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ORIGINAL RESEARCH ARTICLE

Comparative Detection of *Schistosoma haematobium* Using Microscopy, Circulating Cathodic Antigen Test, and qPCR in Northern Nigeria

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ABSTRACT
Urogenital schistosomiasis caused by Schistosoma haematobium remains a major neglected tropical disease in sub-Saharan Africa, particularly in Nigeria, where transmission is sustained by poor sanitation, inadequate water infrastructure, and frequent human contact with infested freshwater bodies. Accurate diagnosis is critical for surveillance, treatment monitoring, and control programs; however, conventional microscopy often underestimates infection prevalence due to low sensitivity in light-intensity infections. A hospital-based cross-sectional study involving 100 participants was conducted between December 2024 and April 2025 at Federal Medical Centre Birnin Kudu, Jigawa State, Nigeria. Urine samples were analyzed using microscopy, point-of-care circulating cathodic antigen (POC-CCA) assay, and quantitative polymerase chain reaction (qPCR). Diagnostic indices, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratios, and 95% confidence intervals, were determined using microscopy as the conventional reference method. The prevalence of *S. haematobium* infection detected by microscopy, POC-CCA, and qPCR was 18%, 23%, and 32%, respectively. qPCR demonstrated the highest sensitivity (77.7%) and NPV (94.1%), while POC-CCA showed sensitivity and specificity values of 72.2% and 87.8%, respectively. Positive likelihood ratios were 5.92 for POC-CCA and 3.55 for qPCR, whereas negative likelihood ratios were 0.32 and 0.29, respectively. Male participants showed higher prevalence rates across all diagnostic methods, with microscopy prevalence of 27.5% among males compared to 8.2% among females. Participants aged 11–20 years had the highest infection prevalence (41.7%). qPCR consistently detected more low-intensity infections than microscopy and antigen-based methods. In conclusion, Molecular and antigen detection methods demonstrated superior diagnostic performance compared to conventional microscopy for detecting S. haematobium infection. The higher sensitivity of qPCR highlights its usefulness in identifying low-intensity and subclinical infections that may be missed by microscopy. Integration of qPCR and POC-CCA into schistosomiasis surveillance and control programs could substantially improve early detection, epidemiological monitoring, and post-treatment evaluation in endemic communities.

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Schistosoma haematobium; urogenital schistosomiasis; microscopy; POC-CCA; qPCR; molecular diagnosis; Nigeria

INTRODUCTION
Schistosomiasis is one of the most important neglected tropical diseases (NTDs) globally and remains a major public health challenge in many low- and middle-income countries, particularly in sub-Saharan Africa where transmission is sustained by poverty, inadequate sanitation, unsafe water sources, and frequent contact with contaminated freshwater bodies (WHO, 2023). Environmental factors such as irrigation practices, climate variability, vector ecology, and poor water resource management also contribute significantly to the persistence of parasitic diseases in endemic regions (Bawale, 2024; Bello et al., 2025; Adejoh et al., 2025). Studies in Nigeria have shown that climatic and ecological conditions strongly influence disease prevalence and vector distribution (Abba et al., 2024; Baba-Adamu et al., 2025; Burga & Mohammed, 2025). The disease is caused by blood flukes of the genus *Schistosoma*, with *Schistosoma haematobium* being the major cause of urogenital schistosomiasis in Africa and the Middle East. Nigeria bears one of the highest burdens of schistosomiasis globally, particularly among rural and semi-urban populations with limited access to potable water and sanitation facilities.

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Urogenital schistosomiasis is associated with haematuria, dysuria, chronic bladder pathology, hydronephrosis, infertility, renal impairment, and increased risk of bladder cancer (Pereira et al., 2022). School-aged children and young adults are disproportionately affected because of frequent occupational, recreational, and domestic exposure to cercariae-infested water bodies. Similar environmentally driven infectious disease patterns have been reported in malaria-endemic and displaced populations in Nigeria, where poor sanitation and water management contribute to disease persistence (Abba et al., 2025). Exposure to contaminated aquatic environments may also increase contact with heavy metals, pollutants, and pathogenic microorganisms that worsen public health outcomes (Aiki et al., 2023; Agboola et al., 2024). Despite mass drug administration (MDA) programs using praziquantel, transmission remains persistent in many endemic areas due to reinfection, inadequate surveillance, poor environmental sanitation, and diagnostic limitations (Hotez et al., 2021).

