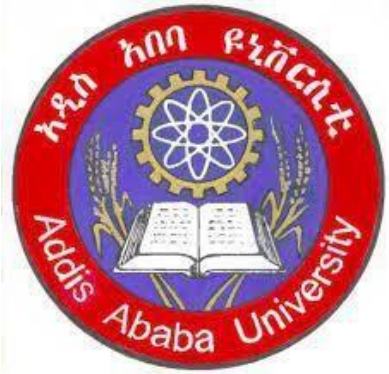

Genetic profile, Epidemiology, and Community Knowledge of *Schistosoma haematobium* in Endemic Regions of Ethiopia

PhD Dissertation

By

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Addis Ababa University

School of Graduate Studies

Aklilu Lemma Institute of Health Research

Tropical and Infectious Diseases Program

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Addis Ababa, Ethiopia

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Molecular Characterization of Schistosoma haematobium, Epidemiology, and Community Knowledge of Urogenital Schistosomiasis in Endemic Regions of Ethiopia

By

Tigist Mohammed Degu

A thesis submitted to the Department of Microbiology, Immunology and Parasitology; College of Health Sciences; Addis Ababa University in the fulfilment of the requirements for the Degree of Doctor of Philosophy in Tropical and Infectious Diseases

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Genetic profile, Epidemiology, and Community Knowledge of *Schistosoma haematobium* in Endemic Regions of Ethiopia

Tigist Mohammed Degu
Addis Ababa University, 2025

Abstract

Background: Schistosomiasis (SCH) is a highly prevalent yet neglected tropical disease (NTD), particularly in sub-Saharan Africa (SSA), where it continues to impose a significant burden on public health and socio-economic development. Urogenital schistosomiasis (UGS), caused by *S. haematobium*, is a significant public health challenge in low-altitude regions of Ethiopia, including Gambella, Afar and Benishangul-Gumuz. While extensive research has focused on the epidemiology and treatment of SCH, critical gaps remain in understanding the genetic makeup of *Schistosoma* populations in Ethiopia and the potential hybridization events that may influence transmission dynamics and treatment outcomes. Additionally, comprehensive epidemiological data on *S. haematobium* infection is lacking across all age groups, particularly among pre-school age children (PSAC). This gap is compounded by limited data on community knowledge, attitudes, and practices (KAP) regarding UGS, which are essential for evaluating the effectiveness of current control measures and designing target-specific interventions.

Objectives: This study aimed to investigate the genetic profile of *S. haematobium* and to identify hybridization events in Afar and Gambella endemic areas; assess the prevalence, intensity, and risk factors of *S. haematobium* infection among PSAC; evaluate community KAP in Gambella, Abobo district; and to determine the prevalence and infection intensity of *S. haematobium* across all age groups in the Kurmuk district of Benishangul-Gumuz.

Methods: A community-based cross-sectional study was conducted between 2021 and 2024 to address all objectives. Multi-locus genetic analysis, targeting the mitochondrial cytochrome oxidase sub-unit I (cox1) gene and the nuclear internal transcribed spacer 2 (ITS2) regions, was performed on miracidia isolated from human urine samples using rapid diagnostic polymerase chain reaction (RD-PCR). Infection status was determined through a urine filtration microscopy (egg detection and count) and dipstick screened for hematuria. Socio-demographic data, risk factors, and outcome variables (KAP) were collected using a pretested structured questionnaire. Data were analyzed using SPSS version 23, with logistic regression and odds ratios employed to assess the strength of associations between dependent and independent variables.

Results: A total of 177 miracidia were analyzed using mitochondrial cox1 and 24 miracidia were analyzed using nuclear ITS2 gene markers, revealing exclusive infection with *S. haematobium* and no evidence of mixed infection/hybridization with *S. bovis* or *S. mansoni*. This suggests mono-specific transmission in the Afar and Gambella regions, likely due to ecological separation, species-specific host preferences, and limited zoonotic transmission pathways. The prevalence of *S. haematobium* infection among PSAC in Abobo, Gambella, was 16.7%, with 20% being heavy-intensity infections. The prevalence of macro and microhematuria were 7.9% and 26.2%, respectively. The prevalence of infection was significantly higher among PSAC playing or bathing in infested water (AOR = 2.9, CI: 1.0–8.1, p=0.041) and residing in Abaro village (AOR = 4.3, CI: 1.6–11.9, p=0.005). The overall prevalence of *S. haematobium* infection was 34.2% in Kurmuk, Benishangul-Gumuz, with the highest prevalence among participants with Swimming habits (AOR = 2.43, CI: 1.19-4.94, p=0.023). Infection intensity varied significantly across villages, with mean egg counts (MEC) ranging from 2.39 in Ogendu (CI: 1.05-3.72) to 14.1 eggs/10 mL urine in Dulshatalo (CI: 4.98- 23.12).

Hematuria prevalence was 39.2%, with a higher odd in Dulshatalo village (AOR = 2.64, 95% CI: 1.43-4.87, P = 0.004). Moreover, microhematuria was significantly associated with *S. haematobium* egg detections (P < 0.001), and a moderate agreement (Kappa = 0.57) was established with the microscope finding. The community KAP assessment in Abobo, Gambella, revealed that most participants (90.6%) had heard of UGS, and over 94% recognized at least one major symptom. However, more than half of the participants perceive (59.9%) drinking contaminated water as a primary way of transmission and understanding prevention measures (15.9% for keeping hygiene, 26.8% for refraining from using contaminated water) were limited. Over 50% did not associate urinating near water to transmission; nearly all had contact with dam/river water. Education and prior infection history were key predictors of better KAP.

Conclusion: This study provided critical insights into the epidemiology and genetic profile of *S. haematobium* infections in endemic regions of Ethiopia, confirming the absence of mixed infection and suggesting mono-specific transmission in Afar and Gambella. The high prevalence of *S. haematobium* infection and heavy intensity, particularly among PSAC and older children (5–12 years), shows the public health burden of UGS in these areas. The study also identified key risk factors, such as water contact behaviors, and reveals significant gaps in community knowledge of UGS transmission and prevention despite high awareness of the disease. To address these challenges, targeted interventions are needed, including providing safe water sources, improved sanitation, and community-based health education programs to reduce risky behaviors and enhance prevention practices. Control programs should also be expanded to include PSAC, which is currently underserved but exhibits significant infection rates. Strengthening surveillance systems to monitor infection trends and genetic diversity and conducting further research to explore ecological and behavioral drivers of transmission will be

essential for designing effective, context-specific strategies to achieve sustainable SCH control in these endemic regions.

Keywords: Genetic profile; Epidemiology; community based; PSAC; *S.haematobium;* knowledge, attitude, and practice; Afar, Gambella, Benishangul-Gumuz; Ethiopia.

Declaration

This is to certify that, I, the undersigned, declare the dissertation titled Genetic profile of *Schistosoma haematobium*, Epidemiology, and Community Knowledge in Endemic Regions of Ethiopia‖submitted to the Aklilu Lemma Institute of Health Research, Addis Ababa University in fulfillment of the requirements for the Doctor of Philosophy (PhD) degree in Tropical and Infectious Diseases is an original piece of work produced by me/my original work. I confirm that this dissertation has not been previously presented at Addis Ababa University or any other academic institutions and that all sources and references used have been duly acknowledged.

Tigist Mohammed (GSR/3676/10) _____ _____

Name of student (ID No.) Signature Date of submission

Dedication

This PhD dissertation is dedicated to my daughters, Hiyam Nasir and Eintisar Nasir, whose love and laughter have been a constant source of strength and motivation throughout this journey.

May this achievement inspire you both to dream big, pursue knowledge, and never give up on your aspirations.

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List of Abbreviations and Acronyms

AOR	Adjusted Odds Ratio
COR	Crude Odds Ratio
KAP	Knowledge, Attitude, and Practice
FMoH	Federal Ministry of Health of Ethiopia
PCR	Polymerase Chain Reaction
SOPs	Standard Operating Procedures
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization
SCH	Schistosomiasis
SCHu	Urogenital Schistosomiasis
PZQ	Praziquantel
NTDs	Neglected Tropical Diseases
MDA	Mass Drug Administration
WASH	Water, Sanitation, and Hygiene
UGS	Urogenital Schistosomiasis
ALIHR	Aklilu Lemma Institute of Health Research
MEC	Mean Egg Count
PC	Preventive Chemotherapy
ELISA	Enzyme-Linked Immunosorbent Assay
LAMP	Loop-Mediated Isothermal Amplification
cPCR	Conventional Polymerase Chain Reaction
RT-PCR	Real-Time Polymerase Chain Reaction
DdPCR	Digital Droplet Polymerase Chain Reaction

Mb	Megabases
CHW	Community Health Workers
SAC	School-Aged Children
PSAC	Preschool-Aged Children
DALYs	Disability-Adjusted Life Years
FGS	Female Genital Schistosomiasis
MGS	Male Genital Schistosomiasis
DRC	Democratic Republic of Congo
EPHI	Ethiopian Public Health Institute
STI	Sexually Transmitted Infection
HPV	Human Papillomavirus
IL	Interleukin
MRI	Magnetic Resonance Imaging
TNF-α	Tumor Necrosis Factor Alpha
IVF/ICSI	In Vitro Fertilization/Intracytoplasmic Sperm Injection
WHOPE	World Health Organization Pesticide Evaluation Scheme
SCI	Schistosomiasis Control Initiative

Chapter 1: Introduction

1.1 Background

Schistosomiasis, also known as Bilharzia, is a highly prevalent neglected tropical disease caused by blood-dwelling fluke worms of the genus *Schistosoma.* Blood flukes are members of the invertebrate phylum Platyhelminthes, which includes multicellular eukaryotic invertebrates with tube-like or dorsoventrally flattened bodies exhibiting bilateral symmetry. Within the phylum, schistosomes belong to the class Trematoda. The term 'Trematoda' is derived from the Greek *trematodes'* (having holes), referring to the ventral and oral suckers, which form the characteristic feature of the group (Smyth, 1994).

Out of the 24 species of schistosomes, the main species known to infect humans are *Schistosoma haematobium, S. mansoni,* S. *japonicum, S. mekongi, S. intercalatum* and *S. guineensis* (Rollinson, 2009*).* A significant number of *S. haematobium* worms tend to be found in the vesicle plexuses of the pelvis, causing UGS. On the other hand, other species primarily occur in blood vessels around the intestine and the liver causing intestinal schistosomiaisis (Nation *et al.,* 2020).

A unique feature of schistosomes that differentiates them from other trematodes is their dioecious nature,i.e., they have separate sexes. The life cycle of the *Schistosoma* species involves two hosts: a mammalian definitive host and a snail intermediate host. The transmission cycle begins when human urine or stool containing parasite eggs contaminate freshwater habitats. In these environments, the eggs hatch into miracidia, which actively seek out and infect specific species of freshwater snails. Inside the snails, the parasites multiply asexually by a process

termed polyembryony, producing thousands of infective cercariae that are eventually released into the water. Humans become infected through domestic, occupational, or recreational contact with contaminated water (WHO, 2023).

The distribution of SCH is highly focal due to the transmission being dependent on the existence of competent intermediate snail hosts. Ecological factors that promote the presence and abundance of these snails also contribute to the local risk of *Schistosoma* spp transmission (Olkeba *et al.,* 2020; Tabo *et al.,* 2022). Poverty-related factors like inadequate sanitation, lack of access to clean water, and limited healthcare exacerbate the disease‘s spread. High-risk groups include children, agricultural and fishing workers, and women performing domestic activities in freshwater (WHO, 2022a; Lackey and Horrall, 2023).

The disease manifests in acute and chronic forms. The acute form of the disease has an incubation period of 14-84 days, while the chronic asymptomatic infection can last for many years. If untreated, chronic SCH can lead to high morbidity and mortality, with the onset of unsolvable complications (King *et al.,* 2008; McManus *et al.,* 2018). Factors such as the age at first infection, infection intensity, duration, and the presence of co-infections influence disease severity (French *et al.,* 2018).

The association between *S. haematobium* and UGS was first identified in the 1850s by German pathologist Theodor Bilharz, who discovered the parasite in a patient‘s blood vessels in Egypt (Gryseels *et al.,* 2006). The genome of *S. haematobium* is approximately 385 megabases (MB), comprising about 13,000 genes (Young *et al.,* 2012). Within mammalian hosts, sexual reproduction promotes hybridization and genetic diversity and enables the parasite to adapt to natural and human induced selection pressures (Gouvras *et al.,* 2018; Rey *et al.,* 2021).

Diagnosing UGS involves clinical evaluation, laboratory testing, and imaging techniques. Laboratory methods include microscopic detection of schistosome eggs in urine, dipstick tests for haematuria and proteinuria, molecular assays to detect parasite DNA in various specimens and immunodiagnostic techniques used to detect parasite antigens (Weerakoon *et al.*, 2018; Chala, 2023). Imaging techniques, including ultrasound and endoscopy, are also used to assess disease complications (Manciulli *et al.*, 2023).

Controlling UGS requires an integrated approach, including regular distribution of praziquantel (PZQ), improved sanitation and access to clean water, and effective snail control measures. Sustained efforts in community health education and strengthening healthcare infrastructure are crucial for reducing the disease burden (McManus *et al.*, 2018; Faust *et al.*, 2020; WHO, 2022b).

Genome sequencing has advanced research on vaccine development, diagnostics, and targeted treatments (Gouvras *et al.*, 2018; Rey *et al.*, 2021). However, the genetic profile and population structure of *Schistosoma* isolates in Ethiopia remain poorly studied, despite UGS being endemic in many low-altitude areas of the country.

1.2 Rationale for the present study

Schistosomiasis remains a significant public health challenge, with the disease being endemic in 78 countries worldwide. In 2021, the World Health Organization (WHO, 2022a) estimated that 251.4 million people required treatment for SCH. In Ethiopia, schistosomiasis is a pressing concern, with approximately 7.2 million PSAC, 16.9 million SAC, and 30.6 million adults residing in endemic regions, emphasizing the need for targeted control and prevention measures (FMoH, 2021).

The impact of SCH on both the economy and health are significant, with the disease causing more disability than mortality. In Africa, in 2021, it caused 1.5 million (0.92 to 2.58 million) disability-adjusted life years (DALYs) and 11,222 (9,749.48 to12,756.83) death (Peng *et al.,* 2025). Due to its gradual and long-lasting nature, the disease often goes unnoticed in its early stages, posing a threat to development as it disables individuals during their most productive years. It can lead to anemia, growth impairment, and hindered learning abilities in young children (King *et al.,* 2008). Chronic SCH may also affect people‘s ability to work and, in severe cases, even result in death (WHO, 2023).

In PSAC, *S. haematobium* infection, which can occur within the first few months of life (Stothard *et al.,* 2013), causes 40- 50 % prevalence of bladder or urinary lesions reported by ultrasound examination, which could intensify if left untreated (Barda *et al.*, 2017; Sánchez-Marqués *et al.,* 2023). Meta-analysis finding shows that a single dose of 40 mg/kg oral praziquantel is safe and effective for PSAC, similar to its use in SAC (Olliaro *et al.,* 2020). WHO‘s 2022 guidelines recognize this and recommend expanding preventive PC to PSAC, until pediatric formulation is widely available (WHO, 2022b). In light of the recent development of a pediatric praziquantel (PZQ) formulation, there are also coordinated initiatives to integrate their treatment into national control programs (Mutapi *et al.*, 2024).

In *S. haematobium* infection, disease outcome varies dramatically, ranging from mild symptoms to severe damage of the kidneys and/or bladder (Brouwer *et al.,* 2003; Lackey and Horrall, 2023). In SSA, it is estimated to affect 112 million individuals, with 70 million individuals experiencing haematuria, 32 million facing difficulties in urination (dysuria), 18 million suffering from bladder-wall pathology, and 10 million experiencing major hydronephrosis (Van der Werf *et al*., 2003).

According to Rollinson (2009), the pathology caused by *S. haematobium* could greatly outweigh that caused by *S. mansoni* since chronic *S. haematobium* infection is associated with severe pathological implications for bladder cancer. In Africa, the incidence rate of schistosome-associated bladder cancer is estimated to be 7.0 per 100,000 in men and 1.8 per 100,000 in women (Adeloye *et al.,* 2019).

Genital schistosomiasis is also common in endemic areas of urinary manifestation of SCH in both genders, with an estimated prevalence rate of 26 to 75% for female genital schistosomiasis (FGS) (Van derWerf *et al*., 2003; Rafferty *et al.,* 2021; Lamberti *et al.,* 2024a) and 1 to 50% for male genital schistosomiasis (MGS) (Kayuni *et al.,* 2018). Genital schistosomiasis is also considered to be a risk factor for HIV and HPV infection. The disease can therefore, have serious sexual and reproductive health consequences in SCH endemic areas (WHO, 2023; Rossi *et al.,* 2024).

Furthermore, the discovery of hybrid human and animal schistosomes in West Africa adds complexity to the situation, as these hybrid species have the potential to infect a varied range of hosts and exhibit increased strength (Secor & Colley, 2018; Sene-Wade *et al.,* 2018; Angora *et al.,* 2020). Hybridization has also already been suggested to affect the success of drug treatment (Leger and Webster, 2017) and shapes the genetic diversity of *S. haematobium* (Teukeng *et al.,* 2022). Genetic diversity plays a crucial role in the transmission dynamics of SCH, as it can affect the parasite‘s ability to adapt to different environments, hosts, and treatment regimens (Gower *et al.,* 2007; Webster *et al.,* 2010).

In Ethiopia, while extensive research has focused on its epidemiology and treatment, there is a critical gap in understanding the genetic makeup of *Schistosoma* populations and the potential

hybridization events that may influence transmission and treatment outcomes (Webster *et al.*, 2020). Therefore, precise identification of the species in areas where they coexist is vital to provide accurate transmission data (Leger and Webster, 2017). In addition, the lack of comprehensive *S. haematobium* epidemiological data across all age groups, particularly among PSAC, limits the ability to design effective control strategies. Addressing these gaps is essential for informing evidence-based policies, improving disease control programs, and ultimately reducing the burden of UGS in Ethiopia. This study aims to bridge these gaps by assessing the genetic profile, epidemiology, and community knowledge of *S. haematobium* infections in endemic localities, providing a foundation for targeted interventions and contributing to SCH elimination efforts in Ethiopia.

1.3 Significance of the study

The significance of this study lies in its integration of genetic analysis to deepen our understanding of UGS. Genetic insights are crucial for monitoring shifts in parasite populations and assessing transmission patterns. Additionally, the study highlights the high prevalence of *S. haematobium* infection among PSAC, a group often neglected in SCH research and control efforts. Epidemiological data on PSAC are vital for understanding disease dynamics and guiding the development of age-appropriate interventions. Thus, the genetic and epidemiological findings provide a strong foundation for future research and support the formulation of evidence based public health policies aimed at the prevention and control of UGS.

Chapter 2: Literature Review

2.1 Epidemiology of urogenital schistosomiasis

2.1.1 Global situation

Globally, SCH affects over 220 million people, with an annual mortality rate of approximately 11,792. Sub-Saharan Africa bears the highest burden, accounting for 90.6% of individuals requiring treatment for the disease and spread across 41 countries in the region (WHO, 2023). The primary causative species contributing to the global disease burden are *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum* (Aula *et al.*, 2021; WHO, 2022a). *S. japonicum* is found in China, the Philippines, and Indonesia; *S. haematobium* is prevalent in Africa, the Middle East, and the French island of Corsica; and *S. mansoni* is endemic in Africa, the Middle East, the Caribbean, Brazil, Venezuela (the Bolivarian Republic of) and Suriname. Meanwhile, the other species known to infect humans, *S. guineensis* and *S. intercalatum* (both endemic in Central and West Africa), and *S. mekongi* (restricted to a short stretch of the Mekong River in southern Lao People‘s Democratic Republic and eastern Cambodia) are of local regional importance (WHO, 2023).

Approximately 100 million individuals are globally affected by UGS caused by *S. haematobium* (WHO, 2020a). Nigeria has the highest prevalence of SCH worldwide, with over 25 million cases, dominated by *S. haematobium*, followed by Tanzania with 19 million cases. The Democratic Republic of Congo and Ghana mutually account for 15 million cases (Aula *et al.*, 2021; Saidu *et al.*, 2023). Among those affected, children are disproportionately impacted, with UGS prevalence reaching as high as 62% in some populations (Masdor *et al.*, 2023).

The *S. haematobium* species group currently includes eight species, some of which have medical importance (*S. haematobium, S. intercalatum, S. guineensis*) and others of veterinary relevance (*S. bovis, S. mattheii, S. curassoni*) (Rollinson, 2009). *Schistosoma bovis* and *S. haematobium* are closely related sister taxa within this group (Rollinson *et al.,* 1990). *S. bovis* causes ruminant SCH in Africa, the Mediterranean, and the Middle East. Both species utilize freshwater snails of the same genus and their distribution overlaps in many parts of Africa (Webster, 2009). A zoonotic hybrid between these species has been reported (Boissier *et al.,* 2016; Angora *et al.,* 2020).

The distribution and transmission of *S. haematobium* depend on specific snail species from the genus *Bulinus*, which includes approximately 36 species within four groups. Endemic regions report variations in intermediate host species, with significant distributions across Africa, Madagascar, the Middle East, and the Mediterranean (Rollinson *et al.*, 2001). Notable snail species include *B. abyssinicus*, *B. africanus*, *B. tropicus*, *B. globosus*, *B. truncatus*, and *B. forskalii* (Rollinson, 2009; Lu *et al.*, 2018). In Africa, *Bulinus* snails have been identified as key hosts, with *B. globosus* exhibiting the highest prevalence of schistosome cercariae (18.4%) in Nigeria (Hailegebriel *et al.*, 2020).

2.1.2 Ethiopia situation

Schistosomiasis is endemic in several regions of Ethiopia and it is one of the NTDs prioritized for control and elimination by the Ethiopian Federal Ministry of Health (FMoH, 2021). Its prevalence varies depending on environmental conditions, sanitation, and access to clean water (Mengistu *et al.*, 2016). Both *S. mansoni* and *S. haematobium* have been known to be endemic since they were first reported in a few foci during the 1950s (Kloos *et al.*, 1988; Ali *et al.*,

2006; Gashaw *et al.*, 2015). According to Woldeyohannes *et al.* (2021), a systemic review among SAC, 28.9% pooled prevalence of SCH is projected.

Schistosoma haematobium is known to occur in lowland areas below 800 meters above sea level, specifically in the Afar, Gambella, Somali, and Benishangul-Gumuz regions of Ethiopia (Deribe *et al.*, 2012; Negussu *et al.*, 2013; Degarege *et al.*, 2015; Geleta *et al.*, 2015). *Bulinus abyssinicus* and *B. africanus* are the two snail species that transmit *S. haematobium* in Ethiopia (Fig 1). In the northeastern part of Ethiopia, particularly in Afar region and Wabe Shebelle area in the southeast, *B. abyssinicus* is the primary intermediate host. Conversely, in the western region, specifically in Kurmuk, *B. africanus* is responsible for transmitting the parasite (Ali *et al.*, 2006). However, the intermediate host species in Gambella remains unidentified (Deribew *et al.*, 2022b).

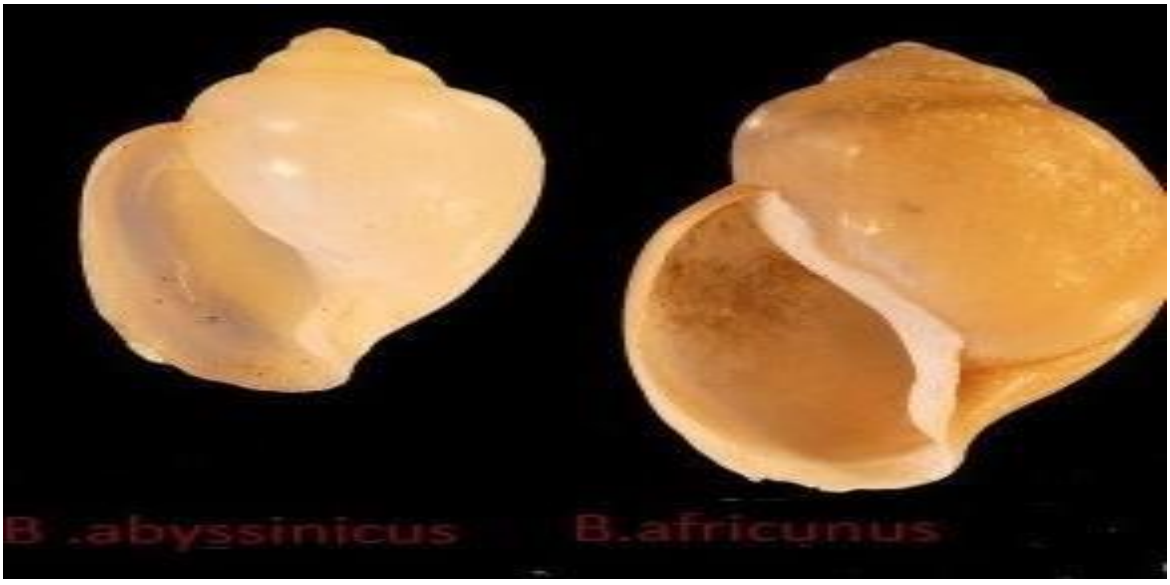

Figure 1: Shells of *Bulinus* species in Ethiopia [Source: (Garba Djirmay *et al.*, 2024)]

Epidemiological studies indicate that UGS poses a significant health challenge in endemic regions of Ethiopia, with SAC being the most affected population segment. In the Afar region,

studies consistently highlight the endemic nature of UGS. Degarege *et al.* (2015) reported an overall prevalence of 20.8%, ranging from 12.5% to 37.0% in the Middle Awash Valley and 0% to 5.3% in the Lower Awash Valley among SAC. In addition, Deribew *et al.* (2022a) reported a prevalence of 7% in Hassoba Buri village, while Degarege *et al.* (2022) reported a prevalence of 24.4% across villages such as Buri, Kusra, Hassoba, Kelhat, and Andada among SAC.

UGS is also a significant public health concern in the western Benishangul-Gumuz region, particularly in the Kurmuk district. Mekonnen *et al.* (2013) reported a community prevalence of 57.8% in DulShatalo and Kurmuk towns and Deribew *et al.* (2022a) found a prevalence of 5.6% among SAC in Kurmuk district.

Similarly, in high-risk areas of Gambella, Ethiopian Public Health Institute (EPHI) reported a UGS with approximately 15% prevalence, with SAC being the most affected group (EPHI, 2021). Additional cross-sectional studies by Geleta *et al.* (2015) and Deribew *et al.* (2022a) revealed prevalences of 35.9% and 24.2% among SAC, respectively. Limited epidemiological studies have also been conducted in southeastern Ethiopia, specifically in the Afdera and Gode zones of the Somali region, where Negussu *et al.* (2013) reported a prevalence of 16.0%.

While research on UGS predominantly focuses on SAC, other age groups remain understudied, leaving gaps in understanding the disease's full impact across communities. Expanding research to include adults and younger children would provide a more comprehensive picture of disease burden and support targeted interventions. Moreover, various KAP studies in Ethiopia have primarily assessed community awareness of intestinal SCH (Legesse *et al.*, 2009; Nyantekyi *et al.*, 2014; Gebreyohanns *et al.*, 2018; Alemu *et al.*, 2024), often overlooking UGS in endemic regions. This narrow focus leaves significant gaps in understanding community knowledge.

Hence, more inclusive studies would address these gaps and inform more effective public health strategies.

2.2. Life cycle and pathophysiology of *S. haematobium* infection

Schistosome parasites are multicellular organisms with a highly complex life cycle, requiring two obligate hosts. The life cycle begins with the release of eggs into freshwater through the urine or feces of the human host. Changes in osmolarity and light trigger the hatching of the miracidia, which swims in the water and enters the tissue of an intermediate host (a snail). Once inside the new host, the miracidia transform into sac-like structures called sporocysts, which reproduce asexually to produce numerous cercariae. The mature cercariae burst through the snail tissue and get into the water, awaiting a mammalian definitive host. Infection occurs when these cercariae penetrate the skin, which can happen within minutes (Nelwan, 2019; Nanes Sarfati *et al.*, 2021).

