MOLECULAR IDENTIFICATION AND CHARACTERISATION OF *SCHISTOSOMA* SPECIES IN FRESHWATER IN BODO COMMUNITY, RIVERS STATE

BY

SAMPSON, MARGRET E.
REG NO: 20214326558

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL
FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

AUGUST, 2025.

MOLECULAR IDENTIFICATION AND CHARACTERISATION OF *SCHISTOSOMA* SPECIES IN FRESHWATER IN BODO COMMUNITY, RIVERS STATE

BY

SAMPSON, MARGRET E.
REG NO: 20214326558

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE (MASTER'S IN PUBLIC HEALTH), MPH

AUGUST, 2025.

CERTIFICATION

This certifies that **"MOLECULAR IDENTIFICATION AND CHARACTERISATION OF *SCHISTOSOMA SPECIES* IN FRESHWATER IN BODO COMMUNITY, RIVERS STATE"** written by **SAMPSON MARGRET E.** Registration Number **20214326558** in partial fulfillment for the award of the degree of Master in Public Health (MPH) in the Department of Public Health Technology of the Federal University of Technology, Owerri.

…………………………	11/02/26 …………………
Prof. U.M. Chukwuocha	**Date**
(Supervisor)	
…………………………	03/02/2026 …………………
Dr. U.G. Ekeleme	**Date**
(Co-Supervisor)	
…………………………	03/02/2026 …………………
Dr. C.C. Iwuala	**Date**
(Head of Department)	
…………………………	16/02/2026 …………………
Prof. (Mrs.) E.A. Nwoke	**Date**
(Dean of SOHT)	
…………………………	…………………
Prof. Mrs. J.N. Nwosu	**Date**
(Dean, PG School)	
…………………………	**27:10:2025** …………………
Dr. B. A. Magaji	**Date**
External Examiner	

DEDICATION

This thesis is dedicated to God Almighty for his infinite mercies upon me.

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to my supervisor and co-supervisor, Prof. Uchechukwu M. Chukwuocha and Dr. U.G. Ekeleme, for their invaluable guidance and support throughout this work. Their willingness to spare time amidst their busy schedules and provide essential counsel and corrections has been instrumental in my journey.

I extend my heartfelt thanks to the Head of Department, Dr. C.C. Iwuala, for his love, care, and the invaluable lessons he imparted to me. His efforts in positioning the department among the elite in the University are commendable.

I am also grateful to the incumbent Dean of the School of Health Technology (SOHT), Prof. Rev. Sr. (Prof.) Oparaocha, for her wisdom in coordinating this prestigious faculty.

To the esteemed professors in the department: Prof. A.N. Amadi, Prof. (Mrs.) U. A. Nwoke, and Prof. (Mrs.) Sally Ibe, as well as the dedicated lecturers: Dr. Chike C. Okereke, Prof. (Mrs.) Blessed Nworuh, Prof. J.C. Nwaokoro, Dr. Obinna Udujih, Dr. C.I.C. Ebirim, Dr. (Mrs.) C. O. Akanazu, Dr. (Mrs.) S. Orji, Dr. (Mrs.) O. Okorie, and my PG Coordinator, Dr. (Mrs.) Winnie U. Dozie, I am deeply appreciative of the positive impact during my time as a postgraduate student in this department. Your contributions have significantly enhanced the success of this great department.

I would also like to acknowledge the non-academic staff, particularly the DAO, for their diligence in ensuring that the department functions optimally.

To my family, Mrs. Gift Davies, Mrs. Ibifuro Perri, Dr. Onari Perri, Mrs. Ibisaki Willie-Wills, Mr. Friday Davies, and Mbari Shedrack, my valued cousins, loved ones and friends, including Dike Joseph, Uywagwu Chisom, Odina Francisca, Anya Donatus, Onyewuchi Leonard, and Miracle Ginika, I am immensely grateful for your unwavering support.

Lastly, I cannot forget to thank my colleagues and course mates for making this journey smooth. Your holistic support has been invaluable, and your encouragement has truly been appreciated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Accurate identification and characterization of Schistosoma species in freshwater are crucial for understanding the transmission dynamics of schistosomiasis and guiding effective control measures. This study molecularly identified and characterized *Schistosoma* species in freshwater from four locations in the Bodo community, Rivers State, Nigeria. A total of 200 water samples were collected from Nuumu-Tekurun, Nuumu-Bari-aage, Bon-Sunday, and Nuumu-Kekpaban rivers. Structured questionnaires were also administered to 200 randomly selected residents to assess behavioural and environmental risk factors associated with infection. Data were analyzed using descriptive statistics and chi-square tests in SPSS version 23.0. Findings revealed that (139) 69% of households relied on pipe-borne water, while (159) 80% of participants frequently engaged in swimming and other water-contact activities lasting more than 15 minutes. Urination or defecation in water bodies was reported by 82% of respondents, and the presence of snails 177 (89%) and 189 livestock (95%), particularly cattle (61%), was common near rivers. The overall prevalence of *Schistosoma* infection was 18.5% ($p < 0.001$), with *S. japonicum* 15 (40.5%) being the most prevalent, followed by *S. mansoni* 12 (32.4%) and *S. haematobium* 10 (27%). Significant predictors of infection ($p \leq 0.027$) included use of rivers as a main water source, daily exposure to water bodies, prolonged contact, and proximity of livestock and snails. The study concludes that schistosomiasis persists as a public health concern in Bodo community. Strengthened health education, improved access to safe water, and regulation of livestock activities near freshwater sources are recommended to curb transmission and protect community health.

Keywords: Schistosomiasis, *Schistosoma* species, freshwater, molecular identification, Bodo community, Rivers State, public health.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Schistosomiasis, a chronic parasitic disease caused by blood flukes of the genus *Schistosoma*, poses a significant global health burden, particularly in resource-limited and freshwater-dependent communities, such as the Bodo community in Rivers State, Nigeria (World Health Organization, 2023). As a Neglected Tropical Disease (NTD), schistosomiasis disproportionately affects impoverished populations lacking access to adequate sanitation and safe water, perpetuating cycles of poverty and ill health (Hotez et al., 2006). NTDs contribute significantly to morbidity, disability, and mortality, hindering socio-economic development and progress toward the Sustainable Development Goals (SDGs) (Fenwick, 2012; World Health Organization, 2021). With its complex life cycle, schistosomiasis is one of the most prevalent NTDs, affecting hundreds of millions globally, with over 251.4 million requiring annual preventive treatment (World Health Organization, 2021, 2023). The long-term consequences, including organ damage, impaired cognitive function, and increased cancer risk, significantly reduce quality of life and economic productivity in affected populations (King & Dangerfield-Cha, 2017; Van der Werf et al., 2003).

Over a billion individuals are thought to be infected by parasitic helminths, which have a terrible effect on both human health and economic advancement. These helminths include schistosomes, filarial worms, and soil-transmitted nematodes. The blood flukes (trematode worms) of the genus Schistosoma are the cause of the acute and chronic parasitic disease schistosomiasis. According to estimates, in 2021, at least 251.4 million people needed preventative care. Morbidity will be decreased and prevented by preventive treatment, which should be repeated over a number of years. There have been reports of schistosomiasis transmission from 78 different nations. However, only 51 endemic countries with moderate-to-high transmission need preventative chemotherapy for schistosomiasis, where individuals and communities are targeted for extensive treatment (World Health Organisation, 2023).

Schistosoma are diverse parasitic flatworms with complex life cycles involving humans and freshwater snails (Gryseels et al., 2006). Unlike other trematodes, *Schistosoma* have separate sexes. Six of the approximately 20 recognized species infect humans, with *S. haematobium*, *S. mansoni*, and *S. japonicum* being the most common and significant (Rollinson et al., 2013).
These three species have distinct geographical distributions, preferred snail hosts, and pathologies. *S. haematobium* causes urogenital schistosomiasis in Africa and the Middle East, leading to symptoms like blood in urine and increased bladder cancer risk (Schistosoma, Control in Egypt Research Project, 1980). *S. mansoni* causes intestinal schistosomiasis in Africa and the Americas, presenting with abdominal pain, diarrhea, and potential liver enlargement. *S. japonicum*, primarily in East Asia, also causes intestinal schistosomiasis but is linked to higher egg output and central nervous system involvement (Gong et al., 2021). Other less common species include *S. mekongi*, *S. intercalatum*, and *S. guineensis* (Southgate et al., 2013). The existence of hybrid *Schistosoma* species (e.g., *S. haematobium* x *S. bovis*) is a growing concern due to potential changes in transmission, pathogenicity, and drug resistance (Huyse et al., 2009; Leger et al., 2018).
The flukes that cause schistosomiasis are first discovered in snails and subsequently released into the water. The parasites can enter the human body through the skin and remain there for years if it comes into contact with tainted water. The forked head of the parasite's human-infecting stage enables it to pierce the skin after growing in the snail. The two primary forms of the disease, intestinal and urogenital schistosomiasis, are caused by the three primary types of schistosomes. Snail fever and bilharzia are other names for this illness.

Schistosomiasis symptoms are mostly brought on by the body's response to the worms' eggs. In tropical and subtropical regions, schistosomiasis is common, particularly in underprivileged areas where access to clean drinking water and proper sanitation is limited. At least 90% of those who need schistosomiasis therapy are thought to reside in Africa. S*chistosoma haematobium, S. japonicum, and S. mansoni* are the three primary species that infect people. *S. mekongi, S. intercalatum, and S. guineensis* (once thought to be the same as S. intercalatum) are three other species that are more geographically localised. A few cases of human infection with cattle-derived hybrid schistosomes *(S. haematobium x S. bovis, x S. curassoni, x S. mattheei*) have also been reported.

Accurate differentiation of Schistosoma species is of paramount importance for effective schistosomiasis control and management. Species-specific variations in drug susceptibility, particularly to praziquantel, the mainstay of schistosomiasis treatment, have been documented (Doenhoff et al., 2008). For instance, *S. japonicum* is often considered more susceptible to praziquantel than *S. mansoni*, while resistance has been reported in some *S. mansoni* populations (Ismail et al., 1999). Moreover, the development of effective control strategies, including snail control interventions, relies on a thorough understanding of the specific snail intermediate hosts involved in the transmission cycle, which vary significantly among Schistosoma species (McCullough & Mott, 1983). Species-specific diagnostic tools, such as polymerase chain reaction (PCR)-based assays, are essential for accurate epidemiological surveillance and monitoring of control program effectiveness (Hamburger et al., 2004). Furthermore, understanding the genetic diversity and population structure of *Schistosoma* species can provide insights into parasite evolution, adaptation to local environments, and the potential for the emergence of drug resistance (Webster et al., 2006). The identification of hybrid species and the investigation of zoonotic transmission pathways are also critical for informing public health policies and preventing the spread of infection from animal reservoirs to humans (Huyse et al., 2009; Leger et al., 2018).

The transmission depends on freshwater contaminated with infective *Schistosoma* cercariae (Cheesbrough, 2006). The cycle begins when eggs, excreted by infected humans, hatch in water, releasing miracidia. These miracidia must infect specific freshwater snail species to develop (Rollinson et al., 2013). Inside the snail, miracidia multiply into thousands of cercariae, which are then released into the water (Cheesbrough, 2006). These cercariae actively swim, penetrate human skin during water contact (e.g., swimming, bathing), and transform into schistosomulae (van der Werf et al., 2003; Hunter et al., 1993). The schistosomulae migrate to the liver to mature into adult worms. Adult worms then settle in specific human veins, continuously producing eggs to perpetuate the life cycle (Gryseels et al., 2006; Rollinson et al., 2013).

In Nigeria, schistosomiasis represents a significant and widespread public health challenge, with the disease being endemic in all 36 states and the Federal Capital Territory (Federal Ministry of Health Nigeria, 2013). The prevalence of schistosomiasis varies across the country, with higher rates often observed in rural communities with limited access to clean water and sanitation and where water-contact activities are common (Oguonu & Okafor, 1993). It is estimated that tens of millions of Nigerians are at risk of infection, and millions suffer from the associated morbidities, placing a substantial burden on the healthcare system and hindering socio-economic development (Ekpo & Mafiana, 2020). The Niger Delta region, including Rivers State, is particularly vulnerable to *schistosomiasis* transmission due to its extensive network of rivers, creeks, and swamps, which provide ideal habitats for the snail intermediate hosts and facilitate frequent human contact with freshwater (Olufemi et al., 2021). Studies conducted in various parts of the Niger Delta have documented high prevalence rates of schistosomiasis in different population groups, including school-aged children, fishermen, and farmers, highlighting the ongoing transmission and the need for targeted interventions (Sowemimo, 2007; Ukoli, 1990). The environmental conditions prevalent in this region, such as the abundance of specific snail species (e.g., *Bulinus globosus* for *S. haematobium*, *Biomphalaria pfeifferi* for *S. mansoni*) and the socio-cultural practices that involve frequent water contact, create a complex and challenging environment for schistosomiasis control.

The Bodo community in Rivers State is characterized by its close proximity to and dependence on a network of freshwater bodies, including rivers, streams, and potentially stagnant pools. The socio-economic activities of the community are intrinsically linked to these aquatic resources, with fishing serving as a primary source of livelihood for many residents, and farming practices often involving irrigation techniques that require frequent water contact (Author's preliminary observations/Community reports). Domestic water use, including bathing, washing, and fetching water directly from these sources, further increases the exposure of the community members to potentially cercariae-contaminated water. The lack of adequate sanitation facilities in many parts of the community exacerbates the problem, as the open defecation and urination into or near freshwater bodies contribute to the contamination of these sources with *Schistosoma* eggs, perpetuating the transmission cycle (Olufemi et al., 2021). The presence of specific freshwater snail species known to serve as intermediate hosts for *Schistosoma* in the region, coupled with the high frequency of human-water contact, creates a particularly conducive environment for the transmission and persistence of schistosomiasis within the Bodo community (Okafor, 1985). The complex interplay of these environmental, socio-economic, and behavioral factors necessitates a comprehensive and targeted approach to schistosomiasis control in this setting.

Despite the recognized endemicity of schistosomiasis in Rivers State and the potential for high transmission rates in communities like Bodo, there remains a significant gap in detailed, species-specific data regarding *Schistosoma* parasites in the freshwater environment of this specific locality. The majority of previous studies conducted in the region have focused on determining the overall prevalence of schistosomiasis in human populations using traditional parasitological methods, such as the Kato-Katz technique for stool examination and urine filtration for *S. haematobium* eggs (Savioli et al., 2017). While these methods are useful for detecting patent infections, they often lack the sensitivity to detect low-intensity infections accurately, and not reliably differentiate between the different *Schistosoma* species circulating in the area (Weerakoon et al., 2018). Furthermore, there is a paucity of information on the genetic and phenotypic characteristics of the *Schistosoma* populations present in the Bodo community's freshwater sources. This lack of granular data hinders a comprehensive understanding of the local epidemiology of schistosomiasis, including the dominant species, their genetic diversity, potential for drug resistance, adaptation to local snail hosts, and the precise transmission dynamics within the community.

Characterizing the *Schistosoma* species present in the freshwater sources of the Bodo community is of paramount importance for several critical reasons. Firstly, different *Schistosoma* species exhibit variations in their susceptibility to praziquantel, the primary drug used for schistosomiasis treatment (Doenhoff et al., 2008). Accurate species identification can inform drug administration strategies, allowing for the selection of the most appropriate treatment regimens and the monitoring for potential drug resistance within specific species. Secondly, effective control strategies, including snail control interventions, rely on a thorough understanding of the specific snail intermediate hosts involved in the transmission cycle, which often exhibit species-specific preferences and ecological requirements (McCullough & Mott, 1983). For instance, targeting the specific habitats of *Bulinus* snails for *S. haematobium* or *Biomphalaria* snails for *S. mansoni* can significantly enhance the effectiveness of snail control measures. Thirdly, molecular characterization of *Schistosoma* species can provide valuable insights into the genetic diversity and population structure of the parasites, which can inform our understanding of parasite evolution, adaptation to local environments, and the potential for the emergence of drug resistance (Webster et al., 2006). Fourthly, the identification of hybrid *Schistosoma* species and the investigation of zoonotic transmission pathways are also critical for informing public health policies and preventing the spread of infection from animal reservoirs to humans (Huyse et al., 2009; Leger et al., 2018). Understanding whether livestock or other animals play a role in schistosomiasis transmission in the Bodo community can have significant implications for control strategies. Finally, species-specific data provides a more accurate baseline for monitoring the impact of control interventions and detecting any changes in species distribution or prevalence over time, allowing for the evaluation of program effectiveness and the adaptation of control strategies as needed.