Accurate diagnosis is essential for surveillance, treatment monitoring, and elimination strategies. Conventional urine microscopy for detecting *S. haematobium* eggs remains the most widely used diagnostic method because it is inexpensive and simple to perform. However, microscopy has reduced sensitivity in low-intensity infections, depends heavily on skilled personnel, and is affected by day-to-day variation in egg excretion. Consequently, it often underestimates the true prevalence of schistosomiasis, especially in areas where control interventions have reduced infection intensity (Beltrame et al., 2023). Sandoval (2006) demonstrated that molecular methods could detect infections missed by conventional parasitological techniques, while van Dam et al. (2004) reported improved diagnostic performance using antigen-based detection methods.

Environmental contamination and poor waste management practices further contribute to disease transmission and public health risks in many Nigerian communities (Adamu et al., 2023; Adepehin et al., 2025). Heavy metal contamination of soils and water bodies resulting from urbanization, mining, and agricultural activities has become an increasing concern (Aiki et al., 2023). Studies on radon contamination in groundwater also highlight the vulnerability of water systems to pollutants that may coexist with parasitic disease transmission (Abubakar et al., 2024; Bashir et al., 2023). In addition, carcinogenic substances and toxic contaminants in aquatic ecosystems may aggravate chronic disease conditions associated with schistosomiasis (Agboola et al., 2024). The occurrence of heavy metal-tolerant bacteria in contaminated environments further demonstrates the persistence of pollutants in endemic communities (Badamasi & Salisu, 2025; Bilyaminu et al., 2025). These findings emphasize the need for integrated environmental monitoring and sensitive disease surveillance systems.

To overcome the limitations of microscopy, alternative diagnostic approaches, including antigen detection assays and molecular techniques, have increasingly been adopted. The point-of-care circulating cathodic antigen (POC-CCA) assay detects schistosome antigens released by adult worms and offers advantages such as rapid turnaround time, field applicability, and ease of use in resource-limited settings (RMD, 2020). El-Ghareeb et al. (2016) reported high sensitivity of POC-CCA in urinary schistosomiasis diagnosis, although its performance may vary with infection intensity.

Molecular diagnostic methods, particularly quantitative polymerase chain reaction (qPCR), have demonstrated improved sensitivity and specificity by detecting parasite DNA in biological samples. qPCR assays targeting repeated genomic sequences such as Dra1 can identify low-intensity and pre-patent infections commonly missed by microscopy (Ten Hove et al., 2019). Gillardie et al. (2021) highlighted the usefulness of molecular epidemiological approaches for urinary schistosomiasis surveillance, while Vinkeles Melchers et al. (2020) reported excellent diagnostic performance of real-time PCR in Kenyan children infected with *S. haematobium*. Bakare et al. (2018) further emphasized that no single diagnostic method serves as a perfect gold standard because of differences in infection intensity and parasite detectability.

Recent advances in environmental and biomedical research in Nigeria have demonstrated the growing application of molecular and analytical techniques in disease surveillance and environmental monitoring (Abubakar et al., 2024; Bashir et al., 2023; Adejoh et al., 2025; Baba-Adamu et al., 2025). Electrochemical and molecular detection technologies have also shown promising applications in identifying contaminants and biological agents in environmental and clinical samples (Cesewski & Johnson, 2020). Furthermore, WHO (2022) emphasized the need for improved diagnostic innovations to support schistosomiasis elimination programs. Combining antigen detection assays with molecular techniques has been shown to improve detection accuracy, particularly in low-endemicity settings and during post-treatment monitoring (Beltrame et al., 2023; Vinkeles Melchers et al., 2020).

