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SCHOOL OF MEDICAL LABORATORY SCIENCES

Epidemiology of *Schistosoma mansoni*, Snail Intermediate Host Fauna and Physico Chemical Characterizations of Snail Breeding Habitats in Mizan Aman, Bench Sheko Zone, Southwest, Ethiopia.

By: Gashaw Temesgen

Thesis paper submitted to the Institute of Health, Faculty of Health Sciences, School of Medical Laboratory Sciences, Jimma University, in partial fulfilment for requirement for Degree of Masters in Medical Parasitology.

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Jimma, Ethiopia

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Declaration of Principal Investigator

I, the undersigned declare that this thesis is my original work, has not been presented for degree in this or other Universities and that all sources of materials used for this have been fully acknowledged.

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Abstract

Background: *Schistosomiasis is snail borne parasitic neglected tropical disease which causes major public health problem and poses a negative economic development in Africa. In Ethiopia numerous studies reported that prevalence of Schistosoma mansoni (S. mansoni) infection was high and the distributions, type of snail intermediate host responsible for transmission and hot spot areas are not well documented. The purpose of this study was to determine the prevalence of S. mansoni infection, determination and distribution of snail intermediate host fauna, hot spot transmission area and physico chemical characterization of snail breeding habitats in Mizan Aman, Bench Sheko Zone, South west Ethiopia.*

Method: *A community based cross sectional study was conducted on 364 household's selected using systematic sampling technique from October 2021 - February, 2022. Stool sample collected from each study participant and examined by Kato-katz technique within 24hours. Snails were collected by scoop net, gloved hand and transported by plastic container containing old water and vegetation to Mizan Aman public health regional laboratory for morphological identification and examination of cercariae shedding and snail infection. Water physico-chemical parameters were characterized on site and in the laboratory, from each snail sampling sites. Data were entered into EPI Data version 3.1 and analysis was carried out by SPSS version 20. Descriptive analysis was conducted and for risk factors and physico chemical parameters, P-value <0.05 were reported as statically significant.*

Results: *The overall prevalence of S. mansoni infection was 23.2% (95% CI: 18.9-28), with 80.3, 16% and 3.7% of light, moderate and heavy infection intensity, respectively. Using river water for domestic use, swimming, bathing habit in river and presence of farm close to river were risk factors. A total of 274 snails were collected, of which Biomphalaria pfeifferi, Biomphalaria sudanica, and Lymnaea natalensis accounted for 187(68.2%), 29(10.6%), and 58(21.2%), respectively. The pooled prevalence of cercariae infection was 34(12.4%). Turbidity and conductivity had significant association with snail abundance.*

Conclusion: *Schistosoma mansoni infection is an ongoing public health problem in Mizan Aman, Bench Sheko Zone, South west Ethiopia. Biomphalaria pfeifferi snail species are the major intermediate host of schistosome responsible for transmission to human. All snail sampling sites except Shonga-1 was infested with one or more types of cercariae. Therefore, integrated control strategy should be followed to attain morbidity control.*

Key: *Schistosomiasis, B. pfeifferi, B. sudanica, L. natalensis, S. mansoni, Trematodes, Cercaria, Ethiopia*

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Abbreviations and Acronyms

AOR……Adjusted odds ratio

COR……....Crud odds ratio

DALY…….Disability adjusted life year

EPG…….Egg per gram

FMOH…….Federal minister of health

GMEC…….Geometric mean egg count

GPS ……Geographical positioning system

IRB ……....Institutional Review Board

NTDs……. ….Neglected tropical diseases

NTU…… Nephelometric turbidity unit

MDA…….....Mass drug administration

SAC…….....School age children

SCH……..Schistosomiasis

SNNPR……....South nation nationality people representative

SWEPR……Southwest Ethiopia people region

SSA……..Sub-Saharan Africa

STH……..Soil transmitted helminthes

PPF……...Periportal fibrosis

WASH……. Water sanitation hygiene

WHO…….. World health organization

1. Introduction

1.1. Background

Schistosomiasis is snail borne neglected tropical disease caused by blood flukes of trematodes parasite of genus *Schistosoma*. Human acquire infection when people come in contact with fresh water infested with freely swimming larval stage of parasite infective to human called cercariae (1). Six known trematode parasite species causing different types of schistosomiasis are *Schistosoma mansoni, Schistosoma intercalatum, and Schistosoma japonicum* are causing (intestinal schistosomiasis), *Schistosoma haematobium* (urogenital schistosomiasis) and *Schistosoma mekongi and Schistosoma guineensis* (Zoonotic schistosomiasis). Globally, schistosomiasis transmission has been reported from 78 countries, and geographical distribution of the disease as a public health problem is mainly noted in tropical and subtropical regions of Africa, Asia, the Caribbean and South America. The main species that cause schistosomiasis to humans in Africa are *S. mansoni* (intestinal schistosomiasis) and *S. haematobium* (urogenital schistosomiasis). Schistosomiasis is a major public health problem and poses negative economic problem especially in developing countries (2,3). About 90% of the disease burden is in Sub-Saharan Africa (SSA) countries with limited access to clean water and sanitary facilities, causing acute or chronic illness, which is leading to serious socio-economic consequences, next to malaria. Ethiopia is SSA country that, intestinal schistosomasis caused by *S. mansoni* are widely distributed in many regions of the country (4). In endemic areas where schistosomiasis is prevalent, infection in children is associated with growth stunting, intellectual disability and in adults with a reduced ability to work and in addition to these significant morbidity and mortality have far-reaching complications in multiple human organ systems, including irreversible pulmonary hypertension, renal, genitourinary, central nervous system conditions, and neoplasia (5,6).

Transmission of the disease depends on the actual freshwater snails that belong to genera *Biomphalaria* (*S. mansoni*), *Oncomelania (S. japonicum*), *Bulinus* (*S. haematobium, S. intercalatum, S. guineensis*), *Neotricula aperta (S. mekongi) and Lymnea species (Faciolla)* which are medically important snail intermediate host, commonly transmit trematodes parasitic disease to human and animals. In Ethiopia, snail species that belong to genus *Biomphalaria, Biomphalaria pfeifferi (B. pfeifferi),*

Biomphalaria sudanica (B. sudanica) and *Bulinus*, *Bulinus abyssinicus (B. abyssinicus), Bulinus africanus (B. africanus), Lymnaea*, *Lymnaea natalensis (L. natalensis) and Lymnaea truncatula (L. truncatula)* are widely distributed in different part of country, and serve as intermediate hosts for the transmission of *Schistosomes* and other trematodes to human and animals. These snails uses different water bodies as breeding habitats including larger lakes, small lakes, rivers, streams, swamps, and temporal ponds. Human acquire infection when come in contact with water infested with cercariae, during domestic, occupational, and recreational activities. Cercariae are the freely swimming trematodes infectious larval stage recovered from intermediate host which can live for up to 48 hours in freshwater and directly piercing the skin of humans, transform in to schistosomula stage that migrate through lung to liver gradually develop into adults and oviposit in the mesenteric vessels of humans. Some of the ova produced remain trapped in liver causing tissue granuloma which leads chronic infection, whereas others join into the intestine, promoted by the host immune complex formed granuloma. Ova release with faeces from infected person which will contaminate the water environment and will hatch into first larval stage of parasite freely swimming for up to 12 hours called miracidum, which is able to penetrate, and infect its specific snail intermediate host, and then parasite undergoes asexual multiplication, and development to primary and secondary sporocysts then in to cercariae the stage infective to humans(7–9).

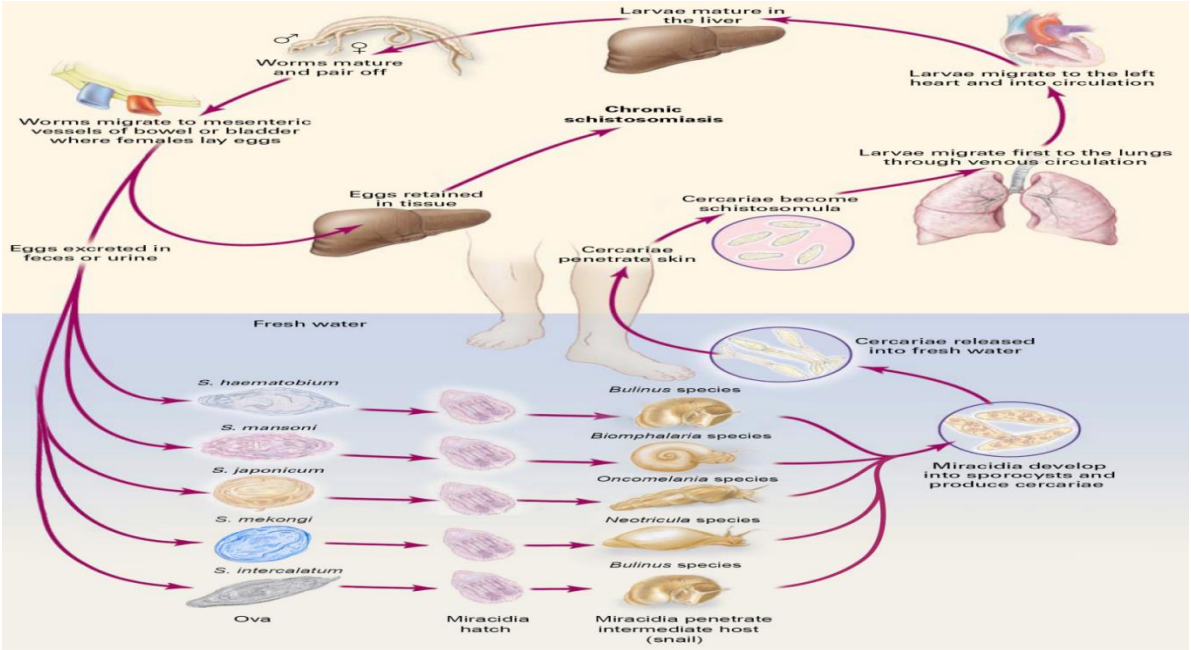

Figure 1: Life cycle of *Schistosomiasis* (Allen *et al.*, 2002)

The distribution of disease is influenced by the environment, water project schemes, and other people's migration (10). Increasing population and movement of people from endemic to none endemic areas where intermediate host snail is present, have contributed to increased transmission and introduction of schistosomiasis to new areas (11). The abundance, occurrence, and infection rates of intermediate host snails were largely influenced by environmental factors including, water physico-chemical parameters, sanitation, and water contact behaviour of the inhabitants (12).