Therefore, this research, which focuses on the identification and characterization of *Schistosoma* species in the freshwater environment of the Bodo community using molecular techniques, holds significant potential to contribute valuable insights for the development of more targeted interventions, the formulation of evidence-based policies, and the enhancement of health education initiatives in this and other similar endemic communities.

1.2 Statement of the Problem

Schistosomiasis, a neglected tropical disease caused by parasitic trematodes of the genus *Schistosoma*, remained a major public health challenge globally, with over 273 million people requiring preventive treatment in endemic areas (World Health Organization, 2023). The burden of schistosomiasis was disproportionately high in Africa, where approximately 90% of those affected resided (Tchuenté et al., 2018). In Nigeria, the disease is endemic across all 36 states, affecting an estimated 20 million people (Ekpo & Mafiana, 2020; Savioli et al., 2017). Particularly in communities such as Bodo in Rivers State, where access to safe drinking water and adequate sanitation was limited, the risk of schistosomiasis transmission was exacerbated (Olufemi et al., 2021).

Despite the high prevalence, detecting and identifying Schistosoma species in freshwater bodies within the Bodo community has remained inadequately explored. Traditional diagnostic methods, such as stool and urine examinations, often lacked the sensitivity required to accurately detect low-intensity infections (Weerakoon et al., 2018). Techniques like the Kato-Katz method and urine filtration could be time-consuming, expensive, and may have yielded biased prevalence estimates due to sample collection limitations (Savioli et al., 2017). These deficiencies underscore the necessity for more reliable diagnostic methods, such as molecular techniques, to enhance the identification of Schistosoma species in environmental samples (Crego-Vicente et al., 2021).

Moreover, limited studies characterized the environmental risk factors and species diversity of Schistosoma in freshwater bodies in Nigeria, particularly in the Bodo community. This lack of localized data impeded understanding of the unique transmission dynamics and ecological determinants influencing schistosomiasis prevalence in these regions (Dorkenoo et al., 2023; Standley et al., 2012). Identification and Characterisation of Schistosoma species in freshwater sources within Bodo provided crucial epidemiological data that informed targeted public health interventions and contributed to the larger global agenda of schistosomiasis elimination.

This research focused on filling the knowledge gap regarding the prevalence and distribution of Schistosoma species in freshwater environments of the Bodo community, utilizing molecular techniques for precise identification. The findings contributed valuable insights that guided control measures and informed community health strategies against schistosomiasis.

1.3 Aim and Objectives

The main objective of this research was to identify and characterize Schistosoma species in freshwater sources using molecular techniques in the Bodo community in Rivers State.

1.3.1 Specific Objectives

i. To determine the prevalence and spatial distribution of different Schistosoma species across sampled freshwater sites in Bodo Community.

ii. To assess environmental risk factors (such as water quality parameters and snail population density) associated with the transmission of Schistosoma species in the sudty area.

iii. To analyze the distribution patterns of Schistosoma species in various freshwater bodies within the Bodo community.

1.4 Research Questions

The specific research question to guide the study on *Schistosomiasis* species detection by molecular techniques in freshwater in Bodo community, Rivers State is:

i. What are the prevalence rates and spatial distribution patterns of *Schistosoma* species across sampled freshwater sites in the Bodo community?

ii. What environmental risk factors are associated with the transmission of *Schistosoma* species in freshwater bodies?

iii. How are *Schistosoma* species distributed across different freshwater bodies within the Bodo community?

1.5 Research Hypotheses

The research hypothesis will be in the form of a null hypothesis and an alternative hypothesis, considering the sample type and the level of significance:

Null Hypothesis (H0)

$H0_1$: There is no significant difference in the prevalence of Schistosoma species across freshwater samples from Bodo community when characterized using molecular methods.

$H0_2$: There is no significant association between environmental risk factors and the prevalence of Schistosoma species in freshwater bodies

Alternative Hypotheses (H_1)

H_{11}: There is a significant difference in the prevalence of *Schistosoma* species across freshwater samples from the Bodo community when characterized using molecular methods.

H_{12}: There is a significant association between environmental risk factors and the prevalence of *Schistosoma* species in freshwater bodies.

1.6 Significance of the Study

The significance of studying *Schistosoma* species identification by molecular techniques in freshwater in Bodo community, Rivers State, lies in several key areas. Accurate species identification is paramount because molecular techniques offer precise differentiation of *Schistosoma* parasite species in freshwater. This precision is vital as various species exhibit differing pathogenicity and treatment responses, enabling tailored interventions and treatments for the specific species prevalent in Bodo community. Furthermore, the molecular detection of *Schistosoma* species in freshwater significantly aids disease surveillance and control. By pinpointing the prevalence and distribution of different species, public health authorities can strategically direct interventions like mass drug administration, snail control programs, and health education campaigns towards high-risk areas or groups.

Moreover, this research is valuable for monitoring treatment responses. Since *Schistosoma* species may react differently to treatment drugs, molecular species detection allows for tracking treatment efficacy and evaluating specific drug effectiveness against particular species. This data provides an evidence base for decision-making on treatment protocols and for surveillance of drug resistance.

molecular techniques empower research and development by providing rich data for further studies and advancements in schistosomiasis. Comprehending the genetic diversity and population dynamics of *Schistosoma* species in Bodo community offers scientists insights into transmission patterns, host-parasite relationships, and potential targets for novel treatments or interventions.

1.7 Justification of the Study

Schistosomiasis is a major public health concern, particularly in tropical and subtropical regions, including Nigeria. The commonly used diagnostic methods, such as the Kato-Katz thick smear technique for microscopic identification of eggs and rapid diagnostic tests for circulating cathodic antigen (POC-CCA), lack sensitivity, especially in low-intensity infections (Weerakoon et al., 2018). This limitation emphasizes the need for more accurate diagnostic approaches. Molecular techniques, particularly polymerase chain reaction (PCR)-based methods, have emerged as valuable tools for detecting schistosome DNA due to their higher sensitivity and ability to identify early pre-patent infections (Cavalcanti et al., 2013; Bergquist et al., 2019; Fernández et al., 2019).

Research has demonstrated that different sample types—such as blood (Pontes et al., 2002; Cnops et al., 2013), urine (Lodh et al., 2014), stool (Pontes et al., 2002; Abbasi et al., 2010), and cercariae in contaminated water (Hertel et al., 2004)—can be utilized for the molecular detection of

Schistosoma species. Given the unique ecological circumstances of the Bodo community, which has abundant freshwater bodies conducive to the transmission of *schistosomiasis*, employing molecular techniques for species identification is particularly relevant.

This study aims to elucidate the prevalence of Schistosoma species in the freshwater sources of the Bodo community in Rivers State. The findings will provide critical information on the distribution and types of schistosome parasites affecting the local population, which is essential for tailoring public health interventions. Moreover, the results could inform local health authorities about the necessary steps to mitigate schistosomiasis transmission. The education sector in Nigeria may also benefit from this study, as it can serve as a basis for developing a comprehensive schistosomiasis policy for educational institutions and creating educational materials that help affected patients and their families understand their situation, thereby promoting prevention strategies.

1.8 Scope of the Study

The scope of this investigation into *Schistosoma* species identification through molecular techniques in freshwater within the Bodo community, Rivers State, is precisely delineated by its geographical confines, the populations under scrutiny, and the interrelationship of its variables. the study is strictly situated within the Bodo community, an area nestled in Rivers State, Nigeria. This encompasses a thorough examination of all freshwater bodies, including rivers, streams, ponds, and canals, that lie within the community's defined borders.

The study centers on two distinct populations. Firstly, it targets the environmental *Schistosoma* population, specifically the DNA of these parasites found within the freshwater sources of Bodo. The primary objective is to pinpoint *Schistosoma mansoni* and *Schistosoma haematobium*, given their prevalence as human-infecting species in Nigeria, particularly in Rivers State. Nevertheless, the study's scope is broad enough to permit the discovery of other, perhaps less common or previously unrecorded, *Schistosoma* species through the application of highly sensitive molecular methods. Implicitly, though not as a direct subject of analysis, the presence and distribution of intermediate host snails, such as *Bulinus* species for *S. haematobium* and *Biomphalaria* species for *S. mansoni*, are also considered integral environmental factors, recognizing their indispensable role in the parasite's life cycle and transmission dynamics.

the independent variable is the detectability of *Schistosoma* species DNA in freshwater samples, which is ascertained using molecular techniques. The dependent variables encompass the specific identity of the *Schistosoma* species (e.g., *S. mansoni*, *S. haematobium*, or others) identified in these samples, the prevalence, indicated by the proportion of samples testing positive for *Schistosoma* DNA, and the distribution, reflecting the spatial patterns of the parasite's occurrence across various sampling sites within Bodo community. While not directly manipulated, environmental factors such as water temperature, water quality metrics, and the density of specific snail intermediate hosts will be acknowledged as potential influences on these dependent variables. Similarly, human interaction patterns with freshwater will be recognized for their role in transmission risk within the community.

the study exclusively employs advanced molecular techniques, including Polymerase Chain Reaction (PCR) and potentially DNA sequencing, to accurately detect and identify *Schistosoma* species DNA in freshwater. The duration of data collection will be adapted to the chosen sampling strategy and available resources, potentially spanning from several months to over a year. The study acknowledges inherent constraints such as financial limitations, time restrictions, challenges in accessing certain sampling locations, and any technical hurdles encountered in the molecular detection and differentiation of *Schistosoma* species.

CHAPTER TWO

LITERATURE REVIEW

2.1 Conceptual Review

2.1.1. Overview of Schistosomiasis

Bilharzia, another name for schistosomiasis, is a parasitic worm-borne illness. Trematodes, or flukes, are the source of this sickness. The parasitic flatworms known as schistosomes (blood flukes) are members of the genus Schistosoma. Parasites are organisms that feed on the nutrients of their host and live in or on it. The host is harmed by this. In 2018, the Global Schistosomiasis Alliance, when schistosomiasis occurs, the flukes are discovered in *Oncomelania hupensis* snails and subsequently released into the sea. The parasites can enter your body and remain there for years if your skin comes into contact with tainted water. The parasite that develops in the snail and then infects humans has a type of forked head that makes it possible for it to pierce your skin. The two primary forms of the disease, intestinal and urogenital schistosomiasis, are caused by the three primary types of schistosomes. Snail fever and bilharzia are other names for this illness.

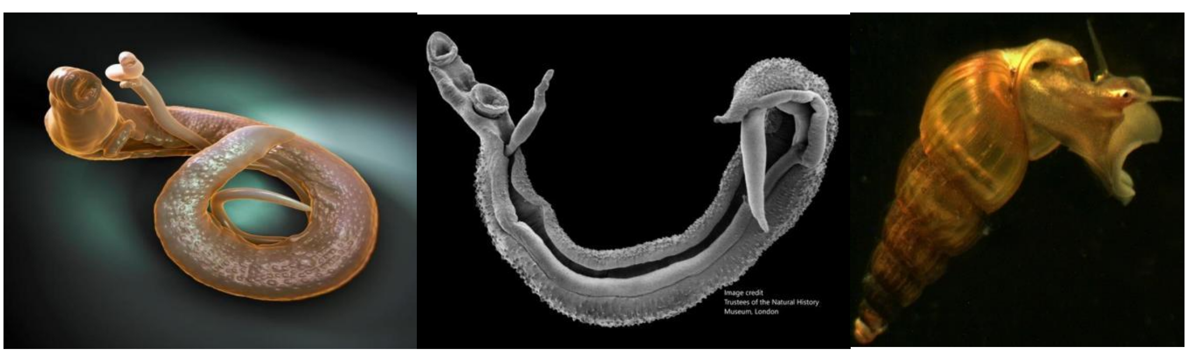

Figure 2.1: Diagram of *Schistosoma* species and *Oncomelaniahupensis*

Source: (Global *Schistosomiasis* Alliance, 2018)

Most human infections can be accounted for by *Schistosoma* haematobium, S. japonicum, and S. mansoni, and a minor contribution from S. intercalcatum and S. mekongi. Ethiopia is one of the endemic countries for both S. mansoni and S. haematobium. (Global *Schistosomiasis* Alliance, 2018)

2.1.2. Causes and Transmission Pathway of *Schistosomiasis*

Certain species of blood trematodes (flukes) belonging to the genus *Schistosoma* are the cause of schistosomiasis (bilharziasis). *Schistosoma haematobium, S. japonicum*, and *S. mansoni* are the three primary species that infect people. *S. mekongi, S. intercalatum*, and *S. guineensis* (once thought to be the same as *S. intercalatum*) are three other species that are more geographically localised. A few cases of human infection with cattle-derived hybrid schistosomes (*S. haematobium, x S. bovis, x S. curassoni, x S. mattheei*) have also been reported. Unlike most trematodes, which are hermaphroditic, *Schistosoma* spp. are dioecous (individuals of separate sexes) (Centers for Disease Control and Prevention, 2018).

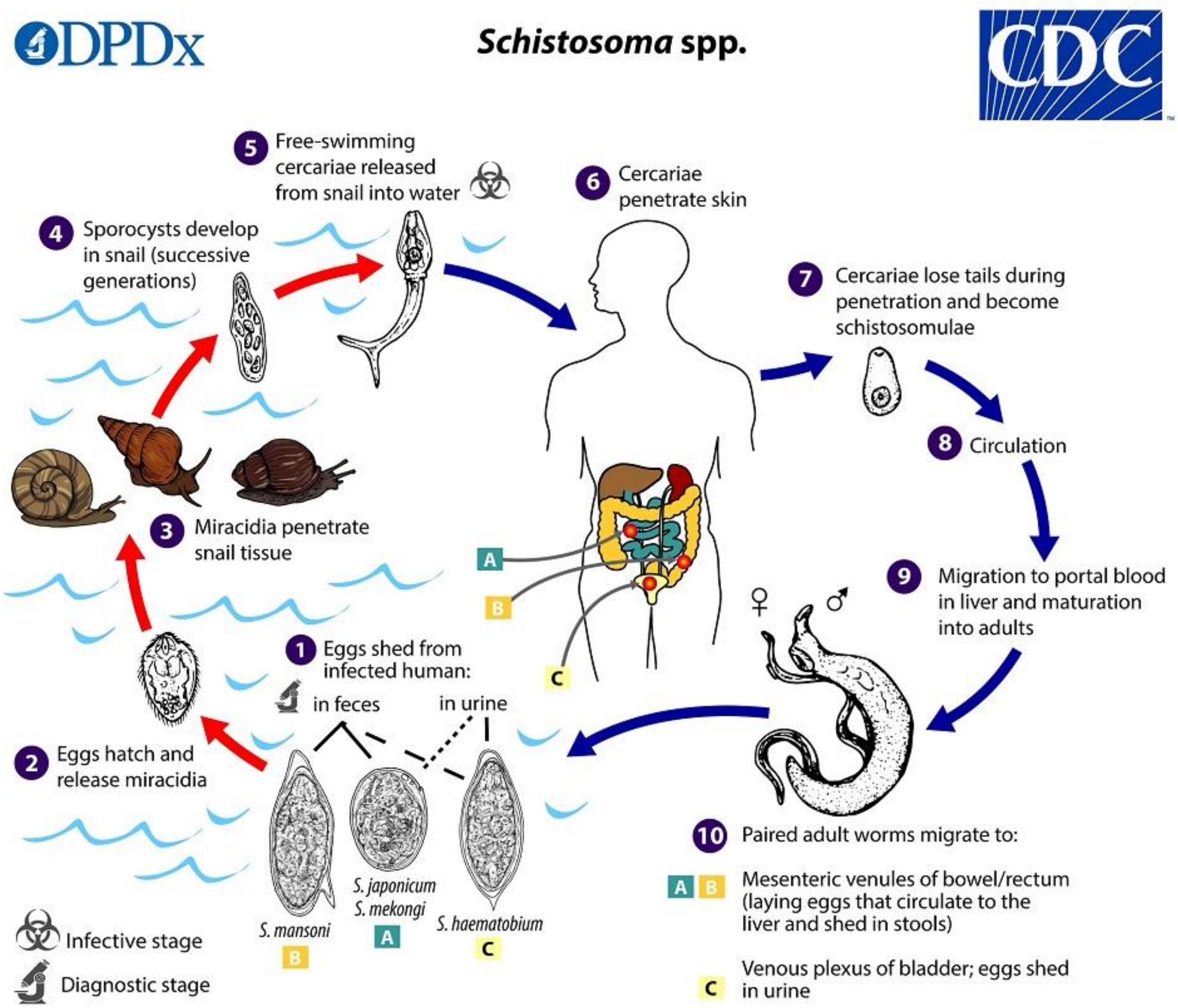

Figure 2.2: Transmission Pathway/Lifecycle of *Schistosomiasis*

Source: (Centers for Disease Control and Prevention, 2018)

Urine or faeces are used to get rid of eggs (1). Miracidia (2), which swim and infiltrate particular snail intermediate hosts (3), are released when the eggs hatch under ideal circumstances. The snail goes through two generations of sporocysts. (4) and cercariae production (5). The infectious cercariae swim, pierce the human host's skin (6), and lose their forked tail to become schistosomulae (7) after being released from the snail. Before settling in the veins, the schistosomulae move through a number of tissues and phases (8,9). Human adult worms live in the mesenteric venules in a variety of places, some of which appear to be species-specific (10). For example, the superior mesenteric veins draining the small intestine (A) are more likely to contain *S. japonicum*, while the superior mesenteric veins draining the large intestine (B) are more likely to contain *S. mansoni*. It is impossible to say with certainty that one species only exists in one place, though, because both species can live in each location or migrate between them. Although it can also be found in the rectal venules, *S. haematobium* is most frequently found in the venous plexus of the bladder (C). The tiny venules of the portal and perivesical systems are where the females (which range in size from 7 to 20 mm; the males are slightly smaller) lay their eggs. According to the Centres for Disease Control and Prevention (2018), the eggs are gradually transported to the lumen of the intestine (*S. mansoni and S. japonicum*) and the bladder and ureters (*S. haematobium*), where they are expelled as urine or faeces, respectively. Dogs are reservoirs for *S. mekongi*, while cattle, dogs, cats, rats, pigs, horses, and goats are reservoirs for *S. japonicum*.