Despite increasing awareness of sensitive diagnostic tools, microscopy remains the dominant diagnostic method in many Nigerian health facilities because of limited laboratory infrastructure and the high cost of molecular assays. Consequently, many low-intensity and asymptomatic infections remain undetected, sustaining disease transmission within endemic communities. This study therefore compared the diagnostic performance of urine microscopy, POC-CCA assay, and qPCR for detecting *S. haematobium* infection among patients attending Federal Medical Centre Birnin Kudu, Jigawa State, Nigeria. The findings are expected to provide evidence for improving schistosomiasis diagnosis, surveillance, and control strategies in endemic communities.

MATERIALS AND METHODS

Study Design and Area

A hospital-based cross-sectional study was conducted between December 2024 and April 2025 at the Federal Medical Centre Birnin Kudu, Jigawa State, Nigeria.

Study Population and Sample Size

A total of 100 consenting participants presenting for routine laboratory investigation were enrolled. The sample size was determined based on feasibility and comparable diagnostic studies in similar endemic settings.

Sample Size determination

Sample size (n) was calculated by single population proportion based on the following assumption, as it is a cross-sectional study with quantitative variables as given by.

The formular $N = Z^2 PQ/d^2$ (Daniel, 1999).

Where

N= minimum sample size based on a single proportion

Z = 1.96 (at 95% interval from Z table)

P = 4.8% = 0.48 = (proportion of the population).

From the previous study (Omoroghe *et al.*, 2019)

Q = (1- p) = Complementary probability = 1 – 0.48 = 0.52

Where d = 0.05 = precision at 95% confidence interval

Therefore, sample size

$N\ 1.96^2 \times 0.48 \times 0.52^2 / (0.05) = 0.95886336/ 0.0025 = 383.5454 = 400$

Since the study population is less than 10,000, the correction formula was used, and the final sample size was found as

nf =no/ (1+ no/N)

Therefore, nf = 400/ (1+400/109) = 1+ 3.6697 = 4.6697

Then nf = 400/4.6697 = 85.65. Then non- response rate of 15% was added nf = 85.65= 100

Therefore, 13.5% was added to 85.65 (is less than or equals to) < = 100

The sample size (n)= 100

Ethical Considerations

Ethical approval was obtained from the Health Research Ethics Committee of the Federal Medical Centre Birnin Kudu. Written informed consent was obtained from all participants prior to sample collection.

