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The Merits of Urine Color Observation as a Rapid Diagnostic Technique to Estimate *Schistosoma Haematobium* Infection in Two Endemic Areas of Benue State, Nigeria

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1. Introduction

Schistosomiasis is the most prevalent parasitic infection in the world after malaria, with nearly 207 people infected, and 779 million currently at risk in 76 countries of the tropics where the disease is endemic (Steinmann *et al.*, 2006).

In sub-Saharan Africa, about 192 million are found to be infected with schistosomiasis (Hotez and Kamath, 2009). The highest prevalence and intensities of human schistosomiasis occur in school-aged children, adolescents, and young adults who also suffer from the highest morbidity and mortality.

Urinary Schistosomiasis due *Schistosoma haematobium* is a significant cause of clinical morbidity and disability in the endemic countries of Africa and the Middle East, where more than 110 million people are infected (van der Werf and de Vlas, 2001).

In sub-Saharan Africa two-thirds of the schistosomiasis cases are due to *Schistosoma haematobium*, which represents an important cause of severe urinary tract disease (van der Werf *et al.*, 2000). They also estimated that 70 million and 32 million individuals out of 682 million people in sub-Saharan Africa had experienced hematuria and dysuria, respectively, within the last two weeks of their reports. *S. haematobium* produces bladder wall pathology in approximately 18 million people in sub-Saharan Africa, and 10 million people suffer from hydronephrosis (van der Werf *et al.*, 2000). Renal failure accounts for a large percentage of the estimated 150,000 deaths from urinary tract schistosomiasis in sub-Saharan Africa, and significant association was observed between major bladder wall pathology and squamous cell carcinoma (Maxwell, 2008). A significant percentage of women and men with urinary schistosomiasis acquire genital ulcers and other lesions (King and Dangerfield-cha, 2008).

Identification of cases or communities for treatment with *Schistosoma haematobium* infection is usually based on microscopic detection of eggs in urine. Haematuria and proteinuria are recognized clinical features of *S. haematobium* infection (Wilkins, 1977). Many epidemiological studies have been conducted to investigate the characteristics of these methods to measure urinary schistosomiasis; this usually involved comparing the outcomes with intensity of infection.

As part of early and successful control with chemotherapy, rapid, cost-effective and reliable diagnostic tools and assay that will play an important role in assessing cases of infection are needed in the present time. Thus, the study was designed to determine if urine colour can be used as a potential diagnostic tool for rapid screening of urinary schistosomiasis in endemic areas and to compare it with other diagnostic methods such as filtration technique and reagent strip tests.

2. Materials and methods

2.1 Study area

The study was conducted in two contiguous local government areas (Buruku and Katsina-Ala) of Benue State endemic for urinary schistosomiasis (Houmsou *et al.*, 2009). The selection of the areas was based on previous reports from local hospitals, clinics and health centers where cases of urinary schistosomiasis were common particularly among school children. Other parasitic infections like onchocerciasis, malaria and parasites of the gastro-intestinal tract were also reported by health officials. The relative position of the two Local Government Areas in Benue State is about the Middle Eastern part of the State. The areas are drained by streams and rivers among which river Katsina-Ala is the biggest; ponds are also found all over the areas (Figure 1). The areas have a monthly temperature ranging from 27-38°C and 900-1000 mm of rain fall annually with two distinct seasons: the dry season starts in late October and usually ends by March, while the rainy season lasts from mid-April to early October.