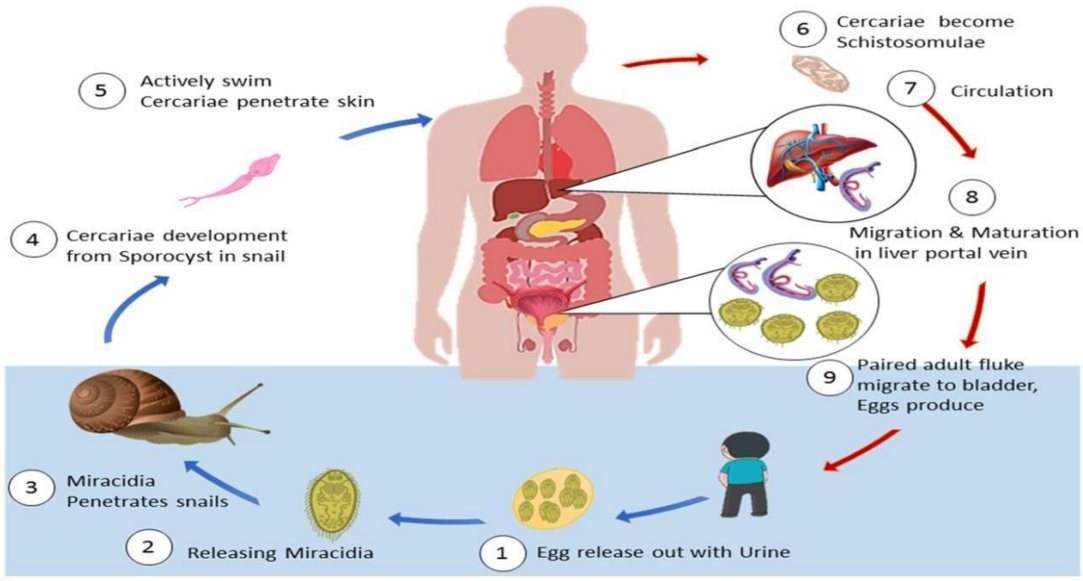

Figure 2: Life cycle of *S. haematobium* [source: (Chatterji *et al.*, 2024)].

Once inside the human body, the young worms embark on an incredible journey. The parasites undergo morphological and biochemical transformations into juvenile forms called schistosomula in the skin. These migrate into and through the dermis, eventually reaching dermal blood vessels around 72 hours after infection (Shebel *et al.,* 2012; Nation *et al.,* 2020). The schistosomula then travel through the circulation, reaching the lungs around 7-8 days post-infection, where they may remain for up to two weeks. After this, the schistosomula migrate from the lungs to the left side of the heart through the pulmonary veins, and from there, they enter the abdominal aorta. From the abdominal aorta, they can reach the liver‘s portal vein via the celiac trunk, inferior and superior mesenteric arteries, or iliac arteries (Nation *et al.,* 2020).

Upon reaching the hepatic portal vein, the parasites experience substantial growth, elongating their bodies toward the posterior end. Their weight doubles approximately every 2-3 days within the first two weeks. The development of sexual reproductive organs is particularly significant during this stage (Nanes Sarfati *et al.,* 2021). Subsequently, the female worms seek a mate and migrate against the blood flow from the liver to reach their preferred egg-laying sites (Mehlhorn, 2021a).

S. haematobium first enters the splenic vein and then proceeds into the inferior mesenteric vein. This vein leads directly to the superior rectal (hemorrhoidal) vein, which connects to a network of vessels known as the rectal venous plexus. This plexus is further interconnected with vessels draining the reproductive organs, such as the uterine and vaginal venous plexuses in females and the prostatic venous plexus in males. These reproductive venous plexuses, in turn, connect with the network of vessels that drain the bladder, known as the vesical venous plexus (Nation *et al.,* 2020).

The female adults lay approximately 300 round, terminally-spined eggs daily (Mehlhorn, 2021a). These eggs are deposited in the vesical venous plexus, where they can traverse the blood vessel bladder wall to reach the bladder lumen. To propagate the transmission cycle, only a third to half of these eggs are excreted in the urine (Costain *et al.,* 2019; Orish *et al.,* 2022). The remaining eggs become entrapped in the capillary beds of the pelvic end organs, particularly in the bladder, ureters, and genital tract, leading to urinary and genital SCH (Santos *et al.,* 2021).

From the liver, *S. haematobium* can also migrate to the superior mesenteric vein and move towards the intestine instead of progressing to the bladder. An autopsy studies in Egypt found adult *S. haematobium* worms in 47% of cases in mesenteric/portal veins and 48% in the bladder, demonstrating their migration beyond the urinary tract (Cheever *et al.,* 1977). They can also enter systemic venous blood flow via the pelvic venous system or the anorectal plexus, reaching the inferior vena cava and potentially being found in other tissue. However, eggs deposited in the intestine and tissues are "dead-end" because they lack an efficient route to exit the host‘s body and continue the parasite‘s transmission cycle (Nation *et al.,* 2020).

2.3. Urogenital schistosomiasis: pathogenesis and immunological response

Schistosoma haematobium causes disease in the urinary tract, commonly called urinary schistosomiasis, due to the migration and deposition of eggs in the bladder and urethra. However, it has been observed that the migration of *S. haematobium* eggs is not confined solely to the urinary tract. The term urogenital schistosomiasis was introduced in 2009 to describe the condition, as *S. haematobium* eggs are often found in the vesical plexuses of the pelvis, which can lead to obstructive disease in both the urinary and reproductive systems (Sturt *et al.,* 2020a).

The first clinical sign typically appears 10–12 weeks after infection, manifesting as haematuria due to damage to the bladder wall, often accompanied by increased frequency of micturition and dysuria in the later stages of the disease (Barsoum, 2013; Mawa *et al.,* 2021). Chronic urinary infections may lead to lesions in the bladder and ureter walls, causing fibrosis in the bladder and lower ureters, calcification in the urinary tract, kidney damage, and, in severe cases, bladder cancer. Furthermore, chronic infection in the genital tract can result in infertility (Mawa *et al.,* 2021; Santos *et al.*, 2021).

The clinical manifestations and complications of chronic SCH are primarily due to the inflammatory responses generated by the eggs laid by female *S. haematobium* in the venous plexus of the bladder and other pelvic organs (Colley & Secor, 2014; Honeycutt *et al.*, 2014). The soluble antigenic glycoprotein secreted by the eggs attracts a significant immunological response that results in granuloma formation around the eggs. These granulomas, which develop at sites of maximal egg accumulation, aim to destroy the eggs but can also cause fibrosis in the surrounding host tissues (Acharya *et al.*, 2021). T-helper-2 lymphocytes primarily mediated the formation of granulomas and the subsequent local inflammatory response (Pearce *et al.,* 2002). Moreover, the schistosomula and their eggs stimulate the secretion of anti-inflammatory cytokines, promote the generation of regulatory T and B cells, and lead to elevated levels of IgE and eosinophils (Molehin, 2020).

2.3.1. Female genital schistosomiasis

Female genital schistosomiasis (FGS) is a severe and chronic gynecological condition; estimates suggest that 20–56 million women and girls in endemic African regions may be affected (Kjetland *et al.*, 2012; Kayuni *et al.*, 2018; Nemungadi *et al.*, 2022).

This condition arises primarily from the deposition of *S. haematobium* eggs in the female genital tract, with rare cases involving *S. mansoni* (Kukula *et al.*, 2019; Nation *et al.*, 2020). The eggs can lodge in various parts of the genital tract (fig 6), including the cervix, uterus, vagina, fallopian tubes, and ovaries, without any consistent predilection for a specific organ (Sturt *et al.*, 2020a).

The resultant granuloma formation leads to characteristic gynecological manifestations, including grainy sandy patches, homogenous yellow sandy patches, and rubbery papules (Randrianasolo *et al.*, 2015; Costain *et al.*, 2018). These lesions, accompanied by abnormal angiogenesis, contribute to symptoms such as vaginal discharge, itching, pre-pubertal and post-coital bleeding, and dyspareunia (Kukula *et al.*, 2019; Nemungadi *et al.*, 2022).

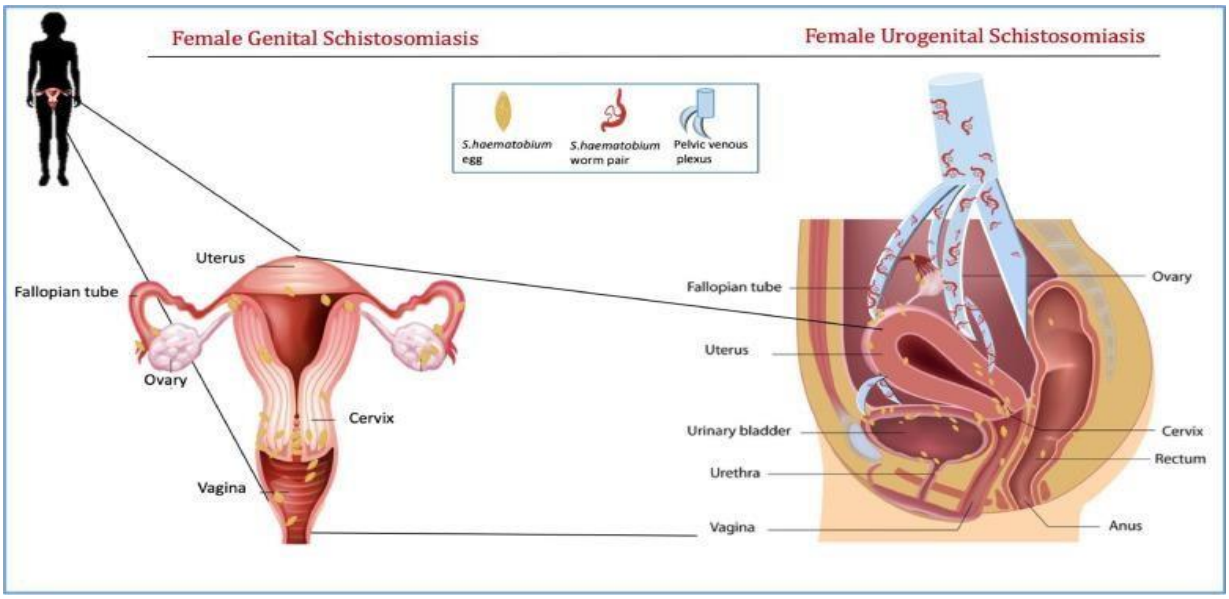

Figure 3: The anatomy of female genital schistosomiasis [Source: (Sturt *et al*., 2020b)].

FGS has been linked to adverse pregnancy outcomes, including ectopic pregnancy, miscarriages, stillbirths, and preterm delivery (Kjetland *et al.*, 2006; Orish *et al.*, 2022). It may also delay

postpartum healing and result in vesicovaginal fistula formation (Misiak *et al.,* 2023), while untreated cases can lead to infertility (Kjetland *et al.,* 2010). A study in SSA reported a 44% prevalence of sub-fertility in *S. haematobium* endemic regions (Miller-Fellows *et al.,* 2017). Moreover, a study in East Africa proved that infertility odds were higher in *S. haematobium* endemic areas (Woodall & Kramer, 2018).

FGS has also been implicated in increased susceptibility to HIV. Schistosomiasis associated loss of mucosal integrity and angiogenesis in the vagina and cervix elevate the risk of HIV acquisition (Sturt *et al.,* 2020b; Patel *et al.,* 2021). Immunological changes, including elevated CD4 receptor and chemokine co-receptor production in response to *Schistosoma* eggs, may further enhance HIV transmission risk (Kleppa *et al.*, 2014; Patel *et al.*, 2021).

Additionally, FGS may act as a co-factor for human papillomavirus (HPV) infection and cervical cancer through altered cervical mucosal gene expression and reduced protective cytokine levels (Kjetland *et al.,* 2010; Dupnik *et al.,* 2019). However, more research is needed to confirm these associations (Umbelino-Walker *et al.,* 2023).

Diagnosis and management of FGS remain challenging due to limited awareness among healthcare professionals and inadequate diagnostic resources in endemic areas (Lamberti *et al.,* 2024b). The clinical presentation often overlaps with sexually transmitted infections (STIs) or cervical cancer, leading to frequent misdiagnoses (Kjetland *et al.,* 2012; Nganda *et al.,* 2023). Diagnosis primarily relies on recognizing clinical features through colposcopy or pelvic examination (Kjetland *et al.,* 2012; Orish *et al.,* 2022). Histological biopsy and PCR analysis of genital specimens can confirm a diagnosis but require specialized facilities often unavailable in endemic regions (Engels *et al.,* 2020).

Regular treatment with PZQ from an early age, either through MDA or test-and-treat strategies, is effective in preventing the progression of SCH to genital damage and FGS progressing to the granuloma stage (Kini *et al.*, 2009; Nemungadi *et al.*, 2022; Nganda *et al.*, 2023). Moreover, introducing a clinical care package at the primary healthcare level to diagnose and treat females presenting symptoms of FGS before the age of 21 could significantly reduce sub-fertility rates (Miller-Fellows *et al.*, 2017) and lower HIV incidence by an estimated 9–22% (Ndeffo Mbah *et al.*, 2014). Furthermore, expanding the scope of healthcare services and improving the overall delivery of care for marginalized women and girls by integrating FGS management with HIV, STIs, and cervical cancer prevention could greatly enhance health outcomes in endemic regions (Engels *et al.*, 2020).

2.3.2. Male genital schistosomiasis (MGS)

Male genital schistosomiasis (MGS) is a poorly recognized illness closely associated with regions endemic to *S. haematobium*. It first came to light in Egypt (Madden, 1911) and now affects millions of males in SSA (Kayuni *et al*., 2018). According to a recent report by kayuni *et al.* (2024), zoonotic and hybrid schistosomes, which include mixed infections of *S. mansoni* and potential hybrids of *S. haematobium* and *S. mattheei*, can lead to MGS that resembles those caused by human schistosomes.

MGS results from the deposition of schistosome ova in various genital organs (epididymis, prostate, seminal vesicles, vas deferens, and testes) and reproductive fluids. This can lead to a range of symptoms, including genital or ejaculatory pain, haemospermia, abnormal ejaculate, organ enlargement, granulomas, fibrosis, and calcifications (Stecher *et al.*, 2015; Lang *et al.*, 2017; Nation *et al.*, 2020; Kayuni *et al.*, 2024).

MGS is a potential cause of male infertility, although the precise incidence is unknown (Kini *et al.,* 2009). Male infertility resulting from SCH can be attributed to various factors, including hormonal imbalances, damage to the testicular tissue, and partial or complete genital ductal system obstruction resulting from granulomas and bilharzial inflammation (Abdel-Naser *et al*., 2018).

MGS may also contribute to increased HIV transmission. Men with MGS exhibit elevated levels of CD4+ lymphocytes and cytokines (e.g., IL-4, IL-6, IL-10, TNF-α) that attract HIV-infected cells into semen, upregulate viral replication, and increase viral load (Leutscher *et al.*, 2005; Stecher *et al.*, 2015). Furthermore, MGS has been linked to genital cancers, though the mechanisms remain unclear (Figueiredo *et al.*, 2015; Choto *et al.*, 2020).

Diagnosing MGS remains challenging as gonadal lesions are not always detectable or specific. Microscopic semen analysis can reveal ova, while imaging techniques such as scrotal or transrectal ultrasonography and MRI may detect inflammation and pathologies linked to infertility (Abdel-Naser *et al.*, 2018).

Higher doses (60 mg/kg) of PZQ administered 2–3 times annually may be more effective in resolving some MGS symptoms, but its ability to reverse chronic pathologies remains uncertain (Alonso *et al*., 2006; Perignon *et al*., 2007; Lang *et al.*, 2017). For extensive testicular or ductal damage, hormonal replacement and assisted reproductive technologies (IVF/ICSI) may be necessary (Abdel-Naser *et al.*, 2018).

In general, genital schistosomiasis is underreported and underappreciated due to limited clinical infrastructure and reliance on insensitive diagnostic methods, such as symptomatology and microscopy (Stecher *et al.*, 2015; Stothard *et al.*, 2020). Low-cost molecular diagnostics and

radiological tools like portable ultrasonography should be implemented in endemic areas to monitor morbidity and outcomes (Kayuni *et al.,* 2018; Engels *et al.,* 2020). In addition, raising awareness among healthcare providers and integrating its management with broader SCH control programs are critical for alleviating chronic infections and associated comorbidities (Faust *et al.*, 2020; Orish *et al.*, 2022).

2.3.4. *Schistosoma haematobium* associated bladder cancer

Schistosoma haematobium was classified as a carcinogenic parasite by the International Agency for Research on Cancer (IARC) in 1994 and reaffirmed in 2012 (Botelho *et al.,* 2017). It is strongly associated with squamous cell carcinoma (SCC) of the bladder, the second leading cause of bladder cancer globally (Saginala *et al.,* 2020). Across Africa, in 2018 alone, approximately 549,393 new cases of bladder cancer and 199,922 deaths were reported (Jalloh *et al.,* 2022). SCC in Africa is primarily linked to *S. haematobium* infection, though it can also arise from other factors (Manley *et al.,* 2017). Significant associations of SCC with *S. haematobium* infection have been reported in Nigeria (41.7%) (Ibrahim *et al.,* 2015), Tanzania (73.5%) (Rambau *et al.,* 2013), and Senegal (29.2%) (Diao *et al.,* 2008).

Bladder SCC linked to SCH exhibits distinct clinical features, including earlier onset, advanced stage diagnosis, poor prognosis, and limited response to chemotherapy (Efared *et al.,* 2022). The disease arises when *S. haematobium* eggs, deposited in bladder tissues by adult worms residing in the venous plexus, trigger chronic inflammation. This inflammation releases growth factors and carcinogenic substances, such as cytokines TNF-α and IL-6, promoting oxidative stress, DNA damage, cellular proliferation, and angiogenesis, key hallmarks of cancer (Honeycutt *et al.,* 2014; Ishida & Hsieh, 2018; Santos *et al.,* 2021). Over time, the persistent inflammatory

response leads to fibrosis, genetic mutations, and ultimately malignant transformation (Jalloh *et al.,* 2022).

In addition, chronic inflammation disrupts the local immune system, making the bladder epithelium more vulnerable to co-infections by bacterial and viral agents, which can further contribute to the carcinogenic process (Efared *et al.,* 2022). Despite these findings, the specific mechanisms by which *S. haematobium* infection leads to bladder cancer remain poorly understood (Ishida & Hsieh, 2018).

The treatment of bladder cancer is typically initiated when hematuria is observed, followed by diagnostic procedures such as cystoscopy, transabdominal ultrasound, and CT urography. Treatment options depend on the disease stage and may include resection, chemotherapy, cystectomy, and radiation (Saginala *et al.,* 2020). However, in many African countries, early diagnosis and treatment are hindered by limited access to cancer screening and diagnostic services. As such, the most cost-effective public health response to bladder cancer in Africa is focused on preventing SCH through effective control measures for *S. haematobium* infection (Botelho *et al.,* 2017; Adeloye *et al.,* 2019).

2.4. Schistosomiasis control strategy and challenges

Numerous strategies and policies have been implemented to combat SCH, but despite substantial progress, the disease continues to affect millions globally (Dioe *et al.*, 2019). The World Health Organization (WHO, 2022b) has updated its guidelines for controlling and eliminating human SCH. These guidelines emphasize the importance of PC through MDA for all age groups and stress the need to improve access to safe drinking water, enhance sanitation, control intermediate hosts, and promote health education and behavior change.

2.4.1. Preventive chemotherapy (PC)

Preventive chemotherapy is the periodic mass distribution of safe, quality-assured medicines to communities and schools (WHO, 2024).The primary goal of SCH control programs is to eliminate the morbidity associated with the disease in target populations, primarily through PC using PZQ (Gordon *et al.*, 2019; Faust *et al.*, 2020). Since its development by Bayer in the 1970s, PZQ has been the first line drug for combating all species of schistosomes (Mehlhorn, 2021b). Administered at a dosage of 40 mg/kg body weight, it has demonstrated remarkable efficacy, with cure rates up to 90% in the population (Kramer *et al.*, 2014; Zwang & Olliaro, 2014; Andrade *et al.*, 2017) and 92% cure rate for 1-5 year old children (Mutapi *et al*., 2011). Its primary target is adult worms, reducing egg excretion and preventing the development of morbidity (Rollinson, 2009; Kayuni *et al.*, 2018). The mechanism of action involves interfering with the parasite‘s muscular activity, leading to paralysis and inhibiting its binding to host tissue (Mehlhorn, 2021b). In addition, PZQ damages the *Schistosoma* tegument (outer surface), which exposes parasite antigens to strong antibody responses (Pearson *et al.*, 2021).

Globally, PZQ, through MDA, has primarily been distributed to SAC aged 5 to 15 years, often through school-based programs (French *et al.*, 2018; Deol *et al.*, 2019; Aruleba *et al.*, 2019). In 2021, 75.3 million people received PC for SCH, of which 58.9 million were SAC, and 94% of all treatments were delivered in the African Region (WHO, 2022a). Between 2000 and 2010 and 2015–2019, there was a significant reduction in the prevalence of SCH among SAC in SSA, with an overall decline of 58.3%. Specifically, the prevalence of *S. haematobium* decreased by 67.9%, while *S. mansoni* prevalence declined by 53.6% during the same period (Kokaliaris *et al.*, 2022). However, the existing approach has consistently failed to reach a significant portion of at-risk individuals, including out of school children, PSAC, and women of reproductive age, and that

could result in an age shift in the prevalence and intensity of infection (Mduluza & Mutapi., 2017; Turner *et al.*, 2017; Faust *et al.*, 2020). WHO's 2022 guidelines recognize this and recommend expanding PC to include PSAC and high-risk adult groups, such as women of reproductive age, pregnant and lactating women (when appropriate), and those with frequent water contact like farmers and fishermen (WHO, 2022b).

Moreover, there is growing recognition that treatment alone will not eliminate SCH (Secor & Colley, 2018; Santos *et al.*, 2020). After each scheduled MDA program, within 1–2 months, the schistosomula gives rise to a new generation of adult worms, which results in the prevalence remaining mainly unaffected (Aruleba *et al.*, 2019; Zacharia *et al.*, 2020). Furthermore, widespread use of PZQ in Africa may lead to the growth of PZQ-resistant strains (Crellen *et al.*, 2016; McManus *et al.*, 2020). The long-term sustainability of PC efforts remains uncertain without complementary interventions that address the root causes of transmission (WHO, 2022b).

In Ethiopia, according to FMoH, (2021), more than 27 million SACs have been treated through MDA as part of broader NTD control efforts since the launch of the programs in 2016. The campaigns are typically conducted annually or biannually in high-risk zones. According to the health authorities of Gambella, Benishangul-Gumuz and Afar region, school-based (MDA) for schistosomiasis and soil-transmitted helminths was conducted for years, except for the first year of the COVID-19 pandemic. Treatment coverage rates often reach over 75.5%; effective drug delivery is hindered by geographic barriers, logistical constraints, cultural resistance, and lack of awareness (Chisha *et al.*, 2020). This requires integrated control methods and an expansion of PC to all populations in need (WHO, 2022b).

2.4.2. Intermediate host control

The ecology of the intermediate host can influence SCH prevalence; hence, controlling the snail can control the disease (Duarte *et al.,* 2020*;* Ahamide *et al.,* 2023). Before the focus of control shifted to anti-schistosomiasis chemotherapy in the 1970s and early 1980s, control of intermediate host snails was the primary strategy for managing SCH (Stothard *et al.,* 2009; Zheng *et al.,* 2021), and it contributed to many successful control outcomes (Rollinson *et al.,* 2013; King *et al.,* 2015).

When snail control programs are combined with MDA, the prevalence and incidence of the disease are reduced more rapidly (Ahamide *et al.,* 2023), and the strategy is also cost-effective (Lo *et al*., 2018). Evidence from various regions shows the effectiveness of such approaches, including a significant reduction in human prevalence in China (Li *et al.,* 2020; Zheng *et al.,* 2021) and in Africa (Morocco and Tunisia) (Amarir *et al.,* 2011; Aula *et al.,* 2021), and its eradication from Japan (Tanaka and Tsuji, 1997).

The primary method of snail control has traditionally involved molluscicides, with Niclosamide being the most commonly used synthetic chemical and the only one recommended by the WHOPE (The WHO Pesticide Evaluation Scheme) since the 1960s (WHO, 2017). However, while effective in reducing snail populations, its use is constrained by toxicity to non-target aquatic organisms and high operational costs, which hinder large-scale implementation (Colley *et al.,* 2014; Duarte *et al.,* 2020). Efforts have been made to develop different formulations of Niclosamide to reduce its environmental impact, but it remains problematic due to its toxicity to fish and other aquatic life (Liu &Yang, 2021).

In response to the drawbacks of chemical molluscicides, natural alternatives have gained attention (Garba Djirmay *et al.,* 2024). Natural molluscicides are less toxic and have a lower environmental impact, making them more sustainable for long-term use in SCH control programs, particularly in rural and environmentally sensitive areas (Lu *et al.,* 2018; Liu &Yang, 2021).

Environmental modification has also played a crucial role in snail control. Techniques such as vegetation removal, eliminating hiding places, and increasing water flow through mechanical disturbance help reduce snail habitats and populations (Lu *et al.,* 2018; King *et al.,* 2020). In China, several successful practices, including lining irrigation ditches with cement, converting paddy fields, and utilizing marshland, have proven effective in controlling snail populations and contributing to SCH control efforts (Li *et al.,* 2016).

Biological control methods have recently gained attention as an alternative to chemical and environmental strategies. Predatory species, such as prawns and water bugs, have been shown to reduce snail populations effectively. A field study conducted in Senegal demonstrated that prawns could significantly decrease snail populations and laboratory experiments on the water bug *Sphaerodema urinator* have highlighted its potential as a natural predator of schistosome intermediate hosts (Sokolow *et al.,* 2014; Younes *et al.,* 2017).

Despite the potential for these strategies, implementing and including snail control in MDA efforts in Africa has proven challenging (Binder *et al.,* 2020). The process requires extensive training, the identification of water contact sites, and customized approaches, all of which require significant resources and personnel (Secor & Colley, 2018).

In Ethiopia, chemical molluscicides like niclosamide are not feasible, primarily due to budget constraints and the high cost of these chemicals (Hailu, 2014). Hence, the FMoH strategic NTD plan for 2021–2025 emphasizes environmental management and snail control in high-risk areas. Key strategies include habitat modification to limit snail growth, and encouraging community involvement in reducing snail populations and improving sanitation (FMoH, 2021).

2.4.3. Water, sanitation, and hygiene (WASH)

Efforts to control SCH transmission have increasingly emphasized the role of WASH interventions. Reducing environmental contamination with schistosome eggs and minimizing human contact with infested water enhance treatment outcomes and offer long-term benefits (Evan Secor, 2014; Gordon *et al.*, 2019).

An effective WASH strategy hinges on integrating water, sanitation, hygiene, and health education. Monitoring and adapting these interventions to local contexts is critical, with strong community involvement ensuring sustainability (Molyneux *et al.*, 2017; López and Fletcher, 2022). By focusing on water treatment methods such as chlorination, filtration, and UV disinfection, as well as providing alternative safe water sources, such as piped water or protected water points, human exposure to cercariae and other schistosome life stages can be minimized (Braun *et al.*, 2018).

In endemic regions, where SCH is associated with occupational or recreational water contact, providing WASH facilities in public areas, including workplaces, is crucial (Torres-Vitolas *et al.*, 2023). Promoting infrastructure such as bridges and safe recreational areas could also reduce infection risks. Achieving this requires substantial initial investment and sustained collaboration across sectors and can lead to long-term cost savings (WHO, 2021).

Well-maintained sanitation systems also prevent water contamination with, urine, vaginal fluids, or stool (Grimes *et al.*, 2014; Phillips *et al.*, 2022). Encouraging hygiene practices, such as handwashing with soap or "*endod*", which is toxic to schistosome larvae, further strengthens control measures (Grimes *et al.*, 2015; Simoonga *et al.*, 2020).

Community participation in monitoring and maintaining water quality and sanitation is essential to ensure the sustainability of WASH programs. This involvement must include local health workers and representatives to promote WASH adoption, maintenance, and proper use (Croll and Rollinson, 2011).

In Ethiopia, much of the rural and small-town population relies on community-managed water supplies and more than half (56%) of rural households use unimproved sanitation facilities (Murungu *et al.*, 2022). Additionally, their reliance on open water sources for domestic use, livelihoods, and recreational activities, with common open-air defecation and urination, highlighted the necessity of WASH facilities (Assefa *et al.*, 2021; Phillips *et al.*, 2022). The Schistosomiasis Control Initiative (SCI), supported by WHO and UNICEF, began addressing these issues in the early 2000s by scaling up WASH interventions alongside chemotherapy programs (Jemal *et al.*, 2019). Ethiopia has integrated WASH interventions into broader health initiatives, significantly contributing to SCH and other NTDs control (Tchuem-Tchuente *et al.*, 2020). However, WASH intervention assessment is essential for tracking progress and ensuring evidence-based decision-making (Worku, 2017; WHO, 2021).

2.4.4. Behaviour change intervention

Behavioral interventions are a key control mechanism in addressing SCH, mainly through changing risky behaviors. These interventions focus on changing practices such as freshwater

contact, open defecation and promoting the use of treated water and safe hygiene practices. By influencing behaviors, these interventions interrupt the transmission cycle of the parasite and enhance the effectiveness of other control measures, such as MDA (Gordon *et al.*, 2019; Torres-Vitolas *et al.*, 2023). A study conducted in Tigray region of Ethiopia from 2009 to 2012 combining MDA campaigns with behavioral change strategies and improved sanitation reduced the disease prevalence from 44.7% in 2009 to 12.3% in 2010 and 2.0% by 2013 (Gal *et al.*, 2022). Moreover, a project integrating MDA, WASH and behavioral change interventions in Wolita zone reports infection level reduction (Mengistu *et al.*, 2024).