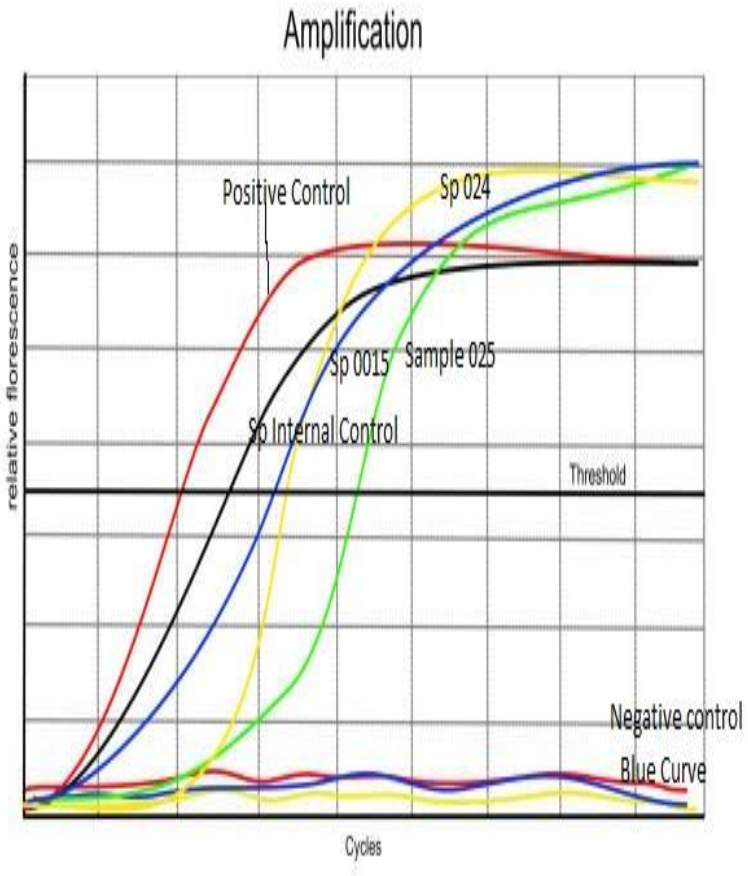

Figure 1: Amplification Curve

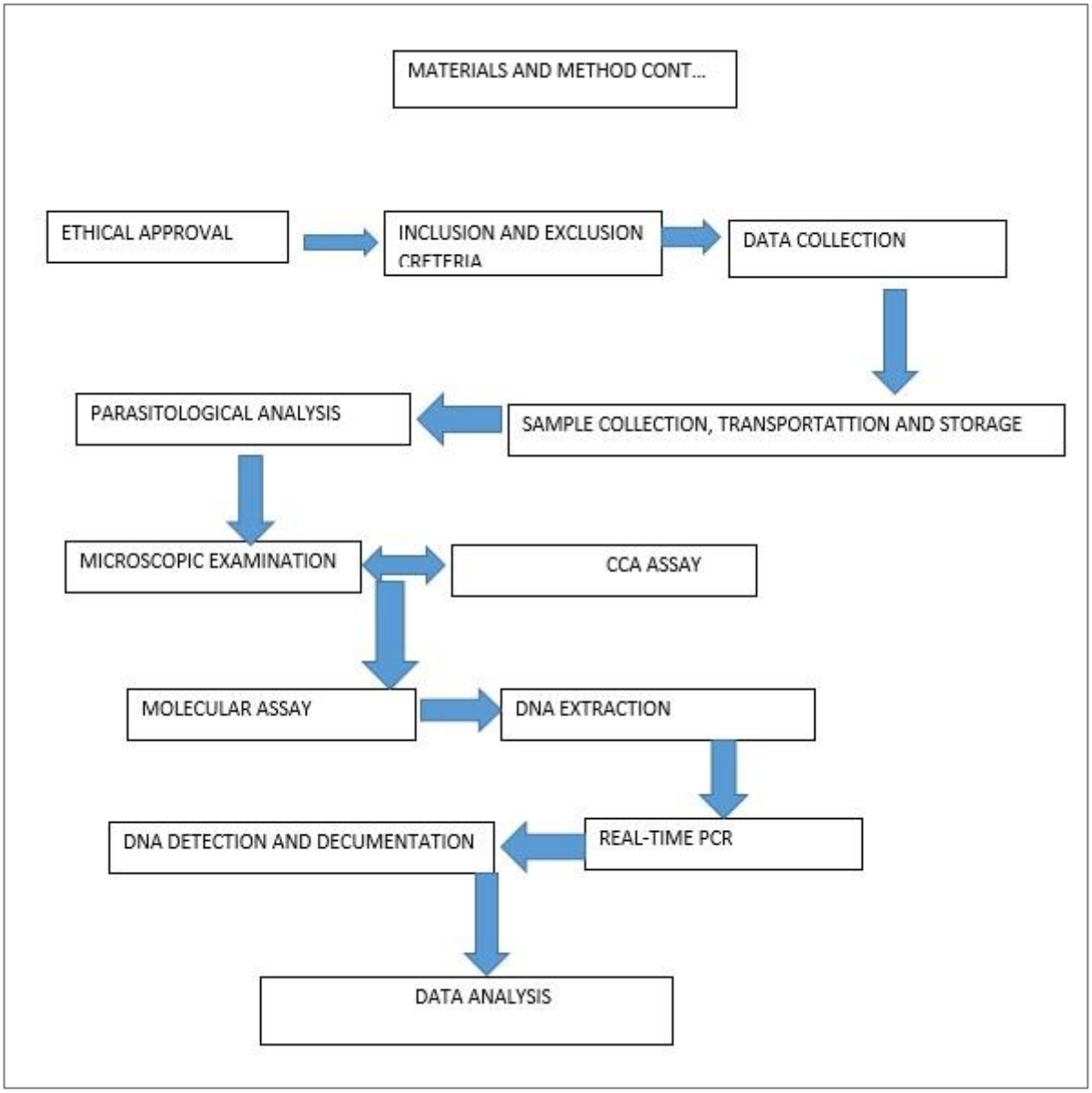

Figure 2: Study Flow Chart

Laboratory Procedures

Urine samples were collected between 10:00 and 14:00 hours and examined using urine microscopy, POC-CCA assay (Rapid Medical Diagnostics, South Africa), and qPCR following manufacturer protocols. Appropriate positive and negative controls were included in the molecular assays (RMD 2020).

Microscopy

About 10 ml of urine was centrifuged at 3000 rpm for 5 min in order to concentrate eggs of schistosomes. The supernatants were discarded. A drop of deposit was placed at a center of clean grease free glass slide, and cover with a cover slip avoids air bubbles and overflowing. The slide will be examined microscopically using x10 and x40 objectives lens with condenser low and diaphragm close for the characteristics eggs. The infection intensity was classified as light (< 50 eggs/10ml urine) or heavy (>50 eggs/10ml urine) following WHO recommendation (WHO 2022).

Circulating Cathodic Antigen (CCA)

The urine POC – CCA cassette test was performed according to the protocol and procedures described by the manufacturer (Rapid Medical Diagnostics, Cape Town, South Africa). Ensure all reagents are equilibrated to room temperature (20 – 25°C) before commencing the assay. Remove the cassette and pipette from their pouches just prior to use. Squeeze the pipette and insert the tip into the urine sample. Allow the sample to fill up by gently releasing the pipette. Transfer 2 drops of urine to the circular well of the test cassette by gently squeezing the pipette. Allow the sample to absorb entirely into the specimen pad within the circular well. Read the result at 20 min (RMD 2020).

DNA Extraction

10 µL of proteinase K (PK) was pipetted into a nuclease-free centrifuge tube containing the pre-treated sample. A 200 µL of lysis buffer was added to the tube and vortex mix for 30 seconds. The mixture was incubated at 56°C for 15 minutes in a heating block, then the 1.5 mL tube