World health organization (WHO) launched mass drug administration (MDA) of praziquantel as a preventive chemotherapy and as a cornerstone policy for the control of schistosomiasis in endemic regions of the world, by setting a goal of morbidity reduction and elimination target by 2020 and 2025, respectively. Ethiopian Federal Minister of Health (FMoH) implemented praziquantel MDA, in 2015 as a national control program, treated over 19 million individuals in endemic areas, and designed to achieve the goal (13). *S. mansoni* infection is still becoming a public health problem since the risk of reinfection and recurrent disease is common, even in areas with high treatment coverage(14). Currently, WHO published new road map to guide action against Neglected tropical disease (NTDs) during 2021-2030, target to elimination of schistosomiasis as public health problem and transmission interruption in humans in the selected countries by 2030. Recommended strategies were integrated control strategies, co-implementation of increased geographical coverage of praziquantel MDA to all at risk groups, improved water sanitation hygiene (WASH), and snail control like molluscicide use and environmental modification expected to hasten achievement of the new 2021-2030 goals for morbidity control and elimination of the disease as a public health problem (15).

1.2. Statement of the problems

Schistosomiasis is a parasitic disease that affects both individuals and the communities in endemic areas. It is the main cause of morbidity and mortality in endemic countries, with major consequences on public health and the economy, especially in SSA (16). According to WHO 2019 report, 779 million peoples are at risk of acquiring infection, 51 countries require preventive chemotherapy total number of 236.6 million people, from which 105.4 million people were received preventive chemotherapy worldwide with total treatment coverage of 44.54% (67.21% in school age children (SAC) and 17.67% adults) and causes about 1.9 million disability-adjusted life years (DALYs) annually (17,18).

In Africa, schistosomiasis morbidity remains a major public health problem, 41 countries required preventive chemotherapy. *S. mansoni* alone affects over 54 million people, 400 million leaving at risk areas and about 20 million people are currently suffering from complications of chronic *S. mansoni* infection and up to 42% of those infected have been found with periportal fibrosis (PPF). About 0.2 million deaths are attributed to chronic *S. mansoni* every year, which is mainly due to varices, death occurs in up to 29% of those who present late with bleeding varices (19). Schistosomiasis is an important public health problem in developing countries, SAC are the most affected and suffers from anaemia, stunting, and low school performance (20). Schistosomiasis is not only public health problems of endemic countries, it is also issue of non-endemic areas due to importation through tourists and migrants from endemic countries (21). In Ethiopia schistosomiasis are major public health problems, population living at risk estimated to be 53.3 million in 480 woredas. Ethiopian, FMoH has launched a national praziquantel MDA as control program in mapped areas since 2015, planned to eliminate by 2020, but still it remains one of a public health problem in many regions of the country (4,22).

Schistosomiasis caused by *S. mansoni* and *S. haematobium* are common in Africa, especially in freshwater environments containing specific snail intermediate host for parasite where there is more direct human contact with cercariae infested freshwater. *Biomphalaria* and *Bulinus* are snail intermediate hosts for *S. mansoni* and *S. haematobium,* respectively. *B. pfeifferi* and *B. sudanica* are the common snail species that are responsible for the transmission of *S. mansoni* in Africa including Ethiopia (23).

The construction of water schemes like dam and irrigation, for hydroelectric power and agricultural requirements development favour transmission, especially of *S. mansoni* (24).

Praziquantel MDA is the strategies implemented to control schistosomiasis in endemic regions of Africa. Treatment targeted mainly to SAC, without the consideration of the rest of community that are equally being infected, and may serve as a reservoir host causes for transmission and reinfection (25). MDA schedule as control and elimination strategies of *S. mansoni* shows progressive morbidity reduction, but not fully successful, even in communities started at the low prevalence of infection. Currently, MDA program target for the elimination of schistosomiasis transmission needs to follow integrated control strategies, because MDA will not prevent reinfection. Co-implementation of MDA, with snail control, improvement in WASH and community behavioural change was recommended to achieve control and elimination program (26).

In Ethiopia, studies conducted about schistosomiasis were focused on SAC. However, limited studies were conducted on community and identification of snail intermediate host, hot spot area responsible for transmission. This study aimed to determine the prevalence of *S. mansoni* infection, distrubition and identification of snail intermediate host fauna, hot spot transmission area and physico-chemical parameters contributing snail abundance and infection in Mizan Aman, Bench Sheko Zone, southwest, Ethiopia.

2. Literature Review

2.1. Schistosomiasis

Schistosomiasis is snail born parasitic NTDs caused by blood flukes of genus *Schistosoma*. The disease, also known as "bilharzia," is prevalent in 78 countries in Africa, South America and Asia. Globally, an estimated 230-240 million people are infected with schistosomiasis. The most common forms of disease in Africa are *S. mansoni* which cause intestinal schistosomiasis, and *S. haematobium* causing urinary schistosomiasis. Freshwater snails, act as intermediate host, after being infected by schistosome miracidiae. The infected snails produce other larvae called "cercariae," which infect humans by penetrating the skin during water contact. Intermediate hosts of Schistosomes in Africa are freshwater pulmonate snails that belong to the *Planorbidae* family, species belong to the two genera, *Biomphalaria* host for *S. mansoni*, and *Bulinus* for *S. haematobium* (27).

2.2. Prevalence of *Schistosoma mansoni* infection and associated risk factors

Ninety percent of disease burden are in Africa, and the prevalence of infection ranges from less than 1% and as high up to 90% depending on different reasons. Studies conducted in northeast coast and Sergipe of Brazil, to determine prevalence and risk factors of *S. mansoni* and other intestinal parasitic infection, reported the prevalence of *S. mansoni* as 16.4%, and 24%, respectively, and being male gender, age 10-30, rice farmer, water contact behavior were identified as risk factors for *S. mansoni* infection (28,29). Similar studies conducted in two different area of Marolambo and sentinel sites on children in Madagascar, to determine the prevalence of *S. mansoni* infection reported 73.6% and 5 %, respectively (30,31).

Geographical distributions and prevalence of schistosome infection varies based on favourability of the ecology to intermediate host and risk factors for disease transmission. Studies conducted in Khartoum, Sudan, Uganda, west Kenya, Mbita and islands of Lake Victoria of west Kenya to determine the prevalence and associated risk factors, results showed that prevalence of *S. mansoni* infection was 2.95%, 25.6%, 11.8%, 60.5% and 82%, respectively. And swimming, bathing, and playing in freshwater bodies were reported as associated risk factors of *S. mansoni* infection (32–35). Another similar study conducted in community of age 1–95 years in Tanzania,

and Malawi the prevalence of *S. mansoni* infection was 68.9% and 9.5%, respectively, and those studies reported that *S. mansoni* infection transmission is influenced by season, swimming, and living near to the rivers (36,37).

In Ethiopia, national wide survey of STHs and schistosomiasis carried out on primary school children from 2013-2015, indicated the prevalence of schistosomiasis was 4.0%, and *S. mansoni* is the prevalent, widely distributed than *S. haematobium* (3.5 *vs* 0.3%). Studies of schistosomiasis conducted in northern part of Ethiopia, at different time in Sanja, Bahirdar, Tigray, Sanja town, and Gorgora, northwest Ethiopia, to determine the prevalence of *S. mansoni* infection and risk factors, reported prevalence as 16.67%, 24.9%, 5.95%, 89.9% and 20.6%, respectively and swimming habits, frequency of swimming, washing clothes, and bathing in rivers were identified as associated risk factors of infection(38–42). Whereas studies conducted in southern part of Ethiopia, Wolaita, Gamo Gofa, Yachi, Jimma and Bench Maji, southwest Ethiopia, aimed to determine the prevalence and risk factors of *S. mansoni* infection, prevalence of *S. mansoni* infection was 81.3%, 14%, 42.9%, 27.6%, 9.3% and 44.8%, respectively and poor knowledge, not wearing shoes were significantly associated factors with *S. mansoni* infection (43–47).

2.3. Snail intermediate host

Several freshwater snails are serving as intermediate host of different species of *Schistosomes* replication and development to an infective free-swimming larval stage (cercariae) infecting human and animals. Human acquire infection when come in contact with cercariae infested freshwater through skin penetration. Freshwater *pulmonate* snails that belong to the *Planorbidae* family are the common intermediate hosts of *Schistosomes* in Africa and species belong to the genera of *Biomphalaria* and *Bulinus*, host for *S. mansoni* and *S. haematobium* respectively. *B. pfeifferi* were the dominant and widely distributed snail intermediate host in Africa. Malacological survey conducted in Kisumu City, western Kenya, reported out of 1,059 snails collected, 425(40.1%) were putatively identified as *B. pfeifferi*, 407(38.4%) as *B. sudanica* and 227(21.5%) as *Bu. globosus*, which is similar with survey from Mara River in Kenya, and Tanzania, where from all snail species identified, *B. pfeifferi* accounting for more than half (61.1%) of the snails, followed by *Bu. africanus* (22.2%) and *Lymnaea species* (16.7%) (45, 46).

B. pfeifferi species were the highly abundant snails intermediate host of schistosome, Malacological survey conducted in Omo Gibe River Basin, southwest Ethiopia, and Sanja, Northwest Ethiopia, which reported distribution of snails as *B. pfeifferi* 66.8%, *B. Sudanica* 0.2%, *Bu. globosus* 4.8%, *B. forskalii* 4.3%, *L. natalensis* 24%, and in Sanja, Northwest Ethiopia, *B. pfeifferi* were the leading snail intermediate host, during January and April, which accounts about 97.4% and 81.4%, respectively(12,48–50). However, in some studies conducted in Kpong Head Pond, Ghana and Niger River Valley West Africa , *Bu. truncates* was the dominant whereas *B. pfeifferi* the second leading snail intermediate host schistosome (51,52).

Prevalence of cercariae infection in snail intermediate host can be determined by cercariae shedding, crushing, or molecular identification. According to the survey conducted in Gombe ecosystem of western Tanzania, the overall prevalence of cercariae infection by cercariae shedding method was 12%, and in western Kenya, the pooled prevalence cercariae infection by cercariae shedding method was 26.5%, in Kisumu City, western Kenya, and these cercariae infections in *B. pfeifferi* and *B. sudanica* was 1.6% and 1.7%, respectively. Similarly in Omo Gibe River Basin, southwest Ethiopia, fresh water snail infection prevalence was 4.6%. Another similar study conducted to determine snail cercariae infection rate in Uganda, along Lake Albert and Lake Victoria, revealed the infection rate of *Biomphalaria* snail in Lake Albert as 8.9% of which 15.8% was *S. mansoni* and 84.2% non-human trematode and in Lake Victoria, infection rate was 2.1%, of which 13.9% were infected with *S. mansoni* cercariae, 85.7% non-human trematode cercariae and 0.4% multiple trematodes. *B. pfeifferi* and *B. stanly* snail species are the most infected by both human and non-human cercariae trematode (12,48,53–55).

Systematic review of *S. mansoni* and *S. haematobium* infection of snail intermediate host in Africa, showed prevalence of snail's cercariae infection as 5.5% (5.2% *S. mansoni* and 5.6% *S. haematobium*) (23). Malacological survey conducted in Gorgora, Northwest Ethiopia, schistosome infection in *Biomphalaria* species, as 0.0% human schistosome cercariae and 1.6% shed bird's schistosome cercariae (42), whereas in Sanja and Ewke Amba, Northern Ethiopia, in February and April schistosome infection in *Biomphalaria pfeifferi* as 16.9% and 0.027%, respectively. The study concluded that *Biomphalaria* snail are known intermediate host for *S. mansoni* and its distribution, infectivity rate vary from season to season(50). Another similar study

conducted in community living near rivers of Jimma town, and Tikur Wuha, Southern Ethiopia, revealed cercariae infection of *Biomphalaria* snails intermediate host by cercariae shedding method was 58.0% and 1.3% respectively (56,57).