Pathogenesis

The length and intensity of infection, the site of egg deposition, and concomitant infection all affect how severe and apparent schistosomiasis is. Initial infection is undetected in people from endemic locations. However, acute febrile illness (also known as Katayama fever or acute schistosomiasis) is frequently the result of an initial Schistosome infection in travellers to endemic locations. This is a sign of the immune system's reaction to the developing Schistosomes and eggs. The site of cercarial penetration may experience skin rash and discomfort (Swimmer's itch). In the mesenteric venules, the cercariae are transferred and mature into the adult stage. By adopting host antigens, the mature flukes are shielded from host immunological responses. An estimated 100 to 300 eggs are produced daily by *S.mansoni,* most of which pass through the intestinal wall and are expelled in the faeces, occasionally along with blood and mucus. Cellular responses to the eggs in the tissue result in persistent inflammation, which damages the liver, digestive system, and other organs. Granulomata, ulceration, and thickening of the intestinal wall are the results of the host's reaction to eggs that have become stuck in the intestinal mucosa. Colonic and rectal polyps are caused by large granulomata. (2, 5) Following an infection with *S. hematobium*, fibrosis of the bladder and ureters develops.

2.1.3. Signs and Symptoms of *Schistosomiasis*

The body's reaction to the worms' eggs is mostly what causes the symptoms of schistosomiasis. Intestinal schistosomiasis may cause fever, diarrhoea, chills, coughing, abdominal pain, and blood in the stool. Liver enlargement is common in more severe cases, and it can occasionally be accompanied by an accumulation of fluid in the peritoneal cavity and hypertension of the abdominal blood vessels. The spleen may also enlarge under these circumstances. Blood in the urine, or haematuria, is a sign of urogenital schistosomiasis. In more severe cases, ureteric and bladder fibrosis as well as kidney impairment may be discovered. Bladder cancer is another possible outcome in the later stages. Women who have urogenital schistosomiasis may have vulva nodules, genital ulcers, vaginal bleeding, pain during sexual activity, and miscarriage. Prostate, seminal vesicles, and other organ diseases can result from male urogenital schistosomiasis. One of the other long-term, irreversible consequences of this disease is infertility.

Figure 2.3: Signs of Intestinal *Schistosomiasis*

Source: (Jauréguiberry & Paris, 2010)

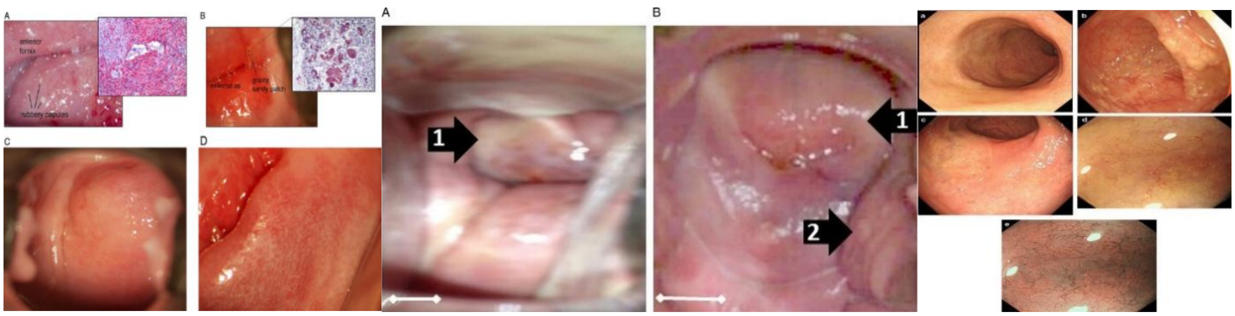

Figure 2.4: Signs of Urogenital *Schistosomiasis*

Source: (EKPO*, et al*., 2017)

2.1.4. Disease Burden of *Schistosomiasis*

As of 2021, *schistosomiasis* was found in 78 countries, predominantly in Africa, Asia, and South America. It was estimated that at least 220.8 million people required preventive treatment for *schistosomiasis* in 2017. The disease is particularly common in poor communities without access to safe drinking water and adequate sanitation (World Health Organization, 2021)

Africa carries the highest burden of the disease, with approximately 90% of those requiring treatment for *schistosomiasis* living there. The most affected African countries include Nigeria, the

United Republic of Tanzania, Ghana, Mozambique, Cote d'Ivoire, and Niger (World Health Organization, 2021)

In the Americas, *schistosomiasis* is found mainly in Brazil, Suriname, and Venezuela. In the Eastern Mediterranean region, it is found in Yemen and Sudan, while in Asia, it is found in the Philippines, Cambodia, Lao People's Democratic Republic, and Indonesia

With approximately 20 million persons afflicted, Nigeria is said to have the biggest number of individuals with the illness. With a broad distribution over the Niger River Basin, the Southwest, Central, and Northeast Highlands, as well as the Chad Basin, *Schistosoma haematobium* is more common in Nigeria. In Nigeria, *Schistosoma mansoni* is widely distributed but less common. The states of Delta, Edo, Ebonyi, Enugu, Nasarawa, and Plateau are recognised to be the most burdened. Both sexes are equally impacted. A significant percentage of the infection affects school-age children, teenagers, and young adults. All year long, transmission takes place.

2.1.5. Epidemiology and Impact on Public Health

Schistosomiasis poses a significant global public health challenge, affecting millions, particularly in tropical and subtropical regions where poverty, inadequate sanitation, and limited access to clean water prevail (World Health Organization, 2023). The disease burden includes both acute and chronic manifestations, leading to various morbidities and, in severe cases, increased mortality (Colley et al., 2014).

Acute schistosomiasis, or Katayama fever, can occur weeks after initial infection, especially in first-time exposures. Symptoms include fever, fatigue, cough, myalgia, headache, and abdominal pain (Li et al., 2021). While often self-limiting, acute schistosomiasis can lead to severe complications, such as pneumonitis (Andrade & Andrade, 2008).

Chronic schistosomiasis arises from long-term exposure, driven by the immune response to trapped *Schistosoma* eggs, leading to granuloma formation and organ damage (Gryseels et al., 2006). *Schistosoma mansoni* and *S. japonicum* primarily affect the hepatointestinal system, causing hepatosplenic schistosomiasis, which can result in portal hypertension and related complications (van der Werf et al., 2003). *Schistosoma haematobium* targets the urogenital system, leading to complications such as hematuria, dysuria, and an increased risk of bladder cancer (Picaud et al., 2010).

Chronic schistosomiasis can also lead to anemia and malnutrition, adversely affecting children's growth and cognitive development (Stephenson et al., 1993). The economic impact includes lost wages and reduced agricultural output, perpetuating poverty in endemic regions (World Health Organization, 2021).

Schistosomiasis has a significant negative impact on health and the economy, and it causes more disability than death. Though the consequences are typically reversible with therapy, schistosomiasis in children can result in anaemia, stunting, and a decreased capacity for learning. In addition to impairing a person's capacity to work, chronic schistosomiasis can occasionally be fatal. It is challenging to determine the exact number of deaths from schistosomiasis because of hidden diseases such bladder cancer, liver and renal failure, and ectopic pregnancies brought on by female genital schistosomiasis. These prominent species and the risks they pose are listed below.

1. *Schistosoma mansoni*

S. mansoni is widely spread in many African countries including the Sudan, Kenya, Madagascar, South America, Middle East, Brazil and India. It is responsible intestinal *schistosomiasis*. In Ethiopia, it can occur in agricultural communities along streams in the altitudes between 1300 and

2000m above sea level. S. mansoni is also reported from all administrative regions. The streams, irrigation schemes and lakes are the major sites harboring the intermediate host like snail. The infection is more common in rural than urban communities. It is more common in developing countries where agricultural products are produced mostly by irrigation. Between 1978 and 1982, the Institute of Pathobiology in Addis Ababa conducted schistosomiasis surveys in all 14 administrative regions, finding that 15% of the population had S. mansoni. According to the 1988–1989 national schistosomiasis survey, the overall prevalence was 25. In Ethiopia, *S. mansoni* is widely dispersed. The prevalence ranged from 10 to 92%, and cases were reported from 225 (62%) and 85 (23%) of the 365 communities investigated for *S. mansoni* between 1961 and 1986 (World Health Organisation, 1984). Streams, lakes, and irrigation systems are the primary sites of transmission. In Ethiopia, the degree of infection varies from place to place and is correlated with its severity.

According to certain research, children and adolescents were more likely to have *S.mansoni* infections (Kloos, Lo, Birrie, Ayele, Tedla, & Tsegay, 1988). Children were therefore more susceptible to environmental pollution. Compared to Christians, the prevalence is higher among Muslims. According to Ayele and Tesfa-Yohannes (1987), the prevalence was 42.4% in males and 26.5% in females. This knowledge is pertinent to the management of schistosomiasis using selective chemotherapy.

Clinical Features

During the cercarial invasion, a type of skin rash called "swimmer's itch" often appears 2 to 3 days later. It causes an itchy, red, bumpy rash on the skin that was exposed. Cercarial dermatitis is a condition that goes away on its own. In the early stage of infection, people might feel feverish, tired, cough, and have general allergic reactions. These symptoms are caused by things the cercariae and schistosomula release. These issues are common in tourists who get infected in areas where the disease is common. In places where the disease is regularly found, people don't usually show these symptoms. Acute schistosomiasis begins when the worms grow and start laying eggs. At this stage, people often have chills, fever, headache, skin rash, high levels of a certain type of white blood cell, swollen liver and spleen, and swollen lymph nodes—this is known as Katayama fever (Braunwald, Fauci, Kasper, Hauser, Longo, & Jameson, 2001).

2. *Schistosoma haematobium* /urinary *Schistosomiasis*

Urinary/vesical schistosomiasis is caused by S. haematobium. The species includes a number of strains. The majority of the 54 nations where S. haematobium is present are located in Africa and the eastern Mediterranean. It may also be found on a few islands in the Indian Ocean as well as on tiny islands off the coasts of eastern and western Africa. Sometimes, infections result from both S. haematobium and S. mansoni because they cohabit in certain areas. The distribution of S. haematobium has increased in many nations as a result of the construction of dams and irrigation systems for flood control and electricity production. Certain regions of Ethiopia are home to S. haematobium. It mostly resides in low-lying areas with elevations of 800 meters or less. It may be found in the Awash Valley, Kurmuk (Western Wolega), and the flood plains of Wabi Shebele close to the Somali border (23, 24, and 25). Although the Awash Valley's infection rate ranges from 5% to 54%, a few cases originate from outside the area. The majority of infections in Kurmuk affect individuals between the ages of 5 and 24. In people between the ages of 10 and 14, the infection rate was 62. 7%. The infection rates were comparable for men and women. The infection rate was higher in males, with 39. 9% in Muslims and 18. 1% in Christians. *S.haematobium* flukes pair up in the blood vessels of the liver and then move to the veins around the bladder, which is called the vesical plexus. The female fluke lays eggs in the small blood vessels of the bladder. These flukes can lay between 200 and 2000 eggs per day. Many of these eggs move through the bladder lining into the bladder itself and are passed in the urine. Eggs can be found in the urine about 12 weeks after infection. About 20% of the eggs stay in the bladder wall and become hard over time. These eggs can sometimes be found in the ureters, rectum, reproductive organs, and even the liver. (Haile-Meskal, Woldemichael, & Lakew, 1985).

3. *Schistosoma japonicum*

S. Japonicum is found in mainland China, parts of the Philippines, and western Indonesia. The signs and effects of S. japonicum infection are similar to those of S. mansoni, but usually worse. This type of worm lays more eggs each day, about 500 to 3500 eggs. Enlarged liver and spleen are common in people of all ages. S. japonicum can infect many animals like water buffaloes, dogs, cats, cattle, pigs, sheep, goats, and wild rats. Doctors usually check for this infection by looking for special eggs in the stool or through a rectal biopsy. These eggs are shaped with a small spine on one side.

4. Schistosoma intercalatum

S. intercalatum is the least common and least harmful type of schistosome that lives in humans. It causes intestinal schistosomiasis. This worm is found in central and western Africa. The most common symptoms are diarrhea and pain in the lower abdomen. *S. intercalatum* lays about 300 eggs each day. The eggs that get stuck in body tissues don't cause as much immune response or damage as eggs from other schistosomes. The highest number of infections and the strongest infections are seen in children aged 5 to 14 years. Doctors can diagnose this by finding the special eggs that have a spine at the end.

5. *Schistosoma mekongi*

S. mekongi is found in Laos, Cambodia, and Thailand, along the Mekong River. Its eggs are similar to those of S. japonicum but a bit smaller and more rounded. Dogs are important in spreading S. mekongi infections.

2.2 Theoretical Framework

The theoretical framework of the research on "*Schistosomiasis* species detection by molecular techniques in Rivers State" provides a conceptual foundation for understanding the relationship between *schistosomiasis*, molecular detection methods, and the specific context of Rivers State. This section will explore key theories and concepts that underpin the research design, data collection, and analysis processes.

2.2.1. Social Determinants of Health:

The Social Determinants of Health (SDH) theory, as proposed by WHO, emphasizes that individual characteristics and broader social and environmental factors influence health outcomes. In the case of *schistosomiasis*, factors such as access to clean water, sanitation facilities, and healthcare services play a significant role in transmission and prevention. By applying the SDH theory, the study will aim to elucidate how social factors impact the prevalence and distribution of *schistosomiasis* species

2.2.2. Health Belief Model (HBM)

The belief model is a psychological framework that helps understand individual behavior and decision-making processes. In this case, it can be applied to explore the beliefs, attitudes, and perceptions of individuals relevant to detecting *Schistosomiasis* species in freshwaters using molecular techniques.

The belief model suggests that an individual's behavior is influenced by their perceived susceptibility to a health issue such as contracting *Schistosomiasis*, the severity of the consequences, the perceived benefits, and barriers to acting (e.g., using molecular techniques for detection), and cues to action (e.g., educational campaigns, awareness programs).

