Determination of Urinary Schistosomiasis among School Children in selected Schools in Bekwarra Local Government Area, Cross River State, Nigeria Using Rapid Diagnostic Methods

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ABSTRACT

Urinary Schistosomiasis is caused by the trematode *Schistosoma haematobium*. The disease is characterized by blood in the urine. This study was conducted to ascertain if self-reported haematuria using questionnaire, visible haematuria and micro haematuria using reagent strips could be used as a rapid diagnostic tool for detection of urinary schistosomiasis in the study area. The study was carried out among school children in Bekwarra Local Government Area of Cross River State, Nigeria. A total of 400 urine samples collected from the students were investigated. Analytical procedure employed was detection of haematuria and presence of *S. haematobium* ova in urine using urine sedimentation. Out of the 400 samples observed, 118(29.5%) were infected with *Schistosoma haematobium*. Overall prevalence of micro haematuria, self-reported haematuria and visible haematuria were 94(23.5%), 87(21.8%) and 39(9.8%) respectively. Self-reported haematuria and visible haematuria was highest among male subjects with a prevalence of 63(26.7%) and 28(11.9%) respectively. However, micro haematuria was highest among the female subjects 40(24.4%) as compared to the male 54(22.9%) ($P>0.05$). Self-reported haematuria was highest among the age group 15-19 years 13(25.5%), micro haematuria was highest among the age group 10-14 years 56(26.3%) while visible haematuria was highest among the age group 5-9 years 15(11.0%). However, the variation observed was insignificant ($P<0.05$). The three diagnostic methods employed were not uniform in terms of the results gotten among the eight schools studied. Self-reported haematuria was highest in Community Primary School, Beten 25(50.0%), followed by micro-haematuria in Sacred Heart Primary School, Nyanya 23(46%), while visible haematuria was highest in Community Primary School, Ijibor 12(24.0%). Diagnostic methods revealed that micro haematuria had the highest sensitivity 65(55.1%), followed by self-reported haematuria 50(42.4%) then visible haematuria, 32(27.1%). The proportion of false positive diagnoses was highest in self-reported haematuria 37(9.3%), followed by micro haematuria 29(7.3%) then visible

haematuria 7(1.8%). The findings suggest that reagent strips are rapid method for detection of micro haematuria for identifying individuals and communities infected with *Schistosoma haematobium.*

1. INTRODUCTION

Urinary Schistosomiasis is caused by a trematode known as *Schistosoma haematobium. Schistosoma haematobium* is common among Nigerians, especially in those that live in proximity to water bodies. The first obvious symptom of the infection is blood in the urine also known as haematuria [1]. Urinary Schistosomiasis is transmitted by fresh water snails belonging to the genus Bulinus comprising of the following species; *B. tropicus, B. truncatus, B. forskalli*, and *B. africanus* [2, 3]. Other groups of snails such as the Africanus group (sub-genus *Physopsis*) are involved in the transmission of the disease in eastern, central and West Africa**.**

The truncatus group (sub-genus *Bulinus)* transmits infection in the near East and in some parts of Africa and the forskalli group transmits infection in Nigeria as well as other places in Africa [4].

The role of water bodies in the transmission of infection cannot be over emphasized [4]. Such water bodies include fresh water streams, water accumulated as a consequence of construction of dams and irrigation projects and slow flowing or stagnant water. Water provides an opportunity for the cercariae to survive and penetrate the definitive host. Humans are also pivotal in the transmission of schistosomiasis.

Through insanitary disposal of urine or faeces, water bodies are contaminated with the eggs of schistosomes. Infection occurs through contact with water infested with the free-swimming larval stages of the parasitic worms (cercariae) that penetrate the skin and develop in the human body to maturity [5, 6]. According to Feldmeier *et al.* [7], diagnosis is pivotal in all aspects of human schistosomiasis.

This is because implementation of programmes for the treatment and control of schistosomiasis requires up-to-date information regarding the prevalence and distribution of the diseases [8, 9]. Two major diagnostic techniques have been described as methods mostly used in the diagnosis of urinary schistosomiasis.

They are the direct parasitological techniques (centrifuging or filtering urine to concentrate the eggs) and indirect techniques which includes symptoms, clinical examination, serology and immunological methods [10].