2.4. Environmental and physico-chemical factors

Freshwater snail intermediate hosts of trematodes have adapted to a wide range of environmental conditions. They are found almost in all types of freshwater bodies ranging from small temporary ponds and streams to large lakes and rivers. Mostly occur in shallow water near the shores of slightly flowing water bodies with moderate organic content, moderate light penetration, little turbidity, a muddy substratum rich in organic matter, submergent or emergent aquatic vegetation, and abundant micro-flora. Snail breeding habitats mainly environmental and physico-chemical quality parameters have its own effect on abundance and trematodes infection of snail's intermediate hosts. According to Malacological survey of schistosomiasis snail intermediate host conducted in western Kenya, to assess the effect of environmental and physico-chemical quality parameters of breeding habitats on distribution and abundance of snail intermediate host, result revealed that the presence of aquatic plant and water temperature have positive significance association with snail abundance and another similar study conducted in western Kenya, also revealed that dissolved oxygen, pH and turbidity were predictive of *Biomphalaria* snail species, whereas pH and turbidity were predictive for *Bulinus* snail species, water turbidity shows negative predictive association with the distribution of *Biomphalaria* and *Bulinus* species abundance (48,58). Malacological survey to identify transmission sites for intestinal schistosomiasis on north-western Tanzania, physico-chemical quality parameter of snail breeding habitats, revealed that water temperature, pH, conductivity and dissolved oxygen doesn't shows correlation with snail abundance but conductivity shows positive significance with the number of trematodes infected *Biomphalaria* species (59). In Ethiopia, study conducted to determine factors affecting the occurrence of freshwater snail species reported that water depth, turbidity, emergent macrophyte cover and human settlement as important factors determining the occurrence of *B. pfeifferi,* whereas water temperature, concentration of dissolved oxygen, submerged macrophyte cover and human settlements as determining factors for occurrence of *B. sudanica* (60).

2.4. Conceptual Frame Work

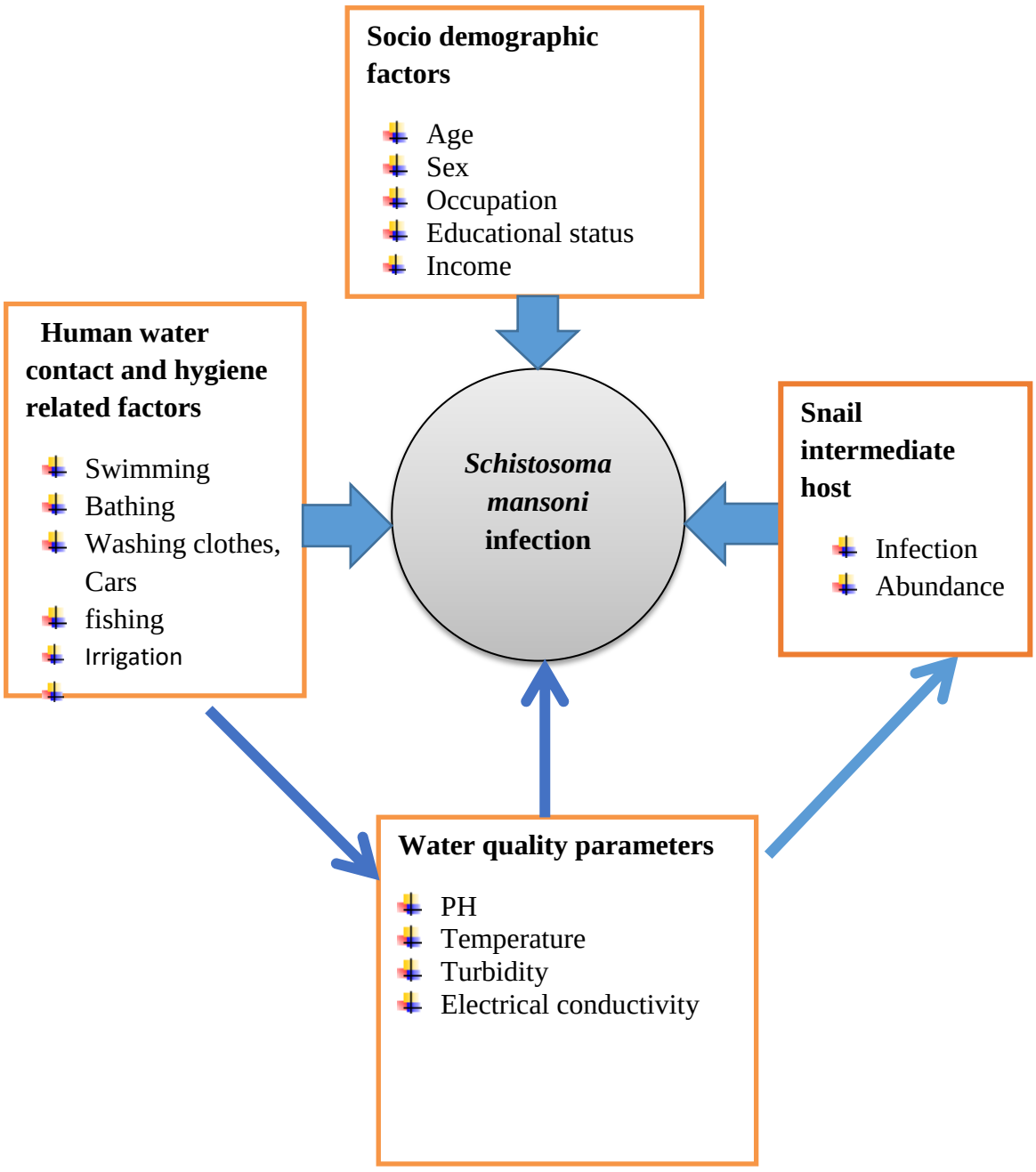

Figure 2: Conceptual frame work of the study

2.5. Significance the study

Human schistosomiasis is a major public health problem in the communities with poor personal and environmental sanitation. Preventive and control measures towards schistosomiasis infections launched in local and national levels, but many peoples are still suffering from the disease in resources limited countries. Mizan Aman is one of *S. mansoni* infection mapped endemic area in Ethiopia, MDA started in 2015/2016 on SAC. However, still peoples are living with infection and the status of community is not fully elucidated and disease is not completely mapped. Findings from this study can provide information about the prevalence of *S. mansoni*, and associated risk factors, identify snail intermediate host fauna, active hot spot transmission area, and water physico-chemical parameters contributing for intermediate host snail abundance and infection. Which is helpful to the concerned governmental, non-governmental bodies and other stakeholders to guide intervention measures and design efficient preventive and control strategies. Furthermore, the findings can also serve as reference baseline data for further research to be conducted in the area.

3. Objectives

3.1. General Objective

To determine the prevalence of *Schistosoma mansoni* infection, Snail intermediate host fauna and physico-chemical characterizations of snail breeding habitats in Mizan Aman, Bench Sheko Zone, Southwest, Ethiopia.

3.2. Specific Objectives

- To determine the prevalence of *Schistosoma mansoni* infection
- To assess associated risk factors for *Schistosoma mansoni* infection
- To identify snail intermediate host fauna
- To determine snail infection rate
- To identify hot spot transmission areas
- To asses associated water physico-chemical parameters for snail abundance

4. Methods and Materials

4.1. Study area and Period

The study was conducted in Mizan Aman, Bench Sheko Zone, Southwest, Ethiopia, from October, 2021 - February, 2022, to determine the prevalence of *S. mansoni* infection, snail intermediate host fauna and physico-chemical characterizations of snail breeding habitats. Mizan-Aman is a Town located in Bench Sheko Zone, Southwest Ethiopia Region (SWEPR) state, Southern part of Ethiopia, which is located at a distance of 561 Km southwest capital city of Ethiopia Addis Ababa and 230Km from Jimma Town. There are five *kebeles* in Mizan Aman city administrative namely; Adis ketema, Hibret, Kometa, Ediget and Shasheka, and the town is found with tropical climatic conditions with elevation of 1451m to 1753m from sea level and its astronomical location is 6^{o} 48'N and 35^{o} 26'E. According to Mizan Aman Town plan commission socio demographic and economic data estimated the total population of Mizan Aman Town to be 123,005 of which 63,963 of them are males and the rest 59,042 are females. Kometa and Shasheka are the two *kebeles* from five *kebeles* found in Mizan Aman, which are crossed with rivers suspected as source of infection, namely Shonga and Agu River, respectively. Total populations of Kometa *kebele* is about 7,903 and with 1,665 households, while Shasheka kebele has 11,118 total populations and 2,334 households (61).

Shonga River which crosses kometa *kebele* drains in the center of Mizan-Teppi University, Mizan campus while Agu is small river crossing shasheka *kebele*. Three snail collection sites along the Shonga River and two from Agu River were purposively selected based on the observation of the direct human water contact behaviours for different routine activities like washing clothes, bathing, fetching, crossing, sanding and swimming purposes.

The five river shoreline sites were named as Shonga (Shonga main road (Shonga-1), Shonga nuhamin café (Shonga-2), Shonga desta mender (Shonga-3) and Agu primary school (Agu-1), Agu lideta church (Agu-2). All snail sampling sites were delineated and located by using GARMIN 72 Geographical positioning system (GPS).