By applying the belief model, researchers can analyze the factors that influence people's behavior towards detecting *Schistosomiasis* species. They can explore factors such as knowledge and awareness of the disease, perceptions of susceptibility and severity, perceived benefits, and barriers to using molecular techniques, and the impact of informational campaigns or educational interventions. Detecting *Schistosomiasis* species in freshwater, the HBM can be applied to understand the factors influencing individuals' engagement in preventive behaviors. For example:

a. **Perceived susceptibility**: Individuals' beliefs about their susceptibility to *Schistosomiasis* can influence their willingness to engage in testing and detection efforts. If individuals perceive themselves to be at high risk due to their exposure to freshwater, they are more likely to act.

b. **Perceived severity**: The perception of the seriousness or severity of *Schistosomiasis* can impact individuals' motivation to detect the disease. Understanding the potential health consequences of the infection can enhance their perceived need for testing.

c. **Perceived benefits**: Awareness of the benefits associated with early detection of *Schistosomiasis*, through molecular techniques, can motivate individuals to participate in testing. For instance, early detection allows for timely treatment, reducing the risk of complications.

2.2.3. One Health Approach

The One Health approach recognizes the interconnectedness of human, animal, and environmental health in addressing complex diseases such as *schistosomiasis*. Given that *schistosomiasis* is a zoonotic disease transmitted through water sources contaminated with parasitic larvae, the One Health perspective is crucial in understanding the ecological factors that contribute to disease transmission. By integrating environmental DNA analysis with human *schistosomiasis* detection, the study aligns with the principles of the One Health approach to provide a holistic understanding of disease dynamics in Rivers State.

2.2.4. Theory of Planned Behavior (TPB)

The Theory of Planned Behavior (TPB) is another theoretical model that can support the study on detecting *Schistosomiasis* species. This model suggests that behavior is influenced by an individual's attitude towards the behavior, the subjective norms surrounding the behavior, and their perceived behavioral control.

In *Schistosomiasis* detection, the TPB can help understand and predict individuals' intentions and actual behaviors related to testing. For example:

a. **Attitude:** Individuals' positive attitudes towards the use of molecular techniques for *Schistosomiasis* detection can increase their intention to engage in such testing. Positive attitudes may stem from the accuracy, efficiency, and reliability of the molecular techniques.

b. **Subjective norms**: The subjective norms surrounding *Schistosomiasis* detection play a role in shaping individuals' intentions. If significant others, such as healthcare providers or family members, positively endorse molecular testing, individuals may be more inclined to participate.

c. **Perceived behavioral control**: Individuals' perception of the ease or difficulty in accessing and undergoing molecular testing can influence their actual behavior. If individuals believe that the process is simple, accessible, and convenient, they are more likely to engage in testing.

By applying the HBM and TPB to the study on *Schistosomiasis* detection in freshwaters using molecular techniques, researchers will gain insights into the factors that influence individuals' behavior and design targeted interventions to encourage participation in testing initiatives. Understanding the beliefs, perceptions, attitudes, norms, and perceived control can guide the development of effective strategies to promote disease detection, prevention, and treatment among at-risk populations.

2.3 Empirical Review

Schistosomiasis is a parasitic disease caused by several species of trematode worms of the genus *Schistosoma* . According to the World Health Organization, *Schistosomiasis* is one of the 20 tropical diseases (World Health Organisation, 2023). This section will focus on different molecular approaches and ways of detecting *Schistosomiasis* species identified by different researchers.

The DNA based examines have recently gained popularity for the detection of infectious diseases, the studies carried out by Ullah, *et al*. (2021) evaluated the sensitivity of cfDNA markers with varying numbers of cercaria and the result suggests that cfDNA markers can be useful for developing a diagnostic tool for the detection of S. japonicum infection. In a similar vein, PCR techniques have exhibited a promising degree of sensitivity and specificity. PCR is also useful in analyzing vaginal lavages which may identify genital *schistosomiasis* (Ajibola, Gulumbe, Eze, & Obishakin, 2118). Okeke, *et al*. (2022) employed the use of polymerase chain reaction (PCR) methods to screen and characterize the Biomphalaria snails from Nkalagu, Nigeria, 212 snails were screened and the result shows (77.4%) of the snails were infected with *Schistosomes*, but only 16 (9.76%) of the snails were positive for S. mansoni infection. Elraheem *et al*., (2021) carried out a cross-sectional study on eighty patients attending the urology clinic of Sohag University Teaching Hospital to detect the molecular prevalence of urine *schistosomiasis* and evaluate microscopic examination vs. PCR technique for detection of *Schistosoma haematobium (S. haematobium)* in urine of patients. The result shows that microscopic examination and PCR were positive among (68.8%) and (87.5%). Microscopy was a good test to rule in cases of urine *schistosomiasis*, with 100% specificity and 100% PPV, but was of limited sensitivity (NPV = 40%) and missed 12.5% of positive cases. They conclude that Urine *schistosomiasis* was highly prevalent in the studied population.

eDNA has equally emerged as one of the powerful molecular techniques for detecting and monitoring various organisms in their natural habitats. The studies conducted by Alzaylaee, *et al*. (2020) on *Schistosoma* species detection by environmental DNA assays in African freshwaters suggest that eDNA monitoring can detect *Schistosomes* in freshwater bodies, but refinement of the field sampling, storage, and assay methods are likely to optimize its performance. They maintained that environmental DNA-based approaches will help to inform epidemiological studies and contribute to efforts to control and eliminate *schistosomiasis* in endemic areas. This accords with Sato, *et al*. (2018) findings in the Maevatanana District of Madagascar, results show one water source with active transmission was identified through the detection of *S. mansonie* DNA in the water and the intermediate host Biomphalariapfeifferi collected from the same water source. In a similar vein, successful detection of *S. mansoni* in freshwater samples by using aquatic eDNA was found by (Sengupta, *et al*., 2019). In laboratory experiments, true eDNA was detected in as few as

10 cercariae per liter of water. The eDNA method furthermore detected schistosome presence at two additional sites where snail shedding failed, demonstrating a higher sensitivity of eDNA sampling. However, they conclude that eDNA provides a promising tool to substantially improve the environmental surveillance of S. mansoni.

Rojas-Caraballo, A., *et al*. (2019) successfully used this design in their study on "Molecular detection of *Schistosoma mansoni* and *Schistosoma haematobium* infections in freshwater snails collected from Kisumu, Western Kenya. Dr. Rojas-Caraballo and colleagues employed a cross-sectional design to investigate the presence of *Schistosoma* parasites in freshwater snails collected from different sites in Kisumu, Western Kenya. which they strategically selected multiple sampling sites, including various types of freshwater habitats, such as rivers, lakes, and ponds, to ensure representation of the diverse ecological settings within the study area. They collected snail samples from these locations during a specified period, following standardized protocols. To detect the presence of *Schistosoma* DNA in the collected snail samples, the researchers employed molecular techniques, specifically PCR amplification and sequencing. The genetic markers targeted by the PCR assays allowed for the identification and differentiation of *Schistosoma* species.

The data obtained from the laboratory analysis were statistically analyzed to determine the prevalence, distribution, and species composition of *Schistosoma* infections in freshwater snails. The findings provided insights into the transmission dynamics of *schistosomiasis* in the study area and contributed to the understanding of the local epidemiology of the disease Weerakoon, Gordon & McManus (2018), reviewed that DNA-based methods represent important screening tools, particularly in those endemic areas with ongoing control where infection prevalence and intensity have been reduced to very low levels. However, they suggest that Nested PCR (nPCR) is a more sensitive and specific approach than cPCR, and has been successfully applied in different instances in the diagnosis of *schistosomiasis*.

Another molecular technique used in detecting *Schistosomiasis* is Loop-mediated isothermal amplification (LAMP) along with other isothermal techniques that appeared in the early 21st century as an alternative to those methods, overcoming some of the aforementioned limitations and achieving a more inexpensive diagnostic (Crego-Vicente, *et al*., 2021). A preliminary study conducted by Crego-Vicen, *et al*. (2021) demonstrated that the genus-specific LAMP assay could be a potential molecular tool to be used for detection, not only for different pure schistosome species but also for hybrids S. haematobium-S. bovis in urine samples. Loop-mediated isothermal amplification (LAMP) methods have been the most popular NA amplification and detection techniques. According to Gray, Ahmad &Kar (2022), LAMP has proven its superiority over PCR by specific amplification of target DNA even in the co-presence of non-target sequences.

(Hind Alzaylaee., 2020) described the creation of new environmental DNA (eDNA) qPCR assays to detect the presence of the human-infecting Schistosoma mansoni, S. haematobium, and S. japonicum. They used genomic DNA preparations to test the assays' specificity across the three species, and the results demonstrated successful target sequence amplification without cross-amplification between the three focal species. Furthermore, we used synthetic DNA from several Schistosoma species to assess the tests' specificity, and the results showed a good overall specificity; nevertheless, the assays for S. japonicum and S. haematobium revealed cross-species amplification with very closely related species. The target species was found to be present in all infected aquaria when we used eDNA samples from aquaria housing infected host gastropods to assess the efficacy of the S. mansoni assay. Lastly, we used eDNA samples from eight distinct natural freshwater sites in Tanzania to assess the efficacy of the S. mansoni and S. haematobium assays. We found that the infection status determined by eDNA and traditional assays of parasite prevalence in host snails showed a strong correlation. According to their findings, eDNA monitoring may be used to identify schistosomes in freshwater environments; however, its effectiveness will probably be maximised by improving field sampling, storage, and assay techniques. Environmental DNA-based methods are expected to support epidemiological research and attempts to eradicate and control schistosomiasis in endemic regions.

Guegan, *et al*. (2019) evaluated two in-house real-time *Schistosoma* PCRs, targeting respectively S. mansoni [Sm] and S. haematobium in excreta, biopsies, and sera as potential tools to diagnose active infections and monitor treatment efficacy. The results depict that *Schistosoma* PCRs clearly outperform standard microscopy on stools and urine and could be part of reference methods combined with WB-based serology, which remains a gold standard for initial diagnosis. In the same line of reasoning.

2.4 Gaps in the Existing Literature.

Despite Substantial efforts, there are critical areas that need further investigation which includes

1. **Limited Application of Molecular Techniques:** Existing literature predominantly relies on conventional diagnostic methods for detecting *Schistosoma* species in freshwater sources, potentially leading to underestimation of prevalence and misidentification of species (Daniel G Colley, Secor, & King, 2015). Applying molecular techniques, such as polymerase chain reaction (PCR) assays, is essential to enhance the sensitivity and specificity of *Schistosoma* detection in environmental samples (Weerakoon, Gordon, & McManus, 2018).
2. **Neglect of Localized Studies:** While national surveys provide valuable epidemiological data, localized studies are crucial for identifying community-specific risk factors and

transmission dynamics (Sukhyun Ryu, 2022). The lack of research focusing on the Bodo Community in Rivers State limits our understanding of this region's unique challenges and opportunities for *schistosomiasis* control.

3. **Inadequate Characterization of Species Diversity:** Current literature often overlooks the diversity of *Schistosoma* species in freshwater bodies, which is critical for designing effective control strategies (Standley, Mugisha, Dobson, & JR, 2012). The Bodo Community may harbor multiple species with varying pathogenicity and transmission dynamics, necessitating detailed molecular characterization.
4. **Sparse Investigation of Environmental Risk Factors:** Studies frequently fail to explore the role of environmental factors in sustaining *Schistosoma* transmission cycles, such as water quality and snail intermediate hosts (Stensgaard *et al*., 2016). Understanding these local ecological drivers is essential for targeted interventions in the Bodo Community.
5. **Lack of data on schistosome prevalence:** There is a lack of data on the prevalence of *schistosomiasis* in Nigeria, particularly in freshwater habitats (El-Nadi, Omran, Ahmed, & Fadel, 2017) (El-Nady *et al*., 2017). This gap in the literature emphasizes the need for more research on the prevalence of *schistosomiasis* in Niger.
6. **Limited Use of Integrated Diagnostic Approaches:** While molecular tools offer high sensitivity, their integration with traditional methods like snail surveys and environmental sampling is underexplored (Bergquist, Zhou, Rollinson, Reinhard-Rupp, & Klohe, 2017). A holistic approach combining molecular techniques with ecological assessments can provide a more comprehensive understanding of *Schistosoma* transmission dynamics

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Design

This study employs a cross-sectional descriptive research design, integrating field sampling and laboratory analysis, to provide a comprehensive investigation of *schistosomiasis* in the Bodo Community. This approach allows for the examination of the current status of *Schistosoma* species diversity, prevalence, and distribution across various aquatic environments within the community. A cross-sectional design is appropriate for this study as it allows for the simultaneous assessment of *Schistosoma* prevalence and distribution across multiple locations within Bodo Community. This approach provides a valuable snapshot of the current epidemiological situation, which is essential for informing targeted interventions and control strategies. The integration of both field sampling and laboratory analysis, incorporating both morphological and molecular techniques, ensures a robust and multi-faceted investigation. Molecular techniques, in particular, offer enhanced sensitivity and specificity for parasite identification, overcoming limitations associated with traditional morphological methods. The study focused on identifying *Schistosoma* species in freshwater rivers, which represent key transmission sites within the Bodo community. A stratified random sampling technique was used to select four major rivers with significant human activity and traffic. This stratification strategy ensured that the sample was representative of the variability in environmental conditions and transmission risk associated with differing levels of human interaction with the riverine ecosystems. Sampling was conducted at designated riverine points, selected based on factors such as water contact patterns, snail habitat suitability, and proximity to human settlements. Samples were then subjected to rigorous laboratory analysis to accurately assess *Schistosoma* prevalence, identification, and distribution. This targeted sampling approach optimized the efficiency of the study and increased the likelihood of detecting *Schistosoma* in locations where it is most likely to be found.

Tembo (2019) successfully utilized a similar design in their study on the molecular detection of *Schistosoma mansoni* and *Schistosoma haematobium* infections in freshwater snails from Kisumu, Western Kenya. Tembo (2019) strategically selected multiple sampling sites, encompassing diverse freshwater habitats (rivers, lakes, and ponds), and employed PCR amplification and sequencing to detect and differentiate *Schistosoma* species DNA in collected snail samples. This study provides a strong precedent for the use of molecular techniques in schistosomiasis research and demonstrates the value of a targeted sampling approach in identifying transmission foci.

3.2. Study Area

Bodo community is located in the southern part of Rivers State, Gokana local government area, Nigeria, specifically in Ogoni land. It is situated in the Niger Delta region, a vast network of rivers, creeks, and mangroves that flow into the Gulf of Guinea, bordered by other towns and villages in Ogoni land and close to the Atlantic Ocean [Neinbarini Zabbey, 2023]. This region is Africa's largest swamp forest habitat after the Congo Basin, characterized by a long rainy season from March-April to October, with precipitation intensifying from 2,500 mm in the north to 4,000 mm along the coast, and a dry season peaking in January and February with a monthly mean of 150 mm rainfall. The Niger Delta is also an extensional rift basin, one of Africa's largest subaerial basins, covering approximately 75,000 square kilometers subaerially and a total of 300,000 square kilometers, with a sediment fill up to 12 kilometers deep, formed by a failed rift junction during the separation of the South American and African Plates. Its unique geology, including the ductile Paleocene Akata Formation, contributes to its immense petroleum wealth. Historically, the delta's inaccessibility protected its rich biodiversity, but the discovery of oil in the 1950s and subsequent infrastructure development opened it up for extensive exploitation, making it highly threatened by human activity.

The unique environmental characteristics of the Bodo community, particularly its low-lying wetland geography and inherent susceptibility to flooding, create conditions highly conducive to the transmission of waterborne diseases such as schistosomiasis. The successful transmission of schistosomiasis depends on suitable climatic conditions and biological events, with its distribution and prevalence significantly influenced by environmental factors affecting both intermediate snail hosts and human hosts.

Figure 3.1: Map of Nigeria showing Rivers state indicating Bodo Community

Source: https://themaritimehub.wordpress.com/tag/bodo-community/

3.3. Population for the Study

The study population comprises the human residents and freshwater ecosystems within the Bodo community, Rivers State, Nigeria. The human population includes individuals residing near and interacting with local water bodies, particularly high-risk groups such as school-aged children, given the reported high prevalence of schistosomiasis in the region. The environmental component consists of freshwater rivers and associated water bodies known to serve as habitats main source of survival such as agriculture, fishing, and trading. The region has fertile land suitable for farming, with crops like cassava, yam, maize, and vegetables being cultivated. Fishing is also a significant economic activity, given the proximity to rivers and water bodies.Bodo community has been reported to have a high prevalence of schistosomiasis, making it a significant area for research.

