

**Original Research Article**

**EVALUATE THE EFFICACY OF MICROSCOPY AND PCR IN THE  
DIAGNOSIS OF URINARY SCHISTOSOMIASIS AMONG PRIMARY  
SCHOOL CHILDREN IN WAMAKKO LOCAL GOVERNMENT AREA  
OF SOKOTO STATE – NIGERIA**

**ABSTRACT**

**Aims:** The aim of this study was to evaluate the efficacy of microscopy and PCR in the diagnosis of urinary schistosomiasis among primary school children in Wamakko Local Government Area. Sokoto, North-western, Nigeria

**Study Design:** This was a cross-sectional, descriptive study designed to evaluate the efficacy of microscopy and Polymerase Chain Reaction in the diagnosis of urinary schistosomiasis among primary school children in Wamakko Local Government Area. Sokoto, North-western, Nigeria

**Place and Duration of Study:** This research was conducted at the centre for advance research and training Usmanu Danfodiyo University, Sokoto, Sokoto State, from May to July, 2016

**Methodology:** Study was carried out on randomly selected 50 urine samples of Primary school children in Wamakko. The DNA extraction was carried out using the phenol-chloroform method and the extracted DNA was amplified by PCR using a set of primers to detect *Dra* 1 repeat gene fragment at 121bp. The PCR products were detected using Agarose gel electrophoresis

**Results:** Among the study population, *S. haematobium* eggs were detected in 5 subjects while *S. haematobium* DNA was detected in 3 subjects. One of the subject was positive with PCR but negative with microscopy. The sensitivity and specificity for PCR were 60.0% and 95.92%, respectively.

**Conclusion:** : It was concluded that microscopy is the gold standard and that it is suitable for diagnosis of acute urinary schistosomiasis in children. Due to high cost, PCR should be reserved for clinically susceptible cases,

**Keywords:** Efficacy of Microscopy and PCR,; Urinary Schistosomiasis; School Children; Sokoto State, Nigeria.

## INTRODUCTION

Schistosomiasis, originally known as Bilharziasis is a parasitic disease caused by the platy helminth worm of the class Trematoda, genus *Schistosoma* (commonly known as blood-flukes) and is relatively common in developing countries, especially on the continent of Africa and in central rural zones of Egypt and China [1]. In 1851, Theodor Bilharz discovered parasites in portal circulation and associated them with being causal agents of cystitis. Pirajá da Silva first described the pathogenic agent in 1908. Ferguson, in 1911, was the first to report the high frequency of bladder cancer and its etiological relation to urinary bilharziasis [2].

Schistosomiasis is the second leading parasitic challenge to public health after malaria. There are 5 species of *Schistosoma* parasites that produce schistosomiasis in humans and each of them has its respective clinical manifestations [3]: *Schistosoma mansoni* and *Schistosoma intercalatum* cause intestinal schistosomiasis, *Schistosoma japonicum* and *Schistosoma mekongi* cause the Asian variety of intestinal schistosomiasis, and *Schistosoma haematobium* causes urinary schistosomiasis and is most frequent in Africa, the Middle East and the Mediterranean [1]. Schistosomes infect approximately 200 million people in the tropics and subtropics, and of these, *S. haematobium* infects close to 100 million people in 53 countries of Africa and the Middle East. Schistosomiasis is widely distributed in Nigeria. It is hyper-endemic in many states of the north and southwest with moderate to low endemicity in the southeast. *S. haematobium* occurs in nearly all endemic foci while *S. mansoni* is predominantly found in the north and some part of the southwest. [4]. Like other neglected tropical diseases, urinary schistosomiasis is endemic in poor and marginalized Communities [5].

Diagnosis is made through the study of urinary sediment in the search for eggs. Urine were collected between 11:00 and 14:00 hours, considered to be the hours in which there is maximum parasite expulsion. Infection is classified as mild when there are less than or equal to 50 eggs per 10mL and serious when there are more than 50 eggs per 10mL [6].

Despite the numerous available diagnostic technique employed in the diagnosis of urinary schistosomiasis, some practical issues are yet to be clarified regarding the basic diagnostic tools associated with this public health important parasitic disease. The direct microscopic sedimentation technique of urine and examination of urine using filtration method for the detection of *S. haematobium* egg, including indirect methods i.e. detection of haematuria, proteinuria, circulating egg antigens, schistosome specific antibodies and ultrasound scans etc are among the diagnostic techniques [7]. Membrane use in urine filtration remains a common egg concentration technique which thereafter, enables counting of eggs under the microscope. Hence, microscopy is the gold standard in the diagnosis of urinary schistosomiasis [8].

