

A WATER QUALITY-BASED PREDICTIVE TOOL FOR DISASTER MANAGEMENT OF WATERBORNE INFECTIONS DURING DROUGHT EVENTS

WORK PACKAGE 2: PREVALENCE, ASSOCIATED RISK FACTORS AND DIAGNOSTIC BIOMARKERS OF SCHISTOSOMIASIS AMONG SCHOOL GOING CHILDREN IN NELSON MANDELA BAY MUNICIPALITY

Final Report to the
Water Research Commission

by

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- Overview of work packages (WRC report no. 3229/1/25)
- Work package 1: Integrated ecological assessment of vegetation, physico-chemical properties and schistosomiasis intermediate host snail distribution in freshwater bodies (WRC report no. 3229/2/25)
- Work package 3: Bacteriological assessment of water sources and retrospective analysis of diarrhoeal prevalence in Nelson Mandela Bay (WRC report no. 3229/4/25)
- Work package 4: Pre- and post-intervention assessment of an educational program on hygiene knowledge and practices among municipal waste and sanitation workers in Nelson Mandela Bay (WRC report no. 3229/5/25)

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EXECUTIVE SUMMARY

BACKGROUND

Schistosomiasis, a parasitic disease caused by *Schistosoma* species, remains a significant public health challenge in South Africa, with 2 to 3 million infections and 20 million at risk. In Nelson Mandela Bay (NMB), water scarcity and infrastructure failures drive reliance on unsafe water sources, exacerbating transmission risks. Despite passive surveillance and underreporting due to limited healthcare access, local studies reveal fluctuating prevalence (13–29% urinary schistosomiasis in KwaNobuhle) and low detection rates ($\leq 2\%$ in schools), underscoring the need for updated prevalence data and targeted interventions. Traditional diagnostics (e.g., Kato-Katz, urine filtration) lack sensitivity for low-intensity infections, while advanced methods like PCR are resource-intensive. Urinary biomarkers (e.g., haematuria, proteinuria) offer a promising, cost-effective alternative for early detection in low-resource settings. Addressing diagnostic gaps, coupled with improved surveillance and community-specific health strategies, is critical to curbing transmission and enhancing disease control in NMB.

AIMS

The aims of the project – work package 2 (WP2):

1. Assess the knowledge, attitude, and practices and risk factors associated with schistosomiasis prevalence and transmission among primary school going children.
2. Determine the prevalence and intensity of *S. haematobium* and *S. mansoni* infection through urine and fecal samples respectively, in school going children.

METHODOLOGY

A comprehensive cross-sectional study was conducted to determine the prevalence, risk factors, and diagnostic biomarkers associated with schistosomiasis among school-going children aged 5–14 years in NMB. The study was structured into three distinct phases: phase 1 included assessing risk factors and KAPs, phase 2 evaluated the prevalence of schistosomiasis and STHs, and phase 3 profiling diagnostic urinary biomarkers. In Phase 1, structured, close ended, interview-administered questionnaires were used to assess socio-demographic characteristics, environmental risk factors, and KAP related to schistosomiasis. Data were analysed to identify factors influencing KAP and their associations with demographic and behavioural variables. In Phase 2, urine and stool samples were collected to diagnose urogenital and intestinal schistosomiasis as well as STHs. Urine analysis involved the use of dipsticks to detect haematuria, followed by urine filtration to identify *S. haematobium* eggs. Haematuria positive samples (2+ and 3+) underwent molecular diagnostics (cPCR) targeting the Dra1 repeat sequence for cell-free DNA (cfDNA) confirmation. Stool analysis focused on detecting *S. mansoni* and STH infections using Kato-Katz and POC-CCA tests, with cPCR targeting the cox-1 gene performed on POC-CCA-positive samples to confirm *S. mansoni*. In Phase 3, urinalysis was conducted using Siemens Multistix 10SG dipsticks to measure urinary biomarkers such as proteins, leukocytes, bilirubin, ketones, and glucose. Correlations among these biomarkers were evaluated to explore their potential association with schistosomiasis.

RESULTS AND DISCUSSION

A total of 759 children (58% males, 42% females; mean age 11 ± 1.5 years) participated in the study. In Phase 1, the study revealed poor KAP among participants, with only 11% demonstrating knowledge about schistosomiasis. Key risk factors included swimming (44%), living near water bodies (21.1%),

using bush toilets (5.1%), lacking access to household tap water (4.3%), and fishing (0.3%). Knowledge scores were significantly influenced by gender ($p = 0.015$) and grade level ($p = 0.045$), with females scoring lower than males ($\beta = -0.15$; $p = 0.018$). Attitude ($p = 0.023$) and practice ($p = 0.001$) scores improved in higher grades, though children in grades 4–7 displayed fewer positive attitudes ($\beta = 0.07$; $p = 0.038$) and practices ($\beta = 0.11$; $p = 0.001$). Unawareness of disease transmission was associated with lower knowledge ($\beta = -0.24$; $p = 0.091$) and attitude scores ($\beta = -0.22$; $p = 0.001$). Using a neighbour's toilet was linked to significantly lower knowledge ($\beta = -0.55$; $p = 0.020$). In Phase 2, haematuria was detected in 33.6% of urine samples, but traditional urine filtration identified only one egg-positive case (0.1%). Molecular diagnostics confirmed *S. haematobium* in 31.4% of haematuria-positive samples. For *S. mansoni*, no infections were detected using Kato-Katz, but POC-CCA indicated a prevalence of 3.2%, with 32.1% of these confirmed by cPCR. Two cases of *Ascaris lumbricoides* (0.3%) were detected using Kato-Katz. In Phase 3, haematuria (33.6%) was the most prevalent biomarker, followed by leukocytes (21.3%) and proteins (15%). Other biomarkers, including bilirubin and ketones, were detected at lower frequencies. A strong correlation was observed between haematuria and leukocytes.

GENERAL

The aims of WP2 were successfully achieved, with multivariate regression analysis identifying key demographic and behavioural predictors of schistosomiasis-related KAP among school-aged children. Diagnostic evaluation of *Schistosoma mansoni* in the NMB study area demonstrated significant variability in infection prevalence between diagnostic methods (Kato-Katz vs. POC-CCA). The findings informed evidence-based recommendations to mitigate transmission through integrated interventions targeting education, sanitation infrastructure, diagnostic optimization, and research prioritization

CONCLUSIONS

This multi-phase study highlights poor KAP and significant environmental risk factors for schistosomiasis transmission. Molecular diagnostics proved more sensitive than traditional methods in detecting schistosomiasis, emphasizing the underestimation of disease burden in low-transmission settings. Urinalysis provided additional insights into biomarkers potentially linked to schistosomiasis, paving the way for targeted control measures and further research in endemic areas. This study highlights the limitations of traditional diagnostic techniques in low-transmission settings, emphasizing the need for sensitive molecular methods like cPCR to detect low-intensity infections. The significant prevalence of urogenital schistosomiasis identified through advanced diagnostics underscores ongoing transmission risks in NMB especially among school-going children. The findings stress the necessity for targeted educational programs to improve KAP, public health interventions tailored to mitigate risk factors, preventive chemotherapy with praziquantel and water sanitation and hygiene practices interventions among the school-going children in the study area. Urine reagent strips show promise as cost-effective initial screening tools, yet further validation with comprehensive diagnostics is recommended to enhance early detection and use in monitoring control efforts.

RECOMMENDATIONS

Key recommendations include

- **Education & Awareness:**
 - Integrate schistosomiasis and soil-transmitted helminth education into school curricula, emphasising transmission cycles and treatment.
 - Develop age-, gender-, and culturally tailored programs, leveraging children as knowledge disseminators to households.

Health System Interventions:

- Implement routine surveillance and advanced diagnostics (e.g., PCR) to detect asymptomatic infections.

- Advocate for mass drug administration (MDA) using affordable generic praziquantel, particularly in areas with limited water/sanitation access.

Environmental & Sanitation Measures:

- Install sanitation facilities in high-risk areas to curb open defecation near water bodies.
- Upgrade waste management systems and monitor freshwater sources for intermediate host snails.

Community Engagement (EHPs):

- Launch hygiene campaigns promoting safe waste disposal and handwashing.
- Use mobile apps, games, and multilingual/culturally relevant materials to improve health literacy.

Future Research Priorities:

- Conduct longitudinal studies to track prevalence trends and seasonal impacts.
- Apply molecular diagnostics to assess genetic diversity of *Schistosoma* strains and cytokine profiles in low-transmission settings.
- Investigate migration patterns, water-contact behaviours, and adaptive WASH interventions

See the conclusions and recommendations Chapter 7 below for more details.

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ACRONYMS & ABBREVIATIONS

BOD	Biological Oxygen Demand
CAA	Circulating Anodic Antigen
CCA	Circulating Cathodic Antigen
CFDNA	Circulating Cell-Free Parasite DNA
cPCR	Conventional Polymerase Chain Reaction
DHIS	District Health Information System
ELISA	Enzyme-Linked Immunosorbent Assay
ERR	Egg Reduction Rate
HIV	Human Immunodeficiency Virus
KAP	Knowledge, Attitudes, and Practices
KCl	Potassium Chloride
KH_2PO_4	Potassium Dihydrogen Phosphate
LMICs	Low- and Middle-Income Countries
MCC	Medicines Control Council
MDA	Mass Drug Administration
mM	Millimolar
NaCl	Sodium Chloride
Na_2HPO_4	Disodium hydrogen phosphate
NHLS	National Health Laboratory Service
NMB	Nelson Mandela Bay
NMBHD	Nelson Mandela Bay Health District
NTDs	Neglected Tropical Diseases
PBS	Phosphate Buffer Saline
cPCR	Conventional Polymerase Chain Reaction
pH	Hydrogen Potential
POC-CCA	Point of Care Circulating Cathodic Antigen Test
PZQ	Praziquantel
SEA	Soluble Egg Antigen
STH	Soil Transmitted Helminths
WASH	Water Sanitation Hygiene
WHO	World Health Organization
USD	United States Dollars

GLOSSARY

Schistosomiasis (Bilharzia): A parasitic infection caused by blood-dwelling *Schistosoma* flatworms (trematodes), transmitted through contact with freshwater contaminated by infectious cercariae.

Schistosoma: A genus of parasitic flatworms (*phylum Platyhelminthes*) causing schistosomiasis. Key species include *S. mansoni* (intestinal) and *S. haematobium* (urogenital).

Trematoda: A class of parasitic flatworms (flukes) that includes *Schistosoma* species.

Digenea: An order of trematodes with complex life cycles involving multiple hosts.

Urogenital Schistosomiasis: Caused by *S. haematobium*, affecting the urinary and reproductive systems.

Intestinal Schistosomiasis: Caused by species like *S. mansoni*, damaging the intestines and liver.

Passive Surveillance: Disease monitoring reliant on voluntary case reporting, often leading to underdiagnosis.

KAP (Knowledge, Attitudes, Practices): A framework to assess community awareness and behaviours influencing disease risk and prevention.

EHPs (Environmental Health Practitioners): Professionals reporting and managing public health risks, including schistosomiasis cases.

Urine Filtration: A diagnostic method for detecting *S. haematobium* eggs in urine; limited sensitivity in low-intensity infections.

Kato-Katz Microscopy: A stool-based technique to identify *Schistosoma* eggs; less effective for low-level infections.

POC-CCA (Point-of-Care Circulating Cathodic Antigen): A rapid test detecting *S. mansoni* antigens in urine; sensitivity declines at low infection levels.

PCR (Polymerase Chain Reaction): A molecular method to detect *Schistosoma* DNA; highly sensitive but costly and technically demanding.

Haematuria: Presence of blood in urine, a biomarker for urogenital schistosomiasis.

Proteinuria: Excess protein in urine, associated with kidney damage from schistosomiasis.

Leukocyturia: Elevated white blood cells in urine, indicating inflammation or infection.

Low-Intensity Infections: Infections with few parasites, often undetected by traditional diagnostics.

Non-Invasive Diagnostics: Methods like urine dipsticks or antigen tests that do not require invasive procedures.

Incubation Period: The 6–8-week delay between infection and egg production, complicating early diagnosis.

CHAPTER 1: BACKGROUND

1.1 INTRODUCTION

Schistosomiasis, commonly known as bilharzia, is a parasitic infection caused by trematodes of the *Schistosoma* species (Njikho et al., 2023a). These blood-dwelling parasites belong to the kingdom *Animalia* and are categorized under the phylum *Platyhelminthes* (Khiani & King, 2023). They are categorised within the class Trematoda, order *Digenea*, family *Schistosomatidae*, and the genus *Schistosoma*. Schistosome parasites encompass various species that cause two main types of disease in humans: gastrointestinal and urogenital schistosomiasis (Kokaliaris et al., 2022). Intestinal schistosomiasis is attributed to *Schistosoma mansoni*, *S. mekongi*, *S. intercalatum*, *S. guineensis*, and *S. japonicum*, while urogenital schistosomiasis is primarily caused by *S. haematobium* (McManus et al., 2018).

In South Africa, an estimated 2 to 3 million people are infected with schistosomiasis, with 20 million at risk (Appleton & Miranda, 2015; Nwoko et al., 2023a). Surveillance largely remains passive, depending on case notifications, which often results in underreporting. This underreporting is due to limited awareness among healthcare professionals and inadequate access to healthcare services in remote areas, hindering accurate case detection and response (de Boni et al., 2021a). In NMB (NMB), water shortages have been exacerbated by increased water usage, poor rainfall, and failing infrastructure. These challenges force residents to rely on unsafe water sources, which heightens the risk of schistosomiasis transmission. A 2022 workshop at Nelson Mandela University highlighted rising schistosomiasis cases in NMB through case reports from environmental health practitioners (EHPs). In addition, a retrospective study done by Hambury, (2021) in KwaNobuhle showed urinary schistosomiasis prevalence fluctuating from 13% in 2014 to 29% in 2017, then slightly declining to 19% in 2018. A 2017 mapping program by the Eastern Cape Department of Health found 2% of schoolchildren infected with *S. haematobium* and 0.9% with *S. mansoni* in limited NMB schools (CDCD, 2019). Given the rising schistosomiasis cases reported by EHPs, fluctuating prevalence rates observed in KwaNobuhle, and the limited data from the 2017 mapping program in KwaNobuhle schools, there is a clear need for a comprehensive prevalence study to better understand the current scope of the disease and guide effective public health interventions. Limited awareness and unfavourable attitudes contribute to the disease's persistence, emphasizing the importance of understanding risk factors and the knowledge, attitudes, and practices (KAP) associated with schistosomiasis for effective prevention.

Eliminating schistosomiasis, requires innovative diagnostic approaches, particularly in low-transmission areas where traditional methods fall short. Methods such as urine filtration and Kato-Katz microscopy frequently overlook low-intensity infections due to their dependence on low egg detection, which takes place only after a 6–8-week incubation period (Siqueira et al., 2021a). This limitation results in numerous cases remaining undiagnosed, allowing the disease to persist without detection and facilitating continued transmission. Advanced diagnostics such as POC-CCA provide improved accuracy by identifying live antigens from *S. mansoni* parasite (Coelho et al., 2016a,); however, POC-CCA sensitivity diminishes at low infection levels (Peralta & Cavalcanti, 2018). Molecular methods using polymerase chain reaction (PCR) is highly sensitive in detecting low concentration *Schistosoma* DNA; however, it is frequently cost-prohibitive, requires specialised technical skills, and lacks accessibility in low-resource environments. Investigating the link between schistosomiasis and non-invasive urinary biomarkers, such as haematuria, proteinuria, and leukocyturia, presents a transformative opportunity for disease detection and control. By leveraging these biomarkers, a cost-effective and accessible diagnostic tools tailored to resource-constrained settings can be developed. Understanding their

relationship with schistosomiasis infections is crucial for improving disease monitoring, enabling rapid interventions, and achieving better health outcomes. This research is important as it is a pivotal step towards breaking the cycle of transmission in NMB. Unlocking the potential of urinary biomarkers will bridge critical diagnostic gaps, strengthen public health responses, and ultimately controlling transmission.

1.2 PROJECT AIMS

The following were the aims of the project:

1. Assessment of knowledge, attitude, and practices (KAPs) and risk factors associated with schistosomiasis prevalence and transmission among primary school going children.
2. Determining the prevalence and intensity of *S. haematobium* and *S. mansoni* infection through urine and fecal samples respectively, in school going children.

1.3 SCOPE AND LIMITATIONS

This study addressed the lack of data on schistosomiasis prevalence in NMB, particularly in the post-coronavirus diseases (COVID)-19 period. School-aged children, who are highly susceptible to both schistosomiasis and STHs, were the primary focus due to their increased vulnerability. The research aimed to provide precise information on the extent of infection and associated transmission factors in a low transmission area, ultimately contributing to better health outcomes, cognitive and physical development, and improved educational performance among children. The involvement of schools in prevalence surveys also fosters community engagement and shared responsibility for health initiatives.

By combining various diagnostic methodologies, the study took a comprehensive approach to determining the distribution of schistosomiasis in the region, enhancing diagnostic accuracy and minimizing false negatives and positives. The use of multiple diagnostic tools allows for the detection of infections at different stages and improves the differentiation between infected and uninfected individuals. The circulating cathodic antigen (CCA) test addresses the limitations of the Kato-Katz method by detecting *S. mansoni* in low-intensity infections, while cPCR provides a sensitive and specific means of identifying minimal levels of *Schistosoma* DNA. Though complex and costly, PCR was included to validate diagnoses through its ability to detect low-level infections and differentiate between false positives and negatives. The profiling of urinary metabolites detected through urinalysis offers novel diagnostic advantages. These biomarkers complement traditional methods, providing a non-invasive, accessible, and cost-effective approach suitable for large-scale use in resource-limited settings. Urinary metabolites, which reflect the body's biochemical response to infection, can aid in diagnosis even in cases of low egg excretion. Identifying these metabolites can also illuminate disease mechanisms, highlight potential drug targets, and support post-treatment monitoring during screening programs.

CHAPTER 2: METHODOLOGY

2.1 STUDY AREA

The study was conducted in five towns within NMB: Motherwell, Despatch, KwaNobuhle, Kariega, and Ibhayi. Located on the shores of Algoa Bay in the Eastern Cape, NMB is one of South Africa's eight metropolitan municipalities, established on December 5, 2000. Covering an area of 1,959 km², it has geographical coordinates of 33.57°S, 25°36'E. According to the 2022 census, NMB's population is 1.2 million, with the majority being Black (60%), followed by Coloured (23.6%) and White (14.4%) (Stats, 2022). The predominant languages spoken are isiXhosa, Afrikaans, and English. NMB experiences long, cool winters and short, warm summers, with a dry, clear, and windy climate year-round. In 2023, the average temperature was 22.7°C, with a high of 35.5°C in April and a low of 12°C in July. NMB is a major seaport with a large automotive manufacturing industry, contributing significantly to the local economy, along with community services. Fishing, agriculture, and recreational activities are key human activities in the area. **Figure 2.1** below present the study areas, population, geographical coordinates, and languages spoken in the five towns included in this study.

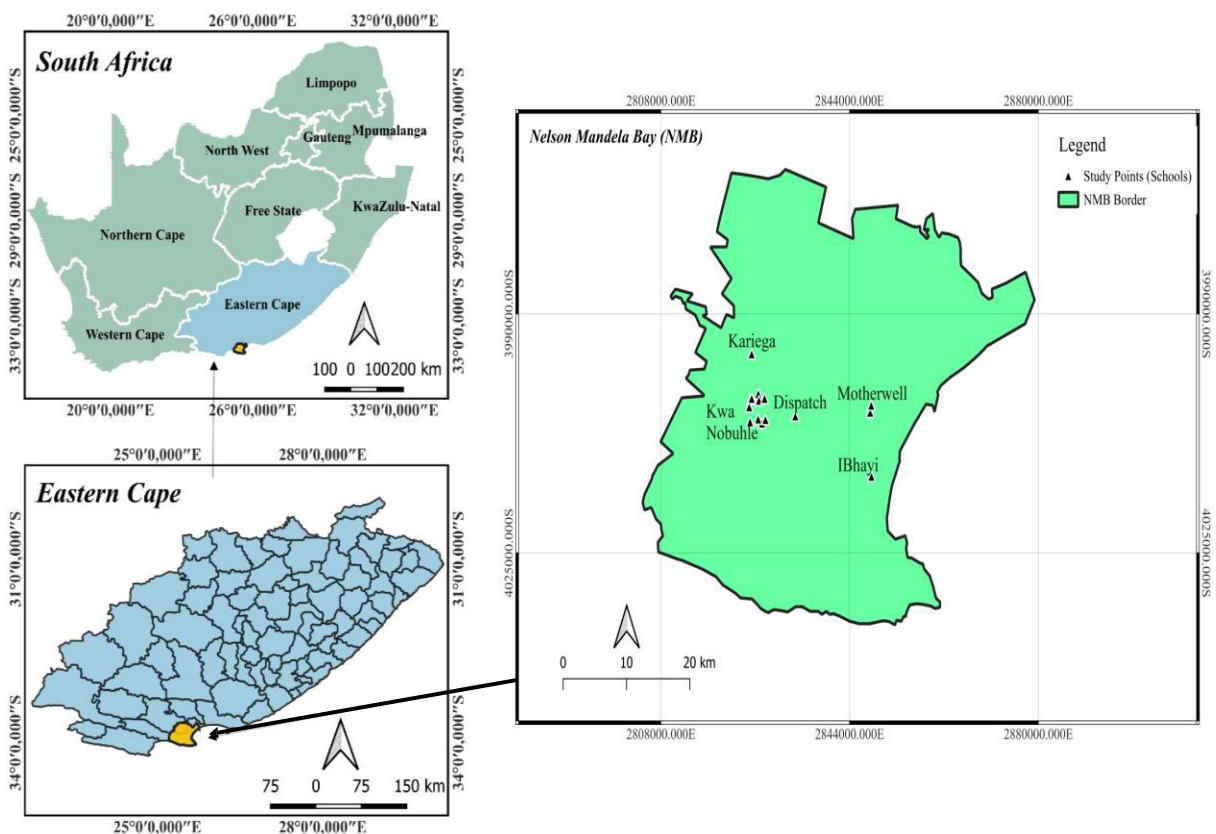


Figure 2.1: A map showing the study area and locations of 15 primary schools sampled in this study as shown by the triangles (QGIS 3.4 Prizren).

2.2 STUDY DESIGN WITH SCHEMATIC DIAGRAMS

An observational cross sectional study design using quantitative methods was utilized in this study. Sample and data collection was done from September 2023 to February 2024. The study was divided into three phases (**Figure 2.2**). Phase 1 was to determine the risk factors and KAP associated with schistosomiasis transmission in the study area. A semi-structured questionnaire survey was used in an interview-based method. Phase 2 was to determine the prevalence of schistosomiasis (*S. haematobium* and *S. mansoni*) and STHs among school-going children in Motherwell, Despatch, Kariega, KwaNobuhle and Ibhayi towns of NMB. Phase 3: urinalysis to profile parameters was performed in all the participants in the study and association between the detected biomarkers and schistosomiasis infections were done. The study sites were purposefully selected due to the existence of water bodies in residential areas that may act as potential transmission sites for schistosomiasis (Sow et al., 2023).

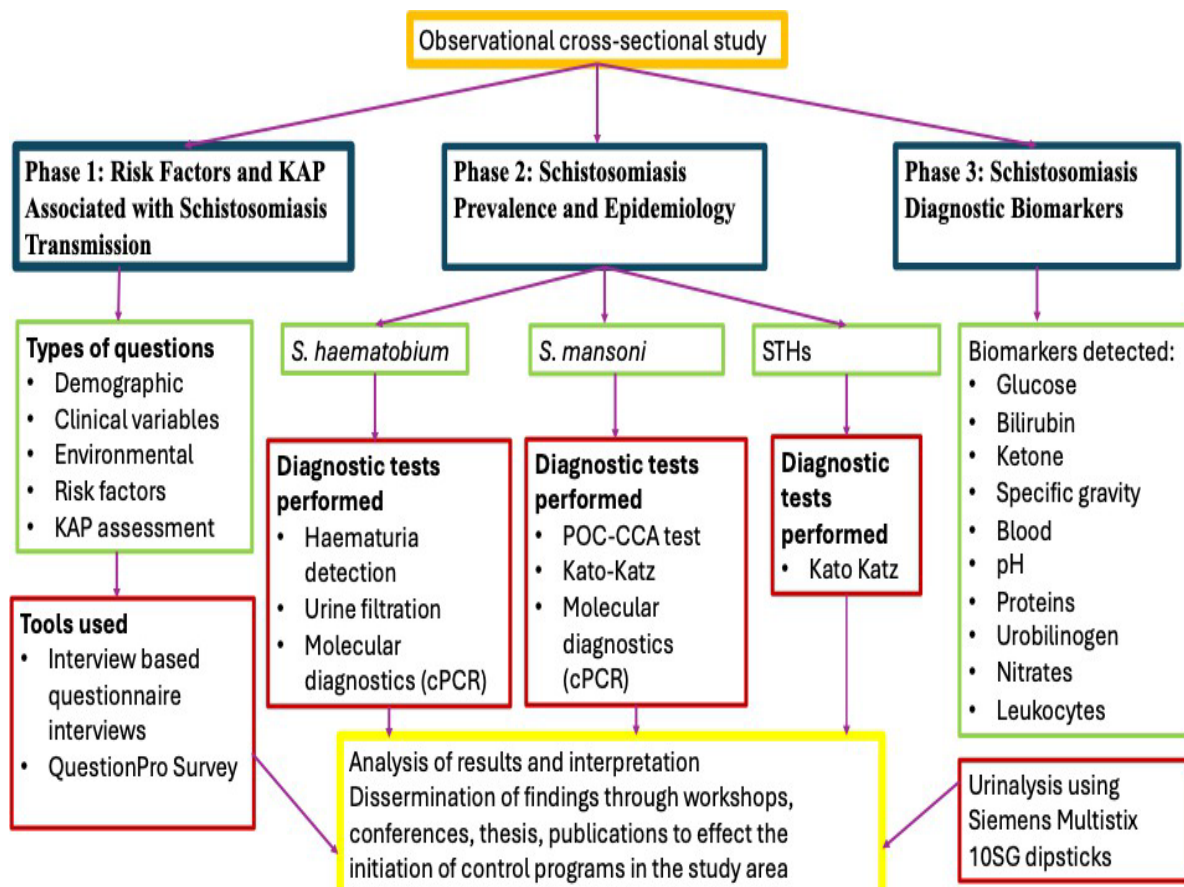


Figure 2.2: Schematic diagram of the study design and methods relative to the three phases.

2.3 POPULATION AND SAMPLING

In South Africa, the public primary education system is generally divided into two distinct stages: the foundation phase, comprising grades R (0) to 3, and the intermediate phase, which includes grades 4 to 7. All primary schools within the study area that encompassed these grade ranges were deemed eligible for inclusion in the study. A stratified cluster-sampling method was employed to select the study participants, wherein the entire student population from each school was divided into the two phases—grades 0-3 and 4-7. Random selection of classes from each grade in every school was then carried out, ensuring a predetermined number of students from each grade level were represented in the study.

This sampling method ensured that each child had an equal opportunity to be selected for participation, while also maintaining proportional representation of gender and grade across the sample, consistent with the overall population. Only participants with parental consent were included in the study. During the data collection process, a total of 64 potential participants were absent and were therefore excluded from the study. As a result, the final sample consisted of 759 participants, as summarised in **Figure 2.3**.

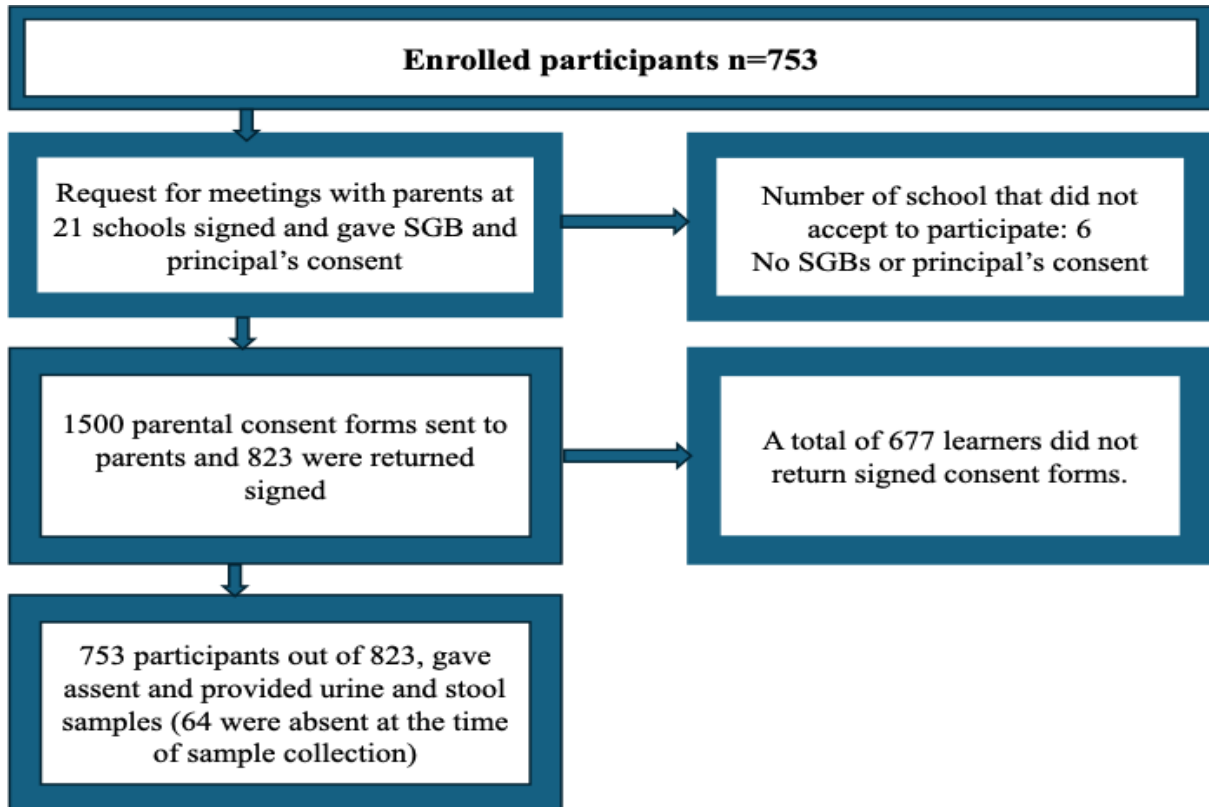


Figure 2.3: Schematic diagram for study participants' recruitment, enrolment and participation.