In the Bodo community, Rivers State, there are several rivers, lakes, and streams such as

- Nuumu-Tekurun Rivers
- Nuumu-Koozo Rivers
- Bon-Sunday River
- Nuumu-Kpe-Nobana Rivers
- Nuumu-Bari-aage Rivers
- Nuumu-Vikpara River
- Nuumu-Kegborozor River.
- Nuumu-Kekpaban River

3.4. Sample and Sampling Method

3.4.1 Sample Size

The sample size for this study comprises of 200 samples size with an average of 50 water samples in each river for Characterization of *Schistosoma* species in freshwater within Bodo community Rivers State, this research involve a sample size selected from eight different rivers and lakes in the area.

To justify the sample size statistically, the following formula was applied:

$n = Z^2 p\ (1\text{-}p)/d^2$

Where

- n = required sample size
- Z = Z-score corresponding to the desired confidence level (1.96 for 95% confidence)
- p = estimated prevalence of schistosomiasis (assumed to be 0.154 based on prior studies)
- d = margin of error (chosen to be 0.05)

$$n = \frac{(1.96)^2 \times 0.154 \times (1-0.154)}{0.0025} = \frac{3.8416 \times 0.154 \times 0.846}{0.0025} = \frac{0.5004990}{0.0025}$$

n =200

The minimum sample size after calculation is 200

3.4.2 Sampling Methods

The research utilizes a stratified random sampling technique to ensure a representative sample from the Bodo community and the selected water bodies. The community was stratified based on critical environmental factors such as proximity to riverbanks, water source types, and socio-economic status. This stratification enhanced the representativeness of the samples and allowed for meaningful comparisons across different groups, as emphasized by Macintyre and Sobel (2001).

This sampling strategy ensured that each river was adequately represented, allowing the research to capture variations in schistosomiasis prevalence across different freshwater environments

Also;

Purposive sampling was also employed to select specific rivers or water bodies exhibiting high human activity. This technique concentrated efforts on locations most likely to have higher concentrations of *Schistosoma* species DNA, optimizing the potential for successful laboratory testing.

Community engagement played a vital role in fostering trust and encouraging participation in the study. Local leaders and community members were actively involved, which promoted ownership of the study and significantly increased response rates (Kumar et al., 2016).

The following rivers are:

i**. Nuumu-Tekurun Rivers:** This River is a vital water source for the community, supporting daily activities like fishing, bathing, and drinking water. It's also a popular spot for social gatherings and ceremonies, fostering community engagement

ii. **Nuumu-Bari-aage Rivers:** This River is a significant water source for the community's agricultural activities, as it provides crop irrigation. It's also a popular spot for fishing and recreation.

iii. **Bon-Sunday River:** This River plays a crucial role in the community's agricultural activities, providing irrigation for farmlands. It's also a popular spot for recreational activities like swimming and fishing.

iv. **Nuumu-Kekpaban River**: This River is an important transportation route for the community, connecting them to nearby markets and trade centers. It's also a popular spot for fishing and recreation

3.5. Instrument for Data Collection

The instruments for data collection comprise of;

i**. Questionnaire:** A structured questionnaire was used to collect data on environmental and behavioral factors that influence exposure to *Schistosoma* infection. The questionnaire, featuring 3 subsections and 19 questions overall, targeted three key research questions, with 200 copies distributed. The questionnaire was administered exclusively in English through face-to-face interaction, allowing for immediate clarification of any ambiguities. It was systematically organized into the following sections: participants' main sources of household water, including protected or unprotected wells, rivers or streams, and piped water (Appendix)It also included questions on whether participants visited water bodies and the frequency of such visits, ranging from daily to monthly. Various water contact activities were evaluated, such as swimming, fetching water, bathing, washing clothes, performing other domestic washing tasks, fishing, participating in religious practices like baptism or worship, and crossing water bodies. Participants were further asked about the frequency of daily water contact whether once, twice, thrice, or more than three times and the duration of each contact, categorized into less than 5 minutes, 5 to 10 minutes, 10 to 15 minutes, or more. The questionnaire also explored sanitation behaviors, including whether individuals urinated in or out of water bodies while swimming and the frequency of defecation or urination in or near water bodies, such as every day, occasionally, once, or uncertain. In addition, participants were asked about the presence of livestock around water bodies, specifying types such as cattle, goats, sheep, or pigs, as well as the presence of freshwater snails, which are known intermediate hosts of *Schistosoma* parasites. This questionnaire was designed to gather comprehensive information on environmental exposures, individual behaviors, and ecological factors that contribute to the transmission of *Schistosoma* in the study population.

ii**. Water Sample Collection and Laboratory Experiment (Materials)**

The instrumentation used include various laboratory apparatus such as Water sample collections (Falcon Conical centrifuge tubes, water sample collector, heparinized bottles (EDTA), cool wool, stains, facemasks, hand gloves, clinical coats (laboratory coats), Giostyle cooling boxes (for sample transportation), ice blocks (to preserve samples at the recommended temperature), and Ziploc bags. DNA extraction was performed using sample preparation and inactivation materials, including pipettes, pipette tips, spin columns, spin tubes, nuclease-free water, viral RNA buffer, viral DNA/RNA shield, a biosafety cabinet, a refrigerated polymerase chain reaction centrifuge (Eppendorf), a mini-spin centrifuge (Eppendorf), facemasks, hand gloves, disposable laboratory coats, 96% ethanol, cotton wool/gauze, and laboratory sample racks. Materials needed for sample elution include Zymo extraction kits, pipettes, pipette tips, spin columns, spin tubes, Eppendorf tubes, nuclease-free water, viral RNA buffer, viral DNA/RNA shield, wash buffer, a biosafety cabinet, a refrigerated polymerase chain reaction centrifuge (Eppendorf), a mini-spin centrifuge (Eppendorf), human blood samples, facemasks, hand gloves, disposable laboratory coats, 96% ethanol, cotton wool/gauze, and laboratory sample racks.

For the preparation of the master mix and template, additional materials used include primers (both forward and reverse), probes, templates (extracted DNAs), pipettes, pipette tips, micro PCR tubes, nuclease-free water, a biosafety cabinet (dead air box), a biosafety cabinet (laminar flow box), a refrigerator, a refrigerated polymerase chain reaction centrifuge (Eppendorf), a mini-spin centrifuge (Eppendorf), a vortex mixer (Fisher brand), facemasks, hand gloves, disposable laboratory coats, 96% ethanol, and cotton wool/gauze. Characterization of species (real-time PCR) will also require micro-PCR tubes and a real-time PCR machine.

3.6. Validity of Instrument

1. **Questionnaire:** Face validity was used to validate this instrument. The study questionnaire was carefully prepared by the researcher and approved by the researcher's supervisors after corrections were made and was deemed suitable for the study, as it able to resolve the research questions and objectives of the study.
2. **Instruments for Water sample collection and Laboratory Experiments**: The validity of the instruments was assured by adhering to standardized protocols for sample collection and analysis since the instruments has been standardized and validated by the manufacturer, therefore requires no further validation as they have been certified and approved to be used for such procedures by relevant professional and regulatory bodies.

3.7 Reliability of Instruments

The Reliability of laboratory materials and equipment was maintained through the use of well-calibrated equipment, training for laboratory personnel, and test replication. Cross-validation with known positive and negative controls was conducted to ensure accurate results.

3.7. Method of Sample Collection

Water samples were collected from freshwater sources in Bodo community (Nuumu-Tekurun, Nuumu-Bari-aage, Bon-Sunday, and Nuumu-Kekpaban River) using containers (Falcon tubes, sterile bottles or flasks)

Representative water samples were collected from various locations (5m apart) within each water body against the water current. Fifty (50) samples (100 cl) were collected from each water body, given a total of two hundred (200) water samples used for the study. The collected water samples were properly preserved using the Tripple packaging system before taken to the laboratory:

3.7.1 Sample Preparation for DNA Extraction and Isolation, Master Mix, Amplification, and Interpretation of PCR Result

3.7.1.1 Sample Preparation Protocol for DNA Extraction and Isolation

The sample preparation protocol consisted of three main steps: buffer preparation, sample preparation, and RNA purification. In the first step, buffer preparation, beta-mercaptoethanol was added to achieve a final concentration of 0.5% (v/v), corresponding to 250 µL or 500 µL per 50 mL or 100 mL of viral RNA buffer. Additionally, 24 mL of 100% ethanol (or 26 mL of 95% ethanol) was mixed with 6 mL of viral wash buffer concentration, or 192 mL of 100% ethanol (or 204 mL of 95% ethanol) was added to 48 mL of the same wash buffer.

In the second step, sample preparation, 200 µL of serum was transferred into an Eppendorf tube, and an equal volume (200 µL) of DNA/RNA Shield (2X concentration) was added. This mixture reacted for 15 minutes before 400 µL was transferred into a new Eppendorf tube for purification. For soil samples, 0.4 g (two scoops) was collected in an Eppendorf tube, and a digester (Proteinase K) was added to convert the soil into liquid. After adding 400 µL of DNA/RNA Shield (2X concentration) to the 200 µL liquid sample, the mixture reacted for 15 minutes before transferring 400 µL into a new Eppendorf tube for purification.

In the final step, RNA purification, 800 µL of viral RNA buffer was combined with each 400 µL sample (2:1 ratio) and mixed well. This mixture incubated for 15 minutes before being transferred to a Zymo-Spin IC Column in a collection tube, followed by centrifugation for 2 minutes at 10,000 rpm. The column was then placed into a new collection tube, where 500 µL of viral wash buffer was added. After centrifuging again for 30 seconds at 10,000 rpm, the flow-through was discarded, and this step was repeated. Subsequently, 500 µL of 95% ethanol was added to the column and centrifuged for 1 minute at 10,000 rpm to ensure complete removal of the wash buffer. The column was then transferred into a nuclease-free tube, and to elute the RNA, 15 µL of DNase/RNase-free water was added directly to the column matrix. After centrifugation for 30 seconds at 10,000 rpm, the filtrate was discarded, and the eluted RNA/DNA was collected in the nuclease-free tube, referred to as the "template" or "eluted RNA/DNA," which was stored at -80°C.

3.7.1.2 Master Mix Protocol

The number of reactions needed was determined to begin the process, and the total volume of master mix required (typically 10-20 µL per reaction) was calculated. In a 1.5-2.0 mL microcentrifuge tube, the master mix was prepared by combining 2X PCR buffer (10 µL per reaction), dNTPs (2 µL per reaction at 10 mM each), $MgCl_2$ (1.5 µL per reaction at 50 mM), forward primer (1 µL per reaction at 10 µM), reverse primer (1 µL per reaction at 10 µM), and Taq polymerase (0.5 µL per reaction at 5 U/µL). Nuclease-free water was then added to achieve the calculated master mix volume. The master mix was vortexed for 10-15 seconds to ensure thorough mixing, followed by centrifugation at 10,000 rpm for 10-15 seconds to collect the liquid at the bottom of the tube. The prepared master mix was aliquoted into individual PCR tubes or a 96-well plate (10-20 µL per reaction), with any remaining master mix stored at -20°C for future use. Next, 1-5 µL of template DNA was added to each PCR tube, and the mixture was gently mixed by pipetting up and down. Finally, the PCR tubes or plates were placed in a thermal cycler, and the PCR program was executed according to the specific protocol.

3.7.1.3 Amplification by real-time PCR

The extracted DNA was amplified using one set of primers, namely ITTS2F (TAA CAA ggT TCC gTA ggT gAA) and ITTS1R (TgC TTA AgT TCA gCg gT), targeting the ITS region of *Schistosoma* , i.e., *S. intercalatum, S. haematobium, S. mansoni* (prevalent in humans), and the SR1 and SR2 regions of *S. mansoni/S. japonicum/S. mekongi* (prevalent in both bovine and human). This was done according to the PCR protocol. The expected amplicon band size was 981 bp. The PCR reaction volume was 20 µL and consisted of 5 ng/µL of the DNA template, 5 µL of nuclease-free water as a non-template control (Sango Biotech amplification kit, China), and 0.5 µM of each primer, performed on a thermal cycler (Magnetic Induction Circular MIC PCR system, Japan). The following PCR conditions were used: 95 °C for 15 minutes of initial denaturation, followed by 45 cycles of 30 seconds at 95 °C, 30 seconds at 56 °C, and 1 minute at 72 °C, using FAM, JOE quencher, and BHQ1.

3.7.1.4 Interpretation of PCR Result

Interpreting PCR results for *schistosomiasis* involved assessing the presence or absence of specific DNA sequences associated with the *Schistosoma* species responsible for the disease. A positive PCR result with a cycle threshold (CT) of less than 45 indicated the presence of *Schistosoma* DNA in the sample, suggesting an active infection, and was further analyzed to identify specific species, such as *Schistosoma* mansoni or *Schistosoma* japonicum. Conversely, a negative PCR result with a CT greater than 45 suggested that *Schistosoma* DNA was not detected, potentially indicating no active infection. However, the timing of sample collection was crucial, as the parasite may not have been present in detectable amounts during certain stages of infection. Negative results could also arise from inadequate sample handling, low parasite load, or mutations in the target DNA sequences that hinder detection. Thus, a comprehensive interpretation required careful consideration of both the PCR results and the clinical context.

3.8 Method of Data Collection

In identifying environmental determinants that potentially influence the prevalence and distribution of schistosomiasis species in freshwater ecosystems, a structured questionnaire was administered to 200 residents living near the studied water bodies (50 individuals per site). The participants were selected from local residents around the freshwater sources to ensure relevance to the study area. The questionnaire was developed through an extensive review of relevant literature.

Separately, 200 water samples were collected from the four rivers (50 samples per river) to analyse for the presence of Schistosoma species. The sample size of 200 participants for the questionnaires was justified using a statistical formula for cross-sectional studies n = Z2p (1-p)/d2. This calculation yielded approximately 196 participants, rounded up to 200 to account for potential variability and non-responses, ensuring adequate statistical power for reliable inferences about environmental factors affecting schistosomiasis in the Bodo Community.

3.9. Method of Data Analysis

Data obtained from questionnaires and freshwater samples were coded and analyzed using the Statistical Package for the Social Sciences (SPSS) version 20.0. Descriptive statistics such as frequencies, percentages, means, and standard deviations were used to summarize participants' characteristics, environmental factors, and *Schistosoma* species prevalence across sites. Inferential statistics were conducted using the Chi-square test to examine associations between categorical variables, including water contact behaviors, environmental risk factors, and infection prevalence. A p-value of less than 0.05 was considered statistically significant. For molecular data, polymerase chain reaction (PCR) results were analyzed by comparing DNA bands with molecular weight markers to identify *Schistosoma* species. The proportion of each species was calculated relative to the total positive samples, and spatial variations across river sites were assessed using cross-tabulations and Chi-square tests.

3.10. Ethical Consideration and Informed Consent

The study and verbal consent process will receive ethical approval from the head of the Department of Public Health, Federal University of Technology Owerri (Appendix……). Furthermore, ethical approval will be sought and obtained from the Rivers State Ministry of Health.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1: Environmental risk factors of *Schistosoma*

Summarized in Table 4.1, figure 4.1 and 4.2 are the environmental risk factors of *Schistosoma* . It was shown that out of the 200 participants used for the study, 138(69%) of them had piped water as their main source of water for the households, 29(15%) used Protected/covered water well, 24(12%) used River /stream whereas 9(5%) used unprotected/uncovered water well as main source of water for the households. The frequency of visits to water bodies was once a week for the majority, with 88 (44%) following, then by those who visit once a month, 58 (29%), once every two days 27(15%), and every day 69(35%). Depicted in Fig 4.1 are the different water contact activities undergone by the respondents. As indicated, the majority of them, 159(80%), swim in the river, followed by those who bathe, 142(71%), and wash clothes, 137(69%), in the river. However, large number of them do not fetch water from the river 115(58%), do other washings in the river 151(76%), fish in the river 114(57%), undergo baptism & worship 179(90%) and cross the water bodies 157(79%). Based on the time taken for activities while in contact with water, it was shown that greater number 101(51%) of the subjects spend more than 15 minutes unlike the least of them 12(6%) that spends less than 5 minutes (Figure 4.2).

In addition, Table 4.1 demonstrated that while swimming in water bodies numerous of them 130(82%) preferred to defecate/urinate in water compared to those that defecate/urinate outside the water 29(18%). How many times of defecation/urination in water bodies was represented as four categories: Every day and when nature calls (63 or 40%), occasionally and when around the river (38 or 24%), cannot remember (37 or 23%) and every day and when nature calls (21 or 13%). Also, almost all the participants revealed that there was presence of livestock around the river 189(95%) which was mostly Cattle (61%) followed by Pigs (17%), Sheep (12%) and Goats (10%). Equally, most of them 177(89%) indicated that there are Presence of snails around the river.

