sokoto (Ref: No. SUBEB/785/VI). Before recruiting study participants, written informed consent was obtained from both the school children and their parents/guardians. A meeting was held to explain the purpose of the study. Participation was voluntary, and children were free to withdraw at any time without obligation.

Samples Collection
Urine
Urine samples from consenting participants were collected following a 20-30 minutes period of physical exercise, between 10:00 and 12:00 hours, when the excretion of *Schistosoma haematobium* eggs is most optimal. Each participant was provided with a pre-numbered sterile wide-mouthed plastic bottle. The participant□s age and gender were recorded on a form corresponding to the bottle number. Each urine sample was preserved with two drops of 40% formalin, tightly sealed, labeled, and then transported to the laboratory for *Schistosoma haematobium* egg detection.

Blood
Three milliliters of venous blood were collected from each willing participant using a plain vacutainer blood collection tube. In the laboratory, serum was extracted from each sample by centrifugation at 3000 revolutions per minute (rpm) for 10 minutes, and then stored at -40°C until analysis.

Laboratory Examination
Estimation of Urine Microhematuria
Uric 10 CF (ACCU-ANSWER, UK) was used for rapid determination of urine biochemicals (blood, protein and leukocytes). The reagent area of the test strip was immersed into a well-mixed, fresh urine for 40 seconds and removed quickly. The strip edge was placed against the rim of the container to wipe excess urine. The strip was laid on a paper towel with the reagent areas upward. The strip was held horizontally and compared the reagent areas on the strip to the corresponding color chart on the bottle label at the exact times specified and in good light. The strips were held close to the color blocks and matched carefully; and the results taken (Monica, 2006).

Filtration Technique:
Urine samples were shaken to resuspend the eggs, and 10 ml of the mixture was withdrawn into a syringe and filtered through a 25 mm Millipore filter membrane with a pore size of 12 µm, secured in a Swinnex support chamber. Schistosome eggs were trapped on the filter membrane. Using forceps, each membrane was carefully removed from the chamber, inverted to expose the side with trapped eggs, and placed onto a microscope slide. The slide was stained with Lugol's iodine and examined microscopically under a ×40 objective for the detection of *Schistosoma haematobium* eggs (Monica, 2006).

Quantification of Schistosoma haematobium Eggs:
A capillary or Pasteur pipette, held at an angle of about 45°, was used to fill one grid of the counting chamber (Improved Neubauer counting chamber with a ruled area of 9 mm^2 and a depth of 0.1 mm) with the well-mixed sediment, avoiding overfilling. The chamber was allowed to sit undisturbed for 2 minutes, dried on the underside, and then examined under a microscope using a ×10 objective. The condenser iris was adjusted to provide good contrast for the identification and counting of *S. haematobium* eggs (Richter, 2000). The egg count per participant was recorded as eggs per 10 ml of urine and categorized into intensity classes: negative, light intensity (1-49 eggs/10 ml), and heavy intensity ($\geq$50 eggs/10 ml).

Extraction of S. haematobium DNA in Urine and Serum Samples
DNA Extraction was carried out using Dongshen genomic DNA extraction kit (GDSBio, China) according to manufacturers' instructions. Four hundred (400) µl of urine/serum sample was dispensed into a microcentrifuge tube and 800 µl of Solution RS was

added, mixed thoroughly by brief vortexing and centrifuged for 3 min at 5,000 rpm. The supernatant was discarded and 200 µl of Solution DS was added, mixed immediately and thoroughly by brief vortexing. Twenty (20) µl of Proteinase K and Solution MS were added, mixed thoroughly by brief vortexing and incubated at 65^0C for 10 min. Then 220 µl of Ethanol (100%) was added to the lysate and mixed thoroughly by brief vortexing. The whole mixture was pipetted into the spin column placed in a 2 ml collection tube, and centrifuged at 12,000 rpm for 1 min. The flow-through was discarded, 500 µl of Wash Buffer PS added and centrifuged for 1 min at 12,000 rpm. The flow-through was discarded, 500 µl of Wash Buffer PE added and centrifuged for 1 min at 12,000 rpm. The flow-through was discarded, 500 µl of Wash Buffer PE was added for the second time and centrifuged for 1 min at 12,000 rpm. The flow-through was discarded and centrifuged for 3 min at 12,000 rpm to dry the column membrane. The flow-through and the collection tube were discarded. The spin column was placed in a clean 1.5 ml microcentrifuge tube, 60 µl of Elution Buffer TE was pipetted directly onto the membrane and incubated at room temperature for 2 min. It was centrifuged for 2 min at 12,000 rpm and stored at -20^0C (Antony *et al.,* 2015).