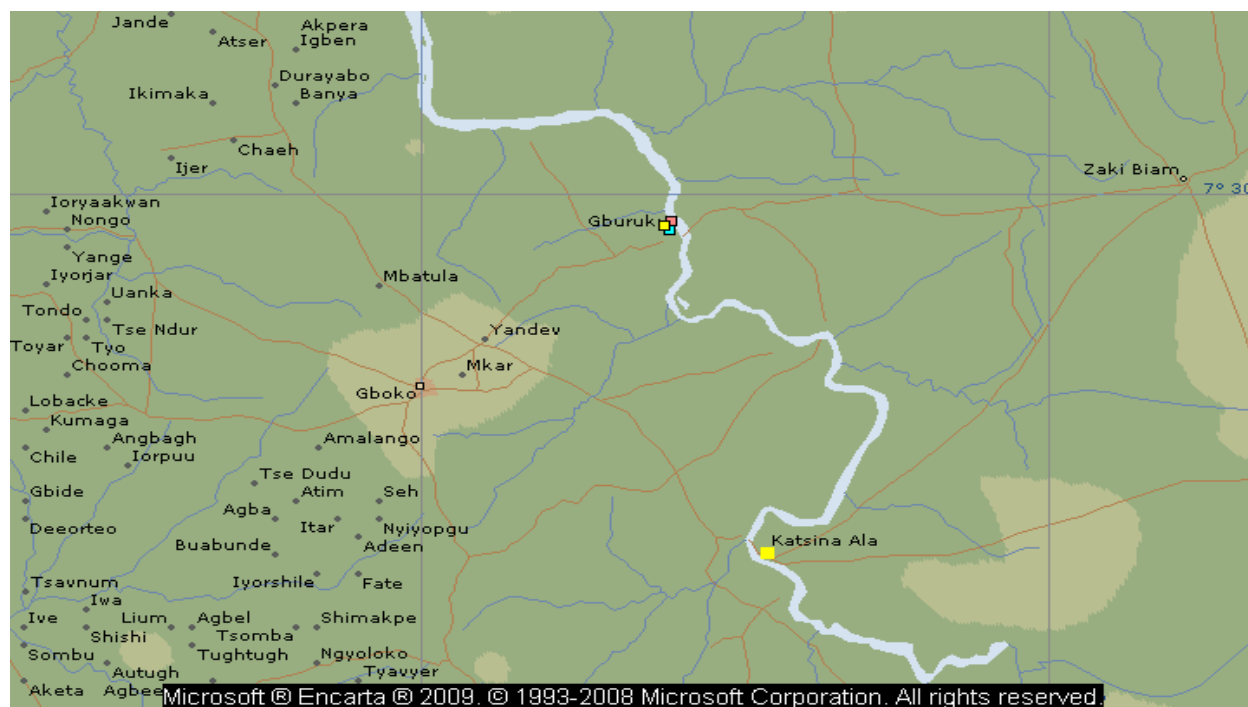


Fig. 1. Physical Map of Buruku and Katsina-Ala LGAs of Benue State, Nigeria (Encarta 2008)

2.2 Study population and samples collection

Prior to the commencement of the research, ethical approval was sought from the Ministry of Health, Benue State and the Local Government Education Authorities of both areas. Parents of the school children were duly informed on the significance of the study.

A total of 1,292 urine specimens were collected from apparently healthy individuals aged 1-30 years, whom were carefully instructed to collect the last part of their urine. About 20 ml of clean-catch, midstream urine samples were collected in a 20 ml capacity autoclaved wide mouthed, leak, proof universal containers. Samples were obtained between 10:00 hrs and 14:00 hrs of the day (Cheesbrough, 2000). Urine specimens were visually inspected for colour and graded, from (1) as urine free of any trace of microhaematuria or proteinuria (light-yellow) to (3) as red urine (blood urine). The specimens were appropriately labeled with identification numbers and placed in a cold box. Where delay in transportation of specimens to laboratory was inevitable, ordinary household bleach was added to the urine samples to preserve any schistosome ova present (Cheesbrough, 2000; W.H.O., 2003).