2.1 SAMPLE SIZE CALCULATION

The 2019 Eastern Cape Report on Communicable Diseases Control Directorate (CDCD) presented findings from a cross-sectional survey carried out in 2017 in the Eastern Cape province. The study included 1972 primary school pupils, with 48% being males and 52% being girls. The overall prevalence of *S. haematobium* throughout all districts was 2% (CDCD, 2019, p. 12). Using this prevalence, the formula from Deribew et al., 2022 was used to calculate the appropriate sample size in the prevalence study as follows:

$$n = \frac{Z^2 \times p \times (1 - p)}{d^2}$$

Where n = sample size, $Z = 1.96$ representing a confidence level of 95% p is the prevalence of schistosomiasis (using the 2% for schistosomiasis in NMB (CDCD, 2019, p. 12), and $d = 1\%$ is the precision error.

That is,

$$n = \frac{1.96^2 \times 0.02 \times (1 - 0.02)}{0.01^2} \cong 753.$$

Adjusting for a 10% nonresponse rate of $753 \times 0.1 = 75$ respondents yield a minimum sample size of 753 and a maximum of 828 respondents that will be considered for this study.

2.2 DATA COLLECTION

2.2.1 Risk Factors Associated with Schistosomiasis Transmission

Data collection was conducted through face-to-face interviews by two trained field workers using a structured questionnaire with 48 closed-ended, multiple-choice questions. Each interview lasted approximately 15-20 minutes. The questionnaire was titled "Risk factors and knowledge, attitudes, and practices regarding schistosomiasis among school-age children in NMB" and was developed after reviewing existing literature (Balogun et al., 2022; Liu et al., 2022; Ude et al., 2024).

The questionnaire was divided into two sections as shown in **Table 2.1** Section A focused on socio-demographic information, clinical symptoms, and risk factors associated with schistosomiasis transmission. Questions in this section addressed activities such as swimming, bathing, crossing rivers without footwear, collecting water from nearby slow-moving water bodies, and sanitation practices, as well as the presence of toilets and sources of household water. Section B aimed at assessing the children's knowledge, attitudes, and practices regarding schistosomiasis, including general awareness, knowledge about snails, causes, routes of transmission, prevention, and treatment of schistosomiasis. Section B explored their sources of knowledge, whether schistosomiasis can be prevented, and if certain practices like wearing shoes, using clean water, and seeking medical treatment are important to reduce the risk of infection. Mixed-format questioning was used in the current survey, with a combination of multiple-choice questions and simple dichotomous "Yes" or "No" or uncertain "I don't know" response options (Martel et al., 2019).

Table 2.1: Components of the questionnaire

Section	Sub-Section	Questions	Type of questions
Section A	Socio-demographic	Age, gender, grade, name of community, religious affiliation	Demographic questions Closed ended
	Clinical Variables	Infected/ not infected, history of infection, clinical symptoms	Close ended Multiple choice
	Risk factors for schistosomiasis transmission	Swimming, bathing, crossing rivers barefooted, collection of water for domestic uses, recreational activities (swimming, fishing), water	Close ended

		sources, sanitation, presence of toilets, identification of snails and if they are harmful	
Section B	Knowledge	Schistosomiasis signs and symptoms, cause, mode of transmission, prevention and treatment, sources of knowledge,	Close ended
	Attitude	If it is a serious disease, does it have a cure, can it be prevented, faeces and urine as sources of infection	Close ended
	Practices	Wears shoes when going outside, washing clothes and bathing in open water sources and freshwater bodies, seeking treatment, use of toilets, urinating/ defecating in rivers.	Close ended

The questionnaire was developed in English and translated into Afrikaans and IsiXhosa, the two most widely spoken local languages in NMB, to ensure younger children could understand the questions. A back translation into English was conducted to verify and validate the accuracy of the translations. The interviews were carried out in private rooms at the schools during break and lunch times to minimise disruption to the participants' learning.

2.2.2 Urine and stool sample collection

A total of 759 urine and stool samples were systematically collected from school children aged 5 to 14 years across the participating schools. Two 50 millilitre (ml) plastic bottles, characterised by a wide mouth and a screw cap, were supplied to the selected participants. Each bottle was labelled and featured a distinct unique identifier (ID) (Esiere et al., 2022a). Midstream urine samples were collected from 10:00 to 14:00, aligning with the peak period of egg excretion in individuals infected with *Schistosoma* (Umar et al., 2017). The timing corresponds with the circadian rhythms of the parasites, thereby facilitating optimal conditions for both egg release and excretion (Rawlinson et al., 2021). The researcher performed a visual demonstration to illustrate the correct method for collecting samples and transferring them into specific individual bottles. Participants were instructed to return with containers that held more than half of a urine sample. Samples were gathered from staff bathrooms at the schools and then transported to a private room to maintain participant confidentiality. Participants received soap to facilitate comprehensive handwashing following the return of the sample containers. The plastic bottles containing stool and urine samples were securely sealed and placed in a cooler box before being transported to the laboratory located at Nelson Mandela University for analysis (Sow et al., 2023). The average duration for transport from the sample collection site to the laboratory was one hour. In the laboratory, parasitological analysis and the POC-CCA test was conducted on the same day, executed by the researcher alongside a laboratory technician.

2.2.2.1 Haematuria detection

On the day of collection, each urine sample (i.e. one single sample per person) was analysed for microhaematuria utilising a urinalysis reagent strip (Siemens Multistix 10 SG Reagent Strips). After briefly immersing the reagent strip into the urine sample for 1 to 2 seconds, the strip was removed and placed on the bench for approximately one minute. The colour of the reagent strip was ultimately compared to the corresponding reference values in the colour fields. The concentration of haemoglobin,

reflecting the intensity of haematuria in urine, was documented and classified as negative (0), trace (\pm), small (1+), moderate (2++), and large (3+++), in accordance with the manufacturer's guidelines (Deribew et al., 2022).

2.2.2.2 Urine filtration

The urine filtration technique was used for the detection of *S. haematobium* eggs in the urine. The urine filtration process was conducted using CHM® MNY Nylon membrane filters catalogue number: MNY010013H, pore size 10 μ m, size 13mm and a 10 ml plastic syringe. Each urine sample was thoroughly mixed, and 10 ml was drawn into the syringe. The Swinnex® filter holder 13 mm catalogue number: SX0001300, was then attached to the syringe, and the urine was filtered through the membrane. After filtration, the membrane filter was carefully removed from the holder and placed on a microscope slide for examination (Alade et al., 2023). A drop of Lugol's iodine was added to the membrane filter, which was then covered with a coverslip (Sow et al., 2023). The slide was examined under a Motic B1 Series light microscope at objective magnifications of $\times 10$ and $\times 40$ by two skilled laboratory technicians. The quantity of *S. haematobium* eggs per 10 ml was enumerated and documented for each participant (Sacolo-Gwebu et al., 2019). The sample underwent analysis, and any *S. haematobium* eggs that were detected were classified as positive for *Schistosoma*. The intensity of *S. haematobium* infection was defined by the quantity of schistosome eggs present in 10 ml of urine, with 1–49 eggs per 10 ml indicating a light infection and 50 or more eggs per 10 ml signifying a heavy infection (Wiegand et al., 2021).

2.2.2.3 Kato-Katz Technique

Duplicate Kato-Katz thick smear slides were prepared for each stool sample using the Helmintex® Kato Katz Kit from Bio-Manguinhos, Oswaldo Cruz Foundation, Rio de Janeiro (RJ), Brazil, following the manufacturer's instructions. Stool samples were sieved through a mesh screen to remove larger particles before being mounted on slides with a template aperture. After the template was removed, a cellophane sheet soaked in glycerol and malachite green was applied. The samples were compressed using microscope slides to ensure even distribution for microscopic examination. The slides were analysed under a Motic B1 Series light microscope at objective magnifications of $\times 10$ and $\times 40$ by two experienced laboratory technicians. To ensure quality control, 10% of the slides were re-examined by the lead technician to confirm positive or negative results. Helminth egg counts were multiplied by a factor of 24 to estimate infection intensity in eggs per gram (EPG) of stool. Positive and negative discrepancies or differences in egg counts exceeding 20% were re-read to maintain consistency and accuracy.

2.2.2.4 POC-CCA Test

Urine samples were tested for *S. mansoni* circulating cathodic antigen (CCA) using the POC-CCA test kits (Rapid Medical Diagnostics, South Africa). All test reagents were equilibrated to room temperature prior to use. Two drops of urine (each equivalent to 40 μ l) were pipetted into the cassette well using a straw dropper. The cassette was incubated for 20 minutes, after which results were visually assessed by two trained laboratory technicians using a scoring guide. Test results were categorized as negative (no test line), trace (faint test line), 1+ (light red test line), 2+ (dark red test line), and invalid (absence of the control line). Test results exceeding 25 minutes were considered invalid and repeated.

2.2.3 Molecular examination for schistosome infection

2.2.3.1 Preparation of urine cell pellet

A volume of 10 ml of urine was placed in a 15 ml falcon tube and subjected to centrifugation in a Eppendorf® Centrifuge 5810, at a speed of 5,000 revolutions per minute (rpm) for a duration of 5 minutes. This procedure entails the extraction of the liquid component, called supernatant, from the solid particles, known as cell pellets, which contain the cell-free DNA, in the urine samples. The rapid velocity and extended duration of centrifugation guarantee the effective sedimentation of cells at the bottom of the tube. After centrifugation, 5 ml of the supernatant (liquid component) above the sediment was poured off. The remaining 5 ml and cell pellet were combined and divided into two 2.5ml Eppendorf tubes. This procedure removed most of the liquid portion of the urine, resulting in concentrated solid cell pellets (Guegan et al., 2019). The samples were stored in a freezer at a temperature of -20°C until the following phase, which involves extracting and concentrating the cfDNA.

2.2.3.2 Preparation of stool samples for DNA Extraction

Stool samples were centrifuged before DNA extraction to enhance detection accuracy. Centrifugation concentrated parasitic DNA and removed contaminants, yielding cleaner samples for molecular testing. Stool samples (pea-sized portions) were suspended in 10 mL phosphate-buffered saline (PBS, pH 7.4) to disintegrate the stool matrix and release parasitic cells. The suspension was vortexed, centrifuged at 5000 rpm for 10 minutes, and divided into aliquots for storage at -20°C until further analysis. This protocol ensured the preservation of parasitic DNA and minimized sample degradation.

2.2.3.3 DNA Extraction from urine samples

The centrifuged urine samples were thawed in preparation for DNA extraction. The ZymoBIOMICS DNA Miniprep Kit designed for purifying DNA from a wide array of sample inputs ranging from faeces, biofilms, and urine samples was used. The DNA extraction was done according to the manufacturer's guidelines. The protocol was as follows: The urine cell-pellets were suspended in 150 microlitres (µl) of DNA elution buffer and then transferred into separate Eppendorf tubes. Next, 200 microlitres of bio-fluid - cell buffer solution (red) and 20 µl of proteinase K enzyme were added to digest the sample. The sample was subjected to vortexing in order to get a thorough mixture and thereafter incubated at 55 °C for a duration of 10 minutes on a heating block. A precisely measured volume of 420 µl of genomic binding buffer was added to the digested sample and properly mixed. Subsequently, the mixture was moved to a Zymo-Spin IIC-XL column placed in a collecting tube and subjected to centrifugation at a speed of 14,000 rpm for a duration of 1 minute. The columns were transferred to fresh collection tubes. Then, 400 µl of DNA pre-wash buffer was added to the columns. The columns were spun at 14,000 rpm for 1 minute, and the contents of the collection tubes were then discarded. Next, 700 µl of g-DNA wash buffer was added and the mixture was centrifuged at 14,000 rpm for 1 minute. After that, the collecting tube was emptied once again. Afterwards, 200 µl of g-DNA wash buffer was added and centrifuged at 14,000 rpm for 1 minute. The collection tube containing the flow through was then discarded. The columns were transferred to sterile micro-centrifuge tubes and 50 µl of DNA elution buffer was added. The mixture was kept at room temperature for 5 minutes and then centrifuged at 14,000 rpm for 1 minute to extract the DNA. Subsequently, the columns were disposed of and the g-DNA, which had already been extracted in the micro-centrifuge tubes, was stored at -20 °C until further analysis by cPCR (Esieré et al., 2022b).

2.2.3.4 *cfDNA Extraction from the stool sample*

DNA extraction from stool samples was performed using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, USA) following the manufacturer's instructions. The centrifuged stool samples were thawed in preparation for DNA extraction. The protocol was as follows: The suspension was suspended in 150 microlitres (μ l) of DNA elution buffer and then transferred into separate Eppendorf tubes. Next, 200 microlitres of bio-fluid - cell buffer solution (red) and 20 μ l of proteinase K enzyme were added to digest the sample. The sample was subjected to vortexing in order to get a thorough mixture and thereafter incubated at 55 °C for a duration of 10 minutes on a heating block. A precisely measured volume of 420 μ l of genomic binding buffer was added to the digested sample and properly mixed. Subsequently, the mixture was moved to a Zymo-Spin IIC-XL column placed in a collecting tube and subjected to centrifugation at a speed of 14,000 rpm for a duration of 1 minute. The columns were transferred to fresh collection tubes. Then, 400 μ l of DNA pre-wash buffer was added to the columns. The columns were spun at 14,000 rpm for 1 minute, and the contents of the collection tubes were then discarded. Next, 700 μ l of g-DNA wash buffer was added and the mixture was centrifuged at 14,000 rpm for 1 minute. After that, the collecting tube was emptied once again. Afterwards, 200 μ l of g-DNA wash buffer was added and centrifuged at 14,000 rpm for 1 minute. The collection tube containing the flow through was then discarded. The columns were transferred to sterile micro-centrifuge tubes and 50 μ l of DNA elution buffer was added. The mixture was kept at room temperature for 5 minutes and then centrifuged at 14,000 rpm for 1 minute to extract the DNA. Subsequently, the columns were disposed of and the g-DNA, which had already been extracted in the micro-centrifuge tubes, was stored at -20 °C until further analysis by cPCR (Esiere et al., 2022b).

2.2.3.5 *Amplification of S. haematobium cfDNA by cPCR.*

The cPCR analysis was conducted using genomic DNA (gDNA) extracted from urine samples to confirm *S. haematobium* infection. A total of 121 urine samples, exhibiting 2+ or 3+ haematuria as detected by dipstick, were purposively selected for cPCR confirmation. Additionally, one urine sample confirmed positive through urine filtration was employed as a positive control for validation of the assay. Central to the research approach was the amplification of the Dra1 repeat sequence, a 121-bp fragment specific to *S. haematobium*. This sequence (Hamburger et al., 2001, pp. 907–911), is present in hundreds to thousands of copies within the *S. haematobium* genome, constituting approximately 15% of its total DNA. Species-specific primers targeting *S. haematobium* were used, with amplification focusing on the 121 bp Dra1 tandem repeats (Esiere et al., 2022b, p. 274). These tandem repeat sequences, characterized by their high copy number, enhance the sensitivity of detection in molecular diagnostics. Primer sequences, synthesized by Inqaba Biotech (Johannesburg, South Africa), were optimised based on previously established protocols (**Table 2.2**).

2.2.3.6 *Amplification of S. mansoni DNA by cPCR*

Given the high sensitivity of molecular methods, cPCR was employed as the confirmatory diagnostic technique. However, due to the high cost of cPCR testing, priority was given to samples that exhibited POC-CCA positivity for detailed molecular evaluation. PCR was conducted using genomic DNA (gDNA) extracted from stool samples. A total of 28 stool samples that tested positive for *Schistosoma* antigen using the POC-CCA test were included. Amplification targeted the *Schistosoma* mitochondrial cytochrome c oxidase subunit 1 (cox1) gene of 251 bp, specific to *S. mansoni* DNA. The reaction utilized species-specific primers for *S. mansoni*, with primer sequences detailed in **Table 2.2**. The oligonucleotide primer pairs were adapted from Sady et al., (2015) (Sady et al., 2015) to target highly repetitive sequences in the *S. mansoni* genome, thereby maximizing the chances of successful amplification and detection of positive cell-free DNA (cfDNA) even at low concentrations. These primers,

obtained from Inqaba Biotech, Johannesburg, South Africa, were carefully selected based on their efficacy in previous studies (Sady et al., 2015).

Table 2.2: Genes amplified by cPCR and their respective primer sequences.

Sequence	Primer name	Primer sequence	Reference
<i>Dra1</i> repeat	ShDra-1 F/R primer	5'GATCTCACCTATCAGACGAAAC-3' F 5'T CACAACGATACGACCAAC3'	(Esiere et al., 2022c, p. 274)

The cPCR reaction utilized 1 µL of gDNA as the template. The reactions were carried out in a final volume of 20 µl constituted with 10 µl OneTaq 2X Master Mix with Standard Buffer, 1 µl of template gDNA (10-30ng/µl), 1 µl of forward primer (10µM), 1 µl of reverse primer (10µM) and 7 µl nuclease free water (reagents were purchased from Inqaba Biotech, Johannesburg, South Africa).

The template DNA was initial denatured by heating the DNA double helix to 94°C for 5 minutes. This process effectively melts the DNA double helix, preparing the template for amplification. This was followed by 35 cycles of denaturation; the DNA double helix melted at 94°C for 30 seconds, allowing the template to become accessible for primers to bind to the complementary strands of the DNA. During annealing the primers were exposed to the target DNA sequence at 50°C for 30 seconds, which facilitated the binding of the primers to the target DNA sequence. The extension step involved heating the reaction to 68°C for a minute. During this step, the DNA polymerase enzyme added nucleotides to the primers, resulting in the synthesis of new DNA strands by *Taq* polymerase enzyme. The last step after the 35 cycles was a final extension where the temperature was set at 68°C for 10 minutes. This step was crucial as it guaranteed the full synthesis of DNA and resulted in the production of the amplified PCR product. At the end of the PCR reaction the temperature was held at 4°C (to reduce enzymatic activity, enabling storage or additional processing of the resulting amplicons). This study utilised a Bio-Rad T100 Thermal Cycler for the cPCR reaction.

2.2.3.7 Agarose gel electrophoresis

The integrity of the cPCR amplicons was verified using a 1% agarose gel. One gramme of agarose powder was added into a conical flask with 100 ml of 1X TBE buffer (Tris Boric Ethylene diamine Tetraacetic acid). The mixture was subjected to microwave heating for 5 minutes to promote the dissolution of agarose powder, resulting in a transparent solution. The mixture was allowed to cool, after which 4 µl of ethidium bromide was added. The solution was introduced into an electrophoretic casting tray, after which a comb was positioned. The solution was subsequently permitted to cool for approximately 30 minutes prior to transfer to an electrophoresis tank. A measured volume of 10 µl of cPCR product, pre-mixed with 2 µl DNA loading dye, was transferred using a pipette. Following the removal of the comb, the mixture was positioned into the appropriate lane of the agarose gel. A 10Kb Fast DNA ladder, along with negative control, was loaded onto the gel. An electric field of 120 V was applied to the gel for 25 minutes. The gel was examined with a UV trans-illuminator to identify the bands. The agarose gel electrophoresis process was adapted from (Esiere et al., 2022).

2.2.4 Schistosomiasis Diagnostic Biomarkers

Urinalysis was performed utilising Siemens Multistix 10 SG Reagent Strips to assess various parameters, including glucose, bilirubin, ketones, specific gravity, blood, pH, nitrites, urobilinogen, and leukocytes, as illustrated in **Figure 2.4**. The procedure adhered to the manufacturer's protocol, confirming that all test strips were within their expiration date and stored correctly. In short: A strip was extracted from the container, and the lid was promptly secured to avoid air exposure risks. The strip was subsequently immersed in the urine sample, guaranteeing that all test pads were adequately saturated. Excess urine was eliminated by carefully and gently dragging the strip along the container's rim and dabbing it on a clean multi-wipe cloth. A timer was started immediately and given that the majority of parameters necessitated an incubation period of 30 to 60 seconds, colour changes were recorded immediately except for leukocytes that required 2 minutes incubation time. Each test pad was analysed in accordance with the designated time for each parameter, starting with those that necessitated the shortest incubation period. The colour changes on the reagent strip were compared to the colour on the reference colour chart provided on the container label for respective biomarkers and results were recorded for each biomarker accordingly. The procedure was carried out on a well-lit surface area to guarantee precise colour matching. Upon completion of the readings, the container containing the remaining strips was securely sealed and stored in a location shielded from light and moisture to maintain the integrity of the reagents and prevent any changes in reactivity. **Figure 2.5** shows the multistix 10SG test strip compared to the colour block on the container label

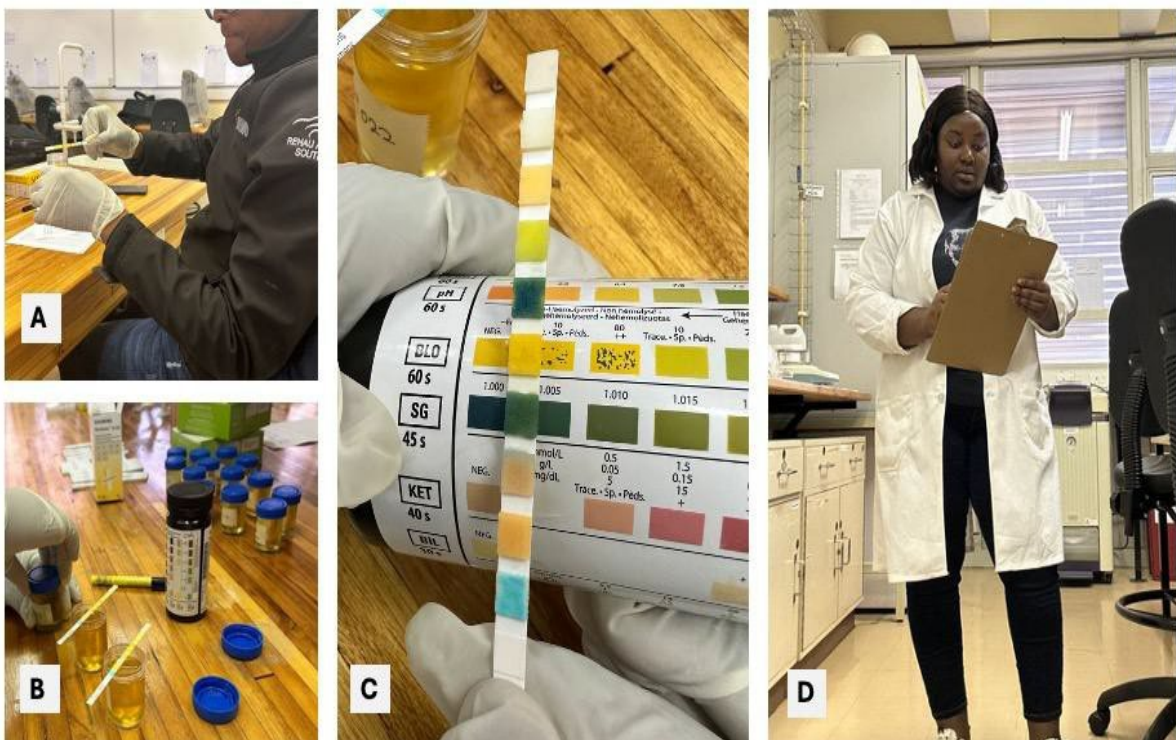


Figure 2.4: Urinalysis. Image A illustrates the dipstick positioned horizontally above the container following its immersion in urine. Image B illustrates the incubation period as the strip undergoes colour development. Image C presents a comparison between the dipstick results and the reference scale indicated on the container. Image D presents the documentation of the results subsequent to the analysis.

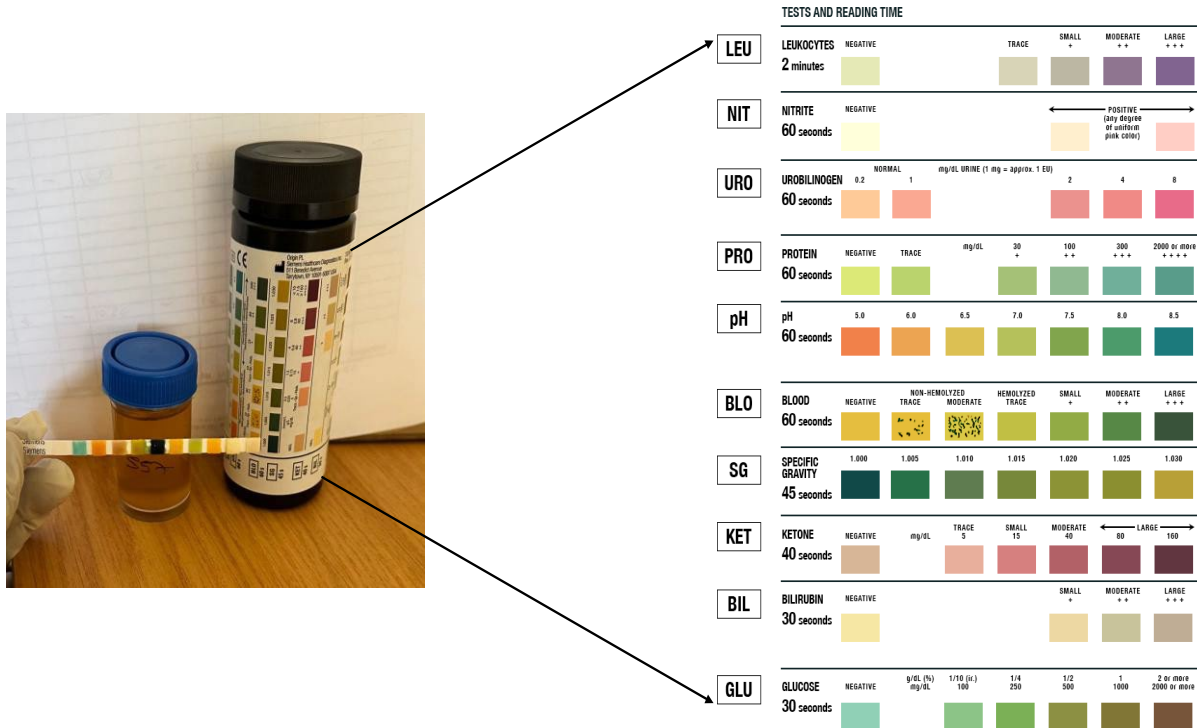


Figure 2.5: Multistix 10SG test strip compared to the colour block on the container label. On the left, a urine dipstick is being held against the reference colour scale on the container to compare results. The right side lists the tests measured by the dipstick, including leukocytes (LEU), nitrite (NIT), urobilinogen (URO), protein (PRO), pH, blood (BLO), specific gravity (SG), ketones (KET), bilirubin (BIL), and glucose (GLU), along with their reading times.

2.2.5 Recruitment

After obtaining ethics approval and authorization from the Eastern Cape Department of Education (ECDOE), permission was requested from 21 schools to participate in the study. Seventeen schools agreed, while four were declined by their governing boards. Meetings were held with parents at these schools to explain the study. However, parental consent could not be obtained from two schools, leaving 15 schools eligible. A total of 1,500 consent forms were distributed, and 823 signed forms were returned, meeting the criteria for participation. Participants were selected using a stratified cluster sampling method across 15 primary schools in five towns within NMB. During school visits, the study's objectives and procedures were clearly explained to principals, teachers, and students. Class rosters of children aged 5–14 in grades 0–7 was compiled, including name, gender, and age, focusing only on those with parental consent and who provided assent. The study was communicated in simple language to ensure participant understanding. Participation was voluntary, and students could withdraw at any time. Assent was obtained both verbally and in writing, with parental or guardian consent required. Eligibility criteria included enrolment in one of the selected schools, age between 5 and 14 years, written parental consent, verbal and written assent, fluency in IsiXhosa or Afrikaans, and presence on the sample collection day.

