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Original Article

Comparative Diagnosis of Schistosomiasis by Standard and Molecular Methods in Turan District of Kwande Local Government Area, Benue State, Nigeria

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ABSTRACT

The study was carried out to compare the diagnosis of schistosomiasis by standard and molecular methods in the Turan District of Kwande LGA. A total of 682 samples were collected, 482 urine and 200 stool samples. The urine samples were filtered on Whatman filter No.3 paper for DNA extraction and amplification. The samples were examined microscopically using centrifugation method for urine and the kato Katz method for stool. Forty (40) samples of the filtered urine were randomly picked irrespective of being positive or negative by microscopy and amplified by Polymerase Chain Reaction (PCR). Chi-square and Fisher's exact test were applied to determine the association of infection in relation to location and water contact activity. The Kappa coefficient was also applied to assess the degree of concordance between the diagnostic techniques. From the 482 urine samples analyzed, 111(23.0%) were infected with S. haematobium; and out of the 200 stool samples examined, 3(1.5%) were infected with S. mansoni. In comparison PCR established a prevalence of 12 (30.0%) for S. haematobium and 6 (15%) for S. mansoni. PCR also detected 3 (7.5%) co-infections with S. mansoni and S. haematobium. Concordance between centrifugation and PCR revealed an almost perfect agreement ($\kappa = 0.81$) while Kato-Katz and PCR showed a substantial agreement ($\kappa = 0.63$). Prevalence of urogenital schistosomiasis by location revealed the highest infection rate of 32 (29.4%) in Yaav, followed by Kumakwagh with prevalence of 31(24.8%) and lowest infection rate of 27 (20.4%) was observed in Mbadura. However, no significant difference was observed in the prevalence of urinary schistosomiasis between the different communities ($\chi^2 = 4.68$, $p = 0.20$). The study revealed that detection of Schistosoma parasite-specific DNA in urine by PCR is an effective tool for the diagnosis of schistosomiasis than processing urine and stool by standard methods.

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INTRODUCTION

Schistosomiasis (also called Bilharzia) is a parasitic disease caused by blood flukes (trematode worms) of the genus *Schistosoma.* It remains one of the most neglected tropical diseases which leads to significant economic and public health consequences, particularly in rural communities (*Angora et al.*, 2020, He *et al*., 2016, Colley *et al*., 2014). It is considered the second most important human parasitic disease after malaria in terms of morbidity and mortality (Percheron *et al*., 2024, Lo *et al*., 2022).

Schistosomiasis is estimated to affect more than 250 million people globally and cause approximately 280,000 deaths annually, while 779 million people are at risk of infection worldwide (LoVerde 2019, McManus *et al*., 2018; Ross *et al*., 2017), with at least 90% of those requiring treatment for schistosomiasis live in Africa (WHO 2023, Aula *et al*., 2021). Five species of Schistosomes infect humans however; *Schistosoma haematobium* and *Schistosoma mansoni* are the most prevalent in Sub-Sharan Africa including Nigeria, where they cause urogenital and intestinal schistosomiasis respectively (Meslo *et al.,* 2022). People are infected during routine agricultural, domestic, occupational, and recreational activities, which expose them to infested water (WHO 2023).

Most epidemiological assessments of the burden of schistosomiasis have relied on parasitological methods, such as identifying eggs in stool samples for intestinal schistosomiasis or urine samples for urinary schistosomiasis. Consequently, direct parasitological detection techniques are most often associated with poor sensitivity which limits both the diagnosis of individuals with early and low-level infections leading to false negative results (Ross *et al.,* 2017; Weerakoon *et al*., 2015). Due to these problems, people are shifting towards molecular techniques such as the polymerase chain reaction (PCR) for the diagnosis of schistosomiasis. The PCR does not only improve diagnosis but also uplifts effective research in current times (Cavalcanti *et al.,* 2019; He *et al.,* 2016). Because of their precision and sensitivity, molecular methods offer significant advantages in terms of accurate and timely parasitic detection (Chala, 2023).