However, since urinary schistosomiasis is a common cause of blood in the urine of people living in endemic areas, the presence of haematuria has been suggested as a rapid assessment method for identifying infected individuals and communities at risk [11]. Examples of such rapid diagnostic methods are the use of macro-haematuria, reagent strips for detection of micro-haematuria and self-reported haematuria [11,12, 13].

The aim of the study was therefore to determine urinary schistosomiasis among school children in Bekwarra Local Government Area, Cross River State, Nigeria using rapid diagnostic methods.

2. MATERIALS AND METHOD

2. 1. Study Area

Bekwarra Local Government area is located in the Northern part of Cross River State, Nigeria with a longitude of 6°41'38N, 8°58'3E and latitude 6°69'89N, 8°96'50E (Fig. 1). It is bounded in the North by Benue State, South by Ogoja Local Government Area, East by Obudu Local Government Area and West by Yala Local Government Area (Fig. 2). It has an area of 306 km^2 and a population of 105,822 at the 2006 census [14]. Average annual rainfall ranges from 859.2 mm to 895.8 mm. The major economic activities of the people in this area include agriculture, cattle/goat and poultry rearing, petty trading. Majority of the indigenes are farmers whose produce are sold within and outside the state. Most communities in Cross River State are typical rural settlements intersected by several fresh water habitats, some of which are man-made ponds, streams and rivers. Members of this community depend largely on the streams, rivers, wells and rainwater to meet their water needs. The schools in the community are scattered in the villages and because of the intersection of streams among villages, the school children usually recreate in these waters during and after school hours, hence the need for this study.

Figure 1. Map of Cross River State, Nigeria showing local government areas
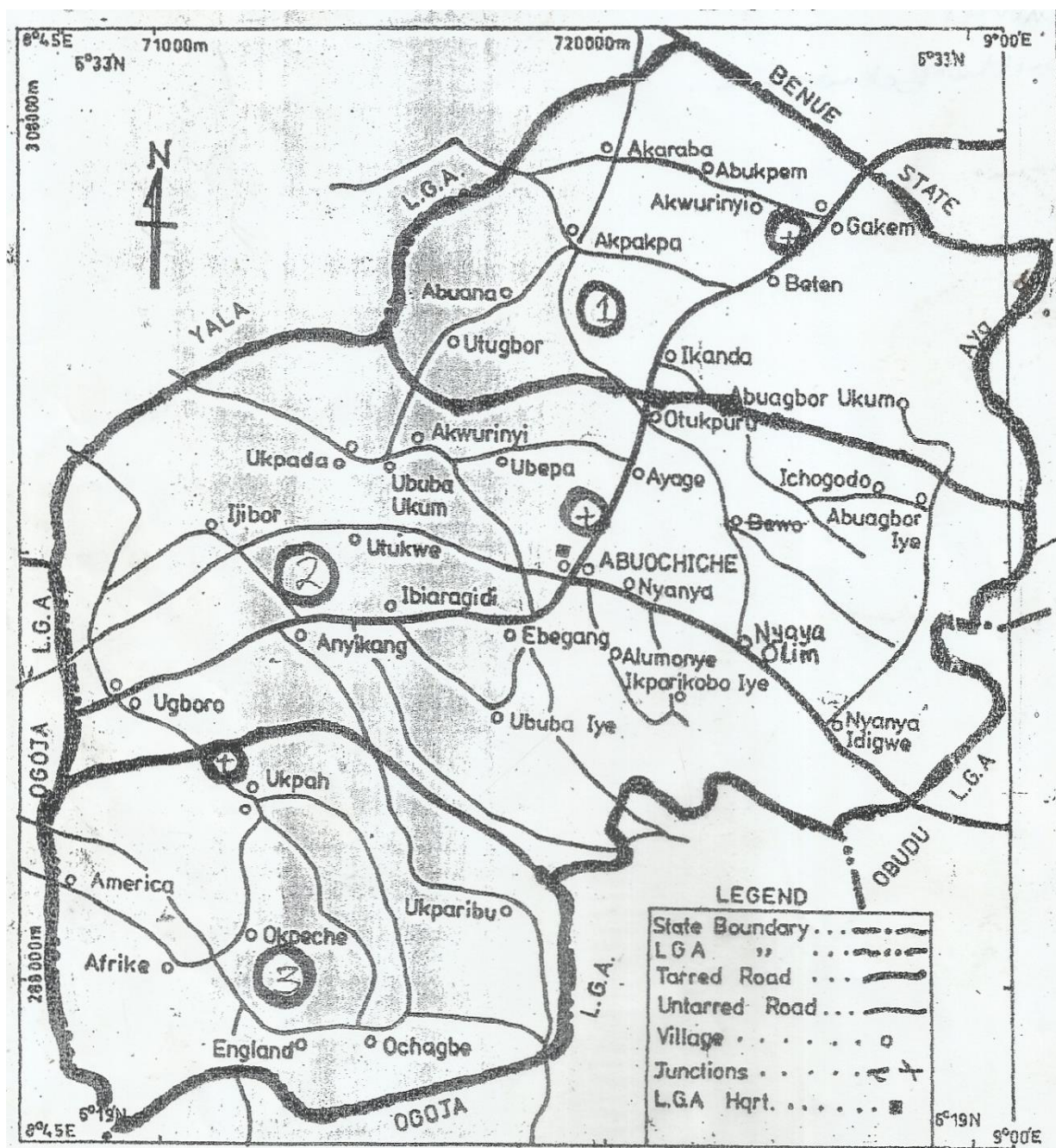
Figure 2. Map of Bekwarra Local Government Area of Cross River State, Nigeria