2.3 Determination of microhaematuria and proteinuria

Reagent strips (Medi-Test Combi 9, Macherey-Nagel GmbH & Co.KG, Germany) were dipped into the urine inside the universal containers. Microhaematuria and proteinuria were evaluated and results were ranked as negative (-ve), trace(+), positive(++), (+++) according to the manufacturer's instructions. Concentrations (Ca) of microhaematuria and proteinuria were measured as erythrocytes/ μ l (ery/ μ l) and mg/dl of albumin respectively. The degree of microhaematuria and proteinuria concentrations were graded as follows: 0 (negative), Ca.5-10 (+), Ca.50 (++) and Ca.250 (+++) and 0 (negative), Ca.30 (+), Ca.100 (++) and Ca.500 (+++) respective.

2.4 Egg counts

Eggs were recovered from urine by the filtration technique. Filtration is the most sensitive, rapid, and reproducible technique for detecting and quantifying *S. haematobium* eggs in urine. Using blunt-ended (untoothed) forceps, a polycarbonate membrane filter (13 mm diameter and 13 μ m porosity) 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 is filled to the 10ml mark with well-mixed urine after which the plunger was replaced. Holding the syringe over a beaker the urine was slowly passed through the filter. The filter holder is removed and unscrewed before a blunt-ended forceps is carefully used to remove the membrane filter. This was transferred face upwards (eggs on surface) to a slide before addition of a drop of Lugol's iodine with subsequent covering using a cover glass. Using the 10X objective with the condenser iris closed sufficiently to give good contrast, the entire filter was examined systematically for eggs of *S. haematobium*. The number of eggs are counted and reported as egg per 10ml of urine, 1-49/10 ml urine was considered as light infection and ≥ 50 eggs/10 ml of urine as heavy infection (W.H.O., 2003).

2.5 Statistical analysis

Microsoft Excel 2007 and PASW (Predictive Analysis software) version 18.0.were used to perform data analysis. Associations between urine colour, microhaematuria, proteinuria and intensity of infection were tested using Spearman correlation (*rho*). The significance level was considered at $p < 0.01$.

2.6 Evaluation of diagnostic performance

The diagnostic performances of urine colour observation, microhaematuria and proteinuria were assessed by calculating sensitivity, specificity, positive and negative predictive values using the following formulae.

- Sensitivity = $\frac{a}{a+b}$ with a = True positive
b = False negative
- Specificity = $\frac{c}{c+d}$ with c = True negative
d = False positive
- Positive predictive value (PPV) = $\frac{a}{a+d}$
- Negative predictive value (NPV) = $\frac{c}{c+b}$

2.7 Results

Urine was visually inspected and assigned a number. Figure 2.0 illustrates the urine colour chart related to the presence of proteinuria and microhaematuria. The urine colour chart ranges from 1 to 3, with 1 indicating urine free of any trace of microhaematuria or proteinuria (light-yellow) and 3 corresponding to red urine (blood urine). Whereas number 2 grouped as brown colour corresponds to visually discernable microhaematuria and proteinuria present in the urine.



Key: 1 = urine free of any trace of haematuria and proteinuria
2 = urine with presence of haematuria and proteinuria
3 = urine with visible blood

Fig. 1. Urine colour observation

The relationship between urine colour observation and microhaematuria among subjects examined in Buruku and Katsina-Ala LGAs is shown in Fig.3. Of the 666 subjects screened having brown colour, 322(48.3%) had microhaematuria with a breakdown of 21.8%, 10.7% and 15.9% for microhaematuria at Ca.5-10, Ca.50 and Ca.250 respectively. Out of the 48 screened having blood urine, 45(93.8%) had microhaematuria at Ca.250, while 3(6.3%) having light yellow colour of urine had microhaematuria at Ca.5-10. A significant relationship was observed between urine colour observation and microhaematuria ($\rho=0.5, p<0.01$).