Table 4.1: Environmental risk factors of *Schistosoma*

Variables	Frequency	Percentage
The main source of water for the households		
River /stream	24	12
Piped water	138	69
Protected/covered water well	29	15
Unprotected/uncovered water well	9	5
Total	**200**	**100**
Frequency of visits to water bodies		
Every day	12	6
Once in two days	29	15
Once a week	101	51
Once a month	58	29
Total	**130**	**100**
Swimming in water bodies, where do you defecate/urinate		
Urinate in water	130	82
Urinate out of water	29	18
Total	**159**	**100**
How many times of defecation/urination in water bodies		
Every day and when nature calls	16	12
Cannot remember	31	24
It was only once	53	41
Occasionally and when around the river	30	23

Total	**130**	**100**
Presence of livestock around the river		
Yes	189	95
No	11	6
Total	**200**	**100**
Type of livestock around the river		
Cattle	115	61
Goats	19	10
Sheep	23	12
Pigs	32	17
Total	**189**	**100**
Presence of snails around the river		
Yes	177	89
No	23	12
Total	**200**	**100**

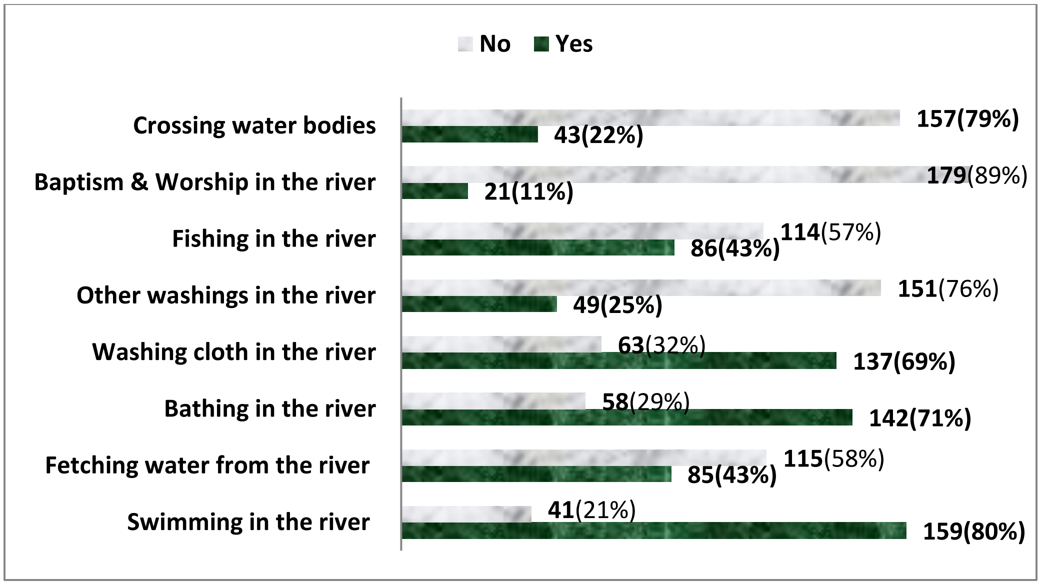

Figure 4.1: Water Contact Activities

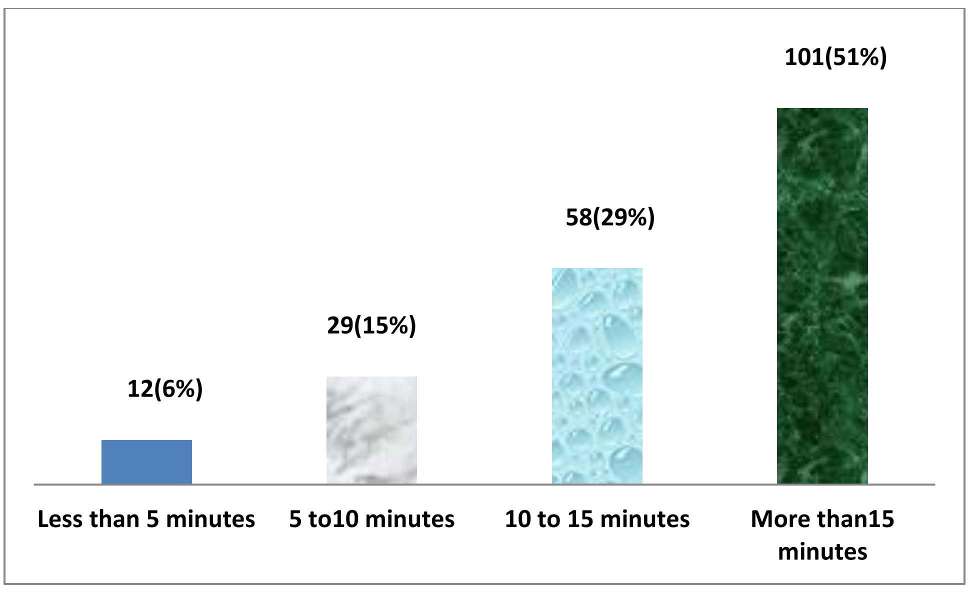

Figure 4.2: Time taken for activities in contact with water

4.1.2: Prevalence of *Schistosoma* species in freshwater

Table 4.2 presents the results of the Prevalence and species of *Schistosoma* identified in the freshwater studied. From the findings, out of the 200 water samples it was indicated that few of them 37(18.5%) showed presence of *Schistosoma* compared to 163(81.5%) without *Schistosoma*, suggesting that the prevalence of *Schistosoma* in the studied water samples is 18.5%.-Moreover, *Schistosoma* species identified in the infected water samples were mainly *Schistosoma* japonicum 15 (40.5%) followed by S. mansoni 12 (32.4%) and S. haematobium 10 (27%).

Table 4.2: Prevalence of *Schistosoma* species in freshwater

Variables	Frequency	Percentage	X^2	p-value
Prevalence of *Schistosoma*				
Positive	37	18.5		
Negative	163	81.5		
Total	**200**	**100**	**79.38**	**< 0.001**
***Schistosoma* species identified**				
Schistosoma mansoni	12	32.4		
S. haematobium	10	27.0		
S. japonicum	15	40.5		
Total	**37**	**100**	**1.03**	**0.598**

4.1.3: Prevalence of *Schistosoma* across different freshwater studies

Summary of the prevalence of *Schistosoma* across the different freshwater studies is depicted in Figure 4.3, whereas Table 4.3 summarizes *Schistosoma* species across different freshwater studies. As illustrated in Figure 4.3, there was an insignificant (X^2= 2.46; p=0.483) difference in the prevalence of *Schistosoma* across the different freshwater studies. However, there was higher prevalence in Nuumu-Tekurun 12(32.4%) compared to Nuumu-Kekpaban 11(29.7%), Nuumu-Bari-aage 8(21.6%) and Bon-Sunday 6(16.2%).

In addition, no significant difference (X^2= 2.46; p=0.483) was observed in the distribution of different species of *Schistosoma* across the different freshwater sites studied (Table 4.3). However, it was shown that there was a greater prevalence of *Schistosoma* mansoni in Nuumu-Bari-aage 5(41.7%) followed by equal distribution in 3(25%) in Nuumu-Tekurun and Nuumu-Kekpaban and 1(8.3%). Comparatively, the prevalence of S. haematobium was higher in Nuumu-Tekurun 4(40%) followed by an equal 2(20%) distribution in Nuumu-Bari-aage, Bon-Sunday, and Nuumu-Kekpaban. In addition, the distribution of S. japonicum was greater in Nuumu-Kekpaban 6(40%) followed compared to 5(33.3%), 3(20%) and 1(6.7%) obtained in Nuumu-Tekurun, Bon-Sunday and Nuumu-Bari-aage respectively.

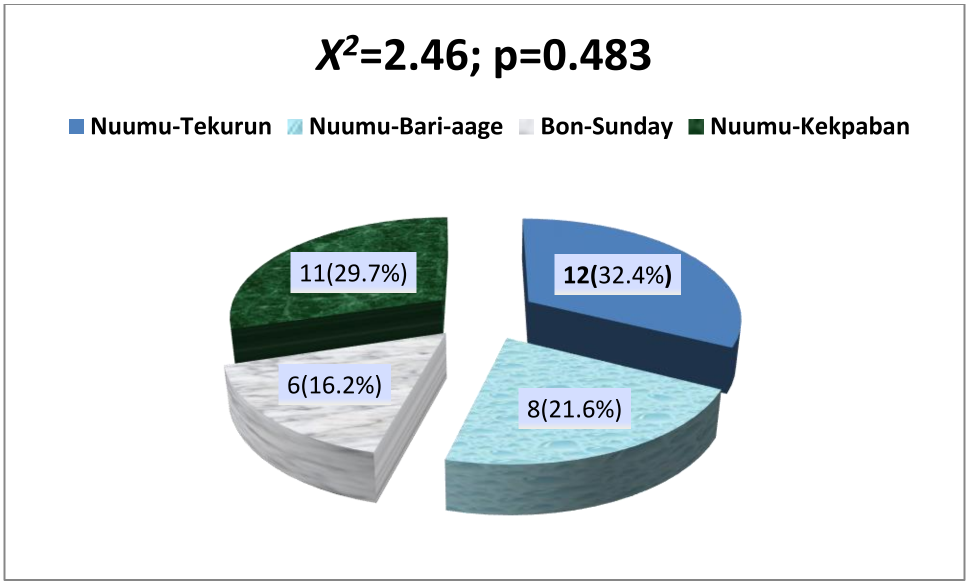

Figure 4.3: Prevalence of *Schistosoma* across different freshwater studied

Table 4.3: ***Schistosoma*** **species across different freshwater studied**

***Schistosoma* species**	**A**	**B**	**C**	**D**	**Total**
Schistosoma mansoni	3(25%)	5(41.7%)	1(8.3%)	3(25%)	12(32.4%)
S. haematobium	4(40%)	2(20%)	2(20%)	2(20%)	10(27%)
S. japonicum	5(33.3%)	1(6.7%)	3(20%)	6(40%)	15(40.5%)
Total	**12(32.4%)**	**8(21.6%)**	**6(16.2%)**	**11(29.7%)**	**37(100%)**
			X^2=5.80; p=0.446;DF=6		

A=Nuumu-Tekurun, B=Nuumu-Bari-aage, C=Bon-Sunday, D=Nuumu-Kekpaban

4.1.4: Environmental risk factors influencing *Schistosoma*

Table 4.4a and b present the results of the association between environmental risk factors and *Schistosoma*. From the results, all the environmental risk factors exhibited strong ($p \leq 0.027$) influence on *Schistosoma* prevalence, the type of livestock around the river ($p=0.068$), and washing clothes in the river ($p=0.360$). Out of 37 freshwaters with *Schistosoma*, greater prevalence of *Schistosoma* occurs among the river locations where participants (38%) use River /stream as main source of water for the households. Prevalence was also greater where the frequency of visit to water bodies was every day (41%) and more than 15 minutes spent during contact with water (38%). Equally, there was a higher (73%) prevalence in freshwater where most participants defecate/urinate, especially every day and when nature calls (41%). More *Schistosoma* (76%) was also found in waters where livestock (76%) visit, particularly Cattle (49%). Presence of snails as well had a significant influence on *Schistosoma* with higher (62%) prevalence recorded in waters with snails around it (Table 4.4a). Furthermore, higher *Schistosoma* was found in freshwater due to swimming (54%), fetching (73%) and bathing (78%) in river. As also shown, other washings in the river (78%), fishing (81%) and baptism & worship (51%) in the river demonstrated serious association with *Schistosoma* ($p < 0.001$). Moreover, prevalence was higher as result of crossing of water bodies (51%) (Table 4.4b).

Table 4.4a: Environmental risk factors influencing *Schistosoma*

Variables	Positive	Negative	Total	X^2	p-value
Main source of water for the households					
River /stream	14(38%)	10(6%)	24(12%)		
Piped water	9(24%)	129(79%)	138(69%)		
Protected/covered water well	5(14%)	24(15%)	29(15%)		
Unprotected/uncovered water well	9(24%)	0(0%)	9(5%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**78.07**	**< 0.001**
Frequency of visit to water bodies					
Every day	15(41%)	54(33%)	69(35%)		
Once in two days	12(32%)	15(9%)	27(14%)		
Once a week	5(14%)	11(7%)	16(8%)		
Once a month	5(14%)	83(51%)	88(44%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**18.12**	**< 0.001**
Time taken for activities in contact with water					
Less than 5 minutes	8(22%)	4(2%)	12(6%)		
5 to10 minutes	9(24%)	20(12%)	29(15%)		
10 to 15 minutes	5(14%)	53(33%)	58(29%)		
More than15 minutes	15(41%)	86(53%)	101(51%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**26.13**	**< 0.001**
Swimming in water bodies where do you defecate/urinate					
Urinate in water	10(27%)	120(98%)	130(86%)		
Urinate out of water	27(73%)	2(2%)	29(15%)		
Total	**37(100%)**	**122(100%)**	**159(100%)**	**96.87**	**< 0.001**
How many times of defecation/urination in water bodies					

Every day and when nature calls	15(41%)	1(1%)	16(12%)		
I do not remember	9(24%)	22(24%)	31(24%)		
It was only once	11(30%)	42(45%)	53(41%)		
Occasionally and when around the river	2(5%)	28(30%)	30(23%)		
Total	**37(100%)**	**93(100%)**	**130(100%)**	**42.05**	**< 0.001**
Presence of livestock around the river					
Yes	28(76%)	161(99%)	189(95%)		
No	9(24%)	2(1%)	11(6%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**30.95**	**< 0.001**
Type of livestock around the river					
Cattle	18(49%)	98(60%)	116(58%)		
Goats	8(22%)	15(9%)	23(12%)		
Sheep	7(19%)	19(12%)	26(13%)		
Pigs	4(11%)	31(19%)	35(18%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**7.11**	**0.068**
Presence of snails around the river					
Yes	23(62%)	154(94%)	177(89%)		
No	14(38%)	9(6%)	23(12%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**30.94**	**< 0.001**

Table 4.4b: Environmental factors influencing *Schistosoma*

Variables	Positive	Negative	Total	X^2	P-value
Swimming in the river					
Yes	20(54%)	139(85%)	159(80%)		
No	17(46%)	24(15%)	41(21%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**18.04**	**< 0.001**
Fetching water from the river					
Yes	27(73%)	58(36%)	85(43%)		
No	10(27%)	105(64%)	115(58%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**17.25**	**< 0.001**
Bathing in the river					
Yes	29(78%)	113(69%)	142(71%)		
No	8(22%)	50(31%)	58(29%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**10.3**	**0.027**
Washing cloth in the river					
Yes	23(62%)	114(70%)	137(69%)		
No	14(38%)	49(30%)	63(32%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**0.85**	**0.360**
Other washings in the river					
Yes	29(78%)	20(12%)	49(25%)		
No	8(22%)	143(88%)	151(76%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**71.24**	**< 0.001**
Fishing in the river					

Yes	30(81%)	56(34%)	86(43%)		
No	7(19%)	107(66%)	114(57%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**26.86**	**< 0.001**
Baptism & Worship in the river					
Yes	19(51%)	2(1%)	21(11%)		
No	18(49%)	161(99%)	179(90%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**80.62**	**< 0.001**
Crossing water bodies					
Yes	19(51%)	24(15%)	43(22%)		
No	18(49%)	139(85%)	157(79%)		
Total	**37(100%)**	**163(100%)**	**200(100%)**	**19.83**	**< 0.001**

4.2 Discussion

Characterization of *Schistosoma* species in freshwater of Bodo community, Rivers State, was conducted by this study. From the findings, piped water was the major source of water for most of the households, contrary to reservoir being the source of water in the study carried out by Kaleson *et al*. (2022) in Shelleng Local Government Area, Adamawa State. However, water contact activities were mainly as a result of swimming (80%), bathing (71%), and washing of clothes (69%) in the river. In similar study in Doma Local Government Area, Nasarawa State, a high number of the respondents 232 (45.5%) use pipe-borne water for domestic purposes in their homes whereas 87 (17.0%) of them utilized well water as the main source of water for domestic purposes while 191 (37.5%) accessed water from rivers, streams and pools (Pam *et al*. 2021).