Various behavioral intervention models aim to modify human behavior, such as health education, social-environmental, physical-environmental, and incentive-based interventions, and target behavior change by modifying social structures and infrastructure and offering material rewards (Torres-Vitolas *et al.*, 2023). Health education is one of the most widely used strategies and has aided SCH control in China (Gordon *et al.*, 2019).

Health education messages can be disseminated through various channels, such as radio, television, film, drama, traditional opera, and print media, including posters, magazines, and daily items like shirts and umbrellas. Community Health Workers (CHWs), students, teachers, village leaders, and parents also can help spread information within the community. These methods can be tailored to fit local contexts, cultural practices, and available resources (Stewart & Franks, 2009).

In Ethiopia, the health extension program, launched in 2003, became a key platform for delivering health education in rural communities. By 2015, school-based health education was

integrated into national efforts to control SCH and linked to MDA campaigns to raise awareness about the benefits of PZQ treatment (Molla *et al.,* 2018; Tchuem-Tchuente, 2020).

Generally, behavior change interventions that focus on water-related behaviors, sanitation, hygiene, and safe water use are essential for controlling SCH and are most effective when integrated with other health measures.

2.4.5. Vaccine

Currently, no commercial vaccine is available against human schistosomes (Yamey *et al.,* 2024). However, vaccines could be crucial for long-term, sustainable control and elimination of SCH. Even a partially protective vaccine, in combination with MDA, could help reduce schistosome infections and interrupt transmission (McManus *et al.,* 2020).

Despite discovering several promising vaccine candidates, only four—Sh28GST, Sm-14, Sm-TSP-2, and Sm-P80 advanced to human clinical trials (Anisuzzaman *et al.,* 2020). Of these, the recombinant 28-kDa glutathione S-transferase (rSh28GST), presented with Alhydrogel (Bilhvax), has shown promise in clinical studies for *S. haematobium* (Naseri *et al.,* 2021).

This antigen, expressed in the tegument and sub-tegument of both adult and larval schistosomes, is safe and immunogenic in Phase 1 and 2 trials, generating a TH2-type immune response. However, a Phase 3 trial revealed insufficient protection, underscoring the need for continued development of alternative candidates (Riveau *et al.,* 2018; Anisuzzaman *et al.,* 2020; Naseri *et al.,* 2021; Hotez & Bottazzi, 2023).

In conclusion, integrated, multi-component approaches targeting various points in the schistosome life cycle are essential for controlling and ultimately eliminating SCH (Gordon *et al.,* 2019). The ongoing reliance on PC must be supplemented with improved access to clean

water, better sanitation and hygiene, health education to support behavior change, environmentally friendly snail control, and the development of a viable vaccine. Achieving these goals will require substantial financial investment, human resources, and long-term political commitment (Chitsulo *et al.*, 2000; French *et al.*, 2018).

2.5. Molecular characterization of *Schistosoma haematobium*

Sophisticated molecular tools have facilitated a greater understanding of the relationships between many of the 24 recognized species of *Schistosoma* (Lockyer *et al*., 2003; Webster *et al*., 2006; Loker and Brant, 2006). Because of their complex, indirectly transmitted life cycle, size, and location within the human, interpreting the patterns of transmission and reproductive patterns of schistosomes requires indirect methods such as population genetic analyses using tools of molecular markers (Webster *et al*., 2020). Molecular characterizations enabled the study of the genetic diversity of schistosome and snail populations as well as the detection of the different hybridizations between *Schistosoma* species. This leads to opportunities to investigate many important topics such as the contribution of parasite genetics to variation in disease burden and pathology, the genetic consequences of various control activities for parasite populations, patterns of recruitment and transmission in endemic areas, and the likely evolution and spread of drug resistance (Rollinson *et al*., 2009; Webster *et al*., 2020).

2.5.1. Genetic markers used in molecular characterization

Microsatellite analyses and mitochondrial DNA sequencing methods have been developed and are contributing to a better understanding of the genetic structure of *schistosoma* and snail populations (Rollinson *et al*., 2009). Advances in the molecular characterization of schistosomes

and their genomes have provided several molecular diagnostic markers for different species (Webster *et al*., 2010).

Mitochondrial cox1 and nuclear ITS1 are used in different hybridization and genetic diversity tests. Sequence variation in the cox1 *gene* is commonly compared between specimens to identify evolutionary differences and similarities. It was expected that such DNA data would reveal any geographical structuring of the parasite populations and the extent of the genetic diversity within and between populations (Webster *et al*., 2012). On the other hand, nuclear ITS data is a powerful marker for introgression detection and confirming species identity (Webster, 2009; Leger and Webster, 2017). Hence, in most molecular studies, multi-locus approaches, combining nuclear and mitochondrial DNA markers are applied.

Microsatellite analysis has been seen as a promising method to acquire a more detailed characterization of genetic variation and schistosome population structure. Microsatellite loci are highly variable DNA markers widely used to investigate how parasite populations change over time and space, bottleneck effects and historical and contemporary population level changes (Webster *et al.,* 2015). Several *Schistosoma* species-specific microsatellite markers have thus been developed and evaluated. One of the first attempts to isolate microsatellite markers for *S. haematobium* was that of Golan *et al.* (2008), who identified 9 polymorphic loci with the number of allelic diversity and gene diversity ranging from two to seven. And another 16 microsatellite loci have been developed by Gower *et al.* (2011). Similarly, Glenn *et al.* (2013) identified 15 polymorphic microsatellite markers for *S. haematobium* that showed high variability in individuals and populations. To reduce cost and time, Webster *et al.* (2015) developed novel multiplex microsatellite polymerase chain reactions using 18 existing *S. haematobium* microsatellite loci, which enable simultaneous amplification across two multiplex

microsatellite PCRs. The principal drawback of microsatellite markers has been the cost and labor associated with the need to genotype multiple loci.

Now whole genome sequences of the three principal schistosome species infecting humans are available, which facilitates the transition from population genetics using limited numbers of markers to population genomics using genome wide marker information, but these are large (363–400 Mb) and prohibitively expensive (Berriman *et al.,* 2009; Young *et al.,* 2012; Luo *et al.,* 2019). To overcome that, exome capture methods are developed, utilizing RNA baits that hybridize with the targeted exome sequences and are isolated from non-coding DNA. The attractive features of exome sequencing for schistosome: (i) this approach is scalable to work with hundreds of individual miracidia, (ii) repetitive regions of the genome that are problematic to align are removed, (iii) contaminating sequences amplified from FTA cards (e.g. bacteria from fecal matter and water) are eliminated, and (iv) reducing the genome size from 363 Mb to 15 Mb allows sequencing to high depth read, providing robust scoring of variable sites (Le Clec'h *et al.,* 2018).

2.5.2. A different stage of the parasite used for DNA detection

In the past, molecular studies involved using adult worms for epidemiological studies; however, adult worms are not available for direct sampling due to their location in the mesenteric system surrounding the intestine or bladder. Getting adults depends on infecting laboratory snails with miracidia and exposing laboratory rodents to cercariae from the infected snails (Webster *et al*., 2010). The inherent problem of laboratory passage is that it is time-consuming and introduces sampling error, bottlenecks and selection biases, such that the resulting isolates may not be representative of genetic variation in the original population (Shrivastava *et al*., 2005; Gower *et al*., 2007).

Later, PCR amplification becomes possible from the larval stage of the parasite. This methodology, first described by Shrivastava *et al*. (2005), enabled the genotyping of individual *S. japonicum* larval samples that require storage by freezing, which does not apply to field conditions. Afterward, further improved into simple, long-term, room-temperature storage of field-collected larval schistosome samples on cards and for multiplex PCR analysis, leading to multilocus genotyping of single larval samples by Gower *et al.* (2007). Even now, whole genome sequencing from this stage becomes possible (Le Clec'*et al.*, 2018).

Another approach to molecularly characterize *Schistosoma* parasites involves extracting DNA from the egg stage, as noted by Webster *et al.* (2019). However, working with eggs presents challenges due to their immature and small size, which limits the available DNA for analysis. Additionally, the egg's tough shell further complicates DNA extraction. Eggs isolated from host feces or urine is also vulnerable to contamination, whether from bacteria or residual host DNA, complicating the purity of the sample and the accuracy of molecular assays (Doyle *et al.*, 2019).

An advanced preservation method was developed to preserve the egg, miracidia and cercarial stages, allowing laboratory and epidemiological investigation without relying on live laboratory animals. Whatman FTA cards were introduced by Gower *et al*. (2007) and Webster (2009) recommended RNAlater as an effective preservation method for these stages. These preservative methods enabled DNA marker analysis by stabilizing parasite samples directly from naturally infected hosts, facilitating more detailed and ethically advantageous studies on parasite heterogeneity (Webster, 2009).

2.5.3. Genomic structure of *schistosoma* species

Schistosoma haematobium, S. mansoni, and *S. bovis* have a diploid set of eight chromosomes, consisting of seven autosomes and a pair of sex chromosomes (ZW system). Males have identical sex chromosomes (ZZ), while females have different ones (ZW) (Buddenborg *et al.,* 2021; Nikolakis *et al.,* 2022; Stroehlein *et al.,* 2022). The genome size of *S. haematobium* is 385 Mb (Young *et al.,* 2012), while *S. mansoni* and *S. bovis* have estimated genome sizes of 363 Mb and 374 Mb, respectively (Berriman *et al.,* 2009; Oey *et al.,* 2019). The cox1 gene is found in the mitochondrial genome, spanning about 14–18 kilobase pairs and including 12–13 protein-coding genes, including cox1 (Le *et al.,* 2000; Lockyer *et al.,* 2003). ITS2 sequences are located in the nuclear genome within the rDNA repeat unit located in chromosome 3, which contains the 18S, 5.8S, and 28S rRNA genes. These sequences are typically arranged in tandem on one or more autosomes, but their exact chromosomal locations in schistosomes may vary and are often poorly mapped (Hirai *et al.,* 1889; Stroehlein *et al.,* 2022).

2.5.4. Population genetic analyses of *S. haematobium*

The increasing use of molecular techniques and advancements in the tools resulted in a growing number of reports on genetic diversity, hybridization and introgression of *S. haematobium* (Leger and Webster, 2017).

2.5.4.1 Genetic diversity of *S. haematobium*

Genetic variability among parasite populations is an imperative factor in their potential for producing harmful effects on human populations. The diversity of this infection may play an important role in pathology, with heterogeneous versus homogeneous infections resulting in different clinical outcomes (Rollinson *et al*., 2009).

There is preliminary evidence of an association of specific parasite genotypes with more intense pathology in *S. haematobium* (Brouwer *et al*., 2003). Various factors can influence the genetic diversity of schistosome populations. Overlapping contact sites and snail and human movements may support the coexistence of many genotypes in a given area. They can also engender the development of new parasitic strains or families through genetic interchange and recombination between local and introduced genotypes (Gower *et al*., 2013; Glenn *et al*., 2013).

Knowledge of the genetic diversity of schistosome and snail populations adds a further dimension to the monitoring and surveillance of disease (Curtis *et al*., 2002; Rollinson *et al*., 2009). *S. haematobium* population genetics investigation is important for understanding its variable disease manifestations, epidemiology, if any development of drug resistance, and for developing new strategies for treatment, vaccination, and diagnosis (Webster *et al*., 2012).

Several studies have shown genetic variation within and between populations of *S. haematobium* in various African countries using microsatellite foci (Table 1). Across 6/5 SSA countries, high levels of genetic diversity were detected in *S. mansoni* and *S. haematobium* populations at the country level (Gower *et al*., 2013). In another study, in Africa's six locations, Glenn *et al*. (2013) detected significant variance in genetic diversity and differentiation of *S. haematobium* with 51%-70% heterozygosity from five sampling locations. The study done in the 2 major African endemic countries, Mali and Nigeria, by Ezeh *et al*. (2015) also demonstrated the significant difference in the genetic variation of the parasite.

On the other hand, a study done on 41 localities representing 18 countries across Africa and the Indian Ocean Islands, using microsatellite and cox1 markers, shows the low polymorphism of *S. haematobium.*

Only two groups can be identified across the parasite‘s range in SSA: Group 1clusters parasites from mainland Africa, while Group 2 clusters parasites exclusively from the Indian Ocean islands and the neighboring African coastal regions (Webster *et al.*, 2012). This is also supported by other studies done in different areas (Sady *et al.*, 2015a; Quan *et al.*, 2015).

Table 1: Genetic diversity of *S. haematobium* across Africa.

Country	**Level of diversity**	**Markers used**	**Reference (year)**
Cameron	Low	Microsatellite loci	Teukeng *et al.*, 2022
Nigeria	no genetic diversity	Microsatellite loci	Onyekwere *et al.*, 2022
Côte d‘Ivoire	Significant genetic Diversity	Microsatellite loci	Angora *et al.*, 2022
Egypt	High	Randomly amplified polymorphic DNA Markers	El-Kady *et al*., 2020
Egypt, Zimbabwe and South Africa	A moderate to high Level	----------	Afifi *et al*., 2016
Yemen	Low	mitochondrial (cox1)	Sady *et al.*, 2015a
Sudan	Low	Nuclear (ITS 2) and mitochondrial (cox1)	Quan *et al*., 2015
Mali and Nigeria	High level	microsatellite loci	Ezeh *et al.*, 2015

Zanzibar	High	Cox1 and the NADH-dehydrogenase subunit 1 (nad1)	Webster *et al*., 2013
Mali, Niger	High levels	Microsatellite loci	Gower *et al*., 2013
Cameroon	Intermediate		
Kenya and Tanzania	Low level		
Zanzibar Malawi, Mauritius	Highest levels of Diversity	Microsatellite loci	Glenn *et al*., 2013
Nigeria and Senegal	Were nearly as diverse		
South Africa	Much low diversity		
Mali	High	Microsatellite loci	Gower *et al*., 2011

2.5.4.2. Hybridization and introgression of *Schistosoma haematobium*

Hybridization and genome introgression in parasites and pathogens can have major impacts on the host and the epidemiology and evolution of disease (Leger and Webster, 2017). New gene acquisition through introgressive hybridization can lead to phenotypic innovation that can profoundly influence disease evolution (Husey *et al*., 2009).

In nature, the distribution of schistosome species and their intermediate snail hosts and definitive host specificity are believed to restrict these hybridization events from occurring. However, the ecological barriers between species can be lost due to natural and anthropogenic changes (Husey *et al*., 2009). Anthropogenic changes, such as dam construction, human and animal migration, and altered agricultural practices, increasing global trade, and climate change, are predicted to

increase opportunities for interspecific exposure and co-infection and, therefore, hybridization (Leger and Webster, 2017). Moreover, allelic exchange among gene pools of sympatric, interbreeding species leads to instant large genetic diversity (Panzner & Boissier, 2021).

Schistosomes undergo a sexual reproductive stage within their mammalian host, enabling interactions between different species (heterospecific crosses), which may result in either parthenogenesis or hybridization depending on the phylogenetic distance of the species involved (Webster *et al*., 2013; Sene-Wade *et al*., 2018). As a result, human populations encounter new infections more frequently, and co-infection by multiple parasites from different lineages or species within individual hosts can occur (Webster *et al.,* 2013).

The different Hybridizations that occur between schistosomes have already been identified using molecular tools between different human specific schistosome species (e.g *S. mansoni* and *S.haematobium*), different animal-specific schistosome species (e.g *S. bovis* × *S. curassoni* hybrids), and between human specific and animal specific schistosome species (e,g *S.haematobium* × *S. bovis* and *S.haematobium* × *S.* mattheei) (Webster *et al*., 2010; Webster *et al*., 2013; Moné *et al*., 2015; Leger and Webster, 2017). Studies have highlighted the existence of introgressive hybridization between human-specific and animal-specific schistosome species as a possible emerging public health problem that may pose a serious challenge for disease control and elimination programs. Since then, hybridization between schistosomes has been known to influence the disease epidemiology and enhance phenotypic characteristics affecting transmission, morbidity and drug sensitivity (Webster *et al*., 2013; Leger and Webster, 2017). In addition, the hybrid status of the parasite may impair the parasitological, serological, and molecular diagnostic (Fernández-Soto *et al*., 2020).

In many endemic areas, *S. haematobium* is sympatric with *S. bovis*, *S. mattheei*, *S. curassoni*, *S. intercalatum*, and *S. magrebowiei*, a closely related species that shares certain characteristics. In such closely related species, when co-infecting a host, introgression can result and whole genome admixture can occur through hybridization (Leger and Webster, 2017). Most studies focus on *S. haematobium* and *S. bovis* hybridizations, since from the other *S. haematobium* group species *S. bovis*, is the most abundant, potentially having the widest geographical overlap (Webster *et al*., 2010). Certain members within the group's ability to hybridize and form viable offspring, especially emphasized in *S. haematobium* and *S. bovis*, had been proved. This is evidenced by hybridization events detected in the laboratory by Taylor (1970) and Webster *et al.* (2013) and hybridization in humans and snails in nature (Table 2). Moreover, according to Platt *et al*. (2019), *S. bovis/S. haematobium* hybridization demonstrates profound consequences of ancient introgression, at least occurring 108-613 generations ago,from a livestock parasite into the genome of *S.haematobium*.And such introgression is unidirectional from *S.bovis* with in *S.haematobium* (Rey *et al.*, 2021).

According to Mathieu-Bégné *et al*. (2024), hybrid schistosomes could exploit traits from both *S. haematobium* and *S. bovis*, potentially improving their survival, infectivity, and ability to adapt to diverse immune environments in humans and livestock.

Table 2: Hybridization between *Schistosoma* species

Host species detected in	**Species**	**Country**	**Genetic markers used**	**Reference (year)**
From human	*S.haematobium* and *S.bovis*	Mali	Cox1 and ITS2	Agniwo *et al.*, 2023
	S.haematobium and *S.bovis*	Cameron	nuclear (ITS2) and mitochondrial (cox1)	Teukeng *et al.*, 2022
	S.haematobium and *S.bovis*	Nigeria	Cox1 and ITS2	Onyekwere *et al.*, 2022

	S.haematobium and *S.bovis*	Côte d'Ivoire	Cox1 and ITS2	Angora *et al.*, 2020
	S.haematobium and *S.bovis*	Malawi	Cox1 and ITS2	Webster *et al.*, 2019
From human (Senegal)	*S.haematobium* and *S.bovis*	Senegal River Basin	Microsatellite, Cox1 and ITS2	Boon *et al.*, 2018; Boon *et al.*, 2019
	S.haematobium and *S.bovis*	Richard-Toll	Cox1 and ITS2	Webster *et al.*, *2013;*Sene-Wade *et al*., 2018
	S.haematobium and *S.bovis*	Delta of the Senegal River	Cox1 and ITS2	Husey *et al*., 2009
	S.haematobium and *S.bovis*	Mali		Soentjens *et al.*, 2016
	S.haematobium and *S.bovis*	Corsica, France	Cox1 and ITS2	Boissier *et al*., 2015
	S.haematobium and *S.bovis*		Cox1 and ITS2	Moné *et al*., 2015
	S.haematobium and S.mansoni	France		Le Govic *et al.*, 2019
From snail	*S.haematobium* and *S.bovis*	Niger	Microsatellite, Cox1 and ITS2 and 18S DNA Regions	Pennance *et al*., 2020
	S.haematobium and *S.bovis*	Côte d'Ivoire	Cox1 and ITS2	Tian bi *et al*., 2019

Furthermore, *S. haematobium* hybridizations with other animal and main human schistosome species were also reported in different areas. *S. haematobium–mattheei* hybrid was detected in Malawi (Webster *et al.*, 2019) and hybrids resulting from *S. mansoni* and *S. haematobium*

schistosome cross-breeding have been documented in northern Senegal (Husey *et al.,* 2013) and from a migrant boy in France (Le Govic *et al.,* 2019).

There is also a report of an infection cluster of prepatent *S. haematobium x S. mattheei* hybrids, with evidence of *S. mansoni* infection, as detected in returned Belgium travelers from South Africa (Cnops *et al.,* 2021). These multi-species co-infection and new hybrids may indicate zygotic species barriers are breaking down, allowing perhaps even broader schistosome species barriers to become eroded (Stothard *et al*., 2020).

The biological, epidemiological, or pathological consequences of this hybridization are yet unknown but certainly warrant further investigation due to the potential risk of zoonotic transmission, reservoirs of infection, increased transmission and pathology related to hybrid vigor (Sene-Wade *et al*., 2018).

Chapter 3: Research Questions and Objectives

3.1. Research questions

Based on the review of the literature, the following research questions were proposed:

i) What was the genetic profile of *Schistosoma* species circulating in Afar and Gambella endemic regions of Ethiopia?

ii) What were the prevalence, infection intensity, and associated risk factors of *S. haematobium* infections among PSAC in Abobo, Gambella, Ethiopia?

iii) What were the prevalence, infection intensity, and factors associated with *S. haematobium* infections among community members in Kurmuk, Benishangul-Gumuz, Ethiopia?

iv) What was KAP levels regarding UGS among community members in Abobo, Gambella, Ethiopia?

3.2. Objectives

3.2.1. General objective

The general objective of the study was to assess the genetic profile, epidemiology, and community knowledge of *S. haematobium* infections in UGS endemic localities in Ethiopia.

3.2.2. Specific objective

i) To molecularly characterize the genetic profile of *Schistosoma* species isolated from human hosts in Afar and Gambella regions of Ethiopia.

ii) To determine the prevalence, intensity and associated factors of *S. haematobium* infection among PSAC in Abobo, Gambella, Ethiopia.

iii) To determine the prevalence and infection intensity of *S. haematobium* among community members in Kurmuk, Benishangul-Gumuz, Ethiopia.

iv) To assess the levels of KAP related to UGS among community members in Abobo, Gambella, Ethiopia.

Chapter 4: Materials and Methods

4.1. Study area and population

The study was conducted in three UGS endemic areas of Ethiopia (Gambella, Afar and Benishangul- Gumuz), which differ in climate and geographical profiles but share common risk factors for transmission, including limited access to safe drinking water, improved sanitation, and inadequate health infrastructure (Leta *et al.*, 2020; FMoH, 2021).

The study was conducted in three regions of Ethiopia, each with a specific focus. In the Gambella Region (Abobo District), the research focused on the molecular characterization of *Schistosoma* species, assessing the prevalence, intensity, and associated risk factors of *S. haematobium* infections among PSAC, as well as evaluating community KAP regarding UGS. In the Afar Region (Haruka District), the study primarily focused on the molecular characterization of *Schistosoma* species. Meanwhile, in the Benishangul-Gumuz Region (Kurmuk District), the research aimed to determine the prevalence, intensity, and risk factors of *S. haematobium* infections among the community.

Gambela is located in southwestern Ethiopia, bordering South Sudan to the west, the Oromia Region to the northeast, and the Southwest Ethiopia Peoples' Region to the southeast. The region is divided into four administrative zones. Abobo Wereda, part of the Anywaa Zone, is situated at 7°51′0″ North, 34°33′0″ East, approximately 822 kilometers southwest of Addis Ababa. Its altitude ranges from 650 to 1,337 meters above sea level. According to 2022 demographic data from the regional office, Abobo Wereda had a population of 28,661, with 14,043 males and 14,618 females in 19 kebeles. The primary economic activities in the area are agriculture and fishing. The Alwero River that flows through Abobo town is a vital resource for domestic,

occupational, and recreational purposes. There is only one catholic health center.

The Afar Region is located in northeastern Ethiopia, spanning coordinates 39°34′–42°28′E and 8°49′–14°30′N. It shares regional borders with the Tigray Region to the northwest, the Amhara Region to the southwest, the Oromia Region to the south, and the Somali Region to the southeast. Internationally, it borders Djibouti to the east and Eritrea to the northeast (CSA, 2021). The region is subdivided into six administrative zones and one special woreda. Haruka District, located in Zone 3 of the Afar Region, lies in the Middle Awash Basin (Haldane river and Belean stream), approximately 300 kilometers northeast of Addis Ababa. The district comprises nine kebeles and has a population of 20,146, most of whom are pastoralists. Livestock, such as cattle, goats, sheep, camels, and donkeys, play a vital role in the district‘s economy, providing the primary source of livelihood for many households. The district's pastoralist communities are heavily dependent on the seasonal movement of livestock, which is influenced by the availability of grazing land and water resources (Megenas *et al.*, 2024). There is no governmental health center.

Benishangul-Gumuz is one of the eleven regional states in Ethiopia, subdivided into three administrative zones. Kurmuk is one of the seven districts in the Assosa Zone of Benishangul - Gumuz Regional State, located approximately 762 kilometers west of Addis Ababa. Kurmuk district is bordered by Menge district to the east, Sudan and South Sudan to the west, Sherkolle district to the north, and Komosha district to the southeast. Kurmuk experiences arid and semi-arid desert climatic conditions, with an annual rainfall ranging from 1,000 to 1,500 millimeters and temperatures between 28°C and 42°C. The altitude of the district varies from 650 to 1,337 meters above sea level. According to the 2020 local health office demographic, the population of the Kurmuk district was 24,346, comprising 12,051 males and 12,295 females in 19 kebeles.

The primary economic activities in Kurmuk include traditional gold mining, trade, and agriculture. The community relies heavily on seasonal streams (Shamade and Dinchaba river) and Kurmuk dam for domestic and occupational activities. The district is served by three health centers: Horazhabe, Dull-Shitalo, and Famatsera.

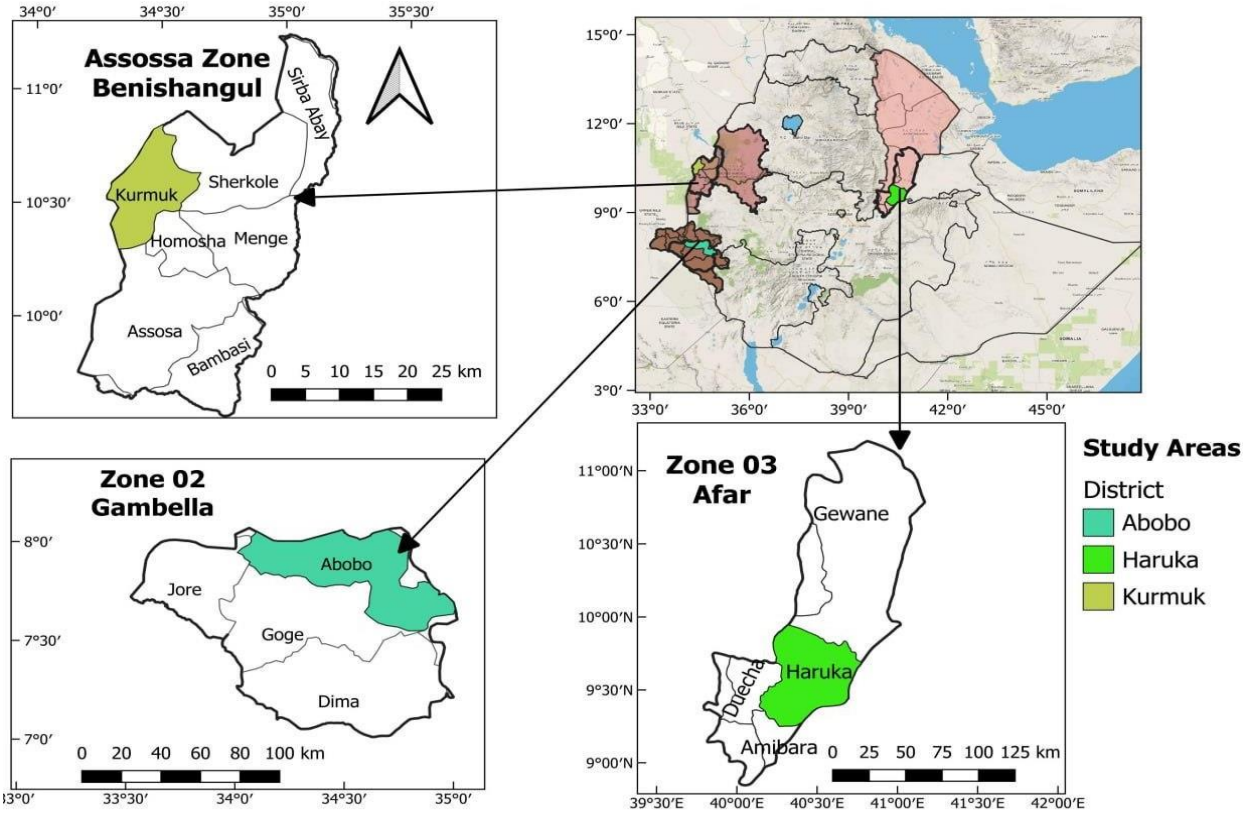

Figure 4: Map of the study areas

4.2. Study design and period

Between June 2021 and July 2024, community-based cross-sectional studies were conducted to assess the prevalence and infection intensity of *S. haematobium*, evaluate KAP and perform miracidia genotyping.