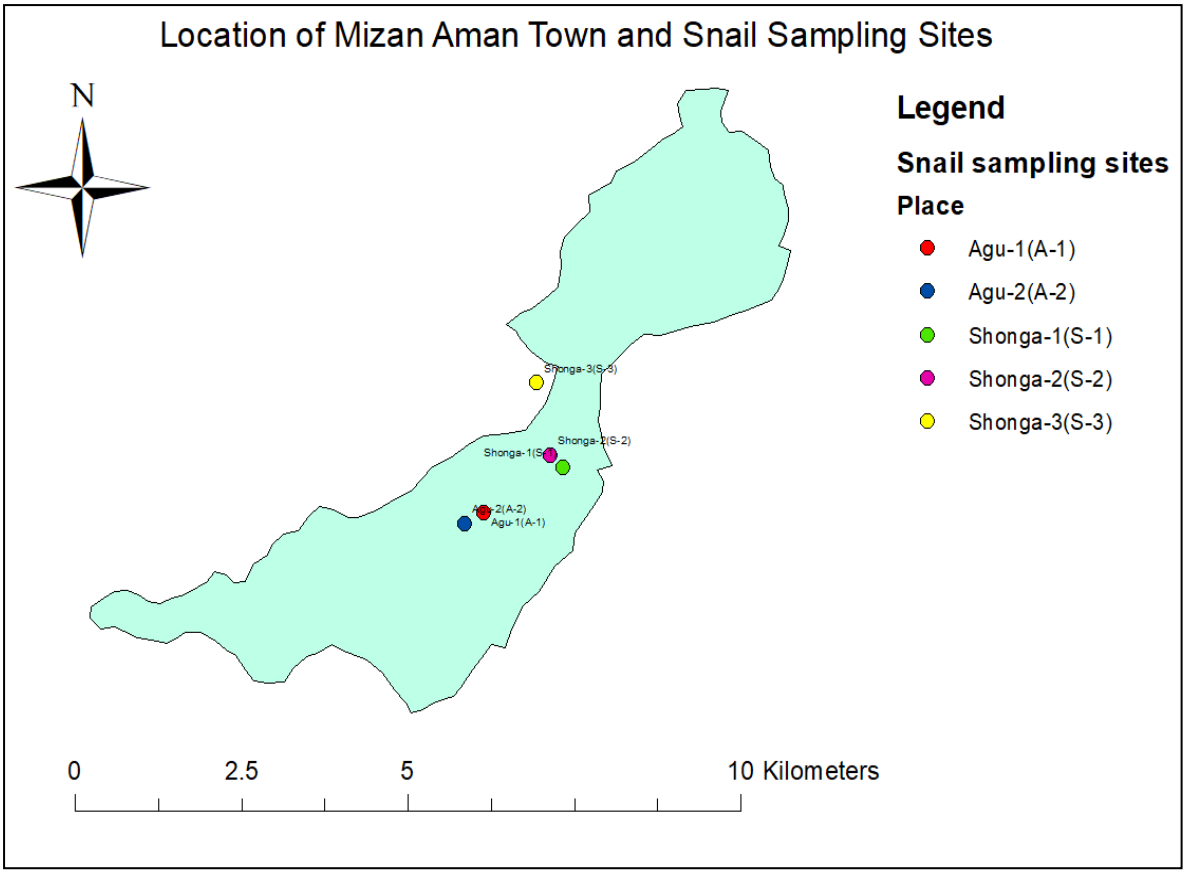

Figure 3: Map of study area

Where, Shonga-1(S-1) is found at geographical elevation of 1359m above sea level, located near Mizan-Aman highway or before entering Mizan main campus of Mizan-Teppi University. This is a site where a car wash discharges its wastes that include grease, car fuel in to the river, sanding and most people lives around and students were bathing and washing clothes. Shonga-2(S-2) is located at 1348m above sea level, in the middle of Mizan main campus, Mizan-Teppi University. Wastes from university directly discharged on both side of river bank and wastes from student cafeteria were very commonly disposed around the river bank, those who work in the cafeteria were bathing and washing their clothes. Shonga-3(S-3) is found at 1326m above sea level in the back of a lounge, near to Desta mender/village in which people around the village uses the river for bathing and washing clothes.

Agu-1(A-1) is found at 1355m above sea level, which is located in the main gateway of Aman Primary School, in which students and the community around the river commonly use it for bathing, washing clothes and drinking their cattle. Agu-2(A-2) is located the elevation of 1372m above sea level, near to Aman Lideta Church in which

peoples come from the rural kebele to the town and in around the village was washing their hands, legs, clothes and motor bicycles (**Plate 1**).

Plate 1: Snail collection sites (Shonga Desta mender (A), Agu Aman Primary School (B).

4.2. Study Design

Cross sectional study design was employed for parasitological and malacological study.

4.3. Population

4.3.1. Source population

All residents of Mizan Aman for parasitological and all river shoreline sites with high human activities were selected for malacological study.

4.3.2. Study Population

Households in selected *kebeles* of Mizan Aman Town for parasitological and snail sampling sites in selected rivers for malacological study.

4.3.3. Study unit

Individuals in selected households and snails collected from each sampling sites

4.4. Eligibility criteria

4.4.1. Inclusion criteria

All residents in selected *kebeles* who consented to participate in the study were included for parasitological study and rivers shorelines which had high direct human water contacts in selected rivers were included for malacological study.

4.4.2. Exclusion Criteria

Individual who lived less than six months in the study area, age less than one year, who did not give consent to participate and those who were on Anti-helminthic drug during or four weeks prior to the study were exclude for parasitological and rivers shorelines which have no high human water contact were excluded for malacological study

4.5. Sample size and sampling technique

4.5.1. Sample Size

Sample size was determined by using single population proportion formula with the following assumptions:

Prevalence (p) of *S. mansoni* was 9.3% from previously conducted community based study in the area, prevalence and intensity of STH and *S. mansoni* infection in Zemika *Kebele*, Bench Maji Zone, Southwest, Ethiopia (47).

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

Where n= sample size, Zα/2 = statistic for a level of confidence (z =1.96 at 95% CI), p = expected prevalence or proportion (p =0.093, d = margin of error (if 3%, d= 0.03). n=sample size =**360.** Since, study population is 3,999 households, which is less than 10,000, it appears necessary to use correction formula n/1+n/N

Where, n=360

N= Total number of households in selected *kebeles*

The resulting sample size =331

After considering 10% none - response rate contingency, the final sample size was =**364**

4.5.2. Sampling technique

Purposive sampling technique was employed to select *kebeles*, Kometa and Shasheka *kebeles* were selected, which are in close proximity to rivers suspected as sources of infection in the area. Then systematic sampling technique was followed to select the households from the selected *kebeles*, and a proportional allocation technique was followed between the two selected *kebeles* to reach the sample size. Accordingly, **152** households from kometa and **212** households from shasheka *kebele* were selected. The first household was selected through lottery method and the next household was selected by the interval of **11** up to the final sample size reach, sampling interval (K) equals to total number of household (N)/sample size (n) = 3999/364=10.98 ~ **11**. One eligible individual was selected randomly from selected household, if there is no eligible individual in the selected household, household next to the selected was selected as a replacement.

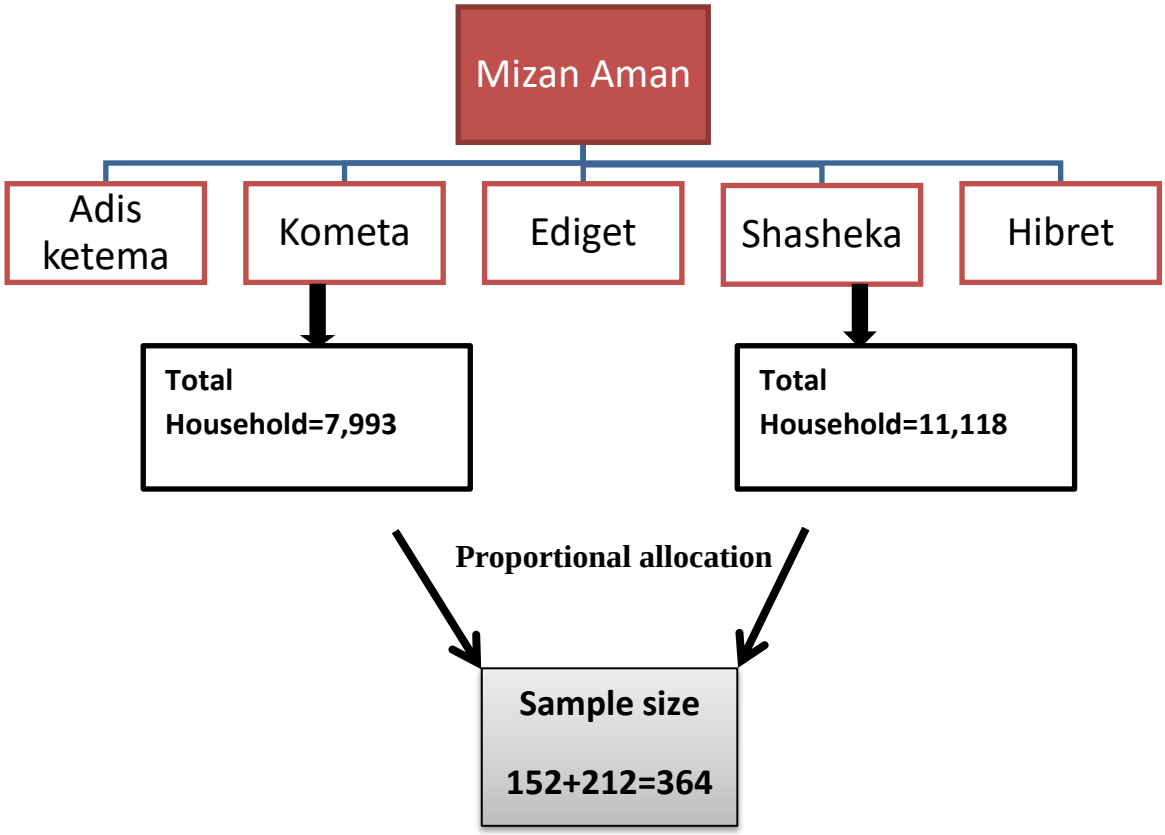

Figure 4: Schematic flow of the study

4.5.3. Snail intermediate host sampling

Snails were collected from Shonga and Agu River crossing the selected *kebeles*. Sites along the rivers shorelines which had high direct human water contacts for daily routine activities like collecting water, washing clothes, cars, bathing, and swimming, playing and agricultural purpose such as fishing, irrigation were selected for snail collection. Five snail sampling sites were selected from the two selected rivers, three from Shonga named and coded as (Shonga-1 (S-1), Shonga-2(S-2), Shonga-3(S-3) and two from Agu, coded as Agu-1(A-1), and Agu-2(A-2). Snails were collected using metallic handed 2mm mesh size scoop net and gloved hand, from all probable sites, including on the leaves and surface of leave debris, under rock and from the vegetation for 30 minute in each selected snail collection sites (12) .

4.6. Variables

4.6.1. Dependent variable

S. mansoni infection

4.6.2. Independent variable

Socio-demographic variables:

Age

Sex

Average monthly income

Educational level

Occupation

Family size

Water source and hygiene related factor

Source of water for drinking

Source of water for domestic use

Human water contact habits

Snail intermediate host related factor

Intermediate snail host abundance

Rate of intermediate snail host infection

Physico chemical characteristics of snail breeding habitats

4.7. Data collection

4.7.1. Socio-demographic and risk factors data collection

Semi-structured questionnaire was prepared in Amharic and then translated to English and again translated back to Amharic. Questionnaire that addressed socio-demographic information of inhabitants and risk factors of *S. mansoni* was used, data were collected using through interview administered questionnaire after taking written consent of participation.

4.7.2. Stool sample collection

Stool specimens (about 2 grams) were collected from each study participants, with unique identification code labelled dry plastic leak proof stool cup and transported with ice bag to Mizan Aman public health regional laboratory. Then it was processed, and examined by experienced laboratory technologists within 24 hours using Kato-Katz technique for parasite detection and quantification following standard protocol. Intensity of eggs was determined and the number of eggs counted multiplied by 24 to determine the eggs per gram (epg) of stool and their intensity level classified according to WHO *S. mansoni* infection intensity classification, as Light (1-99 epg), Moderate (100-399 epg) and Heavy infection(>400 epg) (62).