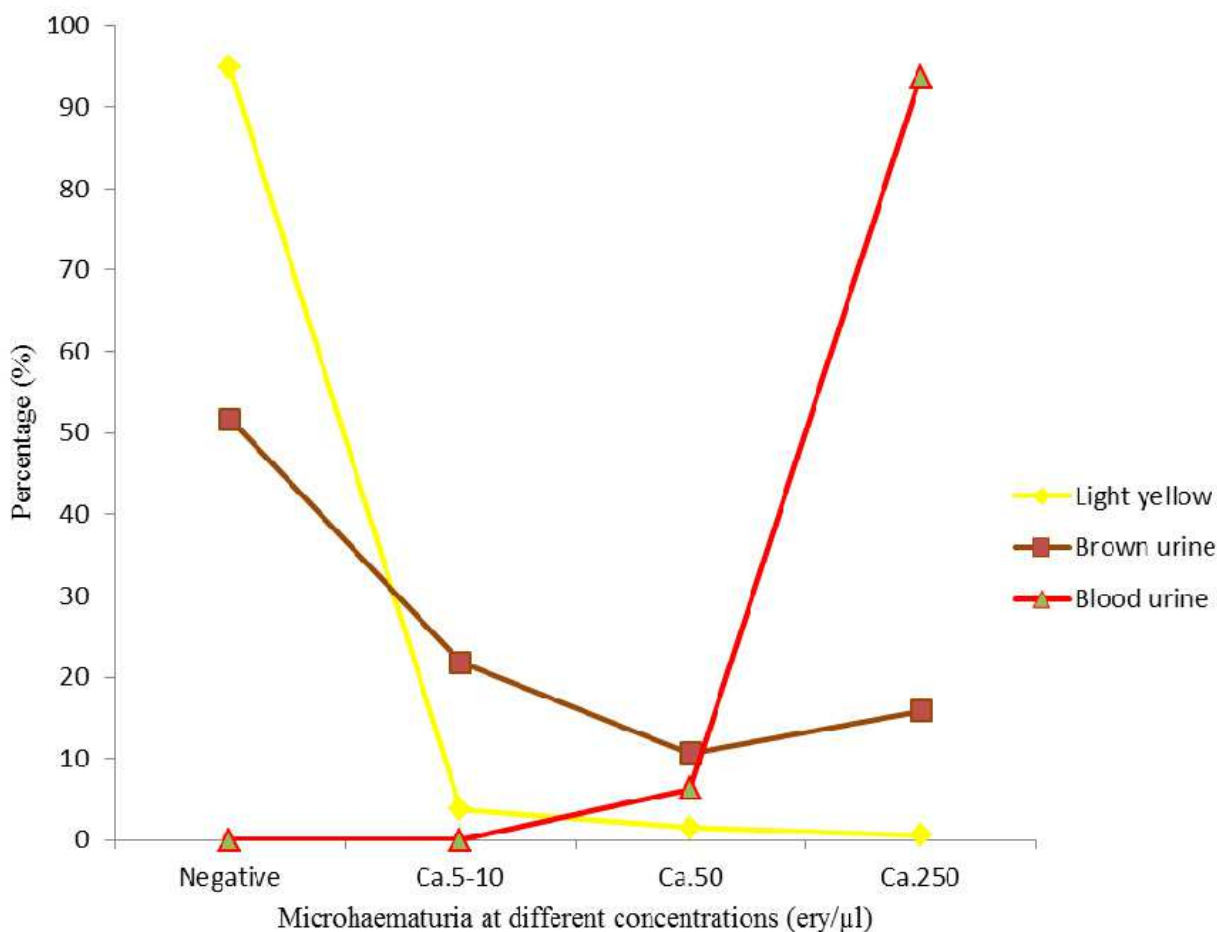


Fig. 2. Relationship between urine colour observation and microhameturia among subjects examined in Buruku and Katsina-Ala LGAs of Benue State

The relationship between urine colour and proteinuria among subjects examined in Buruku and Katsina-Ala LGAs of Benue is shown in Fig.3. Of the 666 screened having brown urine, 604(90.7%) had proteinuria with a breakdown of 301(45.1%), 208(31.2%), 95(14.3%) for proteinuria at Ca.30, Ca.100 and Ca.500 respectively. Out of the 48 screened having blood urine, 33(68.8%) had proteinuria at Ca.500, while 78(15.5%) of the 578 having light yellow colour urine had proteinuria at Ca.30. A significant relationship was observed between urine colour and proteinuria ($\rho=0.7, p<0.01$).