2.2.6 Ethical considerations

Prior to data collection from the schools the researcher received ethical approval to conduct the research project in schools in NMB from the Nelson Mandela University Research Ethics Committee-

Human (REC-H) ID number: [H23-HEA-ENV-001]. Furthermore, the researcher received permission from the relevant gatekeepers including Eastern Cape Department of Basic Education (ECDoBE) and the school principals and the school governing boards for the respective schools. For each phase of the study the ethical considerations were adhered to with special reference to following all the stipulations specified in the approval letters from both REC-H committee and DoBE.

2.2.7 Data analysis

The data was entered into a Microsoft Excel spreadsheet by the researcher, processed to eliminate errors. The statistician subsequently transferred the data to R software (Version 4.31) and analysed the data. Sex, age study site, and grade of participants were reported as frequencies and percentages. The POC-CCA data were recorded in an Excel spreadsheet, with quantitative intensities coded as follows: negative, trace, 1+ and 2+ were transformed into numeric values of 0, 1, 2 and 3 respectively. Results from the agarose gel electrophoresis were read and recorded by the researcher and verified by a team of trained molecular biologists in Microsoft excel. The Cohen's Kappa (κ) statistic was used to assess the magnitude of agreement between the different diagnostic techniques results, with $\kappa < 0$ indicating poor agreement, $0.0 < \kappa < 0.4$ indicating slight to fair agreement, $0.4 < \kappa < 0.8$ indicating moderate to substantial agreement and $0.8 < \kappa < 1.0$ indicating an almost perfect agreement (Knopp et al., 2018, p. 5).

The Cramer's V statistics were further used to measure the strength of the significant association, with 0 indicating no association and 1 indicating a strong association. The study aimed to evaluate the prevalence of *S. haematobium* using haematuria, urine filtration, and cPCR. Continuous data, such as age and egg intensities, were summarized as mean \pm standard deviation (SD), while categorical data (e.g., sex, age, study site, and grade) were reported as frequencies and percentages. Urinalysis results were coded as negative, trace, 1+, 2+, or 3+, transformed into numeric values (0-4), and cleaned for accuracy. Descriptive statistics summarized the distribution of haematuria. Gel results were analysed by the researcher and verified by trained molecular biologists. The Kruskal-Wallis H test compared results from three cPCR primers (Dra1) with $p < 0.05$ considered significant. Chi-square and Cramer's V tests were used to assess and measure the strength of associations between the different diagnostic tests, respectively.

Data from the survey responses of each participant were captured in the QuestionPro® (<https://www.questionpro.com/>) data management system. Upon completion of data entry in the QuestionPro® software, an Excel spreadsheet and a code sheet were generated. The data collected from the questionnaire was processed and analysed using Microsoft Office 365 (2019 edition). The researcher conducted data cleaning and processing, which involved carefully examining the data for any errors in the scores assigned to the risk variables related to transmission and KAP about schistosomiasis items in the questionnaire. The scores for the KAP aspects were calculated by combining the participants' answers to the KAP questions. A score of one was allocated for each Yes response, while a score of two was awarded for "No" and three for "do not know" or erroneous answers. The inclusion of the "I don't know" option was intended to prevent conjecture (Martel et al., 2019). The data was analysed and described using descriptive and inferential statistics. Various tests, such as Pearson's correlations, independent student t-test, analysis of variance (ANOVA), and linear regression analysis, were employed. A multivariate regression analysis was conducted to determine the risk factors that are associated with schistosomiasis transmission and KAP regarding schistosomiasis. A p -value less than 0.05 (p -value < 0.1) was deemed statistically significant.

CHAPTER 3: KNOWLEDGE, ATTITUDE, AND PRACTICES AND RISK FACTORS ASSOCIATED WITH SCHISTOSOMIASIS PREVALENCE AND TRANSMISSION AMONG PRIMARY SCHOOL GOING CHILDREN

3.1 RESULTS

3.1.1 Socio-demographic characteristics

The study recorded a response rate of 92.22%. The response rate is significant, indicating the engagement and interest of participants, parents, and gatekeepers. The study involved a total of 759 participants, with 440 (58%) being males and 319 (42%) being females. The participants were primary school-going children aged 5 to 14 years from KwaNobuhle, Motherwell, Kariega, Despatch and Ibhayi. The majority of the participants fell between the age category of 9 to 14 (89.9%) years, with fewer participants in the 5 to 8 (10.1%) years age category. Participants enrolled in the study were in grades 0 to 7, with the majority of participants in grades 4 to 7 (61.5%) and a few participants in grades 0 to 3 (38.5%).

Participants had different religious affiliations ranging from African Traditional Religion, mainline churches, Apostolic, Pentecostal, Muslim and others, with a descending distribution of 454 (59.8%), 136 (17.9%), 85 (11.2%), 30 (4%), 3 (0.4%) and 51 (6.7%) respectively. The number of people in a family ranged from 1 to 3; 62 (8.2%), 4 to 6; 344 (45.3) and more than 7; 353 (46.5%). Among the total participants, 11 individuals reported traveling to and returning from rural areas during the holiday period, while the remaining 748 participants remained within NMB. The majority of the participants were recruited from schools in KwaNobuhle 439 (57.8%), followed by Motherwell 152 (20%), Despatch 60 (7.9%), both Ibhayi and Kariega, with 54 (7.1%) participants each (refer to **Table 3.1**).

Table 3. 1: Socio-demographic characteristics of study participants

Socio-demographic variable	Number of participants (n)	Percentage (%)
	759	100.0
Age		
5 to 8 years	77	10.1
9 to 14 years	682	89.9
Grade		
0 – 3	292	38.5
4 – 7	467	61.5
Gender		
Male	440	58.0

Schistosomiasis & STH Prevalence, Risk Factors, and Biomarkers in Nelson Mandela Bay School children

female	319	42.0
Religious affiliation		
Mainline	136	17.9
Pentecostal	30	4.0
Apostolic	85	11.2
African tradition	454	59.8
Muslim	3	0.4
Other	51	6.7
How many are you in your family?		
1 to 3	62	8.2
4 to 6	344	45.3
more than 7	353	46.5
Do you travel outside Nelson Mandela Bay during the holidays, or do you stay within the city?"		
Yes	11	1.8
No	720	94.5
N/A	28	3.7
If yes to the question above, from where?		
N/A	748	98.6
Rural areas	11	1.4
Town		
KwaNobuhle	439	57.8
Kariega	54	7.1
Despatch	60	7.9
Ibhayi	54	7.1
Motherwell	152	20

3.1.2 Clinical variables and symptoms

Of the 759 participants, relative to the responses of the questionnaire survey, a total of 4 (0.5%) had schistosomiasis infections. Specifically, 1 (0.1%) participant reported being infected with schistosomiasis during the time of the visit, while 3 (0.4%) had a history of schistosomiasis infection. Of the 759 participants, 51 (6.7%) had cough, 52 (6.9%) had diarrhoea, 5 (0.7%) had muscle aches and pains, 59 (7.8%) had tummy pain, 4 (0.5%) had an itchy red blotchy and raised rash, 9 (1.2%) reported seeing blood in urine while 627 (82.6%) participants did not report to have any of these symptoms at the time of the visit. Of the participants who reported having any of the clinical symptoms, 27 (3.7%) reported that they do nothing to treat the symptoms, 17 (2.2%) reported that they use home remedies, 6 (0.8%) reported that they went to the hospital and 130 (17.1%) did other things to get better (refer to **Table 3.2**).

Table 3. 2: Clinical symptoms reported by the study participants

Variable	Yes (n)	%
Do you have any of these symptoms now?		
Fever	0	0.0
Cough	51	6.7
Diarrhoea	52	6.9
Muscle aches and pains	5	0.7
Tummy pain	59	7.8
Itchy, red blotchy and raised rash	4	0.5
Blood in urine	9	1.2
Blood in stool	0	0.0
Pain during urination	0	0.0
Vaginal bleeding	0	0.0
Chills, fatigue, fever	0	0.0
None	627	82.6
What do you do to treat these symptoms?		
Do nothing	27	3.7
Use home remedies	17	2.2
Go to the hospital	6	0.8
Other	130	17.1
N/A	627	82.6

3.1.3 Availability of hygiene facilities

Out of the 759 participants, 698 (92%) indicated that they obtain water from municipal water sources (municipal water sources) in their homes, while 28 (3.7%) reported having household boreholes. A total of 33 (4.3%) individuals reported that they obtain their water from the community boreholes. In the current study 160 (21.1%) stated that they resided in close proximity to a river, whereas 599 (78.9%) did not. Regarding the presence of toilets in their homes, the majority of the participants, specifically 607 individuals (80%), reported to have functional toilets at home, whereas 152 individuals (20%) reported not having functional toilets. Among the participants who did not have functioning toilets, 39 (5.1%) resorted to using the outdoors, 22 (2.9%) used their neighbour's toilet, and 2 (0.3%) used public toilets as shown in **Table 3.3**.

Table 3.3: Availability of hygiene facilities

Variable	Responses (n)	Responses (%)
	759	100.0
Where do you fetch water to drink?		
Community borehole	33	4.3
household borehole	28	3.7
household tap water	698	92.0
Do you live near a river?		
Yes	160	21.1
No	599	78.9
Do you have a toilet at home?		
Yes	607	80.0
No	152	20.0
If not, where do you urinate or defecate?		
Bush	39	5.1
Toilet	2	0.3
Neighbour's toilet	22	2.9
N/A	696	91.7

3.1.4 Risk factors associated with schistosomiasis transmission

Among the 759 participants in the present study, the majority 663 (87.1%) reported choosing to spend their leisure time at home, either after school or on weekends. A small fraction of participants 3 (0.4%) engaged in fishing in the rivers nearby, whereas a considerably larger proportion 86 (11.3%) mentioned participating in swimming activities in those same rivers. Out of the total, only 7 (0.9%) participants were involved in

other activities, as specified in **Table 3.4**. Among the 86 students who engage in swimming after school, 38 (44%) of them have acknowledged urinating in the water during their leisure activity. Despite these practices, all participants indicated that they engage in bathing activities at their place of residence, utilising running tap water. Regarding the subject of crossing a river on the way to school, it was found that out of the total participants, 81 (9.1%) participants out of the total sample size reported crossing a river. Among the participants who go through a river on their way to school, it was found that half of them reported crossing barefoot, whereas the other half mentioned that they occasionally wore shoes when crossing the water body.

Table 3.4: Risk factors associated with schistosomiasis transmission.

Variable	Responses (n)	Responses (%)
Do you cross a river on your way to school?		
Yes	81	9.1
No	678	90.9
Do you wear shoes when you cross the river?		
Yes	6	0.8
No	38	5.0
Sometimes	30	4.0
N/A	678	89.3
Where do you bathe?		
Home	759	100.0
River	0	0.0
Where do you go play after school or when you are not at school?		
Swimming	86	11.3
Fishing	3	0.4
At home with friends	663	94.1
Other	7	0.9
When you are swimming in the river, do you urinate in the water?		
Yes	38	5.0
No	78	10.3
N/A	649	85.5

3.1.5 Knowledge of schistosomiasis among participants

In the current investigation, a total of 759 participants were enrolled. Among them, 81 (11%) participants reported that they had heard about schistosomiasis (**Figure 3.1**). The acquisition of information on schistosomiasis by the participants was reported to be from both the school and their parents. The study author contends that schistosomiasis is not part of the official curriculum for primary and secondary schools in South Africa, as mentioned in the (Integrated School Health Programme, 2024). However, based on the current research findings, it is indicated that certain schools offer limited education on schistosomiasis. Approximately 51 (62%) of the participants in the study reported having received education on schistosomiasis during their time in school. This is commendable, yet, in order to effectively control the disease, it is important to implement more formalised and targeted awareness programs in schools. The study also revealed an interesting finding that over 28 (35%) of the learners mentioned being educated about schistosomiasis at home.

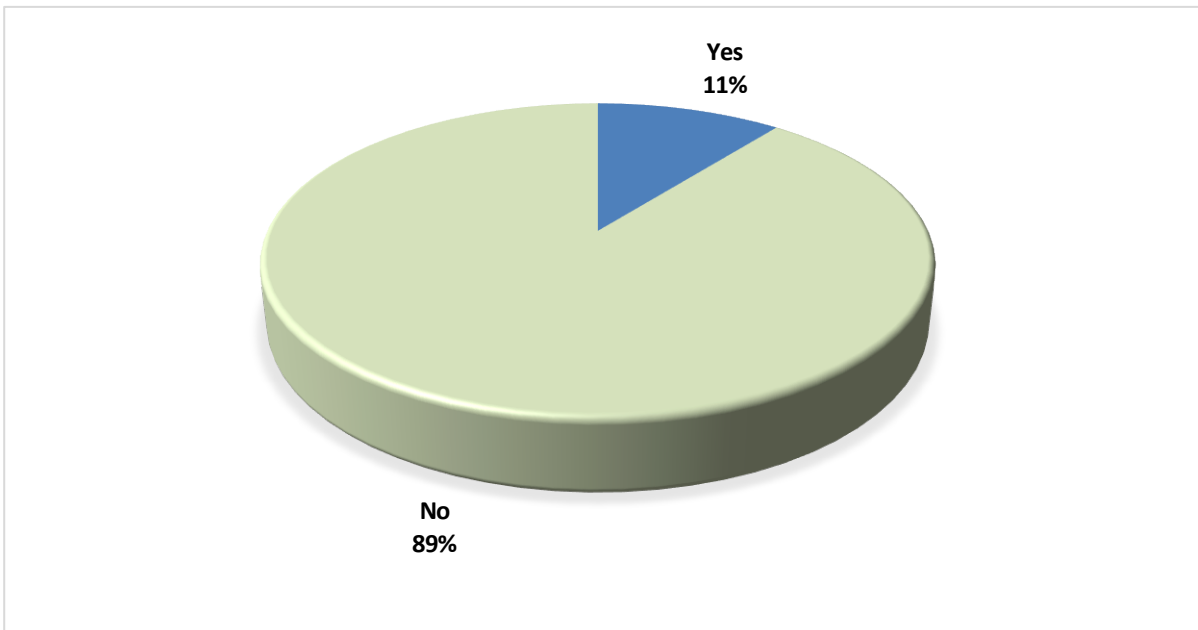


Figure 3.1: Participants' knowledge regarding schistosomiasis

The findings indicate a notable gap in the participants' knowledge of schistosomiasis and its prevention, as shown in **Table 3.5**. The findings reveal that a significant majority of the participants, specifically 741 (97.6%), were not aware of the preventive measures for schistosomiasis, with only 18 (2.4%) acknowledging that the disease can be prevented. This highlights a prevailing lack of understanding regarding the prevention of the disease among the study population. Regarding the understanding of snail intermediate hosts, it was found that only 69 (9.1%) participants were aware that snails can be found in rivers, where they act as carriers for disease-causing parasites. According to the data, 660 (90.6%) participants, were found to be unaware of the presence of snails in their local water sources. Among the individuals who were aware of the snails, a mere 4.7% (36 individuals) perceived them as harmful, while only 17 (2.2%) held the belief that snails could potentially cause disease. Overall, the results indicate a general lack of knowledge about both the disease and its transmission patterns among the participants. The low awareness of preventive measures, the minimal recognition of the role of snails in disease transmission, and the limited understanding of the harmful effects of snails highlight the necessity for further comprehensive education and outreach initiatives.

Table 3. 5: Knowledge and awareness of schistosomiasis among participants

Knowledge	Responses (n)	Responses %
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	759	100.0
Can schistosomiasis be prevented?		
True	18	2.4
False	22	2.9
I don't know	719	94.7
If you are sick with schistosomiasis, you can be treated.		
True	13	1.7
False	13	1.7
I don't know	733	96.6
Not everyone with schistosomiasis will get sick		
True	4	0.5
False	22	2.9
I don't know	733	96.6
Avoiding playing in the river prevents schistosomiasis.		
True	9	1.2
False	23	3.0
I don't know	727	95.8
Do you know about snails in the river?		
Yes	69	9.1
No	690	90.9
Do you think they are harmful?		
Yes	36	4.7
No	469	61.8
I don't know	254	33.5
Do they cause diseases?		
Yes	17	2.2
No	481	63.4

I don't know	261	34.4
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The responses to knowledge questions on schistosomiasis among the 759 participants are depicted in **Figure 3.2**. The figure displays the count and proportion of individuals who answered "True" to specific questions regarding the disease. It is worth mentioning that 24 (3.2%) participants demonstrated accurate knowledge regarding the symptoms associated with schistosomiasis infection, including fever, itchy rash, dysuria, and the presence of blood in urine or stool. It was observed that there were common misconceptions about the disease among the participants. Specifically, 13 (1.7%) participants incorrectly believed that schistosomiasis is transmitted by mosquitoes, while only 8 (1.1%) participants accurately identified that schistosomiasis spreads when an infected person urinates in water bodies. Furthermore, it is worth noting that a mere 7 (0.9%) participants, demonstrated awareness regarding the potential effectiveness of abstaining from activities such as swimming, bathing, or fishing in slow-moving water bodies as a means of preventing infection. In addition, it was found that 5 (0.7%) participants, held the belief that urine and faeces could potentially be sources of schistosomiasis infection. Furthermore, it is worth noting that a minority of only 2 (0.3%) participants mistakenly believed that individuals with schistosomiasis cannot transmit the disease to others. The low percentages observed in this study highlight the limited amount of knowledge that exists regarding schistosomiasis in the study area.

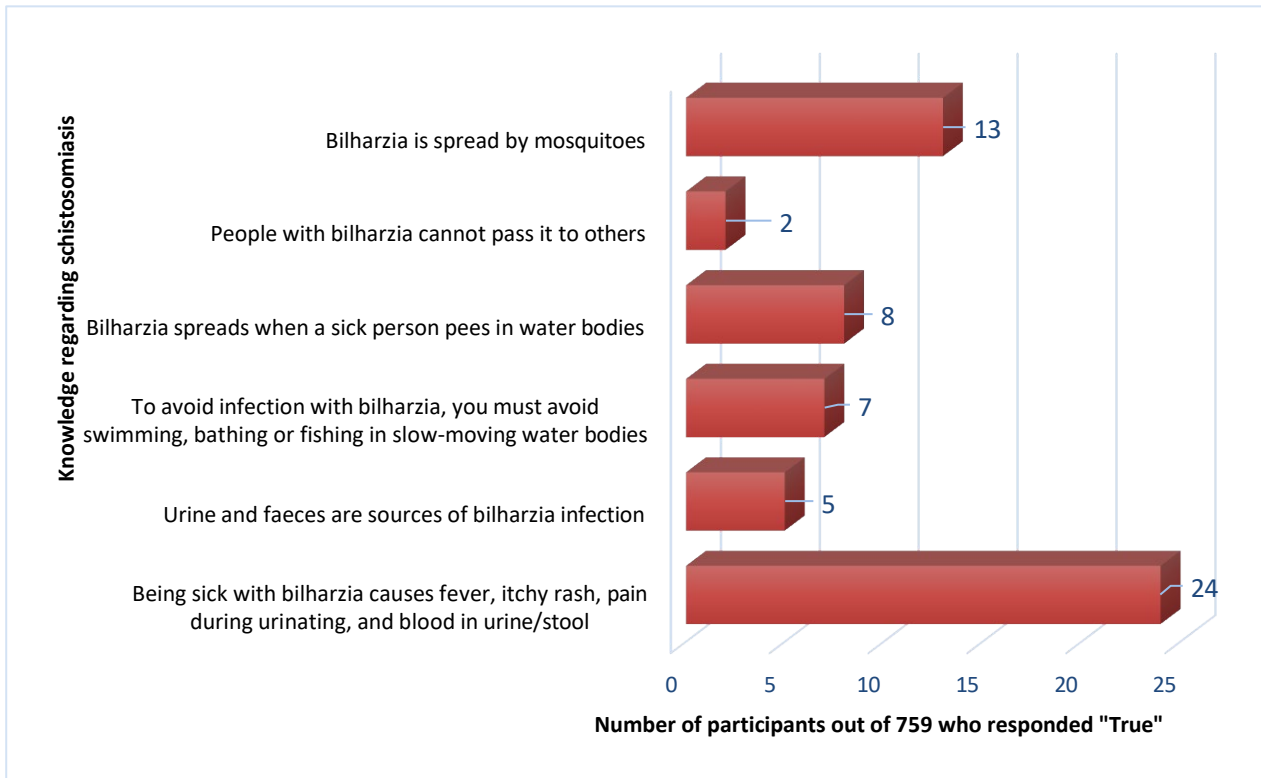


Figure 3.2: Knowledge about schistosomiasis transmission

3.1.6 Attitudes towards schistosomiasis among participants

The data presented in **Table 3.6** represents the responses of 759 participants in relation to their attitudes and perceptions of schistosomiasis. The data indicates that there is a lack of awareness regarding the seriousness of schistosomiasis as an illness. The data suggests a concerning pattern when it comes to attitudes surrounding treatment for schistosomiasis. According to the data collected, it was found that only 5 (0.7%) participants hold the belief that a cure for the condition exists. On the other hand, 15 participants (2.0%) expressed the opinion that there is currently no cure available. The significant absence of positive attitudes

observed in this study highlights a notable deficiency in health education. This deficiency has the potential to result in unwarranted fear or disregard for treatment among individuals who are at risk of infection.

With respect to the impact on daily life, 3 (0.4%) of the participants indicated that their daily life has been disrupted by schistosomiasis, while 86 (11.3%) reported that it has not caused any disturbances in their life. This could indicate either a low prevalence of symptomatic cases among the participants who are infected or a limited understanding of the impact of schistosomiasis on their health and daily activities. According to the data collected in the current study, 107 (14.1%) reported that schistosomiasis is not a serious illness. The majority of participants, 587 (77.3%), expressed uncertainty regarding the severity of schistosomiasis as an illness. Only 65 (8.6%) participants, believe that schistosomiasis is a serious illness.

Interestingly, the level of concern regarding the contraction of schistosomiasis is significantly low. The research findings indicate that a significant majority 665 (87.6%) of participants exhibit uncertainty regarding worrying associated with the disease. Additionally, a notable proportion 93 (12.3%) of participants demonstrate a lack of concern, while a negligible percentage 1 (0.1%) consisting of only one participant expresses worry about contracting the disease. The low level of attitudes among individuals may indicate a lack of awareness regarding the risks associated with the disease or a sense of invulnerability. On the other hand, the high percentage of individuals who do not know about the disease suggests uncertainty or ignorance about its potential impact on health outcomes. Based on the data collected, it is evident that the participants generally lack positive attitudes and concern regarding schistosomiasis.

Table 3.6: Attitudes towards schistosomiasis among participants

Attitudes	Responses (n)	Responses (%)
	759	100.0
Do you think schistosomiasis is a serious illness?		
True	65	8.6
False	107	14.1
I don't know	587	77.3
Do you think there is a cure for schistosomiasis?		
True	5	0.7
False	15	2.0
I don't know	739	97.4
Has your daily life been disturbed by being sick (schistosomiasis) in any way? (if infected)		
True	3	0.4
False	86	11.3
I don't know	670	88.3
Do you worry about suffering from schistosomiasis?		

True	1	0.1
False	93	12.3
I don't know	665	87.6

3.1.7 Practices relating to schistosomiasis among participants.

The findings in **Table 3.7** shed light on the practices associated with schistosomiasis among the participants of the study, indicating noteworthy practices that may play a role in the spread of the disease. According to the current research findings, a total of 173 (22.8%) participants indicated that they do not wear shoes when playing outside the house. This practice is recognised as a known risk factor for exposure to schistosomiasis. This behaviour has been found to increase the likelihood of schistosomiasis infections, especially when the individuals cross slow-moving water bodies contaminated with *Schistosoma* parasites thereby heightening the risk of infection. In addition, 92 (12%) participants reported visiting a river or water body after school, thus increasing their risk of potential schistosomiasis transmission. The frequency of these visits varied among the participants. Specifically, 57 (7.5%) participants visited once a day, 17 (2.2%) visited twice a day, and 9 (1.2%) visited multiple times a day. Such frequent exposure to contaminated water sources significantly increases the vulnerability of individuals to schistosomiasis. In the current study, only 21 (2.8%) participants reported actively avoiding rivers as a preventive measure against contracting schistosomiasis. The low percentages of responses regarding schistosomiasis knowledge observed in the current study suggests a lack of awareness or implementation of preventative measures among the majority of participants.

Table 3.7: Practices relating to schistosomiasis among participants

Practices	Responses (n)	Responses (%)
	759	100.0
Do you wear shoes when going outside?		
Yes	173	22.8
No	166	21.9
Sometimes	420	55.31
How often do you go to the river?		
Never	667	87.9
Every day after school	9	1.2
Whenever I want to play	57	7.5
Once a day	17	2.2
Twice a day	9	1.2
Have you ever been tested for schistosomiasis?		
Yes	1	0.1

No	758	99.9
Is avoiding rivers with snails helpful in preventing schistosomiasis?		
True	21	2.8
False	114	15.0
I don't know	624	82.2

3.1.8 Differences in KAP and demographics

The results obtained from assessing the differences in KAP scores across the participants' demographics are presented in **Table 3.8**. The ANOVA and t-tests were conducted to investigate potential relationships between demographic variables (age, gender, grade, and religious affiliation) and KAP scores. The sample mean scores were compared across different groups in order to identify any significant differences.

Among the participants, a statistically significant difference was observed in the knowledge scores based on their gender (p -value=0.015) Males tend to have higher values ($M = 128.55$) compared to females ($M = 104.07$) and grade (p -value=0.045). These findings align with the results of a systematic review conducted by Sacolo et al. (2018). The review examined 12 papers from sub-Saharan Africa, that investigated the impact of sociodemographic factors on awareness of schistosomiasis. Eight studies consistently found that variables such as age, gender, and level of education were strongly associated with higher levels of knowledge and awareness of schistosomiasis. The results of the review attributed the higher level of knowledge among males to the health education that was provided to fishermen and farmers, as they are more exposed to risky water practices. The difference between the review and the current study is that the respondents in the current study were specifically school-going children, whereas the review focused on both school-going children and adults. However, another study held in Botswana by, Gabaake et al. (2022) solely focused on school-going children and had similar findings to the current study, they found that males had significantly higher knowledge scores compared to females and individuals without health conditions.

In terms of attitude scores, the results of the current study revealed a statistically significant difference between the two grade groups (0-3 and 4-7) ($p = 0.023$). Specifically, participants in the higher-grade group exhibited higher average scores ($M = 138.86$) compared to those in the lower grade ($M = 93.59$). These findings indicate that as children advance in their academic journey, their perspectives on schistosomiasis prevention and associated behaviours tend to become more favourable. This shift in attitude can likely be attributed to a heightened level of knowledge and awareness acquired through education.

There was a notable difference in practice scores between students in grades 0-3 and grades 4-7. On average, students in higher grades achieved higher scores (p -value= 0.001). These findings indicate that older children are more inclined to adopt improved practices in relation to schistosomiasis prevention. This could be attributed to their enhanced knowledge and the development of positive attitudes as they mature. The analysis of the demographic variables indicates that gender and grade level have a notable influence on the knowledge, attitude, and practice scores of the sample population. The findings of the study suggest that male participants, specifically those in grades 4-7, exhibited higher average knowledge scores. This implies that both gender and grade level have a significant influence on the acquisition of knowledge related to schistosomiasis and its associated practices.

Interestingly, in the current study, while knowledge and practice scores did not significantly differ based on religious affiliations, attitude scores did show significant differences (p -value =0.086). This suggests that

religious beliefs might influence participants' attitudes towards schistosomiasis prevention, possibly due to varying cultural perspectives on health and hygiene.

Table 3.8: KAP scores assessment.

Demographic Variable	n	Mean	Std dev	ANOVA (p-value)
Knowledge score				
Age				
5 to 8 years	77	11.00	25.26	0.105
9 to 14 years	682	97.43	207.16	
Gender				
Male	440	62.86	128.55	0.015*
Female	319	45.57	104.07	
Grade				
0 – 3	292	41.71	93.59	0.045*
4 – 7	467	66.71	138.86	
Religious				
Mainline	136	19.43	43.86	0.141
Pentecostal	30	4.29	10.05	
Apostolic	85	12.14	26.49	
African tradition	454	64.86	133.48	
Muslim	3	0.43	0.79	
Other	51	7.29	17.96	
Attitude score				
Age				
5 to 8 years	77	19.25	34.57	0.318
9 to 14 years	682	170.50	292.42	
Gender				
Male	440	110.00	186.68	0.490

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Female	319	79.75	140.36	
Grade				
0 – 3	292	73.00	130.92	0.023*
4 – 7	467	116.75	196.07	
Religious				
Mainline	136	34.00	63.39	0.086†
Pentecostal	30	7.50	13.08	
Apostolic	85	21.25	35.89	
African tradition	454	113.50	190.96	
Muslim	3	0.75	0.96	
Other	51	12.75	22.87	
Practice score				
Age				
5 to 8 years	77	25.67	33.83	0.182
9 to 14 years	682	227.33	266.84	
Gender				
Male	440	146.67	168.36	0.142
Female	319	106.33	132.43	
Grade				
0 – 3	292	97.33	129.26	0.001*
4 – 7	467	155.67	172.34	
Religious				
Mainline	136	45.33	55.58	0.438
Pentecostal	30	10.00	11.79	
Apostolic	85	28.33	33.29	
African tradition	454	151.33	181.80	
Muslim	3	1.00	1.00	

Other	51	17.00	17.52
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*Statistical Significance at a 5% level; (ref)=reference category, †Statistical Significance at a 10% level.