Kato Katz procedure first label a glass slide with the sample identification number and then place a plastic template on top of it. Add a small amount of the stool sample on a newspaper and press a piece of mesh on top and using a spatula, scrape the sieved faecal material through the screen so that only the debris remains. Scrape up some of the sieved stool to fill the hole in the template, avoiding air bubbles and levelling the stool off to remove any excess and carefully lift off the template and place it in a bucket of water mixed with concentrated detergent so that it can be reused. Place one piece of the cellophane, which has been soaked overnight in methylene blue glycerol solution, over the stool sample then place a clean slide over the top and press it evenly downwards to spread the stool in a circle (62) (**Plate 2**).

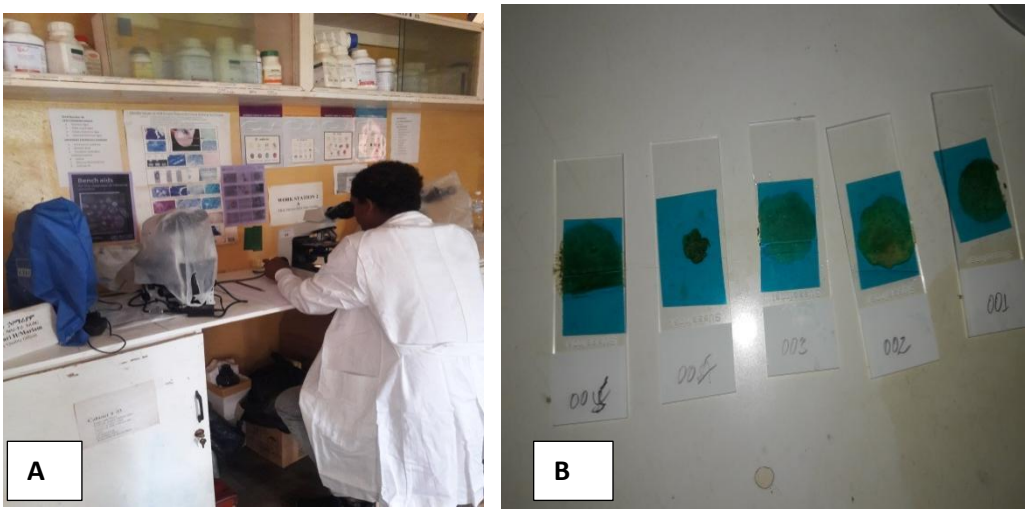

Plate 2: Microscopic examination of *S. mansoni* (A) and prepared Kato Katz slide (B).

4.7.3. Snails collection and Examination for cercariae shedding

Snails were collected using standardized metallic handed 2mm mesh size scoop net from the selected rivers shoreline sampling sites that was identified based on the direct human water contact. Trained individuals about scooping method of snail collection collected snail through deeping the scoop in water of all probable sites including on the leaves and surface of leave debris, under rock and vegetation performed from 9:00-9:30am. Then snails were separated from attached aquatic plant and substrates by gloved hands, store in plastic container containing vegetation and water. Collection site name, date, time and other field information were labelled on container. Snail collected from different sites were stored in different container and transported to Mizan Aman public health regional laboratory. Then snail maintained in plastic container containing old tape water and vegetation for 24 hours, after that each snail was washed with water and its species was differentiated by their shell morphology, following east African freshwater snail shell morphology identification key. The size and direction of the opening, number of shell coiling, shape of the shell and direction of twisting of the shell was taken as morphologic characteristics used for identification of snail species (63). Each individual snail after morphologically identified placed in wide mouth glass vials containing 10ml of clean water, then all the vials containing snail was exposed to artificial light (100 watt) for about 3-4 hours to initiate cercariae shedding. From each snail containing vials water was aspirated by using pastor pipette and then water dropped into clean microscopic slide, one drop in the one end and one drop into the other end of slide and examined using Olympus

compound microscope four times objective and checked for cercariae shedding. When snail shed cercariae, identified to genus level based on cercariae tail, and swimming property(64,65) (**Plate 3**).

Plate 3: Snail collection (A), maintaining (B), washed snails (C), processing and light exposure (D&E) and cercariae (F).

4.7.4. Physico – chemical characterization of snail breeding habitats

The physico-chemical characteristics of snail breeding habitats, in which snail was collected was determined. In situ measurement was carried out for water temperature, conductivity and pH using multi-probe meter (HQ30d single input multi-parameter digital meter). Turbidity was measured using turbidity meter. Water chemistry analysis was performed in each sampling sites before snail collection commenced. The multi probe of each quality measurement was calibrated as recommended by the manufacturer in Mizan Aman public health regional laboratory. After dipping the probe into the water and waiting for a minute, the value of each quality parameter was read and recorded (**Plate 4**).

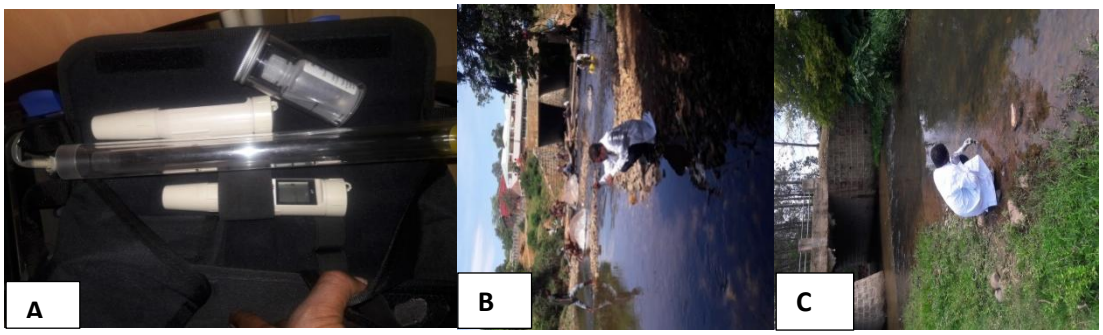

Plate 4: Physico-chemical characterization of snail breeding habitats (Multi parameter (A), Conductivity probe (B) and PH probe(C)

4.8. Data Analysis

Data was coded, cleaned and entered into computer Epi data version 3.0 and analysed using SPSS version 25.0 computer statistical software. Descriptive statistical analysis like percentage, frequency and bivariate and multivariable logistic regression analysis was undertaken to assess the association between explanatory and outcome variables.. In bivariate analysis, variables with p-value < 0.25 were selected as candidate variable for multivariable logistic regression to see the individual effect of each candidate variables and variables with p-value < 0.05 were considered as statistically significant and odds ratio (OR) (crud odds ratio (COR) and adjusted odds ratio (AOR) were used to determine the main predictors and Pearson Correlation was performed to see the significant relationship ($p< 0.05$) between water physico-chemical parameters and snail abundance and infection rate . Finally, the findings were presented by using tables, graphs and charts.

4.9. Data quality control

To ensure data quality, questionnaire was translated to local language and pre-test was conducted on the nearby *kebele*, training was given for laboratory technicians, about the standard processing and examination of stool by kato-katz techniques for parasite detection and quantification and from both positive and negative Kato-Katz smears (10 %) were randomly selected and re-read by two independent senior laboratory experts who are blinded to the primary results. Training also given to snail collectors about the standard scoop net snail collection methods and snail species identification was done by trained senior biologist. Water physico-chemical characterization were analysed by trained environmental health laboratory technician. Data processing, analysis quality was assured by coding and double entry.

4.10. Ethical consideration

The study protocol was reviewed and approved by Institutional Review Board (IRB) of the Institute Health of Jimma University. Supportive letter written from Jimma University Institute of Health was given to Bench Sheko Zone Health Department and then another supportive letter was written from zonal health department and submitted to kometa and shasheka *Kebele* administration office. Before any data collection the purpose, objective and importance of study were explained and written consent was obtained from each study participant, which assured that the participation was on voluntarily basis. Confidentiality was maintained at all levels of the study. Individuals who were positive for *S. mansoni* were treated by referring to nearby health facilities.

4.11. Dissemination of Findings

The findings of the study were submitted and presented to Department of Medical Parasitology, School of Medical Laboratory, Faculty of Health Sciences, Institute of Health, postgraduate and research coordinating office. The results would also submitted to the Bench Sheko Zone Health Department, Mizan Aman city administrative health office and health education would be given to the community, about *S. mansoni*, risk factors and hot spot transmission sites. Finally, an attempt would be made to publish on local or international scientific peer reviewed journal.

5. Results

5.1. Socio demographic characteristics

A total of 349 study participants were participated in this study, to determine the prevalence and associated risk factors of *S. mansoni* infection. The response rate of the study was 96% (349/364). From the total study participants, 182(52.1%) were males and 167(47.9%) females. The minimum, maximum and median age of participants was 1, 80 and 20 years old, respectively. All the participants were more than six month residents of kometa and shasheka *kebele* of Mizan Aman Town administration, at the time of data collection and majority of them 340 (97.4%) lived more than or equal to one year in the area while 9 (2.6%) were residents less than one year (**Table 1**).

Table 1: Socio demographic characteristics of study participants in Mizan Aman, Southwest Ethiopia

Sociodemographic variables		**Frequency**	**Percent**
Sex	Male	182	52.1
	Female	167	47.9
	Total	**349**	**100**
Age	1-4	36	10.3
	5-9	43	12.3
	10-14	49	14.0
	15-19	46	13.2
	20-24	54	15.5
	>25	121	34.7
	Total	**349**	**100**
Length of stay	> 6month	9	2.6
	1year and above	340	97.4
	Total	**349**	**100**
Education	Preschool	48	13.8
	Not formal education	39	11.2
	Primary school	166	47.6
	Secondary school and above	96	27.5
	Total	349	100.0
Religion	Orthodox	110	31.5

	Muslim	92	26.4
	Protestant	147	42.1
	Total	349	100
Average monthly income	301-500	117	33.5
	501-1000	120	34.4
	>1000	112	32.1
	Total	349	100
Occupation	Unemployed	43	12.3
	House wife	67	19.2
	Student	167	47.9
	Labour worker	12	3.4
	Farmer	32	9.2
	Merchant	14	4.0
	Government employee	14	4.0
	Total	349	100

5.2. Prevalence of *S. mansoni,* soil transmitted helminths and other intestinal parasites

The overall prevalence of *S. mansoni* infection in Mizan Aman, Bench Sheko Zone, Southwest Ethiopia was 23.2% (81/349) (95%, CI:18.9-28%). Among the all positive 38 (10.9) were *S. mansoni* mono-infected, and 43(12.3%) were co-infection with other helminths. From the total *S. mansoni* infected 48 (59.3%) them were males and 33 (40.7%) were females.

The prevalence of at least single intestinal parasitic infection was 177/349 (50.7%) (95%, CI: 43.3-54.4%). The prevalence of any STHs mono-infection was 20.3% of which *T. trichuria* was the leading 49 (14%), and prevalence of any other intestinal parasite mono-infection was 1.4% where the least was *H. nana* 2(0.6%). The prevalence of double intestinal parasitic infection was 57(16.3%) of which the highest co-infection is seen between *S. mansoni* and *T. trichuria* 36 (10.3%), and then the least co-infection is seen between *A. lumbricoides* and *S. mansoni* 1(0.3%) and *S. mansoni* and *H. worm* 1(0.3%). About 6 (1.8%) individuals had multiple intestinal parasitic infections (**Table 2**).