Figure 4 shows the relationship between urine colour observation and intensity of *Schistosoma haematobium* eggs amongst subjects examined in Buruku and Katsina-Ala LGAs of Benue State. Of the 1,292 subjects screened for urine colour, 578 had light yellow (normal colour) among which 46(8.0%) and 3(0.5%) had 1-49eggs/10ml of urine and ≥ 50

eggs/10ml respectively. Of the 666 having brown colour urine, 378(56.8%) and 80(12.0%) had 1-49eggs/10ml and ≥ 50 eggs/10ml of urine respectively. Out of the 48 having blood urine, 38(79.2%) had ≥ 50 eggs/10ml of urine. A significant relationship was found between urine colour and intensity of *Schistosoma haematobium* eggs among participants examined ($\rho=0.6, p<0.01$).

Table 1.0 compares urine colour observed as an indirect test and the true disease status as determined by filtration technique in Buruku and Katsina-Ala LGAs of Benue State. When Compared to the true disease status, light yellow (normal colour) of urine is considered as negative and this was observed in 529(91.5%) participants having no *S. haematobium* eggs (true negative) and 49(8.5%) having *Schistosoma haematobium* eggs (false negative). Of the 714 participants considered positive (brown + blood urine), 504(70.6%) had *S. haematobium* eggs (true positive), while 210(29.4 %) had no *S. haematobium* eggs (false positive).

Table 2 validates observed urine colour as an indirect test in the screening of urinary schistosomiasis in Buruku and Katsina-Ala LGAs of Benue State. The ability of the observed urine colour to accurately identify all those with the disease (sensitivity) was 91.1%, while its ability to correctly sort out all those without the disease (specificity) was 71.6 %. The positive predictive value (PPV) was (70.6 %), while the Negative Predictive Value (NPV) was 91.5%.