was briefly centrifuged to remove any droplets from the inside of the lid. A 250 µL of ethanol was added to the sample, the cap was closed, and the mixture was mixed thoroughly by pulse vortexing for 15 seconds. The tube was briefly centrifuged again to remove droplets from the lid. The mixture was transferred to a Spin Column and centrifuged at 10,000 × g for 1 min.

The flow-through was discarded, 500 µL of wash buffer 1 was added to the Spin Column and centrifuge at 10,000 × g for 1 minute. The flow-through was discarded, and 500 µL of wash Buffer 2 was added to the Spin Column and centrifuged at 10,000 × g for 1 minute; the flow-through was discarded. 500 µL of wash buffer 2 was added and centrifuged at 10,000 × g for 1 minute. The flow-through was discarded. The Spin Column was placed into a clean 1.5 mL collection tube and centrifuged at 10,000 × g for 2 minutes to completely dry the membrane. The Spin Column was transferred to a new clean 1.5 mL collection tube, 100 µL of Elution buffer was added directly to the center of the membrane, and incubated at room temperature for 2 minutes. The spin column was removed and discarded. The eluate in the microcentrifuge tube contains the purified DNA.

DNA Amplification

A total of 35 µL of reaction mix was dispensed into a clean, sterile 1.5 mL Eppendorf tube, followed by the addition of 0.4 µL of enzyme mix and 1 µL of internal control, bringing the volume to 36.4 µL per sample. This mixture was then transferred into a microwell plate, to which 4 µL of extracted DNA was added, resulting in a final volume of 40 µL per well. The microwell plate was sealed with an appropriate sealant before being placed into a thermocycler programmed as follows: an initial incubation at 37°C for 2 minutes, followed by 94°C for 2 minutes for 1 cycle; then 40 cycles of 93°C for 15 seconds and 60°C for 1 minute.

