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Comparison of Polycarbonate Filter Paper and Sedimentation Methods in Diagnosing *Schistosoma haematobium* Infection in Makurdi, Benue, Nigeria

V. U. Obisike[1*], E. U. Amuta[1], A. B. Audu[2] and S. A. Kwenev[2]

[1]Department of Zoology, University of Agriculture, Makurdi, Nigeria.
[2]Department of Biological Sciences, Benue State University, Makurdi, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Schistosomiasis affects about 204.6 million people in 78 countries of the world, out of which 58 countries are endemic with moderate to high intensity. Different diagnostic tools (Rapid Diagnostic Test kit) have been used in the diagnosis of *Schistosoma haematobium* infection hence, there is the need to ascertain the most sensitive RDT for proper screening of *Schistosoma haematobium*. This Study was undertaken to comparatively examine whether there is an association between urine colour observation and intensity of *Schistosoma haematobium* infection using polycarbonate membrane filtration method and sedimentation methods. A total of 202 (118 males and 84 females).urine samples were examined from some selected primary schools in Makurdi. Of these, 52 (25.74%) tested positive of *Schistosoma haematobium* using filtration technique. The findings of this research demonstrated that urine colour observation was significantly associated with infection intensity (X^2 = 0.721, p<.01). Three (3) Out of 67 urine sample screened having brown colour, 19(32.8%) tested positive for *Schistosoma haematobium* ova with for light, mild and heavy infection (14, 5 and 3 respectively). The study also revealed a significant association between membrane

**Corresponding author: E-mail: talk2vu@gmail.com;*

filtration and sedimentation techniques (r= 0.964). However, the findings of this research revealed that membrane filtration technique is more sensitive RDT than conventional sedimentation technique.

Keywords: Schistosoma haematobium; diagnostic; techniques; Membrane filtration; sedimentation.

1. INTRODUCTION

Schistosomiasis is a disease caused by infection with schistosome parasites. Schistosomes are important digenetic trematode parasites of man and livestock. Globally, schistosomiasis rank second among parasitic diseases of public health importance and is found in 48 African countries. It is estimated that 200 million people are infected, of which 120 million people are symptomatic and 20 million have severe disease. In 74 countries, 600 million people are at risk of infection [1]. Schistosomiasis is one of the majorly diseases of tropical and subtropical regions and is found in South and Central America, Africa, Asia and South-East Asia. Estimate suggests that 85% of all schistosomiasis cases are now in sub Saharan Africa sub-region [1]. In Nigeria, one of the most severally affected countries in Africa, it is estimated that 101.28 million people are at risk of the infection while 25.8 million people are infected with *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma intercalatum* [1].

This Study was designed to comparatively examine whether urine colour observation could be correlated with intensity of infection in urinary schistosomiasis as measured by the gold-standard parasitological diagnosis.

Makurdi is the capital of Benue State of Nigeria. The city is located in central Nigeria along the Benue River and holds the base for the Nigerian Air force aircraft squadrons. Makurdi has an estimated population of 500,797 persons [2]. It holds the Benue State University and the Federal University of Agriculture. It is located on latitude 7.7306^{o}N and longitude 8.5561^{o}E along the banks of the Benue River which is a major tributary to the Niger River. It is an agricultural catchment area and has a variety of potentials in human capital and material resource [2]. Although populations living within the township area of Makurdi Local Government Area depend largely on boreholes for their drinking water, other rural populations living in rural areas have to depend on the surrounding streams.

2. MATERIALS AND METHODS

The study was carried out between September 2017 and December 2017. Farming is the major occupations. Membrane filtration techniques and Sedimentation methods were compared as methods of evaluating the prevalence of *Schistosoma haematobium* infection in Makurdi Benue State-Nigeria.

2.1 Study Population and Samples Collection

Ethical consent was sought from the Local Government Education Authorities and the Benue State Ministry of Health. Head Teachers of the school children were enlightened on the importance of the study.

Urine samples were collected from 202 primary school pupils. About 20 ml of clean-catch, mid stream urine samples were collected in a 20 ml capacity autoclaved wide mouthed, leakproof universal bottles. Samples were obtained between 10AM hrs and 14PM each day, according to the method of Cheesbrough [3]. Samples were screened for urine colour and samples were appropriately labeled with unique identification numbers, ordinary household bleach was added to the urine samples to preserve any schistosome ova before placing in a cooler. These samples were transported to laboratory for proper examination for the present of *Schistosoma haematobium* ova [3].

2.2 Indirect Reagent Strip Technique

Reagent strips (Medi-Test Combi 10) were dipped into the urine inside the universal containers. Various parameters such as Glucose, Protein, Blood, Urobilinogen, Nitrite, Bilirubin pH were evaluated according to the manufacturer's instructions.

Ova were recovered from the urine samples by the filtration technique. Using blunt-ended (untoothed) forceps, a polycarbonate membrane filter was placed carefully on the filter support of the filter holder (13 mm diameter) and attached

to the end of a 10ml Luer syringe. The plunger was removed from the syringe before the syringe was filled to the 10ml mark with well-mixed urine after which the plunger was replaced.