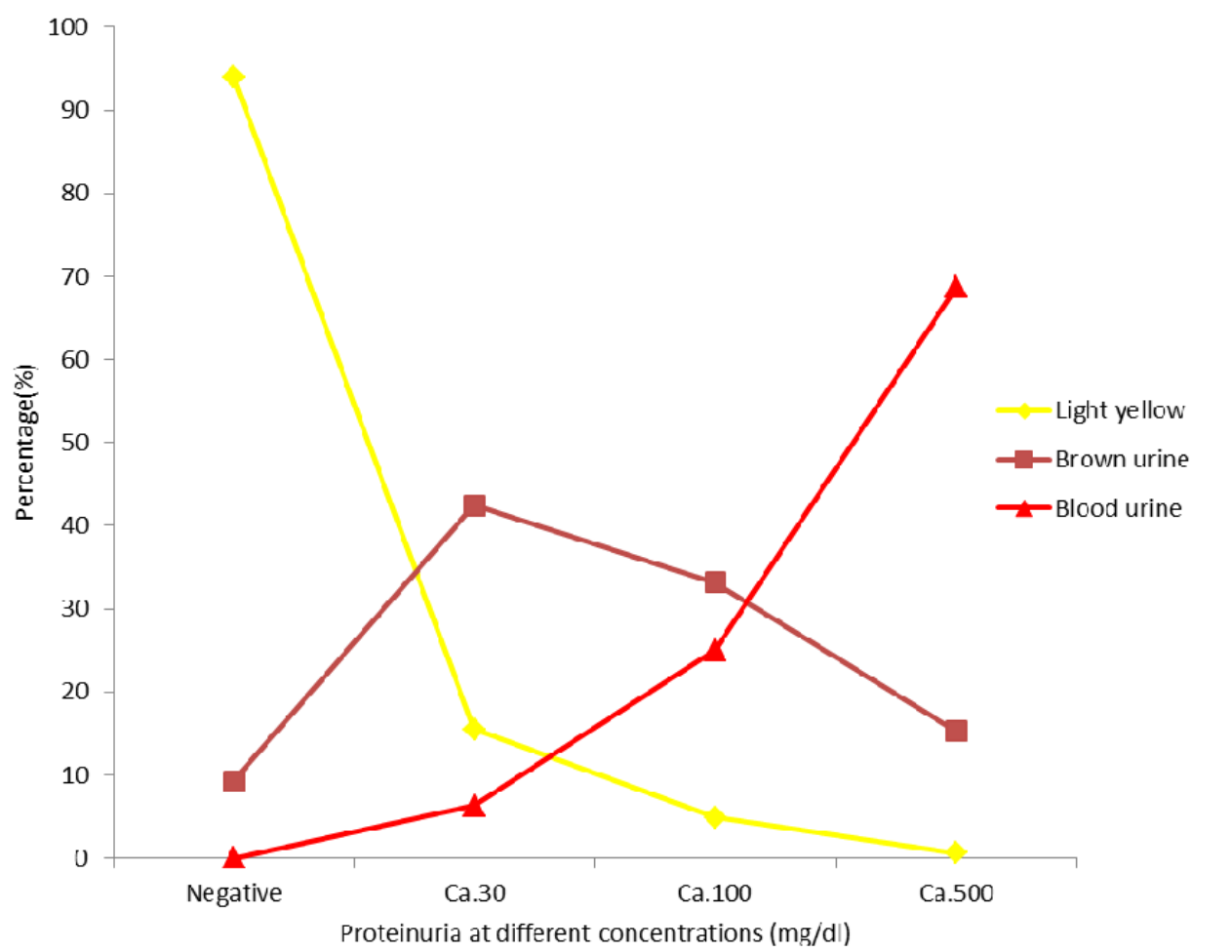


Fig. 3. Relationship between urine colour observation and proteinuria among subjects examined in Buruku and Katsina-Ala LGAs of Benue State, Nigeria