4.3. Source of population and study participants

Source population and study participants for each objective

Molecular characterization of *Schistosoma* (Afar and Gambella)

- **Source population**: All circulating *Schistosoma* genetic profiles in Afar and Gambella regions during the study period.
- **Study participants**: Were purposively selected children aged 5 to 15 years, residing in Haruka and Abobo districts.
- **Inclusion criteria:**
  - Children aged 5 to 15 years
  - Volunteered to participate in the study
  - Tested positive for hematuria (≥ +2) using a urine dipstick test
  - Had hatched miracidia
  - Residing in Haruka or Abobo districts
- **Exclusion criteria:**
  - Children outside the age range of 5 to 15 years
  - Those who did not volunteer to participate
  - Hematuria negative or hematuria score less than +2 by dipstick test
  - Unsuccessful hatched miracidia

Prevalence, infection intensity, and risk factors of *S. haematobium* among PSAC (Abobo, Gambella)

- **Source population**: All healthy PSAC aged 1–5 years living in Abobo district during the study period.

- **Study participants**: From four purposively selected kebeles/villages (Abaro, Tagni, Mender 17, and Wankak), randomly selected PSAC were included in the study.

Inclusion criteria:

- PSAC aged 1 to 5 years
- Living in the study area
- One child per household
- Mothers /caregivers were available and willing to participate in an interview

Exclusion criteria:

- Children outside the age range of 1 to 5 years
- Not residing in one of the selected kebeles
- More than one eligible child per household
- Urine sample less than 10ml
- Mothers or caregivers who were unavailable or refused the interview

Prevalence and infection intensity of *S. haematobium* among community members (Kurmuk, Benishangul-Gumuz)

- **Source population**: All individuals residing in Kurmuk district during the study period.
- **Study participants:** From purposively selected four kebles\villages (Dulshatalo, Belehu- jibla, Ogendu, and Kurmuk), randomly selected individuals aged 5 years and above who volunteered participated in the study.

Inclusion criteria

- Individuals aged less than five years

- Residents of Dulshatalo, Belehu-Jibla, Ogendu, or Kurmuk kebeles
- Volunteered and provided informed consent (or assent for minors with parental/guardian consent)

Exclusion criteria

- Individuals who are temporarily visiting the selected kebeles
- Individuals with severe disease
- Individuals unwilling to provide consent/assent

KAP related to UGS (Abobo, Gambella)

- **Source population**: All community members residing in Abobo district during the study period.
- **Study participants**: From purposively selected Abobo town and mender 4 and 5 kebele, household heads and household members were the study participants

Inclusion criteria:

- Residents of Abobo Town and Mender 4 and 5 kebeles
- Household heads or household members aged 12 years or older
- Volunteered to participate in the study

Exclusion criteria:

- Individuals younger than 12 years
- Not residing in the study kebeles
- Unwilling to participate

4.4. Sample size estimation

The sample size for estimating the prevalence of *S. haematobium* infection and KAP study was calculated using the following formula (Charan & Biswas, 2013):

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

Where:

- N is the sample size.

- Zα/2is the Z-score corresponding to the desired confidence level (for a 95% confidence level, Zα/2=1.96).
- P is the estimated prevalence, as no recent population-based study exists (50% (or p=0.5))
- D is the desired absolute precision or margin of error (5%).

$$n= \frac{(1.96)^2 \cdot 0.5.\ (1-0.5)}{(0.05)^2}$$

$$n= \frac{(3.8416)\ \cdot 0.25}{0.0025}$$

$$n= \frac{(0.9604)}{0.0025}$$

n= 384

A 5% non-response rate was added to the initial sample size. Thus, the final sample size was 403.

4.5. Sampling technique

The study areas (districts and kebeles) were selected purposively from Gambella, Afar and Benishangul-Gumuz regions of Ethiopia based on the endemicity of UGS (Birrie *et al.,* 1995; Mekonnen *et al.,* 2013; Geleta *et al.,* 2015; Degarege *et al.,* 2022) and proximity to water sources. The study targeted Abobo Wereda (Gambella) near the Alwero River, Kurmuk District (Benishangul-Gumuz) near seasonal streams (Shamade and Dinchaba River) and dam, and Haruka District (Afar) in the Middle Awash Basin, where *S. haematobium* is prevalent.

Molecular characterization study

- Two districts purposively nominated from Afar and Gambella regions and again study participants purposively chosen based on having hatched miracidia.

Prevalence studies (Abobo and kurmuk Districts)

- The study districts were stratified by kebeles based on their administrative boundaries. The sample size was allocated proportionally to each kebele according to its population size. Study participants were randomly selected from each kebele using a simple random sampling technique.

KAP study (Abobo district)

- A list of all households in the selected kebeles of Abobo district was obtained from the respective kebele leaders. The total sample size (n=403) was proportionally distributed among the kebeles (323 particpants from abobo town and 80 participants from Mender 4 and 5) based on the number of households (1043 vs 260) in each study site. Households were then selected using a systematic random sampling technique. The study participants from each selected household were recruited using a simple random sampling technique (one person from each household).

4.6. Sample collection and investigation

4.6.1. Sample collection and processing for molecular characterization (Afar and Gambella)

4.6.1.1. Collection and preservation of miracidia on Whatman FTA cards

Urine samples were first filtered. After thorough mixing, 10 ml urine was taken by syringe and pushed gently through the filter (polycarbonate filter with a 12 µm pore size and Swinnex polypropylene filter holder (SterliTech corporation, USA). To facilitate miracidia hatching, the filter was removed with forceps, placed in a petri dish filled with bottled water and exposed to sunlight for 30 minutes (Webster *et al.,* 2010).

Once miracidia hatched, 10 to 20 miracidia were carefully collected from each child using a micropipette under a dissecting microscope to ensure precise collection. Each miracidium was placed onto a separate area of the Whatman FTA card (QIAGEN, Germany), allowing for proper absorption and preventing sample mixing. The FTA card was left to air-dry for one hour to stabilize the genetic material before storage. Once dried, the FTA cards were labeled with essential sample information, including the collection date, location, and number of miracidia. The labeled cards were then placed in a sealed plastic bag to protect them from moisture and contamination (Gower *et al*., 2011). The sealed bags were stored at room temperature until further molecular analysis.

4.6.1.2. DNA extraction and quality control

DNA extraction from each miracidium was carried out using the protocol outlined by Beltran *et al.* (2008) with a little modification. A 4-mm Harris Uni-core micro-punch (Qiagen, Hilden, Germany) was used to excise the specific area on the Whatman FTA card where each miracidium had been deposited. The punch was then placed into a 1.5-ml Eppendorf tube

containing 20 µl of 250 mM NaOH. The tube was left at room temperature for 1 hour to facilitate cell lysis and DNA release.

Following this, the sample was incubated at 99°C for at least 8 minutes without agitation to ensure proper cell disruption. Afterward, 10 µl of 250 mM HCl, 5 µl of 500 mM Tris-HCl, and 5 µl of 2% Triton X-100 were added and then incubated at 99°C for an additional 8 minutes with agitation at 12,000 rpm to extract the DNA. Finally, the DNA was eluted with 50 µl of nuclease-free water and stored at -20 °C until further analysis.

The quality of the DNA was assessed through two quality control steps to ensure its suitability for downstream molecular analyses. The concentration of the DNA was measured using the Qubit 4 Fluorometer (Thermo Fisher Scientific, USA), which provided accurate quantification based on fluorescent dyes that specifically bind to nucleic acids. The integrity and purity of the DNA were further evaluated using gel electrophoresis. The extracted DNA and molecular weight

ladder were loaded in a 2% agarose gel, then visualized under UV light for clear, distinct bands and the absence of smearing, indicating high-quality DNA suitable for molecular applications.

4.6.1.3. PCR amplification

1. Cox1 genotyping

DNA from each miracidium was molecularly characterized by rapid diagnostic multiplex PCR (RD-PCR) on cox1 (Webster *et al.,* 2010). For each *Schistosoma* species, species-specific primers (Table 3) were used (*S. haematobium* 120 bp, *S. mansoni* 215 bp, and *S. bovis* 260 bp) (Angora *et al.,* 2020) (Table 3).

Table 3: Primer Sequences for species identification

Primer type	**Primer name**	**Sequence**
Universal reverse	Shmb.R	5′ -CAA GTA TCA TGA AAY ART ATR TCT AA -3′
Species-specific forward primers	Sb.F	5′-GTT TAG GTA GTG TAG TTT GGG CTC AC-3′
	Sm.F	5′-CTT TGA TTC GTT AAC TGG AGT G-3′
	Sh.F	5′-GGT CTC GTG TAT GAG ATC CTA TAG TTT G-3′

2. Nuclear internal transcribed spacer II (ITS2) genotyping

ITS2 genotyping on a randomly selected sample of 24 miracidia was assessed for all sites using a previously described PCR protocol (Webster *et al.,* 2013; Webster *et al.,* 2010). *Schistosoma* genus-specific primers amplifying a 505 bp fragment of the internal transcribed spacer-2 (ITS2) sub-unit consisting of using one set of primers, namely ITS2F (5′-GGCTGCAGCGTTAACCATTA-3′ and ITS2R: (5′-ACACACACCATCGGTACAAA-3′).

Each PCR was carried out in a total volume reaction of 20 µl. The reaction mix included 10 µL of 2X Platinum™ SuperFi II PCR Master Mix (Thermo Fisher Scientific, USA), 3 µL of extracted DNA, 2 µL of 1X primer mix (comprising 1 µL of 10 µM reverse primer and 1 µL of 10 µM forward primer), and 5 µL of nuclease-free water. After gently mixing the components and briefly spinning them down to ensure they were at the bottom of the tube, the tubes were placed into the thermal cycler.

The thermal cycling conditions were as follows: an initial denaturation step at 98°C for 30 seconds to denature the DNA, followed by 30 cycles of denaturation at 98°C for 10 seconds, primer annealing at 52°C for 30 seconds, and extension at 72°C for 2 minutes. After amplification, a final extension was performed at 72°C for 5 minutes, followed by holding the reactions at 4°C. The PCR reactions were run on a GeneAmp PCR System 9700 (Applied Biosystems, USA).

After amplification, 5 µL of the PCR product, a positive control and a ladder (100bp) were loaded onto a 2% agarose gel stained with 10 µL of GelRed dye. The gel was then subjected to electrophoresis at 130 V for 45 minutes. Following electrophoresis, the gel was visualized under a UV transilluminator, and an image was captured for analysis (Onyekwere *et al.*, 2022).

The COX1 and ITS2 gene amplified fragment size was compared with the molecular weight marker, and the expected amplicon size was confirmed based on the primers used and the organism's genetic profile. The PCR products were stored at -20°C for future further genotyping.

Quality control measures were implemented, using negative control to prevent contamination and a positive control to ensure the PCR reaction was functioning correctly.

4.6.2. Data collection and laboratory examination for the prevalence studies (Abobo and Kurmuk Districts)

4.6.2.1. Urine sample collection

A community announcement about urine examinations was made by *kebele* leaders (local authorities), health extension workers and instructors before the start of the sample collection. Then, in the following days, the objectives of the study were explained and consent obtained; and using a structured format first, socio-demographic information and water contact behavior

data were collected from study participants with the help of NTD nurses and health extension workers, who know the local language (Aguak, Kenbata and Berta). Then, at the end of the interview, the participants brought urine samples between 10:00 am and 2:00 pm in 50ml containers. The containers were checked for at least 10 ml; if insufficient, the participants drank water and tried again. The presence of blood in the urine was tested by using urinalysis reagent strips (LABOQUICK Urine Testing Strips, 10 Parameter Urinalysis Test Strips, Turkey), afterward, the sample was preserved using formalin (0.1ml for 10ml of urine) and transported to Aklilu Lemma Institute of Health Research (ALIHR) laboratory for microscopic examination.

4.6.2.2. Questionnaire survey

Socio-demographic and water contact behavior information was collected through close-ended questionnaires. Following demographic information, taking the PSAC to water source, PSAC bathing or playing and urinating in water, and warming water to wash the child at home were among water contact practice questions forwarded for mothers/caregivers in Abobo district. In Kurmuk district, for the communities‘ prevalence study, swimming, playing and washing habits in open water were the questions raised.

4.6.2.3. Macro and microhaematuria detection

In this study, macrohaematuria refers to visible blood in urine, while microhaematuria indicates red blood cell (RBC) detection through urinalysis (Leslie *et al.,* 2024). First, a visual inspection of the urine was done to see a red or pink discoloration in the urine sample then further investigated by using urinalysis strip test by briefly immersing the complete area of the strip into the urine sample (upto 5 seconds), the strip removed and hold in horizontal position for about a minute. Then the reagent strip was compared with corresponding color blocks and the intensity

of microhaematuria was recorded according to the manufacturer‘s instruction (LABOQUICK 10 parameter Urinalysis Test Strips, Turkey, bach No. LURS.021.004), (negative; trace; +; ++; and +++).

4.6.2.4. Urine filtration and microscopy

The urine filtration technique was used to identify *S. haematobium* eggs in urine samples. The urine sample was drawn into a 10 ml disposable syringe, and a polycarbonate filter with a 12 µm pore size (SterliTech corporation, USA) was attached to the syringe using a Swinnex polypropylene filter holder (SterliTech corporation, USA). The urine was gently pushed through the filter using the syringe plunger to avoid damaging the filter or losing eggs. After filtration, the filter was removed using forceps and placed on a clean microscope slide (Ahmed, 2015). The slide was then examined under a light microscope, with the 10x objective, by trained laboratory technicians and quality control was performed on 10% of the slides. The *S. haematobium* eggs were identified based on their characteristic oval shape and terminal spine. The number of eggs is counted, and the level and intensity were determined per 10ml of urine and categorized as light (< 50 egg) and heavy infections (≥ 50 egg) (WHO, 1991). In this study, *S. haematobium* infection and UGS were defined as the presence of *S. haematobium* eggs in urine.

4.6.3. Data collection and processing for assessing KAPs (Abobo district, Gambella)

Data were collected using structured and some open-ended questionnaires. The questionnaire developed through conducting a scoping review of existing literature and evaluating similar questionnaires (Kabatereine *et al.,* 2014; Sady *et al.,* 2015; Koffi *et al.,* 2018; Dejon- Agobe *et al.,* 2021; Essien-Baidoo *et al.,* 2023). Questionnaire includes a structured introduction and sequenced logically, starting with general socio demographic questions to more specific ones (Boynton *et al.,* 2004; Ranganathan & Caduff, 2023).

The questionnaire was designed to assess various aspects of participants, knowledge (on symptoms, mode of transmission, prevention, and control), attitudes (toward its severity, prevention and sanitation practice), and practices (health-seeking behavior and risky practice) toward UGS. Most questions were structured, offering single or multiple response options. "I don‘t know" and "Other" options were included in the questionnaire to minimize guessing. In addition, the questionnaire featured open-ended questions to capture participants' perspectives and thoughts regarding the prevention and treatment of UGS. The questionnaire was initially developed in English, translated into Amharic and pre-tested for clarity and acceptability in the study districts. Through a house–to-house visit, each interview was done by trained NTD nurses fluent in the local language and selected from each of the study kebeles. To complement the questionnaires, direct observations were made regarding the participants' behaviors related to open-water contact, the presence of *Bulinus* snails, and human waste near the dam. Each correct answer on the questionnaire was assigned one point, while incorrect or ―I don‘t know‖ responses received zero points (Dejon- Agobe *et al.,* 2021). The total score for each participant was calculated by summing up the points across various sections of the questionnaire, amounting to 24, 2, 2 and 5 points for knowledge, attitudes, for treatment-seeking practice, and risky practice (open water contact practice and urinating in an open field/ bush/by open water), respectively.

4.7. Dependent and independent variables

For molecular characterization of *Schistosoma* Species (Afar and Gambella)

- **Dependent variable:**
    - Genetic profile of *Schistosoma* miracidia
- **Independent variables:**

- Geographic location (Afar and Gambella)
- Age and sex of participants

For prevalence of *S. haematobium* among PSAC (Abobo, Gambella)

- **Dependent variables:**
  - Prevalence of *S. haematobium* infection
  - Infection intensity (mean egg count per 10 mL of urine)
- **Independent variables:**
  - Socio-demographic factors: sex, age, education, occupation, residing village
  - Water contact behavior

For prevalence of *S. haematobium* among community members (Kurmuk, Benishangul-Gumuz)

- **Dependent variables:**
  - Prevalence of *S. haematobium* infection
  - Infection intensity (mean egg count per 10 mL of urine)
- **Independent variables:**
  - Socio-demographic factors: Age, gender, education level, occupation, and kebele\villages.
  - Water contact behavior

For KAP related to UGS (Abobo, Gambella)

- **Dependent variables:**
  - Knowledge about UGS
  - Attitude towards UGS
  - practices related to UGS

- **Independent variables:**
    - Socio-demographic factors: sex, age, education, occupation
    - History of UGS (yes/no)

4.8. Data analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) software version 23.0 after the completion of data collection, cleaning, editing, and coding. The descriptive statistics (mean, percentages, or frequency) were calculated and summarized in tables and figures. Bivariate and multivariate logistic regressions were used to determine the associations as well as the strength of associations of the outcome variable of interest with the independent variable. Variables with *P*- values less than 0.25 in the binary logistic test were included in the multivariable model to determine the key correlates of *S. haematobium* infections. Odds ratios (OR) at 95% confidence intervals (CI) were computed to measure the strength of association between different exposure variables and the outcome variable of interest. A p-values of less than 0.05 was considered statistically significant. Mean egg count association with independent variables was analyzed using the Mann-Whitney U and Kruskal-Wallis non- parametric test. Agreement between microscopic examination and urine dipstick test results was determined using Kappa statistic.

4.9. Quality control

Various measures were taken to ensure that data collected from the study subjects was accurate and reliable. The data collectors received a thorough orientation on the study's objectives, which helped them understand the importance of maintaining data integrity. Any errors discovered during the pre-analytical, analytical, and post-analytical processes were promptly corrected. The

investigators closely monitored the analytical procedures throughout the data collection period, including laboratory investigations, to ensure consistent and valid results. Standard operating procedures (SOPs) were developed for each task and strictly followed to guarantee the quality of all activities described in this study.

4.10. Ethical consideration

The protocol was reviewed and ethically approved (Ref No: ALIPB IRB/13/2019/20) by the Institutional Review Board (IRB) of former Aklilu Lemma Institute of Pathobiology, Addis Ababa University (ALIPB) presently Aklilu Lemma Institute of Health Research (ALIHR). Permission to conduct the study was obtained from the regional, the head of the district health offices and from the kebele authority. The participants were informed in the local language about the aim of the research and assured that they had full right to participate or withdraw from the study. Urine samples were collected after obtaining informed written consent and assent from all potential candidates or their parents/caregivers. Participants who were positive for UGS were treated with praziquantel (at 40 mg/kg body weight). Confidentiality was ensured by anonymizing data, removing personal names, and securely storing information in password-protected files. Data were used solely for study purposes, and results were presented without identifying study participants.

Chapter 5: Results

5.1. Characteristics of study participants involved in molecular analysis

The study included 44 participants aged 5–15 years, all microscopy positive. Among them, 28 (63.6 %) showed +3 haematuria and 16 (36.4%) had +2 haematuria. Most study participants were males (68.2%) and 9-13 years old (43.2 %). Participants were nearly evenly distributed between Afar (47.7%) and Gambella (52.3%) (Table 4).

Table 4: Socio-demographic **c**haracteristics of participants involved in molecular analysis

Variables	**Categories**	**No.**	**Percent**
Age (yrs)	5-8	13	29.5
	9-13	19	43.2
	14-15	12	27.3
Sex	Male	30	68.2
	Female	14	31.8
Region	Afar	21	47.7
	Gambella	23	52.3

5.2. Genotyping of *Schistosoma* using the Cox1 and ITS2 gene

Two hundred eighty samples were genotyped, from which only 177 gave consistent results using the RD-PCR, targeting the COX1 mitochondrial gene. In addition, 24 (12 from Afar and 12 from Gambella) *Schistosoma* miracidia genotyped targeting ITS2 nuclear gene (Table 5).

Table 5: Number of Miracidia collected from participants and analyzed

Region	**Villages**	**Altitude/longitude**	**No of children**	**Miracidia genotyped (N)**
Gambella	Abobo	N 7°51′0, 34°33′0″ E	23	81
Afar	Kelati Buri	N 09 28.691, E 04015.993, Altitude 726mt	8	53
	Buri	N 09 29.866 E 040 18.549, Altitude 731mt	5	19
	Kusra	N 09 32.421, E 040 23.441, Altitude 700mt	8	24

None of the miracidia analyzed exhibited the COX1 and ITS2 profile characteristic of *S. mansoni* or *S. bovis*, suggesting that these species were absent within the sample. Instead, all miracidia displayed the COX1 and ITS2 profile specific to *S. haematobium* (Fig. 5&6), indicating that the entire sample was composed exclusively of this species and the absence of mixed infection.

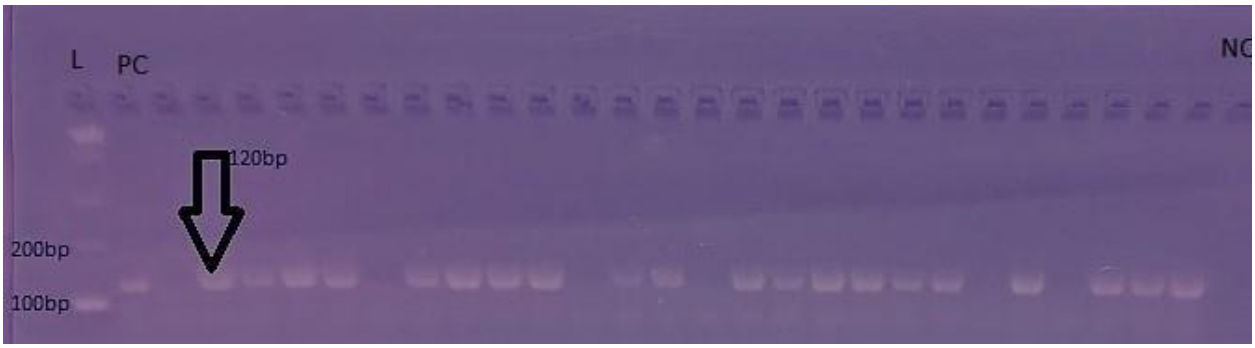

Figure 5: Mithochondrial COX1 gel image; molecular weight marker (L) Positive control of *S. haematobium* (PC), Negative control (NC), *S. haematobium* (120 bp).

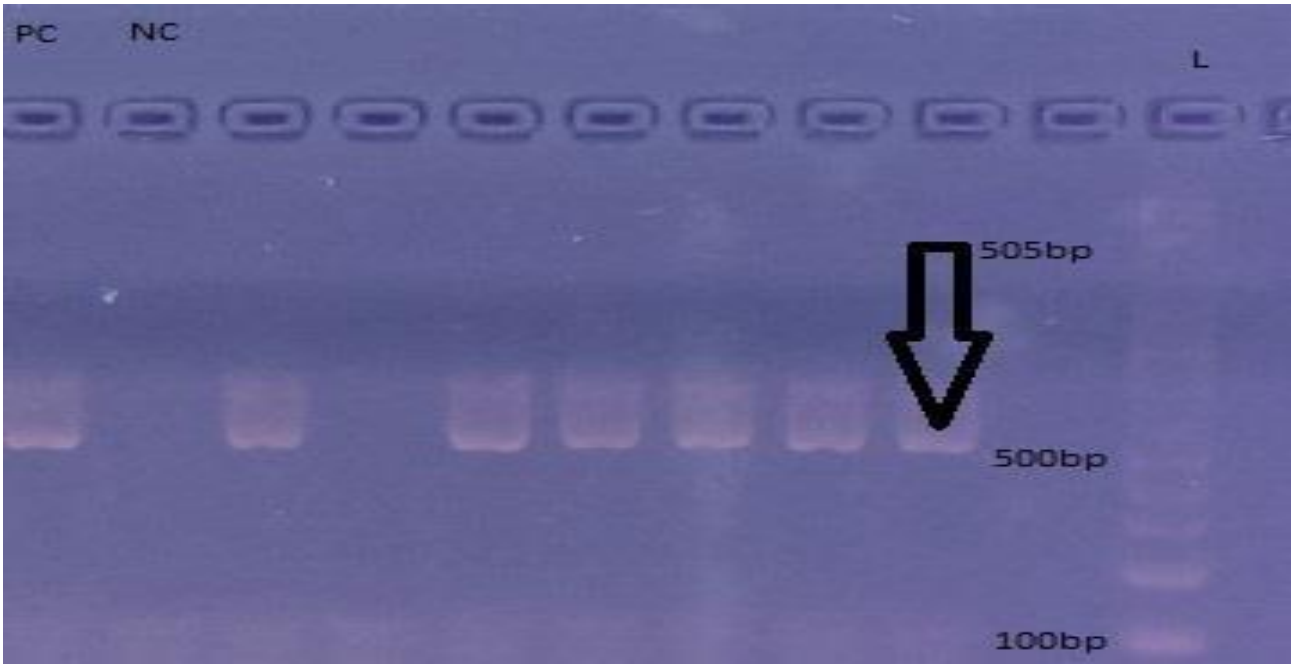

Figure 6: Nuclear ITS2 gel image; 100bp molecular weight marker (L), Positive control (PC), Negative control (NC), *S. haematobium* (505bp).

5.3. Characteristics of study participants involved in PSAC prevalence study

A total of 390 PSAC provided urine samples, and 390 mothers participated in interviews, resulting in a 2% non-response rate. The mean (standard deviation, SD) age of the PSAC was 3.8 (1.2) and this study included 209 (53.3) male and 181 (46.4) female PSAC. The majority of mothers/caregivers were non-educated (67.9%), housewives (53.3%), and used freshwater for bathing and washing clothes (97.2%) (Table 6).

Table 6: Characteristics of the study participants involved in PSAC prevalence study, Abobo district, Gambella

Variables	**Categories**	**Frequency**	**Percent**

Age of PSAC in years	1	11	2.8
	2	54	13.8
	3	74	19.0
	4	105	26.9
	5	146	37.4
Sex of PSAC	Male	209	53.6
	Female	181	46.4
Villages/residence	Abaro	137	35.1
	Mender 17	119	30.5
	Wankak	91	23.3
	Tagni	43	11.0
Marital status of parents	Married	383	98.2
	Single	4	1.0
	Window	2	0.5
	Divorced	1	0.3
Parents education	No formal education	265	67.9
	Primary	70	17.9
	Secondary	38	9.7
	Higher education	17	4.3
Parents occupation	Farmer	137	35.3
	Housewife	208	53.5
	Employed	24	6.2
	Merchants	21	5.4
Presence of toilet at	Yes	243	62.3

Home	No	147	37.7
Washing and bathing in open water	Yes	379	97.2
	No	11	2.8

5.4. Prevalence and intensity of *S. haematobium* infection

The overall prevalence of *S. haematobium* infection, macro and microhaematuria was 16.7%, 7.9%, and 26.2%, respectively (Table 7). Microscopic prevalence was varied among the study villages with high prevalence shown in Abaro (27.7%; 38/137) followed by Tagni village (20.9%; 9/43) (Table 8). Of those *S.haematobium* infected, 32.3% (21/65) exhibited macrohaematuria, while 73.8% (48/65) presented with microhaematuria. A significant association (*P =0.000*) was observed between the infection and haematuria.

Table 7: Prevalence of *S. haematobium* infection determined by macrohaematuria, microhaematuria and urine filtration among preschool children, Gambella, Ethiopia, 2024

Prevalence	**N/%**	**95 % CI**
S. haematobium infection by microscopy	65 (16.7)	13.1-20.3
Microhaematuria	102 (26.2)	21.8-30.8
Macroheamaturia	31 (7.9)	5.4-10.5

Of the 65 PSAC who were positive for *S. haematobium* infection, 52 (80%) had light-intensity infection (< 50 egg counts/10 ml of urine) and 13 (20%) had heavy-intensity infections (≥ 50 egg counts/10 ml of urine). The heavy intensity of infection was higher among females (69.2%; 9/13) and among five years old (46.2%; 6/13) (both genders combined). Moreover, the intensity of infection was higher among PSAC who lived in Abaro village than those in Mender 16, Tagni and Wankak villages (Fig.7).