Several Studies had been conducted in detecting *S. haematobium* using polymerase chain reaction. Hamburger and his colleague cloned *S. haematobium* genome repeated sequence, using the Dra I repeated sequence [9].

48 The polymerase chain reaction is a powerful technique that has rapidly become one of the  
49 most widely used techniques in molecular biology because it is quick, sensitive and specific.  
50 The technique utilises DNA polymerase enzymes and synthetic oligonucleotides to produce  
51 billion copies of specific regions of DNA. Polymerase Chain Reaction have proven to be  
52 highly sensitive and specific molecular tool for detecting *S. haematobium* ([10]).

53 Investigators have launched molecular approaches for detecting schistosomiasis; detection of  
54 parasite (schistosome) DNA in clinical human samples such as faeces, sera and urine,  
55 confirms the existence of parasite DNA in the host and provide evidence of the infection.  
56 Parasite DNA may be a helpful guideline for selecting the appropriate treatment for  
57 schistosomiasis [11]. A considerable amount of literature has been published on PCR  
58 methods and is proven to have improve the direct detection of *S. haematobium*. DNA seems  
59 to be prepared from egg prior to PCR amplification from urine, stool or organ biopsy as  
60 samples. Mitochondrial genes have been used in molecular analysis to demonstrate the  
61 relationship between different species, because they show high mutation rates compared to  
62 nuclear markers and exist in high number. More-over, this led to their exploitation for  
63 studying phylogenetic variation in these parasites [12].

64

## 65 **Methodology**

### 66 **Study Design:**

67 Cross sectional study was conducted in Wamakko Local Government Area, Sokoto State,  
68 from May to July, 2016. The study was performed by collecting urine samples as well as the  
69 administration of questionnaire to the participants.

### 70 **Study Area:**

71 Wamakko is located in Sokoto State of Nigeria. It is one of the 23 Local Government Area  
72 that make up the present Sokoto State. The selected area for this study is located at the  
73 extreme North West of Nigeria between longitude 05° 11<sup>1</sup> to 13° 03<sup>1</sup> East and Latitude 13° 00  
74 to 13° 06<sup>1</sup> North. It covers an area of 60.33km square, with an annual rainfall ranging  
75 between 500mm to 1300mm[13] . Dry season starts from October and last up to May and wet  
76 season begins in May and last up to September every year [13]. The predominant tribes of  
77 Wamakko are: Fulani, Hausa and some of the minority tribes such as Yoruba, Igbo etc. the  
78 inhabitants major occupation include: rearing of animals, fishing and petty trading[14] .

### 79 **Study Population**

80 The study population includes both male and female children attending primary school in  
81 Wamakko whose age range between 5 to 16 years old.

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83

84 **Inclusion Criteria:**

85 Participation in the study was based on pupils' willingness and interest. Also, random  
86 sampling method was employed to include primary school children between 5 – 16 years of  
87 age.

88 **Exclusion Criteria:**

89 Primary school children that were unwilling and uninterested to participate, as well as pupils  
90 outside the age range of 5 – 16 years old.

91 **Ethical Consideration**

92 Objectives and procedures of the research were explained to the various school heads and the  
93 participants. An informed consent was obtained from all the respondents and the various  
94 school heads before inclusion using approved protocol. Approval was also obtained from the  
95 ethical committee of Sokoto State Ministry of Health.

96 **Sample selection**

97 Urine samples were collected into well labelled, sterile, leaked proof screw caped universal  
98 container between 10:00 to 14:00 hours for optimal egg passage.

99 **Filtration Technique:**

100 Approximately, 10ml of urine sample passed through What-man No 1 (What-man  
101 international, Maidstone England) filter paper. The filtration was accomplished by vacuum  
102 pump filtration. (Bedford cooperation, Masachusset, USA. The exposed quadrant of the filter  
103 paper which contain the eggs were stained with 1% ninhydrine in acetone solution as well as  
104 lugols iodine and allowed to develop overnight. This helped to increase the visibility of the  
105 eggs. Thereafter, the filter paper was examined under the microscope using x10 objective  
106 lens to check for the presence or absence of the ova of *S. haematobium* [15].