3.1.9 Differences in KAP and risk factors

The results obtained from assessing the differences in the scores for KAP across the participants' risk factor characteristics are presented in Table 10. The participants who utilised municipal tap water from their homes had the highest average knowledge score ($M = 99.71$), compared to those who relied on communal borehole water ($M = 4.71$) and household borehole water ($M = 4.00$). The ANOVA indicated a statistically significant difference in knowledge means among the three groups being compared (p -value = 0.078). These findings suggest that individuals who have access to safer and more reliable water sources, such as household municipal water sources, had higher levels of knowledge about schistosomiasis and its prevention. This is likely because households with better infrastructure have improved information dissemination and awareness regarding the disease.

3.1.10 Impact of risk factors of schistosomiasis transmission on knowledge scores

According to the current research findings, individuals who had a toilet in their home had significantly higher knowledge scores ($M = 86.71$) compared to those who did not have a toilet ($M = 21.71$). This difference in scores was found to be statistically significant ($p = 0.006$). This emphasises the significance of sanitation infrastructure in enabling the acquisition of health-related knowledge, potentially because of the decreased likelihood of disease transmission and improved availability of health education in households equipped with toilets.

The study observed significant differences in knowledge scores among participants who did not have toilets in their households and the alternative locations they utilised for defecation. The participants who did not have access to a toilet and resorted to using the bush had an average score of ($M = 5.57$). On the other hand, those who used their neighbours' toilets had a lower average score of ($M = 3.14$). It is worth mentioning that participants who reported having a toilet at home and selected 'Not Applicable' had the highest average knowledge score ($M = 99.43$). The p -value (0.009) suggests that the observed differences are statistically significant, indicating a strong association between access to proper sanitation facilities and higher knowledge levels. The possible explanation for this phenomenon could be the decreased exposure to disease risks and improved health education among individuals who have access to toilets. These findings are consistent with previous research that emphasises the significance of sanitation in preventing schistosomiasis.

The current study results on knowledge about snails in rivers, which are important carriers of schistosomiasis, found significant differences among the participants. The participants who were knowledgeable about snails exhibited a lower mean score ($M = 9.86$), whereas those who had no knowledge had significantly higher mean scores ($M = 98.57$; $p = 0.001$). Insufficient knowledge about snails, an important intermediate host of the disease, could lead to a lack of good practices required for successful prevention strategies. The literature emphasises the importance of comprehending intermediate hosts in the transmission of schistosomiasis.

Reports from participants on extracurricular activities after school showed significant differences in knowledge scores. The mean score for individuals who swam was ($M = 12.29$), while those who did not swim had a significantly higher mean score ($M = 96.14$; $p = 0.004$). Similarly, there were significant differences between individuals who played at home with friends and those who engaged in other activities. The mean score for those who played at home was ($M = 102.00$), whereas it was much lower for those who did not ($M = 6.43$; $p = 0.003$). These findings suggest that children involved in activities that increase exposure to water bodies or outdoor environments, where schistosomiasis transmission is more likely, had lower awareness of schistosomiasis-related risks.

Additionally, a statistically significant difference ($p = 0.001$) was observed in the knowledge levels of participants who engaged in urinating in the river while swimming. The participants who chose to urinate in the river exhibited a significantly lower mean score ($M = 5.43$) in comparison to those who refrained from doing so ($M = 103.00$). This significant difference highlights how certain risky behaviours are associated with lower knowledge levels, likely due to inadequate education on the health risks posed by such practices.

3.1.11 Impact of risk factors of schistosomiasis transmission on attitudes scores

There were significant differences in attitude scores among participants based on their sources of drinking water. Household tap water had the highest mean score ($M = 174.50$), while community borehole and household borehole had values of ($M = 8.25$) and ($M = 7.00$), respectively. The p -value (<0.001) shows a statistically significant difference in attitudes among participants who were in these categories.

The study revealed several significant findings regarding the influence of environmental and behavioural factors on attitudes toward schistosomiasis. One of the key insights was the statistically significant difference in attitude scores based on proximity to a river (p -value = 0.016). Individuals living near a river had a significantly lower mean attitude score ($M = 40.00$) compared to those living farther away ($M = 149.75$). This suggests that participants living close to rivers, where exposure to schistosomiasis is likely higher, may have more complacent or less informed attitudes toward preventive measures. The lower scores could reflect a sense of inevitability exposure or a lack of effective educational outreach in these areas.

The presence of a toilet at home did not result in significant differences in attitude scores. Interestingly, individuals who used bush toilets had a slightly higher average score ($M = 9.75$) compared to those who used neighbouring toilets ($M = 5.50$), though both scores were notably low. However, those who responded with "Not Applicable", indicating they had a toilet at home, attained the highest average score ($M = 174.00$). The statistically significant differences in scores between these three categories (ANOVA p -value = 0.025) point to the possibility that access to better sanitation at home correlates with more positive attitudes toward schistosomiasis prevention. Households with proper sanitation may be more aware of health risks and more motivated to engage in preventive behaviours.

The results reveal a surprising trend in attitude scores, where individuals who were aware of the role of snails in the transmission of schistosomiasis had significantly lower attitude scores ($M = 17.25$) compared to those who were unaware ($M = 172.50$; $p < 0.001$). This indicates a gap between knowledge and attitude toward prevention. Despite knowing that snails contribute to schistosomiasis transmission, those with awareness still displayed a weaker attitude toward prevention, suggesting that mere knowledge of the risk does not always lead to protective behaviours. These results indicate that although being aware of transmission vectors such as snails is crucial, having knowledge alone is inadequate for promoting positive attitude towards schistosomiasis infection. The lower attitude ratings seen among those with knowledge indicate the necessity for educational initiatives that not only communicate factual information regarding the transmission of schistosomiasis but also indicate the significance of taking preventive measures. Comprehensive health education should prioritise bridging the divide between information and attitude, promoting behavioural modifications that mitigate the risk of schistosomiasis transmission.

These findings indicate the importance of tailoring interventions to the specific conditions and attitudes of different groups. For example, communities living near rivers or relying on bush toilets may benefit from targeted awareness programs focussing on the risks associated with water contact and inadequate sanitation. Additionally, campaigns that address both awareness of snails and the behavioural changes needed to mitigate the risks could help bridge the gap between knowledge and practice, improving overall attitudes toward schistosomiasis prevention.

The findings highlight significant differences in attitude scores based on various extracurricular activities and behaviours related to schistosomiasis risk. For instance, participation in swimming showed a clear disparity in

attitudes, with swimmers having a much lower mean score ($M = 21.50$) compared to non-swimmers ($M = 168.25$; p -value < 0.001). This suggests that individuals who regularly swim, likely in contaminated freshwater sources, may have more complacent or less proactive attitudes toward schistosomiasis prevention. This could be due to a lack of awareness or a sense of invulnerability despite frequent exposure to risk. Non-swimmers, on the other hand, demonstrate significantly better attitudes, likely because they are less exposed to high-risk environments.

Similarly, fishing activities revealed significant differences in attitude scores, with individuals who engage in fishing having an extremely low mean score ($M = 0.75$), while those who do not fish had a much higher mean score ($M = 189.00$; p -value = 0.001). This finding is consistent with the understanding that fishing often involves prolonged contact with freshwater, increasing the risk of exposure to schistosomiasis. Those who participate in fishing may either be unaware of the risks or may not take preventive measures seriously, leading to much lower attitude scores. In contrast, those who avoid fishing may have better attitudes toward disease prevention, likely because they are less exposed to the water sources where schistosomiasis transmission occurs.

The study also found significant differences in attitudes based on social behaviours, such as playing at home with friends after school. Individuals who engaged in this activity had a notably higher mean score ($M = 178.50$) compared to those who did not ($M = 11.25$; p -value < 0.001). This suggests that staying at home may limit exposure to contaminated water bodies and promote healthier attitudes toward schistosomiasis prevention. In contrast, those who do not engage in such activities may spend more time near rivers or lakes, leading to riskier behaviours and lower attitude scores.

Another critical finding was the significant difference in attitude scores based on whether individuals urinated in the river while swimming. Those who engaged in this behaviour had a notably low mean score ($M = 9.50$), while those who did not urinate in the river had a significantly higher mean score ($M = 180.25$; p -value < 0.001). This behaviour, which is closely linked to the lifecycle of schistosomes, demonstrates a lack of awareness or disregard for the disease transmission process. Individuals who do not engage in this behaviour likely possess a better understanding of the risks and therefore, have more positive attitudes toward preventing schistosomiasis.

Overall, these findings indicate the importance of addressing specific behaviours and activities that put individuals at higher risk of schistosomiasis. Tailored interventions that target high-risk groups, such as swimmers and fishers, should focus on raising awareness about the dangers of water contact and promoting practical preventive measures. Educational programs could also focus on social activities, emphasising the importance of staying away from contaminated water sources and discouraging risky behaviours, such as urinating in rivers. By addressing these factors, public health initiatives can foster more positive attitudes toward schistosomiasis prevention and ultimately reduce disease transmission in the community.

3.1.12 Impact of risk factors of schistosomiasis transmission on practices scores

In comparing sources of drinking water, there were significant differences in the practice scores, with a p -value of 0.001 . The mean score for domestic municipality tap water was notably higher ($M = 232.67$) when compared to community borehole water ($M = 11.00$) and household borehole water ($M = 9.33$). The study revealed significant differences in practice scores between individuals who fish ($M = 1.00$) and those who do not ($M = 252.00$; p -value = 0.077), suggesting that environmental factors may play a role in schistosomiasis-related practices (**Table 3.9**).

Table 3. 9: Comparative evaluation of risk factors associated with schistosomiasis infections.

Risk factor	n	Mean	Std dev	ANOVA	p-value
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Knowledge score				
Where do you fetch water to drink?				
Community borehole	33.00	4.71	9.57	0.078†
household borehole	28.00	4.00	9.27	
household tap water	698.00	99.71	213.97	
Do you live near a river?				
Yes	160.00	22.86	44.14	0.530
No	599.00	85.57	188.74	
Do you have a toilet at home?				
Yes	607.00	86.71	186.73	0.006*
No	152.00	21.71	46.08	
If no where do you urinate or defecate?				
Bush	39.00	5.57	10.37	0.009*
Public toilet	2.00	0.29	0.76	
Neighbour's toilet	22.00	3.14	7.47	
Not Applicable	696.00	99.43	213.96	
Do you know about snails in the river?				
Yes	69.00	9.86	18.27	0.001*
No	690.00	98.57	214.22	
Do you cross a river on your way to school?				
Yes	69.00	9.86	21.41	0.727
No	690.00	98.57	211.01	
Where do you go to play after school or when you are not at school: Swimming?				
Yes	86.00	12.29	22.28	0.004*
Not Applicable	673.00	96.14	210.21	
Where do you go to play after school or when you are not at school: Fishing?				
Yes	3.00	0.43	0.53	0.177

Not Applicable	756.00	108.00	232.13	
Where do you go to play after school or when you are not at school: At home with friends?				
Yes	714.00	102.00	221.50	0.003*
Not Applicable	45.00	6.43	10.98	
When you are swimming in the river do you urinate in the water: Yes?				
Yes	38.00	5.43	9.20	0.001*
Not Applicable	721.00	103.00	223.28	
Attitude score				
Where do you fetch water to drink?				
Community borehole	33.00	8.25	11.44	<0.001*
Household borehole	28.00	7.00	8.76	
Household tap water	698.00	174.50	306.95	
Do you live near a river?				
Yes	160.00	40.00	63.67	0.016*
No	599.00	149.75	263.38	
Do you have a toilet at home?				
Yes	607.00	151.75	263.35	0.684
No	152.00	38.00	63.70	
If no, where do you urinate or defecate?				
Bush	39.00	9.75	14.97	0.025*
Public toilet	2.00	0.50	0.58	
Neighbour's toilet	22.00	5.50	7.85	
Not Applicable	696.00	174.00	303.96	
Do you know about snails in the river?				
Yes	69.00	17.25	19.14	<0.001*
No	690.00	172.50	308.21	
Do you cross a river on your way to school?				

Yes	69.00	17.25	27.93	0.117
No	690.00	172.50	299.10	
Where do you go to play after school or when you are not at school: Swimming?				
Yes	86.00	21.50	24.69	<0.001*
Not Applicable	673.00	168.25	302.93	
Where do you go to play after school or when you are not at school: Fishing?				
Yes	3.00	0.75	0.50	0.001*
Not Applicable	756.00	189.00	326.79	
Where do you go to play after school or when you are not at school: At home with friends?				
Yes	714.00	178.50	316.30	<0.001*
Not Applicable	45.00	11.25	11.00	
When you are swimming in the river do you urinate in the water?				
Yes	38.00	9.50	8.58	<0.001*
Not Applicable	721.00	180.25	320.30	
Practice score				
Where do you fetch water to drink?				
Community borehole	33.00	11.00	11.00	0.001*
Household borehole	28.00	9.33	8.14	
Household tap water	698.00	232.67	284.81	
Do you live near a river?				
Yes	160.00	53.33	61.76	0.661
No	599.00	199.67	238.80	
Do you have a toilet at home?				
Yes	607.00	202.33	241.43	0.679
No	152.00	50.67	59.08	
If not, where do you urinate or defecate?				
Bush	39.00	13.00	15.13	0.500

Public toilet	2.00	0.67	0.58	
Neighbour's toilet	22.00	7.33	8.08	
Not Applicable	696.00	232.00	276.94	
Do you know about snails in the river?				
Yes	69.00	23.00	28.58	0.573
No	690.00	230.00	271.95	
Do you cross a river on your way to school?				
Yes	69.00	23.00	26.91	0.748
No	690.00	230.00	273.61	
Where do you go to play after school or when you are not at school: Swimming?				
Yes	86.00	28.67	35.73	0.625
Not Applicable	673.00	224.33	264.89	
Where do you go to play after school or when you are not at school: Fishing?				
Yes	3.00	1.00	1.00	0.077†
Not Applicable	756.00	252.00	300.23	
Where do you go to play after school or when you are not at school: At home with friends?				
Yes	714.00	238.00	284.84	0.193
Not Applicable	45.00	15.00	15.72	
When you are swimming in the river do you urinate in the water?				
Yes	38.00	12.67	15.53	0.767
Not Applicable	721.00	240.33	284.98	

*Statistical Significance at a 5% level; (ref)=reference category, †Statistical Significance at a 10% level.

3.1.13 Multivariate regression analysis of demographic factors related to the KAP of schistosomiasis among study participants

Table 3.10 presents the results of the multivariate regression analysis examining the associations between demographic factors and knowledge, attitudes, and practices (KAP) related to schistosomiasis among 759 participants. This table displays estimates, standard errors, and *p*-values for participants' demographic characteristics, including age, gender, grade, and religious affiliation, in relation to their KAP scores. The study highlights a significant gender disparity in schistosomiasis awareness, with females showing lower knowledge scores than males ($\beta = -0.15$; $p = 0.018$). Boys, who are more exposed to outdoor activities such as swimming

and fishing, are more likely to receive educational messages about schistosomiasis risks, while girls may face social restrictions limiting their awareness. The study revealed that children from African traditional religions had significantly higher knowledge scores about schistosomiasis compared to those from other religious affiliations ($\beta = 0.22$; $p = 0.010$). The study found that participants in grades 4 to 7 had fewer positive attitudes toward schistosomiasis prevention compared to those in grades 0 to 3 ($\beta = -0.07$; $p = 0.038$), highlighting the need for continuous, age-appropriate health education. Religious affiliation significantly influenced attitudes towards schistosomiasis prevention. Children from the Apostolic faith ($\beta = 0.12$; $p = 0.041$) and African traditional religions ($\beta = 0.11$; $p = 0.014$) demonstrated more positive attitudes compared to those from mainline religions. Regarding practices, there was a notable decline in positive practices among children in grades 4 to 7 ($\beta = -0.11$; $p = 0.001$). This grade group is at higher risk due to their involvement in activities like swimming and fishing in rivers, which increase their exposure to schistosomiasis.

Table 3.10: Multivariate regression results on association between risk factors and KAP scores.

Variable	Knowledge score			Attitude score			Practice score		
	Estimate	Standard error	P-value	Estimate	Standard error	P-value	Estimate	Standard error	P-value
Age									
5 to 8 years									
(ref)									
9 to 14 years	0.17	0.11	0.141	0.02	0.06	0.721	0.01	0.05	0.818
Gender									
Male (ref)									
Female	-0.15	0.07	0.018*	-0.02	0.03	0.56	-0.05	0.03	0.131
Grade									
0 - 3 (ref)									
4-Jul	0.09	0.07	0.179	0.07	0.03	0.038*	0.11	0.03	0.001*
Religious									
Mainline (ref)									
Pentecostal	0.01	0.18	0.94	0.05	0.09	0.547	0.03	0.09	0.704
Apostolic	0.18	0.12	0.145	0.12	0.06	0.041*	0.03	0.06	0.669
African tradition	0.22	0.09	0.010*	0.11	0.04	0.014*	0.01	0.04	0.879
Muslim	0.14	0.51	0.788	0.27	0.26	0.289	0.11	0.25	0.664

Other	-0.03	0.15	0.836	0.05	0.07	0.466	0.12	0.07	0.101
Knowledge				0.66	0.07	<0.001	0.11	0.07	0.11
(Intercept)	0.02	0.12	0.891	0	0.06	0.994	0.16	0.06	0.009

*Statistical Significance at a 5% level; (ref)=reference category, †Statistical Significance at a 10% level.

Table 3.11 presents the results of a multivariate regression analysis conducted to assess the associations between various risk factors and knowledge, attitudes, and practices (KAP) regarding schistosomiasis transmission among school-going children in Nelson Mandela municipality. The risk factors analysed included water source, proximity to a river, toilet availability, knowledge about snails in the river, and recreational activities such as swimming and fishing. The table provides estimates, standard errors, and *p*-values for each risk factor's effect on KAP scores, with a *p*-value of less than 0.10 ($p < 0.10$) considered significant.

The findings provide critical insights into the interplay between sanitation access and schistosomiasis-related knowledge and behaviours. Children without a toilet at home demonstrated significantly higher knowledge about schistosomiasis compared to those with a toilet ($\beta = 0.19$; $p = 0.055$). Conversely, children who relied on a neighbour's toilet exhibited significantly lower knowledge about schistosomiasis ($\beta = -0.55$; $p = 0.020$). This implies that indirect access to sanitation might limit exposure to health education or reduce engagement with health information. Understanding the link between children's awareness of river snails and their knowledge of schistosomiasis highlights the crucial role of focused education on these specific vectors to enhance prevention efforts. The current study reveals that children who are unaware of snails in rivers have significantly lower knowledge scores ($\beta = -0.24$; $p = 0.091$), pointing to a critical gap in their understanding. This finding aligns with the broader literature on schistosomiasis transmission.

The result indicating that children with access to household tap water had less positive attitudes towards schistosomiasis prevention compared to those using community boreholes ($\beta = -0.12$; $p = 0.099$) suggests an intriguing relationship between perceived water safety and preventive behaviour. Children with household tap water might perceive their water source as safe and clean, which could reduce their sense of urgency regarding schistosomiasis prevention. This perception might lead to complacency, where the perceived lower risk of infection diminishes their motivation to engage in preventive practices. On the other hand, children using community boreholes might be more aware of the risks associated with less controlled water sources, thus fostering more proactive attitudes towards prevention.

Interestingly, children with access to household tap water exhibited less positive attitudes toward schistosomiasis prevention, possibly due to a perceived lower risk and reduced concern. In contrast, children using public toilets, due to a lack of home facilities, showed significantly more positive attitudes ($\beta = 0.81$; $p = 0.007$). Children who were not aware of the presence of snails in the river had considerably lower positive attitude scores compared to those who were aware. In a comparable manner, it was found that children who did not participate in fishing after school displayed noticeably less positive attitudes ($\beta = -0.56$; $p = 0.024$). This implies that being involved in activities related to transmission is connected to a better comprehension of the risks, ultimately influencing attitudes. Similarly, individuals who participated in playing at home with friends ($\beta = -0.31$; $p < 0.001$) and those who chose not to swim but instead participated in other activities ($\beta = -0.24$; $p = 0.004$) displayed less favourable attitudes. These findings indicate that children who engage in outdoor activities, especially around water sources have heightened awareness of the risks associated with schistosomiasis.

When it comes to practices, children who have household boreholes showed better practices than those who depend on community boreholes ($\beta = 0.20$; $p = 0.066$). This improvement is most likely a result of the increased control that these children have over water quality and usage. As a result, they are able to implement more

consistent and effective preventive practices. However, students who had access to household tap water had slightly lower practice scores compared to those who used community boreholes ($\beta = -0.14$; $p = 0.074$).

Furthermore, a positive correlation between knowledge and attitude scores was observed; every 1% increase in knowledge score was associated with a 0.66-point increase in attitude score. This indicates that as children's understanding of schistosomiasis improves, their attitudes towards prevention become more positive, highlighting the critical role of knowledge in shaping health-related behaviours. These findings align with the research objective of identifying risk factors associated with schistosomiasis transmission among school-going children. By understanding the factors that influence KAP, interventions can be more effectively designed to target areas where knowledge is lacking, attitudes are less positive, and practices are inadequate, ultimately contributing to better prevention and control of schistosomiasis in this population.

Table 3.11: Regression analysis results on association between risk factors and KAP scores.

Variable	Knowledge score			Attitude score			Practice score		
	Estimate	Standard error	P-value	Estimate	Standard error	P-value	Estimate	Standard error	P-value
Where do you fetch water to drink?									
Community borehole (ref)									
Household borehole	-0.17	0.23	0.469	0.09	0.11	0.392	0.2	0.11	0.066*
Household tap water	-0.18	0.16	0.257	-0.12	0.07	0.099†	-0.14	0.08	0.074*
Do you live near a river?									
Yes (ref)									
No	0.01	0.09	0.876	0.04	0.04	0.382	-0.04	0.04	0.333
Do you have a toilet at home?									
Yes (ref)									
No	0.19	0.1	0.055*	-0.05	0.05	0.259	-0.02	0.05	0.718
If not where do you urinate or defecate?									
Bush (ref)									
Public toilet	-0.54	0.64	0.401	0.81	0.3	0.007*	0.06	0.31	0.836
Neighbour's toilet	-0.55	0.23	0.020*	0.09	0.11	0.387	-0.01	0.11	0.945

Not Applicable	-0.21	0.17	0.21	-0.04	0.08	0.58	-0.07	0.08	0.41
			4			4			5
Do you know about snails in the river?									
Yes (ref)									
No	-0.24	0.14	0.09	-0.22	0.07	0.00	0.09	0.07	0.21
			1†			1*			6

*Statistical Significance at a 5% level; (ref)=reference category, †Statistical Significance at a 10% level

3.2 DISCUSSION

This study provides a comprehensive analysis of the risk factors and KAP associated with schistosomiasis transmission among 759 participants in NMB. The findings highlight significant connections between environmental conditions, sanitation practices, and the prevalence of schistosomiasis, offering critical insights into the disease's transmission dynamics in the region. The study identified several symptoms consistent with both acute and chronic schistosomiasis, including cough (6.7%), diarrhoea (6.9%), abdominal pain (7.8%), and haematuria (1.2%). These findings align with previous research, which has linked schistosome infections to a range of symptoms such as muscle aches, itchy rashes, and fever (Dejon-Agobé et al., 2021). The presence of these symptoms, particularly the classic signs of acute schistosomiasis – such as fever, abdominal pain, and haematuria – further confirms the endemicity of the disease in NMB (Anyolitho et al., 2022). Notably, a study by Gabaake et al. (2022) (Gabaake et al., 2022) within a similar age group revealed that 75% of participants lacked knowledge of schistosomiasis symptoms, suggesting that age may significantly influence awareness of the disease.

Acute schistosomiasis, often manifesting as Katayama syndrome, is characterised by systemic allergic reactions, including pulmonary symptoms such as dyspnoea, bronchospasm, and cough (Carbonell et al., 2021). This syndrome, which occurs 3 to 8 weeks post-exposure, has a prevalence rate exceeding 50% among infected individuals and typically resolves spontaneously within 2 to 10 weeks (Nelwan, 2019). However, a subset of individuals may develop chronic symptoms, including weight loss, hepatosplenomegaly, and persistent gastrointestinal discomfort (Carbonell et al., 2021). The study underscores the importance of seeking medical care when symptoms such as itchy rashes or hematuria arise after exposure to freshwater bodies, as these are indicative of potential schistosomiasis infection (Anyolitho et al., 2022; Sánchez-Marqués, Salvador, et al., 2023).

The study revealed poor health-seeking behaviour among participants, with only 20% reporting schistosomiasis symptoms to healthcare facilities, compared to 30% for fever. This aligns with findings by Danso-Appiah et al. (2010) (Danso-Appiah et al., 2010), who noted that individuals with urinary schistosomiasis symptoms were less likely to seek medical care than those with intestinal symptoms or fever. Socio-economic status (SES) emerged as a critical determinant of healthcare access, with higher SES individuals more likely to utilize health facilities (Omondi et al., 2021). However, the study did not directly assess SES among participants, as the focus was on paediatric populations. Nevertheless, the socio-economic status of parents, which reflects that of their children, is a significant factor. The rising poverty rate in NMB, which increased by 15.98% from 2006 to 2016, exacerbates healthcare disparities, with underprivileged populations bearing a disproportionate burden of poor health outcomes (McMaughan et al., 2020; Nelson Mandela Bay Metro 2 Profile). Financial constraints and delayed healthcare-seeking behaviour among socio-economically disadvantaged groups further perpetuate schistosomiasis transmission in the region (Gordon et al., 2020).

Key risk factors identified in this study include recreational water activities, with 11.3% of participants reporting swimming in rivers. Among these, 44% admitted to urinating in the water, a significant contributor to schistosomiasis transmission. Swimming in freshwater bodies inhabited by *Schistosoma* parasites is a well-documented risk factor (Joof et al., 2021a; Nkya, 2023). A study in uMkhanyakude district, South Africa, identified swimming as the primary recreational activity linked to schistosomiasis transmission (Kabuyaya et al., 2017). Additionally, urinating in water bodies by infected individuals directly deposits *Schistosoma* eggs into the water, perpetuating the disease cycle (Nenzhelele et al., 2020).

While 92% of participants had access to municipal water, 20% lacked functional toilets at home, and 5.1% resorted to using bush toilets, increasing transmission risks. Access to safe water and adequate sanitation is strongly correlated with reduced schistosomiasis prevalence (Grimes et al., 2015; Mwai et al., 2021). However, the presence of sanitary infrastructure does not guarantee its proper use, as highlighted by Poague et al. (2023) (Mingoti Poague et al., 2023). Inadequate disposal of human excrement, even in the presence of toilets, remains a significant challenge, particularly in informal settlements where children may avoid using latrines due to fear or cultural norms (Huda et al., 2021; Soares Magalhães et al., 2011). This behaviour poses a major risk, as open defecation and urination can introduce *Schistosoma* eggs into water bodies, perpetuating the parasite's lifecycle (Hailu et al., 2020).

Gender and age were significant determinants of schistosomiasis risk and knowledge. Boys, who are more exposed to outdoor activities such as swimming and fishing, exhibited higher knowledge levels about schistosomiasis risks compared to girls, who often face social restrictions limiting their awareness (Hajissa et al., 2018; Joof et al., 2021b). Conversely, girls, who typically engage in household chores and rely on tap water, face lower exposure but may lack critical knowledge about prevention (Amuta & Houmsou, 2014; Sumbele et al., 2021). Age also played a role, with children aged 10–15 years being 3.8 times more likely to be infected than those aged 5–10 years (M'Bra et al., 2018). This underscores the importance of sustained educational efforts targeting older children, who are at higher risk due to increased independent water-related activities (Favre et al., 2021; Molehin, 2020b).

Religious affiliation influenced schistosomiasis knowledge and exposure, with certain practices, such as frequent ablutions among Muslims and Apostolic groups, increasing water-related activities and transmission risks (Otineme et al., 2019; Yusuf et al., 2022). These findings highlight the need for culturally sensitive educational strategies to address knowledge gaps and reduce exposure among religious groups. Awareness of snail vectors and their role in schistosomiasis transmission was a critical factor influencing preventive attitudes. Children who recognized the presence of snails in water bodies exhibited more positive attitudes toward prevention (Mberekko et al., 2020; Nwoko et al., 2023b). However, perceptions of water safety also played a role, with children using household tap water often perceiving it as safe, potentially leading to complacency in preventive practices (Angora et al., 2019; Grimes et al., 2015). This underscores the need for continuous education about the risks associated with all water sources, regardless of perceived safety.