Oligonucleotide Primers

The following primers were adopted from Hamburger *et al.* (2001) and used for PCR amplification of *Schistosoma haematobium* Dra1
Forward: 5'-GATCTCACCTATCAGA CGAAAC-3' and
Reverse: 5'-TCACAACGATACGACCAAC-3'

Amplification of S. haematobium Dra1 in Urine and Serum Samples

Each of the PCR reaction mixture, sums up to a total volume of 20 micro liters (µL), that consist of the following: 10 micro liter of PCR master mix (2x Taq mix, manufactured by GDSBio, China), 1 µL each of the forward and reverse primers, 6 µL of nuclease-free water, and 2 µL of the extracted DNA sample. The extracted DNA sample serves as template. Each of the PCR tubes containing the reaction mixture was placed on a thermo cycler machine for amplification. Thermocycler amplification occurs in different stages, at different temperature and time as follows: There was an initial reaction at 95 °C for 10 minutes, this was followed by 33 cycles of DNA template denaturation at 95 °C for 30 seconds, annealing of primers at 53 °C for 1.5 minutes, then the elongation process, whereby new DNA strand are synthesized, at 72 °C for 60 seconds, after the last cycle there was a final elongation stage at 60 °C for 5 minutes. Both the negative and positive controls were integrated in the PCR process. PCR procedures used by Antony *et al.* (2015) was adopted and used for this study.

Agarose Gel Electrophoresis

Amplified PCR products for the detection *Schistosoma haematobium Dra1* were separated by running 5 µl each of the product and the 100 bp ladder on a 2% agarose gel containing 3 µl ethidium bromide in 1 x TAE running buffer for 25 min at 100V and 300A. The product was visualized with UV light in a gel documentation chamber. The size marker 100 bp ladder was used to estimate band sizes (Antony *et al.,* 2015).

Statistical Analysis

Data entry and analysis was performed using SPSS software for Windows (version 22.0, SPSS Inc, Chicago, USA). A $p< 0.05$ was considered statistically significant. Demographic, environmental and behavioral characteristics were considered as nominal variables and presented as percentages. Pearson's Chi square test was used to determine the associations of *Schistosoma haematobium* infection with demographic factors. The relationships of haematuria with age and gender of the subjects were examined using Pearson correlation co-efficient. The diagnostic accuracy tests (sensitivity, specificity, the negative predictive value, and the positive predictive value) were calculated using PCR as gold standard.

Results

A total of 335 school children were enrolled in this study. Among them, 197 (58.8%) were males and 138 (41.2%) were females, comprising of 32 (9.6%) aged 5-7 years, 142 (42.4%) 8-10years and 161 (48.0%) 11-13years.

The percentage distribution of different haematuria categories by gender, age and intensity of infection of the study participants was assessed. The haematuria categories are negative (0), trace (10ery/µl), small (25ery/µl), moderate (80ery/µl) and large (200ery/µl) respectively. Generally, there were high negative values of haematuria among all the gender and age

groups of participants except for the intensity of infection. Haematuria was observed to be higher in males (23.4%) than females (18.8%). Age group specific distribution indicated children aged 5-7years significantly ranked higher in haematuria (28.1%; $p = 0.046$). According to the intensity of infection, participants with heavy infection (>50eggs) significantly recorded the highest percentage of haematuria (100%) ($p = 0.000$) (Table 1).

All the 335 urine samples were evaluated for *S. haematobium* by dip stick (haematuria), microscopic, and PCR techniques, of which 72 (21.5%) were positive for haematuria, 66 (19.7%) and 73 (21.8%) positives with microscopy and PCR respectively. Similarly, serum samples collected from all the 335 participants were subjected to PCR for the detection of *Schistosoma haematobium* cell free DNA (ShCFDNA). Seventy-one (21.2%) were found to be positive which included all the 66 participants (positive by microscopy) and 71 participants (positive by urine PCR) as shown in table 2.

The prevalence of urinary schistosomiasis in this study was not associated with gender ($P = 0.374$), even though males were more infected than the female subjects. The highest prevalence was observed among younger participants aged 5-7years using all the four techniques employed in this study namely, strip haematuria (28.1%), microscopy (25%), urine PCR (31.3%) and serum PCR (34.4%) as depicted in table 3.