107

108 **Extraction of DNA**

109 The DNA extraction was carried out using the phenol-chloroform method [16](Massimiliano  
110 *et al.*, 2006).1ml of urine sample was spun in an Eppendorf tube at 12,000rpm using high  
111 speed refrigerated micro-centrifuge (Model MX-301 Tokyo, Japan). The supernatant was  
112 discarded and the deposit re-suspended. 250µl of lysis buffer was placed in another  
113 Eppendorf tube and 250µl of the re-suspended sediment was added. The lysate was  
114 centrifuged at 12,000rpm for 5 minutes. 250µl of the lysate was added to another Eppendorf  
115 tube containing 250µl of buffered phenol/chloroform/isoamylalcohol and 25µl of 3M sodium  
116 acetate. It was vortexed (XH-B) for 1 minute. It was incubated on ice for 15 minutes. It was  
117 centrifuged at 12,000rpm for 10 minutes. 250µl of the aqueous layer was picked and added to  
118 a tube containing 250µl of isopropanol. It was incubated at -20°C for 5 minutes to precipitate  
119 DNA. The supernatant was discarded and the pellets were suspended in 500µl of 70%

ethanol. It was vortexed and centrifuged at 12,000rpm. The supernatant was discarded and the deposit was spun quickly to remove residual ethanol. The deposit was air dried in a half opened tube for 30 minutes. 20µl of Tris EDTA was added to the tube and re-suspended to elute the DNA. The purity and concentration of extracted DNA was checked using Spectrophotometer (Bioscience Nano, Shimadzu Corporation).

# **Materials for PCR Amplification of Schistosome *Dra* 1 Repeat Sequences**

The following materials were used: Nuclease free water, 5X master-mix. *Dra* 1 repeat: Forward primers 5<sup>1</sup>GATCTCACCTATCAGACGAAAC3<sup>1</sup> and reverse primers 5'TCACAACGATACGACCAAC 3<sup>1</sup> (Jena Bioscience, Germany).

A modified technique of [17] Hassan *et al.*, (2014), was used for the PCR in this study. The genomic DNA was subjected to PCR amplification of the schistosome *Dra*1 repeat sequence, using forward primers 5<sup>1</sup> GATCTCACCTATCAGACGAAAC3<sup>1</sup>, and reverse primers, 5'TCACAACGATACGACCAAC 3<sup>1</sup>.

These were used to detect the presence of *S. haematobium*, yielding a PCR product of 121bp. For specific amplification of *S. haematobium*, the mix for the PCR was performed in a 50µl reaction mixture containing 10µl of 5X master mix (Jena Bioscience, Germany), 1.5µl of forward primers, 1.5µl reverse primers, 1.5µl of dimethylsulphoxide, 15.5µl nuclease free water and 20µl of the DNA template.

The PCR cycling conditions consisted of initial denaturation at 95°C for 5 minutes, followed by 33 cycles of 95°C for 30 seconds (denaturation), renaturation at 53°C for 90 seconds and extension at 72°C for 1 minute and final extension at 72°C for 5 minutes. It was thereafter, kept on hold at 4°C. The DNA amplification was carried out using Kyrattec Super cycler thermal cycler (Model SC 300, Australia).

# **Agarose Gel Electrophoresis**

The PCR products were electrophoresed in 2% agarose gel (the gel containing 5µl of 10mg/ml of ethidium bromide solution) at 120V for 90 minutes. 100 bp (DNA marker (Promega Madison Wi, USA) was used as the molecular size marker. The documentation was done using Scope 21 UV transilluminator Desktop Gel Imager (Model UVT1513, Tokvo-Japan).

# **RESULT**

Cross sectional experimental study was conducted to evaluate the efficacy of Polymerase Chain Reaction in the diagnosis of urinary schistosomiasis among primary school children in Wamakko Local Government Area of Sokoto state, Nigeria.

**Table 1- Shows the** Sensitivity and specificity of PCR in comparison with microscopy, using 50 urine samples of primary school children. The table showed that; Sensitivity was 60.0%, Specificity (95.92%), Positive predictive value (60.0%) and Negative predictive value (51.09%)

**Figure 1: Shows** M-100bp Molecular marker(Promega), NC –Negative control, 1-10 PCR products of DNA samples from urine of primary school children targeting DRA-1 repeat gene of *Schistosoma Hematobium*; samples 7 and 10 are positive showing the amplified 121bp segment homologous to the target sequences.

**Figure 2: Shows** M-100bp Molecular marker (Promega), NC –Negative control, sample 11 was negative with microscopy but positive with PCR, showing the amplified 121bp segment homologous to the target sequences of DRA-1 repeat gene of *Schistosoma Hematobium*.