The study has several limitations, including its cross-sectional design, which precludes establishing causal relationships, and reliance on self-reported data, which may introduce biases. Additionally, the findings are specific to NMB and may not be generalizable to other regions. Future research should explore the role of rural-to-urban migration in introducing schistosomiasis into urban areas, as rural regions often have higher prevalence due to inadequate sanitation and exposure to contaminated water. Investigating this dynamic could inform targeted interventions and enhance surveillance efforts.

CHAPTER 4: PREVALENCE AND INTENSITY OF *S. MANSONI* INFECTION THROUGH URINE AND FECAL SAMPLES RESPECTIVELY, IN SCHOOL GOING CHILDREN

4.1 RESULTS

4.1.1 Sociodemographic characteristics of the study population

A total of 759 participants, comprising 438 males (57.7%) and 321 females (42.3%), participated in the study on the diagnostics of *S. mansoni* infections among school-aged children in NMB (see **Table 4.1**). Of these, 467 participants (61.5%) were in grades 4 to 7, while 293 (38.5%) were in grades 0 to 3. The average age was 11.00 ± 1.50 years, with a range of 5 to 14, indicating a predominantly pre-adolescent group. KwaNobuhle had the highest participation with 439 students (57.8%), followed by Motherwell with 152 (20.0%), Ibhayi and Kariega with 54 each (7.1%), and Despatch with 60 (7.9%).

Table 4.1: Demographic characteristics of participants

Demographic characteristic	Total sample size (n=759)	%
Gender		
Male	438	57.7
Female	321	42.3
Grade		
0-3	292	38.5
4-7	467	61.5
Age (years)		
5-8	76	10
9-14	683	90
Town		
KwaNobuhle	439	57.8
Ibhayi	54	7.1
Kariega	54	7.1
Motherwell	152	20.0
Despatch	60	7.9

4.1.2 Assessment of *S. mansoni* Prevalence Using Kato-Katz, POC-CCA, and cPCR Techniques

The prevalence of *S. mansoni* in this study was evaluated using three diagnostic methods: the conventional Kato-Katz technique, the POC-CCA test, and molecular diagnostics via cPCR. Each method yielded varying levels of sensitivity and diagnostic accuracy, particularly in the low-transmission setting under investigation.

4.1.3 Kato-Katz Method

The Kato-Katz technique, widely regarded as a standard method for detecting helminth infections in stool samples, failed to identify any egg-positive cases of *S. mansoni* among the 759 participants analysed. This result shows the limitations of Kato-Katz in low-transmission areas, where infection intensities are often below the detection threshold of microscopy.

4.1.4 POC-CCA Test

In contrast, the POC-CCA test, which detects *S. mansoni* antigens in urine, identified 28 positive cases out of 759 participants, corresponding to a prevalence rate of 3.7% (95% CI: 2.35%–5.03%). Among these positive results, the antigen levels were categorized as follows: 23 participants (82.1%) exhibited trace levels, three (10.7%) were classified as low intensity (1+), and two (7.1%) demonstrated medium intensity (2+). These findings highlight the POC-CCA test's superior sensitivity in detecting low-intensity infections, particularly in comparison to the Kato-Katz method. The predominance of trace-level results in the POC-CCA data suggests a higher sensitivity for detecting subclinical or low-intensity infections. However, the reliability of these trace results warranted further investigation which was done by cPCR (13).

4.1.5 Validation of POC-CCA Findings Using cPCR for *S. mansoni* Detection

To further validate the POC-CCA findings, cPCR was performed on a subset of 28 POC-CCA-positive samples, targeting the *S. mansoni* *cox-1* mitochondrial gene. This method detected *S. mansoni* DNA in 9 samples, yielding a positivity rate of 32.1% (95% CI: 14.8%–49.4%). The relatively wide confidence interval reflects the small sample size used for cPCR analysis. The cPCR findings not only confirmed the presence of *S. mansoni* DNA but also highlighted the capacity of molecular diagnostics to identify infections that may be missed by both microscopy and antigen detection methods. The use of degenerate primers (ShmF and ShmR), adapted from (31), ensured the specificity of the assay. Amplified DNA fragments were approximately 0.766 Kb in size see **Figure 4.1**.

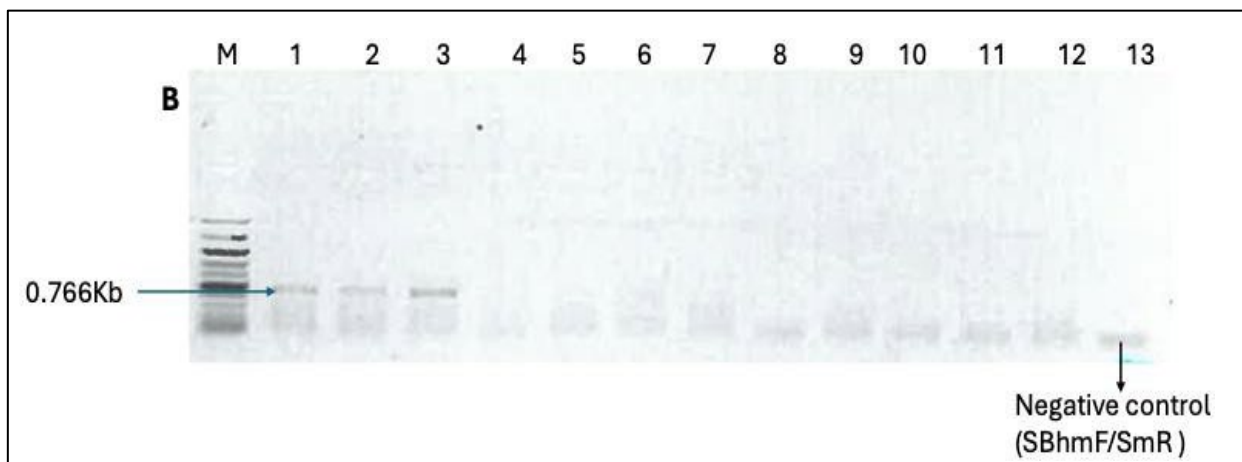


Figure 4.1: Agarose gel electrophoresis image showing amplified repetitions of *S. mansoni* *cox 1* gene sequence from POC-CCA positive samples. Lane "M" contains the DNA molecular weight marker (ladder) for estimating the size of the PCR products. The lane marked "Negative control (SBhmF/SmR)" contains no amplified product, confirming the absence of contamination. The

expected product sizes are approximately 0.766 Kb. Overall, the results indicate the detection of the *cox 1* gene sequence from *S. mansoni* in POC-CCA positive samples, with faint bands visible at the expected sizes.

4.1.6 Demographic Trends in cPCR-Positive Cases

Further analysis of cPCR-positive cases revealed demographic trends (**Table 4.2**). Younger participants aged 5–8 years exhibited a higher prevalence (50%, 95% CI: 10.0%–90.0%) compared to the 9–14 years age group (31.8%, 95% CI: 12.4%–51.3%). Gender-specific prevalence rates indicated slightly higher infection rates in males (40%, 95% CI: 9.6%–70.4%) compared to females (27.8%, 95% CI: 7.1%–48.5%). Similarly, participants in lower grades (0–3) displayed higher prevalence rates (40%, 95% CI: 9.6%–70.4%) than those in grades 4–7 (27.8%, 95% CI: 7.1%–48.5%). These findings suggest that younger children and males may be at greater risk of infection, potentially due to behavioral or environmental factors that warrant further exploration.

Table 4.2: Cox-1 Gene PCR Results by Age, Gender, and Grade for *S. mansoni* detection

Demographic Variable	N	Positive	(%)	95% CI
Age Group				
5 to 8 years	6	3	50	10.0-90.0
9 to 14 years	22	7	31.8	12.4-51.3
Gender				
Male	10	4	40	9.6-70.4
Female	18	5	27.8	7.1-48.5
Grade				
Grade 0-3	10	4	40	9.6-70.4
Grade 4-7	18	5	27.8	7.1-48.5

4.1.7 Summary of Diagnostic Methods for *S. mansoni* Detection

The results of this study emphasize the need for integrating sensitive diagnostic tools like POC-CCA and cPCR into schistosomiasis surveillance programs, especially in low-endemic settings. While the Kato-Katz method remains widely used due to its affordability and simplicity, its limited sensitivity highlights the need for alternative approaches. POC-CCA offers a practical solution for field settings, while cPCR provides critical confirmatory insights, particularly for low-intensity infections. **Table 4.3** compares the effectiveness of three diagnostic methods for detecting *S. mansoni* infections in school-aged children in NMB. Kato-Katz (stool microscopy) identified no cases, highlighting its limitations in low-transmission areas. POC-CCA (antigen detection) detected *S. mansoni* antigens in 3.7% of participants, demonstrating improved sensitivity over microscopy. cPCR, performed on 28 CCA-positive samples, confirmed *S. mansoni* DNA in 32.1% of these cases, showing its superior ability to identify infections, particularly those missed by both Kato-Katz and POC-CCA.

Table 4.3: Diagnostic test results for *S. mansoni* detection among school-aged children, comparing stool microscopy (Kato-Katz), antigen detection (POC-CCA), and molecular diagnostics (cPCR) in a low-transmission setting.

Test method	Number of participants tested	Positive cases (n)	Prevalence (%)	Remarks
Kato Katz (Stool Microscopy)	759	0	0	Highlighted the limitations of traditional diagnostics in low-transmission settings.
POC-CCA (Antigen Detection)	759	28	3.7	Detected <i>S. mansoni</i> antigen in participants, indicating improved sensitivity over microscopy.
cPCR (cox-1 gene)	28 (CCA-positive samples)	9	32.1 (of CCA-positive)	Confirmed <i>S. mansoni</i> DNA, demonstrating superior effectiveness in identifying infections.

4.1.8 Sensitivity and specificity of cPCR to detect Schistosoma infections

The table 4.4 compares the diagnostic performance of POC-CCA and cPCR for detecting *S. mansoni* based on 28 POC-CCA-positive samples. POC-CCA showed 100% sensitivity, identifying all positive cases, but specificity could not be assessed due to the absence of negative samples. The Kappa score was 0, indicating no agreement beyond chance, largely due to the lack of negative data. Precision (positive predictive value) for POC-CCA was 100%, meaning all positive predictions were correct.

For cPCR targeting the *S. mansoni* Cox 1 gene, sensitivity was 32.1%, confirming 9 of the 28 POC-CCA-positive samples. Like POC-CCA, cPCR’s specificity and negative predictive value couldn’t be assessed, and the Kappa score remained 0. Nonetheless, the precision was maintained at 100%, signifying that every positive prediction from the cPCR was accurately identified as a true positive. The cPCR results demonstrated reliability in identifying positive cases; however, its reduced sensitivity resulted in a lower detection rate of infections compared to the POC-CCA test, thereby underscoring its limitations in identifying low-level infections. Both tests demonstrated high precision for positive results; however, they exhibited limitations in specificity and negative predictive value due to constraints associated with sample size and methodology. The precision of positive and negative test results was 100% for positives and 0% for negatives across all tests calculated using the formula:

$$PPV = \frac{\text{True Positives}}{\text{True positives} + \text{False positives}} \times 100$$

This indicates that although positive PCR results were dependable, negative results could not be reliably forecasted, highlighting the need for enhancements in the diagnostic procedure. A definitive infection was confirmed if either Kato-Katz or PCR yielded positive results (32). Overall, the POC-CCA showed perfect sensitivity but was limited by the lack of specificity data, while cPCR was less sensitive, detecting *S.*

mansoni DNA in only about a third of the POC-CCA-positive samples. Both methods had high precision for predicting positive cases but lacked information on negative predictive value and specificity.

Table 4.4: Sensitivity, specificity, precision and Kappa values of PCR using different primers

Predicted***	Observed		Sensitivity (95% CI)	Specificity (95% CI)	Kappa score	Precision (positive & negative predictive)
	Negative	Positive				
POC-CCA (n=28)						
Negative	0	0	100.0 (100.0-100.0)	0	0.0	100.0 & 0
Positive	0	28				
Cox 1 (n=28)						
Negative	0	19	32.1 (14.8-49.4)	0	0.0	100.0 & 0
Positive	0	9				

* Average of the three primers; *** After testing

4.2 DISCUSSION

The aim of this study was to determine the prevalence of *S. mansoni* among school-going children in NMB, utilising a range of advanced diagnostic techniques to identify cases that may have been missed by traditional methods. This population is particularly relevant for schistosomiasis studies, as school-aged children are at a higher risk due to their frequent exposure to contaminated water sources. By focusing on this vulnerable group, the study addresses the limitations of conventional diagnostic tools, especially in low-endemic areas, and reinforces the urgent need for more sensitive and specific methods to improve detection and provide accurate prevalence estimates (Sacolo-Gwebu et al., 2019).

The prevalence of *S. mansoni* infection among school-aged children varies significantly across different geographical regions within endemic areas. In the present study, no cases of *S. mansoni* were detected using the Kato-Katz method, highlighting the challenges of diagnosing low-intensity infections in a low-transmission setting. These results are consistent with expectations, as the Kato-Katz method, while known for its specificity, has well-documented limitations (Espírito-Santo et al., 2014, p. 2; Vonghachack et al., 2017a,). In low-endemic areas like NMB, the parasite load is often minimal, leading to low egg counts (Ajibola et al., 2018; Vinkeles Melchers et al., 2014). Multiple samples typically enhance test sensitivity by increasing the likelihood of detecting infections; however, this study was cross-sectional in nature, meaning that samples were collected at a single time point (Vonghachack et al., 2017b). Despite this limitation, additional diagnostic techniques were employed to confirm *S. mansoni* cases within the study population, thereby strengthening the overall diagnostic accuracy and reliability of the findings (Ajibola et al., 2018).

This finding contrasts with a previous study conducted in KwaZulu-Natal among preschool-aged children (1-5 years), which reported a prevalence of 0.9% (Sacolo-Gwebu et al., 2019). The current study area has experienced significant environmental changes, such as altered rainfall patterns, leading to the drying up of

many transmission sites. These changes have likely contributed to the reduced intensity and prevalence of schistosomiasis among school-aged children (WATER OUTLOOK REPORT NOVEMBER, 2021). A systematic review by Tavakoli-Pirzaman et al. (2024) (Tavakoli Pirzaman et al., 2024) reported higher *S. mansoni* prevalences in several Sub-Saharan African countries, including Madagascar, Guinea, and Tanzania, with rates ranging from 9.5% to 13.2%. In comparison, South Africa exhibited lower prevalence, with reports of 0.8% across multiple studies between 2011 and 2018, emphasizing the need for sustained control efforts in specific areas despite overall lower infection rates (de Boni et al., 2021).

On the other hand, the POC-CCA test demonstrated superior sensitivity compared to Kato-Katz in identifying infections of low intensity. A prevalence of 3.7% was determined using urine samples and the rapid diagnostics point of care test strips. A previous study by Coelho et al. (2016a) (Coelho et al., 2016a) examined the potential for cross-reactivity in the POC-CCA test, particularly regarding antigens from other helminth infections. The study found that infections with helminths such as hookworms, *Hymenolepis nana*, *Enterobius vermicularis*, and *Ascaris lumbricoides* could lead to false positives in the POC-CCA test, even in the absence of *Schistosoma mansoni* eggs in stool samples (Coelho et al., 2016a; Peralta & Cavalcanti, 2018). This research highlights the importance of considering cross-reactivity when interpreting POC-CCA results, especially in areas where multiple helminth infections are prevalent (Bezerra et al., 2018; Favre et al., 2022).

The POC-CCA test has been reported to lack specificity for *S. mansoni* due to antigenic similarities with human components, such as Lewis-X structures, tetra-saccharides composed of sialic acid, fucose, and N-acetyl glucosamine, which are excreted in urine and can lead to cross-reactivity (Diab et al., 2021). Various factors, including diabetes mellitus, urinary tract infections, haematuria, alcohol abuse, diuretics, dehydration, and certain medications (such as rifampin, which affects test strip colour), may influence the results of the POC-CCA test, particularly in cases with trace or weak positives (Ferreira et al., 2017). Although some research suggests that hookworm infection might affect POC-CCA results, (Coelho et al., 2016b, p. 10), this has been contested by other studies (Shane et al., 2011; Tchuem Tchuente et al., 2012). Previous research has similarly found that POC-CCA detects higher prevalence rates in low-endemic settings compared to Kato-Katz (Ochodo et al., 2015; Worrell et al., 2015). Coelho et al., 2016 (Coelho et al., 2016b) also observed that POC-CCA trace results could become positive after concentrating urine samples, highlighting its utility in challenging diagnostic scenarios. Despite its lower specificity and the subjective nature of interpreting trace results, POC-CCA remains valuable due to its better sample compliance and the rapid decline of circulating antigens post-treatment (Vonghachack et al., 2017b; Worrell et al., 2015). Furthermore, POC-CCA is cost-effective compared to Kato-Katz, which often requires multiple tests for accurate diagnosis (Worrell et al., 2015).

In this investigation, cPCR was employed to identify cfDNA, serving as a control for specificity of POC-CCA assessment. DNA-based assays have demonstrated greater reliability in detecting infections, particularly in regions with low endemicity. The process of DNA extraction from urine was essential, and the application of ZYMO BIOMICS enabled the extraction of high-quality DNA from stool samples prior to cPCR analysis. The cPCR method was used on all the POC-CCA positive samples in consistence with the observations by Lodh et al., (2013) (Lodh et al., 2013), who advised against employing sophisticated cPCR methods in low-transmission and resource-limited environments. They concluded that since thermocycler machines for DNA amplification are expensive and not easily accessible for field conditions where electric power is unreliable or

inconsistent, other tests such as POC-CCA could be useful in disease surveillance and monitoring. Stool samples from participants who tested positive for POC-CCA were subjected to PCR analysis for confirmation. Of the 28 samples analysed, 9 (32.1%) were found to be positive for *S. mansoni* DNA.

Prior investigations corroborate these findings. According to Pontes et al., (2003), for example, samples investigated by the Kato-Katz technique had a comparatively lower prevalence of schistosome infection (30.9%) than samples analysed by the PCR technique (38.1%). A study conducted in Kenya found that the prevalence of *S. mansoni* infection, as measured by the POC-CCA test before and after treatment, was 26.5% and 21.4%. In contrast, the Kato-Katz technique reported prevalence rates of 4.9% and 1.5%, respectively (Okoyo et al., 2018). Interestingly, Al-Shehri et al., p. (2018) reported that the overall prevalence of schistosomiasis identified through Kato-Katz smears was 44.1%, while the prevalence in samples assessed by CCA dipstick reached 67.4%, and those evaluated by PCR showed a prevalence of 75.1%. Lodh et al., (2013), pointed out that the Kato-Katz method analyses only approximately 50 mg of faecal matter, representing a small sample size, which increases the likelihood of overlooking some positive cases in instances of low-level infections.

The cPCR results revealed a higher infection rate of *S. mansoni* among males, a finding consistent with studies conducted in Nigeria (K. N. Opara et al., 2021) and Senegal (Senghor et al., 2022), which also reported a greater prevalence in males. This is likely attributed to increased exposure to water sources through activities such as swimming, fishing, and agricultural work, which are more commonly undertaken by males in these regions. The difference in infection rates by gender can be partly attributed to societal roles and behaviour, with males more likely to engage in water-contact activities, thereby increasing their risk of exposure to contaminated water (Anto, 2013).

The recent schistosomiasis outbreak in Tzaneen underscores the critical need for comprehensive diagnostic methodologies in detecting *S. mansoni* infections (SABC News - Breaking News, ., n.d.). The outbreak, which affected over 140 learners at Malwandla Primary School, highlights the limitations of traditional diagnostic methods in low-transmission settings (*Bilharzia-Outbreak-Media-Statement*, n.d.). Implementing advanced diagnostic techniques, such as POC-CCA and cPCR, is essential for accurate detection, enabling timely interventions and effective control measures. This research emphasizes the importance of adopting sensitive and specific diagnostic tools to enhance schistosomiasis surveillance and response efforts in South Africa.

Several recommendations are proposed to enhance future research and public health interventions. Incorporating multiple diagnostic methods, including traditional techniques (Kato-Katz, urine filtration) alongside advanced approaches (PCR, POC-CCA), is crucial for obtaining accurate prevalence estimates, particularly in low-transmission environments. Routine screening programs, along with community education on schistosomiasis prevention, are essential for decreasing infection rates. Furthermore, enhancing access to molecular diagnostic tools in endemic areas may facilitate improved detection and enable more prompt interventions. In conclusion, a comprehensive understanding of the local snail intermediate hosts is essential for addressing the ecological dimensions of schistosomiasis transmission.

CHAPTER 5: PREVALENCE AND INTENSITY OF *S. HAEMATOBIIUM* INFECTION THROUGH URINE AND FECAL SAMPLES RESPECTIVELY, IN SCHOOL GOING CHILDREN

5.1 RESULTS

5.1.1 Demographic Characteristics of Study Participants

The study enrolled a total of 759 school-going children aged 5–14 years from NMB to assess the prevalence of schistosomiasis infections. The cohort comprised 438 males (57.7%) and 321 females (42.3%), with a mean age of 11.00 ± 1.50 years, reflecting a predominance of older children and pre-adolescents. Participants were stratified by grade level, with 467 (61.5%) in grades 4–7 and 293 (38.5%) in grades 0–3. Geographically, the participants were distributed across five principal areas within NMB. KwaNobuhle contributed the largest proportion of participants, representing 439 individuals (57.8%), followed by Motherwell with 152 participants (20.0%). Ibhayi and Kariega each accounted for 54 participants (7.1%), while Despatch contributed 60 participants (7.9%). This demographic distribution ensured a representative sample of the study population, facilitating a comprehensive analysis of infection patterns across different age groups and geographic locations within NMB (refer to **Table 5.1**).

Table 5.1: Demographic characteristics of participants.

Demographic characteristic	Total sample size (n=759)	%
Gender		
Male	438	57.7
Female	321	42.3
Grade		
0-3	292	38.5
4-7	467	61.5
Age (years)		
5-8	76	10
9-14	683	90
Town		
KwaNobuhle	439	57.8
Ibhayi	54	7.1
Kariega	54	7.1

Motherwell	152	20.0
Despatch	60	7.9

5.1.2 Diagnostic Techniques used to diagnose *S. haematobium*

5.1.2.1 Dipstick Urinalysis Test for Haematuria

The dipstick urinalysis test for haematuria identified a prevalence of 33.6% (255 out of 759 participants), with 143 (56.1%) of the haematuria-positive cases occurring in males and 112 (43.9%) in females. Semi-quantitative analysis of microhaematuria revealed varying levels of haematuria: trace haematuria (10 erythrocytes/ μ L) was detected in 96 (12.6%) samples, mild haematuria (+, 25 erythrocytes/ μ L) in 37 (4.9%) samples, moderate haematuria (++, 80 erythrocytes/ μ L) in 81 (10.7%) samples, and high levels of haematuria (+++, 200 erythrocytes/ μ L) in 41 (5.4%) samples (refer to **Figure 5.1**).

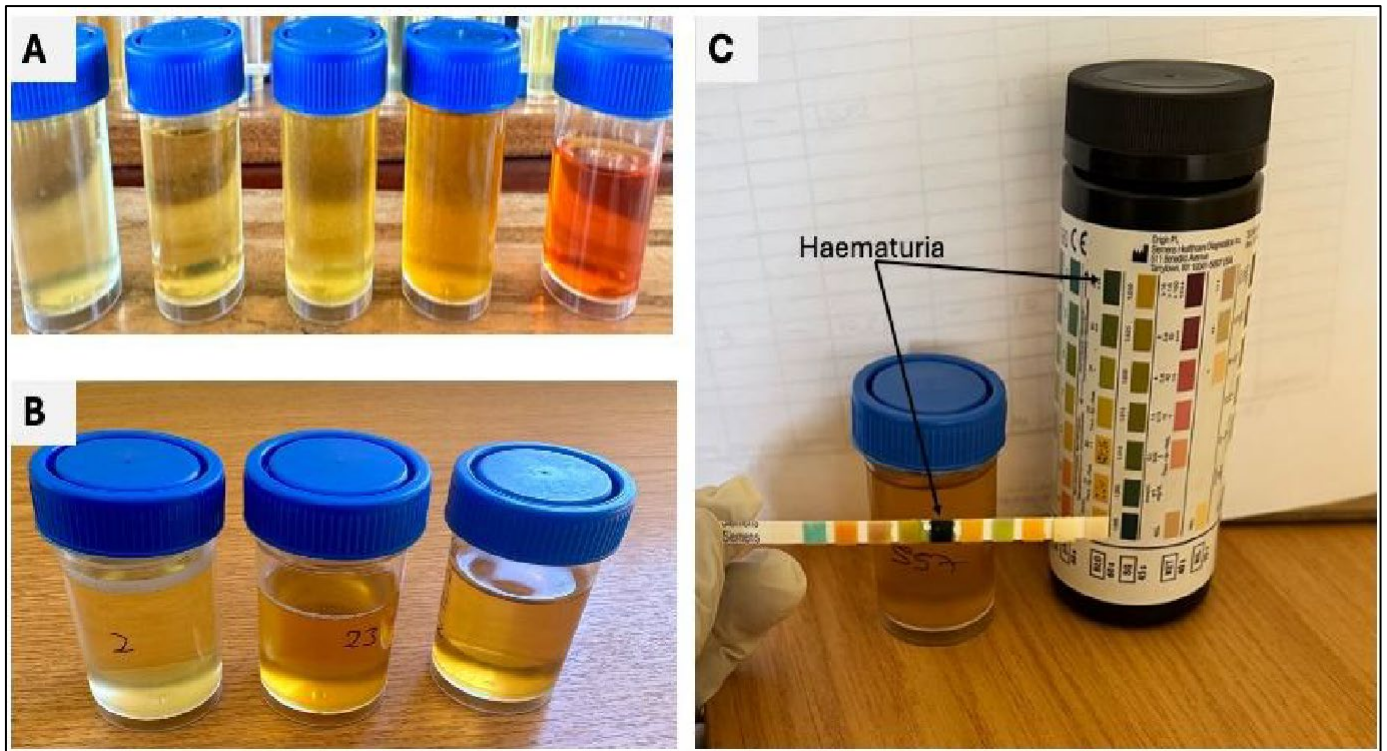


Figure 5.1: Different levels of visual haematuria. Picture A and B shows samples with different levels of visual haematuria. Picture C shows the reagent strip showing 3+ positive haematuria.

Visual haematuria was observed in some samples, further emphasizing the utility of dipstick urinalysis as an effective diagnostic tool for identifying *S. haematobium* infections. These findings highlighted the potential severity of *S. haematobium* infections among the school-aged children examined and highlighted the importance of integrating dipstick urinalysis into diagnostic protocols for schistosomiasis in endemic populations.

5.1.2.2 Urine Filtration

The prevalence of schistosomiasis, as determined by urine filtration, was 0.1% (1 out of 759 participants), indicating an exceptionally low prevalence within the study population. The single positive sample was obtained from a male participant aged 9–14 years from Kariega. **Figure 5.2** presents a microscopy photograph

of a *S. haematobium* egg at 40x magnification, captured from the urine sample of the positive case. This finding confirmed the presence of *S. haematobium* in the study area and in consideration of the limitations of urine filtration as a diagnostic tool in low-prevalence settings the researchers proceeded to do cPCR on haematuria positive samples to confirm diagnosis.

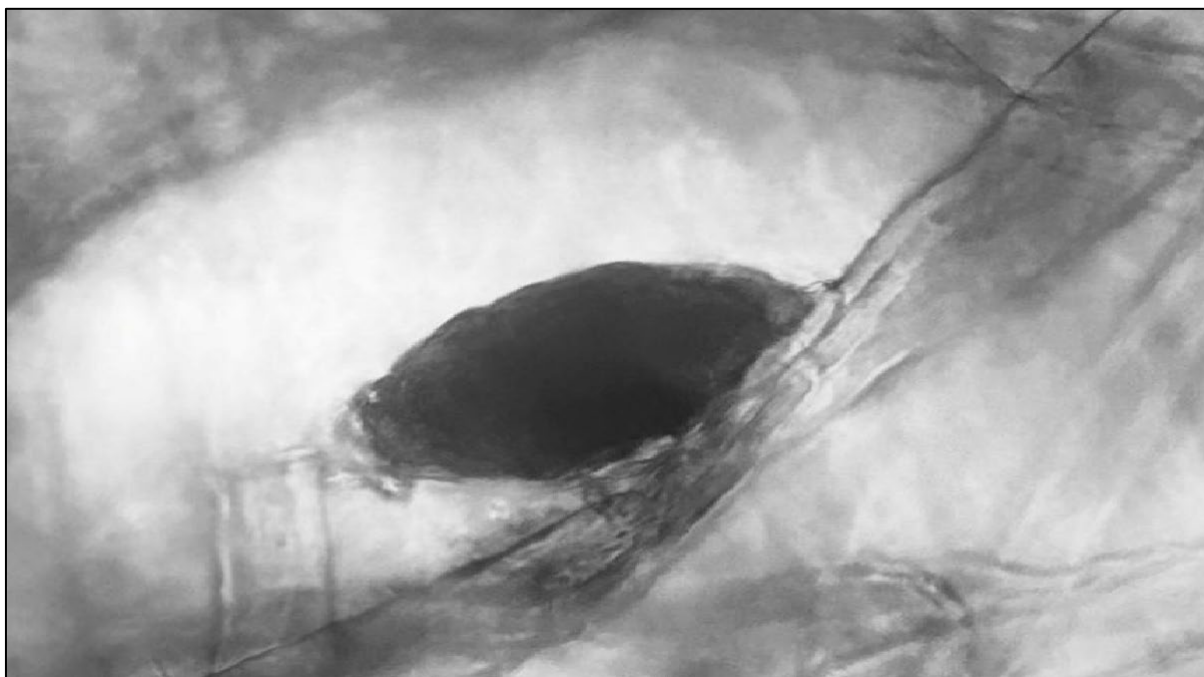


Figure 5. 2: This is a microscopy photograph of a *S. haematobium* egg at 40x magnification, taken from a male learner in the study area.