Table 2: *Schistosoma mansoni* and other intestinal parasite mono and co-infection, in Mizan Aman, southwest Ethiopia

Mono and Co-infection of *S. mansoni* and STH		**Frequency (N)**	**Percent (%)**
S. mansoni	Mono- infection	38	10.9
	Co-infection with other helminths	43	12.3
	Total	**81**	**23.2**
STH Mono-infection	*A. lumbricoides*	15	4.3
	T. trichuria	49	14
	H. worm	7	2
	Total	**71**	**20.3**
Other intestinal parasite Mono-infection	*Teania species*	3	0.9
	H. nana	2	0.6
	Total	**5**	**1.4**
Co- infection of STH and other intestinal parasites	*A. lumbricoides & T. trichuria*	13	3.7
	A. lumbricoides & S. mansoni	1	0.3
	H. worm & T. trichuria	6	1.7
	S. mansoni & T. trichuria	36	10.3
	H. worm & S. mansoni	1	0.3
	Total	**57**	**16.3**
Multiple parasitic infection	*A. lumbricoides, S. mansoni & T. trichuria*	3	0.9
	A. lumbricoides, T. trichuria & H. worm	1	0.3
	S. mansoni, T. trichuria & Teania species	1	0.3
	A. lumbricoides, S. mansoni, T. trichuria & Teania species	1	0.3
	Total	**6**	**1.7**

5.3. Assessment of *S. mansoni* infection associated factors

In Binary Logistic Regression analysis of socio demographic characteristics and risk factors of *S. mansoni* infection, factors such as sex, age and water sources for domestic use, river bathing, crossing, swimming habit and farm land near to river were candidate variables (p-value < 0.25) had significant association. Results of multivariable logistic regression analysis show that river as sources of water for domestic use, bathing, swimming, and farmland near to river were significantly associated are main predictors of *S. mansoni* infection (p-value < 0.05). The odds of having *S. mansoni* infection among the study participants who use river water as source water for domestic use were six times (AOR: 5.86, 95% CI:2.897-11.826, p= <0.001), bathing in rivers three times (AOR: 3, 95%CI: 1.318-6.738, p= 0.009), habit of swimming three times (AOR: 2.6, 95%CI: 1.284-5.336, p=0.008), and farm close to river two point three time (AOR:2.28, 95%CI: 1.095-4.745, p= 0.028) more likely to have *S. mansoni* infection(**Table 3**).

Table 3: Results of binary and multivariable logistic regression analysis of *S. mansoni* infection and associated risk factors, Mizan Aman, Southwest Ethiopia

Variables		***S. mansoni* infection**		**Frequency**	**OR (95% CI)**		**p-value**
		Positive N (%)	**Negative N (%)**	**N (%) n=349**	**COR**	**AOR**	
Sex	Male	48(26.4)	134(73.6)	182(52.1)	1.455 (.879-2.407)	.810(.418-1.571)	0.533
	Female	33(19.8)	134(80.2)	167(47.9)	**1**		
Age	1-4	5(13.9)	31(86.1)	36(10.3)	**1**		
	5-9	19(44.2)	24(55.8)	43(12.3)	4.908(1.601-15.044)	1.366(.356-5.249)	0.650
	10-14	15(30.6)	34(69.4)	49(14)	2.735(.890-8.409)	.571(.145-2.252)	0.423
	15-19	15(32.6)	31(67.4)	46(13.2)	3.000(.971-9.268)	.597(.151-2.368)	0.463
	20-24	11(20.4)	43(79.6)	54(15.5)	1.586(.500-5.027)	.545(.138-2.144)	0.385
	=>25	16(13.2)	105(86.8)	121(34.7)	.945(.320-2.785)	.302(.082-1.118)	0.073
Source of water and contact behaviour							
Water source for	Pipe	4(20)	16(80)	20(5.7)	1.937(.599-6.263)	2.562(.672-9.763)	0.168

domestic use	Springe	19(26)	54(74)	73(21)	1.660(.520-5.299)	.997(.274-3.634)	0.997
	River	42(53.8)	36(46.2)	78(22.3)	9.851(5.400-17.969)	5.853(2.897-11.826)	**<0.001****
	Well	16(9)	162(91)	178(51)	**1**		
Bathing	Yes	69(35.9)	123(64)	192(55)	6.778(3.509-13.094)	2.979(1.318-6.730)	**0.009****
	No	12(7.6)	145(92.4)	157(45)	**1**		
Swimming	Yes	34(40.5)	50(59.5)	84(24)	4.934 (2.893-8.413)	2.617(1.284-5.336)	**0.008****
	No	47(17.7)	218(82.3)	265(75.9)	**1**		
Crossing river	Yes	42(38.5)	67(61.5)	109(31.2)	3.231 (1.928-5.413)	1.418(.692-2.908)	0.340
	No	39(16.3)	201(83.8)	240(68.8)	**1**		
Farming close to river	Yes	38(42.7)	51(57.3)	89(25.5)	3.760(2.208-6.404)	2.280(1.095-4.745)	**0.028****
	No	43(16.5)	217(83.5)	260(74.5)	**1**		

Note: AOR (Adjusted odds ratio), COR (Crud odds ratio), CI (Confidence interval) 1(Reference category) and ** = significant at p < 0.05

5.4. Intensity of *S. mansoni* and other intestinal parasite infections

Schistosoma mansoni infection intensity were classified according to WHO classification. Among the total participants with *S. mansoni* infection 65 (80.3%), 13 (16%), and 3 (3.7%) had light, moderate and heavy infection, respectively.

5.5. Intermediate Snail Host

A total of 274 freshwater snails that belongs to two families *Planorbidea* and *Lymnaeidea,* and three species namely *B. pfeifferi, B. sudanica,* and *L. natalensis* were collected from the five snail sampling sites of Shonga and Agu Rivers of Mizan Aman, Bench Sheko Zone, Southwest Ethiopia. From all snail sampling sites, at least one snail intermediate host had been collected except Shonga-1(S-1). The highest number of snails were collected from Shonga-3(S-3) which accounted 124 (45.3%), followed by Agu-1(A-1) 78 (28.5%), and Agu-2(A-2) 60 (21.9%) and the least collection was from Shonga-2 (S-2) 12 (4.4%). From the three medically important snail intermediate host species collected and identified in this study area, *B. pfeifferi* is the most abundant and highly distributed snail species which accounts for 187 (68.2%), followed by *L. natalensis* 58 (21.2%), and *B. sudanica* 29 (10.6%) (**Table 4**).

Table 4: Abundance and distributions of freshwater snail intermediate hosts, in Shonga and Agu Rivers, Mizan Aman, Southwest Ethiopia

Collection sites name	**Snail species**			**Total n (%)**
	B. pfeifferi	***B. sudanica***	***L. natalensis***	
Shonga-1(S-1)	0	0	0	**0 (0)**
Shonga-2(S-2)	9	0	3	**12 (4.4)**
Shonga-3(S-3)	69	14	41	**124 (45.3)**
Agu-1(A-1)	63	7	8	**78 (28.5)**
Agu-2(A-2)	46	8	6	**60 (21.9)**
Total	**187(68.2%)**	**29(10.6%)**	**58(21.2%)**	**274 (100)**

5.6. Prevalence of *Schistosomes* infection in freshwater snail intermediate host

All sampling sites in which snails were collected, snails were infected with one or more types of cercariae. The overall prevalence of freshwater snail infection in this study by cercariae shedding was 34/274 (12.4%) (95%, CI: 8.4-12.4%). The minimum and maximum cercariae shedding rate across the snail collection sites ranged from 2.9 – 52.9 %, the highest cercariae shedding rate was recorded from Agu-2 (A-2) which accounted for 18 (52.9%), followed by Shonga-3 (S-3) 11 (32.4%), Agu-1(A-1) 4 (11.8%) and the least cercariae shedding was recorded from Shonga-2 (S-2) 1(2.9%). From the total snail positive for cercariae 23(67.6%) were human schistosome cercariae, 8 (23.5%) none human schistosome cercariae and 3 (8.8%) mixed cercariae (human schistosome and non-human schistosome). *Biomphalaria pfeifferi* was with the highest cercariae shedding snail species in the study area, which accounted for 26 (76.5%), followed by *L. natalensis* 5 (14.7%) and *B. sudanica* were 3 (8.8%) (**Table 5)**.

Table 5: Prevalence of snail intermediate host species infection, in Mizan Aman, Southwest Ethiopia

Site	Species	Number of infected snail	Cercariae			Infection rate (%)
			Number of human schistosome	Number of non-human schistosome	Number of mixed	
S-1	*B. pfeifferi*	-	-	-	-	-
	B. sudanica	-	-	-	-	
	L. natalensis	-	-	-	-	
S-2	*B. pfeifferi*	1	1	-	-	2.9
	B. sudanica	-	-	-	-	
	L. natalensis	-	-	-	-	
S-3	*B. pfeifferi*	5	4	-	1	32.4
	B. sudanica	1	1	-	-	

	L. natalensis	5	-	5	-	
A-1	*B. pfeifferi*	4	4	-	-	11.8
	B. sudanica	-	-	-	-	
	L. natalensis	-	-	-	-	
A-2	*B. pfeifferi*	16	12	3	1	52.9
	B. sudanica	2	1	-	1	
	L. natalensis	-	-	-	-	
Total		34 (12.4%)	23 (67.6)	8 (23.5%)	3 (8.8%)	100

5.7. Physio-chemical characterization of snail breeding habitats

Descriptive analysis of water chemistry parameters in the selected sites of Agu and Shonga rivers identified for snail collection based on water human direct contact. The results of physico-chemical parameters in the study area revealed the mean temperature was 21.06^{o}c, the lowest water temperature was 18.9^{o}c from Shonga -1(S-1) and the maximum was 22.2^{o}c in Shonga -3(S-3). With regard to pH, the mean pH value in this study was 7.574, the minimum and maximum pH was recorded 7.46 from Shonga -3(S-3) and 7.67 from Agu-1(A-1), while, the mean of water turbidity in this study was 6.05 NTU, the lowest turbidity value was 5.5 NTU which is recorded from Shonga-3 (S-3) and the maximum was 6.38 NTU from Shonga-1(S-1). The other physico-chemical parameter conducted to characterize snail breeding habitats in this study was water conductivity (µS/Cm), the mean conductivity was 63.78 µS/Cm, whereas the minimum and maximum conductivity of 60.55 and 68.99 µS/Cm recorded from Shonga-1(S-1) and Shonga-3(S-3), respectively. The mean, minimum and maximum water flow rates of rivers in the snail sampling sites were 0.2042, 0.089 and 0.628m/s, respectively (**Table 6**).