Besides, fewer proportions of the participants visit the river for fetching water (43%), fishing (43%), other washings (25%), crossing of the water bodies (22%), and baptism & worship (11%). This result of the least of the population having water contact via baptism & worship was in congruence with the report of Ntajal *et al*. (2021) that only one percent of the entire sample population had water contact for cultural/religious reasons, e.g., during the "holy baptism". Moreover, previous workers (Ahmad and Bassey, 2010) found that in the exposure water contact activities, washing clothes and utensils had 561(14.1%), collecting water 548(13.8%), crossing water or fording 143(3.6%) and fishing had 58(1.5%). Moreover, these visitations were mostly done once a week (44%), and activities lasted more than 15 minutes (51%). Lower exposure duration (6 and 30 min) was recorded in the study of Sidibé *et al*. (2024). However, in the findings of Ahmad and Bassey (2010), the time spent as well the intensity of different exposure water contact activities varies and ranged from (30minutes -1hour 30 minutes), for washing clothes and utensils, collection of water (5- 10minutes), crossing of water or fording (2- 5minutes), fishing (1-2hours).

Out of 159 that swim in the rivers, most of them (82%) had defecated/urinated at least once in water bodies while swimming. In contrast, the contaminative activities (defecation and urination) were only 49(1.2%) in the study of Ahmad and Bassey (2010). Furthermore, in the present study, there was the presence of livestock (95%), especially Cattle (61%) around the river, and there was also the presence of snails (89%). Both *S bovis* and *S curassoni* can infect a range of livestock hosts, and the ability of parasites and pathogens to infect multiple host species is a risk factor for disease emergence in humans (Taylor *et al*., 2001). Moreover, previous studies have hypothesised that, if *Schistosomes* were to infect livestock species, they might migrate to the urogenital system, and eggs might be transmitted via the urinary route (Webster *et al*., 2013; Djuikwo-Teukeng *et al*., 2019). Additionally, according to Colley *et al*. (2014) and Machacek *et al*. (2018), transmission to humans occurs when the free-swimming larval form (cercaria) is shed from infected freshwater snails and penetrates the skin of the definitive host. Once mature, the male and female *Schistosomes* mate, reproduce, and produce eggs that are excreted via urine or faeces, depending on the species. Eggs excreted into freshwater environments hatch and release mobile miracidia which infect snails to continue the cycle (Colley *et al*., 2014).

Based on the PCR results (Appendix 1) that stated that cycle threshold values (*Ct*) less than 45 is positive, ct above 45 is negative, *ct* below 10 is negative, *ct* 10 to 8 is borderline negative, *ct* 43 to 45 is borderline positive., it was shown that out of the 200 water samples analyzed, few of them 37(18.5%) shows presence of *Schistosoma* with significant association (X^2= 79.38; p< 0.001). Moreover, *Schistosoma* species identified in the infected water samples was mainly *Schistosoma* japonicum 15 (40.5%) followed by S. mansoni 12 (32.4%) and S. haematobium 10 (27%), indicating the weak (X^2=1.03*; p*=0.598) dominance of S. japonicum. In similar work, when evaluated by qPCR, Worrell et al. (2014) reported that 7 of the 108 field water samples were positive for *S. japonicum*, with threshold cycle (*CT*) values corresponding to a cercarial count ranging between ~1 cercaria and 2.5 cercariae. Comparatively, total prevalence of cercarial infections in the study of Chontananarth and Wongsawad (2013) was 17.27% in freshwater snails. Both the miracidia which emerges from the eggs in fresh water and the cercaria which emerges from infected snails are non-feeding larval stages and they target the molluscan and mammalian hosts respectively (Nelwan, 2019). Whilst the cercaria can infect several mammals, including mice, humans, livestock and ruminants, the miracidia infect only snails, and they are highly specific to the genus of snails they infect (McManus *et al*., 2018). However, in the present study, there was an insignificant (X^2= 2.46; p=0.483) difference in the prevalence of *Schistosoma* across different freshwater studies even though higher prevalence was in Nuumu-Tekurun 12(32.4%). In addition, no significance difference (X^2= 2.46; p=0.483) was observed in the distribution of different species of *Schistosoma* across the different freshwater studied. However, there was greater prevalence of *Schistosoma* mansoni, S. haematobium and S. japonicum in Nuumu-Bari-aage (41.7%), Nuumu-Tekurun (40%) and Nuumu-Kekpaban (40%) respectively.

Furthermore, all the environmental risk factors exhibited strong (p≤0.027) influence on *Schistosoma* prevalence with the exception of type of livestock around the river (p=0.068) and washing cloth in the river (p=0.360). Greater prevalence of *Schistosoma* occurred among the river locations where participants (38%) use River /stream as main source of water for the households, when frequency of visit to water bodies was every day (41%) and more than15 minutes spent during contact with water (38%). This correlates with a study by Kiran *et al*. (2016), who reported a prevalence of 75% among those whose source of water contact is the river in Sokoto state. This is because rivers and dams provide grasses and shrubs, which serve as a suitable substrate for snail vectors, hence encouraging a rapid increase in the population of snails with a subsequent increase in cercarial load Onosakponome *et al*. (2022). Moreover, there was higher (73%) prevalence when most participants defecate/urinate in water, especially every day and when nature calls (41%). Higher rate of *Schistosoma* also occurred when livestock (76%) in particularly Cattle (49%) and snails (62%) were found around the river. The schistosome parasite has a complex life cycle that involves two hosts: a freshwater snail, which acts as the intermediate host in which the parasite undergoes larval development, and the definitive hosts (humans or animals) in which the parasite matures into an adult (Boissier *et al*., 2019).

In addition, higher *Schistosoma* was due to swimming (54%), fetching (73%), bathing (78%), other washings in the river (78%), fishing (81%), baptism & worship in the river (51%) and crossing of water bodies (51%). The prevalence of infection was substantially linked ($P < 0.01$) with activities including swimming, bathing or playing in water, washing, and collecting edible snails from streams, ponds, or rivers. These activities could be considered risk factors in the area (Houmsou et al., 2009). However, even when the effect size was unadjusted, self-reported contact with open freshwater sources was not significantly linked to schistosome infection, according to a nationally representative cross-sectional survey conducted in Uganda by Exum et al. (2019) (prevalence ratio 1.08; 95% CI: 0.9–1.26). Similarly, Codjoe and Larbi (2016) also out that children who frequently came into touch with water, including collecting water for family use, had comparatively high levels of cercariae exposure in Ghana's Densu Basin. In the Eastern area of Ghana and the Migori county in Kenya, where schoolchildren traverse water bodies barefoot on their way to and from school, occupational water-contacts including fishing and water crossing have been identified as exposure factors (Martel et al., 2019; Ng'ang'a et al., 2016). Thus, it is clear that water interaction occurs frequently and is often inevitable. Furthermore, it was discovered that the frequency and duration of water contact, which are correlated with exposure level, are connected to the type of water contact activity. Recreational water-contact activities happen less frequently but typically last longer, whereas domestic water-contact activities are associated with more frequent (albeit brief) water-contact activities. Comparable trends were noted in a study conducted in Tanzania's Shinyanga District, which found that swimming increased exposure to schistosomiasis since it was positively connected with longer periods of water contact (Angelo et al., 2018).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The identification and characterization of Schistosoma species among the population of Bodo, located in Rivers State, revealed significant associations between water contact activities and the incidence of schistosomiasis. The survey identified piped water as the most common household source; however, regular activities such as swimming and bathing, which typically last over 15 minutes, increase exposure to Schistosoma. The presence of livestock, particularly cattle and snails, near the river also substantially impacted infection rates. Schistosoma japonicum was the most prevalent species detected, followed by S. mansoni and S. haematobium. Contact with various environmental factors, including daily interactions with water bodies, defecation or urination in the water, and using the river for various activities such as washing, fishing, and bathing, significantly influenced the prevalence of Schistosoma infection. Overall, the findings emphasize the importance of improving sanitation, promoting public health education, and implementing effective water resource management techniques to reduce the transmission of schistosomiasis within the population.

This study successfully identified and characterized Schistosoma species in freshwater sources within Bodo Community, Rivers State, establishing clear associations between water contact activities and schistosomiasis prevalence. Despite the predominance of piped water as a household source, frequent swimming, bathing, and washing in local rivers significantly increased exposure risk. The presence of livestock particularly cattle and freshwater snails near the rivers further contributed to transmission. Schistosoma japonicum emerged as the most prevalent species, *followed by S. mansoni and S. haematobium. Overall, the findings highlight the role of environmental and behavioral factors in sustaining schistosomiasis transmission.*

5.2 Recommendations

From the findings of this research, the following were recommended;

- Although there was a low prevalence of *Schistosoma*, constant surveillance is needed to avoid the emergence of massive increase in *Schistosoma* and associated disease in Bodo community, Rivers State.
- There is a need to enhance health education programmes by environmental health workers and civic societies among local inhabitants about the potential risk of contact with water body. Education campaigns about schistosomiasis should concentrate on the disease's causes, transmission, prevention, and treatment, as well as how the infection affects local residents' health and diet.

  • Reducing the need to use surface water for household functions, which lowers the frequency of exposure, requires ensuring a sufficient and safe supply of drinking water.
- There is now a need to extend the survey into other bodies not investigated by the present study as this will assist in the generalization of findings and information with respect to prevalence of *Schistosoma* in Rivers State.

Recommendations

Based on the findings of this study, the following recommendations are made:

1. **Sustained Surveillance**

Although the prevalence of *Schistosoma* infection in Bodo Community was relatively low, continuous surveillance and routine monitoring of freshwater sources are essential to prevent future outbreaks and detect emerging transmission early.

2. **Health Education and Community Engagement**

Environmental health officers and community-based organizations should intensify health education programs focusing on the risks of contact with infested water, modes of transmission, prevention, and treatment of schistosomiasis. Public awareness campaigns should also emphasize the impact of the infection on health and nutrition.

3. **Improved Water Supply and Sanitation**

The government and relevant agencies should ensure access to adequate and safe water for domestic use to minimize dependence on contaminated surface water sources. Provision of sanitation facilities will further help reduce contamination of local water bodies.

4. **Expanded Epidemiological Surveys**

Further studies should be extended to other freshwater bodies within Rivers State to provide a more comprehensive understanding of *Schistosoma* distribution and to support effective public health interventions across the region.

5. **Integrated Control Measures**

A holistic approach combining snail control, environmental sanitation, and health education should be adopted to interrupt the transmission cycle and promote long-term control of schistosomiasis in the community.

Contribution to knowledge

Contribution Area	Description
1. Local Evidence of Species Distribution	The study establishes the presence and distribution of *Schistosoma japonicum*, *S. mansoni*, and *S. haematobium* in Bodo freshwater bodies, providing baseline molecular evidence of species diversity in the Niger Delta region.
2. Integration of Molecular Diagnostics in Field Surveillance	Demonstrates the application of molecular techniques for accurate detection and characterization of *Schistosoma* species, improving upon traditional microscopy-based identification methods.
3. Link Between Environmental Factors and Transmission	Identifies key environmental and behavioral risk factors such as livestock presence, snail abundance, and human water-contact activities influencing schistosomiasis transmission in the community.
4. Public Health Implications for Control Strategies	Provides evidence to inform public health policies and control programs, emphasizing safe water supply, sanitation improvement, and health education as effective interventions.
5. Baseline for Future Research	Offers molecular and epidemiological data that can serve as a reference point for future research and surveillance efforts on schistosomiasis in Rivers State and similar endemic areas.

REFERENCES

Ahmad, M. M., & Bassey, S. E. (2010). Study on the pattern of water contact activities in *Schistosomiasis* endemic area of Wudil, Kano, Nigeria. Biological and Environmental Sciences Journal for the Tropic (BEST), 7(2), 176-180.

Andrade, Z. A., & Andrade, S. G. (2008). Pathogenesis of *Schistosoma* mansoni infection. Memórias do Instituto Oswaldo Cruz, 103(7), 724-734.

Angelo, T., Buza, J., Kinung'Hi, S. M., Kariuki, H. C., Mwanga, J. R., Munisi, D. Z., ... & Wilson, S. (2018). Geographical and behavioral risks associated with *Schistosoma* haematobium infection in an area of complex transmission. Parasites & Vectors, 11, 1–9. https://doi.org/10.1186/s13071-018-3064-5.

Bergquist, N. R., Fulford, A. J. C., Vennervald, B. J., & Dunne, D. W. (2005). *Schistosomiasis* vaccine development: prospects and challenges. Immunological Reviews, 206(1), 175-196.

Bergquist, R., & Xiao, S. H. (2019). *Schistosomiasis*: A global overview of the control efforts. Infectious Diseases of Poverty, 8(1), 16.

Boissier, J., Mouahid, G., & Moné, H. (2019). *Schistosoma* spp. Global Water Pathogen Project, Michigan State University. 10.14321/waterpathogens.45. hal-02378853

Brown, D. S. (1994). Freshwater snails of Africa and their medical importance. CRC press.

Cavalcanti, M. G., Melo, A. L., & Pontes, L. A. (2013). Molecular detection of *Schistosoma* spp. in fresh water: Implications for public health. BMC Infectious Diseases, 13, 16.

Cheesbrough, M. (2006). District laboratory practice in tropical countries, Part 1. Cambridge University Press.

Cheever, A. W., & Andrade, Z. A. (1967). Pathologic lesions in rhesus monkeys infected with *Schistosoma* mansoni. The American Journal of Tropical Medicine and Hygiene, 16(1), 34-46.

Chontananarth, T., & Wongsawad, C. (2013). Epidemiology of cercarial stage of trematodes in freshwater snails from Chiang Mai province, Thailand. Asian Pacific Journal of Tropical Biomedicine, 3(3), 237-243.

Cioli, D., Bottero, L., & Lizzi, S. (2006). The chemotherapy of *schistosomiasis*: new drugs and perspectives. Pharmacology & Therapeutics, 112(1), 143-162.

Cnops, L., & Van der Werf, M. J. (2013). The role of molecular methods in the diagnosis of intestinal *schistosomiasis*. Journal of Infectious Diseases, 207(1), 75-80.

Colley, D. G., Bustindil, A. L., Secor, W. E., & King, C. H. (2014). Human *schistosomiasis*. The Lancet, 383(9936), 2253-2264.

Colley, D. G., Bustinduy, A. L., Secor, W. E., & King, C. H. (2014). Human *schistosomiasis*. Lancet, 383(9936), 2253–2264. https://doi.org/10.1016/S0140-6736(13)61949-2

Crego-Vicente, M. et al. (2021). Loop-mediated isothermal amplification: A novel molecular tool for the detection of *schistosomiasis*. Trends in Parasitology, 37(1), 87-96.

Creswell, J. W., & Plano Clark, V. L. (2011). Designing and conducting mixed methods research. Sage Publications.

Djuikwo-Teukeng, F. F., Kouam Simo, A., Allienne, J-F., et al. (2019). Population genetic structure of *Schistosoma* bovis in Cameroon. Parasites & Vectors, 12, 56.

Doenhoff, M. J., Cioli, D., & Utzinger, J. (2008). Praziquantel: mechanism of action, resistance and new derivatives for *schistosomiasis*. Current Opinion in Pharmacology, 8(6), 778-785.

Dorkenoo, M. A., et al. (2023). Environmental factors affecting the transmission of *schistosomiasis* in Africa. Environmental Research and Public Health, 20(1), 123-135.

Ekpo, U. F., & Mafiana, C. F. (2020). *Schistosomiasis* in Nigeria: A review on its prevalence and socioeconomic impact. Journal of Infectious Diseases and Epidemiology, 6(1), 1-7.

Ekpo, U. F., & Mafiana, C. F. (2020). *Schistosomiasis* in Nigeria: An update. Parasitology, 147(1), 1-18.

Evans, D. B., Maxwell, L., Anderson, R. M., & Donnelly, C. A. (2003). The economic impact of *schistosomiasis* in sub-Saharan Africa. Acta Tropica, 86(2-3), 173-195.

Exum, N. G., Kibira, S. P. S., Ssenyonga, R., Nobili, J., Shannon, A. K., Ssempebwa, J. C., ... & Makumbi, F. E. (2019). The prevalence of *schistosomiasis* in Uganda: A nationally representative population estimate to inform control programs and water and sanitation interventions. PLoS Neglected Tropical Diseases, 13(8), e0007617.