5.1.2.3 cPCR for *S. haematobium* cfDNA Detection

Given that NMB is a low prevalence area, for cPCR samples positive after screening for haematuria were further analysed for cfDNA detection. The cPCR analysis was done using the 121 randomly selected haematuria-positive samples (2+ and 3+ haematuria positive samples). The Dra1 F/R primers successfully amplified *S. haematobium* DNA in 38 samples (31.4%; 95% CI: 23.1–39.7). The single sample that tested positive via urine filtration was used as served as the positive control to validate the efficacy of the cPCR assay and primer specificity. This positive control, derived from a confirmed *S. haematobium* ovum-positive sample, yielded the expected positive cPCR result, corroborating the reliability of the reagents, equipment, and procedural protocols employed. The concordance between urine filtration and cPCR results supported the diagnostic accuracy of cPCR in detecting *S. haematobium* infections.

5.1.2.4 Detection Rates Across Demographic Subgroups

The results in **Table 5.2** indicate that the prevalence of *S. haematobium* infection varies across demographic groups. Among age groups, the prevalence was slightly higher in the 9–14 age group (31.7%, 95% CI: 22.8–40.7%) compared to the 5–8 age group (29.4%, 95% CI: 7.8–51.1%), but the overlapping confidence intervals suggest this difference is not statistically significant. By gender, the prevalence was marginally higher in males (32.4%, 95% CI: 21.2–43.5%) than in females (30.2%, 95% CI: 17.8–42.5%), though the overlapping confidence intervals indicate this difference is also not statistically significant. In terms of grade levels, the prevalence was significantly higher in the 4–7 grade group (34.6%, 95% CI: 24.1–45.2%) compared to the 0–3 grade group (25.6%, 95% CI: 12.5–38.6%). The non-overlapping confidence intervals suggest this difference may be statistically significant, pointing to a potential trend of increasing *S. haematobium* infection prevalence with higher grade levels. These findings show the importance of sample size in interpreting prevalence

estimates, as smaller samples result in wider confidence intervals and greater uncertainty. Further research with larger sample sizes is needed to confirm these trends and explore potential associations more robustly, particularly in the context of *S. haematobium* transmission dynamics and risk factors.

Table 5.2: Prevalence of *Schistosoma haematobium* Infections Detected by the Dra1 Repeat Sequence Across Demographic Subgroups.

Demographic	Category	n	Positive	Prevalence (%)	95% CI
Age	5 to 8 years	17	5	29.4	7.8–51.1
	9 to 14 years	104	33	31.7	22.8–40.7
Gender	Male	68	22	32.4	21.2–43.5
	Female	53	16	30.2	17.8–42.5
Grade	0–3	43	11	25.6	12.5–38.6
	4–7	78	27	34.6	24.1–45.2

n, number of participants; *CI*, confidence interval.

5.1.2.5 Agarose Gel Electrophoresis Validation

Figure 5.3 presents agarose gel electrophoresis images (A, B, and C) depicting amplified *S. haematobium* Dra1 sequences from haematuria-positive urine samples. Lanes labelled "M" represent the DNA molecular weight marker, used to estimate the size of PCR products. The band marked "UF+ve" corresponds to the urine filtration-positive sample, serving as the positive control for *S. haematobium* DNA amplification. Lane 13, labelled "Negative control (Dra1 F/R)," exhibited no amplification, confirming the absence of contamination. The expected product sizes ranged between 0.050 Kb and 1.5 Kb, consistent with successful amplification of the Dra1 repeat sequence. Each band confirmed the presence of *S. haematobium* DNA, validating the specificity and sensitivity of the Dra1 primers in detecting the parasite in haematuria-positive samples.

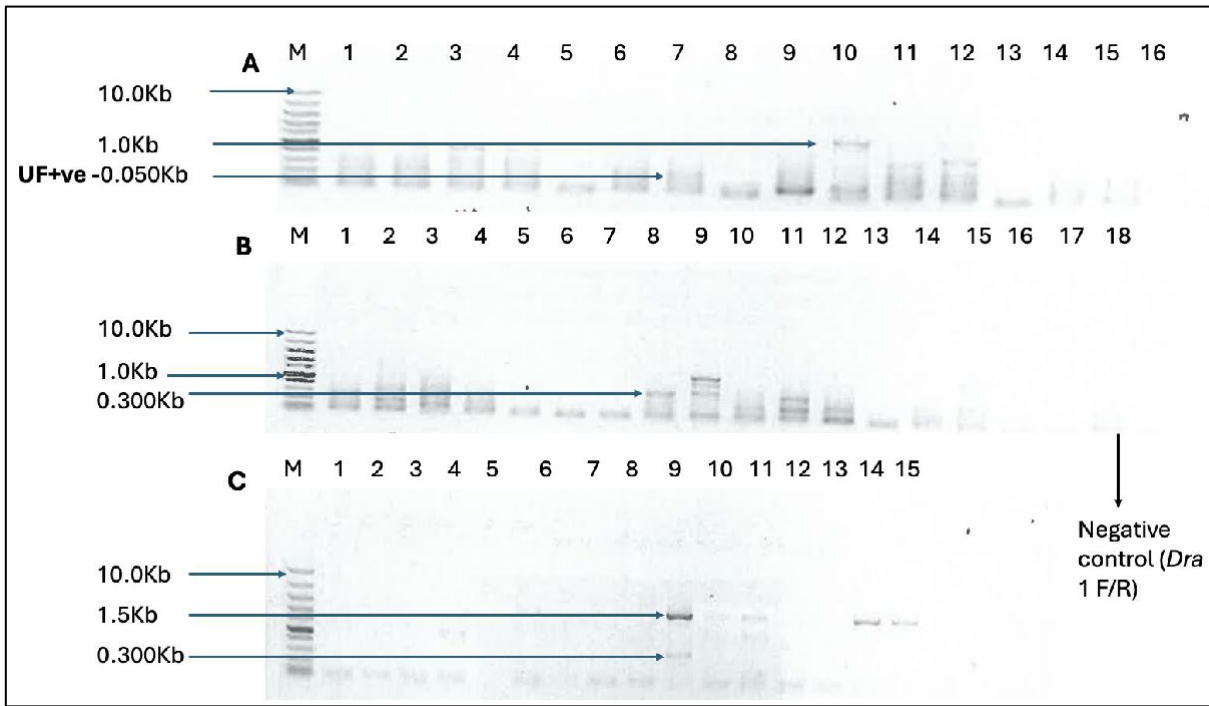


Figure 5.3: Agarose gel electrophoresis image showing amplified repetitions of *S. haematobium* Dra1 sequence from haematuria positive urine samples.

In summary, the diagnostic results for *S. haematobium* show the critical importance of employing a multifaceted diagnostic approach to accurately assess schistosomiasis prevalence, particularly in low-transmission settings such as the NMB area. Utilizing a combination of methods, including haematuria detection, urine filtration, and molecular diagnostics via Cpcr, provided a more comprehensive understanding of infection dynamics, as each method offered unique strengths and limitations. Notably, the prevalence rates varied significantly depending on the diagnostic tool used: urine filtration indicated a very low occurrence of *S. haematobium* (0.1%), while haematuria and cPCR revealed substantially higher infection rates 33.6% and 31.4% respectively.

5.1.3 Specificity and sensitivity of the Dra1 Repeat Sequence for *S. haematobium* Detection

The diagnostic performance of the Dra1 repeat sequence was evaluated using a subset of 121 haematuria-positive samples (refer to **Table 5.3**). The Dra1 repeat exhibited a sensitivity of 31.4% (95% CI: 23.1–39.7), indicating its ability to correctly identify 38 out of 121 true positive cases. However, its specificity was also 31.4% (95% CI: 23.1–39.7), reflecting its limited capacity to correctly classify 83 out of 121 true negative cases.

The Kappa score for the Dra1 repeat was 0, indicating no agreement between the test results and the true infection status. Precision analysis revealed a positive predictive value (PPV) of 100%, confirming the reliability of positive results, while the negative predictive value (NPV) was 0%, highlighting the inability to reliably predict true negatives. These results underscore the limitations of the Dra1 repeat as a diagnostic tool, particularly in accurately identifying true negative cases and achieving concordance with true infection status.

Table 5. 3: Diagnostic Performance of the Dra1 Repeat Sequence for Detecting *Schistosoma haematobium* Infections in Haematuria-Positive Samples (n = 121)

Metric	Dra1 Repeat (n=121)
Observed Negative	83

Observed Positive	38
Sensitivity (95% CI)	31.4% (23.1–39.7)
Specificity (95% CI)	31.4% (23.1–39.7)
Kappa Score	0
Precision (PPV & NPV)	100.0% & 0%

CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

5.2 DISCUSSION

This study aimed to assess the prevalence of *S. haematobium* infections among school-going children aged 5–14 years in NMB, employing a comprehensive diagnostic approach that included dipstick urinalysis, urine filtration, and molecular confirmation via cPCR. School-aged children were selected as the study population due to their heightened vulnerability to schistosomiasis, driven by frequent exposure to contaminated water sources during recreational and daily activities. For instance, a study in Gabon reported a 21% prevalence of haematuria among children aged 5–19 (Dejon-Agobé et al., 2021), while research in Nigeria found a 7.6% prevalence in preschool-aged children (Salawu & Odaibo, 2014). These findings underscore the susceptibility of younger populations to schistosomiasis, likely due to increased water contact behaviours. NMB, a historically endemic region for schistosomiasis, was chosen to evaluate ongoing transmission dynamics and the effectiveness of current control measures in a low-endemic setting. The study illuminates the persistent transmission of *S. haematobium* in NMB, despite its classification as a low-prevalence area, and emphasizes the need for sensitive diagnostic tools to accurately identify infections and inform targeted interventions. This contributes to the broader goal of schistosomiasis control and elimination in South Africa and similar settings.

In regions where access to the gold standard diagnostic method, urine microscopy, is limited, urine dipstick tests have emerged as an effective screening tool for *S. haematobium* (Craik et al., 2023). Haematuria, a clinical manifestation of *S. haematobium* infection, serves as a key indicator of urinary tract morbidity (Vinkeles Melchers et al., 2014). However, its non-specific nature means it can also result from other conditions such as urinary tract infections or kidney stones, limiting its diagnostic reliability when used in isolation (Sánchez-Marqués, Bocanegra, et al., 2023). Despite this limitation, the detection of haematuria through urine dipstick tests in this study signalled potential schistosomiasis transmission in NMB, warranting further investigation due to its strong association with *S. haematobium* as demonstrated in prior research.

The prevalence of haematuria in this study (33.6%) aligns with findings from other regions, demonstrating its utility as a screening tool. For example, in Tanzania, the prevalence of haematuria (0.9%) closely matched the 0.83% prevalence detected by urine filtration, suggesting comparable effectiveness between the two methods (Mazigo et al., 2021). However, in Ethiopia, a significant difference was observed, with urine filtration detecting a prevalence of 0.14% compared to 1.03% using urinalysis dipsticks (Mohammed et al., 2022). The difference shows the higher sensitivity of dipstick tests, particularly in low-endemic settings, a trend mirrored in the current study where 33.6% participants tested positive for haematuria, while only 0.1% tested positive via urine filtration.

The underestimation of schistosomiasis prevalence by urine filtration is further supported by studies in Ethiopia and Nigeria. Deribew et al. (2022) (Deribew et al., 2022a) reported a urogenital schistosomiasis prevalence of 12.2% using urine filtration, which increased to 22.5% with urinalysis reagent strips. Similarly, in Nigeria, reagent strips yielded higher positive results compared to urine filtration (Babatunde et al., n.d.). These findings suggest that while urine filtration is reliable for detecting schistosome eggs, it may miss low-intensity infections that reagent strips can identify. The superior sensitivity (99.3%) and specificity (88.1%) of reagent strips, as

demonstrated by (Deribew et al., 2022b), further validate their use in schistosomiasis surveillance, particularly in low-transmission areas.

The low prevalence of schistosomiasis detected by urine filtration (0.1%) in this study contrasts sharply with the high haematuria rates, underscoring the method's limitations in low-endemic settings. The single positive case detected via urine filtration aligns with historical data from Kariega, where the last documented case was reported in 1959 (C. C. Appleton et al., 2012). The current study results on urine filtration align with findings from a 2016 study in the same area by Müller et al. (2016) (Müller et al., 2016), where one primary school child tested positive for *S. haematobium*. Müller et al. (2016) (Müller et al., 2016) used diagnosed schistosomiasis using microhaematuria detected by Hemastix® strips as proxies for infection. In 2017, the Eastern Cape Department of Health conducted a mapping study using urine dipstick and filtration methods, reporting a 2% prevalence of *S. haematobium* among school-going children in NMB (CDCD, 2019). A retrospective study by Hambury (2021) (Dorné Hambury, 2021) recorded a 20% prevalence of urinary schistosomiasis from 2014 to 2018, with 19% in 2018, significantly higher than the current study findings. This inconsistency and fluctuations in prevalence stresses the challenges of diagnosing schistosomiasis in low-transmission areas like NMB, where retrospective data from symptomatic individuals may not reflect the broader community prevalence, as many infections are asymptomatic.

A study in the Eastern Cape's Buffalo City Municipality by Peters et al. (2023) (Peters et al., 2023) identified a single case of *S. haematobium* infection among pregnant women, reflecting low transmission in urban areas. However, the study populations differed, limiting direct comparisons. Schistosomiasis transmission varies significantly between urban and rural areas, as evidenced by a 73% prevalence among school children in the Mbashe District (Meents & Boyles, 2010). The cases identified by Peters et al. (2023) (Peters et al., 2023) and the current study may indicate migrated transmissions, as other South African provinces report higher prevalences among children, emphasizing the need for further investigations using highly sensitive diagnostic techniques.

Schistosomiasis transmission is influenced by environmental conditions, access to water, sanitation, and hygiene (WASH) practices, and human behaviour. For example, a retrospective study in Mpumalanga's Ehlanzeni District found a 71% prevalence among children aged 10–14 years, with higher rates among males (Njikho et al., 2023). Similarly, in KwaZulu-Natal's Ugu District, rural female pupils had a 32% prevalence (Zulu et al., 2020). In 2024, the South African government reported a schistosomiasis outbreak in Tzaneen, Limpopo, highlighting the variability of infections across endemic areas (*Several Areas near Tzaneen Hit by Bilharzia Outbreak - SABC News - Breaking News, Special Reports, World, Business, Sport Coverage of All South African Current Events. Africa's News Leader.*, n.d.). Migration patterns also play a crucial role in disease spread, as rural-urban migration can facilitate regional transmission, particularly in interconnected communities (Silva-Moraes et al., 2014).

The limitations of traditional diagnostic methods, such as microscopy, are evident in studies from Brazil (Lindholz et al., 2018; Siqueira et al., 2021), the Philippines (Belizario et al., 2024), and Angola (Mediavilla et al., 2024), which demonstrated their insufficient sensitivity in detecting mild to low-intensity infections. The use of highly sensitive diagnostic tools, such as molecular methods like cPCR, was critical in our study to accurately determine schistosomiasis prevalence in low-transmission regions. In such settings, traditional diagnostic methods often fall short due to challenges like low parasite burden, intermittent egg excretion, and variable host immune responses (Cavalcanti et al., 2019; Espírito-Santo et al., 2014). By employing cPCR, we addressed these limitations, ensuring a more precise and reliable assessment of infection rates. For instance, Molehin (2020) (Molehin, 2020a) notes that individuals with frequent infections, such as school-aged children, may develop stronger immune responses, leading to reduced egg excretion.

Furthermore, cPCR specific primers provided the ability to differentiate between *Schistosoma* species, offering valuable insights for targeted epidemiological interventions. Its use significantly reduced the likelihood of false negatives, ensuring that even low-level infections were identified (Sengupta et al., 2019). Ultimately, the integration of cPCR into our diagnostic framework was essential for generating accurate data, which is crucial

for effective disease surveillance, control, and resource allocation in low-endemic areas. Out of 121 samples with moderate to high haematuria levels, cPCR confirmed *S. haematobium* DNA in 31.2%, contrasting sharply with the single positive case (0.1%) detected via urine filtration. This emphasizes the superior sensitivity of molecular diagnostics in identifying low-intensity infections, aligning with studies from Nigeria, Zanzibar, Senegal, and Ghana (Esiere et al., 2022d; Keller et al., 2020; Sow et al., 2023b). The high positivity rate observed with cPCR underscores its potential as a gold standard for schistosomiasis diagnosis in low-endemic regions.

There is need for urgent public health interventions to prevent outbreaks, such as the recent schistosomiasis outbreak in Tzaneen, Limpopo (*Several Areas near Tzaneen Hit by Bilharzia Outbreak – SABC News – Breaking News, Special Reports, World, Business, Sport Coverage of All South African Current Events. Africa’s News Leader.*, n.d.). As a neglected tropical disease (NTD), schistosomiasis often lacks attention, leading to missed cases and delayed interventions. This study emphasizes the necessity of prompt measures, including mass drug administration (MDA), to protect at-risk populations. The primary limitation was the low sensitivity of urine filtration, which identified only one positive case, preventing direct comparisons with cPCR and haematuria. Using a single urine sample per participant may have underestimated prevalence (Santos et al., 2021). In low-transmission areas, where egg excretion can be intermittent and parasite loads are often low, relying on a single sample reduces the likelihood of detecting infections. For more effective diagnostics in such settings, collecting multiple urine samples over an extended period is recommended. This approach increases the probability of capturing *Schistosoma* eggs in urine, thereby providing a more accurate estimation of true prevalence.

Although cPCR demonstrated high sensitivity, its cost, technical complexity, and infrastructure demands limit feasibility in resource-poor settings. The small positive control sample also restricted precise evaluation of cPCR’s sensitivity and specificity. Future studies should enhance diagnostic accuracy by incorporating multiple tests, including antibody detection assays, and increasing urine sample collection per participant, as recommended by the WHO. Establishing reference laboratories with molecular diagnostic capabilities in endemic areas would improve detection. Expanding targeted screening and surveillance beyond school-aged children to other high-risk groups is crucial. Integrating cPCR and molecular tools will enable timely treatment and better control of disease transmission.

CHAPTER 6: EVALUATION OF URINARY METABOLITE MARKERS FOR DETECTING *S. HAEMATOBIIUM* INFECTIONS

6.1 RESULTS AND DISCUSSION

6.1.1 Sociodemographic Data

A total of 759 school-age children provided urine samples, which were analysed through urinalysis. The study population comprised 57.7% male participants and 42.3% female participants, indicating that males constituted the majority of the total number of participants. The mean age of the participants was 11.00 ± 1.50 years, with ages ranging from 5 to 14 years, indicating that the group predominantly consisted of older children or pre-adolescents.

6.1.2 Analysis of Urine Biomarker Distributions and Variability

Figure 6.1 shows the central tendency, variability, and occurrence of outliers for each parameter, offering a detailed overview of the measurements of the urine samples. Observations indicated low median values for glucose, bilirubin, and nitrates, suggesting that the majority of the samples are within the anticipated range for these parameters. However, parameters such as specific gravity and ketone exhibit a higher median value in comparison to others, indicating elevated levels of these parameters in the samples. The blood, specific gravity, and ketone levels exhibit significant variability, as indicated by the extensive interquartile range (IQR). This indicates that there were greater fluctuations in the measurements of these parameters across all samples. In contrast, parameters such as glucose, bilirubin, and nitrates exhibit narrow interquartile ranges, indicating reduced variability within the sample regarding their measurements. All parameters exhibit outliers, defined as values that deviate significantly from the primary distribution range. This indicates that, within this particular demographic, some participants exhibited abnormal readings for specific parameters. The identification of outliers in blood, protein, and urobilinogen levels indicates potential significant deviations from established normal values, which may suggest the presence of underlying health issues in the affected samples. The majority of parameters are within normal ranges; however, notable variability and outliers exist in certain samples. Caution is advised when interpreting these results, and appropriate follow-up actions should be implemented in clinical settings.

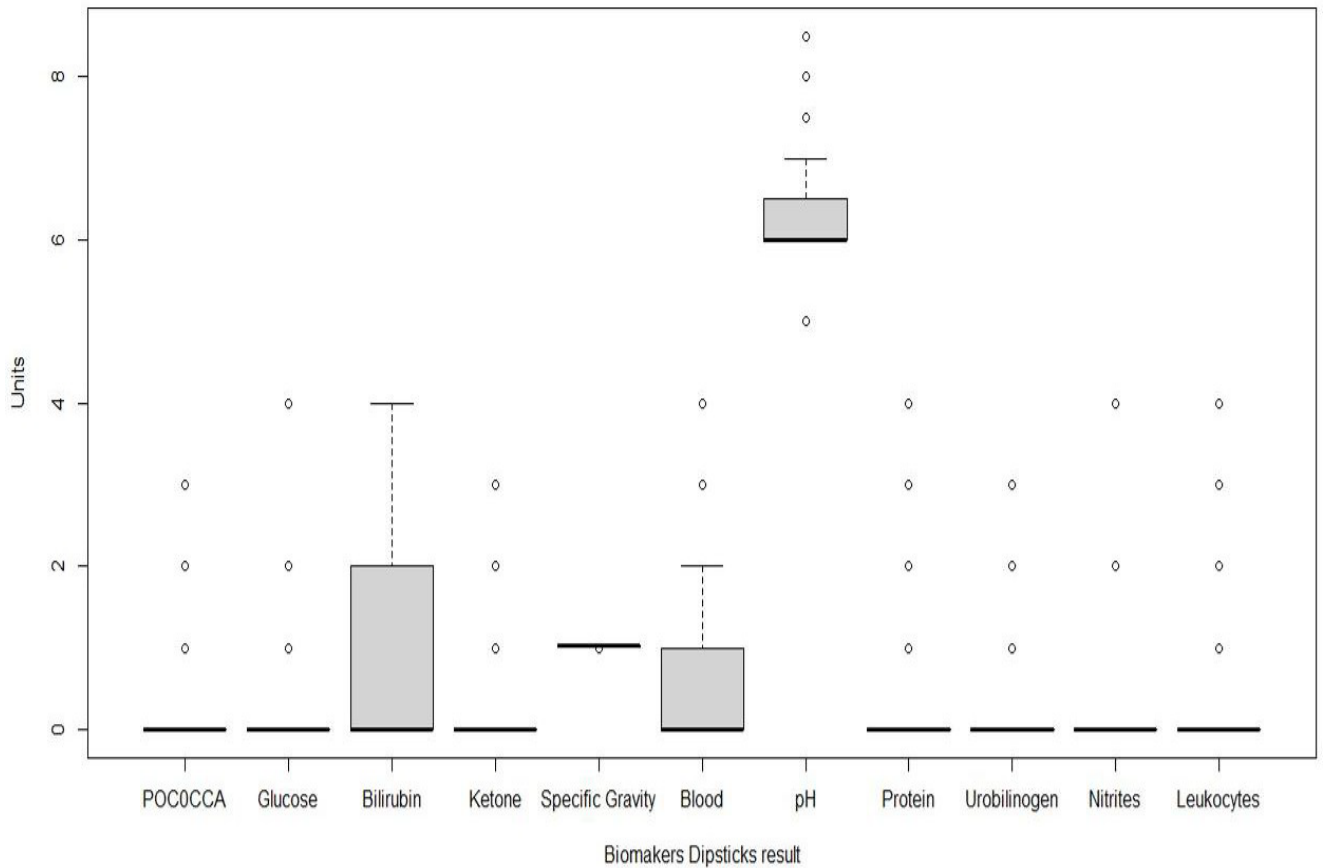


Figure 6.1: Distribution of urine parameters measured by urinalysis. A box plot illustrating the distribution of various urine parameters including glucose, bilirubin, ketone, specific gravity, blood, pH, protein, urobilinogen, nitrates and leukocytes.

6.1.3 Characteristics Of Individual Urine Biomarkers

A comprehensive examination of frequency of all the urine parameters identified via urinalysis, outlining their normal ranges and the clinical implications of any deviations with regards to urogenital schistosomiasis was done. The detected urine parameters provide valuable insights into hydration status, renal and liver function, bladder and ureter fibrosis, and potential schistosomiasis infections. The findings are examined in relation to prior studies, emphasising similarities, differences, and the broader implications of the current data. Cutoffs were established in accordance with the manufacturer's guidelines. The biomarker results, organised by the frequency of positive detections from highest to lowest, demonstrate a clear hierarchy of prevalence among study participants refer to **Table 6.1**. The frequency distribution was specific gravity (99.3%) > bilirubin (39.9%) > blood (33.6%) > leukocytes (21.3%) > protein (15.0%) > pH (13.4%) > ketone (7.2%) > glucose (7.1%) > urobilinogen (1.3%) > nitrates (0.3%).

Table 6.1: Descriptive statistics of urine biomarkers measured by dipsticks results.

Urine Parameter	Normal values	Urine Parameter	Volume measured	Count (n=759)	%
Glucose	<30mg/dL	Negative		705	92.9
		+	250mg/dL	22	2.9

Schistosomiasis & STH Prevalence, Risk Factors, and Biomarkers in Nelson Mandela Bay School children

		++	500mg/dL	31	4.1
		+++	1000mg/L	1	0.1
Bilirubin	0.02mg/dL	Negative	-	456	60.1
		+	na	12	1.6
		++	na	216	28.5
		+++	na	75	9.9
Ketone	<2mg/dL	Negative		704	92.8
		+	15mg/dL	30	4.0
		++	40mg/dL	18	2.4
		+++	80mg/dL	7	0.9
USG		1.000g/ml	-	2	0.3
		1.005g/ml		18	2.4
		1.010g/ml		55	7.2
		1.015g/ml		56	7.4
		1.020g/ml		145	19.1
		1.025g/ml		99	13.0
		1.030g/ml		384	50.6
Blood	<3 Ery/ul	Negative		504	66.4
		trace	10 Ery/ul	96	16.9
		+	25 Ery/ul	37	4.9
		++	80 Ery/ul	81	10.7
		+++	200Ery/ul	41	5.4
pH		5.0	-	107	14.1
		6.0		461	60.7
		6.5		89	11.7
		7.0		31	4.1
		7.5		37	4.9

		8.0		13	1.7
		8.5		21	2.8
Protein	<15mg/dL	Negative		645	85.0
		+	30 mg/dL	74	9.7
		++	100 mg/dL	27	3.6
		+++	300 mg/dL	7	0.9
		+++	>1000 mg/dL	6	0.8
Urobilinogen	1.0mg/dL	Negative		749	98.7
		+	2mg/dL	1	0.1
		++	4 mg/dL	4	0.5
		+++	>8 mg/dL	5	0.7
Nitrates		Negative	-	757	99.7
		2	na	1	0.1
		4	na	1	0.1
Leukocytes	>10Leu/ul	Negative		597	78.7
		+	70 Leu/ul	45	5.9
		++	125 Leu/ul	45	5.9
		+++	500 Leu/ul	62	8.2

Key_ Leu/ul -leukocytes per microliter, USG-Urine specific gravity, pH- potential of hydrogen, Ery-erythrocytes, mg/dL- milligrams per decilitre, g/ml-grams per millilitre.