The use of PCR to detect *Schistosoma* DNA in urine and stool samples is a highly sensitive and specific method that offers significant improvement in diagnosing schistosomiasis in non-endemic areas with low parasitic burden (Cnops *et al.,* 2013; Obeng *et al.,* 2008; Pontes *et al.,* 2003). When used to detect schistosomiasis in various sample types, PCR consistently shows exceptional sensitivity and specificity (Gomes *et al.,* 2010; ten-Hove *et al.,* 2008). Despite its higher accuracy, the practicality

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and cost-effectiveness of PCR in resource-limited settings need careful consideration in its application for the routine diagnosis of schistosomiasis.

MATERIALS AND METHODS

The Study Area

The study was carried out in the Turan District of Kwande Local Government Area, Benue State. Kwande Local Government has an estimated population of about 248,697 (2006 National Population Census). The Local Government is located between latitude $6^{0}53^{'}$ North and longitude $9^{0}14^{'}$ East. The Local Government is bordered by Vandeikya Local Government to the West, Ushongo Local Government to the North and Katsina- Ala in the Northeast, Cross River State to the South and the Republic of Cameroon to the East. The District shares a border with the Takum Local Government Area of Taraba State.

The study area was considered due to the low level of social amenities such as potable water and health facilities. Additionally, the study area is surrounded by large surface water bodies and most inhabitants are farmers and fishermen. The majority of inhabitants of this district depend largely on these surface waters for their domestic and agricultural purposes, especially during the dry season. The study area has a typical climate marked by dry and rainy seasons, the rainy season commences in April and ends in October while the dry season starts in November and ends in March.

Ethical Considerations

Prior to the commencement of sample collection, an introductory letter was obtained from the Head Zoology Department followed by ethical clearance from the Benue State Ministry of Health and Human Services, Makurdi. A survey visit was made to the selected villages to seek consent from the Village Heads, Headmasters, and Parents/Guardians of the participating children. The significance of the research was also explained to the village heads, parents, headmasters of the participating schools and the school children. Informed consent of both parents and enrolled children was obtained.

SAMPLE SIZE DETERMINATION

The sample size was calculated using the formula for public health studies as described by Pourhoseingholi *et al.,* (2013). A urinary schistosomiasis prevalence rate of 55.0% as reported by Amuta and Houmsou (2014) for Benue State was considered. The error margin was set at 0.05 and a sample size of at least 381 was estimated for the study.

Study Population

The study involved primary school children aged 5-15 years in four of the five council wards of Turan district. Four villages with proximity to surface water bodies were selected one from each of the council wards. A sample size of at least 381 was estimated for the study, but for better representation of the population and for more reliable results, a total of six hundred and eighty-two (682) samples were randomly collected from primary school children in the selected villages, which consisted of 482 urine and 200 stool samples.

Urine Sample Collection

Labelled sterile specimen containers (100ml) with screw-cap were given to each of the participating children and they were instructed to pass a single terminal urine into it. The urine samples were collected between 10.00 AM – 14.00 PM hours for optimum egg passage (Mduluza *et al.,* 2020). A structured interview was carried out to obtain information about the children's water contact activity. The specimen containers were collected and placed in an ice pack flask and transported to the laboratories of Primary Health Care, Boagundu, Tomatar and General Hospital Jato Aka for analysis.

Stool Sample Collection

Labelled sterile specimen containers with tight-fitting lids, two applicator sticks and newspapers were given to the participating children. They were

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instructed to pass the early morning stool onto the newspaper and use the applicator sticks to transfer a small amount of the stool to the specimen container. The samples were collected and immediately processed in the field by the Kato-Katz technique.

Parasitological Analysis of Urine Samples

Urine samples were examined microscopically by centrifugation method as described by (WHO 1991). The urine samples were properly agitated by shaking them, poured into a conical flask and allowed to sediment for one hour. The supernatant was withdrawn and the sediment was transferred into a centrifuge tube and centrifuged at 2000 rpm for 2 minutes. The deposits of the centrifuged samples were examined for the presence *of Schistosoma haematobium* ova, using x10 objective to screen the whole deposit.