Federal Ministry of Health Nigeria. (2013). National guidelines for the prevention and control of *schistosomiasis*.

Fenwick, A. (2012). The global burden of neglected tropical diseases. The Lancet, 379(9829), 1960-1969.

Fernández, S. A., O'Neill, S. M., & McMahon, D. F. (2019). Progress in the detection of *Schistosoma* DNA in fecal and urinary samples. Parasites & Vectors, 12, 75.

Gong, Y., McManus, D. P., & Qian, Y. J. (2021). *Schistosomiasis* japonica. The Lancet, 397(10285), 1641-1654.

Grimes, J. E. T., Croll, D., Garn, J. V., , S. L., Templeton, M. R., & Gray, D. J. (2015). The roles of water, sanitation and hygiene in reducing *schistosomiasis*. PLoS Neglected Tropical Diseases, 9(3), e0003571.

Gryseels, B., Polman, K., Clerinx, J., & Kestens, L. (2006). Human *schistosomiasis*. The Lancet, 368(9547), 1602-1618.

Hamburger, J., Ramzy, R. M. R., Ismail, M., El-Baz, T., & Schwartz, E. (2004). Evaluation of PCR for the diagnosis of *Schistosoma* infections in urine, stool, and blood samples. The American Journal of Tropical Medicine and Hygiene, 70(4), 348-353.

Hotez, P. J., Bottazzi, M. E., Franco-Paredes, C., Aultman, E. R., & Roses Periago, M. (2006). The neglected tropical diseases of Latin America and the Caribbean: A review of disease burden and distribution and a roadmap for control and elimination. PLoS Neglected Tropical Diseases, 2(9), e300.

Houmsou, R. S., Amuta, E. U., & Tsar, T. (2012). Profile of an epidemiological study of urinary *Schistosomiasis* in two Local Government Areas of Benue State, Nigeria. International Journal of Medicine and Biomedical Research, 1(1), 39-48.

Hunter, G. W., Swartzwelder, J. C., & Clyde, D. F. (1993). Tropical Medicine. Lea & Febiger.

Huyse, T., Van den Broeck, F., , M., Ploch, S., Loots, C., Coenye, T., ... & Volckaert, F. A. (2009). Natural hybridization between a human and a bovine schistosome species. Molecular Ecology, 18(16), 3479-3489.

Ismail, M. M., Botros, S. S., Metwally, A., Farghaly, A. M., Tao, Y. X., Day, T. H., ... & Bennett, J. L. (1999). Resistance to praziquantel: evidence from *Schistosoma* mansoni isolates in Egypt. The American Journal of Tropical Medicine and Hygiene, 60(6), 932-938.

Juranek, D. D. (1995). *Schistosomiasis*. In Strickland's Tropical Medicine (9th ed., pp. 603-628). W.B. Saunders Company.

King, C. H., & Dangerfield-Cha, M. (2017). The unacknowledged impact of chronic *schistosomiasis*. Chronic Illness, 13(1), 65-79.

Kiran, S., Dalhatu, M., & Jitendra, S. (2016). Current status of *schistosomiasis* in Sokoto, Nigeria. Journal of Parasitic Epidemiology and Control, 1(3), 239-244.

Kumar, P., et al. (2016). Community engagement in public health research: A review. Global Health Action, 9(1), 29875.

Leger, E., Garba, A., Hamidou, A. A., Webster, J. P., & Rollinson, D. (2018). Cattle contribute to urogenital *schistosomiasis* transmission. The Lancet Infectious Diseases, 18(3), 258-260.

Machacek, T., Turjanicova, L., Bulantova, J., Hrdy, J., Horak, P., & Mikes, L. (2018). Cercarial dermatitis: a systematic follow-up study of human cases with implications for diagnostics. Parasitology Research, 117(12), 3881–3895. https://doi.org/10.1007/s00436-018-6095-0

Macintyre, K., & Sobel, H. (2001). Involving the community in the design and implementation of health-related research. Community Health Research, 1(4), 65-72.

Martel, R. A., Osei, B. G., Kulinkina, A. V., Naumova, E. N., Abdulai, A. A., Tybor, D., & Kosinski, C. K. (2019). Assessment of urogenital *schistosomiasis* knowledge among primary and junior high school students in the Eastern Region of Ghana: A cross-sectional study. PloS One, 14. https://doi.org/https://doi.org/10.1371/journal. pone.0218080 June.

McCullough, F. S., & Mott, K. E. (1983). Perspectives on the role of molluscicides in *schistosomiasis* control. Acta Tropica, 40(1), 57-69.

McManus, D. P., Dunne, D. W., Sacko, M., Utzinger, J., Vennervald, B. J., & Zhou, X.-N. (2018). *Schistosomiasis*. Nature Reviews Disease Primers, 4(1), 13. https://doi.org/10.1038/s41572-018-0013-8

Nash, T. E., Cheever, A. W., Ottesen, E. A., & Cook, J. A. (1982). Schistosome infection in humans: evidence for an association between intensity of infection and morbidity. The American Journal of Tropical Medicine and Hygiene, 31(3), 556-563.

Nelwan, M. L. (2019). *Schistosomiasis*: Life cycle, diagnosis, and control. Current Therapeutic Research, 91, 5–9.

Ng'ang'a, M., Matendechero, S., Kariuki, L., Omondi, W., Makworo, N., Owiti, P. O., ... & Omondi-Ogutu, (2016). Spatial distribution and co-infection with urogenital and intestinal *schistosomiasis* among primary school children in Migori County, Kenya. East African Medical Journal, 93, S22–S31.

Ntajal, J., Evers, M., Kistemann, T., & Falkenberg, T. (2021). Influence of human–surface water interactions on the transmission of urinary *schistosomiasis* in the Lower Densu River basin. Ghana Social Science Medicine, 288, 113546.

Oguonu, T. A., & Okafor, F. C. (1993). *Schistosomiasis* in Anambra State, Nigeria. The Nigerian Journal of Parasitology, 14, 67-73.

Okafor, F. C. (1985). Freshwater snails of medical and veterinary importance in southeastern Nigeria. Hydrobiologia, 121(2), 153-157.

Olufemi, A. F., et al. (2021). Water, sanitation, and health impact on the prevalence of *schistosomiasis* in Nigeria. Journal of Environmental Health Science & Engineering, 19(1), 495-503.

Olufemi, O. S., Eniyansoro, F. G., & Ayotunde, E. O. (2021). Water, sanitation and hygiene practices and *schistosomiasis* prevalence in a rural community in Rivers State, Nigeria. Journal of Water, Sanitation and Hygiene for Development, 11(2), 287-295.

Onosakponome, E. O., Ezeanyagu, O. C., Ejinaka, O. R., Obeta, M. U., & Agbalaka, P. I. (2022). Impact of water sources on *schistosomiasis* transmission and urine indicators. African Journal of Health Sciences, 35(6), 697-704.

Pam, V. A., Daramola, O. S., Uzoigwe, N. R., Ombugadu, A., Maikenti, J. I., Ahmed, H. O., ... & Dogo, K. S. (2021). Freshwater snails infection status and predisposing risk factors to *schistosomiasis* in Doma local government area, Nasarawa State, Nigeria. Biomedical Journal of Scientific & Technical Research, 37(2), 29208-29214.

Picaud, O., Rossignol, M., & Vennervald, B. J. (2010). Female genital *schistosomiasis*: a review. Parasitology, 147(S1), S77-S91.

Pontes, L. A., & de Souza, C. S. (2002). Perspectives on the molecular characterization of *Schistosoma* species. International Journal for Parasitology, 32(1), 45-55.

Ritchie, J., et al. (2014). Qualitative research practice: A guide for social science students and researchers. Sage Publications.

Rollinson, D., Knopp, S., Levitz, S. M., Mazigo, H. D., Sleigh, A. C., & Utzinger, J. (2013). Time to set the agenda for *schistosomiasis* elimination. Acta Tropica, 128(2), 423-440.

Savioli, L., Engels, D., Montresor, A., & World Health Organization. (2017). WHO model list of essential medicines: 19th list (April 2015). World Health Organization.

Savioli, L., et al. (2017). *Schistosomiasis*: A global perspective. Infectious Disease Reports, 9(1), 2.

Schistosoma Control in Egypt Research Project. (1980). The epidemiology and morbidity of *schistosomiasis* haematobia in an Egyptian village. The American Journal of Tropical Medicine and Hygiene, 29(4), 736–742.

Sidibé, B., Agniwo, P., Diakité, A., Savassi, B. A. E. O. S., Doumbo, S. N., Akplogan, A., ... & Dabo, A. (2024). Human-water interactions associated to cercarial emergence pattern and their influences on urinary *schistosomiasis* transmission in two endemic areas in Mali. Infectious Diseases of Poverty, 13(1), 62.

Southgate, V. R., , P. D., , J. P., , D. A., , J. A., , J. A., ... & J. A. (2013). Human *schistosomiasis* in West Africa: the current situation and future challenges. Parasitology, 130(S1), S1-S20.

Sowemimo, O. A. (2007). Urinary *schistosomiasis* in school children in a rural community in the Niger Delta area of Nigeria. Nigerian Journal of Parasitology, 28(1), 1-4.

Standley, C. J., et al. (2012). Characterization of *schistosomiasis* in freshwater bodies: A review. Journal of Parasitology, 98(4), 704-713.

Stephenson, L. S., Latham, M. C., & Ottesen, E. A. (1993). Malnutrition and parasitic helminth infections in children. Parasitology, 107(S1), S63-S78.

Taylor, L. H., Latham, S. M., & Woolhouse, M. E. (2001). Risk factors for human disease emergence. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 356, 983–989.

Tchuenté, L. A. T., et al. (2018). *Schistosomiasis*: A neglected tropical disease. Advances in Parasitology, 99, 1-18.

Ukoli, F. M. A. (1990). Parasitology in focus. University of Ibadan Press.

Utzinger, J., van der Werf, M. J., Xiao, Y., Tanner, M., & Engels, D. (2003). Sustainable *schistosomiasis* control: the way forward. The Lancet, 362(9399), 1963-1966.

Van der Werf, M. J., de Vlas, S. J., Brooker, S., Looman, C. W. N., Nagelkerke, N. J. D., Habbema, J. D. F., & Engels, D. (2003). Quantification of clinical morbidity associated with schistosome infection: a systematic review and meta-analysis. Bulletin of the World Health Organization, 81, 738-745.

Warren, K. S., Reboucas, G., & Teesdale, C. H. (1967). Portal and circulatory pressures in chronic murine *schistosomiasis* mansoni. Transactions of the Royal Society of Tropical Medicine and Hygiene, 61(5), 621-631.

Webster, B. L., Diaw, O. T., Seye, M. M., Webster, J. P., & Rollinson, D. (2013). Introgressive hybridization of *Schistosoma* haematobium group species in Senegal: Species barrier break down between ruminant and human *Schistosomes*. PLoS Neglected Tropical Diseases, 7, e2110.

Webster, J. P., Southgate, V. R., Littlewood, D. T. J., & Rollinson, D. (2006). Phylogenetic relationships within the genus *Schistosoma* inferred from nuclear ITS rDNA sequence data. Molecular Phylogenetics and Evolution, 39(3), 643-655.

Weerakoon, K. G., et al. (2018). Molecular approaches for the diagnosis of human *schistosomiasis*. Trends in Parasitology, 34(10), 837-847.

Weerakoon, K. G., Hamid, I., & McManus, D. P. (2018). An update on the diagnosis and treatment of *schistosomiasis*. Current Infectious Disease Reports, 20(5), 12.

World Health Organization. (2021). *Schistosomiasis*: Progress report 2011–2020 and strategic plan 2021–2030. World Health Organization.

World Health Organization. (2021). *Schistosomiasis*: Progress report 2011–2020 and strategic plan 2021–2030. https://www.who.int/news-room/fact-sheets/detail/schistosomiasis

World Health Organization. (2023). *Schistosomiasis*. Retrieved from WHO website

World Health Organization. (2023). *Schistosomiasis*: Key facts. Retrieved from https://www.who.int/news-room/fact-sheets/detail/schistosomiasis. Accessed on 20 July 2025.

QUESTIONNAIRE

SCHOOL OF POST GRADUATE STUDIES, DEPARTMENT OF PUBLIC HEALTH

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

RESEARCH TOPIC: CHARACTERISATION OF *SCHISTOSOMA* SPECIES IN FRESHWATER IN BODO COMMUNITY, RIVERS STATE

This survey is designed to collect demographic information about the four rivers in the Bodo community to inform the collection of freshwater samples for detecting *schistosomiasis* species. The information collected will help us understand the rivers' characteristics and identify potential sampling sites.

Environmental factors influencing *Schistosoma*

1. Main source of water for the households

   (a) Protected/covered water well [ ] (b) River /stream [ ] (c) Piped water [ ] (d) Unprotected/uncovered water well

2. Stream Visitation

   (a) Visit Water bodies [ ] (b) Do not Visit Water bodies [ ]

3. Frequency of visit to Water bodies

(a) Every day [ ] (b) Once in two days [ ] (c) Once a week [ ] (d) Once a month

4. Water Contact Activities
5. Swimming (a) Yes [ ] (b) No [ ]
6. Fetching (a) Yes [ ] (b) No [ ]
7. Bathing (a) Yes [ ] (b) No [ ]
8. Washing clothes (a) Yes [ ] (b) No [ ]

9. Other washings (a) Yes [ ] (b) No [ ]

10. Fishing (a) Yes [ ] (b) No [ ]

11. Baptism & Worship (a) Yes [ ] (b) No [ ]

12. Crossing water bodies (a) Yes [ ] (b) No [ ]

13. Number of times for daily contact with water

(a) Once [ ] (b) Twice [ ] (c) Thrice [ ] (d) More than 3 times

14. Time taken for activities in contact with water

(a) Less than 5 minutes [ ] (b) 5 to 10 minutes [ ] (c) 10 to 15 minutes [ ] (d)

15. Swimming in water bodies, where do you urinate

(a) Urinate in water [ ] (b) Urinate out of water [ ]

16. How many times of defecation/urination in water bodies

(a) Every day and when nature calls [ ] (b) I do not remember [ ] (c) It was only once [ ] (d) Occasionally and when in the field

17. Presence of livestock around the rivers (a) Yes [ ] (b) No [ ]

18. Type of livestock around the rivers (a) Cattle [ ] (b) Goats [ ] (c) Sheep [ ] (d) Pigs

19. Presence of snails around the rivers (a) Yes [ ] (b) No [ ]

RESULT

LABORATORY ANALYSIS

Run Profile

Hold Steps
Reverse transcription at 95°C for 10:00

Cycling		
45 cycles	1)	95°C for 15s
	2)	60°C for 60s acquiring on Green, Yellow, Orange, Red

Cycling: ITTS2F → ITTS2F Probe 1

Target	Sango Schitos Assay → ITTS2F → ITTS2F Probe 1
Normalisation	Dynamic
Ignore	1
Exclusion	None
Threshold	0.573 starting at cycle 1

W1					
48		-	-	-	
W1-4					
2		-	-	-	
W2-4					
3		-	-	-	
W3-4					
4		-	-	-	
W4-4					
5		-	-	-	

W1					
48		-	-	-	
W1-4					
2		-	-	-	
W2-4					
3		-	-	-	
W3-4 POSITIVE					**x̄ = 19.19 σ = 0.00**
4		19.19	-	-	
W4-4					
5		-	-	-	

W1 POSITIVE					**x̄ = 40.72 σ = 0.00**
9		40.72	-	-	
W1-2					
21		-	-	-	
W1-3					
35		-	-	-	
W2					
11		-	-	-	

W2-2					
22		-	-	-	
W2-3					
36		-	-	-	
W3					
12		-	-	-	
W3-2					
23		-	-	-	
W3-3					
37		-	-	-	
W4	**POSITIVE**				**$\bar{x}$ = 17.70 σ = 0.00**
10		17.70	-	-	
W4-2					
24		-	-	-	
W4-3					
38		-	-	-	

W1							
9				-	-	-	

W1-2							
21				-	-	-	
W1-3							
35				-	-	-	
W2							
11				-	-	-	
W2-2							
22				-	-	-	
W2-3							
36				-	-	-	
W3							
12				-	-	-	

W3-2							
23				-	-	-	
W3-3							
37				-	-	-	
W4							
10				-	-	-	
W4-2							
24				-	-	-	
W4-3							
38				-	-	-	