The diagnostic performance of the 3 techniques were calculated and compared by considering PCR technique as the criterion standard test, 90.4% sensitivity for microscopy, whereas for DNA amplification in serum (97.3%) and urine (100%) sensitivity respectively. The specificity and positive predictive value (PPV) remained 100% for all the methods. The negative predictive value (NPV) for egg detection was 97.4%, 99.2% for serum PCR and 100% for urine PCR (Table 4).

Table 1: Percentage Distribution of Different Hematuria Categories by Gender and Age of the Participants.

Variables	No. Examined	Hematuria					
		Neg (%)	Trace (%)	Small (%)	Moderate (%)	Large (%)	Total (%)
Gender				$\chi^2 = 7.629$,	p = 0.106		
Male	197	151(76.6)	12(6.1)	14(7.1)	2(1.0)	18(9.1)	46(23.4)
Female	138	112(81.2)	8(5.8)	6(4.3)	6(4.3)	6(4.3)	26(18.8)
Total	335	263(78.5)	20(6.0)	20(6.0)	8(2.4)	24(7.2)	72(21.5)
Age Group (Years)				$\chi^2 = 15.770$,	p = 0.046**		
5-7	32	23(71.9)	3(9.4)	4(12.5)	2(6.2)	0(0.0)	9(28.1)
8-10	142	118(83.1)	4(2.8)	4(2.8)	4(2.8)	12(8.5)	24(16.9)
11-13	161	122(75.8)	13(8.1)	12(7.5)	2(1.2)	12(7.5)	39(24.2)
Total	335	263(78.5)	20(6.0)	20(6.0)	8(2.4)	24(7.2)	72(21.5)
Intensity of Infection				$\chi^2 = 360.120$	p = 0.000**		
Light (1-50egg)	56	1(1.8)	13(23.2)	20(35.7)	6(10.7)	16(28.6)	55(98.2)
Heavy (>50eggs)	10	0(0.0)	0(0.0)	0(0.0)	2(20.0)	8(80.0)	10(100)
Negative	269	262(97.4)	7(2.6)	0(0.0)	0(0.0)	0(0.0)	7(2.6)
Total	335	263(78.5)	20(6.0)	20(6.0)	8(2.4)	24(7.2)	72(21.5)

Neg = Negative, ** = statistically significant $P \leq$

Table 2: Prevalence of *Schistosoma haematobium* Infection Evaluated by Microscopy, Haematuria and Polymerase Chain Reaction

Diagnostic Test	Number Examined	Number Positive	Prevalence (%)
Hematuria	335	72	21.5
Microscopy	335	66	19.7
Urine PCR	335	73	21.8
Serum PCR (CFDNA)	335	71	21.2

PCR = Polymerase Chain Reaction, CFDNA = Cell Free Deoxyribo Nucleic Acid

Table 3: Positive Results (Percentages) for the Four Diagnostic Tests Stratified by Gender and Age Group.

Variable	Total (%)	+ve by Hematuria	+ve by Microscopy	+ve by Urine PCR	+ve by Serum PCR
Gender					
Male	197 (58.8)	46 (23.4)	42 (21.3)	47 (23.9)	44 (22.3)
Female	138 (41.2)	26 (18.8)	24 (17.4)	26 (18.8)	27 (19.6)
Age (years)					
5-7	32 (9.6)	9 (28.1)	8 (25.0)	10 (31.3)	11 (34.4)
8-10	142 (42.4)	24 (16.9)	22 (15.5)	24 (16.9)	23 (16.2)
11-13	161 (48.0)	39 (24.2)	36 (22.4)	39 (24.2)	37 (22.9)

Key: +ve = Positive, PCR = Polymerase Chain Reaction

Table 4: Estimation of Disease Prevalence, Sensitivity, Specificity, Predictive Values and Diagnostic Accuracy

Diagnostic Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diag. Accuracy (%)
Hematuria	98.5	97.4	91.7	99.6	97.9
Microscopy	90.4	100	100	97.4	97.9
Urine PCR	100	100	100	100	100
Serum PCR (CFDNA)	97.3	100	100	99.2	99.4