2. 2. Ethical Consideration

Identification letter was collected from the Head of Department, Biological Sciences, University of Agriculture Makurdi for the Education Secretary, Bekwarra Local Government Area, Cross River state. Consents to carry out the study in the schools was obtained from Local Government Education Secretary and school heads. Approval to collect urine samples of children were also obtained from parents or guardians of the school children one week before

sample collection. A Pre-survey visit was made to the school in order to educate the school authorities and the children about the importance of the study.

2. 3. Sample Collection

Four hundred sterile sample bottles were given to subjects from eight schools who were instructed on how to collect the samples. Each Sterile sample bottle has provisions for number, sex, age and school of each subject. Information on self reported haematuria, water supply as well as water contact activities of children was collected using questionnaires. Using the form, primary school pupils in lower classes (primary 1-4) were interviewed individually. Some of the questions were translated through the assistance of an interpreter and communicated to them in the local language for better understanding, while those in higher classes (primary 5-6 and those in secondary schools) were grouped in their respective classes and were directed on how to fill the form themselves. Urine samples were therefore collected from each subject in a 20 ml sterile universal bottle by children themselves following the provided instructions. All collections were done between 10:00 am and 2:00 pm within the premises of each school [15]. Samples were immediately moved to Model Primary Health Centre Abuochiche, for analysis.

3. ANALYTIC PROCEDURE

3. 1. Detection of visible-haematuria (Visual observation)

Detection of visible-haematuria was done by careful visual examination of the bottled urine specimens for the specific colour. Results were then reported in a form [16].

3. 2. Detection of micro-haematuria (Reagent strips)

Micro haematuria was tested chemically using the urine chemical reagent strip, medi-test combi-9. The chemical reagent strip was dipped into each of the urine samples collected, and each strip was checked for colour change after 1 min. The colour change of the reagent strip was compared with the manufacturer's colour chart to estimate the amount of blood in the urine.

3. 3. Detection and counting of of schistosoma ova

Each urine sample was thoroughly mixed and 10mls of the samples were centrifuged to concentrate the Schistosoma ova if present. The supernatant was removed with a pipette leaving about 0.5 ml of the fluid with the sediment at the bottom of the tube undisturbed. The remaining fluid and sediment were mixed using a pipette and a drop of the mixture was transferred to a microscope slide and covered with a cover slip. The slide was examined for *S. haematobium* eggs using the x10 objective. The number of eggs were counted and reported as egg per 10ml of urine. 1-49 eggs/10 ml urine was considered as light infection and ≥50 eggs/10 ml of urine as heavy infection [17]

3. 4. Data Analysis

Data analysis was done using statistical package for social sciences (SPSS). Chi-square test was used to compare differences in the various age groups, sexes and schools. Correlation test was used to assess the association between intensity of infection and morbidity indicators of urinary Schistosomiasis. The diagnostic accuracy of micro haematuria, visible haematuria

and self reported haematuria were calculated by computing their Sensitivity (SS), Specificity (SP) and Predictive values (PV) against the 'gold standard' of parasitological diagnosis (microscopy).