A total of 100 extracted samples were processed following the kit manufacturer's protocol, and the reaction mixtures were loaded onto an Azure Biosystems PCR RT machine for amplification. Amplification, amplicon detection and related data analysis were performed with the Azure Biosystems real-time PCR system (Molecular Biosciences, China). The qPCR output from this system consisted of a cycle–threshold (CT) value, representing the amplification cycle in which the level of fluorescent signal exceeded the background fluorescence, and indicating the parasite-specific DNA load in the urine sample tested (Figure 1; Ten Hove *et al*; 2019).

Statistical-Analysis

Data were analyzed using IBM SPSS version 27.0. Sensitivity, specificity, and positive predictive values were calculated using microscopy as the reference method. Chi-square tests were used to assess associations between infection status and sociodemographic variables. Statistical significance was set at $p < 0.05$. Figure 2 shows the methodology flow chart.

RESULTS

A total of 100 urine samples were analyzed using urine microscopy, point-of-care circulating cathodic antigen (POC-CCA) assay, and quantitative polymerase chain reaction (qPCR) for the detection of *Schistosoma haematobium* infection. The prevalence of infection varied according to the diagnostic technique employed, with qPCR detecting the highest number of positive cases, followed by POC-CCA and microscopy.

Table 1: Demographic Distribution and Microscopy Results

Variable	Category	n	Positive	Prevalence (%)	95% CI
Gender	Male	51	14	27.5	15.3–39.7
	Female	49	4	8.2	0.6–15.8
Age	11–20	36	15	41.7	25.6–57.8
	21–30	35	2	5.7	0–13.3

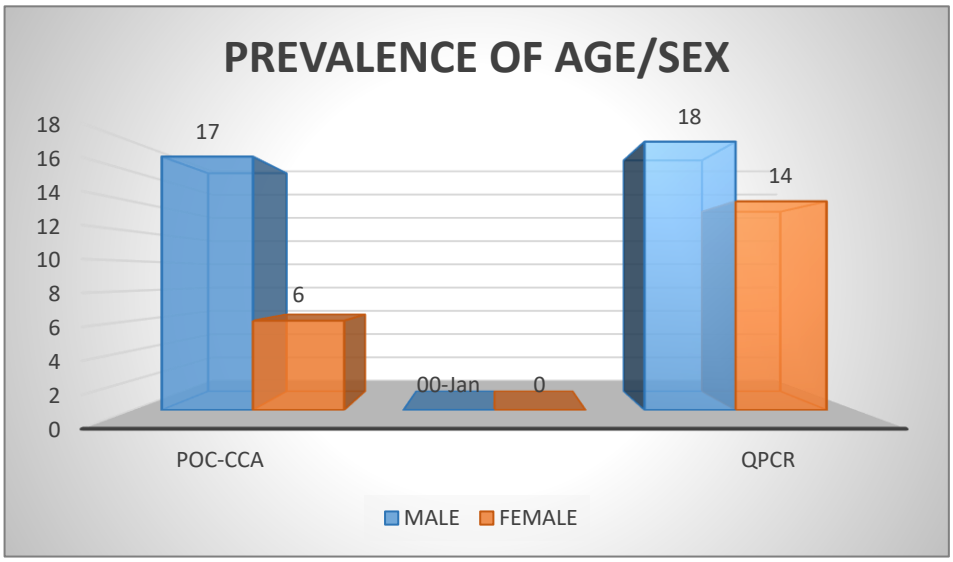

Figure 3: Age/Sex Prevalence chart

Table 2: POC-CCA Results

Variable	Category	n	Positive	Prevalence (%)	95% CI
Gender	Male	51	17	33.3	20.4–46.2
	Female	49	6	12.2	3.0–21.4

Table 3: qPCR Results

Variable	Category	n	Positive	Prevalence (%)	95% CI
Gender	Male	51	18	35.3	22.1–48.5
	Female	49	14	28.6	15.9–41.3