Key: PPV = Positive Predictive Value, NPV = Negative Predictive Value

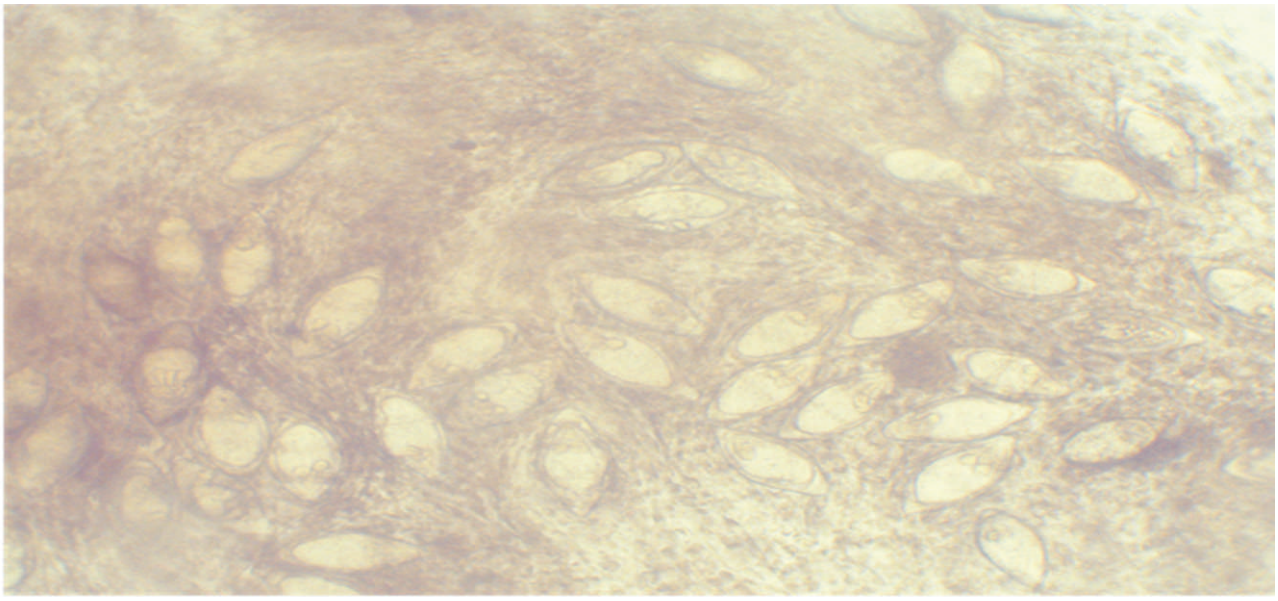

Figure 1: A representative of microscopy results showing ova of Schistosoma haematobium in urine sample

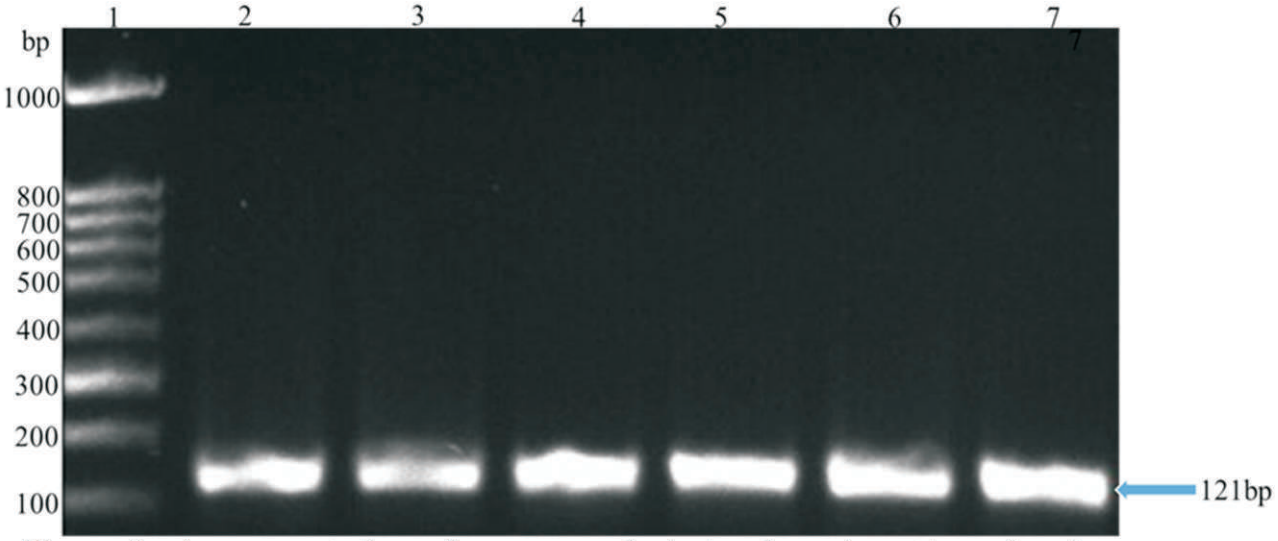

Figure 2: A representative of agarose gel electrophoresis pattern showing PCR amplification products of DRA1 gene from Schistosoma haematobium urine sample. Lane 1: Molecular weight size marker (DNA Ladder). Lane 2 to 7: Positive for DRA1 gene as indicated by 121 base pairs