The syringe was held over a beaker and the urine sample was gently passed through the filter. The filter holder was removed and unscrewed before a blunt-ended forceps was carefully used to remove the membrane filter [3]. This was transferred in such a way that recovered ova were intact on the filter paper by facing the filter paper up and placed to a slide. A drop of Lugol's iodine was added to the slide and covered with a cover slip. Using the × 10 objective with the condenser iris closed sufficiently to give good contrast, the entire filter was examined systematically by adjusting the microscope until a clear image of the ova of *S. haematobium* were seen. The number of eggs are counted and reported as egg per 10ml of urine, 1-10 eggs/10 ml urine was considered as light infection, 11-49 eggs/10 ml of urine as mild infection and >50 eggs/10 ml of urine as heavy infection.

2.3 Detection of ova of Schistosome Flukes by Sedimentation

Detection of the ova of *S. haematobium* was done by microscopic examination of urine samples [4]. Concentration method was done where 10 mls of urine was centrifuged at 2000rpm for 3 minutes in order to concentrate eggs of *Schistosoma*. The was supernatant decanted and deposit tabbed gently and a drop was placed on a clean slide which was carefully covered with cover slip and viewed under the microscope first with x10 and x40 objective lens. Eggs were preserved in refrigerator.

Microscopic examination of the samples was performed at the Laboratory of Biological Sciences Department, Benue State University, Makurdi. Using direct microscopic method [3].

2.4 Data Analysis

Chi-square was used to determine the association between intensity of infection and urine colour while correlation was used to determine relationship in sensitivity between Polycarbonate membrane and sedimentation methods. Simple percentage was used to determine the prevalence of *Schistosoma haematobium* infection.

3. RESULTS

Urine colour was observed from urine samples of 202 pupils aged 7-18 years (118 males and 84 females).Of these, 52 (25.74%) tested positive for *Schistosoma haematobium* ova using the filtration technique. Urine was observed and a unique number was assigned to each of the colours observed using urine colour chart , numbers ranging from 1 to 4, with colur 1 (light-yellow) , colour 2 (light brown), colour 3 (brown) and 4 (dark red urine or bloody urine)..

Table 1 shows the sensitivity of membrane filter paper and sedimentation techniques. Of the 202 urine samples screened, filter paper detected 52 positive for *Schistosoma haematobium* eggs while sedimentation technique detected 41 positive of *Schistosoma haematobium* eggs. Correlation was significant, r= 0.964. Prevalence was higher in males (27.1%) than in females (23.8%) as shown in Table 2.

Fig. 1 and Table 3 show the relationship between urine colour and the intensity of *S. haematobium*. Out of 67 urine screened having brown colour, 19 (32.8%) tested positive of Schistosoma *haematobium* eggs with 14, 5 and 3 for light, mild and heavy infection respectively. Of the 43 screened having bloodbrown colour, 11(25%) were found with *Schistosoma haematobium* eggs with 4, 2 and 5 light, moderate and heavy infection respectively. While, of the 64 urine screened having light yellow colour, 19 (29.7%) were found positive for *Schistosoma haematobium* eggs with 11, 8 and 0 light, moderate and heavy infection respectively.

Table 1. Sensitivity of filter paper/ sedimentation technique

Sedimentation	Polycarbonate membrane filter		
	Positive	**Negative**	**Total**
Positive	41	0	41
Negative	11	150	161
Total	**52**	**150**	**202**

r= 0.964.

Table 2. Sex related prevalence *Shistosoma haematobium*

Sex	Number examined	Number positive (Prevalence)
Female	84	20 (23.8)
Male	118	32 (27.1)
Total	**202**	**52 (25.7)**

Table 3. Intensity of infection in relation to Urine Colour

		Intensity		
Urine colour	**Number examined**	**Light**	**Moderate**	**Heavy**
Light yellow	64	11 (17.2)	8 (12.5)	0
Brown	67	14 (20.9)	5 (7.5)	3(4.5)
Dark brown	43	4 (9.3)	2 (4.7)	5(11.6)