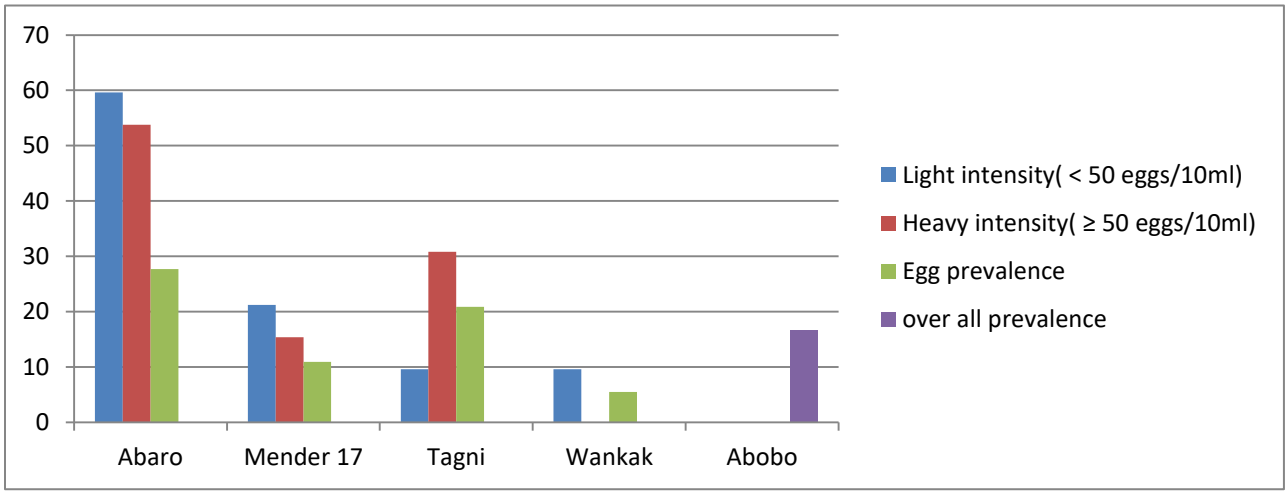

Figure 7: Prevalence and intensity of *S. haematobium* infection by study sites, Abobo, Gambella, 2024.

The mean (SD) count of eggs (MCE) per 10 ml of urine was 5.64 (34.04) and ranged from 1 to 504. The MEC was significantly greater among PSAC with parent who had no formal education (P = 0.004) than those with parents who had formal education. Additionally, MEC was higher among PSAC who had visited (P < 0.000), played and bathed (P= 0.005) and urinated (P= 0.002) in freshwater, compared to those who did not engage in open water-related risky activities (Table 8).

Table 8: Mean egg count and associated factors in Abobo district, Gambella, Ethiopia (2024)

Variables	**Categories**	**Mean egg Count**	**95% CI**	**P –value**
Age in years				0.825[a]
	1	0.6	0.0-1.7	
	2	3.2	0.2-7.1	
	3	2.9	0.4-7.4	
	4	4.6	0.9-9.6	
	5	9.1	2.3-19.0	
Sex				0.561[b]
	Male	2.5	1.3-3.9	
	Female	9.3	3.4-16.8	
Kebeles				0.000[a]
	Abaro	10.5	4.4-20.7	
	Mender 17	3.9	0.3-10.3	
	Tagni	6.5	1.5-12.8	
	Wankak	0.1	0.0-0.2	
Mothers/caregivers education status				0.004[b]
	Formal education	0.6	0.2-1.4	
	No formal education	8.0	3.8-13.9	
Presence of latrine at home				0.000[b]
	No	11.0	5.0-20.0	
	Yes	2.4	0.4-5.8	

Mothers taking the child when going to open water				0.000[a]
	Yes Always	10.5	4.8-19.7	
	Yes sometimes	3.5	0.4-8.7	
	No	1.3	0.0-3.9	
PSAC playing and bathing in open water				0.005[a]
	Yes Always	7.4	2.8-13.0	
	Yes sometimes	3.5	0.4-8.7	
	No	1.3	0.0-3.9	
PSAC urinating in the water				0.002[b]
	Yes	7.2	3.4-12.2	
	No	1.3	0.1-3.8	
Source of water for bathing the PSAC at home				0.000[a]
	open water	9.0	3.5-15.7	
	open and pump	10.7	3.0-24.5	
	Safe water	2.2	0.3-5.6	
Habit of boiling water for bathing the child				0.766[b]
	Yes sometimes	0.6	0.0-1.5	
	No	5.8	2.9-9.8	
Mother/caregiver place of urination while in open water				0.028[b]
Source				
	Open field	6.1	3.3-9.1	

	Toilet at Home	0.0	0.0-0.1	
[c] Kruskal-Wallis test [b] Whitney U Test				

5.5. Risk factors associated with *S. haematobium* infection in Abobo district

Open water contact and sanitation practices of the mothers/caregivers and PSAC were among the possible risk factors identified. In this study, 74.9% of PSACs frequently visited open water, while 74.1% played or bathed in it, and the same percentage urinated in open water. Only 55.9% of mothers or caregivers consistently used safe water to wash their children at home, while 91.8 % reported urinating in an open field near water sources.

In the univariate analysis, villages, absence of toilet at home, PSAC visiting the freshwater bodies, bathing/playing and urinating by water and bath by open water at home, and education status of the mother/caregiver were among the variables that were significantly associated with the prevalence of *S. haematobium* infection (Table 9).

After entering those chosen variables ($p < 0.25$) into the multivariable logistic regression model, to control confounding variables, residence (AOR = 3.7, 95% CI: 1.1 – 12.4) was significant explanatory risk factor for *S. haematobium* infection.

The young children who bathed and played in open water were 89% more likely to have *S. haematobium* parasites as compared with PSAC that did not get into the water. PSAC living in Abaro village were four times more likely to be infected by the parasite compared to those living in Wankak village, with a P-value of 0.005 (Table 9).

Table 9: *S. haematobium* infection and associated factors in Abobo District, Gambella

Variables	**Catagories**	***S. haematobium* infection**	**COR (95 % CI)**	**P-value**	**AOR (95%CI)**	***P**-value**
Age (years)						
	1	18.2 (2/11)	Ref			
	2	16.7 (9/54)	0.9 (0.2-4.9)	0.903		
	3	13.5 (10/74)	0.7 (0.1-3.7)	0.679		
	4	15.2 (16/105)	0.8 (0.2-4.1)	0.798		
	5	19.2 (28/146)	1.1 (0.2-5.2)	0.935		
Sex						
	Male	15.8 (33/209)	0.9 (0.5-1.5)	0.618		
	Female	17.7 (32/181)	Ref			
Villages						
	Abaro	27.7 (38/137)	6.6 (2.5-17.5) *	0.000	4.3 (1.6-1.9)	0.005
	Mender 17	10.9 (13/119)	2.1 (0.7-6.2)	0.172		
	Teigni	20.9 (9/43)	4.5 (1.4-14.6) *	0.011		
	Wankak	5.5 (5/91)	Ref			
Mothers/caregivers education status						
	Formal education	9.6 (12/125)	0.4 (0.2-0.8) *	0.006		
	No formal education	20 (53/265)	Ref			
Presence of toilet at home						
	No	27.2 (40/147)	3.3 (1.9-5.7) *	0.000		

	Yes	10.3 (25/243)	Ref			
mothers taking the child when going to open water						
	Yes always	27.2 (41/151)	5.7(2.3-14.1) *	0.000		
	Yes sometimes	12.8(18/141)	2.2 (0.9-5.9)	0.100		
	No	6.1 (6/98)	Ref			
PSAC playing and bathing in open water						
	Yes always	18.1(27/149)	3.0 (1.2-7.1) *	0.015	2.9 (1.0-3.1)	0.041
	Yes sometimes	22.1 (31/140)	3.8 (1.6-9.1) *	0.002	2.6 (1.1-5.5)	0.037
	No	6.9 (7/101)	Ref			
PSAC urinating in the water						
	Yes	20.1 (58/289)	3.4 (1.5-7.7) *	0.004		
	No	6.9 (7/101)	Ref			
Source of water for bathing the PSAC at home						
	open water	22.4 (17/76)	2.6 (1.3-5.2) *	0.011		
	open and pump	27.1 (26/96)	3.3 (1.8-6.2) *	0.001		
	Safe water	10.1 (22/218)	Ref			
Habit of boiling water for bathing						

the child						
	Yes sometimes	14.3 (2/14)	1.8 (0.2-3.8)	0.808		
	No	16.8 (63/376)	Ref			
Mother/caregiver place of urination while in open water source						
	Open field	17.9 (64/358)	6.7 (0.9-50.3)	0.063		
	Toilet at home	3.1 (1/32)	Ref			
* Significance in the final model was set and reported at p* < 0.05 AOR, adjusted odds ratio; COR, crude odds ratio						

5.6. Socio-demographic characteristics of study participants in Kurmuk district, Benishangul-Gumz Regional State, Ethiopia

A total of 403 participants older than 5 years were included in the study. Of these, 296 (73.4%) were males and 107 (26.6%) were females. The majority of them were Berta by ethnicity (71.7) followed by Amhara (13.6%) and Oromo (10.2%). Also, most of the participants were students (62.3%) (Table 9).

5.7. Prevalence and risk factors of *S. haematobium* infection in Kurmuk District, Benishangul-Gumz Regional State, Ethiopia

The overall prevalence of *S. haematobium* infection, as determined by the urine filtration technique was 34.2% (138 out of 403 samples). Infection rates varied across villages, with the highest prevalence in DulShatalo at 43% (COR = 1.95, 95% CI: 1.06–3.59) and the lowest in Kurmuk at 27.8%.

In the univariate analysis, the age group 5–12 years had the highest prevalence of 45.4% (COR = 4.16, 95% CI: 1.36–12.67), followed by the 13–20 years age group (COR = 3.23, 95% CI: 1.01–10.35). Students and those who swam or played and washed clothes in open water had a higher chance of infection.

In multivariable analysis, when adjusting for confounding factors, swimming in open water remained significantly associated with infection, with an AOR of 2.43 (95% CI: 1.19–4.94). However, while washing clothes in open water showed a significant association in the bivariate analysis (COR=1.95, 95% CI: 1.07–3.56), this association became non-significant in the multivariable model (AOR = 1.05, 95% CI: 0.51–2.17) (Table 10).

These findings showed that swimming in open water is a key factor contributing to *S. haematobium* infection in the study population.

Table 10: Prevalence and risk factors of *S. haematobium* infection in Kurmuk District, Ethiopia: Univariate and multivariable analysis

Variables		**Participants**	**Positive**	**COR (IC95%)**	**AOR (IC95%)**	**P –values**
		403	138 (34.2%)			
Sex	Female	107	40 (37.4%)	1.21 (0.76-1.91)	1.48 (0. 89-2.46)	0.211
	Male	296	98 (33.1%)	Reference	Reference	

Age (yrs)	5–12	174	79 (45.4%)	4.16 (1.36-12.67) *	1.07 (0.59-1.94)	0.607
	13–20	79	31 (39.2%)	3.23 (1.01-10.35) *	0.59 (0.24-1.46)	0.638
	21–28	72	13 (18.3%)	1.10 (0. 32-3.77)	0.81 (0.27-2.39)	0.635
	29–36	36	8 (22.2%)	1.43 (0. 38-5.40)	0.44 (0. 10-1.85)	0.875
	37–44	18	3 (16.7%)	1.00 (0. 19-5.15)	0.56 (0.16-1.97)	0.798
	≥45	24	4 (16.7%)	Reference	Reference	
Villages	Ogendu	87	26 (29.9%)	1.10 (0.56-2.16)	1.02 (0. .49-2.10)	0.882
	Belehu-j	116	38 (32.8%)	1.26 (0.68-2.36)	1.20 (0. .61-2.38)	0.534
	DulShatalo	121	52 (43%)	1.95 (1.06-3.59) *	1.84 (0.96-3.52)	0.051
	Kurmuk	79	22 (27.8%)	Reference	Reference	
Occupation	Student	251	110 (43.8%)	11.7 (1.52-89.96) *	6.63 (0.75-58.84)	0.063
	Farmer	71	10 (14.1%)	2.46 (0.29-20.73)	2.24 (0.25-20.01)	0.390
	Daily laborer	25	9 (36.0%)	8.44 (0. 95-74.85)	6.41 (0.71-57.84)	0.067
	Merchant	22	5 (22.7%)	4.41 (0. 46-42.13)	3.23 (0.32-32.38)	0.204

	Unemployed	18	3 (16.7%)	3.00 (0. 28-32.21)	1.99 (0.17-22.98)	0.470
	Employed	16	1 (6.3%)	Reference	Reference	
Swimming	Yes	247	110 (44.5%)	3.67 (2.27-5.93) *	2.43 (1.19-4.94) *	0.023
	No	156	28 (17.9%)	Reference	Reference	
Washing clothes	Yes	333	122 (36.6%)	1.95 (1.07-3.56) *	1.05 (0.51-2.17)	0.858
	No	70	16 (22.9%)	Reference	Reference	

COR: Crude Odds Ratio; AOR: Adjusted Odds Ratio; CI: Confidence Interval. * Significant association

5.8. Intensity of *S. haematobium* infection in Kurmuk

The study on the intensity of *S. haematobium* infection revealed significant findings across different demographic and behavioral factors. The mean egg count (MEC) recorded for the study participants was 7.33 (95% CI: 4.35-10.31), with a standard deviation (SD) of 30.45 in 10 ml of urine. Out of 138 participants infected with *S. haematobium*, 89.1% displayed a light infection, while only 10.9% exhibited a heavy infection. The number of eggs found per 10 ml of urine varied from a minimum of 1 to a maximum of 500. Participants who swam had a higher heavy infection rate ($P \leqslant .005$) than those without swimming habits, who recorded zero of heavy infection ($\geqslant$50 egg/ml).

A statistically significant difference in MEC was observed between villages (P = .028), ranging from 2.39 CI; 1.05 to 3.72 in Ogendu village to 14.1 CI: 4.98 to 23.12 in Dulshatalo village. When categorized by age group, the highest MEC was noted in the 5-to-12-years age group, followed by the 13-to-20-years group (P ⩽ .001).

Furthermore, the mean egg count varied significantly based on occupation, swimming, and washing habits. In contrast, the MEC and intensity levels were similar for females, averaging 9.98 (CI: 0.38-19.59), and males, averaging 6.38 (CI: 4.21-8.54), as summarized in Table 3 of paper 1 (annex 4).

5.9. Prevalence of haematuria and its associations with demographic factors and water contact behaviors

The prevalence of microhematuria, as detected by dipstick testing, was 38.5% in males and 41.1% in females. The highest rates were in the 13–20 years age group (48.1%) and among students (45.0%), with Dulshatalo village having the highest prevalence (56.2%). Infection was more common in those who swam in open water (46.2%) than in those who did not (28.2%).

Sex, age, and occupation were not significantly associated with infection prevalence. However, residents of Dulshatalo had higher odds of infection than those in Kurmuk (COR = 2.34, 95% CI: 1.30–4.19; AOR = 2.64, 95% CI: 1.43–4.87).

Swimming in open water was significantly associated with higher odds of haematuria (COR = 2.18, 95% CI: 1.42–3.35; AOR = 2.79, 95% CI: 1.43–5.45), even after adjusting for confounders. However, washing clothes in open water showed no significant association with haematuria prevalence (COR = 1.04, 95% CI: 0.61–1.75; AOR = 0.61, 95% CI: 0.32–1.17) (Table 11).

Table 11: Prevalence of microhaematuria and its association with demographic characteristics and water contact practices among participants in Kurmuk District, Ethiopia

Variables		**Prevalence (N\%)**	**COR (95CI)**	**AOR (95%CI**	**p-value**
Sex	Female	44 (41.1)	1.2 (0.76-1.91)	1.4 (0.8-2.4)	0.919
	Male	114 (38.5)	Reference	Reference	
Age Group (Years)	5–12	74 (42.5)	1.48 (0.60-3.64)	0.56 (0.19-1.67)	0.030
	13–20	38 (48.1)	1.85 (0.71-4.83)	1.00 (0.35-2.86)	0.196
	21–28	21 (29.2)	0.82 (0.31-2.22)	0.78 (0.28-2.19)	0.358
	29–36	11 (30.6)	0.88 (0.29-2.66)	1.07 (0.34-3.41)	0.786
	37–44	6 (33.3)	1.00 (0.27-3.66)	0.87 (0.22-3.48)	0.814
	≥45	8 (33.3)	Reference	Reference	
Villages	Ogendu	30 (34.5)	0.96 (0.50-1.81)	0.99 (0.51-1.92)	.985
	Belehu-j	32 (27.6)	0.69(0.38-1.28)	0.68(0.36-1.31)	.223
	DulShatalo	68 (56.2)	2.34(01.30-4.19) *	2.64(1.43-4.87) *	.005
	Kurmuk	28 (35.4)	Reference	Reference	
Occupation	Student	113 (45.0)	1.80 (0.61-5.34)	1.87 (0.51-6.92)	0.108
	Farmer	19 (26.8)	0.80 (0.25-2.62)	0.61 (0.17-2.19)	0.773
	Daily laborer	11 (44.0)	1.73 (0.46-6.47)	1.34 (0.35-5.15)	0.252
	Merchant	5 (22.7)	0.65 (0.15-2.77)	0.52 (0.11-2.40)	0.950
	Unemployed	5 (27.8)	0.85 (0.19-3.71)	0.59 (0.12-2.94)	0.950
	Employed	5 (31.3)	Reference	Reference	
Swimming	Yes	114 (46.2)	2.18 (1.42-3.35) *	2.79 (1.43-5.45) *	0.001

	No	44 (28.2)	Reference	Reference	
Washing clothes	Yes	131 (39.3)	1.039 (0.61-1.75)	0.61 (0.32-1.17)	0.267
	No	27 (38.6)	Reference	Reference	

5.10. Prevalence of microhematuria and diagnostic agreement between microscope and urine reagent strip

Among the 403 participants tested, the prevalence of microhematuria detected by the dipstick test was 39.2% (158/403). In microscopy-negative samples, 18.9% (50/265) tested positive for hematuria by dipstick, while 76.4% (94/123) of those with light infection and 93.3% (14/15) of those with heavy infection were positive for hematuria.

There was a significant association between hematuria and the presence of schistosome eggs in urine (P <0.001). Of the participants with detectable *S. haematobium* eggs in their urine, 108 showed hematuria, resulting in a positive predictive value of 68.4%, with a sensitivity of 78.3% and a specificity of 81.1%. The agreement between dipstick and microscopy diagnosing was moderate, with a Kappa value of 0.57 (Landis and Koch, 1977) (Table12).

Table 12: Association between hematuria and *Schistosoma haematobium* infection among participants in Kurmuk District, Ethiopia

Microscope		**Dipstick result**		
Result		**Positive**	**Negative**	**Total**
	Negative	50 (18.9%)	215 (81.1%)	265
	1-49egg/10ml	94 (76.4%)	29 (23.6%)	123

	≥50egg/10ml	14 (93.3%)	1 (6.7%)	15
Total		158 (39.2%)	245 (60.8%)	403

5.11. Characteristics of study participants involved in the KAP study in Abobo district, Gambella

A total of 403 participants were enrolled in the study, with 308 (76.4%) males and 95 (23.6%) females. Most participants were between 21 and 30 years (43.7%), and the average age was 35.1 years (SD = 11.88), with a median age of 32.0. The survey was conducted through household interviews, mainly targeting household heads, resulting in higher male participation. A significant proportion of participants were farmers (44.2%), followed by government/private employees (33.7%). Atotal of 239 participants (59.3%) had attended school at various levels, including primary (23.8%), secondary (28.5%), and college/university (60.3%).

Regarding housing conditions, most participants (73.9%) lived in homes with tin roofs, cement walls, and cement floors, while 23.6% lived with thatched roofs, mud walls, and floors. Most participants (93.3%) had access to toilets, with 77.1% using private toilets and 22.9% relying on public toilets. Nearly all participants (93.5%) used public hand pumps for drinking water, while the remaining 6.5% depended on open water sources. Additionally, over half of the households reported a history of UGS affecting at least one household member (Table 13).

Table 13: Socio-demographic and environmental characteristics of participants in Abobo District, Ethiopia

Variables	**Categories**	**N (%)**
Age (Year)	12-20	12 (3.0)
	21-30	176 (43.7)
	31-40	127 (31.5)
	41-50	63 (15.6)
	51-60	13 (3.2)
	> 60	12 (3.0)
Sex	Female	95 (23.6)
	Male	308 (76.4)
Education level		
	Illiterate	164 (40.6)
	Primary level	57 (14.1)
	Secondary level	68 (16.9)
	College /university	114 (28.3)
Occupation	Farmer	178 (44.2)
	Merchant	15 (3.7)
	Daily laborer	43 (10.7)
	Employee	136 (33.7)
	Housewives	18 (4.5)
	Other	13 (3.1)
Drinking water source	pump water	377 (93.5)
	open water	28 (6.5)
Housing conditions	Grass roof, mud wall, and floor	95 (23.6)
	Tin roof, cement wall, and floor	298 (73.9)
	Other	10 (2.5)
presence and use of toilet	Private toilet	290 (77.1)
	Public/communal toilet	86 (22.9)
	Nearby /dam /river/bush/ open field	31(7.7)
History of UGS	Yes	302 (74.9)
	No	101 (25.1)

5.12. Knowledge, attitudes, and practices on UGS (Abobo district, Gambella)

Awareness of UGS

Out of 403 participants, the majority of respondents (90.6%) had heard of UGS, with health institutions being the primary source of information (52.1%), followed by family (16.2%) and friends (15.3%) (Table 14).

Knowledge of symptoms

Most participants (96%) mentioned at least one symptom of UGS. The crucial symptom mentioned by the participants is bloody urine (81.9%), while fewer participants identified difficulty or burning sensation during urination (13.2%). A small proportion (1.1%) was unaware of the symptoms (Table 14).

Knowledge of transmission routes

Regarding the transmission pathways, only 107(28.7%) respondents knew skin penetration as a means of infection. Over half of the participants, 59.6%, pointed out drinking unclean water as a source of infection, and 8.6% thought other routes were involved. The awareness of transmission through various water contact activities, such as swimming (33.4%), bathing (42.5%), and washing clothes (9.6%), indicated some understanding of the risk associated with open water contact. However, only 0.3% of participants knew that all forms of infested water contact practice were a source of infection (Table 14).

Knowledge of intermediate host

Over half of the participants (54.0%) did not know intermediate host involvement in the infectious process. Among those who were aware, 19.5% correctly identified the snail, while fewer mentioned mosquitoes (18.6%) and houseflies (8.5%) (Table 14).

Knowledge of treatment and prevention

Over 97% of participants believed that UGS is treatable, with 84.2% identifying modern medicine (seeking treatment at a health facility) as the primary method. However, only two participants mentioned MDA as a preventive strategy. Regarding prevention, 92.6% believed UGS is preventable, with the most commonly mentioned prevention measure being treatment (32.9%), followed by avoiding open water contact (26.8%). However, 5.2% of respondents had misconceptions about prevention methods (Table 14).

School-based MDA awareness and participation

Regarding community-level interventions, over 81% of participants had heard of school-based MDA programs, and 66.5% reported that children in their households participated (Table 14).

Table 14: Participants' knowledge of UGS symptoms, transmission, prevention, and control measures

Knowledge area	**Response**	**Number (%)**
Have you ever heard about bilharzia?	Yes	365 (90.6)
	No	38 (9.4)
From where have you heard	Media (TV/radio/magazine)	25 (6.8)
	Friends	56 (15.3)
	School	41 (11.2)
	Family	59 (16.2)
	Health institution	190 (52.1)
	Other	1 (0.3)
Symptoms [a]	Bloody urine	298 (81.6)
	Stomach pain	17 (4.7)
	Burning/difficulty in Urination	48 (13.2)
	Other symptoms	7 (2.0)
	I don‘t know	4 (1.1)
Transmission Route [a]	Skin penetration	108 (26.8)
	Drinking contaminated water	222 (59.5)
	Other [a]	32 (8.6)

	I don‘t know	12 (3.2)
What practices involving open water contact are UGS transmission routes [a]	Washing clothes	35 (9.6)
	Crossing the river barefoot	90 (22.3)
	Swimming	122 (33.4)
	Bathing	155 (42.5)
	Any open water contact	11 (3.0)
	I don‘t know	3 (0.8)
	Others [b]	9 (2.4)
Vector/intermediate host	Mosquitoes	68 (18.6)
	House fly	31 (8.5)
	Snail	71 (19.5)
	I don‘t know	197 (54.0)
UGS is treatable	Yes	355 (97.3)
	No	10 (2.7)
Ways of treatment [a](N355)	Traditional medicine/treatment	18 (4.6)
	modern medicine	325 (84.2)
	Buying drugs from a Pharmacy	42 (10.9)
	Other	1 (0.3)
UGS is preventable	Yes	338 (92.6)
	No	27 (7.4)
Means of prevention [a] (N338)	Getting treatment	120 (32.9)
	Health education	32 (8.8)
	Hygiene	58 (15.9)
	Avoid contact /use of open Water	99 (26.8)
	Misconception	19 (5.2)
Heard about school-based MDA (N403)	Yes	327 (81.1)
	No	38 (18.9)
Children participate/take drugs from the MDA program (N403)	Yes	268 (66.5)
	No	97 (33.5)
Other [a] Ingestion of spoiled food, Contaminated soil and Unhygienic hand Other [b] drinking dam/river water and nothing [a] more than one answer is possible		

5.13. Participants attitudes and practices towards UGS

Nearly two-thirds of the participants (64.7%) believed UGS could be fatal. Less than half (45.7%) correctly identified and agreed that urinating in open fields or near open water is a key transmission route.

While a significant portion, (31.2%) did not believe this was a valid transmission method.

In terms of risky practices, most participants (92.3%) reported using toilets for defecation and urination, but 7.7% still practiced open defecation nearby dams, rivers, bushes, or open fields. Regarding swimming, 32.9% of participants swam regularly in river or dam water, while a small proportion (13.9%) never swam in open water. Many participants also used dam water for washing clothes (52.1%) and bathing (36.5%).

Regarding health-seeking behaviors, most participants (86.0%) with a history of UGS preferred modern medicine as their treatment method, 12.8% bought medicine from a pharmacy, and 1.5% used traditional medicine (Table 15).

Table 15: Attitudes, risky practices, and health-seeking behaviors related to UGS

Variables	**Frequency**	**Percent**
UGS could kill (N=365)		
Yes	236	64.7
No	60	16.4
I don‘t know	69	18.9
Urinating in an open field or near open water is a means of transmission (N=365)		
Yes	184	45.7
No	114	31.2
I don‘t know	69	18.9
Risky practices (N 403)		
Frequently used means of defecation/urination (N403) [a]		
Toilet	376	92.3

Nearby, dam/river/ bush/ open field	31	7.7
Swimming habit in a river/dam water		
Yes always	114	32.9
Yes sometimes	233	67.1
No	56	13.9
Use of dam water [a]		
Bathing	147	36.5
Swimming	64	15.9
Washing clothes	210	52.1
Health-seeking practice Treatment preference among respondents who have a history of infection (N264) [a]		
Traditional medicine	4	1.5
Modern medicine	227	86.0
Buying medicine from a pharmacy	34	12.8
Other	2	0.8

[a] more than one answer is
possible N number of respondents

5.14. Level of knowledge, attitude and risky practice

The mean score for knowledge was 8.19 out of 24 points, or 34.1% level of knowledge, with a minimum score of 4 and a maximum score of 15. The most frequent score (mode) was 9. The attitude mean score was 1.04 out of 2 points. The mean score on risky practice was 1.97 out of 5 points. Of the 403 individuals, 324 (80.4%) were involved in at least 2 risky practices (ie, mode = 2).

5.15. Assocation between participants' KAP on UGS with sex and age

Univariate analysis result: Male participants were more likely to be informed about UGS (P = 0.046) and less likely to engage in indiscriminate urination or defecation (COR: 0.5, CI: 0.2-0.9). However, males were also more inclined to swim or play in open water (COR: 2.4, CI: 1.3-4.4) and less likely to recognize that all forms of open water contact, such as washing, swimming, bathing, and crossing the river barefoot, could lead to contracting the disease (COR: 0.2, CI: 0.1-0.8). Regarding age, participants aged over 40 years were more likely to identify snails as intermediate hosts (COR: 2.5, CI: 1.4-4.4) but less likely to consider UGS a fatal disease (COR: 0.5, CI: 0.3-0.9) compared to those aged 40 years or younger.

Multivariable analysis results: The multivariable analysis adjusted for occupation status, education, and history of UGS revealed several significant associations. Older participants (aged >40 years) were more likely to recognize snails as intermediate hosts and less likely to believe that SCH is a deadly disease, with an AOR of 0.5 (95% CI: 0.3–1.0; p=0.043). compared to younger participants (AOR=2.3, 95% CI: 1.3–4.2; p=0.007). On the other hand, being male (sex) continued to be the key predictor for lack of understanding about the risks associated with unsafe cercariae infested water contact as a means of contracting biliharzia (P <0 .05). Association of participants KAP about UGS to sex and age is shown in Table 4 of paper 2 (annex 5).

5.16. Association of participant's KAP about UGS with their education and occupation status

Univariate Analysis: In terms of awareness, both educated (90.5%) and non-educated (90.7%) participants had high recognition of bilharzia, with no significant difference (p = 0.461). However, working individuals (farmers, merchants, daily laborer and employees) were three times more informed about schistosomiasis (COR: 3.8, CI: 1.4–10.4) and mass drug

administration (MDA) programs (COR: 3.0, CI: 1.2–7.2) compared to non-working individuals.

Knowledge of symptoms varied by education and occupation. Educated participants were significantly more likely to recognize painful urination as a symptom of UGS (COR: 3.8, CI: 1.7–8.3), while working individuals were more likely to know hematuria as a symptom (COR: 2.8, CI: 1.2–6.5). Regarding transmission routes, educated participants had a greater likelihood of identifying swimming or playing in open water as an exposure factor (COR: 2.1, CI: 1.3–3.4) and were more likely to recognize urinating in open fields or near open water as a source of transmission (COR: 2.7, CI: 1.6–4.6).