Discussion

Urinary schistosomiasis is a major debilitating disease characterized by haematuria (blood in the urine). In the worst situation urinary schistosomiasis will cause bladder cancer. The greatest burden of the disease is found in school age children in sub-Saharan Africa (Elagba *et al*., 2006). According to Anosike *et al.* (2001), Nigeria is one of the highly endemic countries where the disease has been unsystematically recorded and several areas remain where the disease status is unknown.

Strip haematuria is considered an indirect demonstration of infection. It stands as a quite good alternative to both other techniques that gave sensitivity and specificity in agreement with previous reports (Obeng *et al.,* 2008; ten

Hove *et al.,* 2015). It has a reasonable cost with ease and speed of use that make it useful for monitoring the efficacy of control programmers, although the results are often confounded by hematuria of non-schistosomal origin. The cause of haematuria can be serious, for example, bladder cancer or it may be due to a trivial cause like vigorous exercise (Ibironke *et al.,* 2011). Despite the high sensitivity of strip tests in comparison with microscopy, some authors (Van Lieshout *et al.,* 2000) refused to use them alone for rapid mapping surveys because of their moderate specificity. Variation in sensitivity and specificity of strip tests during *S. haematobium* infection has been reported in a previous study (Kjetland *et al.,* 2012). The reported high negative predictive value (NPV) of reagent strips (100%) in comparison with microscopy criterion standard test was in range with a previous study (van Delft *et al.,* 2007). In addition, strip test reported NPV more than microscopy when compared with PCR criterion standard test. This gives strip test more value especially in poor endemic areas where PCR is not available.

The present study revealed that the prevalence of *Schistosoma haematobium* infection in the study area was 19.7% using microscopy. This prevalence is in accordance with the prevalence rates reported by previous studies; 18.7% in Plateau state (Damen *et al.,* 2018), 17.8% in Kano state (Dawaki *et al.*, 2015) and 17.4% in Oyo state (Okoli and Odaibo, 1999); and contrary to 37.7% reported by Bello *et al.* (2014) in Wurno Local Government, Sokoto State, 32.0% by Hassan *et al.* (2017) in Kebbi State and 60.8% recorded by Kiran and Muddasir (2014) in Sokoto state. This difference in the prevalence rate may be influenced by peculiar ecological characteristics, the degree of exposure of people to water bodies through some indigenous water contact activities, and presence of intermediate snail hosts in local river. In this study, there was no significant difference in the prevalence of urinary schistosomiasis between the male and female participants ($p = 0.374$) which implied that infection was not dependent on gender. This is in line with the reports of some researchers (Opara *et al.,* 2007; Christopher *et al.,* 2016; Nwachukwu *et al.,* 2018), but at variance with the reports of others (Ejima and Odaibo, 2010; Dawaki *et al.,* 2016 and Hassan *et al.,* 2017) who reported significantly higher prevalence in males than in females. The high prevalence in males is due to the fact that the males tend to go to river water on regular bases to fetch water for domestic use, play or bath, to swim and to fish unlike the females that may necessarily attach any importance to such water contact activities but rather stay close their mother at home while assisting with domestic chores. The high prevalence of *S. haematobium* as we observed was evident within the age bracket of 5-7 years old. This is in contrast to what was reported by previous study (Christopher *et al.,* 2016). It can be suggested that this possibly was observed because of the low response of the immune status of these age group.

There is strong evidence that detection of parasite specific DNA in urine and CFDNA is more sensitive and specific than processing urine by standard methods (Lodh *et al.,* 2013). Without highly sensitive and specific tests, it will be difficult to ensure the objective control of schistosomiasis. The species-specific DNA detection by PCR appears to be more sensitive than microscopy and haematuria for diagnosis of *S. haematobium* (Lodh *et al.,* 2014). This study agrees with the previous finding of Cnops *et al.* (2013) that PCR amplification of DRA1 fragments could serve as a target for detection of low levels of urinary schistosomiasis because of the high level of the repeat in the parasite genome.