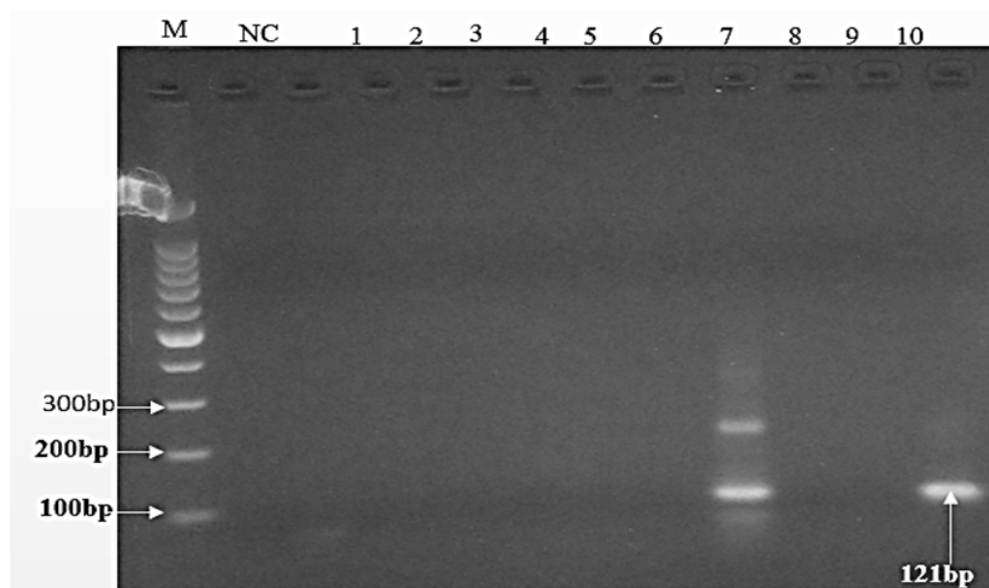
**Table 1: Sensitivity and specificity of PCR in comparison with microscopy, using 50 urine samples of primary school children**

| Variable   | Number   |          | Total |
|------------|----------|----------|-------|
|            | Positive | Negative |       |
| Microscopy | 5        | 45       | 50    |
| PCR        | 3        | 47       | 50    |

Sensitivity = 60.0%

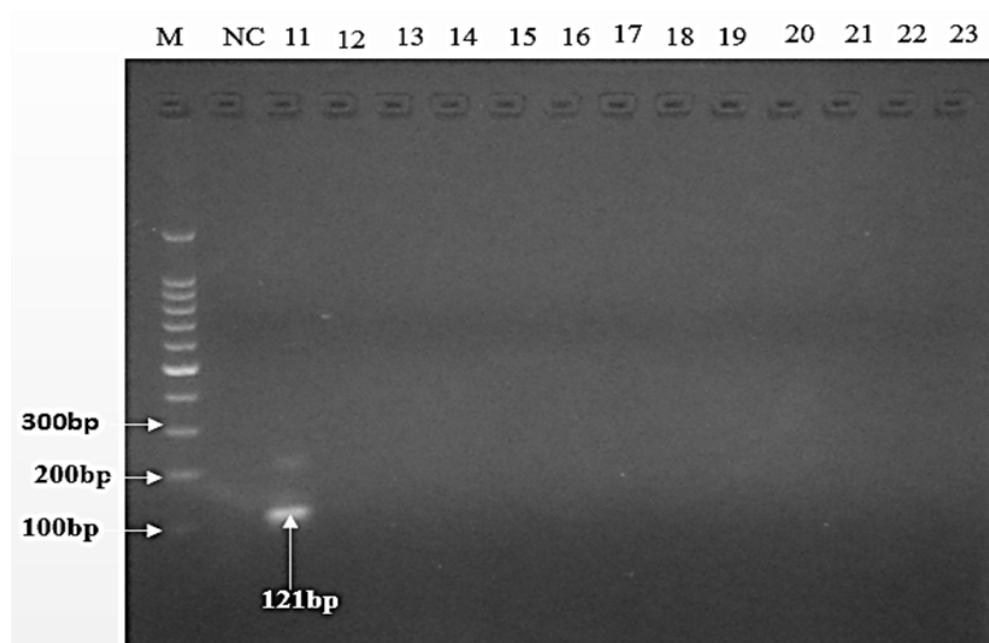
Specificity = 95.92%

177



178

179 **Figure 1:** M-100bp Molecular marker(Promega), NC –Negative control, 1-10 PCR products  
 180 of DNA samples from urine of primary school children targeting DRA-1 repeat gene of  
 181 *Schistosoma Hematobium*; samples 7and 10 are positive showing the amplified 121bp  
 182 segment homologous to the target sequences.