Table 4: Diagnostic Performance of POC-CCA and qPCR

Test	Sensitivity	Specificity	PPV	NPV	95% CI (Sensitivity)	95% CI (Specificity)
POC-CCA	72.2%	87.8%	56.5%	92.3%	46.5–90.3	78.5–94.3
qPCR	77.7%	78.1%	42.4%	94.1%	52.4–93.6	67.1–86.7

Table 5: Additional Diagnostic Performance Metrics

Test	LR+	LR−
POC-CCA	5.92	0.32
qPCR	3.55	0.29

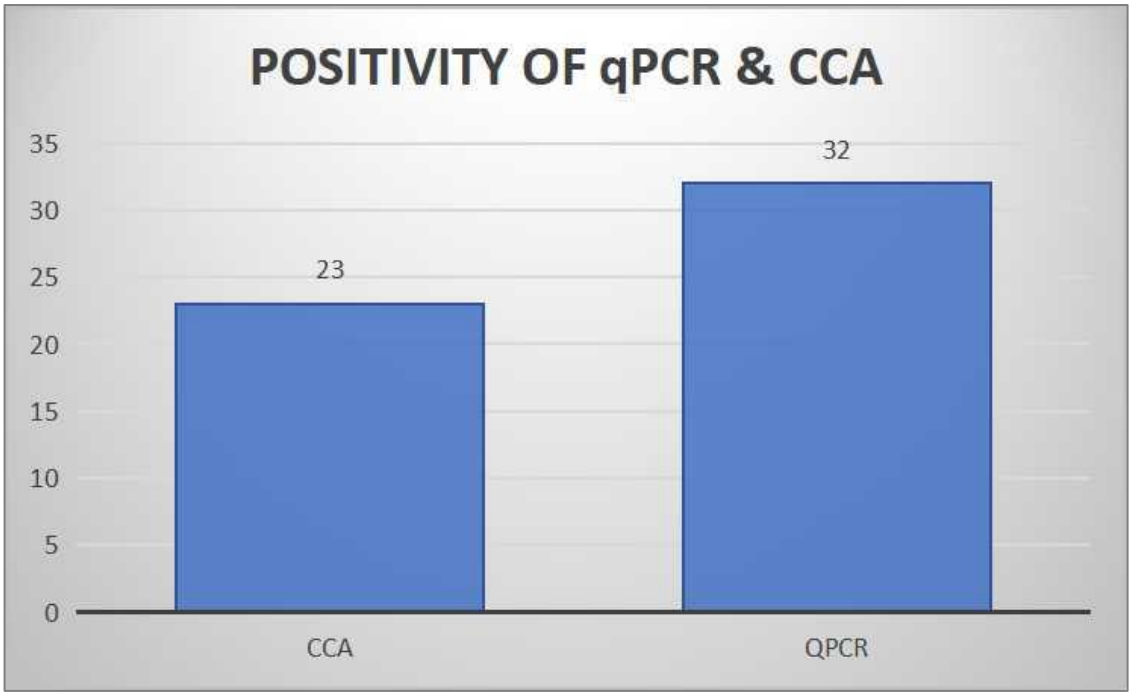

Figure 4- Diagnostic Performance of qPCR and CCA

The demographic distribution of participants and microscopy findings are presented in Figure 3 and Table 1. Out of the 100 participants examined, 51 were males, and 49 were females. Microscopy detected *S. haematobium* eggs in 18 participants, giving an overall prevalence of 18.0%. Male participants showed a substantially higher prevalence of infection (27.5%) compared to females (8.2%). Age-specific analysis revealed that participants aged 11–20 years had the highest prevalence (41.7%), while those aged 21–30 years recorded the lowest prevalence (5.7%). The confidence intervals indicated greater variability among younger age groups, reflecting higher infection heterogeneity.

The results of the POC-CCA assay are shown in Table 2. Using antigen detection, 23 participants tested positive, corresponding to an overall prevalence of 23.0%, which was higher than that obtained by microscopy. Similar to microscopy findings, male participants had a higher prevalence (33.3%) than females (12.2%). The broader detection rate observed with POC-CCA suggests improved sensitivity in identifying infections, including low-intensity infections that may have been missed by microscopy.