In the present study, the high levels of sensitivity and specificity reported by PCR technique in comparison with microscopy criterion standard were previously confirmed (Emery *et al.,* 2012; Cnops *et al.* 2013). Microscopy has long been adopted as the criterion standard technique for diagnosing schistosomiasis. It provides a relatively easy cheap tool that does not need much trained personnel to identify the big characteristic of eggs in urine. However, it requires repeated sampling and careful examination to give high sensitivity, particularly when the infections are light, which are impractical in many epidemiological studies (Espy *et al.,* 2013). Some authors refused to accept microscopy as the only criterion standard for diagnosing urinary schistosomiasis because of its low sensitivity,

making it unsuitable for evaluation of schistosomiasis control programs (ten Hove *et al.,* 2008 and Xu *et al.,* 2010; Espy *et al.,* 2013).

At the same time, the result of this study showed that PCR technique has added 7 new cases over microscopy from 8 non-egg excretory patients that were highly suspected for urogenital schistosomiasis based on recent freshwater exposure. For these reasons, this study considered PCR as the gold standard test and compared the other 2 (microscopy and dipstick hematuria) tests to it. The results agreed with previous studies (Emery *et al.,* 2012 and Espy *et al.,* 2013). The relatively low sensitivity of the other 2 techniques in relation to PCR especially for microscopy confirmed that microscopy should not be considered as the only standard for diagnosing urinary schistosomiasis. The results showed that PCR could serve as an alternative to parasitological diagnostic techniques. However, the only limitations for this are its relatively high cost, especially for developing countries where the disease prevails.

Besides the excellent performance of the draPCR on urine samples, the most striking result of this study is the specific detection of *S. haematobium* in serum. All but two (2) of the serum samples from patients with a confirmed *S. haematobium* infection, were positive with the draPCR. This clearly demonstrates the diagnostic potential of the draPCR to detect *S. haematobium* in serum during the acute phase of the infection.

Conclusion

In the present study, the prevalence of hematuria revealed no significant difference between the male and female participants. It finding reflected the prevalence of the infection and corroborated the fact that infection rate is gender independent**.** Strip test is a good alternative in poor areas where microscopy and PCR are not available because of its good diagnostic accuracy recorded in this study. Our results suggest that urinary schistosomiasis is still prevalent among school children in rural communities in Sokoto State. The recorded prevalence was low compared to previous studies, and this may signify the success of the National Schistosomiasis Control Program. Polymerase chain reaction amplification of DraI-121-bp tandem repeat sequence of *S. haematobium* from urine samples of patients is a recent promising non-invasive test for diagnosing the disease and may serve as a criterion standard in determining the accurate disease prevalence because of its increased sensitivity and specificity over microscopy. Our findings suggest that the draPCR in serum is suitable for diagnosis of urinary schistosomiasis and might be of value in diagnosing patients during the acute phase of infection before eggs excretion and seroconversion, and in light infections.

References

Anosike, J.C., Nwoke, B.E.B., and Njoku, A.J. (2001). The validity of haematuria in the community diagnosis of urinary schistosomiasis infections. *Journal of Helminthology*; ***7(5)****:* 223-225.

Antony, J.S., Ojurongbe, O., Meyer, C.G., Thangaraj, K., Mishra, A. and Kremsner, P.G. (2015). Correlation of Interleukin-6 levels and lectins during *Schistosoma haematobium* infection. *Elsevier*; **76(2)**: 152-155.

Bello, A., Jimoh, O.A., Shiitu, S.B., and Hudu, S. A. (2014). Prevalence of urinary schistosomiasis and associated haemato-proteinuria in Wurno Rural Area of Sokoto State, Nigeria. *Orient Journal of Medicine*; ***26(3□40)***: 114-121.

Christopher, I., Jude, A.O., Japhet, M.O., and Tope, A. (2016). Urinary Schistosomiasis among School Pupils in Ilie Community, Southwestern Nigeria. *Saudi Journal of Medical and Pharmaceutical Sciences;* ***2(7)***:176-180.

Cnops, L., Soentjens, P., Clerinx, J., and Van Esbroeck, M. (2013) A Schistosoma haematobium-Specific Real-Time PCR for Diagnosis of Urogenital Schistosomiasis in Serum Samples of International Travelers and Migrants. *PLoS Neglected Tropical Diseases; **7(8)***: e2413.