Table 6: Physico-chemical parameter values of water in snail intermediate host breeding sites in Mizan Aman, Southwest Ethiopia

Parameters	**Site code**					**Mean**	**Minimum**	**Maximum**
	S-1	**S-2**	**S-3**	**A-1**	**A-2**			
Temperature (^{o} C)	18.9	21	22.2	21.7	21.5	21.06	18.9	22.2
pH	7.6	7.51	7.46	7.67	7.63	7.574	7.46	7.67
Turbidity (NTU)	6.38	6.22	5.5	6.0	6.15	6.05	5.5	6.38
Conductivity (µS/Cm)	60.55	62.02	68.99	63.64	63.70	63.78	60.55	68.99
Flow rate (m/s)	0.628	0.119	0.094	0.089	0.091	0.2042	0.089	0.628

5.8. Association of snail abundance, infection and physico-chemical parameters

In Pearson correlation analysis of water physico-chemical parameters such as temperature, pH, turbidity, conductivity and water flow rate, only turbidity (r = -0.943, N = 5, p= 0.016) negatively and conductivity (r = 0.946, N = 5, p = 0.015) positively correlated with snail intermediate host abundance. No physico-chemical parameters were significantly correlated with snail cercariae infection in this study (**Table 7**).

Table 7: Results of Pearson correlation analysis on the association of water chemistry with snail abundance and snail infection in Mizan Aman, Southwest Ethiopia

Variables	Temperature	pH	Turbidity	Conductivity	Flow rate
Snail Abundance	.838	-.232	-.943*	.946*	-.636
	.076	.708	**.016**	**.015**	.249
Variables	Temperature	pH	Turbidity	Conductivity	Flow rate
Snail Infection	.590	.028	-.419	.553	-.527
	.295	.964	.483	.334	.362

Note: *significant at p < 0.05

6. Discussion

The prevalence of *S. mansoni* infection in this study was 23.2%. This is higher when compared with the findings of studies conducted in northeast coast of Brazil 16.4%, Khartoum, Sudan 2.95%, West Kenya 11.8%, Malawi 9.5% (28,32,34,37). And in Ethiopia, Sanja 16.67%, Tigray 5.95%, Gamo Gofa 14%, and Bench maji 9.3% (38,40,44,47). This variation might be due to difference in socio-economic, demographic factors, control and prevention program, sample size, distribution of intermediate snail hosts.

The findings of the current study is in line with the reports of western Uganda 25.6%, Bahirdar, Ethiopia 24.9%, Jimma, Southwest Ethiopia 27.6% (33,39,46). The present finding is low when compared to studies conducted in Madagascar 73.6%, Mbita 60.5% and islands of Lake Victoria of west Kenya 82%, Tanzania 68.9% (30,35,36). In Ethiopia, Sanja 89.9%, Yachi 74.9%, Wolaita 81.3%, and Bench maji 44.8% (41,43,45,66). This variation might be due to difference in sample size, socio demographic factors, season of study, and geographical distribution of intermediate snail hosts, control and prevention program, and local prevalence of parasite, level of awareness about transmission and prevention.

Schistosoma mansoni intensity from the current study was 80.3%, 16%, and 3.7% for which is classified as light, moderate and heavy infection, respectively. Heavy infection accounted for the least proportion from total *S. mansoni* infected individuals. However, the heavy infection was higher when compared to the study conducted at sentinel sites of Madagascar 0.9% (31). The variation might be due to socio economic and demographic factors, control and prevention program, and poor hygienic practice.

In contrast, this finding was lower as compared to the studies conducted in Marolambo, Madagascar 32.1%, Mbita and islands of Lake Victoria, western Kenya15.2%, Sanja, Northwest Ethiopia 18.7%, and Wondo Genet, Southern Ethiopia 42.4% (30,35,38). This variation might be due to difference in year of MDA round given and sanitation and hygienic practice.

The odds of getting *S. mansoni* among study participants using river as a source of water for domestic purpose and bathing in river was 5.9 and 3 times more likely to have risk of infection, than those didn't use river water for domestic purpose and bathing. This result is in agreement with studies done in Gorgora, Northern Ethiopia (7.7), Jimma, Southwest Ethiopia (8.8), and Wolaita, Southern Ethiopia (3.3) (42,43,46). This might be due to difference in socio economic and demographic factors, hygiene, sanitations practice and frequency of water contact.

Moreover, the odd of getting *S. mansoni* infection among these study participants with habit of swimming was 2.6 times more likely to have the risk of infection than participants not have habit of swimming. This finding was in agreement with the studies conducted in Western Kenya(3.99), Gorgora, Northwest Ethiopia(10), Guangua District, Northwest Ethiopia (11.35), and Jimma, Southwest Ethiopia(2.59) times more likely to get *S. mansoni* infection than participants have not habit of swimming (34,42,56,67). This variation might be due to difference in frequency of swimming, hygiene and sanitation practice. The odds of getting *S .mansoni* infection among individuals who had farm near to river in this study were 2.3 times more likely to happen than those who have no farm near to river. This result was in agreement with the study conducted in Gorgora, Northwest Ethiopia 7.9 (42). This difference might be due to the frequency of water contact, sanitation and hygiene practice.

A total of 274 freshwater snail intermediate hosts were collected from river shorelines selected for snail collection. Of the total snails collected, more than half, were putatively identified as *B. pfeifferi* 187 (68.2%), *B. sudanica* 29(10.6%) and *L. natalensis* 58 (21.2%). This finding was in agreement with the malacological surveys conducted in Kisumu city, western Kenya where *B. pfeifferi* was found to be the most abundant species which accounted for 40.1%, followed by *B. sudanica* 38.4%, and in Mara River Kenya and Tanzania, *B. pfeifferi* accounted for 61.1%, followed by *B. africanus* 22.2% and *Lymnaea species* 16.7%, (48,49). *Biomphalaria pfeifferi* snail was the most abundant intermediate host in this study area, this result is consistent with malacological survey conducted in Omo Gibe River Basin, Southwest Ethiopia, *B. pfeifferi* was the dominant species which accounted for 66.8%, followed by *L. natalensis* 24%, *B. sudanica* 0.2%, *B. globosus* 4.8%, and *B. forskalii* 4.3%. Similarly in Sanja, Northwest Ethiopia, *B. pfeifferi* was the leading snail intermediate host species, which accounted for 97.4% and 81.4% during January and April

respectively, (12,50). This variation might be due to the abundancy and distribution of snail species were influenced by climatic condition, environmental factors and physico chemical parameters of breeding habitat.

This finding was in contrast with the survey reports from Kpong Head Pond, Ghana, where the dominant snail species were *B. truncates* 71%, followed by *B. pfeifferi* 12% and *B. globosus* 1%. Moreover, in Niger River Valley West Africa, *B. truncates* was the most abundant snail species, followed by *B. forskalii, L. natalensis* and *B. pfeifferi* (51,52). This variation might be due to difference in geographical distribution, season and physico chemical parameters of breeding habitats.

The overall prevalence of cercariae infection in snail intermediate host was 34(12.4%). *Biomphalaria pfeifferi* was the most frequently shedding species which accounted for 26(76.5%), followed by *L. natalensis* 5(14.7%) and *B. sudanica* 3(8.8%). This finding was in lined with study conducted in Gombe ecosystem of western Tanzania, the overall prevalence of cercariae infection in snail intermediate host was 12%, and *B. pfeifferi* was also the highest infection frequently cercariae shedding snail species (53). Similarity might be due to socio-economic and demographic factors, poor hygiene and sanitation practice. This prevalence of snail infection was low as compared to with result of Western Kenya 26.5% (54). Variation of infection prevalence might be due to difference in hygiene and sanitation practice or other organic and inorganic debris favouring snail infection. On the other hand, prevalence of cercariae infection in snail intermediate host was higher as compared with freshwater snail survey conducted in Kisumu city, western Kenya, prevalence of cercariae infection in *B. pfeifferi* and *B. sudanica* was 1.6% and 1.7%, respectively, and in Omo Gibe River Basin, southwest Ethiopia 4.6% (12,48). This variation might be due to difference in hygiene and sanitation practice, abundance and distribution of snail intermediate hosts.

In the current study of the total cercariae infected snails, about 23(67.6%) snails were shedding human schistosome cercariae, 8(23.5%) nonhuman schistosome cercariae and 3(8.8%) were shedding both type of cercariae. These result was in line with study conducted in Khartoum State, Sudan, more than half of shedding from *B. pfeifferi* was human schistosome cercariae (68). However, this finding was in contrast with study conducted in Lake Albert and Victoria, pooled prevalence of cercariae infection in

Biomphalaria snails were 8.9% and 2.1% respectively. In Albert, from total cercariae shedding 15.8% was *S. mansoni* cercariae and 84.2% nonhuman schistosome cercariae, whereas in Victoria 13.9% *S. mansoni*, 85.7% nonhuman schistosome and 0.4% shedding both type of cercariae (55). The reason for these variations might be associated with hygiene and sanitation practice of inhabitants, seasonality of snail infection and in general snail intermediate hosts were found in freshwater body contaminated with human and animal excreta.

In the current study physico-chemical parameters such as turbidity and conductivity showed significant association with overall snail abundance and none of the conducted parameters showed significant association with snail cercariae infection. Where turbidity was negatively correlated with snail abundance, this result was in agreement with the finding in western Kenya (69). Conductivity was positively associated with snail abundance in this study, which is in agreement with finding in the Niger River Valley. In contrast with the findings in Uganda population dynamics of *Biomphalaria* in Lake Albert and Lake Victoria conductivity negatively associated, and Ijinga Island, Mwanza, North-Western Tanzania not significantly associated with snail intermediate host abundance. In this study, none of the conducted physico chemical parameters were significantly associated with snail cercariae infection, this is in agreement with finding in Ijinga Island, Mwanza, North -Western Tanzania (52,55,59). This variation might be due to difference in ecology, climatic condition, human water contact and contamination.

6.1. Limitation of the study

- Study design used was cross sectional parasitological and malacological study, determine during at the data collection time.
- Method used for identification of snail species and cercariae was morphological characterizations which is less specific and sensitive than molecular technique.

6.2. Conclusion

Schistosoma mansoni infection was the major public health problem in community of Mizan Aman, Bench Sheko Zone, South west Ethiopia. The prevalence of *S. mansoni* infection was 23.2%. Risk factors such as using river as source of water for domestic use, bathing, swimming and farming close to rivers were major risk factors of *S. mansoni* infection. Three morphologically distinguishable fresh water snail species serve as intermediate hosts potentially transmitting disease of human schistosome and non-human schistosome infection were identified. Snail sampling sites except Shonga-1(S-1) have collected at least one snail intermediate host and infested with one or more type of human schistosome and non-human schistosome cercariae. Shonga-3 (S-3) snail collection site was the highest number of snails were collected 124(45.3) and the least was from Shonga -2(S-2) 12(4.4). The overall prevalence of snail infection was 34/274(12.4), of which 23(67.6) was human schistosome cercariae, 8(23.5) none human schistosome cercariae, and 3(8.8%) mixed type of cercariae. The highest cercariae shedding rate was recorded from Agu-2(A-2) 18/34 (52.9) and the least was in Shonga-2(S-2) 1/34(2.9). *B. pfeifferi* was the highly abundant and cercariae shedding snail species responsible for the transmission of *S. mansoni* and physico-chemical parameters, turbidity and conductivity shows significant association with snail abundance in the study area.