4. RESULTS

4. 1. Prevalence of self-reported haematuria, visible haematuria and micro haematuria in the Study Population in relation to Sex, Age and School

The overall prevalence of self-reported haematuria, visible haematuria and micro haematuria were 21.8 %, 9.8 % and 23.5 % respectively (Table 1). In relation to sex, highest prevalence of self-reported haematuria and visible haematuria were found among male participants 63 (26.7%), 28 (11.9 %) than female participants 24 (14.6 %), 11 (6.7 %) respectively. However, prevalence of micro haematuria was higher among the female children 40 (24.4%) than male children 54 (22.9 %). There was a significant difference in sex related prevalence between the three morbidity indicators of urinary schistosomiasis ($\chi^2 = 5.235$, $P = 0.073$).

Self-reported haematuria was highest in the 15-19 years 14 (27.5 %) age group while highest prevalence of visible haematuria was found among the 5-9 years age group 15 (11.0 %). However, Visual haematuria showed a decrease in prevalence with an increase in age. Micro haematuria on the other hand was found to be most prevalent in the 10-14 years age group 56 (26.3 %). zWhile the 15-19 years age group recorded the lowest prevalence of micro-haematuria 8(15.7 %). However, the variations observed among the different age groups was not significantly different ($\chi^2 = 4.293$; $P = 0.368$).

There was non-uniformity in the prevalence of self-reported haematuria, visible haematuria and micro haematuria among the eight schools studied; Community Primary School Beten had the highest prevalence of self-reported haematuria 25 (50.0 %) while the lowest was reported in St Clement Primary School Ugboro 2 (4.0 %). On the other hand, higher cases of visible haematuria were recorded in Community Primary School Ijibor 12 (24.0 %), followed by Sacred Heart Primary School Nyanya 10 (20.0 %). Ujia Community Secondary School however, had no visible haematuria recorded. The highest prevalence of micro haematuria infection was found in Sacred Heart Primary School 23 (46.0 %) with Ujia Community Secondary School having the lowest prevalence 4 (8.0 %). The variation observed was significantly different ($\chi^2 = 22.234$; $P = 0.074$; Table 1).

Similarly, the intensity of schistosomiasis infection varied with the different morbidity indicators of schistosomiasis used. 9 (75.0 %) of the participants with self-reported haematuria had high intensity infection. Heavy infection ≥50 eggs/10 ml of urine was found in 12 (100 %) of the participants with micro haematuria while 53(50.0%) had light infection <50 eggs/10 ml of urine. Out of 39 subjects having visible haematuria, 26 (24.5 %) had light intensity infection ≤50 eggs/10 ml of urine (Table 2).

The relationship between self reported haematuria as indicator of urinary schistosomiasis and the true disease status as determined by sedimentation technique showed that self reported haematuria was detected in 87(21.8%) participants out of which 50 (12.5%) had both self reported haematuria and presence of eggs (true positive) in their urine while 37(9.3 %) had self reported haematuria with no presence of eggs (false negative) in their urine. Of the 313 (78.3%) samples screened for not having self reported haematuria, 68 (17.0%) had *Schistosoma*

haematobium eggs (false positive) and 245 (61.3 %) were devoid of eggs (true negative). The ability of self-reported haematuria to be used in identifying all those with the disease (sensitivity) was 50(42.4%), while the ability to correctly sort out all those without the disease (specificity) was 245(86.9%). Positive predictive value (PPV) was 50(57.5%) and Negative Predictive Value (NPV) was 245(78.3%). The ability of visible haematuria to identify all those with the disease (sensitivity) was 32(27.1%), while their ability to correctly sort out all those without the disease (specificity) was 275 (97.5%). Positive predictive value (PPV) was 32 (82.1%) while Negative Predictive Value (NPV) was 275(76.2%; Table 3).

The relationship between micro haematuria as indicator of urinary schistosomiasis and the true disease status as determined by sedimentation technique showed that micro haematuria was detected in 94 (23.5%) out of which 65 (16.3%) had both micro haematuria and presence of eggs (true positive) and 29(7.3%) had micro haematuria with no presence of eggs (false negative). Of the 306 (76.5%) samples screened for not having micro haematuria, 53 (13.3 %) had *Schistosoma haematobium*e ggs (false positive) and 253 (63.3%) were devoid of eggs (true negative).The probability of micro haematuria to identify all those with the disease (sensitivity) was 65(55.1%), while their probability to correctly sort out all those without the disease (specificity) was 253(89.7 %). Positive predictive value (PPV) was 65(69.1%) showing that 30.9% of participants not tested positive have the disease. The Negative Predictive Value (NPV) was 253 (82.7%; Table3).