Damen, J.G., Kopkuk, E.D., and Lugos, M.D. (2018). Prevalence of urinary schistosomiasis among irrigation farmers in North Central Nigeria. *Journal of Medical and Health Sciences*; ***7(3)***: 1-4.

Dawaki, S., Al-Mekhlafi, H.M., Ithoi, I., Ibrahim, J., Abdulsalam, A.M., and Ahmed,

A. (2015). The Menace of Schistosomiasis in Nigeria: Knowledge, Attitude, and Practices Regarding Schistosomiasis among Rural Communities in Kano State. *PLoS ONE;* ***10(11)***: 143-148.

Dawaki, S., Al-Mekhlafi, H.M., Ithoi, I., Ibrahim, J., Abdulsalam, A.M., Ahmed, A., and Surin, J. (2016). Prevalence and risk factors of schistosomiasis among Hausa communities in Kano State, Nigeria. *Revista do Instituto de Medicina Tropical de São Paulo*; ***5(8)****:* 54-58.

Ejima, I.A.A. and Odaibo, A. B. (2010). Urinary schistosomiasis in the Niger-Benue Basin of Kogi State, Nigeria. *International Journal of Tropical Medicine;* ***5(3)***: 73-80.

Elagba, H.A.M., Mohamed, E., and Hikmat, I. (2006). Haematological and biochemical morbidity of schistosoma haematobium in school children in Sudan. *Sultan Qaboos University Medical Journal*; ***6(2)****:* 59 - 64.

Emery, A.M., Allan, F.E., Rabone, M.E., and Rollinson D. (2012). Schistosomiasis collection at NHM (SCAN). *Parasitology Vectors*; **5**: 185.

Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, and Jones MF. (2013). Real-time PCR in clinical microbiology: applications for routine laboratory testing. *Clinical Microbiology Review;* ***19***: 165-256.

Hamburger, J., He, N., Abbasi, I., Ramzy, R.M., Jourdane, J., and Ruppel, A. (2001). 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*; **65(6**): 907-911.

Härter, G., Frickmann, H., Zenk, S., Wichmann, D., Ammann, B., Kern, P., Fleischer, B., Tannich, E., and Poppert, S. (2014). Diagnosis of neuroschistosomiasis by antibody specificity index and semi-quantitative real-time PCR from cerebrospinal fluid and serum. *Journal of Medical Microbiology;* ***63***:309-312.

Hassan, J., Mohammed, K., Opaluwa, S.A., Adamu, T., Nataala, S.U., Garba, M.K., and Bunza, N.M. (2017). Diagnostic potentials of haematuria and proteinuria in urinary Schistosomiasis among school-age children in Aliero Local Government Area, Kebbi State, North-Western Nigeria. *Asian Journal of Research in Medical and Pharmaceutical Sciences;* ***2(4)***: 1-9.

Ibironke, O.A., Phillips, A.E., Garba, A., Lamine, S.M., and Shiff, C. (2011) Diagnosis of Schistosoma haematobium by detection of specific DNA fragments from filtered urine samples. *American Journal of Tropical Medicine and Hygiene*, 84: 998-1001.

Kiran, S., and Muddasiru, D. (2014). Epidemiology of schistosomiasis in school aged children in some riverine areas of Sokoto, Nigeria. *Journal of Public Health and Epidemiology;* ***6(6)***: 197-201.

Kjetland, E.F., Leutscher, P.D.C. and Ndhlovu, P.D. (2012). A review of female genital schistosomiasis. *Trends in Parasitology;* ***28(2)***:58-65.

Koukounari, A., Webster, J.P., Donnelly, C.A., Bray, B.C., Naples, J., Bosompem, K., and Shiff, C. (2016). Sensitivities and specificities of diagnostic tests and infection prevalence of *Schistosoma haematobium* estimated from data on adults in villages northwest of Accra, Ghana. *American Journal of Tropical Medicine and Hygiene;* **80(4):** 435-441.

Lodh, N., Mwansa, J.C.L., Mutengo, M.M., and Shiff, C.J. (2013). Diagnosis of *Schistosoma mansoni* without the Stool: Comparison of Three Diagnostic Tests to Detect *Schistosoma mansoni* Infection from Filtered Urine in Zambia. *American Journal of Tropical Medicine Hygiene;* **89**: 46-50.

Lodh, N., Naples, J.M., Bosompem, K.M., Quartey, J., and Shiff, C.J. (2014) Detection of Parasite-Specific DNA in Urine Sediment Obtained by Filtration Differentiates between Single and Mixed Infections of *Schistosoma mansoni* and *S. haematobium* from Endemic Areas in Ghana. PLoS One;**9(3)**:e91144.

