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DEPARTMENT OF ZOOLOGICAL SCIENCES

STUDIES ON ZWAI WETLANDS AS RESERVOIRS OF GASTROINTESTINAL PARASITES OF SHEEP AND HUMANS AND AS HABITATS OF SNAIL VECTORS.

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Zoological Sciences (Fisheries and Aquatic Sciences Stream)

By Ayalew Sisay

Adviser: Professor Brook Lemma

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GRADUATE STUDIES

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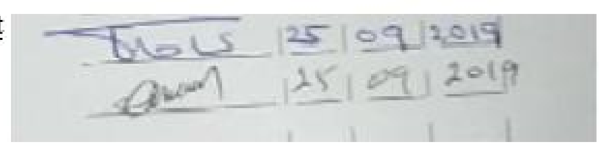

Advisor Prof. Brook Lemma Signature______________Date___________

Chairman Prof. Seyoum Mengistou Signature______________Date___________

Declaration

I, Ayalew Sisay, declared that this dissertation is my original PhD work done by me under the supervision of Professor Brook Lemma from April 2015 to July 2017 at Zoological Sciences of Addis Ababa University. The study and data involved in this PhD thesis have not been submitted for the award of any other degree. All other contributions and sources of information cited herein are duly acknowledged.

Abstract

In tropical countries like Ethiopia where the wet seasons are restricted to less than five months of the year, wetlands such as those found around Lake Zwai provide ideal environments for water-related diseases of sheep and humans. As a consequence, this study was conducted over a 2-year period to study the gastro-intestinal parasites of sheep and humans (schoolchildren) that frequently visit the wetlands of Lake Zwai, the intermediate hosts (snails) inhabiting the same wetlands and the effectiveness of drugs on the gastro-intestinal parasites identified in the wetlands. The results generally showed that the wetlands of Lake Zwai are infested with gastro-intestinal parasites as shown in the tracer animals used (sheep) and by a different set of parasites picked by schoolchildren visiting the same wetlands. The snail vectors surveyed in these wetlands were generally found to host the diseases of the sheep rather than those diseases of the schoolchildren. The drug effectiveness trials conducted of the livestock gastro-intestinal nematode parasites were found to be susceptible in Tetramizole and Ivermectine, with lower resistance in only one drug (Albendazole). It was concluded that Zwai wetlands that provide fresh herbage to sheep throughout the year and attractive playing grounds to schoolchildren of a nearby school, provide conducive environments mostly to waterborne gastro-intestinal parasites of sheep and to a lesser extent to humans (schoolchildren). In all cases of the definitive hosts (sheep and humans), their performance in life is severely affected, if in rare cases deaths do not occur. It was, therefore, recommended that visits of sheep and humans to the wetlands should be restricted particularly at peak infection months, inflow of animal wastes with parasite eggs be diverted for treatment, regimes of prophylactic treatments should be introduced and generally awareness of the disease cycles, herbages for animals should be collected and treated before feeding the sheep, safe wetland playing-grounds for schoolchildren should be identified and safe waste-handling methods should be introduced into the local population and sheep owners.

Keywords: *Gastrointestinal parasites, Lake Zwai, Schoolchildren, Sheep, Wetland.*

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
AOR	Adjusted Odds Ratio
m.a.s.l.	meters above sea level
CL	Confidence Limit
CR	Crude Ratio
DO	Dissolved Oxygen
EPG	Eggs Per Gram
FECRT	Faecal Egg Count Reduction Test
GI	Gastro-intestinal
GIP	Gastro-intestinal Parasites
H'	Shannon Weiner Index
ILCA	International Livestock Control Association
kg	Kilogram
L	Liter
mg	Milligram
ml	Milliliter
MoWR	Ministry of Water Resources, now Ministry of Water, Irrigation and Energy
OD	Odds Ratio
ohms	The unit of electrical resistance
PAST	Paleontological Statistics
PC1	Principal Component
PCA	Principal Component Analysis
PCV	Packed Cell Volume
*p*H	A measure of acidity or alkalinity of the solution
RESO	Regression Software
S.E.	Standard Error
SPSS	Statistical Program for Social Science
VAR	Variance

WAAVP	World Association for the Advancement of Veterinary Parasitology
WHO	World Health Organization
μm	Micrometer
χ^2	Chi-square

DEDICATION

This thesis is dedicated to my mother, W/o Zufan Gezahegn Ali for her love, unselfish support, life-long advice and day and night prayers throughout her life that laid a strong foundation for my career and a determination to complete this work.

CHAPTER 1.

GENERAL INTRODUCTION

1.1 Introduction

Wetlands are among the world's most productive environments (Burlakova *et al*., 2008) with high level of biodiversity and relatively high biological production potentials (Brouwer *et al*., 2014). They provide a stable habitat for aquatic organisms due to their low water velocity and shallow depth (Jeong *et al*., 2014). In wetlands, the primary factor controlling the environment and the associated plant (macrophytes) and animal life is water (Lan *et al*., 2010; Ruto *et al*., 2012). Wetlands are usually places where there is extensive plant growth because of the abundance of water and nutrients, which eventually provide food and shelter for the visiting animals.