6.3. Recommendations

- Extend preventive chemotherapy to all population in need
- Health education should be established for local community
    - Risk factors of *S. mansoni* infection
    - Prevention and transmission way of the disease
    - Avoidance of human contact with water infested with schistosome cercariae, identified as source of infection
- Reducing environmental contamination by improving local water sanitation and hygiene
- Snail control measures should be applied
- Apply integrated control strategies (Co-implementation of Praziquantel MDA, snail control, WASH and health education about water contact behavioural change) for achievement of WHO goals for morbidity control and elimination as a public health problem
- Further research required that focus on snail intermediate host, mapping of hot spot transmission areas and identification of effective snail control methods.

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Annex 1

Information sheet and consent

Information sheet

Introduction: Hello! My name is Gashaw Temesgen

I am a student at Jimma University, School of Medical Laboratory Sciences, doing my MSc thesis. **Title**: **Prevalence of Schistosoma mansoni infection, snail intermediate host fauna and physico-chemical characterization of breeding habitats, in Mizan Aman, Bench Sheko zone, Southwest, Ethiopia**. I am collecting information about the effect of socio demographic variable on the prevalence of *S. mansoni* infection. *S. mansoni* parasite is among the most common cause of human infections which is distributed throughout the world and cause thousands of avoidable outpatient morbidity and mortality, especially in school-age children. They are also the leading cause of gastrointestinal pain, malnutrition, malabsorption, anaemia, mental retardation and other diseases. The diagnosis of these infections commonly relies on the detection of parasites egg in stool. So, you are chosen to participate in this study by systematic Sampling technique and other participants will be selected for this study from Mizan Aman.

Purpose: The objective of this study is to determine the prevalence of *S. mansoni* infection, snail intermediate host fauna and physico-chemical characterization of breeding habitats, in selected kebeles of Mizan Aman.

Benefit: This study will determine the magnitude of *Schistosoma mansoni* infection, identify the major snail vector responsible for the transmission of *S. mansoni* infection and identify the active transmission site (hot spot area), provide information to the concerned bodies to design effective control strategies and community to take action from that identified site.

Risk: by participating in this study, you may sacrifice your time, otherwise you may not face any risk due to participating in the study.

Confidentiality: The result of study each participant will be kept confidentially, instead of writing the name, coding will be used.

Right to refuse or withdraw: you have a full right to refuse from participating in this research.

Person to Contact: If you want to know more information, you can contact me by:

Gashaw Temesgen: Telephone number: **0917164237**

E-mail address: **gashutemesgen2012@gmail.com**

Consent form

He/she willing to participate in the study entitled **"Prevalence of *Schistosoma mansoni* infection, Snail Intermediate Host Fauna and Physic-Chemical Characterization of Snail Breeding Habitats, in Mizan Aman, Bench Sheko Zone, Southwest, Ethiopia"**. Well informed that, this study requested to give single fresh stool sample about 2gm, doing this no any reasonably foreseeable discomforts, disadvantages and risks will happen to you. Being participating on this research has many advantages, from these you can know your health situation concerning about this intestinal parasite and get appropriate free laboratory diagnosis without seeking health institution.

All the information that we collect about you during the course of the research will be kept strictly confidential. You will not be able to be identified in any reports or publications. Finally I would like to thank you for taking time to hear the information given and willing to participate.

Participant Name ……Signature……Date……...

Investigator Name…….....Signature.....Date…….

Part I Socio demographic variables

ID NO, _____

Kebele: _____

1. Age: _____

2. Sex: 1. Male 2. Female

3. Religion: 1.Orthodox 2. Muslim 3.Protestant 4.Others(specify)_____

4. Educational Level 1.Not formal education 2.Primary school 3.Secondary school and above

5. Length of stay in area 1. >6 month 2. 1 year and above

6. Average monthly income(Birr) 1. 301-500 2. 501-1000 3. >1000

7. Occupation

1. Unemployed
2. House wife
3. Student
4. Daily laborer
5. Farmer
6. Merchant
7. Employed
8. Pensioned

Part II: Personal hygiene

8. House hold has latrine in their compound? 1 .Yes 2. No

8.1. If **No** for ***Q8***, where do you defecate? 1. Communal latrine 2. Open field

9. Does your compound have communal latrine? 1 .Yes 2.No

9.1. If **No** for ***Q9***, Are you defecating open field? 1. Yes 2.No

10. How often do you take shower? 1. Daily 2. Twice a week 3. Weekly

11. Habit shoe wearing 1. Always 2. Sometimes 3. Never

Part III: Water sources

12. Source of water for drinking 1.Pipe 2. Spring 3. River 4. Pond

13. Source of water for domestic purpose? 1. Pipe 2. Spring 3. River 4. Pond

14. Habit basing in river water 1. Yes 2. No

14.1. If **Yes** for ***Q14***, frequency of basing 1. Daily 2. Twice a week 3. Weekly

15. Habit of fishing 1. Yes 2. No

16. Habit of crossing rivers 1. Yes 2. No

16.1. If **Yes** for ***Q16***, frequency of crossing river 1. More than twice per day 2. Daily 3.Weekly 4.Sometimes 5.Never

17. Habit of swimming in rivers 1. Yes 2. No

17.1.If **Yes** for ***Q17***, frequency of swimming 1. Twice per day 2. Daily 3. Weekly 4. Some times

18. Farming land near to river 1.Yes 2.No

Part IV: Laboratory results

19. Result of Kato-katz

    1. No ova/ parasites seen
    2. *S. mansoni*
    3. Other helminthes specify_____

20. Infection intensity 1. Low 2. Moderate 3. Heavy

Annex 2

Physic-chemical quality parameters and cercariae shedding data recording tool

Form for field data collection of snails

Collection site/ code: _____ Date: _____(dd/mm/yy)

Water bodies type: _____

Collection time: Started: _____ Ended: _____

Collector's name: _____

Environmental and physico-chemical parameters

1. Temperature: _____

2. pH: _____

3. Conductivity(ms): _____

4. Turbidity: _____

5. Flow rate:_____

6. Season (1). Dry ☐ (2). Rainy ☐

Malacological data collection form

Collection site/Code: ________________ Date examination: ___________ (dd/mm/yy)

Time of shedding: Started: ____________ Ended: __________

Examiner name: ___________________________

Sample ID	Cercariae shedding		Species of snail	Date of Shedding	Time of shedding (Morning /Evening)	Fate of snail specimen (preserved/ discarded)
	Yes	No				

Sample ID	Cercariae shedding		Species of snail	Date Shedding	Time of shedding (Morning /Evening)	Fate of snail specimen (preserved/ discarded)
	Yes	No				

Annex 3

Photo and video of snail collection and laboratory works

Figure: Snail collection in Agu and Shonga River Mizan Aman, southwest Ethiopia 2022.

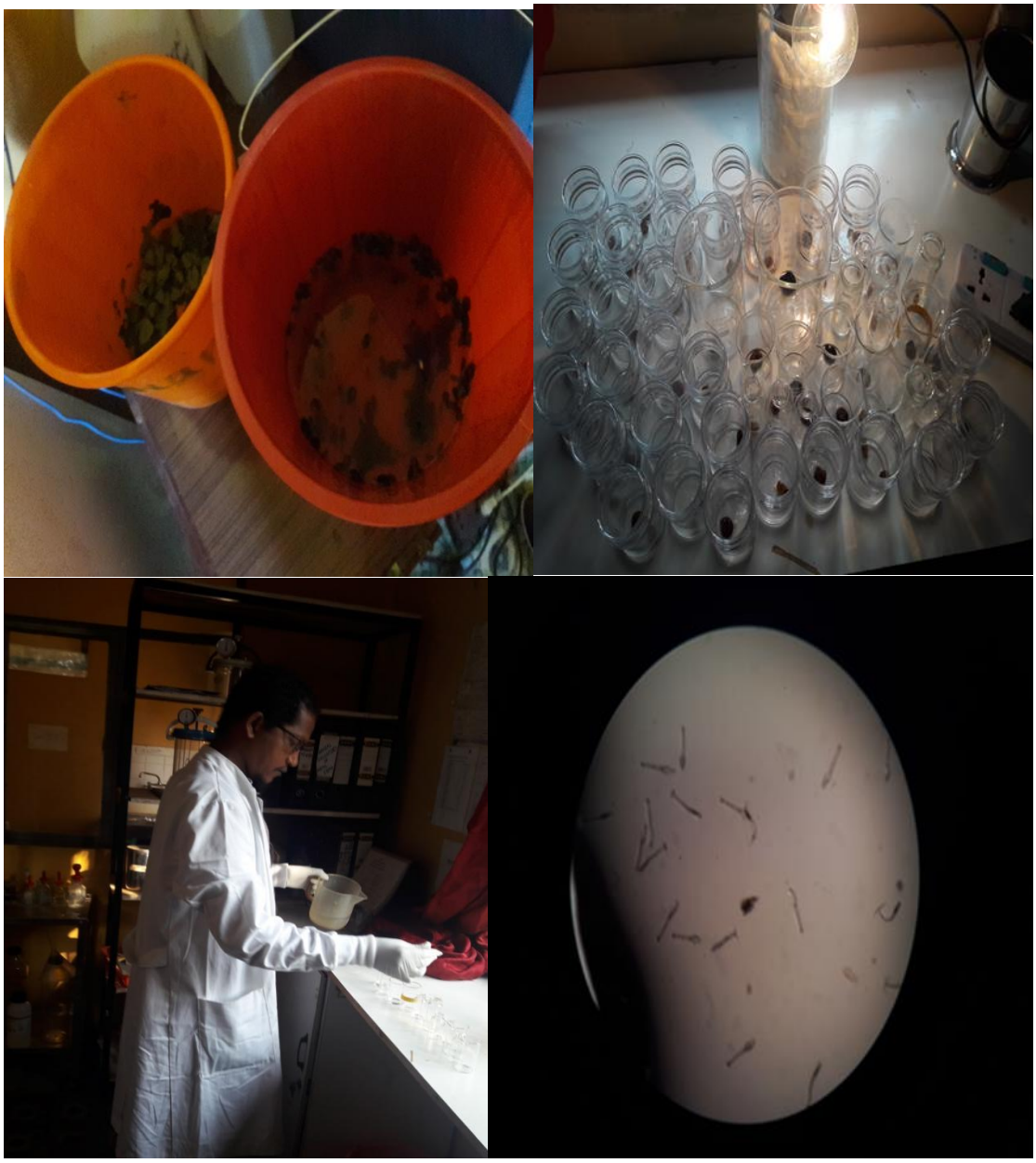

Figure: Fresh water snail cercariae shedding techniques in the laboratory Mizan Aman southwest, Ethiopia 2022.