Regarding prevention, educated participants had a significantly higher likelihood of recognizing getting treatment (COR: 1.7, CI: 1.1–2.6) and valuing health education (COR: 3.1, CI: 1.3–7.7) as preventive measures. Occupation status also played a role, as working individuals were significantly more likely to perceive UGS as a potentially fatal disease ($p < 0.05$).

Risky practices differed significantly across groups. Educated participants were less likely to use dam water for washing clothes (COR: 0.6, CI: 0.4–0.9). Working individuals were significantly less likely to engage in open defecation or urination (COR: 0.2, CI: 0.1–0.4) compared to non-working participants (Table 5, paper 2 (Annex5).

Multivariable analysis: After adjusting for cofounders, several associations remained significant. Educated participants continued to have greater awareness of symptoms, with significantly higher odds of recognizing burning or painful urination (AOR = 3.8, 95% CI: 1.6–8.8, $p = 0.002$). Similarly, they were more likely to identify swimming or playing in open water as a transmission route (AOR = 2.1, 95% CI: 1.3–3.6, $p = 0.002$) and acknowledge the importance of treatment (AOR = 2.1, 95% CI: 1.3–3.4, $p = 0.004$) and health education (AOR = 3.1, 95% CI: 1.2–8.3, $p = 0.023$) in UGS prevention.

For practices, non-working individuals remained significantly more likely to practice open defecation compared to working individuals (AOR = 0.2, 95% CI: 0.1–0.7, p = 0.012). Education status influenced water use behaviors, with non-educated participants more likely to use dam water for washing clothes (AOR = 0.6, 95% CI: 0.4–0.9, p = 0.016).

Regarding attitudes, working individuals were significantly more likely to perceive UGS as a deadly disease (AOR = 0.2, 95% CI: 0.1–0.7, p = 0.007). Educated participants had higher odds of perceiving urinating in open fields as a transmission route (AOR = 3.2, 95% CI: 1.8–5.8, p = 0.000). KAP's association with education and occupation status is shown in Table 5 of paper 2 (Annex 5).

5.17. Association of participant's KAP about UGS with a history of infection

Univariate logistic regression analysis

Participants with a history of UGS demonstrated a significantly higher likelihood of being knowledgeable about bilharzia and its control measures than those without prior infection. Specifically, individuals with past UGS were more aware of the disease (COR: 5.5, CI: 2.7–11.6) and MDA programs (COR: 3.6, CI: 2.1–6.0). Additionally, they exhibited greater awareness of hematuria as a symptom of UGS (COR: 2.4, CI: 1.5–3.8) and recognized the role of snails in disease transmission (COR: 2.2, CI: 1.2–4.1). Their understanding of preventive measures was also better, as they were more likely to acknowledge the importance of avoiding unsafe water sources (COR: 1.6, CI: 1.0–2.6).

Despite their higher awareness of the disease, participants with a history of UGS were paradoxically less likely to agree that urinating in open water contributes to the spread of the disease. Furthermore, they had a significantly higher tendency to engage in risky behaviors, such

as swimming or playing in open water (COR: 6.3, CI: 3.4–11.7). This suggests that while previous infection may enhance knowledge, it does not necessarily translate into safer practices, highlighting potential gaps in behavioral change interventions.

Multivariate logistic regression analysis confirmed that past infection history remained a significant predictor of bilharzia-related knowledge and behaviors even after adjusting for potential confounders ($p < 0.05$). Participants who had previously contracted UGS were likely to have heard about the disease (AOR: 3.6, CI: 1.6–8.0) and MDA (AOR: 2.4, CI: 1.4–4.2). They also had increased odds of identifying hematuria as a key symptom (AOR: 1.8, CI: 1.0–3.0) and acknowledging the importance of avoiding unsafe water sources as a preventive strategy (AOR:1.9, CI: 1.0–3.3). Participants' KAP regarding UGS in association with their history of the disease is shown in Table 6 paper 2 (Annex 5).

Chapter 6: Discussion

6.1. Summary and interpretation of major findings

The key findings of this study in study areas include the following: (1) All miracidia analyzed showed mitochondrial cox1 gene profiles specific to *S.haematobium*; (2) In addition, nuclear ITS2 data from miracidia confirmed the exclusive presence of *S. haematobium* group; (2) A high prevalence and intensity of *S. haematobium* infection among PSAC, with maternal practices such as taking children to open water sources and engaging in unhygienic activities significantly contributed to the risk of infection; (3) A high general awareness of UGS and MDA programs within the community, but the level of understanding regarding the disease's transmission, prevention, and control measures is inadequate and with continued risky behaviors. Education, gender, age, occupation, and infection history, influenced KAP outcomes, highlighting the need for targeted health education; (4) *S. haematobium* remains highly prevalent in Kurmuk district, particularly in DulShatalo village, with children /adolescents being the most affected groups and swimming\playing being significant risk factor. Targeted interventions focusing on reducing water contact and improving sanitation could control transmission; (5) Diagnostic agreement between microscopic examination and urine dipstick test results.

6.2. Genetic profile of *Shistosoma* miracidia

Schistosoma miracidium was characterized, first using the mitochondrial marker (Cox1), which is haploid and inherited from the mother and then using the nuclear marker (ITS2), which is diploid and bi-parentally inherited (Huyse *et al.,* 2009; Boissier *et al.,* 2016). All miracidia exhibited a genetic profile specific to *S.haematobium* without any Sh x Sb or Sh x Sm mixed infection.

The absence of mixed infections in this study may reflect true biological dominance of *S.haematobium* in Afar and Gambella region and may also reflect ecological or host-specific factors that restrict the co-occurrence or cross-breeding of *S. haematobium* with other *Schistosoma* species (Huyse *et al.,* 2009; Webster *et al.,* 2012; Mathieu-Benge *et al.,* 2024). In addition , the absence of mixed infections using species-specific singleplex PCR assays suggests a low likelihood of hybridization within the sampled population (Webster *et al.,* 2012; Angora *et al.,* 2020) and aligns with the geographic and epidemiological context of study regions, where coinfection and hybridization events between these species have not been documented.

In contrast to our findings, studies from several African countries, based on mitochondrial-nuclear markers, have reported that hybridization between *Schistosoma* species is relatively common in natural populations (Cunin *et al.,* 2003; Huyse *et al.,* 2013; Angora *et al.,* 2020; Fall *et al.,* 2021; Onyekwere *et al.,* 2022; Teukeng *et al.,* 2022).

However, whole genomic studies indicate that contemporary hybridization is rare or absent in natural populations. Berger *et al.* (2022) investigated *S. bovis* and *S. curassoni* in Senegal and found that hybridization between these species and *S. haematobium* was constrained by strong prezygotic barriers, particularly the limited geographical and host overlap. Similarly, Platt *et al.* (2019) analyzed 96 miracidia from Niger and Zanzibar and found no evidence of contemporary hybrids, but showed 3.3–8.2% Nigerien *S. haematobium* retained *S. bovis* ancestry from an ancient introgression event (~108–613 generations ago). Thus according, to Platt *et al*., 2019, hybridization occurred historically but is not ongoing. This shift in understanding highlights the importance of whole-genome sequencing over marker approaches, to exclude the possibility of backcrosssed hybrids or introgression events (Platt *et al.,* 2019; Berger *et al.,* 2022).

In conclusion, this study emphasizes the need for ongoing genomic surveillance to monitor the genetic structure and evolutionary dynamics of *Schistosoma* populations in Ethiopia. Although environmental modifications like dam construction and irrigation expansion (Steinmann *et al.,* 2006; Erko *et al.,* 2013; Alemayehu *et al.,* 2015; Aula *et al.,* 2021), along with shifts in human and livestock movements (Patz *et al.,* 2019), are expected to increase the risk of co-infections, our findings suggest that such dynamics may be localized or have not yet significantly impacted the studied area. Advanced genomic tools, such as whole-genome sequencing, should be employed to detect cryptic hybridization events and to understand the factors driving genetic isolation or admixture. Such insights will be invaluable for guiding targeted interventions and mitigating the risks associated with UGS transmission and hybridization.

6.3. *S. haematobium* infection and risk factors among PSAC in Gambella, Ethiopia

Investigating the prevalence and risk factors of *S. haematobium* infection in PSAC is crucial for understanding the disease burden and identifying key factors for targeted prevention and early intervention, ultimately aiding in reducing disease transmission and long-term health issues (Stothard *et al.,* 2011; Osakunor *et al.,* 2018a; Macklin *et al.,* 2018). The overall prevalence based on urine filtration was 16.7%, with 20% experiencing a heavy intensity of infection. The mean infection intensity was 5.64 (SD 34.0), ranging from 0.1 to 10.5 eggs/10 ml of urine across different study locations.

The observed prevalence of *S. haematobium* infection was consistent with findings from Tanzania (16.9%) (Mushi *et al.,* 2022) and the overall SSA pooled prevalence (15%) (Kalinda *et al.,* 2020). However, this prevalence was higher than that found in Malawi (10.7%) (Poole *et al.,* 2014) and Senegal (2.3%) (Sow *et al.,* 2023), but lower than in Angola (37.1%) (Mediavilla *et al.,* 2024) and Zimbabwe (35.1%) (Mduluza-Jokonya *et al.,* 2020).

These differences may be influenced by variations in socio-demographic characteristics, WASH practices, and environmental conditions, which all play a role in the spread and prevalence of UGS.

The prevalence was notably higher in Abaro (27.7%) and Tagni (20.9%) compared to Wankak (5.5%) and Mender 17 (10.9%). This geographic variability is likely due to proximity to water bodies and socio-economic differences, consistent with other studies in Ethiopia among SAC (Woldeyohannes *et al.,* 2021).

The heavy intensity of infection (20%) found in this study was higher compared to similar studies conducted in other African countries, such as 6.3% in Ghana (Osakunor *et al.,* 2018b) and 6.8% in Zimbabwe (Mutsaka-Makuvaza *et al.,* 2019a), and significantly higher than in Malawi, where no heavy-intensity infections were reported (Mayo *et al.,* 2016). However, it aligns with findings from Angola, where a 20.3% infection rate was observed (Sánchez-Marqués *et al.,* 2023). The heavy infection intensity and haematuria observed indicate that this group is at risk of morbidity (Lwambo *et al.,* 1997). If PSAC continues to be overlooked in prevention and control efforts, their growth and development could be compromised (Mutapi *et al.,* 2021; Terer *et al.,* 2013), and the disease may worsen until they reach school age (Stothard & Gabrielli, 2007; Santos *et al.,* 2021). Therefore, local public health authorities should implement regular screening and treatment using current PZQ to protect children's health and limit transmission (WHO, 2011; Coulibaly *et al.,* 2012).

This study found no significant differences in the prevalence and intensity of infection related to the age or gender of PSAC. This could be because PSAC in the study area have similar exposure to infection, as their mothers or caregivers typically take them to freshwater sources.

This aligns with previous reports (Ekpo *et al.,* 2010a; Mushi *et al.,* 2022), which found no association between age or gender and infection prevalence. However, other studies have reported significant age (Poole *et al.,* 2014; Mayo *et al.,* 2016; Mutsaka-Makuvaza *et al.,* 2019a) and gender differences (Mazigo *et al.,* 2022).

The significant association between visiting and playing in open water and the prevalence and intensity of infection was expected, as skin contact with cercaria-infested water is a known transmission route and aligns with findings from other studies (Ekpo, 2012; Sacolo- Gwebu *et al.,* 2019a; Mushi *et al.,* 2022). These results highlight the need for targeted education and awareness programs for mothers to promote behavioral changes, particularly particularly regarding the risks associated with of visiting the water bodies with their children and engaging unhygienic practices by the waterside.

Overall, the findings show that *S. haematobium* infection in the Gambella area could occur very early in life, and PSAC is particularly vulnerable to morbidity. To address this, the national disease control initiative in Ethiopia, as WHO recommended, should, include ≥2 years ages PSAC in annual PC program and testing and taking provisional measures for under-2-year-olds to avert *S. haematobium* associated morbidity and downstream complications later in life (Mutapi *et al.,* 2011; Reinhard-Rupp& Klohe, 2017; WHO, 2022b).

6.4. Prevalence of UGS among communities in Kurmuk district, Benishangul - Gumuz

Population-based studies offer valuable insights into the patterns of SCH across different age groups, with prevalence and infection intensity serving as indirect indicators of morbidity. This information is crucial for shaping treatment strategies and assessing the effectiveness of mass drug administration programs.

In Kurmuk district, the overall prevalence of *S. haematobium* infection was 34.2%, with children (45.4%) and adolescents (39.2%) exhibiting higher rates. The mean intensity of infection was 7.33 eggs/10 mL of urine (SD = 30.5), with values ranging from 2.39 to 14.1 across study sites, reflecting the typical spatial heterogeneity of SCH.

Most *S. haematobium* infections in this study were categorized as light, consistent with findings from other regions in Ethiopia (Negussu *et al.,* 2013; Geleta *et al.,* 2015; Degarege *et al.,* 2015; Deribew *et al.,* 2022a) and neighboring Sudan (Ismail *et al.,* 2014). The overall prevalence observed here was higher than rates documented among SAC in other Ethiopian regions, including Horzehab, Beshir, Kurmuk, and Amenzibeni in Benishangul-Gumuz (12.2%) (Deribew *et al.,* 2022a), Afdera Gode in the Somali National Regional State (16.0%) (Negussu *et al.,* 2013), the Middle and Lower Awash Valley (20.8%) (Degarege *et al.,* 2015), and Amibera District in Afar (7.4%) (Awoke *et al.,* 2013). Conversely, it was lower than prevalence figures reported in Gambella Regional State (35.9%) (Geleta *et al.,* 2015) and Hassoba village in the Afar region (47.6%) (Ayele *et al.,* 2008) and higher than those observed elsewhere (Manz *et al.,* 2020) across all age groups. These variations in prevalence can be attributed to differences in environmental settings, population density, WASH practices, and the age distribution of participants.

The prevalence of *S. haematobium* infection in Dulshatalo (43%) was lower than the 57.8% reported in 2007 (Mekonnen *et al.,* 2013), although the mean egg intensity was similar (14.90 vs. 15.32 eggs/10 ml). In Kurmuk town, the prevalence increased more than fourfold (27.8%) compared to the 5.8% found in 1996 (Birrie *et al.,* 1995).

These findings indicate that *S. haematobium* infection rates remain high in both villages, despite MDA rounds conducted in 2015, 2017, and 2019 (Leta *et al.,* 2020). This may be due to their location on the Ethio-Sudan border, where transboundary transmission is likely, and the community's reliance on nearby Shamade and Dinchaba rivers and dams for domestic activities like bathing, washing clothes, and traditional gold mining. These practices increase exposure to contaminated water, leading to ongoing infection and re-infection despite repeated treatment campaigns.

Children and adolescents were the most affected age groups in this study, though multivariable analysis adjusting for socio-demographics and open water contact practices did not find age to be a significant predictor, differing from a population study in Tanzania (Manz *et al.,* 2020), where age remained significant. This discrepancy may be attributed to differences in sample size and variables.

Participants who swam or played in open water had significantly higher *S. haematobium* infection rates and mean egg intensity. However, while washing clothes in open water was associated with high prevalence and significant egg intensity, it was not a key predictor of infection. This may be due to detergent use and limited skin exposure during washing, as suggested by other studies (Rudge *et al.,* 2008; Zida *et al.,* 2016).

Both sexes showed comparable rates of *S. haematobium* infection and intensity, consistent with previous findings in Dulshatalo and other studies (Awoke *et al.,* 2013; Hajissa *et al.,* 2018; Angora *et al.,* 2019; Manz *et al.,* 2020; Deribew *et al.,* 2022a). However, some studies report higher prevalence in males (Negussu *et al.,* 2013; Senghor *et al.,* 2014; Geleta *et al.,* 2015), while others indicate higher rates in females (Koukounari *et al.,* 2009; Nkegbe, 2010).

The agreement between *S. haematobium* egg detection and hematuria tested by dipstick, accordance with other research (Birrie *et al.,* 1995; Rudge *et al.,* 2008; Ugbomoiko *et al.,* 2009; Koukounari *et al.,* 2009; Bogoch *et al.,* 2012; Deribew *et al.,* 2022a) reinforces the urine dipstick as a viable community level diagnostic tool (King *et al.,* 2013).

6.5. KAP toward UGS in Abobo district, Gambella, Ethiopia

Understanding the factors influencing community knowledge, perceptions, and practices regarding UGS is critical to effective, sustainable disease control strategies (Manderson *et al.,* 2009). Community-based research forms the foundation of successful interventions, as emphasized by the World Health Organization (WHO, 2022a). This study investigates the specific KAP of communities in Abobo, a UGS endemic area in Ethiopia with prevalence rates between 22.2% and 35.9% (Geleta *et al.,* 2015; Deribew *et al.,* 2022a).

Knowledge and awareness

The study revealed a high level of community awareness of UGS (90.6%), surpassing awareness levels reported in Ethiopian regions, such as Benishangul-Gumuz (Assefa *et al.,* 2021), where the disease is prevalent but poorly recognized. This finding aligns with studies conducted across Africa (Kabatereine *et al.,* 2014; Rassi *et al.,* 2016; Sacolo *et al.,* 2018; Nenzhelele *et al.,* 2020; Dejon- Agobe *et al.,* 2021). Additionally, a significant proportion of participants (81.1%) were able to recognize hematuria (blood in urine) as a primary symptom of UGS. This high level of symptom recognition may be attributed to past infection experiences, as a history of infection was significantly associated with awareness of UGS symptoms ($P < 0.05$).

Knowledge gaps and misconceptions

Despite this high awareness of UGS, the community's knowledge of *S. haematobium* transmission was inadequate. Many participants were unaware of how the parasite enters the body, the role of snails in the disease's lifecycle, and the risks associated with unsafe water contact and unhygienic practices. These knowledge gaps are consistent with findings from other studies, such as in Gabon (Dejon- Agobe *et al.,* 2021), where communities recognized symptoms like hematuria but lacked an understanding of transmission mechanisms. Similarly, in South Africa, Sacolo-Gwebu *et al.* (2019b) reported that while awareness and knowledge of the symptoms were high, knowledge of the transmission cycle was minimal. Such gaps in understanding transmission pathways can increase exposure to the parasite, perpetuating transmission and contributing to high infection and morbidity rates.

Understanding of preventive measures was also notably inadequate. Only 15.9% of participants recognized the importance of maintaining environmental hygiene, 26.8% acknowledged the need to avoid freshwater contact, and 49.6% did not understand the risks associated with open-air urination as a source of water contamination. These findings align with similar gaps reported in other studies (Sacolo *et al.,* 2018; Koffi *et al.,* 2018; Nenzhelele *et al.,* 2020), emphasizing the necessity of increasing awareness of effective prevention strategies.

Misconceptions as seen in other studies (Sady *et al.,* 2015b; Dawaki *et al.,* 2015; Yirenya *et al.,*2016; Koffi *et al.,* 2018; Sacolo-Gwebu *et al.,* 2019b; Mutsaka-Makuvaza *et al.,* 2019b; Dejon- Agobe *et al.,* 2021) about disease prevention further showed these knowledge gaps. Some participants erroneously believed that not drinking contaminated water, removing stagnant water, or avoiding sunlight exposure could prevent UGS. These misconceptions and inadequate

understanding of the prevention and transmission cycle of the disease highlight a lack of health education and promotion of preventive measures in the research area.

Awareness of MDA

The study showed a high level of awareness regarding school-based MDA programs, with 81.1% of participants being familiar with the initiative. This level of awareness exceeds that reported in other regions, such as Benishangul-Gumuz in Ethiopia. Additionally, two-thirds of participants reported that their families had received praziquantel through MDA programs, a rate higher than those reported in Mozambique (9%) (Rassi *et al.,* 2016) and Gabon (10%) (Dejon- Agobe *et al.,* 2021). This may show an effective advocacy of the program or be attributed to consistent MDA campaigns conducted over the years, which should be sustained and expanded.

Attitudes on severity and prevention

The majority of respondents demonstrated a positive attitude toward recognizing that UGS is preventable and treatable and its fatal potential, indicating a strong foundation for promoting preventive behaviors and treatment-seeking (Ekpo *et al.*, 2010b). However, only half of respondents acknowledged that urinating near water sources contributes to transmission, stressing a gap in awareness that could sustain the disease cycle (Mazigo *et al.,* 2012; Lai *et al.,* 2015).

Risky practices and health-seeking behavior

Regardless of the community's primary drinking water source being public hand pumps, many participants relied on dam or river water for washing, bathing, and recreational activities like swimming, as previously reported (Deribew *et al.,* 2022b). Additionally, open-air urination and defecation were shared practices, further exacerbating the risk of transmission. These show the

need for integrated control strategies to effectively break the transmission cycle in the study area (WHO, 2022b).

Encouragingly, the study found that over 81% of participants sought medical treatment when infected, reflecting positive health-seeking behaviors. This trend is consistent with findings from Yemen (Sady *et al.,* 2015b) and Gabon (Dejon- Agobe *et al.*, 2021), where 81.1% and 78.2% of participants sought medical care in health institutes, respectively. The perception of UGS as a fatal disease (reported by 65% of participants) likely contributed to this behavior, as individuals who view the disease as severe are more likely to seek treatment. This attitude and practice are beneficial and should be reinforced to reduce transmission and prevent complications (Koffi *et al.,* 2018).

Factors influencing KAP

Formal education emerged as a significant determinant, with educated individuals demonstrating greater awareness of symptoms (dysuria), prevention strategies (seeking treatment and health education), and risk behaviors (swimming or playing in contaminated water). Educated participants also recognized the role of urination or defecation near water sources in disease transmission. These findings underscore the critical role of education in fostering informed attitudes and practices (Mutsaka-Makuvaza *et al.,* 2019b), aligning with similar results from studies in Yemen (Sady *et al.,* 2015b), where education was strongly linked to comprehensive KAP, and in Nigeria (Dawaki *et al.,* 2015), where formal education enhanced understanding of contaminated water as source of infection and deworming as preventive measures. Furthermore, Koffi *et al.* (2018) also highlighted that individuals with higher educational levels were more aware of symptoms like painful urination and the risks of swimming in contaminated water.

Gender differences also occurred, with male participants showing less knowledge about the risks of freshwater contact, potentially explaining their higher participation in activities like swimming, as knowledge is the foundation and a key factor influencing people‘s attitudes and practices (Guang *et al.*, 2018). A similar pattern was observed in prior studies near the Alwero Dam, where males had higher exposure to freshwater (Deribew *et al.*, 2022b).

Additional factors, such as age, occupation, and infection history, were found to impact participants knowledge, perception, and risky or safe practices in the area and these findings align with research conducted by Dawaki *et al.* (2015) and Angelo *et al.* (2019).

In conclusion, understanding the complex interplay of various factors across different contexts in social findings is a challenging endeavor (Manderson *et al.*, 2009). However, this study enabled us to identify protective and risk factors influencing KAPs related to UGS. These insights into the community's health behaviors can guide the development of targeted interventions, particularly in designing effective educational messages tailored to their needs.

6.6. Limitations of the studies

To the best of our knowledge, this is the first study to provide molecular data on the genetic characteristics of *S. haematobium* in an endemic region of Ethiopia. Nevertheless, the interpretation of our findings should consider the following limitations.

The community and PSAC prevalence study utilized a single urine filtration from a single-day collection, which may have reduced the sensitivity in detecting light infections, potentially leading to an underestimation of the overall prevalence. In addition, molecular techniques, such as PCR, could offer greater sensitivity than light microscopy.

The study faced challenges in assessing intermediate host factors. Since it took place during the rainy season, flooding likely displaced intermediate host snails from their natural habitats, making their collection and analysis difficult. This constraint may have affected the study‘s ability to fully evaluate the role of these hosts in transmission dynamics.

The KAP survey lacked a qualitative component that could have enriched the findings by offering deeper insights into UGS.

The genetic analysis was based on a relatively small number of miracidia, which may limit the robustness of conclusions regarding mixed infection/hybridization events. A larger sample size would provide more definitive insights into the genetic structure of *Schistosoma* populations in these regions. In addition, the lack of restriction enzyme analysis in ITS2 gene detection, which could have improved the resolution and specificity of *Schistosoma* species characterization, and sequencing, is also required for phylogenetic and haplotype analysis, warrants more detailed molecular approaches in future studies.

Chapter 7: Conclusion and Recommendations

7.1. Conclusion

This study offers important insights into the absence of mixed infection even in areas where both human and animal SCH coexist in UGS endemic regions. Despite the potential for cross-species transmission, the findings demonstrate that no significant mixed infection or genetic interchange. This aspect is critical for understanding the epidemiology of SCH in these areas, as it highlights the persistence of the human strain of *S. haematobium* in an environment where animal reservoirs may also be present. In addition, this study revealed the concerning prevalence and intensity of *S. haematobium* infections among PSAC. Maternal practices, such as accompanying children to open water sources for daily activities and engaging in unhygienic behaviors, emerged as major contributors to the heightened risk of infection and associated morbidity. Furthermore, despite the widespread endemicity and awareness of the disease in the Gambella region, the level of understanding regarding its transmission, prevention, and control measures remains critically low. Misconceptions and lack of practical understanding of preventive measures perpetuate risky behaviors, thereby facilitating the ongoing spread of the disease. Our findings also highlight the continuing high prevalence of *S. haematobium* infections in Kurmuk town and surrounding villages, with children and adolescents disproportionately affected. Despite repeated MDA initiatives, the high prevalence rates suggest that MDA alone has been inadequate to interrupt transmission. The continued transmission is likely influenced by environmental factors and the community's reliance on open water sources for domestic and recreational activities. These results collectively indicate that the community remains entrenched in a cycle of UGS transmission, with insufficient progress toward breaking this cycle. The high

prevalence and intensity of *S. haematobium* infection, combined with inadequate community awareness and continued risky behaviors, place the population at significant risk.

7.2. Recommendations

We forward the following recommendations, based on the findings of the study and the conclusion drawn

- ✓ Including aged 2 years and older PSAC in annual PC programs, and adopt a test and treats‖ approach for children under 2 year to ensure targeted treatment.
- ✓ Investing in reliable water sources to reduce dependence on infested water bodies and ensure access to safe water for domestic use.
- ✓ Install low-cost, durable sanitation facilities in water accessible Areas
- ✓ Expand and sustain MDA campaigns to ensure high coverage and participation with a particular focus on high-risk communities and groups, where infection rates and intensity are notably elevated and populations are vulnerable.
- ✓ Community-based health education programs should be strengthened to address gaps in knowledge about UGS transmission and prevention. Specifically, the primary mode of transmission (contact with cercarial infested water) and the role of the intermediate snail host in the transmission cycle. Educational campaigns should emphasize the risks of urinating near water sources, the importance of proper hygiene practices, and the role of environmental contamination in sustaining transmission. Engaging local leaders, schools, and community health workers in these efforts could enhance their reach and effectiveness.

- ✓ Promote cross-border collaboration with neighboring regions and countries to control the trans-boundary spread of UGS through shared surveillance and intervention strategies.
- ✓ Further research should be conducted to explore the role of intermediate hosts and environmental factors to better understand and address infection dynamics. Further molecular studies in the area are highly recommended to see any changes in parasite population and to determine the genetic polymorphism of *S.haematobium* to understand the broader epidemiological patterns and inform targeted control strategies.
- ✓ Implement multifaceted strategies that include health education, improved WASH infrastructure, regular screening, and community engagement to reduce ongoing transmission and achieve the goal of SCH elimination.

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Annex 1: Study Participants' Information Sheet

Title: - Molecular Characterization of *Schistosoma* species, Epidemiology, and Community Knowledge of Urogenital Schistosomiasis in Endemic Regions of Ethiopia

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Background information

Schistosoma haematobium is a parasitic worm that causes urogenital schistosomiasis, a neglected tropical disease affecting millions of people worldwide, particularly in sub-Saharan Africa, the Middle East, and parts of Asia. It is a major public health issue in Ethiopia, particularly in rural areas with limited access to clean water and sanitation. The disease is endemic in regions like the Afar Region, Benishangul- Gumuz and Gambella, where freshwater bodies such as lakes, rivers, dam and irrigation schemes facilitate transmission. People become infected through contact with water contaminated by parasite larvae released from infected snails. High-risk activities include farming, fishing, washing clothes, and swimming. Despite mass drug administration (MDA) campaigns using praziquantel, challenges such as population movement, poor sanitation, and suitable snail habitats sustain transmission. Hence this PhD research project which will be conducted by researchers from the Aklilu Lemma Institute of Health Research (ALIHR), Addis Ababa University (AAU) and aimed to carry out molecular analysis ,epidemiological and KAP study on *S.haematobium* infection in UGS endemic area of Ethiopia. This study has been reviewed and approved by Institutional Review Board (IRB) of Aklilu Lemma Institute of Pathobiology(presently Aklilu Lemma Institute of Health Research), AAU, ensuring that it meets ethical standards for research involving human participants.