6.1.3.1 Glucose

Individuals with detectable glucose were 2.9% (22/759) had 250mg/dL, 4.1% (31/759) had 500mg/dL and only one (0.1%) participant had 1000 mg/dL. Glycosuria is a condition that occurs when the amount of glucose filtered by the kidneys is greater than the kidneys' ability to reabsorb it. This typically happens when the concentration of glucose in the blood is around 180 mg per dL. A study by (Rodrigues et al., 2022, p. 3), assessed the alterations in peripheral blood glucose levels caused by *S. mansoni* infection in *Holochilus sciureus* rodents, which serve as a natural host for the parasite. The plasma glucose concentration was determined by performing a colorimetric enzymatic assay on blood samples. The study revealed that *S. mansoni* infection in *H. sciureus* leads to a decrease in glucose levels in the peripheral blood of rodents, specifically in males. Female rodents that were infected showed consistent glucose levels and effectively regulated the hepatic granulomatous response. Schistosomiasis is characterized by significant tissue damage, hence the potential to enhance glucose tolerance and decrease its concentration in the peripheral blood (Wolde et al., 2019, p. 4). Adult worms metabolize host glucose for their survival, which may result in a

decrease in blood glucose levels (Rodrigues et al., 2022, p. 3). This decrease in glucose levels may be due to the competition for glucose between the host and the parasite (Duan et al., 2018, p. 40). Additionally, the hepatic granulomatous response in female individuals may play a role in regulating glucose levels and preventing significant decreases. Further research is needed to fully understand the mechanisms behind these findings and their implications for the treatment of schistosomiasis.

Infection with schistosomiasis triggers a strong type 2 and regulatory immune response, which effectively decreases chronic inflammation commonly linked to metabolic disorders such as obesity (McManus et al., 2018, p. 15). Gaining insight into the correlation between schistosome development and host glucose metabolism is essential for comprehending the mutual evolution of these two organisms and potentially developing novel therapeutic strategies (Echeverry et al., 2010). Understanding how schistosomiasis influences glucose levels could lead to the development of new treatments that target metabolic pathways affected by the infection. Diabetes mellitus, Cushing syndrome, Fanconi syndrome, glucose infusion, pregnancy are some of the medical conditions that can cause constant glycosuria (Milani & Jialal, 2023).

6.1.3.2 *Bilirubin*

There were quite a number of participants with detectable bilirubin in urine, 1.6% (12/759) had +1, followed by 28.5% (216/759) with 2+ and 9.9% (75/759) had 3+ graded bilirubin. Usually there is normally no bilirubin in urine. Bilirubin is a yellow pigment, present in bile, a liver-produced fluid. Elevated levels of bilirubin, known as bilirubinuria, indicate the presence of liver associated diseases, including cirrhosis, hepatitis, gallstones, and biliary tract disease. Moreover, it functions as an early indication of the beginning of jaundice. Even the smallest amounts of bilirubin are deemed abnormal and warrant further scientific investigation. In a study by (Egoro, 2017) patients with *S. mansoni* infection, bilirubin levels were found to be statistically significant ($p \leq 0.05$), indicating changes in liver function attributable to the infection. The study showed that infected patients had higher levels of total bilirubin than the control group. This suggests that the liver is involved in the long-term or chronic *S. mansoni* infection.

The association between liver metabolism and liver fibrosis injury has garnered interest with the identification of glucuronic acid metabolism as a separate metabolic pathway associated with schistosomiasis infection (de Morais et al., 2010; Egoro et al., 2017). The hepatic bilirubin UDP-glucuronosyltransferase 1A1 (UGT1A1) enzyme, which is the sole enzyme responsible for catalysing bilirubin metabolism in the body, is linked to the advancement of several disorders including *S. mansoni* infections (Xue et al., 2024, p. 9). Schistosome egg granulomas formed in the liver are responsible for the development of liver fibrosis and occur as a result of an inflammatory reaction caused by persistent stimulation from the egg antigen (Leite et al., 2015, p. 7; Xue et al., 2024, p. 11). The formation of schistosome egg granulomas and liver fibrosis seems to occur at the same time as the suppression of hepatic UGT1A1 expression. It has been proposed that the activation of signalling pathways linked with inflammation can hinder the production of UGT1A1 thereby inhibiting bilirubin metabolism (Xue et al., 2024).

Liver fibrosis in *S. mansoni* infections is caused by a vicious cycle involving two routes: the hepatic inflammatory response and metabolic abnormalities. These pathways mutually reinforce each other, driving the progression of liver fibrosis. Enhancing the expression of hepatic UGT1A1 to facilitate the metabolism of bilirubin could play a crucial role in reducing liver fibrosis caused by *S. mansoni* (Xue et al., 2024, p. 11). Infection with *S. mansoni* causes damage to the liver membrane, which is likely responsible for the notable increase in liver bilirubin observed and excreted in the plasma and urine respectively of infected individuals (Egoro, 2017).

6.1.3.3 Ketone

Ketones are acidic chemicals produced in the liver. Although ketones can be detected in everyone, they are mostly found in individuals who practice fasting or adhere to low-carb/high-fat diets. Increased ketone levels signify insufficient insulin levels and are observed in circumstances such as diabetes, malnutrition, vomiting, gastrointestinal disorders, pregnancy, and feverish conditions. Ketones are produced via the process of fat oxidation in the body, serving as a source of energy or fuel. They are also produced when there is a decrease in body weight or when there is not enough insulin to enable the body to use sugar for energy. Urinary ketones are generally not present. The results of this study show that 4% (30/759) had 4mg/dL, 2.4% (18/759) had 40mg/dL and 0.9% (7/759) had 80mg/dL ketones in urine. No association between urogenital schistosomiasis and ketones was identified, consistent with the findings reported by (Midzi et al., 2024, p. 4). The examination of ketone levels in the urine of participants infected with *S. haematobium* indicated no statistically significant correlation with the infection ($p > 0.05$) (Midzi et al., 2024, p. 3). Individuals with infections exhibited a lower frequency of ketones in comparison to markers such as haematuria and proteinuria, which are more directly linked to the morbidity associated with schistosomiasis. The lack of association between ketone bodies and urogenital schistosomiasis, the presence of these metabolites in infected individuals could reveal the pathophysiological effects of protein-energy malnutrition (Mduluzza-Jokonya et al., 2020, p. 20). Ketones are produced when there is an inadequate supply of sugar or glucose necessary to fulfil the body's requirements, particularly during fasting and dieting periods.

6.1.3.4 Specific Gravity

Specific gravity, widely used to assess hydration status, is a non-invasive biomarker for schistosomiasis infection (Armstrong, 2007, p. 583; Rosinger et al., 2018, p. 2). The Multistix 10SG dipstick test measures a range of 1.005-1.030 g/ml, with variations reflecting urine concentration influenced by fluid intake (Rosinger et al., 2018, p. 6). Specific gravity between 1.005-1.010 g/ml suggests dilution due to excessive fluid intake, while 1.010-1.020 g/ml indicates normal hydration, and 1.020-1.030 g/ml points to concentrated urine, often due to dehydration or kidney stress. In our study, most participants had specific gravity values within the normal range (1.010-1.030 g/ml), indicating adequate urine concentration based on manufacturer guidelines. Specific gravity below 1.020 g/ml was associated with healthy hydration for most participants.

However, studies by Armstrong, 2007, p. 583) and (Rosinger et al., 2018, p. 6) identified a specific gravity threshold of >1.020 g/ml as indicative of hypohydration, correlating with more concentrated urine. Based on this threshold, a significant proportion of our participants were dehydrated, with 13% (99/759) showing specific gravity of 1.025 g/ml and 50.6% (384/759) having values of 1.030 g/ml, as shown in **Figure 6.2**.

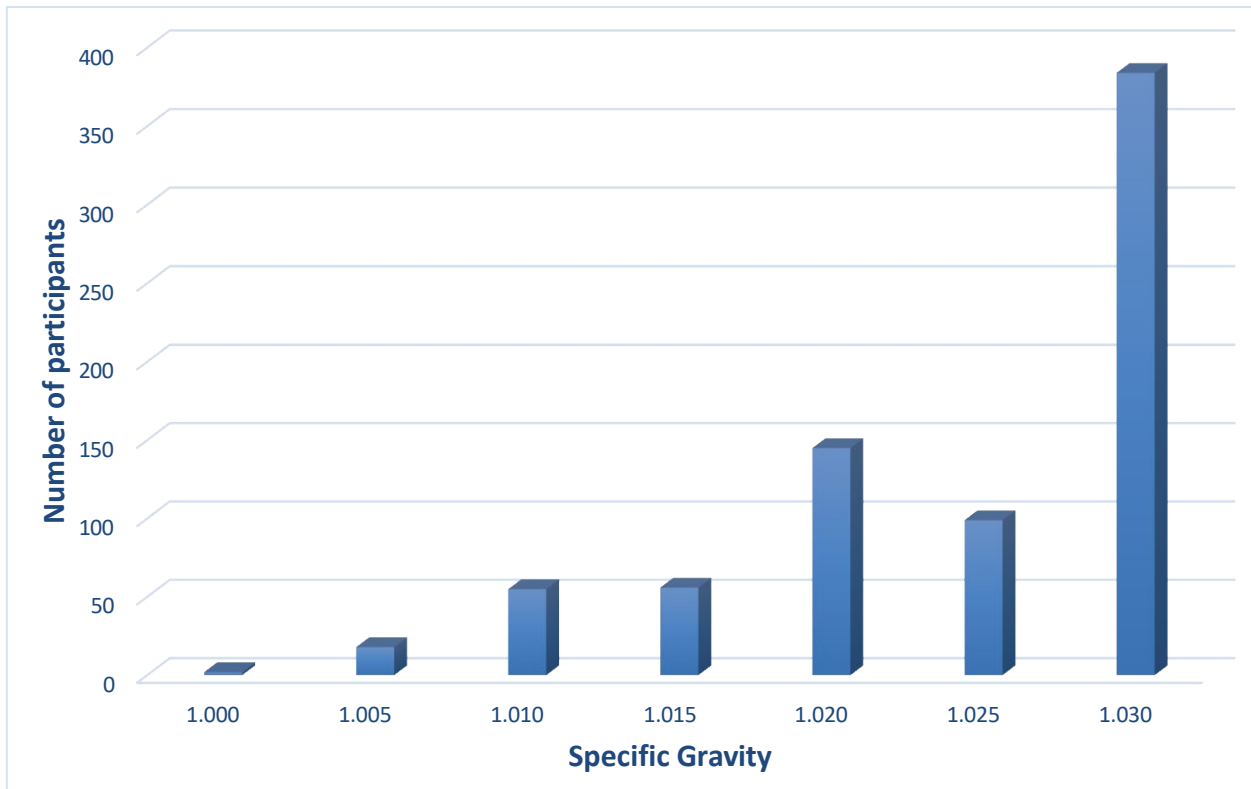


Figure 6.2: Specific gravity levels in study participants.

Schistosomiasis has the potential to influence specific gravity measurements through the release of eggs into urine or by imposing extra stress on the kidneys. The relationship between heightened egg production and specific gravity remains ambiguous. Infections caused by *S. haematobium* may interfere with hydration, as the kidneys must juggle waste filtration while addressing the infection, ultimately reducing the body's capacity for effective hydration regulation (Colley et al., 2014; De Lorenzo et al., 1997).

(Rosinger et al., 2018) proposed that women infected with *S. haematobium* would have significantly higher urinary specific gravity compared to both uninfected women and those infected with *S. mansoni*. While *S. mansoni*-infected women also showed elevated specific gravity, their levels were lower than those with *S. haematobium*. The study confirmed that *S. haematobium* was associated with notably higher specific gravity levels, often exceeding 1.020 g/ml. This suggests that specific gravity measurement could be a valuable tool for detecting and monitoring schistosome infections, particularly in women (Nmorsi et al., 2005; Rosinger et al., 2018).

6.1.3.5 Blood (Haematuria)

Table 6.1 presents the results of blood presence in urine samples also called haematuria, illustrating the distribution of erythrocyte (red blood cell) concentrations in urine samples. The majority of the samples, accounting for 66.4% (504/759), tested negative for haematuria, indicating no detectable red blood cells. A smaller proportion of the samples, 16.9%(96/759), showed trace amounts of erythrocytes, about 10 Ery/ μ l. Slightly fewer samples, 4.9%(37/759), exhibited a low positive result with 25 Ery/ μ l. Moderate and high levels of haematuria were less common, with 10.7%(81/759) of the samples showing 80 Ery/ μ l and 5.4%(41/759) displaying a high concentration of 200 Ery/ μ l. Out of the 255 (%) participants with detectable haematuria, 143 were males and 112 were females. These results suggest that while the majority of the population tested does not exhibit haematuria, a significant minority does, ranging from trace to high levels of red blood cells in their urine. Some of the samples had visual haematuria.

Haematuria serves as the most significant predictor for *S. haematobium* infection (Kildemoes et al., 2015). In a study by Elom et al., (2017) children aged 12 to 15 years had a significant prevalence of *S. haematobium* at 35%, and 92.5% of the infected children had haematuria. The study by Elom et al., (2017) also noted that the degree or severity of haematuria was influenced by the intensity of the infection, specifically the number of eggs excreted per 10ml of urine. The degree or severity of haematuria in 36 infected children excreting 51-60 eggs per 10ml of urine was higher, whereas children excreting 1-10 eggs per 10ml of urine exhibited less severity of haematuria (Elom et al., 2017; El-Ghareeb et al., 2015). In areas lacking access to urine microscopy, the urine dipstick test serves as a feasible screening tool for *S. haematobium* (Craik et al., 2023). Haematuria, a clinical sign of *S. haematobium* infection, indicates urinary tract damage and morbidity, though its low specificity can lead to misleading results if used alone (Vinkles Melchers et al., 2014). Dipstick detection of haematuria in the study area suggests potential schistosomiasis infection and/or other urinary tract infections, warranting further investigation. In Tanzania, haematuria prevalence was 0.9%, aligning closely with the 0.83% prevalence found through urine filtration, showing both methods' similar effectiveness (Mazigo et al., 2021).

6.1.3.6 pH

The results show a normal variation in the urine pH among the participants, **Figure 6.3**. A number of participants; 14.1% (107/759) individuals had slightly acidic pH= 5 which is typical for a healthy population. Common/normal range of pH which is 5.5-6.5 had more individuals (550/759), whilst 13.4% (102/759) had alkaline pH range 7.0 to 8.5. The average urine pH in most individuals is 6.0 but it usually fluctuates due to diet, medicine and other health conditions (Milani & Jialal, 2023). Causes of low acidity levels in the body include cranberry juice, dehydration, diabetes mellitus, diabetic ketoacidosis, diarrhoea, emphysema, high protein diet, hunger, potassium depletion, drugs such as methionine and mandelic acid, and a potential inclination towards the development of kidney or bladder stones (Patel, 2006).

Typically, urine has a slightly acidic pH due to metabolic processes. If the urinary pH is below 5.5 and there is systemic acidaemia (serum pH below 7.35), it indicates renal failure caused by the kidneys' inability to eliminate hydrogen ions (Duarte et al., 2014). Contrarily, the primary reason for alkaline urine is a stagnant urine sample caused by bacterial development and the decomposition of urea, which releases ammonia (Utsch & Klaus, 2014). Alkaline urine can be caused by various factors including stale or old urine specimens, hyperventilation, the presence of urease-producing bacteria, renal tubular acidosis, a vegetarian diet, and vomiting. Measuring urine pH is beneficial for diagnosing and treating urinary tract infections and the production of crystals or calculi (Echeverry et al., 2010).

There has not been a direct indication that urine pH is directly associated with schistosomiasis infections. However, urinary tract infections due to *S. haematobium* infections can alter the pH of schistosomiasis (Colley et al., 2014). Schistosomiasis infection can cause renal tubular dysfunction, affecting urinary acidification and concentration. A study done by (Duarte et al., 2014), showed a defect in the urinary concentrating ability defect was observed in 85% of the study population which was individuals with hepatosplenic schistosomiasis. They were investigating this issue in schistosomiasis patients under water deprivation conditions. The study found higher mean values of urine pH (pH = 5.5) in hepatosplenic schistosomiasis patients, with 45% showing distal tubular acidosis. This abnormality is not usually reported in schistosomiasis, unlike other parasitic diseases like cutaneous and visceral leishmaniasis (Asfaw et al., 2023). Therefore, changes in the urine pH are not a diagnostic marker or indication to specific morbidities even other than schistosomiasis (Lima Verde et al., 2009). Chronic bladder inflammation and bacterial infections in the genitourinary tract can alter the urine pH.

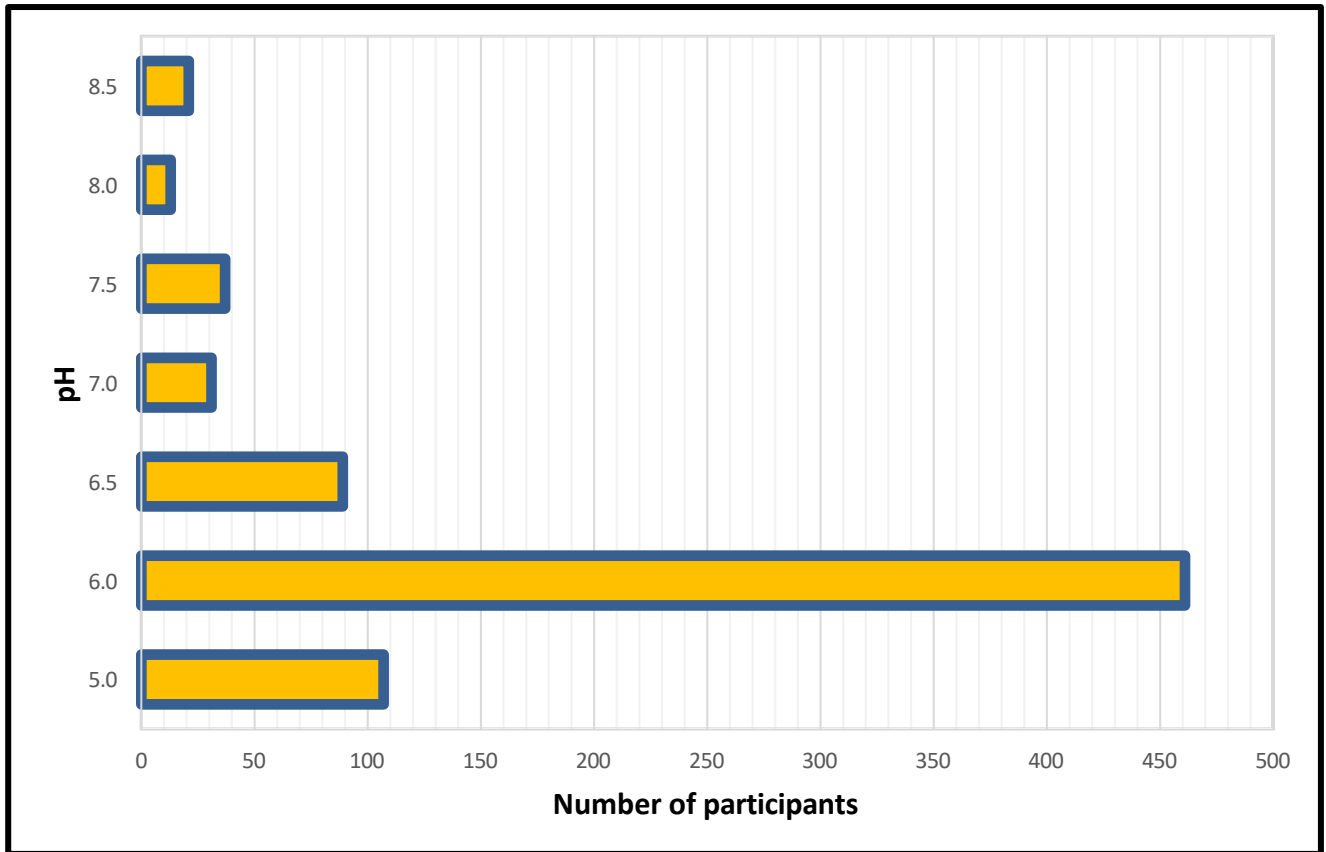


Figure 6.3: The variability in pH levels in study participants.

6.1.3.7 Protein

According to the manufacturer the normal protein in urine should be <15mg/dL. In our study we found 85% (645/759) had no detectable protein in urine samples and, for the participants who were infected 9.7% (74/759) had 30mg/dL, whilst 3.6% (27/759) had 100mg/dL followed by 0.9% (7/759) with 300mg/dL, lastly 0.8% (6/759) had >1000mg/dL as shown in Table 5.1. Proteinuria is the medical term for the disorder characterised by elevated quantities of protein in the urine. Persistent rise of protein levels in urine might be a result of urinary or renal system issues and require medical intervention (Milani & Jialal, 2023).

Typically, the glomerular capillary wall allows molecules with a molecular weight less than 20,000 Daltons to pass through. The majority of the minute portion of filtered proteins are absorbed again and broken down by the cells of the proximal tubule (Milani & Jialal, 2023). Consequently, proteins are typically seen in urine in small quantities. Around 33% of the protein found in urine is albumin, another 33% is Tamm-Horsfall glycoprotein, which is secreted by tubular cells, and the rest is made up of plasma proteins such globulins (Patel, 2006).

Proteinuria can be categorised as either temporary or chronic (K. Opara et al., 2021). Temporary proteinuria is usually a harmless condition, such as orthostatic proteinuria caused by extended standing (Milani & Jialal, 2023). Persistent proteinuria can be classified into three specific patterns: glomerular, tubular, and overflow (Kayange et al., 2020). The first situation arises when proteins that are typically impermeable, like as albumin and transferrin, traverse a compromised glomerular capillary wall. Therefore, this pattern is observed in cases of low blood albumin, subsequent generalised edoema, and increased serum lipids, as observed in nephrotic syndrome (Kayange et al., 2020; Milani & Jialal, 2023).

Various research examining the correlation between proteinuria and *S. haematobium* infection have repeatedly shown a robust connection, suggesting that the presence and/or severity of *S. haematobium* infection is directly linked to proteinuria (Elom et al., 2017; Oyeyemi & Odaibo, 2017; Tetteh-Quarcoo et al., 2022; Yameny, 2017). For example, a study in Kenya showed that after administering treatment to students for schistosomiasis, there was a reduction in the occurrence of proteinuria in the entire study population the following year (Kayange et al., 2020). To be more precise, it decreased from 56.0% before the intervention to 26.0% after the intervention. Similarly, the prevalence of schistosomiasis decreased from 69.4% before therapy to 19.0% after treatment.

6.1.3.8 Nitrites

In our results 99.7% (757/759) were negative whilst 0.3% (2/759) were positive for nitrates. Hence, the detection of nitrite in the urine signifies the existence of bacteria (Utsch & Klaus, 2014,). The primary harmful characteristic of nitrite is its capacity to be converting specific amino groups into mutagenic and carcinogenic N-nitroso compounds. Epidemiological studies have identified a link which involves the consumption of nitrate in individuals with stomach cancer. The underlying mechanism of the reaction is the infection caused by *S. japonicum* (Patel, 2006,). Secondary bacterial colonisation of the bladder causes the conversion of nitrate in urine to nitrite by these microbes (Milani & Jialal, 2023). The nitrite formed reacts with amines to produce hazardous and carcinogenic N-nitroso compounds (Patel, 2006,). Nitric oxide (NO), a crucial mediator molecule, can significantly impact the physiology of *S. japonicum* worms by causing damage to the mitochondria and inhibiting mitochondrial respiration, which is essential for parasite metabolism. This is crucial for the production of eggs and the creation of biological mechanisms, which help protect worms against host immune system attacks (Shen et al., 2017).

6.1.3.9 Urobilinogen

Urobilinogen is produced by the process of reducing bilirubin. For urobilinogen 98.7% (749/759) had negative <1.0mg/dL, whilst 0.1% (1/759) had 2mg/dL followed by 0.5%(4/759) had 4mg/dL, lastly 0.7%(5/759) had >8mg/dL. Bilirubin is a hepatocellular pigment that aids in the decomposition of erythrocytes (Echeverry et al., 2010, p. 11). Typical urine contains a little amount of urobilinogen, with levels reaching up to 1.0 mg/dL. The absence or minimal presence of urobilinogen in urine may indicate impaired liver function. Elevated levels of urobilinogen (> 2 mg/dL) in urine can be indicative of liver disorders such as hepatitis or cirrhosis (Milani & Jialal, 2023). A study done by Midzi et al., (2024) in Zimbabwe, found that there were negative associations between schistosomiasis infections and urobilinogen (p-value > 0.05). Therefore, in terms of schistosomiasis diagnostics urobilinogen proved to be a poor biomarker for detecting schistosomiasis infection, indicating limited effectiveness in reliably identifying cases (Midzi et al., 2024; Panic et al., 2019).

6.1.3.10 Leukocytes

Leukocytes, often known as white blood cells, are the immune system's cells responsible for defending the body against infectious diseases and foreign intruders. Typically, a small number of white blood cells can be seen in urine and usually produce bad outcomes. A positive result will occur when there is a substantial rise in the number of white blood cells (WBCs) in the urine during this screening test. If the number of leukocytes in urine is greater than 10 Leu/ μ l, it could be a sign of infections in the urinary tract or kidneys. In our study leukocytes had >10Leu/ μ l 78.7% (597/759) negative participants whilst 5.9% (45/759) had 70Leu/ μ l and 125Leu/ μ l each, lastly 8.2% (62/759) had 500Leu/ μ l. Presence of leukocytes in urine indicates inflammation in the urinary system. Empirical research demonstrates that the presence of leukocytes in urine escalates during the body's immune response to an infection.

Leukocyturia is commonly recognised as an indirect indicator for urine schistosomiasis in screening protocols (Kildemoes et al., 2015; Yameny, 2017). In addition to haematuria and proteinuria, leukocyturia serves as a

morbidity marker for urinary schistosomiasis in *S. haematobium* infections (Kildemoes et al., 2015). The elevated level of total leukocytes count seen is associated with the host immune response to the presence of adult schistosomes in the bloodstream. It is widely acknowledged that eosinophils (white blood cells) play a significant role in defending against helminthic infections (Dejon-Agobé et al., 2021).

6.1.4 Assessment of correlations between urine biomarkers

The associations among different urine biomarkers were explored (refer to **Table 6.2**). Gaining an understanding of these interactions can offer useful insights into the fundamental human physiological processes and probable mechanisms of disease morbidity including schistosomiasis. The correlation matrix of urine biomarkers revealed weak associations among most biomarkers, indicating limited statistical significance. Glucose demonstrated weak positive correlations with; bilirubin ($r = 0.03$), ketone ($r = 0.03$), specific gravity ($r = 0.06$), haematuria ($r = 0.07$), protein ($r = 0.06$), and leukocytes ($r = 0.06$), while exhibiting a weak negative correlation with; pH ($r = -0.06$) and nitrites ($r = -0.01$). Similarly, bilirubin showed moderate positive correlations with; ketone ($r = 0.17$), urobilinogen ($r = 0.13$), and haematuria ($r = 0.09$), with weaker correlations to pH ($r = -0.09$) and specific gravity ($r = 0.09$). In line with these findings, Midzi et al., (2024) reported a positive correlation between bilirubin and urogenital schistosomiasis ($r = 0.080$, $p = 0.476$), although this correlation was statistically insignificant. This finding indicates that the role of bilirubin in schistosomiasis may require further examination. pH exhibited a weak positive correlation with urobilinogen ($r = 0.12$), but no significant relationships were found with other biomarkers.

Ketone displayed weak positive correlations with urobilinogen ($r = 0.14$) and protein ($r = 0.10$). Specific gravity had a negative correlation with pH ($r = -0.37$) but did not exhibit any notable associations with other parameters, including haematuria ($r = 0.06$) and protein ($r = 0.04$). Notably, haematuria showed its strongest association with leukocytes ($r = 0.23$), though other relationships, such as with protein ($r = 0.01$), were negligible. This correlation aligns with findings from Utsch & Klaus, (2014) who noted that haematuria accompanied by proteinuria and leukocyturia indicates renal disease and necessitates additional diagnostic assessment. Correlations of these three biomarkers has been successfully associated with schistosomiasis infections. In line with these findings, Tetteh-Quarcoo et al., (2022) in Ghana assessed the association between haematuria, proteinuria and leukocyturia with schistosomiasis infections. They followed the trend of presence of these biomarkers in individuals before treatment with praziquantel and after treatment. The results indicated a clear relationship stated: as the occurrence and severity of *S. haematobium* infections declined, the associated morbidity markers also showed a reduction. Sixth week post-treatment, most participants with no and/or low intensity infections by microscopy, exhibited undetectable levels of proteinuria and haematuria, with a single individual showing leukocyturia (Tetteh-Quarcoo et al., 2022). This trend illustrates the relationship between usefulness of haematuria, proteinuria and leukocyturia, as biomarkers of schistosomiasis infection also, indicating the effectiveness of using urinalysis as a tool for monitoring *S. haematobium* infections during mass drug administration.

Haematuria showed moderate effectiveness for identifying heavy infections ($\kappa = 0.434$), followed by proteinuria ($\kappa = 0.317$). There was no agreement for urobilinogen and ketones. In line with the correlations found in the current study, Midzi et al., (2024,) found that in light *S. haematobium* infections, haematuria ($\kappa = 0.262$) and proteinuria ($\kappa = 0.154$) indicated only slight agreement, with no agreement with urobilinogen (Midzi et al., 2024).

Finally, protein, urobilinogen, nitrites, and leukocytes exhibited weak or negligible associations among themselves, with the majority of correlation coefficients approximating zero. The majority of these biomarkers exhibited negligible linear relationships, indicating a lack of significant correlation among them within the dataset. The findings highlight the difficulty in establishing definitive links between prevalent urine biomarkers and particular parasitic infections as indicated by this statistical analysis.