Parasitological Analysis for Stool Samples

Stool samples were examined by the Kato-Katz method as described by (WHO 1991). Cellophane stripes were soaked in 50% glycerol-malachite green for at least 24 hours before use. A small amount of faces was transferred onto a newspaper and the screen was pressed on top of the faecal samples. Using a spatula, the upper surface of the screen was scraped across to sieve the fecal sample then a clean microscope slide was labeled with the sample identification number and a plastic template was placed on top of it. A small amount of sieved faecal sample was transferred into the hole of the template and the hole was carefully filled and leveled with a spatula. The template was carefully removed so that the entire faecal sample was left on the slide. The faecal sample on the slide was covered with a glycerol-soaked cellophane stripe. The microscope slide was inverted and the faecal sample was pressed against the cellophane on a smooth surface to spread the sample evenly. The microscope slide was gently slid sideways holding the cellophane to avoid separating the cellophane stripe. The slide was air dried then placed under the microscope and examined using x10 and x40 objectives for the presence of ova of *S. mansoni*

Urine Preparation for DNA Detection (PCR)

Urine filtration and DNA extraction were carried out using the method described by Ibironke *et al.,* (2012). Approximately 50 ml of urine was filtered through a cone-shaped Whatman No. 3 filter paper disc (Whatman International, Maidstone, England). This grade of paper was selected because it maintains a cone shape when folded, and it retains both schistosome eggs and DNA fragments from urine during filtration (Ibironke *et al.,* 2011). Each filter paper was marked with a unique identification number of the participant. The cone was set in a plastic funnel and the urine was filtered. To avoid contamination, plastic cups and funnels were used only once. After filtration, the paper disc was opened and air-dried under a fly-proof net and then packed individually with desiccant in a Ziploc bag and transported to the laboratory of the Zoology Department, Joseph Sarwuan Tarka University, Makurdi for DNA extraction and amplification by PCR with species-specific primers.

DNA Extraction from Urine

Forty samples of the filtered urine were randomly picked irrespective of the result by microscopy (positive or negative), ten samples from each of the communities and were subjected to DNA extraction and amplification. The DNA was extracted from the filtered urine on a Whatman No. 3 filter paper disc using the methanol and heat extraction method as described by Ibironke *et al.,* (2012). The central portion of the filter paper was excised by a regular paper punch and sliced into fragments before being subjected to DNA extraction. The paper punch and razor were cleaned with 70% ethanol and dried before processing any new sample to avoid contamination. For each specimen, the sliced fragments were placed into a 1.5 mL Eppendorf tube and labelled accordingly, then 140 µL of methanol was added into the Eppendorf tubes to fix the DNA for 40 minutes after which the methanol

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was aspirated and the template was allowed to air dry for 20 minutes. The templates were fixed with methanol for the second time and incubated at 37 ^{0}C for 40 minutes; it was fixed with methanol for the third time and incubated at 37 degrees Celcius for another 40 minutes. The dried blots were transferred to 1.5 µL Eppendorf tubes containing 7.5µL of 10x PCR buffer, 0.7 µL of proteinase K and 66.75 µL of double distilled sterile water and the tubes were incubated at 56 degrees Celcius for 3 hours. The incubating temperature was raised to 99.9 degrees Celcius for 10 minutes thereafter the tubes were cooled to 4 degrees Celcius and kept for further use.

Polymerase Chain Reaction Amplification

Amplification was carried out using *Schistosoma* species-specific multiplex PCR primers previously used by Sady *et al.,* (2015). Forward primer SHBM- F (5'- TTTTTTGGTCATCCTGA GGTGTAT-3') and reverse primer SH-R (5'- TGATAATCAATGACCCTGCAATAA-3') were used for *Schistosoma haematobium*. Reverse primer SM- R (5'- TGCAGATAAAGCCACCCCT GTG-3') was used for *Schistosoma mansoni.* The reaction volume of 20 µL consisted of 1 unit Taq DNA polymerase, 1x PCR buffer, 1.5 mM of MgCl2, 200 µM of dNTPs, 0.25 µM of forward primer, 0.25 µM of each of the reverse primers, 0.1µg/ml of BSA (Bovine serum albumin), 5 % DMSO (dimethyl sulfoxide), 5 µL of DNA template and nuclease-free water to complete the final volume. There was initial denaturation at 95 degrees Celcius for 3 minutes, followed by 33 cycles of 95 degrees Celcius for 30 sec, an annealing temperature of 58 degrees Celcius for 1 minute and an extension temperature at 72 degrees Celcius for 1 minute followed by a final extension at 72 degrees Celcius for 7 minutes. Amplification was conducted in 0.2 mL PCR tubes in a thermal cycler and electrophoresis of amplified PCR products was analyzed on a 2 % agarose gel stained with ethidium bromide at 75v for 50 minutes and was visualized with UV light and photographed to estimate band sizes.