Your participation is voluntary, and this information sheet provides details about the study, including the specimens required, procedures, risks, benefits, and your rights as a participant. We kindly request you or your family to take the time to read or have someone read to you and ensure you fully understand these details before deciding to participate.

Objectives: To assess the epidemiology and community KAP, genetic profile, and population structure of *Schistosoma* isolates from humans in selected UGS endemic areas of Ethiopia.

Study area: The study will be conducted in three Regions Ethiopia namely: Gambella (Abobo district), Afar (Haruka district), and Benishangul-Gumuz (Kurmuk district). The study area is selected because of UGS endemicity and their proximity to infested water sources.

Study Participants: The study will enroll preschool-aged children (1–5 years) to assess early schistosomiasis prevalence, community members (≥5 years) for broader prevalence, household heads for knowledge, attitude, and practice (KAP) evaluation, and children (5–15 years) to characterize the parasite molecularly.

Specimens Required: A 10 ml urine sample will be collected from each participant. The sample will be collected by trained and experienced technicians using standard, non-invasive procedures. The process is simple, painless, and typically takes only a few minutes. The urine sample will be securely transported to a designated laboratory, which may be located abroad, for analysis. The screening will involve three techniques: Dipstick Test: A rapid diagnostic tool to detect the presence of blood, Microscopic Examination: To visually identify parasite eggs under a microscope and Advanced methods molecular Techniques:, such as PCR (Polymerase Chain Reaction).

Risks Associated with Specimen Collection: The collection of a urine sample is a routine and safe procedure. It is non-invasive and does not involve any needles or instruments that could cause pain. There are no significant physical risks associated with participating in this study.

Confidentiality: Your privacy and confidentiality are of utmost importance. All information collected from you will be handled with strict confidentiality. Your participation will remain anonymous. Your name, address, or any other identifying information will not be linked to your sample or test results. Data will be securely stored and accessible only to authorized members of the research team. It will not be shared with third parties or organizations without your explicit consent. Any published results from this study will be presented in aggregate form, ensuring that

no individual can be identified.

Benefits of the Study: There will be no direct financial compensation for participating in this research. However, if you test positive for *S. haematobium*, you will receive appropriate treatment at no cost.

Withdrawal Rights: Participation in this study is entirely voluntary. You are free to decide whether or not to participate. You have the right to withdraw from the study at any time, without providing a reason, and without any penalty or loss of benefits. Your decision to participate or withdraw will not affect your access to healthcare, treatment, or other services

Contact Information: If you have any questions, concerns, or need further clarification about the study, please feel free to contact the principal investigator.

Principal Investigator: [Tigist Mohammed]

Phone Number: [0911816144]

Thank You:

Thank you for considering participation in this important research study. Your contribution will help advance our understanding of *S. haematobium* and improve public health outcomes for the community. If you agree to participate, please confirm your consent by signing the consent form provided by the research team.

Amharic Version of Study Participants' Information Sheet

ለጥናቱ ተሳታፊዎች የሚሰጥ መግለጫ

መረጃ ወረቀት

ርዕስ፡ የበ ብልሀርዚያ ጥገኛ ትላትልና ተዋስያን ሞለኪውላር ባህሪያት፣ ኤፒዴሚዮሎጂ እና የህብረተሰብ እውቀት፣ አመለካከት እና ልምድ (KAP) በኢትዮጵያ ቢልሃርዚያ በሚገኝባቸው ክልሎች

ተቋም፡ አክሊሉ ለማ ኢንስቲትዩት ኦፍ ፓቶባዮሎጂ (ALIHR)፣ አዲስ አበባ ዩኒቨርሲቲ (AAU)፣ አዲስ አበባ፣ ኢትዮጵያ

ፖ.ሳ.ቁ፡ 1176፣ ስልክ፡ +251 11 276 30 91፣ ፋክስ፡ +251 11 275 52 96

ዋና ተመራማሪ፡ ትግስት መሀመድ (የዶክትሬት ተማሪ)

ተቋም፡ ALIHR, AAU, አዲስ አበባ, ኢትዮጵያ

አድራሻ፡ ፖ.ሳ.ቁ. 1176, ስልክ: +251 11 276 30 91, ፋክስ: +251 11 275 52 96, ሞባይል: +251 911 816 144

ኢሜል፡ tgdeg43@gmail.com

የጥናቱ ዳራ፡ Schistosoma haematobium ይህ ተህዋስያን ቢልሃርዚያ የሚባል በሽታ የሚያስከትል ሲሆን፣ ይህ በሽታ በተለይ በንዑስ-ሰሃራ አፍሪካ፣ መካከለኛው ምስራቅ እና በእስያ ክፍሎች ውስጥ ሚሊዮኖችን ሰዎች የሚጎዳ ነው። በኢትዮጵያ፣ ይህ በሽታ በገጠር አካባቢዎች ውስጥ ንፁህ ውሃ እና የጤና አጠባበቅ አገልግሎቶች በጣም የተዳከሙባቸው ቦታዎች ውስጥ ትልቅ የጤና ችግር ነው። በሽታው በአፋር፣ ቤንሻንጉል-ጉሙዝ እና ጋምቤላ ክልሎች ውስጥ በተለይ የሚገኝ ሲሆን፣ ይህም በሐይቆች፣ ወንዞች፣ ግድቦች እና የተለያዩ የውሃ ምንጮች የተሞሉባቸው ቦታዎች ላይ የሚገኝ ነው። ሰዎች በተህዋስያኑ ሕዋሳት (larvae) የተሞሉ ውሃ ሲነኩ እና ሲገቡ፣ በሽታው ይዛቸዋል። እንደ ፣ ዓሣ ማጥመድ፣ ልብስ ማጠብ እና መዋኘት ያሉ ከክፍት ውሃ የሚያገናኙ የሥራእንቅስቃሴዎች ለበሽታው መጋለጥን ይጨምራሉ።

በፕራዚኳንተል (praziquantel) የሚሰጡ የጅምላ ህክምና ዘመቻዎች ቢኖሩም፣ የህዝብ መንቀሳቀስ፣ የንፁህ ውሃ እጥረት እና ለተህዋስያኑ መራባት ተስማሚ የሆኑ (snail) የቢልሃርዚያ ቬክተር እና የውሃ ምንጮች በመኖራቸው በሽታው እንዲቀጥል ያደርጋል። ስለዚህ፣ ይህ የዶክትሬት ምርምር ፕሮጀክት በአክሊሉ ለማ ኢንስቲትዩት ኦፍ ፓቶባዮሎጂ (ALIHR) እና አዲስ አበባ ዩኒቨርሲቲ (AAU) በተሳተፉ ተመራማሪዎች በኢትዮጵያ ይህ በሽታ በሚገኝባቸው ክልሎች ውስጥ የሞለኪውላር ትንታኔ፣ የኤፒዴሚዮሎጂ ጥናት እና የህብረተሰብ እውቀት፣ አመለካከት እና ልምድ (KAP) ጥናት ለማካሄድ ያለመ ነው። ይህ ጥናት በአክሊሉ ለማ ኢንስቲትዩት ኦፍ ፓቶባዮሎጂ (ALIHR) የሥነ ምግባር ኮሚቴ (IRB) ተፈትሽቶ እና ተፈቅዷል፤ ይህም ሰዎችን የሚያካትት ምርምር ሥነ ምግባራዊ መስፈርቶችን እንደሚያሟላ ያረጋግጣል።

የጥናቱ ዓላማ፡ የጥናቱ ዋና ዓላማ በኢትዮጵያ ውስጥ በሽንት ቢልሃርዚያ (የወሊድ አካል ቢልሃርዚያ) በሚገኝባቸው ክልሎች ውስጥ የበሽታውን ስርጭት፣ የህብረተሰብ እውቀት፣ አመለካከት እና ልምድ (KAP) እንዲሁም የተህዋስያኑን የጄኔቲክ ባህሪያት ለመመርመር ነው።

የጥናቱ ተሳታፊዎች የሚሰጡት መረጃና ሂደት፡ በዚህ ጥናት ላይ መሳተፍ ሙሉ ፈቃደኝነት ላይ የተመሠረተ ነው። ስለሆነም በመጀመሪያ በጥናቱ እንዲሣተፉ ፈቃደኝነትዎን በትህትና እንጠይቃለን።በዚህ ጥናት ለመሳተፍ ከፈቀዱ እስከ ሃያ ደቂቃ ያህል ለጥያቄዎች ምላሽ ይሰጡናል። ከዚያም 10 ሚሊ ሊትር የሽንት ናሙና ይሰጡናል ፡ ናሙናው በሙያተኞች ይሰበሰባል እና ወደ ላቦራቶሪ ተወስዶ ይመረመራል።

የጥናቱ ተሳታፊዎች፡ በዚህ ጥናት ዉስጥ የሚሳተፉት በጋምቤላ (አቦቦ ወረዳ) አፋር (ሀሩካ ወረዳ) እና ቤንሻንጉል-ጉሙዝ (ኩርሙክ ወረዳ) ክልሎች ዉስጥ ያሉ ነዋሪዎች ይሆናሉ ።

በጥናቱ በመሳተፍ የሚመጣ ጉዳት፡ የጥናቱ ተሳታፊ በመሆኖ ምንም ችግር ይከሰታል ተብሎ አይጠበቅም።

በጥናቱ በመሳተፍ የሚገኝ ጥቅም፡ በጥናቱ በመሳተፍ ምክንያት የሚገኝ የገንዘብ ወይም ሌላ ጥቅም የለም። ነገር ግን የእርሶ ወየም የልጆ በዚህ ጥናት ውስጥ መሳተፍ በጥናቱ ቢልሃሪዚያ ከተገኝዎበት ፣ በነጻ ህክምና ይሰጥዎታል።ከዚህም ተጨማሪ ቀደም ብሎ ማወቅ እና ማከም ከተህዋስያኑ ጋር የተያያዙ ውስብስብ ህመሞችን ለመከላከል ይረዳል።

ምስጢርን ስለመጠበቅ: በጥናቱ ውስጥ የተሰበሰቡ ማናቸውም ግላዊ መረጃዎች ሚስጥራዊነታቸው የተጠበቀ ይሆናል። ከማንነትዎ ጋር በቀጥታ ተያያዥነት ያላቸው መረጃዎች ለምሳሌ ስምና ሌላ ነገሮች በጥናቱ ዉጤት ላይ አይካተትም።

ከጥናቱ ስለመውጣትና ስለማቋረጥ: ይህ ጥናት በፈቃደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን በማናቸውም ወቅት በፈቃደዎ ከጥናቱ መውጣት ይችላሉ። ከጥናቱ ቢወጡም እንኳ በማንኛዉም የጤና ተቐሙ የመገልግል መብቶ የተጠበቀ ነዉ።

ጥያቄ ካለዎት፡ ከጥናቱ ጋር በተያያዘ ማናቸውም ጥያቄ ቢኖርዎ ከላይ በተጠቀሰዉ አድራሻ መሰረት ለጥናቱ ተመራማሪዎች ጥያቄዎን ማቅረብ ይቻላል።

አመሰግናለሁ!

ይህን ጥናት ለመሳተፍ ከወሰኑ በኋላ፣ የፈቃድ ፎርም በመፈረም ፈቃድዎን ያሳውቁ።

Annex 2: Consent form for Participation

Name of the Participant: _____

Age: _____Sex: _____

District: _____Kebele: _____Code: _____

I understand that and got adequate information about the research, **Molecular Characterization of *Schistosoma haematobium*, Epidemiology, and Community Knowledge of Urogenital Schistosomiasis in Endemic Regions of Ethiopia**.I, or my family, may be requested to provide a 10 ml urine sample and participate in an interview as part of this study on bilharziha. The researchers have informed me that there is no risk associated with participating in the study, providing the requested samples, or taking part in the interview. I acknowledge that the results of laboratory diagnosis, as well as any information shared during the interview, will be used solely for research purposes, and all information related to myself or my family will be kept strictly confidential. I am fully aware that participation in this study is entirely voluntary, and I have the right to withdraw at any time without providing a reason. Additionally, I understand that

choosing not to participate will not affect my aaccess to medical care or any other benefits. I confirm that I have been provided with all necessary information, that I have had the opportunity to ask questions, and that my concerns have been addressed. This informed consent form has been conveyed by the language that I fully understand. By signing below, I voluntarily agree to provide a urine sample and participate in the interview for this study.

Name of Participant: _____ Signature: _____Date: _____

Name of Researcher Obtaining Consent: _____

Signature: _____Date: _____

CONSENT LETTER: Amharic version

<u>የጥናቱ ተሳታፊዎች የስምምነት መግለጫ (ዕድሜያቸዉ 18 እና ከዛ በላይ ለሆኑ)</u>

የተሳታፊዉ ስም _____ ዕድሜ _____ ጾታ_____

እኔ ስሜ ከላይ የተጠቀሰዉ ይህን ጥናት የሚጠናው በ ብልሀርዚያ ጥገኛ ትላትልና ተዋስያን አማካኝነት የሚከሰቱ በሽታዎች ጥናቱ በሚካሄድበት ቦታ በምን ደረጃ ላይ እንዳሉ እና የማህበረሰብ ዕውቀት ለማወቅ እንደሆነ በቂ መረጃ ተሰቶኛል። በእኔ ወይም ቤተሰቦቼ የጥናቱ ተሳታፊ ለመሆን ከተስማማሁ ቃለመጠይቅ እንደሚጠየቅ እንድሁም 10 ሚሊ ይተር የሽንት ናሙና እንደሚሰጥ ተገልጾልኛል። የተጠየቁትን ናሙናዎች በመስጠት ወይም በቃለ መጠይቁ ውስጥ በመሳተፍ ምንም አይነት አደጋ እንደማይገጥመኝ ተገንዝቤያለሁ። የላቦራቶሪ ምርመራ ውጤቶች እንዲሁም በቃለ መጠይቁ ወቅት የተጋሩ ማንኛውም መረጃ ለጥናታዊ ዓላማዎች ብቻ እንደሚውል እንዲሁም እኔ ወይም ቤተሰቦቼ በተመለከተ ያለው ማንኛውም መረጃ በጥብቅ ሚስጥር እንደሚያዝ ተረድቻለው። ስለ ጥናቱ በሚገባኝ መንገድ ገለፃ ከተደረገልኝና በጉዳዩ ላይ በአግባቡ እንዳስብበት ጊዜ ከተሰጠኝ በኃላ እንደዚሁም ግልፅ ያልሆኑ ነገሮችን

የመጠየቅና ከፈለኩም በማንኛውም ጊዜ ያለመሳተፍና የማቋረጥ መብቴ የተጠበቀ መሆኑንተገልጾልኛል። ከዚህ በታች በመፈረም በጥናቱ ለመሳተፍ ፈቃደኛ መሆኔን አረጋግጣለሁ።

የተሳታፊዉ ስም

_____**ቀን** _____**ፊርማ** _____

የተሳታፊዉን ማረጋገጫ የተቀበለዉ ሰዉ ስም

_____**ቀን** _____**ፊረማ** ____

የጥናቱ ተሳታፊዎች የስምምነት መግለጫ ቅጽ (ዕድሜያቸዉ ከ 18 በታች ለሆኑ)

የተሳታፊዉ ህጻን ቤተሰብ/አሳዳግ ስም _
ዕድሜ _____**ጾታ** _____

እኔ ስሜ ከላይ የተጠቀሰዉ በ ብልሀርዚያ ጥገኛ ትላትልና ተዋስያን አማካኝነት የሚከሰቱ በሽታዎች ጥናቱ በሚካሄድበት ቦታ በምን ደረጃ ላይ እንዳሉ ለማወቅ ስለሚደረገዉ ጥናት በቂ መረጃ የተሰጠኝ ሲሆን ልጄ የጥናቱ ተሳታፊ ለመሆን ከተስማማ/ች ቃለመጠይቅ እንደምጠየቅ እንድሁም 10ml የ ውሃ ሽንት ናሙና ልጄ እንደሚሰጥ/እንደምትሰጥ ተገልጾልኛል። ልጄ በጥናቱ በመሳተፉ/በመሳተፏ ምክንያት ልደርስበት/ልደርስባት የሚችል ችግር እንደሌለም ተገልጾልኛል። በተጨማሪም የሚሰበሰበዉ ናሙና በ ብልሀርዚያ ጥገኛ ትላትልና ተዋስያን አማካኝነት የሚከሰቱ በሽታዎች ጥናትና ምርምር ብቻ እንደሚዉል ተገልጾልኛል። ከእኔ እና ከልጄ ጋር ቀጥተኛ ግንኙነት ያላቸዉ ነገሮች እነደ ስም የመሳሰሉት በሚስጢር እንደሚያዝና በጥናቱ ዉጤት ላይ እንደማይታተም ተገልጾልኛል።በጥናቱ መሳተፍ ሙሉ ለሙሉ በፈቃደኝነት ላይ የተመሰረተ መሆኑ ተገልጾልኛል። ስለዚህ ልጄ በጥናቱ እንድሳተፍ/እንድትሳተፍ መስማማቴን በፊርማዬ አረጋግጣለሁ።

የተሳታፊዉ ህጻን ስም

_____ፆታ _____ዕድሜ _____

ህጻን _____በጥናቱ ለመሳተፍ ፈቃደኛ ነህ/ነሽ?

1. **አዎ ለመሳተፍ ፈቃደኛ ነኝ**
2. **አይ ለመሳተፍ ፈቃደኛ አይደለሁም**

የተሳታፊዉ ህጻን ቤተሰብ/አሳዳግ ስም

_____ቀን _____ፊርማ ____

የተሳታፊዉን ህጻንና ቤተሰብ/አሳዳግ ማረጋገጫ ቃል የተቀበለዉ ሰዉ ስም .

_____ፊረማ_____ ቀን_____

Annex 3: Questionnaires

Questionnaire 1: Developed for the Study – *Schistosoma* genetic profile and genetic diversity in urogenital schistosomiasis endemic area of Ethiopia

Code: _____No----------

Region_____Date _____/_____/_____altitude \latitude _____

Part I: Socio-demographic Data: Please kindly provide the following information.

Q. No.	Questions	Variables
	Sex	1. Male 2. Female
	Age	_____years
	Kebele /villages	_____
	Number of miracidia	

	Date of collection	
	Dipstick result	
	Microscopic result	

Questionnaire 2: A questionnaire developed for the Study on the risk factors for Bilharzia exposure among preschool-aged children (aged 1 to 5 years who provided urine samples), collected from mothers or caregivers.

Code: _____No----------

Date _____/_____/_____

Part I: Socio-demographic Data: Please kindly provide the following information.

No.	Question	Response Options	Skip to
1	Locality/Kebele Name:		
3	Child's Gender:	1. Female 2. Male	
4	Child's Age:		
5	Mother's/Caregiver's Age:		
6	Mother's/Caregiver's Education Level:	1. No formal education 2. Can read and write 3. Primary education 4. Secondary education 5. College/University 6. Other (Specify)--------	
7	Mother's/Caregiver's Marital Status:	1. Single 2. Married 3. Widowed 4. Divorced	
8	Mother's/Caregiver's Religion:	1. Orthodox 2. Muslim 3. Protestant 4. Other (Specify)----------	
9	Mother's/Caregiver's Ethnicity:		

10	Mother's/Caregiver's Occupation (Multiple answers possible):	1. Farmer 2. Farmer & trader 3. Daily laborer 4. Housewife 5. Government or private employee 6. 7 Housemaids. Other (Specify)------	
11	What is your main source of drinking water? (Multiple answers possible):	1. Borehole/Pump 2. Dam/Water reservoir 3. Well 4. Tap water 5. River 6. Spring 7. Pond 8. Other (Specify)-------	
12	Do you have a latrine/toilet facility at home?	1. Yes 2. No	

Part II: Open water contact and sanitation practice of Mothers and PSAC

1	Do you use water from a reservoir, river, well, or spring?	1. Always 2. Sometimes 3. Never	
2	For what purpose do you use water from these sources? (Multiple answers possible):	1. Bathing 2. Swimming 3. Washing clothes 4. Watering animals 5. Other (Specify)--------	
3	Do you take your child with you when you go to collect water or wash near a river or reservoir?	1. Always 2. Sometimes 3. Never	If no got to Q 6
4	Does your child play or bathe in river or reservoir, water?	1. Always 2. Sometimes 3. Never	
5	When your child plays or bathes in river or reservoir water, do they submerge themselves?	1. Always 2. Sometimes 3. Never	
6	Where do you and your child urinate when you visit a water source? (Multiple answers possible):	1. Nearby bushes 2. Near or in the water 3. Return home 4. Other (Specify)----	
7	What type of water do you use to bathe your child at home? (Multiple answers possible):	1. River water 2. Reservoir/Dam water 3. Pond water 4. well or	
		Pump water 5. Other (Specify)-----	

8	Do you prepare warm water for bathing your child at home?	1. Always 2. Sometimes 3. Never	

Questionnaire 3: Developed for the Study – Community Knowledge, Attitude, and Practice about Urogenital schistosomiasis in Abobo, Ethiopia

Name _____Villages _____

Date ____/____/_____

Dear study participants, please kindly provide the following information.

Section 1: Socio demographic Information

No.	Questions	Response options	Skip
1	What is your name?	----------	
2	Gender	1. Male, 2. Female	
3	What is your age?	(Open-ended)	
4	How many family members do you have?	(Open-ended)	
5	What is your level of education?	1. No education, 2. Can read and write, 3. Primary education, 4. Secondary education, 5. College/University	
6	Marital status	1. Single, 2. Married, 3. Divorced, 4. Widowed	
7	What is your family's monthly income?	1. Below 500, 2. 501–1000, 3. 1001–2000, 4. 2001–3000, 5. Above 3000, 6. Other (Specify)	
8	What is your religion?	1. Orthodox, 2. Muslim, 3. Protestant, 4. Other (Specify)	
9	What is your ethnicity?	1. Aguak, 2. Nuware, 3. Mejenger, 4. Kanbata, 5. Amhara, 6. Oromo, 7. Other (Specify)	
10	What is your occupation?	1. Farming, 2. Farming and trade, 3. Daily laborer, 4. Houseworker, 5. Government/Private employee, 6. Housewife, 7. Other (Specify)	
11	What is your source of drinking water?	1. Pump water, 2. Spring/River water, 3. Well water, 4. dam water, 5. Other (Specify)	
12	What type of house does your family live in?	1. Thatched roof, mud walls, and mud floor, 2. Corrugated iron roof, cement walls, and cement floor, 3. Other (Specify)	
13	Do you have a toilet?	1. Yes, 2. No	If no goes to the next section

14	If yes to question 13, what is the floor made of?	1. Wood, 2. Mud, 3. Cement, 4. Other (Specify)	

Section 2: Participants' Knowledge about Bilharzia (Schistosomiasis)

No.	Questions	Response
1	Have you heard about Bilharzia?	1. Yes, 2. No (If No, skip to next section)
2	If yes to question 1, where did you hear about it?	1. TV, 2. Radio, 3. Newspaper, 4. Friends, 5. School, 6. Family, 7. Health institution, 8. Other (Specify)
3	What are the symptoms of Bilharzia?	1. Blood in urine, 2. Abdominal pain, 3. Cough, 4. Fever, 5. Burning sensation during urination, 6. Nausea, 7. Vomiting, 8. Swollen face, 9. Fatigue, 10. Loss of appetite, 11. Diarrhea, 12. Don't know
4	How is Bilharzia transmitted?	1. Drinking untreated water, 2. Swimming/Playing in contaminated water, 3. Eating contaminated food, 4. Other (Specify), 5. Don't know
5	How does the Bilharzia parasite enter the human body?	1. Through the skin, 2. Drinking contaminated water, 3. Eating contaminated food, 4. Contact with contaminated soil, 5. Poor hand hygiene
6	What transmits Bilharzia?	1. Snails, 2. Flies, 3. Mosquitoes, 4. Other (Specify), 5. Don't know
7	Has anyone in your family been affected by Bilharzia?	1. Yes, 2. No
8	Can Bilharzia be treated?	1. Yes, 2. No (If No, skip to question 10)
9	If yes to question 8, how can it be treated?	1. Traditional medicine, 2. Modern medicine, 3. Buying medicine from a pharmacy, 4. Other (Specify)
10	Can Bilharzia be prevented?	1. Yes, 2. No (If No, skip to question 12)
11	If yes to question 10, how can it be prevented?	1. Taking medicine, 2. Health education, 3. Hygiene, 4. Vaccination, 5. Other (Specify)
12	Have you heard about the school-based drug distribution program for Bilharzia control?	1. Yes, 2. No
13	Do your children participate in this drug distribution program?	1. Yes, 2. No
14	Which activities involving water are transmission routes for Bilharzia?	1. Washing clothes, 2. Crossing rivers barefoot, 3. Swimming, 4. Bathing, 5. Other (Specify)

Section 3: Participants' Attitudes and Practices Regarding Bilharzia

No.	Question	Response Options
1	Is Bilharzia a deadly disease?	1. Yes, 2. No, 3. Don‘t know
2	Can Bilharzia be treated?	1. Yes, 2. No, 3. Don‘t know(If No, skip to question 4)
3	If yes to question 2, how can it be treated?	----------
4	Can Bilharzia be prevented?	1. Yes, 2. No, 3. Don‘t know(If No, skip to question 6)
5	If yes to question 4, how can it be prevented?	----------
6	Do you think urinating\defecating in fields or near water sources is a transmission route for Bilharzia?	1. Yes, 2. No, 3. Don‘t know
7	Where do your family members usually defecate\urinate?	1. Private toilet, 2. Shared toilet, 3. Under trees/fields/riverbanks, 4. Other (Specify)
8	Do you or your children swim in rivers/dams?	1. Yes, 2. No (If No, skip to question 10)
9	If yes to question 8, how often?	1. Always, 2. Occasionally
10	Do you or anyone in your family who has been infected with Bilharzia?	1. Yes, 2. No(If No, skip to question 12)
11	If yes to question 7, what solution did you seek?	1. Traditional medicine, 2. Modern medicine, 3. Buying medicine from a pharmacy, 4. Other (Specify)
12	What do you use dam\river water for?	1. Washing clothes, 2. Swimming, 3. Bathing

Questionnaire 4: Developed for the study – prevalence of *S. haematobium* infection in Kurmuk district, western Ethiopia

Name of Participant: _____No----------

Village_____Date _____/_____/_____

Part I: Socio-demographic Data: Please kindly provide the following information.