Table 1. Prevalence of schistosomiasis in relation to sex, age and schools using the three diagnostic methods

Studied Parameters	Number examined	Self-reported haematuria	Visible haematuria	Micro haematuria	χ^2	P-value
		Number positive	Number positive	Number positive		
Sex						
Male	236	63 (26.7)	28 (11.9)	54 (22.9)	5.235	0.073
Female	164	24 (14.6)	11 (6.7)	40 (24.4)		
Total	400	87 (21.8)	39 (9.8)	94 (23.5)		
Age						
5 – 9	136	22 (16.0)	15 (11.0)	30 (22.1)	4.293	0.368
10 – 14	213	51 (23.9)	20 (9.4)	56 (26.3)		
15 – 19	51	14 (27.5)	4 (7.8)	8 (15.7)		
Total	400	87 (21.8)	39 (9.8)	94 (23.5)		
Schools						
Sacred heart's primary Sch. Nyanya	50	21 (42.0)	10 (20.0)	23 (46.0)	22.234	0.074
St David primary Sch. Afrike	50	7 (14.0)	5 (10.0)	10 (20.0)		

St Clement Pry Sch. Ugboro	50	2 (4.0)	1 (2.0)	6 (12.0)		
Community pry Sch. Beten	50	25 (50.0)	6 (12.0)	17 (34.0)		
Community pry Sch. Ijibor	50	8 (16.0)	12 (24.0)	15 (30.0)		
Community Sec Sch. Afrike	50	11 (22.0)	2 (4.0)	8 (16.0)		
Community Sec Sch. Ukpah	50	5 (10.0)	3 (6.0)	11 (22.0)		
Ujia Community Sec Sch.	50	8 (16.0)	0 (0.00)	4 (8.0)		
Total	400	87 (21.8)	39 (9.8)	94 (23.5)		

Table 2. Sensitivity of the three diagnostic methods among subjects as compared to the presence of eggs in urine.

Intensity of infection (Egg/10 ml)	Self-reported haematuria	Visible haematuria	Micro-haematuria	χ^2	P-value
Light(<50/10 ml urine) N = 106	41(38.7)	26(24.5)	53(50.0)	14.695	0.001
Heavy (>50/10 ml urine) N = 12	9(75.0)	6(50.0)	12(100.0)	8.000	0.018
Total N = 118	50 (42.4)	32 (27.1)	65 (55.1)		

Table 3. Sensitivity, specificity and predictive values of the three diagnostic methods among subjects studied.

Parameter	Presence of self-reported haematuria (%)	Presence of Visible haematuria (%)	Presence of Micro haematuria (%)	χ^2	P-value
Sensitivity	50 (42.4)	32(27.1)	65 (55.1)	11.056	0.000
Specificity	245(86.9)	275(97.5)	253(89.7)	15.709	0.000
Positive Value	50(57.5)	2(82.1)	65(69.1)	7.740	0.021
Negative Value	245(78.3)	275(76.2)	253 (82.7)	4.303	0.116

5. DISCUSSION

The results of this study showed that there is a moderate prevalence of urinary schistosomiasis in this study area. The rapid diagnostic method used in this study which are the visual observation for haematuria, the diagnostic chemical reagent strip and self-reported haematuria using questionnaire gave different results for the same group of people with micro haematuria having the highest prevalence and intensity of infection. The prevalence of self reported haematuria in this study was higher than the report of Adie *et al*. [18] and lower

compared to reports of Okon *et al*. [9] and Atalabi *et al*. [20]. According to Chitsulo *et al*. [21] true prevalence of self reported haematuria is usually underestimated since the method relies on re-call as those who are mildly infected may not recall having had haematuria. This could explain the low prevalence recorded in this study since most the infection is of low intensity.

Visual observation for haematuria produced a low prevalence which is similar to the prevalence reported by Ibidapo *et al*. [22] and lower than what was observed by Babatunde *et al*. [23] The difference in the prevalence recorded in this present study may be due to individual variation in assessing different shades of red and the time of the day at which observations were made. The prevalence recorded using chemical reagent strips technique was low as compared to report of Amaechi, [24] but higher than observation made by Robinson *et al*. [25] in Southern Sudan. In the present study, self reported haematuria and visible haematuria showed highest prevalence among males than female children. The prevalence of self reported haematuria for males and females reported by Okon *et al*. [19] was however higher than our findings. The Prevalence of visible haematuria among females as reported by Babatunde *et al.* [23] was higher than our observations. However highest prevalence of micro haematuria was found among the female than the male children. This observation is similar to findings of Morenike *et al*. [26] who also observed higher prevalence among females than male children. The prevalence among males and females in our observation is lower than findings of Amaechi, [24].