Statistical Analysis

Data were entered into Microsoft Office Excel 2019 and analyzed using the SPSS version 23 statistical package. Chi-square and Fisher's exact test were applied at a 5 % (0.05) level of significance to determine the association of schistosomiasis prevalence by location and water contact activity. Cohen's Kappa (κ) coefficient was used to assess the level of agreement between the two diagnostic methods, with a κ value of $\leq$ 0 indicating no agreement, 0.01-0.20 indicating slight agreement, 0.21-0.40 indicating fair agreement, 0.41-0.60 indicating moderate agreement, 0.61-0.80 indicating substantial agreement, and 0.81-1.00 indicating almost perfect agreement.

RESULTS

Out of the 482 urine samples examined microscopically by centrifugation, 111(23.0%) were infected with *Schistosoma haematobium*; and from the 200 stool samples examined by the Kato-Katz method, 3(1.5%) were infected with *Schistosoma mansoni*. In comparison, PCR detected 12(30.0%) positives for *Schistosoma haematobium* and 6(15.0%) for *Schistosoma mansoni* from the 40 filtered urine samples randomly picked and amplified. PCR also detected 3(7.5%) co-infections with *Schistosoma mansoni* and *Schistosoma haematobium.*

The prevalence of urinary schistosomiasis by location is shown in Table 1. The highest prevalence 29.4% was recorded in Yaav followed by Kumakwagh with an infection rate of 24.8% while the lowest infection of 18.6% was observed in Mbadura. There was no significant difference observed in the prevalence of urinary schistosomiasis between the different communities (χ^2 = 4.68, p= 0.20).

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Table 1: Prevalence of *S. haematobium* in the Study Area by Urine Centrifugation and Microscopy

Location	No. Examined	No. Positive	No. Negative
Kumakwagh	125	31(24.8%)	94(75.2%)
Mbadura	145	27(18.6%)	118(81.4%)
Mbaikyo	103	21(20.4%)	82(79.6%)
Yaav	109	32(29.4%)	77(70.6%)
Total	482		

111(23.0%) 371(77.0%)

$\chi^2 = 4.68$, df =3, p = 0.20

The prevalence of intestinal schistosomiasis in the study area by location is demonstrated in Table 2. Prevalence of 2.0% and 4.0% were recorded in Mbadura and Mbaikyo respectively while no infection was recorded in Kumakwagh and Yaav. However, Fisher's exact test revealed no significant difference in the prevalence of intestinal schistosomiasis between the different communities (p=1.0).

Table 2: Prevalence of *Schistosoma mansoni* in the study area by Kato-Katz and microscopy

Location	No. Examined	No. Positive	No. Negative
Kumakwagh	50	0	50
Mbadura	50	1(2.0%)	49(98.0%)
Mbaikyo	50	2(4.0%)	48(96.0%)
Yaav	50	0	50
Total	200	3(1.5%)	197 (98.5%)

The comparative prevalence of *S. haematobium* by urine centrifugation and microscopy using PCR as a reference test from the randomly picked 40 filtered urine samples is shown in Table 3. All the 9 samples that were positive by centrifugation were confirmed as true positive by PCR while out of the 31 samples that were negative by centrifugation, PCR detected 3 as false negatives. The Kappa coefficient was applied to assess the degree of agreement between centrifugation and PCR which showed an almost perfect agreement (κ= 0.81).