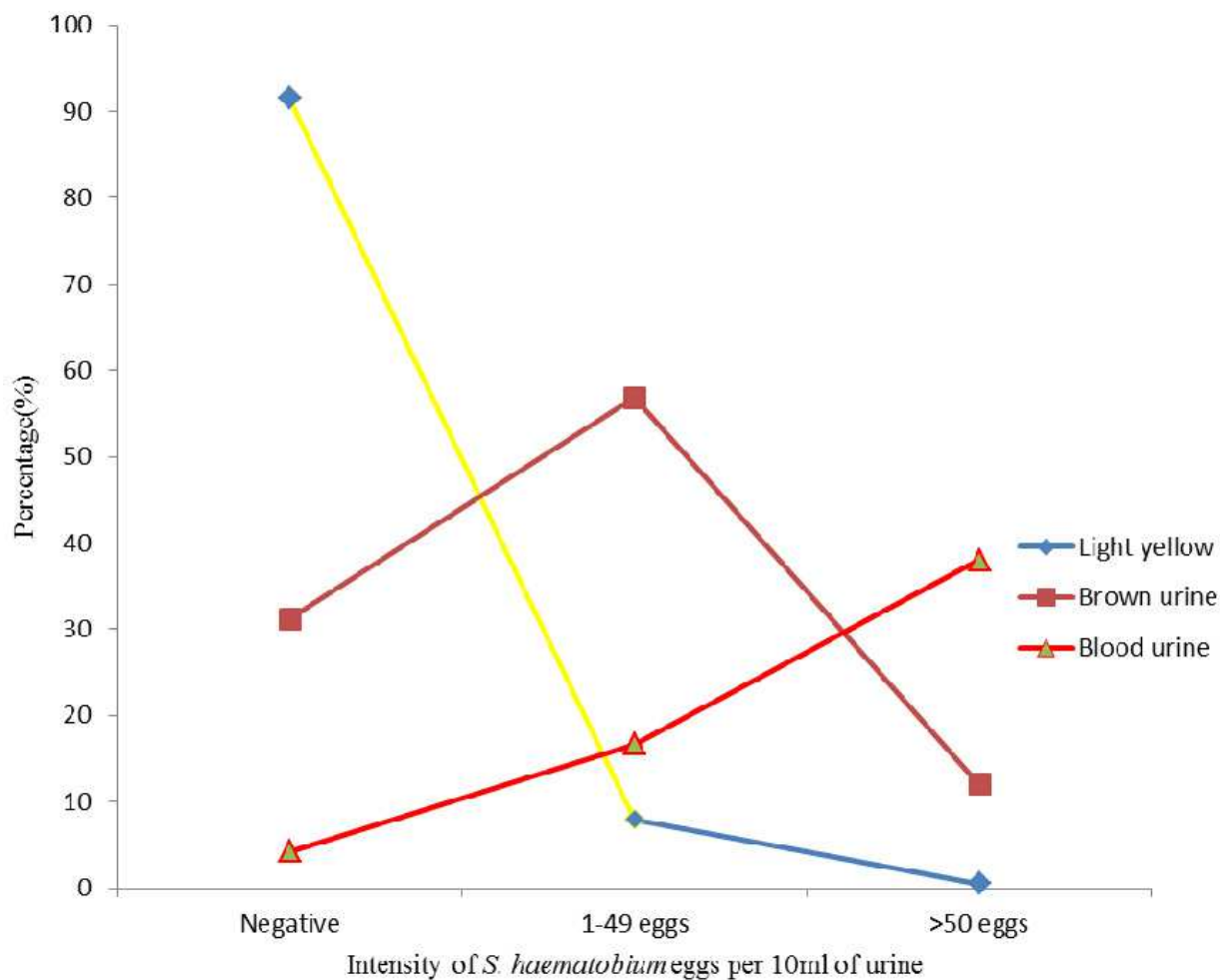


Fig. 4. Relationship between urine colour observation and intensity of *Schistosoma haematobium* eggs among subjects examined in Buruku and Katsina-Ala LGAs of Benue State

Urine colour	Filtration technique (gold standard method) (%)		Total
	Negative	Positive	
Negative (light-yellow)	529(91.5) ^c	49(8.4) ^b	578
Positive (Brown+Red)	210(29.4) ^d	504(70.6) ^a	714
Total	739	553	1292

Key: c= True negative
b= False negative
d= False positive
a= True positive

Table 1. Comparison of urine colour observation as rapid screening test and the true disease status as determined by filtration technique in Buruku and Katsina-Ala LGAs of Benue State

Parameters	Percentage (%)
Sensitivity	91.1
Specificity	71.6
Positive predictive value (PPV)	70.6
Negative predictive value (NPV)	91.5

Table 2. Validation of urine colour as rapid screening test for urinary schistosomiasis in Buruku and Katsina-Ala LGAs of Benue State

3. Discussion

It was demonstrated that assessing urine colour through simple observation significantly estimated the prevalence of infection and correlated with infection intensity as measured by egg counts (Filtration technique) known as the gold standard test. This shows that urine colour observation, if used as rapid screening tool in an endemic area is capable of assessing the endemic community for urinary schistosomiasis prevalence.