$X^{2=}$0.721, df= 4, P>0.05

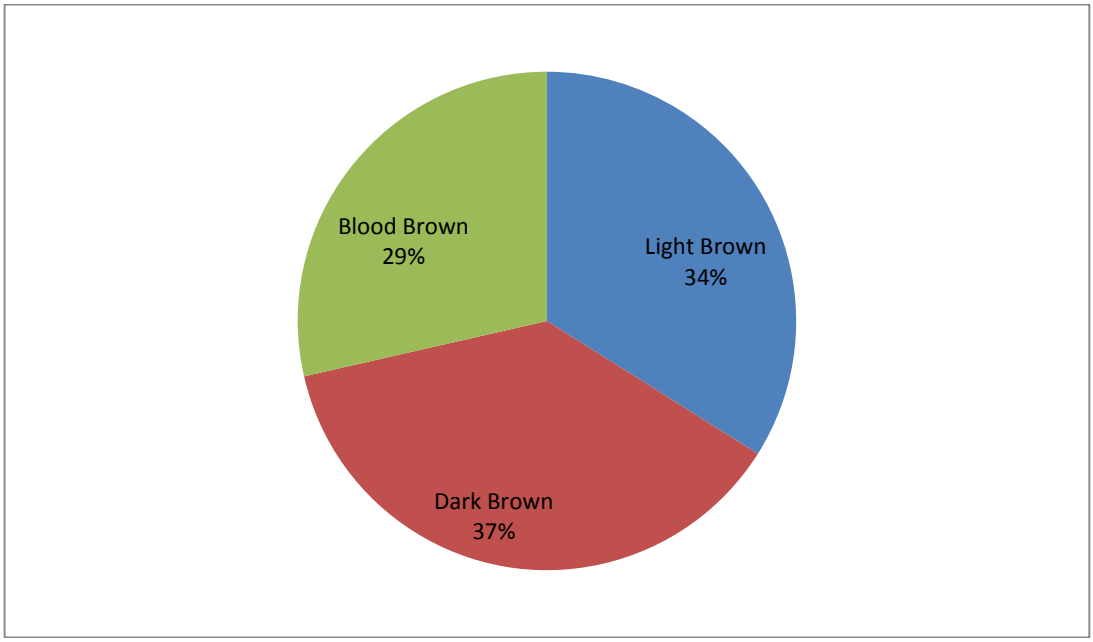

Fig. 1. Prevalence of *Schistosoma haematobium* in relation to urine colour

4. DISCUSSION

The focus of this study was to compare the different filed diagnostic methods evaluating infection status with *Schistosoma haematobium*. The result has demonstrated that filtration technique is more sensitive diagnostic method than the urine, colour and sedimentation techniques. Correlation revealed the significant association between filtration and sedimentation technique. In the field, filtration technique gives a rapid result and is less cumbersome. The higher sensitivity and high egg count recorded with filtration technique could be linked to the fact that the 10ml urine is made to pass through the filter thereby, making it possible for any Schistosoma egg to be trapped unlike the filtration where some of the eggs may either be decanted alongside with the supernatant or may stick to the wall of the test tube.

Some of the blood-brown and brown coloured urine that showed negative result may be an indication of other possible Urinary Tract Infection (UTI). The brown colour of urine might be the result of excreted protein and red blood cells in the urine from the damaged of urinary tract and kidney. Inconclusive evidence suggests that *Schistosoma haematobium* affects the glomeruli, the units of the kidney that function to separate out wastes and extra fluid from the blood. When the glomeruli are damaged, protein and often red blood cells leak into the urine as this might be the case in this study. Previous study stated thus “athough, at the present time the precise origin and clinical significance of the proteinuria observed in *S. haematobium* infection remains unknown claimed an association between glomerulonephritis, the inflammation of the membrane tissue of the kidney and *S. haematobium* infection in human” [5].

This result shows the current prevalence of urinary schistosomiasis in Makurdi as 49 (24.5%). The prevalence is low when compared to the rates reported in other parts of Nigeria as

reported by Ogwumike a high prevalence of 45.4% in Aninri LGA in Enugu State, similarly a prevalence of 42.3% was reported in Abia State, South Eastern Nigeria [6,7].

More so, other studies reported prevalence rates comparable or even lower than our findings. For Example, the prevalence rates in some parts of Borno and Ebonyi State were 24.3% and 21.5% respectively [8] also recorded an overall prevalence rate of 17.8%, [9], recorded a prevalence rate of 15.7%; and [10] reported a prevalence rate of 17% of the infection within Gboko town. Socio-economical and geographical differences may be responsible for the differences in the prevalence. Other conditions that may affect the prevalence of urinary schistosomiasis such as contact with water contaminated with the cercariae of the parasite, the age of study subjects may have also played a vital role in the findings.

Prevalence of urinary schistosomiasis was higher amongst males than in females. The gender of study participants could influence the prevalence of schistosomiasis due to differences in attitude of such persons regarding water use and contact. More males than females are predisposed to the infection due to regular and longer contact with the breeding site of the disease vectors through farming and swimming. Among other reasons the high value of prevalence among males shows that males are more exposed to this parasite either through swimming, or they are engage in other activities that make them have contact with the river and other water bodies, aside the domestic chores of washing and fetching water which exposes both sexes to infection. The prevalence of parasites infection between male and female school children was not significant statistically. This difference in prevalence is however statistically not significant. This could mean that within Makurdi LGA prevalence of urinary schistosomiasis or contact with contaminated water is not gender related.

5. CONCLUSION

The colour observed in the urine sample screened showed a strong association with egg count and intensity. The urine colour could serve as a pointer in field diagnosis of *Schistosoma haematobium* infection. There is need for mass drug administration as it will go a long way to reduce infection and morbidity associated with urinary schistosomiasis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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