Table 3: Comparative diagnosis of *S. haematobium* by urine centrifugation and microscopy using PCR as reference test

	PCR		
Centrifugation	**Positive**	**Negative**	**Total**
Positive	9(22.5%)	0(0%)	9(22.5%)
Negative	3(7.5%)	28(70%)	31(77.5%)
Total	12(30%)	28(70%)	40(100%)
Kappa = 0.81			

The comparative prevalence of S. mansoni by Kato-Katz-microscopy using PCR as a reference test from the randomly picked 40 filtered urine samples is indicated in Table 4. All the 3 samples that were positive by Kato-Katz were confirmed as true positive by PCR while out of the 37 samples that were negative by Kato-Katz PCR detected 3 as false negatives. The Kappa coefficient was used to assess the level of agreement between Kato-Katz and PCR, which revealed a substantial agreement (κ =0.63).

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Table 4: Comparative diagnosis of S. mansoni by Kato-Katz and microscopy using PCR as reference test

	PCR		
Kato-Katz	**Positive**	**Negative**	**Total**
Positive	3(7.5%)	0(0%)	3(7.5%)
Negative	3(7.5%)	34(85.0%)	37(92.5%)
Total	6(15.0%)	34(85.0%)	40(100%)
Kappa = 0.63			

The prevalence of *S. haematobium* in relation to water contact activities is summarized in Table 5. The highest prevalence 36.0% was observed among those subjects that went swimming followed by those who went to fetch water 25.9% while no infection was recorded among the subjects that went washing. No significant difference was observed in the prevalence of urinary schistosomiasis by water contact activities ($\chi^2 = 5.74$, $p = 0.22$).

Table 5: Prevalence of urinary schistosomiasis by water contact activities

Activity	**No. Examined**	**No. Positive**	**No. Negative**
Fishing	10	2(20.0%)	8(80.0%)
Swimming	58	21(36.0%)	37(63.8%)
Fetch water	27	7(25.9%)	20(74.1%)
Bathing	48	9(18.8%)	39(81.2%)
Washing	4	0(0.0%)	4(100.0%)
Combined activities	293	72(24.6%)	221(75.4%)
Total	440	111(25.2%)	329(74.8%)
($\chi^2 = 5.74$, $p = 0.22$).			

DISCUSSION

Evaluation of schistosomiasis prevalence and geographic distribution is vital in planning effective intervention measures. Therefore, accurate and sensitive diagnostic techniques are required for patient management, monitoring of disease transmission, assessment of treatment efficiency and evaluation of the success of the control strategies (He *et al*., 2016).

WHO recommended standard methods for faecal and urine examination for *S. mansoni* and *S. haematobium,* and are currently used for mapping and field-based control of schistosomiasis. Due to the typical size and shape of the lateral and terminal spines, the eggs of *S. mansoni* and *S. haematobium* are easily detected and identified. This method is cost-effective and easy to use, especially in areas where the prevalence of schistosomiasis infection is very high. However, standard methods lack sensitivity for low-intensity infections and after control intervention because the eggs occur sporadically. At least three samples are necessary for Kato-Katz diagnosis in some patients to detect the true infection state (Lodh *et al.*, 2013; Lodh *et al.*, 2014; Meningher *et al.*, 2017). Therefore, in such areas where the intensity of schistosomiasis infection is very low, there is a need for more sensitive diagnostic methods.

The present study was carried out to compare the diagnosis of schistosomiasis by standard and molecular methods in order to ascertain which method will be most applicable for schistosomiasis surveillance, in areas with different rates of schistosomiasis infection.