Mamman, A.B. and Kudu, R. (2001) *Giant of the Tropics*. Sokoto State. Lagos: *Gabamo Publishers:* 437-438.

Monica, C. (2006). *Discrete Laboratory Practice in Tropical Countries. Part 1*. 2nd edn. *Cambridge Press Syndicate of the University of Cambridge*: 247258.

Nwachukwu, P.C., Ohaeri, C.C., Ukpai, O.M., Irole-Eze, O.P., and Amaechi, E. C. (2018). Prevalence of *Schistosoma haematobium*

infection among school-aged children in Afikpo North Local Government Area, Ebonyi State, Nigeria. *Sri Lankan Journal Biology*; ***3(2)***: 1-8.

Obeng, B.B., Aryeetey, Y.A., De Dood, C.J., Amoah, A.S., and Larbi, I.A. (2008). Application of a circulating-cathodic-antigen (CCA) strip test and real-time PCR, in comparison with microscopy, for the detection of Schistosoma haematobium in urine samples from Ghana. *Annals of Tropical Medicine and Parasitology;* ***102***: 625-33.

Okoli, E.I., and Odaibo, A.B. (1999). Urinary schistosomiasis among schoolchildren in Ibadan, an urban community in south-western Nigeria. *Tropical Medicine and International Health;* ***4(4)***: 308-315.

Opara, K.N., Udoidung, N.I. and Ukpong, I.G. (2007). Genitourinary schistosomiasis among preprimary school children in rural community within the Cross-River Basin, Nigeria. *Journal of Helminthology;* ***81(8)***: 393-397.

Pontes, L.A., Dias-Neto, E., and Rabello, A. (2012). Detection by polymerase chain reaction of *Schistosoma mansoni* DNA in human serum and feces. *American Journal of Tropical Medicine and Hygiene;* ***66***:157-162.

Richter, J., Hatz, C., Campagne, G., Bergquist, N.R. and Jenkins, J.M. (2000). *Ultrasound in schistosomiasis: a practical guide to the standard use of ultrasonography for assessment of schistosomiasis-related morbidity*. 2nd international workshop, October 22-26, Niamey, Niger: World Health Organization.

Shuaibu, U., Saadatu, H.S., Shuaibu, A.H., Vasanthakumari, N., Kumar, S. and Syafinaz, A.N. (2017). Prevalence and molecular characterisation of *Schistosoma haematobium* among primary school children in Kebbi State, Nigeria. *Annals of Parasitology*; **63(2)**: 133-139.

Steinmann, P., Keiser, J., Bos, R., Tanner, M. and Utzinger, J. (2019). Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *The Lancet Infectious Diseases*; **6(5)**: 411-425.

Taofiq, S., Bunza, M.D.A., Majeed, Q., Abubakar, M.B. and Ladan, M.U. (2017). Studies on snail vectors of helminth disease agents along Rima River Valley at Kwalkwalawa Village, Wamakko Local Government Area, Sokoto State, Nigeria. *SM Tropical Medicine Journal*; **2(1)**: 1011.

ten Hove, R.J., Verweij, J.J., Vereecken, K., Polman, K., and Dieye, L. (2015). Multiplex real-time PCR for the detection and quantification of *Schistosoma mansoni* and *S. haematobium* infection in stool samples collected in northern Senegal. *Transactions of the Royal Society of Tropical Medicine and Hygiene;* ***102***: 179-185.

van Delft, F., Visser, L., Polderman, A., and van Lieshout, L. (2007) Cough and alterations in semen after a tropical swim. *Netherland Journal Medicine;* **65:** 304-306.

Van Lieshout, L., Polderman, A.M., and Deelder, A.M. (2000). Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. *Acta Tropica; 77*: 69-80.

Wichmann, D., Panning, M., Quack, T., Kramme, S., Burchard, G.D., Grevelding, C., and Drosten, C. (2019). Diagnosing schistosomiasis by detection of cell-free parasite DNA in human plasma. *PLoS Neglected Tropical Diseases;* **3**: e422.

Xu, J., Rong, R., Zhang, H.Q., Shi, C.J., and Zhu, X.Q. (2021). Sensitive and rapid detection of Schistosoma japonicum DNA by loop-mediated isothermal amplification (LAMP). *International Journal of Parasitology;* ***40***: 327-231.

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