In addition to being a useful rapid field diagnostic for *Schistosoma haematobium* infection, urine colour as assessed by observation may also prove to be an indicator of morbidity through proteinuria and microhaematuria. Both proteinuria and microhaematuria correlated well with urine colour, $\rho = 0.7$ and $\rho = 0.5$ respectively at 0.01 significance level. However, the brown colour of urine should be the result of excreted protein and red blood cells into the urine from the damage of urinary tract and kidney. However, 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. Although, at the present time the precise origin and clinical significance of the proteinuria observed in *S. haematobium* infection remains unknown (Elissa, 2004). Nonetheless Sabour *et al.* (1972), Ezzat *et al.* (1974 and Ezzat *et al.* (1978) claimed an association between glomerulonephritis, the inflammation of the membrane tissue of the kidney and *S. haematobium* in human. Chugh and Sakuja (1990) reported that glomurelonephritis is highly prevalent in areas of the tropic where urinary schistosomiasis is also common, however its relationship to *S. haematobium* remain unclear. Sobh *et al.* (1991) showed that infected hamsters and not control animals developed significant glomerular damage related to the presence and intensity of *Schistosoma haematobium* infections.

The sensitivity of urine colour for screening test was high (91.1%) and with a specificity of 71.6%. This high sensitivity may be an indication of the predisposition of these rural dwellers to renal complications associated with urinary schistosomiasis. When compared to other indirect tests used in other studies like reagent strips, sensitivity of urine colour observation in this study was higher, but a specificity lower than results obtained by Ugbomoiko *et al.* (2009) who reported sensitivity of 68.3% and specificity of 83.2% using only microhaematuria, and sensitivity of 67.7% and specificity of 79.6% using only proteinuria respectively as indirect tests. However, variation in sensitivity and specificity of indirect tests during *Schistosoma haematobium* infection has been reported in several studies conducted in different African settings. They have been reported to vary from 41.0 % to 93 % and from 67 % to 99 % for sensitivity and specificity respectively (Anosike *et al*, 2001;

Takougang *et al.*, 2004; Brooker and Utzinger, 2007; Robinson *et al.*, 2009; French *et al.*, 2009; Ugbomoiko *et al.*, 2009).

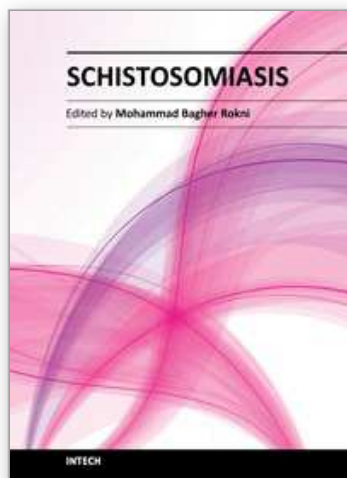
The results obtained, however, agree with preliminary reports undertaken earlier to find its possibility as a rapid screening method in endemic areas (Houmsou *et al.*, 2009). Furthermore it is recommended that additional researches should be conducted in order to elucidate its feasibility in other endemic areas.

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Schistosomiasis

Edited by Prof. Mohammad Bagher Rokni

ISBN 978-953-307-852-6

Hard cover, 310 pages

Publisher InTech

Published online 13, January, 2012

Published in print edition January, 2012

In the wake of the invitation by InTech, this book was written by a number of prominent researchers in the field. It is set to present a compendium of all necessary and up-to-date data to all who are interested. Schistosomiasis or blood fluke disease, also known as Bilharziasis, is a parasitic disease caused by helminths from a genus of trematodes entitled *Schistosoma*. It is a snail-borne trematode infection. The disease is among the Neglected Tropical Diseases, catalogued by the Global Plan to combat Neglected Tropical Diseases, 2008-2015 and is considered by the World Health Organization (WHO) to be the second most socioeconomically devastating parasitic disease, next to malaria. WHO demonstrates that schistosomiasis affects at least 200 million people worldwide, more than 700 million people live in endemic areas, and more than 200.000 deaths are reported annually. It leads to the loss of about 4.5 million disability-adjusted life years (DALYs).

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