Table 6.2: Pearson product-moment correlations of urine biomarkers dipsticks results

	Bilirubin	Ketone	Specific Gravity	Blood	pH	Protein	Urobilinogen	Nitrites	Leukocytes
Glucose	0.03	0.03	0.06	0.07	-0.06	0.06	0.01	-0.01	0.06
Bilirubin	-	0.17	0.09	-0.03	-0.09	0.13	0.10	-0.01	0.07
Ketone		-	0.01	0.03	-0.01	0.10	0.14	-0.01	0.07
Specific Gravity			-	0.06	-0.03	0.04	-0.05	0.01	-0.05
Blood				-	-0.10	0.01	0.00	-0.03	0.23
pH					-	0.05	0.12	0.01	-0.01
Protein						-	0.09	0.01	0.00
Urobilinogen							-	-0.01	0.00
Nitrites								-	-0.02

6.1.5 Urinary Biomarkers in the Diagnosis and Monitoring of Urinary Schistosomiasis

Morbidity markers demonstrated limited comparability to other reference tests, including Kato-Katz and urine filtration. The results from these methods were minimal or absent, with only one case identified out of 759 for urine filtration (Chapter 4) and no cases detected by Kato-Katz (Chapter 5). The restricted data from conventional reference methods obstructed the evaluation of the diagnostic efficacy of morbidity markers, leading to statistically insignificant results. Urine and stool examinations for schistosome ova are commonly employed for diagnosis, but they are less effective in detecting low-intensity infections, where egg counts are low (Corstjens et al., 2020).

The comparison of morbidity markers with PCR results was notably limited because of the selective characteristics inherent in PCR testing. This study involved PCR analysis exclusively on a subset of samples that yielded positive results for POC-CCA or haematuria. Consequently, a significant portion of the study population, those who tested negative by these methods, was excluded from additional molecular analysis. The selective approach constrained the thorough evaluation of schistosome DNA across the entire cohort, thereby diminishing the ability to make direct comparisons between morbidity markers, including haematuria, proteinuria, and leukocyturia, and confirmed schistosomiasis infections.

The purposive sampling approach for haematuria introduced additional complexities in making comparisons. Individuals with 2+ and 3+ haematuria were selected for PCR testing, while those presenting with 1+ haematuria were excluded from further molecular testing. This omission may have resulted in the exclusion of cases involving mild or early-stage infections, consequently distorting the comparison between haematuria and PCR-confirmed schistosomiasis. Narrowing the selection of haematuria-positive participants for PCR reduced the opportunity to investigate the relationship between different intensities of haematuria and schistosome infections, thereby weakening the overall comparison of diagnostic methods. The methodological limitations significantly impaired the capacity to effectively evaluate the diagnostic efficacy of morbidity markers in relation to PCR-confirmed infections.

Despite the limitations in comparing reference gold standard methods with morbidity markers in the current study, previous research has established a link between these markers and schistosomiasis infections. Among participants, various biomarkers associated with *S. haematobium* infection were present at differing levels: haematuria was the most prevalent at 33.6%, followed by leukocyturia at 21.3% and proteinuria at 15%. These biomarkers have been extensively studied for their association with schistosomiasis. Previous studies have established that haematuria and proteinuria are highly sensitive and specific biomarkers for diagnosing *S. haematobium* infections (Kayange et al., 2020). Although leukocyturia is less definitive in diagnosing these infections, it remains an important indicator of underlying pathology (Elom et al., 2017). Notably, Vaillant et al., (2024) highlighted that haematuria and proteinuria demonstrate superior diagnostic performance compared to leukocyturia. While Ochodo et al., (2015) also supported this conclusion, their findings did not reach statistical significance. These results underscore the critical role of haematuria and proteinuria in the detection and monitoring of urogenital schistosomiasis, reinforcing their importance as key biomarkers in clinical practice.

A study by (Elom et al., 2017) established a significant correlation between the excretion of *S. haematobium* ova and the occurrence of proteinuria, haematuria, and leukocyturia, with correlation coefficients recorded at 0.85%, 0.86%, and 0.86%, respectively. All children who excreted more than 40 ova in 10 ml of urine demonstrated the presence of proteinuria, haematuria, and leukocyturia. The results are consistent with the research conducted by Doehring et al., (1985), which indicated comparable strong correlations, yielding coefficients of 0.86 for proteinuria, 0.84 for haematuria, and 0.83 for leukocyturia. Another study done in Zimbabwe by Midzi et al., (2024) showed a strong positive correlation between haematuria and *S. haematobium* infection intensity ($r = 0.592$, $p = 0.0001$) and a moderate correlation with proteinuria ($r = 0.448$, $p = 0.0001$) using Pearson bivariate correlation analysis. Although bilirubin was positively correlated with urogenital schistosomiasis ($r = 0.080$), it was not statistically significant ($p = 0.476$). Additionally, negative correlations for urobilinogen and ketones were also not significant ($p > 0.05$). These results highlight haematuria, proteinuria and leukocyturia as a critical biomarker for detecting heavy *S. haematobium* infections, while emphasizing the limitations of other biomarkers in clinical assessments. However, similar comparisons could not be made in the current study due to limited sensitivity of the conventional methods of schistosomiasis diagnosis (urine filtration and Kato Katz) in the study area.

This study identified additional urinary biomarkers alongside haematuria, proteinuria, and leukocyturia. The prevalence of these biomarkers includes bilirubin at 39.9%, ketones at 7.2%, and glucose at 7.1%. Urobilinogen and nitrates were observed at lower frequencies, with values of 1.3% and 0.3%, respectively. Current research is investigating potential associations between these biomarkers and schistosomiasis infections, although a conclusive link has not yet been established. Panic et al., (2019) reported low frequencies of urobilinogen and nitrates, which is consistent with our findings.

In conclusion, while research has demonstrated a significant correlation between schistosomiasis and haematuria, proteinuria, and leukocyturia, the overlap of biomarkers in infected and uninfected individuals illustrates the need for comprehensive diagnostic criteria. This is particularly important during schistosomiasis surveillance to avoid potential misinterpretation of prevalence based solely on these biomarkers.

CHAPTER 7: CONCLUSIONS & RECOMMENDATIONS

7.1 CONCLUSIONS

In conclusion, this study reveals a concerning but latent prevalence of schistosomiasis among school-aged children in NMB, highlighting a complex interplay of risk factors that contribute to transmission in a low transmission area. Schistosomiasis outbreaks, such as the recent case report in South Africa, could be averted in various provinces through the implementation of effective diagnostics and robust disease surveillance systems (Department of Health SA, 2024). Timely case detection using advanced molecular diagnostic techniques in low-endemic areas, along with consistent surveillance in high-risk areas, enables prompt intervention and treatment, thus reducing the chances of individuals migrating and spreading infections to non-endemic regions. The findings highlight the critical need for targeted public health interventions that address environmental conditions, improve sanitation, and enhance health education tailored to the unique experiences and understanding of different age groups. The identification of inadequate toilet facilities and high-risk behaviours among school-going children emphasizes the urgency of improving hygiene practices within the community. Furthermore, the high prevalence of haematuria suggests that undiagnosed or asymptomatic infections may be contributing to ongoing transmission, necessitating the need for control program and preventive chemotherapy using praziquantel.

7.2 RECOMMENDATIONS

This study outlines multiple recommendations designed to improve public health strategies for decreasing schistosomiasis transmission among school-aged children in NMB, considering the findings and limitations identified. Based on the findings, the subsequent recommendations for the Environmental Health Practitioners (EHPs), Department of Education, NMB municipality, and additional Environmental Health Research are proposed:

NMB municipality and Department of Education

- The Department of Education should incorporate health education that covers transmission cycles, symptoms, and treatment of neglected tropical diseases, such as schistosomiasis and soil-transmitted helminths, into the school curriculum. This process may facilitate the transfer of knowledge from children to their parents, and subsequently, to the wider community.
- The Department of Education should develop and implement tailored educational strategies that address the specific knowledge gaps and behaviours identified among the various age groups and genders within the study area. Interventions should consider cultural backgrounds, with a specific focus on individuals engaged in water-related activities, while also promoting awareness among children from African traditional religious communities.

Department of Health

- Implement frameworks for ongoing assessment and analysis of schistosomiasis prevalence and the efficacy of interventions. This will facilitate the modification of strategies as necessary and guarantee that resources are allocated efficiently to address the disease.
- Allocate resources to local clinics and medical practitioners, and encourage the implementation of advanced molecular diagnostic techniques, especially PCR in the study area or other low transmission

areas, to guarantee precise identification of schistosomiasis. This approach facilitates the identification of asymptomatic and low-level infections that might remain unnoticed otherwise.

- The recent outbreak of schistosomiasis in September 2024 Tzaneen, Limpopo Province, underscores the pressing need for MDA to control and prevent the spread of this neglected tropical disease in high and low endemic areas. Schistosomiasis continues to pose a significant public health risk, particularly in areas with limited access to clean water and adequate sanitation. MDA, using generic praziquantel, is a cost-effective and proven strategy to reduce the prevalence of schistosomiasis and interrupt its transmission cycle. Currently, the lack of accessibility to affordable praziquantel in resource restrained areas, hampers efforts to control and prevent schistosomiasis. Therefore, implementing a policy change that facilitates the use of generic praziquantel would enable widespread preventive treatment, significantly reducing the risk of transmission and the potential for outbreaks.

Environmental Health Practitioners (EHPs)

- EHPs should establish community-led programs aimed at promoting effective hygiene practices, including proper handwashing techniques and the safe disposal of human waste. Educational materials should highlight the significance of utilising toilets and refraining from outdoor defecation to reduce transmission risks.
- In NMB, EHPs may engage in regular monitoring of freshwater bodies and gather samples to assess the presence of intermediate host snails associated with schistosomiasis, as well as other snails that hold medicinal and veterinary importance.
- To effectively deliver health education, EHPs should consider incorporating alternative media sources, such as mobile applications. Additionally, programs that promote interactive platforms like educational games, videos, and animated content should be done and disseminated in schools to help capture children's interest and enhance learning.
- EHPs should ensure that the health education material especially for diseases prevalent in the study area are available in multiple languages and culturally relevant to improve accessibility and understanding. Regular feedback from children and caregivers can also guide continuous improvement in educational approaches.

Nelson Mandela Municipality

- The absence of sanitation facilities in particular locations in the study area led individuals to engage in open defecation near water bodies and vegetation. To mitigate open defecation and decrease the risk of disease transmission by infected individuals, the NMB municipality should evaluate the installation of adequate sanitation facilities in these communities.
- Work in conjunction with local authorities to upgrade sanitation infrastructure, especially in areas where 20% of respondents indicated insufficient access to toilet facilities. This involves the design and implementation of public lavatory facilities in conjunction with enhancements to waste management systems aimed at minimising the risk of environmental contamination.

The implementation of these recommendations has the potential to substantially mitigate the impacts of schistosomiasis and enhance the overall health and well-being of school-aged children even older age groups in NMB.

Future studies

- **Determining the Prevalence and Risk Factors of Schistosomiasis among School-Aged Children in Nelson Mandela Bay: A Longitudinal Study:** This study would follow a cohort of school-aged children over several years to track changes in the prevalence of schistosomiasis, accounting for seasonal variations, environmental factors, and long-term exposure. By collecting multiple urine samples at various time points, the study would enhance the sensitivity of conventional diagnostic techniques, improving the chances of detecting schistosomiasis eggs, particularly in low transmission areas, where egg presence can vary. A longitudinal study improves the accuracy of infection detection by capturing eggs that may be missed with a single sample.
- **Evaluating Schistosomiasis and Soil-Transmitted Helminths Prevalence in Nelson Mandela Bay: A Molecular Diagnostics Approach:** A study that uses molecular diagnostics, PCR reactions, which are highly sensitive and specific in determining the prevalence of schistosomiasis and STHs in NMB and expanding the study population to fisherman and farmers who reside in the study area.
- **Genetic Diversity of Schistosoma Strains:** Sequence and analyse local Schistosoma strains to understand genetic diversity and how it may relate to transmission dynamics, treatment resistance, and infection outcomes.
- **Cytokine Profiles and Disease Progression:** Study the cytokine response profiles in children with varying intensities of Schistosoma infection to understand their role in disease progression and morbidity in low-transmission settings.
- **Impact of Migration on the Introduction and Spread of Schistosomiasis:** Perform a study investigating the role of migration in the introduction and spread of schistosomiasis in the low-endemic or previously non-endemic areas, focusing on both human movement and potential changes in water source contamination.
- **Water Contact Behaviour and Its Influence on Schistosomiasis Infection Rates:** Analyse how the frequency and type of water contact activities (e.g., swimming, fishing, laundry) contribute to schistosomiasis transmission, and propose targeted water sanitation and hygiene (WASH) interventions.
- **The Influence of Seasonal Variations on WASH Conditions and Schistosomiasis Transmission:** Explore how seasonal changes affect water availability, sanitation conditions, and the risk of schistosomiasis transmission, proposing adaptive WASH measures.

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APPENDIX A: QUESTIONNAIRE ON SCHISTOSOMIASIS RISK FACTORS AND KAP IN ENGLISH AND AFRIKAANS

STUDY TITLE: PREVALENCE, ASSOCIATED RISK FACTORS AND DIAGNOSTIC BIOMARKERS OF SCHISTOSOMIASIS AMONG SCHOOL GOING CHILDREN IN NELSON MANDELA BAY MUNICIPALITY

STUDIE TITLE: VOORKOMS, GEASSOSIEERDE RISIKO FAKTORE EN DIAGNOSTIESE BIOMERKERS VAN SKISTOSOMIASIE ONDER SKOOLGAANDE KINDERS IN NELSON MANDELABAAI MUNISIPALITEIT

Study Site/ Studie webwerf:

District/ Distrik _____

Community/Village/ Gemeenskap/Dorp _____

School/ Skool _____

Type of Visit: Enrolment/ Tipe besoek: Inskrywing

Date of Visit/ Besoekdatum: □□ □□□ □□□□

Interviewer Initials/ Onderhoudvoerder Voorletters: _____

Unique Identifier/ Unieke Identifiseerder: _____

Clinical observations/ Kliniese waarnemings

Temperature/ Temperatuur _____

Section A

Sociodemographic, environmental, clinical variables and risk factors associated with the transmission of schistosomiasis.

Afdeling A

Sosiodemografiese, omgewings-, kliniese veranderlikes en risikofaktore wat verband hou met die oordrag van skistosomiase.

Socio-demographic information for participant Sosio-demografiese inligting vir deelnemer	
Age/ ouderdom	-----
Sex/ seks	Male/ manlik Female/ vroulik
Grade/ graad	-----
Name of community Naam van gemeenskap	-----
Parental Occupation Ouerberoep	-----
How many are you in your family? Hoeveel is jy in jou gesin?	-----
Religious affiliation Godsdienstige affiliasie	1. Main line church/ Hooflyn kerk 2. Pentecostal/ Pinkster 3. Apostolic/ Apostoliese 4. African Tradition/ Afrikaanse tradisie 5. Muslim/ Moslem 6. None/ Geen 7. Other(specify)/ Ander (spesifiseer)
Migration Background Migrasie agtergrond	
If yes to question eight, from where?	Country / land

Indien ja by vraag agt, van waar	City/ stad District/ Distrik: Province/ Provinsie
Have you travelled to outside this community in the past 3 months? Het jy die afgelope 3 maande buite hierdie gemeenskap gereis?	Yes/ Ja No/ Nee
How do you normally spend your free time? (more than one answer can apply) Hoe spandeer jy gewoonlik jou vrye tyd? (meer as een antwoord kan van toepassing wees)	At home/ tuis At school/ by die skool In the fields/ in die velde Swimming/ swem Fishing / visvang Other, specify/ Ander (spesifiseer)
Environmental factors/ Omgewings faktore	
Where do you fetch water to drink? Waar gaan haal jy water om te drink?	1.Community borehole/ Gemeenskap boorgat 2.Household borehole/ Huishoudelike boorgat/ 3.Well/ wel 4.River/ rivier 5.Piped household water/ Huishoudelike water met pype 6. Other specify/ Ander (spesifiseer) ...
What is your household main water supply? Wat is jou huishoudelike hoofwatertoevoer?	1.Community borehole/ Gemeenskap boorgat 2.Household borehole/ Huishoudelike boorgat/ 3.Well/ wel 4.River/ rivier 5.Piped household water/ Huishoudelike water met pype 6. Other specify/ Ander (spesifiseer) ...
Do you live near a river? Woon jy naby 'n rivier?	Yes/ Ja No/ Nee
If yes what is the name of the river? Indien ja, wat is die naam van die rivier?	
Do you cross a river on your way to school? Gaan jy 'n rivier oor op pad skool toe?	Yes/ Ja No/ Nee
Do you have a toilet at home? Het jy 'n toilet by die huis?	Yes/ Ja No/ Nee
Where do you urinate/ defecate, if no? Waar urineer/ontlas jy, indien nee?	Bush/ bossie Neighbours' toilet/ buurman se toilet Toilet Other/ ander..... specify/ spesifiseer.....
Do you know about snails in the river? Weet jy van slakke in die rivier?	Yes/ Ja No/ Nee
Do you think they are harmful? Dink jy hulle is skadelik?	Yes/ Ja No/ Nee
Do they cause bilharzia? Veroorsaak hulle bilharzia?	Yes/ Ja No/ Nee

Clinical Variables/ Kliniese veranderlikes	
Infection status/ Infeksiestatus	Infected/ Besmet Not infected/ Nie besmet nie
Have you ever been infected with bilharzia? Was jy al ooit met bilharzia besmet?	Yes/ Ja No/ Nee
Have you ever suspected you had bilharzia? Het jy al ooit vermoed jy het bilharzia gehad?	Yes/ Ja No/ Nee
Have you had any of these symptoms in the past 3 months? Het jy enige van hierdie simptome in die afgelope 3 maande gehad?	Fever/ koors Cough/ hoes Diarrhea / diarree Muscles aches and pains/ spierpyne en pyne Tummy pain/ maag pyn Itchy, red blotchy and raised rash/ juekerig, rooi vlekkerig en verhoogde uitslag Blood in urine/ bloed in urine Blood in stool/ bloed in stoelgang Pain during urination/ Pyn tydens urinering Vaginal bleeding/ Vaginale bloeding Chills, fatigue, fever / koue rillings, moegheid, koors
If yes to the above, what are you doing?	Did nothing/ Het niks gedoen nie Used home remedies, specify the/ ingredients/ Gebruikte boererate, spesifiseer die Seek medical care/ Soek mediese sorg Other specify/ Ander (spesifiseer)
Do you have any of these symptoms now? Het jy nou enige van hierdie simptome?	Fever/ koors Cough/ hoes Diarrhea / diarree Muscles aches and pains/ spierpyne en pyne Tummy pain/ maag pyn Itchy, red blotchy and raised rash/ juekerig, rooi vlekkerig en verhoogde uitslag Blood in urine/ bloed in urine Blood in stool/ bloed in stoelgang Pain during urination/ Pyn tydens urinering Vaginal bleeding/ Vaginale bloeding Chills, fatigue, fever / Koue rillings, moegheid, koors
If yes to the above, what did you do? Indien ja op bogenoemde, wat het jy gedoen?	Did nothing/ Het niks gedoen nie Used home remedies, specify the/ ingredients/ Gebruikte boererate, spesifiseer die Seek medical care/ Soek mediese sorg Other specify/ Ander (spesifiseer)
Risk factors for schistosomiasis transmission Risikofaktore vir oordrag van skistosomiase	
Laundry/ wasgoed	1. Community borehole/ Gemeenskap boorgat 2. Household borehole/ Huishoudelike boorgat/

	3.Well/ wel 4.River/ rivier 5.Piped household water/ Huishoudelike water met pype 6. Other specify/ Ander (spesifiseer) ...
Gardening/ tuinmaak	1.Community borehole/ Gemeenskap boorgat 2.Household borehole/ Huishoudelike boorgat/ 3.Well/ wel 4.River/ rivier 5.Piped household water/ Huishoudelike water met pype 6. Other specify/ Ander (spesifiseer) ...
Swimming / swem	Yes/ Ja No/ Nee
Bathing/ bad	Home/ tuis River/ rivier
Crossing river barefooted Kruis rivier kaalvoet oor	Yes/ Ja No/ Nee
Collection of water for domestic use Versameling van water vir huishoudelike gebruik	1.Community borehole/ Gemeenskap boorgat 2.Household borehole/ Huishoudelike boorgat/ 3.Well/ wel 4.River/ rivier 5.Piped household water/ Huishoudelike water met pype 6. Other specify/ Ander (spesifiseer) ...
Recreational activities Ontspannings aktiwiteite	Swimming/ swem Fishing/ visvang Canoeing/ kanovaart
Urinate/defecate in rivers	Yes/ Ja No/ Nee

Section 2

Afdeling 2

Knowledge Attitude and Practice

Kennishouding en -praktyk

Now I am going to ask a few questions about your knowledge, attitude and practice on Bilharzia

Nou gaan ek 'n paar vrae vra oor jou kennis, houding en praktyk oor Bilharzia

Knowledge/ Kennis	
K1. Do you know about bilharzia? Weet jy van bilharzia?	True/ Waar False/ Onwaar I don't know/ Ek weet nie
K2. Where have you heard about it? Waar het jy daarvan gehoor?	School/ skool At home/ tuis Other/ ander specify/ spesifiseer
K3. Can it be prevented? Kan dit voorkom word?	Yes/ Ja No/ Nee I don't know/ ek weet nie

<p>K4. The main clinical symptoms of bilharzia are fever, itchy rash, pain during urinating and blood in urine <i>or stool</i> Die belangrikste kliniese simptome van bilharzia is koors, jeukerige uitslag, pyn tydens urinering en bloed in urine of stoelgang</p>	<p>True/ Waar False/ Onwaar I don't know/ Ek weet nie</p>
<p>K5. Bilharzia is treated with praziquantel. Bilharzia word met praziquantel behandel.</p>	<p>True/ Waar False/ Onwaar I don't know/ Ek weet nie</p>
<p>K6. Not all person's bilharzia will develop to severe cases. It can however have chronic effects and with coinfections can be deadly? Nie alle mense se bilharzia sal tot ernstige gevalle ontwikkel nie. Dit kan egter chroniese effekte hê en kan met ko-infeksies dodelik wees?</p>	<p>True/ Waar False/ Onwaar I don't know/ Ek weet nie</p>
<p>K7. Being bitten by mosquitoes causes Bilharzia? Om deur muskiete gebyt te word veroorsaak Bilharzia?</p>	<p>True/ Waar False/ Onwaar I don't know/ Ek weet nie</p>
<p>K8. Persons with bilharzia cannot transmit the disease to others when symptoms are not present? Persone met bilharzia kan nie die siekte aan ander oordra wanneer simptome nie teenwoordig is nie?</p>	<p>True/ Waar False/ Onwaar I don't know/ Ek weet nie</p>
<p>K9. Bilharzia spreads when infected people pass eggs into water bodies with snail intermediate hosts? Bilharzia versprei wanneer besmette mense eiers in watermassas met slak-tussengashere inlaat?</p>	<p>True/ Waar False/ Onwaar I don't know/ Ek weet nie</p>
<p>K10. Avoiding contact with infested water bodies prevents bilharzia? Vermy kontak met besmette water liggame voorkom bilharzia?</p>	<p>True/ Waar False/ Onwaar I don't know/ Ek weet nie</p>
<p>K11. It is not necessary for children and young adults to take measures to prevent the infection by <i>Schistosoma</i> parasite Dit is nie nodig vir kinders en jong volwassenes om maatreëls te tref om die infeksie deur <i>Schistosoma</i> parasiet te voorkom nie</p>	<p>True/ Waar False/ Onwaar I don't know/ Ek weet nie</p>
<p>K12. To prevent the infection by bilharzia, individuals should avoid swimming, bathing and fishing in infested water bodies? Om die infeksie deur bilharzia te voorkom, moet individue swem, bad en visvang in besmette waterliggame vermy?</p>	<p>True/ Waar False/ Onwaar I don't know/ Ek weet nie</p>
<p>K13. Treatment of people who are infected with bilharzia is an effective way to reduce the spread of the virus? Behandeling van mense wat met bilharzia besmet is 'n doeltreffende manier om die verspreiding van die virus te verminder?</p>	<p>True/ Waar False/ Onwaar I don't know/ Ek weet nie</p>
<p>K14. Are urine and faeces sources of bilharzia infection? Is urine en ontlasting bronne van bilharzia-infeksie</p>	<p>1: Agree/ stem saam 2: Disagree/ Verskil</p>

	3: I don't know/ Ek weet nie
Attitudes	
A1. Do you think bilharzia is a serious illness? Dink jy bilharzia is 'n ernstige siekte	1: Agree/ stem saam 2: Disagree/ Verskil 3: I don't know/ Ek weet nie
A2. Do you think there is a cure for bilharzia? Dink jy daar is 'n kuur vir bilharzia?	Yes/ Ja No/ Nee
A3. Do you feel that bilharzia is a dangerous disease? Voel jy dat bilharzia 'n gevaarlike siekte is?	Yes/ Ja No/ Nee
A4. Is there enough available information about bilharzia being disseminated at school or at home? Is daar genoeg beskikbare inligting oor bilharzia wat by die skool of by die huis versprei word?	1: Agree/ stem saam 2: Disagree/ Verskil 3: I don't know/ Ek weet nie
A5. Has your daily life been disturbed by bilharzia in anyway? (if infected) Is jou daaglikse lewe op 'n manier deur bilharzia versteur? (indien besmet)	1: Agree/ stem saam 2: Disagree/ Verskil 3: I don't know/ Ek weet nie
A6. IF yes explain how? Indien ja verduidelik hoe?	
A7. Do you worry about suffering from bilharzia? Bekommer jy jou om aan bilharzia te ly?	Yes/ Ja No/ Nee I don't know
A8. Is avoiding rivers with snails helpful in preventing bilharzia? Is die vermyding van riviere met slakke nuttig om bilharzia te voorkom?	1: Agree/ stem saam 2: Disagree/ Verskil 3: I don't know/ Ek weet nie
Practices/ Praktyke	
P1. Do you wear shoes when going outside? Dra jy skoene wanneer jy buite gaan?	Yes/ Ja No/ Nee
P2. Do you wear shoes whilst fetching water/ washing clothes at the river? Dra jy skoene terwyl jy water gaan haal/klere was by die rivier?	Yes/ Ja No/ Nee
P3. How often do you go to the river? Hoe gereeld gaan jy na die rivier?	1. Not at all/ glad nie 2. Once a day/ Een keer 'n dag 3. 2-3 times per day/ 2-3 keer per dag 4. More than 5 times per day/ Meer as 5 keer per dag

	5. Whenever I want to play/ Wanneer ek wil speel
P4. Do you bath in open water sources and fresh water bodies? Bad jy in oop waterbronne en vars water liggame?	1. Not at all/ glad nie 2. Sometimes/ soms 3. Always/ altyd
P5. Do you go to swim or fish in the river? Gaan jy swem of visvang in die rivier?	1. Not at all/ glad nie 2. Sometimes/ soms 3. Always/ altyd
P6. Do you drink river water? Drink jy rivierwater?	Yes/ Ja No/ Nee
P7. Do you seek treatment when suspecting bilharzia? Soek jy behandeling wanneer jy bilharzia vermoed?	Yes/ Ja No/ Nee
P8. In the past month have you visited medical personnel after experiencing the bilharzia symptoms? Het jy die afgelope maand mediese personeel besoek nadat jy die bilharzia-simptome ervaar het?	Yes/ Ja No/ Nee
P9. Have you ever been tested for bilharzia? Is jy al ooit vir bilharzia getoets?	Yes/ Ja No/ Nee
P9a. If yes where and when? Indien ja, waar en wanneer?	

APPENDIX B: *S. HAEMATOBIIUM* DIAGNOSTICS

Haematuria detection by dipstick test

Urine filtration- detection of *S. haematobium* eggs

Agarose gel electrophoresis: cPCR results

APPENDIX C: ADVANCED DIAGNOSTICS FOR INTESTINAL HELMINTHS

POC-CCA test for *S. mansoni*

Kato-Katz technique for *S. mansoni* and STHs

Agarose gel electrophoresis results for *S. mansoni*

The composite image illustrates three diagnostic methods for *S. mansoni*. The top left shows three panels (A, B, C) of a POC-CCA test, where a person in a white lab coat and gloves is performing the test on a wooden table. Panel A shows the initial setup with a yellow liquid in a well. Panel B shows the person using a stick to mix the sample. Panel C shows the person reading the results. The top right shows the Kato-Katz technique, with a person in a blue glove using a stick to mix a sample in a small container. Below this is a slide preparation step, showing a slide with a sample on it. The bottom right shows a person in a white lab coat looking through a microscope to visualize the sample. The bottom left shows an agarose gel electrophoresis image with two panels (A and B) and lane labels (M, 1-13). Panel A has a 0.300Kb marker, and Panel B has a 0.766Kb marker. A negative control (SBhmF/SmR) is also shown.