Q. No.	Questions	Categories
1	Sex	Male Female

2	Age	_____years
3	Ethnicity	_____
4	Religion	1)Orthodox 2)Muslim 3)Protestant 4)Others(specify) _____
5	Educational Status	1) Not reading or writing 2)Primary 3)Secondary 4) College or university
6	Occupation	1)Farmer 2)Student 3)Housewives 4)Merchants 5)Daily laborers 6)Government/private employe 7)Others(specify) _____
7	Residence/kebeles	1)Dulshitalo 2)Beleho-J 3)Ougundu 4)Kurmuk
8	Presence of toilet at home	1) Yes 2) No
9	Drinking water source	1) Pump 2) Open water
10	Swimming habit	1) Yes 2) No
11	Washing clothes in open water	1) Yes 2) No

Annex 4:አማርኛ መጠይቅ

ጥያቄ 1፡ በኢትዮጵያ ውስጥ የ የጂኔቲክ ብልሀርዚያ መገለጫና ያለው የጂኔቲክ ልዩነት ለማወቅ የተዘጋጀ መጠይቅ

ኮድ፡ _____ቁጥር----------
መንደር፡ _____ቀን፡ //_____ከፍታ (Altitude) እና ላቲቲዩድ (Latitude)

ክፍል I: የማህበረሰብ-ዘርፍ እና የምርመራ ዝርዝር መረጃ

ቁጥር	መለያ ቁጥር	ስም	የክልል /መንደር ስም	እድሜ	ጾታ	የዲፕስቲክ ውጤት	የማይክሮስኮፕ ውጤት	ሚራሲዲያ የተሰበሰበበት ቀን	ቁጥር
1									
2									

ጥያቄ 2፡በቅድመ ትምህርት ቤት ህጻናት (ከ1 እስከ 5 አመት እድሜ ያላቸው ፣ የሽንት ናሙና የሰጡ) ላይ (ከእናቶች ወይም አሳዳጊዎች የሚሰበሰብ) ለቢልሃርዚያ ተጋላጭነት መንስኤዎች ላይ የተዋቀረ መጠይቅ

	የአካባቢ ስም/ቀበሌ		
1	የልጅዎ ስም		
2	የልጅዎ ጾታ	1. ሴት 2. ወንድ	
3	የልጅዎ ዕድሜ		
4	የእናቶች/የአሳዳጊዎች እድሜ		
5	የእናቶች/የአሳዳጊዎች የትምህርት ደረጃ	1. ያልተማረ/ መሃይም 2. ማንበብ እና መፃፍ. 3.የመጀመሪያ ደረጃ ትምህርት 4. የሁለተኛ ደረጃ ትምህርት 5. ኮሌጅ/ዩኒቨርሲቲ 6. ሌላ ----------	
6	የእናቶች/የአሳዳጊዎች የጋብቻ ሁኔታ	1፣ ያላገባች 2፣ ያገባች 3፣ ባል የሞተባት 4፣ የተፋታች	
7	የእናቶች/የአሳዳጊዎች ሃይማኖት	1. ኦርቶዶክስ 2. ሙስሊም 3. ፕሮቴስታንት 4. ሌላ ----------	

8	የእናቶች/የአሳዳጊዎች ጎሳ/ ብሄር ----------		
9	የእናቶች/የአሳዳጊዎች ሥራ(ከአንድ በላይ መልስ ሊኖር ይችላል)	1. ገበሬ 2. ገበሬ እና ነጋዴ 3. የቀን ሰራተኛ 4. የቤት ሰራተኛ 5. የመንግስት ወይም የግል ተቀጣሪ 6. የቤት እመቤት 7. ሌላ----------	
10	የመጠጥ ውሃ ከየት ታገኛላችሁ ወይም ትጠቀማላችሁ? (ከአንድ በላይ መልስ ሊኖር ይችላል)	1. የቧንቧ / የፓምፕ ውሃ 2. የግድብ/ ውሃ ማጠራቀሚያ 3. የኩሬ ውሃ 4. የመስኖ ውሃ 5. የወንዝ ውሃ 6. የ ምንጭ ውሃ 7. የጉድጓድ ውሃ 8. የወንዝ, የግድብ እና የኩሬ ውሃ 9. ሌላ----------	
11	እርስዎ መጸዳጃ/ሽንት ቤት አለዎት	1. አዎ 2. አይ	
12	ከግድብ/ከወንዝ//ከኩሬ ምንጭ ውሃ ትጠቀሚያለህሽ/ትቀጅአለሽ	1. አዎን ሁል ጊዜ 2. አዎ አንዳንዴ 3. አይ	
13	የግድብ/ወንዝ/ ኩሬ/ ምንጭ ውሃ ለምን ዓላማ/ ምክንያት ትጠቀሚያለህሽ/ ወይ ትሄጅአለሽ/(ከአንድ በላይ መልስ ሊኖር ይችላል)	1. ገላን ለመታጠብ 2 . ለመዋኛ 3. ልብስ ለማጠብ 4. ለእንስሳት የሚጠጡት ውሃ ለ መቅዳት 5.ሌላ------	
14	ወደ ግድብ/ወንዝ/ኩሬ/ ውሃ ለመቅዳት ወይም ለማጠብ በሚሄዱበት ጊዜ ልጁን ይዘሽው ትሄጅአለሽ	1.አዎን ሁል ጊዜ 2. አዎ አንዳንዴ 3. አይ	አይ" ከሆነ ወደ ጥያቄ 18 ይሂዱ
15	ለጥያቄ 14 አዎ ከሆነ መልሱ ልጅዎ በወንዝ ወይም በግድብ ማጠራቀሚያ/ ውሃ ውስጥ ይጫወታል ወይም ገላውን ታጥቢዋለሽ	1.አዎን ሁል ጊዜ 2. አዎ አንዳንዴ 3. አይ	
16	ልጅዎ በግድብ/ወንዝ/ኩሬ/ ምንጭ ውስጥ ሲጫወቱ ወይም ሲዋኙ በውሃ	1.አዎን ሁል ጊዜ 2. አዎ አንዳንዴ 3. አይ	

	ውስጥ ይሸናሉ።		
17	ግድብ/ወንዝ/ኩሬ/ ውሃ ለመቅዳት ወይም ለማጠብ ትሄጅበት ጊዜ እርስዎ እና ልጅዎ የት ነው የሚሸኑት?(ከአንድ በላይ መልስ ሊኖር ይችላል)	1.በአቅራቢያው በሚገኝ ቁጥቋጦ ውስጥ 2. ከውኃው አጠገብ ወይም ውስጥ 3. ወደ ቤት እመለሳለሁ 4. ሌላ----------	
18	ልጅሽን ለማጠብ ምን አይነት ውሃ ትጠቀሚያለህሽ (ከአንድ በላይ መልስ ሊኖር ይችላል)	1.የወንዝ ውሃ 2. የ ግድብ ወይም ማጠራቀሚያ ውሃ 3. የጉድጓድ ውሃ 1. 4. የፓምፕ ወይም የቧንቧ ውሃ 5. ሌላ----------	
19	ልጅሽን ገላ ለማጠብ ሙቅ / የፈላ ውሃን ያዘጋጃሉ	2. አዎን ሁል ጊዜ 2. አዎ አንዳንዴ 3. አይ	3.

<u>ጥያቄ 3፡በቢልሃርዚያ በሽታ ዙሪያ በ አበቦ ወረዳ የወላጆች ግንዛቤ እና የክፍት ውሃ አጠቃቀም ለመረዳት የተዘጋጀ መጠይቅ</u>

ክፍል 1. የማህበረ-ህዝብ መረጃ

ተ.ቁ	መጠይቅ	መልስ
1.	ስምዎን ቢነግሩን	
2	ፆታ	
3	እድሜዎን ቢነግሩን	
4	የቤተሰብዎን አባላት ብዛት ቢነግሩን	
5	የትምህርት ደረጃዎን ቢነግሩን	1. ያልተማረ\ች 2. ማንበብ እና መጻፍ የሚችል፣ 3. የመጀመሪያ ደረጃ ትምህርት፣ 4. ሁለተኛ ደረጃ ትምህርት፣ 5. ኮሌጅ/ዩኒቨርሲቲ
6	የጋብቻ ሁኔታ	1. ያላገባ\ች፣ 2. ያገባ\ች፣ 3. የፈታ\ች፣ 4. የሞተባት\በት
7	ሥራ	1) ገበሬ 2) የቤት እመቤት 3) ነጋዴ 4) የቀን ሠራተኛ 5) የመንግስት/ግል ተቀጣሪ 6) ሌላ (ይግለጹ) _____

8	የቤተሰብዎን ወርሃዊ ገቢ ስንት ነዉ	1. ከ500 በታች፣ 2. 501--1000፣ 3. 1001--2000፣ 4. 2001--3000፣ 5. ከ3000 በላይ፣ 6. ሌሎች (ይግለጹ)-
9	የምን ሀይማኖት ተከታይ ነዎት	1. ኦርቶዶክስ ፣ 2. ሙስሊም፣ 3. ፕሮቴስታንት፣ 4. ሌሎች (ይግለጹ)--
10	የየትኛዉ ብሄረሰብ አባል ነዎት	1. አጉዋክ፣ 2. ኑዋሬ፣ 3. መዠንገር፣ 4. ከንባታ፣ 5. አማራ፣ 6. ኦሮሞ፣ 7. ሌሎች (ይግለጹ)----
11	መተዳደሪያዎ ምንድነዉ	1. ግብርና፣ 2. ግብርና ንግድ፣ 3. የቀን ሰራተኛ፣ 4. የቤት ሠራተኛ፣ 5. የመንግስት/የግል ሠራተኛ፣ 6. የቤት እመቤት፣ 7. ሌላ (ይግለጹ)-----
12	ለመጠጥ የሚጠቀሙት ዉሃ ከምን ነዉ	1. የፓምፕ ውሃ፣ 2. የምንጭ \የወንዝ ውሃ፣ 3. የኩሬ ውሃ፣ 4. የሐይቅ ውሃ፣ 5. ሌሎች (ይግለጹ)----
13	የቤተሰብዎ ቤት ምን አይነት ነዉ	1. የሣር ጣሪያ፣ ጭቃ ግድግዳ እና የጭቃ\አፈር ወለል፣ 2. የቆርቆሮ ጣሪያ፣ የሲሚንቶ ግድግዳ እና የሲሚንቶ ወለል፣ 3. ሌላ ከሆነ (ይግለጹ)----
14	ሽንት ቤት አሎዎት	1. አዎ፣ 2. የለኝም
15	ከላይ ቁጥር 13 አዎ ከሆነ፣ ወለሉ ከምን የተሰራ ነው?	1. እንጨት፣ 2. ጭቃ፣ 3. ሲሚንቶ፣ 4. ሌሎች (ይግለጹ).....

ክፍል 2. የጥናቱ ተሳታፊዎች ለቢልሃርዚያ በሽታ ዙሪያ ስላላቸዉ ዕዉቀት ስለምልክቶቹ ስለ መተላለፊያና መከላከያ መንገዶች መጠይቅ

1	ስለ ቢልሃርዚያ ሰምተዉ ያውቃሉ?	1. አዎ፣ 2. የለም	ዝለል ወደ ቀጣይ ጥያቄ
2	ከላይ ቁጥር 1 አዎ ከሆነ ከምን ሰሙ	1. ቴሌቪዥን፣ 2. ራዲዮ፣ 3.ጋዜጣ፣ 4. ጓደኞች፣ 5. ትምህርት ቤት፣ 6. ቤተሰብ፣ 7. የጤና ተቋም፣ 8. ሌሎች (ይግለጹ) ----------	አልሰማውም ከሆነ መልሱ እዚጋር መጠየቀውን ያቁሙ
3	የቢልሃርዚያ በሽታ ምልክቶች ምን ምን ናቸው?	1. ደም የተቀላቀለ ሽንት፣ 2. የሆድ ህመም፣ 3. ተቅማጥ፣ 4. ትኩሳት፣ 5. ሽንት ማቃጠል፣ 6. ማስታወክ፣ 7. ማሳከክ፣ 8. የገረጣ ፊት፣ 9. ድካም፣ 10. የምግብ ፍላጎት መቀነስ፣ 11.	

		ሳል፣ 12. አላውቅም |	
4	ቢልሃርዚያ በምን ይተላለፋል?	1. ያልተጣራ ውሃ በመጠጣት፣ 2. በተበከለ ውሃ ውስጥ በመዋኘት/መጫወት፣ 3. የተበከለ ምግብ በመመገብ፣ 4. ሌሎች (ይግለጹ)፣ --------- 5. አላውቅም	
5	የቢልሃርዚያ ተውሳክ\ትል ወደ ሰውነት እንዴት ይገባል?	1. በቆዳ በመብሳት፣ 2. የተበከለ ውሃ በመጠጣት፣ 3. የተበከለ ምግብ በመመገብ፣ 4. ከተበከለ አፈር፣ 5. ንፅህና በጎደለው እጅ	
6	ቢልሃርዚያን የሚያስተላልፍ ምንድ ነዉ ?	1. ትንኝ፣ 2.ዝንብ ፣ 3.ቀንድ አዉጣ፣ 4. ሌሎች (ይግለጹ)፣ ---- 5.አላውቅም	
7	ከቤተሰብዎ ቢልሃርዚያ የተጠቃ ልጅ ነበር	1.አዎ፣ 2. የለም	
8	ቢልሃርዚያን ማከም ይቻላል?	1.አዎ፣ 2. የለም	የለም ከሆነ መልሱ ወደ ቁጥር 10 ጥያቄ ይሂዱ
9	ከላይ ቁጥር 8 አዎ ከሆነ፣ በምን መንገድ ማከም ይቻላል	1. ባህላዊ ህክምና፣ 2. ዘመናዊ ህክምና፣ 3. ከፋርማሲ መድሃኒት በመግዛት፣ 4. ሌሎች (ይግለጹ)-------	
10	ቢልሃርዚያን መከለከል ይቻላል?	1.አዎ፣ 2. የለም	የለም ከሆነ መልሱ ወደ ቁጥር 12 ጥያቄ ይሂዱ
11	ከላይ ቁጥር 10 አዎ ከሆነ\ከተቻለ፣ በምን መንገድ መከላከል ይቻላል	1. መድሃኒት በመውሰድ፣ 2. የጤና ትምህርት፣ 3. ንፅህና. ክትባት፣ 5. ሌሎች (ይግለጹ)----------	
12	ቢልሃርዚያን ለመቆጣጠር በየትምህርት ቤቱ ስለሚታደለው መድሃኒት ሰምተዋል ያዉቃሉ?	1. አዎ፣ 2. የለም	
13	ልጆችዎ በዚህ መድሃኒት እደላ መርሃ ግብር ይሳተፋሉ	1.አዎ፣ 2. የለም	
14	ከዉሃ ጋር ከሚደረጉ ግንኙነቶች የትኞቹ የቢልሃርዚያ መተላለፊያ መንገዶች ናቸው	1. ልብስ ማጠብ፣ 2. ወንዝ በባዶ እግር ማቃረጥ፣ 3. መዋኘት፣ 4. ገላ ማጠብ፣ 5. ሌሎች (ይግለጹ).....	

ክፍል 3. የጥናቱ ተሳታፊዎች ስለ ቢልሃርዚያ በሽታ ስላላቸው አመለካከት እና ሰለሚያደርጉት ድርጊት የተደረገ መጠይቅ

1	ቢልሃርዚያ ገዳይ በሽታ ነው	1. አዎ፣ 2. አይደለም፣ 3. አላውቅም	
2	ቢልሃርዚያን ማከም ይቻላል?	1. አዎ፣ 2. አይደለም፣ 3. አላውቅም	
3	ከላይ ቁጥር 2 አዎ ከሆነ እንዴት ማከም ይቻላል	----------	
4	ቢልሃርዚያን መከለከል ይቻላል?	1. አዎ፣ 2. አይደለም፣ 3. አላውቅም	
5	ከላይ ቁጥር 4 አዎ ከሆነ አንዴት መከላከል ይቻላል	----------	
6	ሜዳ ላይ ወይም ዉሃ ዳር መሸናት የቢልሃሪዚያ መተላለፊያ መንገድ ነው ብለው ያስባሉ	1. አዎ፣ 2. አይደለም፣ 3. አላውቅም	
7	የቤተሰብ አባላት ብዙውን ጊዜ የት ይፀዳዳሉ	1.በግል ሽንት ቤት 2.በጋራ ሽንት ቤት3.ዛፍ ስር\ሜዳ\ወንዝ አጠገብ 4.ሌሎች-(ግለፅ)------	
8	እርስዎ ወይንም ልጆችዎ ወንዝ\ግድብ ውስጥ ይዋኛሉ	1.አዎ፣ 2. የለም	የለም ከሆነ መልሱ ወደ ቁጥር 10 ጥያቄ ይሂዱ
9	ከላይ ቁጥር 8 አዎ ከሆነ በምን ያህል ጊዜ	1.ሁል ጊዜ 2. አልፎ አልፎ	
10	ቢልሃሪዚያ በሽታ ተይዘው ያውቃሉ		
11	ከላይ ቁጥር 7 አዎ ከነበረ ምን መፍትሄ ፈለጉ	1. ባህላዊ ህክምና፣ 2. ዘመናዊ ህክምና፣ 3. ከፋርማሲ መድሃኒት በመግዛት፣ 4. ሌሎች (ይግለጹ)-------	
12	የግድብን ውሃ ለምን አገልግሎት ይጠቀሙታል	1. ልብስ ለማጠብ፣ 2.ለመዋኘት ፣ 3.ገላን ለመታጠብ፣	

ጥያቄ 4፡ ፡በቢልሃርዚያ በሽታ ዙሪያ በኩመሩክ ወረዳ የሚካሂድ የዳሰሳ ጥናት (5 አመት እድሜ በላይ) ፣ የሽንት ናሙና የሰጡ ተሳታፊዎች ላይ የማህበረ-ህዝብ መረጃ እና ለቢልሃርዚያ ተጋላጭነት መንስኤዎች ላይ የተዋቀረ መጠይቅ

የጥያቄ ቁጥር	ጥያቄዎች	መልስ
1.	ጾታ	1) ወንድ
		2) ሴት
2.	እድሜ	_____ዓመት
3.	ብሄር	
4.	የሚከተሉት ሃይማኖት	1) ኦርቶዶክስ 2) ሙስሊም 3) ፕሮቴስታንት 4) ሌላ (ይግለጹ) _____
5.	የትምህርት ደረጃ	1) ማንበብ እና መጻፍ የማይችል 3) የመጀመሪያ ደረጃ 4) ሁለተኛ ደረጃ 5) ኮሌጅ ወይም ዩኒቨርሲቲ
6.	ሥራ	1) ገበሬ 2) ተማሪ 3) የቤት እመቤት 4) ነጋዴ 5) የቀን ሠራተኛ 6) የመንግስት/ግል ተቀጣሪ 7) ሌላ (ይግለጹ) _____
7.	የሚኖሩበት ቀበሌ	1) ዱልሺታሎ 2) ቤለሆ-ጄ 3) ኦገንዱ 4) ኩምሩክ
8.	የመጸዳጃ ቤት አሎዎት	1) አዎ
		2) የለኝም
9.	ለመጠጥ የሚጠቀሙት ዉሃ ከምን ነዉ	1) ፓምፕ(የጉድጋድ) ወይም የባንባ ውሃ
		2) ክፍት ውሃ(የወንዝ፣የኩሬ፣የግድብ ውሃ)
10.	በወንዝ ወይም በግድብ ወሃ ውስጥ ይዋኛሉ	1) አዎ
		2) አይ
11.	ልብስ በወንዝ ወይም በግድብ ወሃ ውስጥ ያጥባሉ	1) አዎ
		2) አይ

Annex 5: Paper I

Current Status of Urinary Schistosomiasis Among Communities in Kurmuk District, Western Ethiopia: Prevalence and Intensity of Infection

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Sage

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ABSTRACT

BACKGROUND: Schistosomiasis is a highly prevalent but neglected tropical disease, particularly in sub-Saharan Africa. In Ethiopia, urogenital schistosomiasis due to *Schistosoma haematobium* has been known to be endemic in several lowland areas. This study was designed to determine the current prevalence and intensity of the urogenital schistosomiasis among communities in Kurmuk District, western Ethiopia.

METHODS: Urine filtration technique and urine dipstick test were used to screen for *S. haematobium* eggs and hematuria, respectively. The data were analyzed with SPSS version 23. Logistic regression and odds ratio were used to measure associations and strength between prevalence, intensity, and independent variables. *P*-values <.05 at 95% CI were considered statistically significant.

RESULTS: The overall prevalence of *S. haematobium* infection as determined by urine filtration was 34.2% (138/403). In bivariate analysis, the most infected (45.4%) age groups were 5 to 12 years (odds ratio [OR] = 4.16, 95% CI: 1.36-12.67), followed by 13 to 20 years (OR = 3.23, 95% CI: 1.01-10.35) with higher significant mean egg count (MEC). The mean egg intensity ranged from 2.39 in Ogendu (CI: 1.05-3.72) to 14.1 in Dulshatalo (CI: 4.98-23.12) villages. The main predictor of infection was swimming habits (adjusted odds ratio [AOR] = 2.43 [CI: 1.19-4.94]). The prevalence of hematuria was 39.2% (158/403), the odds being 2.64 times higher among participants who resided in Dulshatalo than those who resided in Kurmuk (AOR 2.64 [95% CI: 1.43-4.87], *P* = .004).

CONCLUSION: To reduce the infection and interrupt transmission, the PC in place in the area using PZQ should be strengthened and continued, alongside with provision of sanitary facilities, safe alternative water supplies and health education. The Federal Ministry of Health of Ethiopia should also collaborate with the health authorities of the Sudan government for the control of trans-boundary transmission of the disease as the transmission foci are shared between the 2 countries.

KEYWORDS: Urinary schistosomiasis, prevalence, intensity, Kurmuk district, Ethiopia

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Introduction

Schistosomiasis is a highly prevalent and important neglected tropical disease in sub-Saharan Africa, the main burden of the disease being attributed to *Schistosoma mansoni* and *S. haematobium*.[1,2] *S. haematobium* is transmitted by freshwater snail species belonging to the genus *Bulinus*, which contains around 36 species within 4 species groups.[3]

Disease outcome in persons infected with *S. haematobium* varies dramatically, ranging from mild to severe damage of the kidneys and/or bladder.[4,5] The characteristic clinical presentation is terminal hematuria, usually associated with increased frequency of micturition and dysuria.[6] The pathology of urinary schistosomiasis is primarily caused by the eggs laid by female *S. haematobium* adult worm residing in the venous plexus of the bladder and other pelvic organs. On their way to exiting the body through the urinary stream, eggs transit through the bladder mucosal tissue *cause* substantial damage and initiate granulomatous inflammation that can progress over many years to complications including fibrosis and bladder cancer.[7-9]

Genital schistosomiasis is also common in areas endemic for urinary schistosomiasis in both females and males. Females are more prone to the disease and may result in serious complications including ectopic pregnancy and infertility[3,10] and this complication develops in 33% to 75% of infected females.[11] In addition, female genital schistosomiasis is suggested as a risk factor for HIV transmission.[10,12] There are also indications that cervical schistosomiasis lesions could become cofactors for HPV-cervical cancer.[7,13,14]

Urogenital schistosomiasis has been known to be endemic in several lower altitude areas of Ethiopia: in Afar, Gambella,

Annex 6: Paper II

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Assessment of Urogenital Schistosomiasis Knowledge, Attitudes and Practices Among Abobo Communities in Gambella Regional State, Southwestern Ethiopia

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ABSTRACT

BACKGROUND: Knowledge about community awareness and practices related to a disease, among other things, helps to plan relevant control strategies. This study assessed the knowledge, attitude, and practices (KAPs) of the community in Abobo district of Gambella Regional State in Southwestern Ethiopia about urogenital schistosomiasis (UGS), which is endemic to the region.

METHODS: A community-based cross-sectional study was conducted in 2022. A pretested structured questionnaire was administered. Multivariable logistic regression was used to examine factors associated with KAPs variables.

RESULTS: Most study participants (90.6%) responded they have previously heard of UGS. Over 95% of the participants knew at least 1 symptom of UGS and 30% knew the transmission cycle. About 15.9% and 26.8% knew keeping environmental hygiene and refraining from using cercariae infested water, as preventive methods, respectively. Over half of the participants (50.1%) disagreed or did not know that urinating close to the river/dam water contributes to transmission, and almost all participants had contact with the dam/river water in one way or another. Education status and history of having the infection were the key significant correlates of most KAPs variables.

CONCLUSION: Most of the community members had poor knowledge and attitudes about the mode of transmission and preventive measures of UGS. Thus, besides school-based mass drug administration (MDA), UGS control efforts in the region should incorporate health education in conjunction with safe water supplies, and provision of sanitary facilities to effectively reduce the transmission of the disease.

KEYWORDS: Knowledge, attitude, practice, urogenital schistosomiasis, Abobo, Gambella, Ethiopia

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Introduction

Schistosomiasis is one of the neglected tropical diseases (NTDs) caused by blood flukes of the genus *Schistosoma*. There are 6 main species of *Schistosoma* that can infect humans, namely *Schistosoma haematobium, S. mansoni, S. japonicum, S. mekongi, S. intercalatum, and S. guineensis*. Schistosomiasis is estimated to affect over 220 million people globally, with an annual mortality rate of approximately 11 792.[1] Sub-Saharan Africa bears the highest burden of the disease, with 90.6% of individuals requiring treatment.[2,3]

The World Health Organization (WHO) has targeted schistosomiasis for elimination as a public health problem by 2030.[4] To achieve this goal, the WHO strategies include preventive chemotherapy of at-risk groups, increasing access to safe drinking water, and improved sanitation, hygiene education, environmental improvement and intermediate host control.[2,3]

Schistosomiasis is one of the NTDs that the Ethiopian Federal Ministry of Health has designated as a priority for control and elimination.[5] According to a national survey of the country,[6] 265 districts are endemic for schistosomiasis, and 14 million school-age children need regular deworming through mass drug administration (MDA) campaigns. Intestinal schistosomiasis caused by *S. mansoni* is widely distributed in Ethiopia, including Addis Ababa,[6,7] whereas UGS caused by *S. haematobium* has been known to occur in lower areas below 800 m above sea level in Afar (Amibara district), Gambella (Abobo district), Somalia (Afder Gode zone) and Benishangul Gumuz (Kurmuk district) regions.[6-11] However, despite the high burden of UGS in Ethiopia, there is limited information on the community's KAPs regarding the disease and its prevention and control mechanisms.

MDA in Ethiopia was launched in 2015 to control helminths infection-associated morbidity by reducing moderate-to-heavy intensity infections. In addition, the NTD sector, which focuses on preventive treatment, and the Water Sanitation and Hygiene (WASH) sector have been established to break parasite transmission and reach elimination.[6,12]

Annex 7: Paper III

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Schistosoma haematobium infection and associated risk factors among pre-school age children in Gambella, Ethiopia

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S. Haematobium infection and risk factor
Gambella
Ethiopia

ABSTRACT

Background: *Schistosoma haematobium* can infect and cause morbidity in pre-school age children (PSAC) and due to its gradual nature, often goes unnoticed in its early stages and can result in long-term irreversible consequences during their most productive years. This study aimed to determine the prevalence, intensity, and associated risk factors of *S. haematobium* infection among PSAC in Gambella, Ethiopia.

Method: A cross-sectional study was conducted from July to August 2024 among 390 PSAC in four localities in Gambella, Ethiopia. Urine specimens were examined microscopically and with urinalysis reagent strips. Data on associated factors and socio-demographic characteristics were collected from the PSAC mothers/caregivers using a structured questionnaire.

Result: The overall prevalence of *S. haematobium* infection was 16.7 %, of which 20 % (13/65) had heavy-intensity of infection. The prevalence of macro and microhematuria were 7.9 %, and 26.2 %, respectively. The prevalence of infection was significantly higher among PSAC who visited open water sources (90.8 %, $P < 0.001$), bathed in open water (89.2 %, $P = 0.004$), and urinated in open water (89.2 %, $P = 0.004$), as well as among residents of Abaro and Tagni villages (72.3 %, $P < 0.001$) and who had mothers with no formal education (81.5 %, $P = 0.006$). The odds of infection were higher among children who played or bathed in infested water (AOR: 2.9, CI: 1.0–8.1) and those living in Abaro village (AOR: 4.3, CI: 1.6–11.9) compared to those who did not engage in these behaviors or lived in other villages, respectively.

Conclusion: The study showed that *S. haematobium* infection is prevalent among PSAC in Gambella region in Ethiopia, and contact with an open water while playing, bathing or urinating may increase the risk of acquiring infection with the parasite in this population group. These findings will contribute to a better understanding of the epidemiology of the disease among PSAC.

Annex: Photos

Photos from Benishangul Gumuz sites

River between Sudan and kurmuk boarder

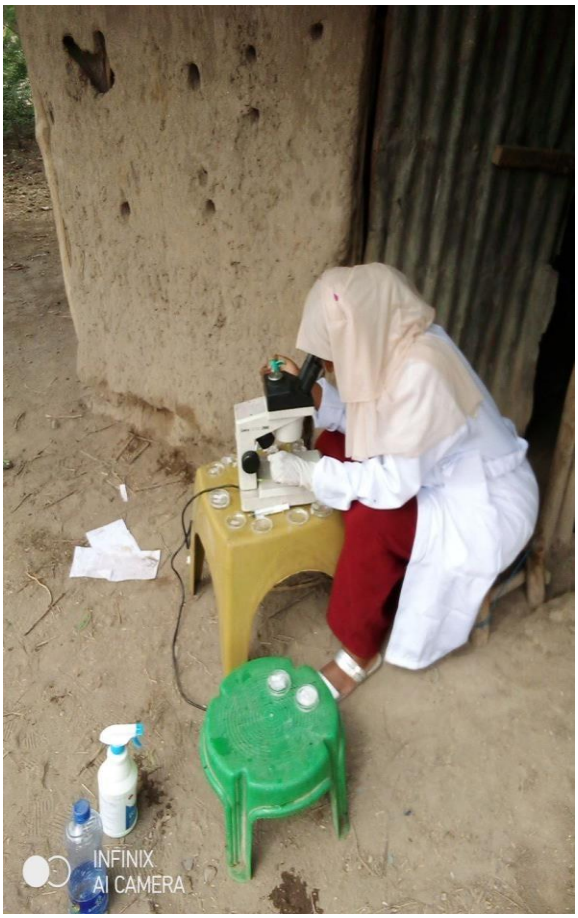
Photo from Gambella sites


INFINIX
AI CAMERA


AI CAMERA


INFINIX
AI CAMERA

Photo from Afar sites



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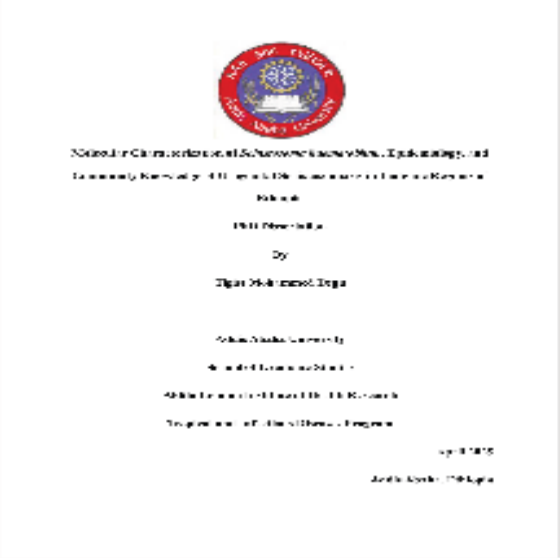

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