183

184 **Figure 2:** M-100bp Molecular marker (Promega), NC –Negative control,  
 185 sample 11 was negative with microscopy but positive with PCR, showing the  
 186 amplified 121bp segment homologous to the target sequences of DRA-1 repeat  
 187 gene of *Schistosoma Hematobium*.

# DISCUSSION

Diagnostic performance of microscopy and PCR: it is obvious that any environmental setting aimed at eradicating schistosomiasis should consider issues related to detection. This is because most of the commonly used methods may lack necessary sensitivity and specificity to accurately come with prevalence of schistosomiasis or parasitic burden [18].

50 urine samples were randomly selected for the PCR study. Samples 6 – 10 (Figure 2) were positive for urinary Schistosomiasis. While others were negative. M-100bp Molecular marker (Promega USA), NC –Negative control, 1-10 PCR products of DNA samples from urine of primary school children targeting DRA-1 repeat gene of *S. hematobium*; samples 7 and 10 were positive showing the amplified 121bp segment homologous to the target sequences. M-100bp Molecular marker (Promega), NC –Negative control, sample 11 was negative with microscopy but positive with PCR, showing the amplified 121bp segment homologous to the target sequences of DRA-1 repeat gene of *S. Hematobium*.

Findings of the study shows that, the Sensitivity of PCR was 60.0% while the specificity was 95.9%. This does not mean that PCR is not sensitive. Sensitivity, specificity, as well as positive predictive value and negative predictive value are influenced by the prevalence of disease in the population that is being tested. From this study, out of the 50 school children that were randomly selected, 5 were positive with microscopy while 3 were positive with PCR. This very low prevalence is responsible for the low sensitivity value of PCR in the study. A similar study was conducted by [19] who reported a sensitivity of 95.4% and specificity of 96%. And a sensitivity of 100% and a specificity of 60.5% were also reported.

From the results of the study, out of the 5 samples were positive with microscopy, 2 were positive with PCR. Furthermore, one of the samples was positive with PCR but negative with microscopy. It could be that the eggs of *S. haematobium* may not always be the only species of *Schistosomes* that might be found in the urine samples of primary school children in Wamakko Local Government Area, The following *Schistosomes* might be implicated; *S. bovis*, *S. intercalatum*, etc. microscopically the ova of *S. bovis*, *S. intercalatum* look alike under the microscope. Also, in Wamakko Local Government Area where this research was conducted, majority of the Farmers were herdsmen and their cattles drink from the same river where the primary school child have contact with. This is in corroboration with the report of [21] *Schistosoma bovis* is a blood fluke in cattle. It is one of the *Schistosoma* species that share a similar life cycle with *S. haematobium* group of *Schistosomes*. Hybrids between *S. bovis* and *S. haematobium* were first described in 2009 in Northern Senegalese so they are epidemiologically important. Other species of *Schistosoma* include: *S. mansoni*, *S. japonicum*, *S. intercalatum*, *S. mekongi*, *S. malayensis* etc. [22]. The *Dra* 1 repeat primers that were used for this study, are very sensitive and specific to only *S. haematobium*. Sensitivity is the probability that a test result will be positive when the disease is present, specificity is the probability that a test result will be negative when the disease is not present. Positive predictive value is the probability that the disease is present when the test is positive

while negative predictive value is the probability that the disease is not present when the test is negative.

## CONCLUSION

It was concluded that microscopy is the gold standard and that it is suitable for diagnosis of acute urinary schistosomiasis in children. Due to high cost, PCR should be reserved for clinically susceptible cases, areas of low endemicity and light infection, adult samples, where there is doubt about the result of microscopy, presence of other species of *Schistosoma* (*S. bovis*, *S. intercalatum*) other than *S. haematobium*, as well as where there is cross breeding between *S. haematobium* and other species.

Polymerase Chain Reaction (PCR) remains most reliable and highly efficacious laboratory investigative tool for the diagnosis of urinary schistosomiasis caused by *S. haematobium*. The eggs of *S. haematobium* may not always be the only species of *schistosomes* that might be found in the urine samples of primary school children in Wamakko Local Government Area, The following schistosomes might be implicated; *S. bovis*, *S. intercalatum*, etc.

## RECOMMENDATIONS

The recommendations are as follows:

Where possible, PCR should be used to confirm the result obtained from microscopy in order to be able to ascertain the species of schistosome that is responsible for such urinary schistosomiasis. This is because the eggs of *S. haematobium*, *S. bovis*, *S. intercalatum* etc. may be implicated. Furthermore, encouragement of molecular research and studies that will employ the use of different primers for the diagnosis of the various schistosomes that could be found in urine. Sequencing of the urinary schistosomes found in Wamakko Local Government Area.

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