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The study revealed a moderate prevalence of 23.0% and 30.0% by microscopy and PCR respectively for urogenital schistosomiasis while a low prevalence of 1.5% was established by microscopy but a moderate prevalence of 15% by PCR for intestinal schistosomiasis. This prevalence is in accordance with the infection rates reported by previous studies, 23.69% in Benue, Nigeria (Okita *et al.,* 2023), 20.0% in Benue-Nigeria (Chikwendu *et al.,* 2019), 17.8% in Kano-Nigeria (Dawaki *et al*., 2016) and 16.4% in Benue, Nigeria (Iboyi *et al.,* 2018) for urogenital schistosomiasis and 9% in two communities of Southwestern Nigeria (Ojo *et al.,* 2021), 8% in Ogun, Nigeria (Alade *et al.,* 2023), 2% in Yobe State, Nigeria (Bigwan *et al.,* 2012) and 8.7% recorded in Kano-Nigeria (Dawaki *et al*., 2016) for intestinal schistosomiasis. However, the higher prevalence of 55.0% was earlier reported in Benue-Nigeria (Amuta and Houmsou 2014) and 58.54% prevalence was reported in Taraba, Nigeria (Houmsou *et al.,* 2016). The lower prevalence reported by this study could be attributed to efforts of the Government and Non-governmental Organizations (NGOs) through continuous community-based treatments in all the states of the federation

The study recorded a high prevalence of urinary schistosomiasis in Yaav and Kumakwagh, this could be attributed to inhabitants' predominately involvement in fishing activities and lack of safe water supply in the communities which predispose them to the infested water bodies. Intestinal schistosomiasis recorded in Mbadura and Mbaikyo could be attributed to a lack of safe water sources, inadequate health education and indiscriminate defecation in the communities due to insufficient sanitary facilities thereby contaminating the environment and enhancing the transmission of the disease. The environment is also an important determinant of transmission, influencing parasite development and the life cycle of snail intermediate hosts. This agrees with the findings previously conducted by Wiegand *et al.,* (2021), Colley *et al.,* (2014) and Weerakoon *et al.,* (2015).

The study also showed that the detection of *S. haematobium* and *S. mansoni* parasite-specific DNA in urine by polymerase chain reaction was more sensitive than processing urine by centrifugation and stool by Kato-Katz, as false negative cases were observed from the standard methods by PCR technique. This could be due to the ability of PCR to detect early or low-level infections and to exponentially amplify a minute amount of DNA too small for direct analysis. This finding agrees with the studies carried out by Edward *et al.,* (2024) Sow *et al.,* (2023), Hessler *et al.,* (2017) and Lodh *et al.,* (2014).

The PCR method recorded 3 (7.5%) co-infections with *Schistosoma mansoni* and *Schistosoma haematobium* which is similar to the study by Anyan *et al., (*2020), which concluded that PCR was a highly sensitive and specific approach for detecting underlying multiple schistosome infections and is an effective means to detect low-intensity infections. Thus, PCR can be used for early surveillance of schistosomiasis infection where the disease is suspected to be present but has not yet been confirmed by standard methods such as the Kato-Katz for intestinal schistosomiasis, and urinary schistosomiasis by urine centrifugation and examination by microscopy.

The higher prevalence recorded among the swimming subjects may be due to higher risks of becoming infected as their bodies are more exposed to water and probably the infective cercariae. This study is in accordance with the studies previously carried out by Nwachukwu *et al.,* (2018) and Anzaku *et al.,* (2017).

CONCLUSION

Schistosomiasis is still a disease of public health importance in the Kwande area of Benue State; therefore, early diagnosis and treatment will mitigate the transmission and the burden associated with the disease in the area. The rate of infection recorded in the study area could be attributed to a lack of safe water supply which predisposed the

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children to the available infested surface water bodies for their domestic, recreational and farming activities, thereby enhancing their risk of infection.

In this study, PCR proved to be an effective technique in epidemiological surveillance of schistosomiasis providing more precise and sensitive results than standard methods of schistosomiasis diagnosis based on microscopy. The study also showed that the DNA of *Schistosoma mansoni* can be detected from filtered urine samples using Whatman No. 3 filter paper by polymerase chain reaction.

Recommendations

Based on the results of the study, the following recommendations are made:

- There should be provision of a safe water supply in the study area to reduce contact with the existing infested water.
- PCR is a better tool for the diagnosis of schistosomiasis, though it is expensive and its application requires the services of highly trained personnel.
- Enlightenment campaigns and health education should be carried out in the study area about the disease and its risk factors.
- There should be persistent community-based treatment using praziquantel and an enlightenment campaign about the drug as some parents/guardians stop their children from taking the drug even when mass community treatment is carried out due to side effects they experience after taking it.

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