The qPCR assay demonstrated the highest detection rate among all diagnostic methods, as presented in Table 3. Overall, 32 participants tested positive, representing a prevalence of 32.0%. Infection prevalence among males was 35.3%, while females recorded 28.6%. The relatively smaller difference between male and female prevalence observed by qPCR compared to microscopy suggests that molecular methods are capable of detecting subclinical and low-parasite-density infections that may not be detectable using conventional parasitological methods.

The comparative diagnostic performance of POC-CCA and qPCR using microscopy as the conventional reference

method is presented in Table 4. qPCR demonstrated the highest sensitivity (77.7%) and negative predictive value (94.1%), indicating superior ability to detect true positive infections and exclude false negatives. POC-CCA showed a sensitivity of 72.2% and specificity of 87.8%, with a higher positive predictive value (56.5%) compared to qPCR (42.4%). The high negative predictive values observed for both methods suggest strong reliability in identifying uninfected individuals.

Additional diagnostic performance metrics are summarized in Table 5. The positive likelihood ratio (LR+) for POC-CCA was 5.92, indicating a relatively strong ability to confirm infection when a positive result is obtained. In contrast, qPCR showed a lower LR+ value of 3.55 but demonstrated a lower negative likelihood ratio (LR− = 0.29), suggesting better performance in ruling out infection among negative cases.

Overall, qPCR demonstrated superior sensitivity and the highest detection rate among the evaluated diagnostic methods, followed by POC-CCA and microscopy (Figure 4). The findings indicate that molecular diagnostic techniques may provide improved detection of low-intensity and asymptomatic *S. haematobium* infections that are frequently underestimated by conventional microscopy.

DISCUSSION

This study demonstrates that both POC-CCA and qPCR detected a higher prevalence of S. haematobium infection than urine microscopy (van Dam *et al.*, 2004). The superior sensitivity of qPCR underscores its ability to identify low-intensity infections that are commonly missed by conventional parasitological methods. Although microscopy was used as the reference method in this study, it is recognized that no single diagnostic test can be considered a true gold standard for schistosomiasis (Bakare *et al.*, 2018). Therefore, the observed differences in diagnostic performance likely reflect variations in infection intensity and antigen or DNA detectability (Vinkeles *et al.*, 2020). POC-CCA offers operational advantages in endemic, resource-limited settings due to its ease of use and rapid turnaround time, making it suitable for large-scale screening and surveillance programs (RMD, 2020).

The sensitivity of the CCA cassette in this study was 72.2% lower than that reported in previous studies, including 96.0% and 96.7% reported by El-Ghareeb et al. (2016). The sensitivity of qPCR in this study was 77.7%. These values were lower than the 89.0% reported by van Dam et al. (2004) and the 95.2% reported in Kenya by Vinkeles et al. (2020). The higher detection rate observed with qPCR is consistent with previous studies showing that molecular techniques are more sensitive than antigen-based and microscopy methods. qPCR can detect low-intensity and pre-patent infections because it amplifies parasite DNA, whereas CCA relies on antigen release, which may be insufficient in light infections. Circulating Cathodic Antigen (CCA) combined with qPCR should be used to improve detection, especially in low-endemicity areas or for post-treatment evaluation.

The different prevalence in this study compared to previous studies could be attributed to variations in geographic and environmental settings as well as socio-cultural practices. Schistosomiasis is known to be focally distributed, and prevalent rates vary widely between communities and locations within Nigeria. The higher sensitivity of qPCR (77.7%) compared to POC-CCA (72.2%) reflects its ability to detect low-intensity infections. Likelihood ratio analysis indicates that POC-CCA has stronger rule-in capacity (LR+ ~6), whereas qPCR demonstrates better rule-out utility (higher NPV).

CONCLUSION

The findings indicate that qPCR and POC-CCA are more sensitive than urine microscopy for detecting S. haematobium infection. While microscopy remains useful in routine settings, integration of antigen detection and molecular diagnostics may improve surveillance, case detection, and schistosomiasis control efforts in endemic regions.

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