The age group 15-19 years had the highest prevalence of self reported haematuria compared to the age group 5-9 years that showed highest prevalence with visible haematuria. Prevalence of micro haematuria however, was found to be highest in the age group 10-14 years. The prevalence of self reported haematuria in our observations contrast the finding of Okon *et al*. [19] where peak prevalence was reported in females 7-8 years and for males, it was reported in the 11-13 years age group. On the other hand, the prevalence of visible haematuria in our study was lower than that reported by Hassan *et al*. [6] among the age group 9-14 years. The authors also reported that the prevalence varied across the age groups. Age related prevalence of micro haematuria detected by reagent strip was similar to what was observed in infection due to *S. haematobium* reported by Morenike *et al*. [26] with highest prevalence recorded in age groups 12-14 years. However, our finding is higher than the findings of Sam-wobo *et al*. [27] Notably, the results of this study deviates slightly from the finding of Ekpo *et al*. [3] who recorded the highest prevalence in children aged 5 years, even though the variation he observed was not significant ($\chi^2 = 4.293$; $P = 0.368$).

The overall prevalence of self reported haematuria, visible haematuria and micro haematuria according to schools varied across the eight schools visited. The reason for this could be that most of the populace in this area engages in rice farming and irrigation where they come in contact with water infected with cerceriae.

The sensitivity and specificity of self-reported haematuria, visible haematuria and micro haematuria varied among the three diagnostic methods used. Micro haematuria had the highest sensitivity, followed by self reported haematuria while visible haematuria had the lowest sensitivity but with high specificity and highest positive predictive valve.The sensitivity and specificity of self-reported haematuria in the schools under survey was similar to what was reported by Bassiouny*et al*. [28], but lower than that obtained by Adie *et al.* [18], and Useh & Ejezie [29]. Our specificity was higher than the observation of Useh & Ejezie [29]. According to Ugbomoiko *et al*. [13] sensitivity of visible haematuria is usually low, but may increase in the presence of heavy infection. This is similar to our finding were we observed low sensitivity

and high specificity of visible haematuria. Our finding is closely related to the observation by Morenike *et al*. [26] who reported low sensitivity and high specificity in an endemic rural area of Nigeria. Sensitivity and specificity of visible haematuria in this study are lower than that reported by Ugbomoiko *et al*. [13] in two rural communities in Osun State, Nigeria. The rapid diagnosis of micro haematuria as indicator of urinary schistosomiasis showed low sensitivity and specificity as compared to findings of Houmsou *et al*. [30] who reported high sensitivity and specificity in Katsina-Ala and Buruku LGAs of Benue State, Nigeria.

This study is related to findings of Hassan *et al*. [6] who reported both low sensitivity and specificity among school children in endemic areas. However, the sensitivity and specificity of micro haematuria in our study is higher than that of Okeke and Ubachukwu [31] in lshielu LGA of Ebonyi, Nigeria. Several observations on high sensitivity and specificity had been reported in different African settings such as those reported by Rollinson *et al*. [9] in southern Sudan and French *et al*. [8] in a longitudinal study of school children from Zanzibar. Also a research from Ghana showed high sensitivity and specificity for urine reagent strips positive for haematuria and *S. haematobium* infection (Kosinskiet *et al*. [32].

Variations observed in the sensitivity and specificity values of micro haematuria in our studies could be due to regional differences of prevalence and the intensity of infection of urinary schistosomiasis as well as the varying quality of the reagent strips from different producers. According to Hassan *et al*. [6] since the predictive values depend on the prevalence of disease and intensity of infection, in higher endemic settings the positive predictive value may be different, this agrees with our finding as we also observed different predictive value among the three methods used.

6. CONCLUSION AND RECOMMENDATION

The results of this study showed that there is a moderate prevalence of urinary schistosomiasis in the study area. The findings also suggest that reagent strips are rapid method for detection of micro haematuria for identifying individuals and communities infected with *Schistosoma haematobium*. Therefore, it can be used as a rapid diagnostic tool for detecting infection with S. *haematobium* in patients.

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