Many civilizations have been built largely on the basis of wetland resources (Shine and Klemm, 1999). Rich variety of resources of wetlands make them highly valuable to the surrounding people. Wetlands in arid and semi-arid regions are very important for the diversity of the surrounding dryland and for humans living around it (Brouwer *et al*., 2014). Wetlands provide economic and social values, and this indicates that wetlands and people are ultimately interdependent (Kansiime *et al*., 2007).

Water is a major determinant of sheep distribution. The distinctive capacity of wetlands to retain water far into the dry season of each year has resulted in increased use of wetlands for sheep grazing during the dry season areas in many developing countries. In wetlands, the availability of forage and water is higher as compared to adjacent drylands to attract sheep for grazing. However, wetlands do not only serve as a feed and water sources for sheep; but unfortunately, they are also serve as sources of parasite and disease caused by organisms. Wetland environments that provide the grazing space for sheep through most of the year are also favorable habitats for the survival and development of helminth parasites of animals and humans. Hence, wetlands are potential habitats for helminth parasites (Jejaw Muluneh *et al*., 2014).

Water-related gastrointestinal parasites of sheep cause diseases of major socio-economic importance worldwide (Roeber *et al*., 2013). In wetland areas, tropical environment is an ideal habitat for helminth parasite species (Jiregna Dugassa *et al*., 2018); particularly in Africa (Demelash Biffa *et al*., 2007). The most common gastrointestinal helminth parasites that affect the grazing sheep in tropical areas belong to the genera *Haemonchus*, *Trichostrongylus*, *Oesophagostomum*, *Bunostomum*, *Ostertagia*, *Cooperia*, *Nematodirus*, *Chabertia* and *Trichuris* (Tembely *et al*., 1997) that require fresh water such as found in wetlands to complete part of their life cycles. However, the severity of infection by these gastrointestinal helminth parasites of sheep vary from region to region related with the difference in ecological and climatic conditions (Molento *et al*., 2016; Jiregna Dugassa *et al*., 2018).

There are several factors that affect the distribution of gastrointestinal helminth parasites on the grazing sheep in wetlands. Some of the factors are the types of helminth present, the burden of infective larval stages, climatic conditions of the grazing area (Demelash Biffa *et al*., 2007), herbage type, stocking density (Roeber *et al*., 2013) and grazing behavior of the host. Some species of gastro-intestinal nematode parasites are capable of producing thousands of eggs per day, which can lead to fast herbage larval contamination (Roeber *et al*., 2013) in wetlands. In Ethiopia, wetland resources are public property and hence everyone has free access to them. In the Central Rift Valley areas, wetlands serve as communal grazing grounds, which predispose sheep to continuous exposure to gastrointestinal helminth infections. Research conducted using tracer sheep experiments in different areas at different seasons of the year confirmed the occurrence of gastrointestinal helminth parasites (Brook Lemma, 1985; Desalegn Lidetu, 2005). Hence, this study was designed to assess the gastrointestinal helminth parasites using tracer sheep experiments in the wetlands of Lake Zwai.

Zoonotic infections are the most common diseases on earth responsible for over 60% of human infectious diseases (Ekong *et al*., 2012). Human evolution and parasitic infections run hand in hand. Environmental changes, human behavior and population movement have a great effect on the transmission, distribution and prevalence of parasitic disease within a community. In developing countries, human population growth demands intimacy with wetlands in search of natural resources for survival. These interactions lead to the emergence of intestinal parasitic zoonosis. Intestinal parasitic infections, which are caused by helminths and protozoa are among the most widespread of human infections (Tadesse Hailu, 2014).

Ingestion and faeco-oral contact of infective parasitic stages are important route of human infection for waterborne zoonosis (Macpherson, 2005; Ekong *et al*., 2012). Factors like eating poorly washed raw vegetables, lack of personal or environmental hygiene, unsafe drinking water, lack of toilet facilities, walking barefoot, fishing, irrigation activities, swimming habits, playing with moist soil, open defecation, and educational status of parents may increase the prevalence and distribution of gastrointestinal parasites in tropical and subtropical countries (Mengistu Legesse and

Berhanu Erko, 2004) of wetland areas. The practice of eating raw or improperly cooked meat and fish has been a source of infection for many parasitic zoonosis in different cultures where this is practiced (Macpherson, 2005).

In wetland areas, the distribution and prevalence of gastrointestinal parasites vary from region to region because of several environmental, social, behavioral and geographical factors (Mengistu Legesse and Berhanu Erko, 2004). In recent decades, the custom of eating raw, or partially cooked foods has grown worldwide and has led to the emergence of parasitic zoonosis (Macpherson, 2005). People in developing countries living in lake or river bordering areas, the tradition of eating raw fish is becoming increasingly fashionable and this has led to the occurrence of fish borne zoonotic parasitic infections (Macpherson, 2005). The prevalence of zoonotic gastrointestinal helminth and protozoan parasites are directly linked with the prevalence of infection in sheep and its pet animals (Ekong *et al*., 2012). Prevalence of gastrointestinal helminths and protozoan intestinal parasites have been studied in different countries of the tropics and subtropics including Ethiopia (Mengistu Legesse and Berhanu Erko, 2004; Tadesse Hailu, 2014). Some of the common zoonotic helminth parasites are *Ascaris*, *Teania*, *Schistosoma*, hookworms, *Strongyloides*, *Trichuris* and others (Ekong *et al*., 2012). These diseases are more prevalent in school age children (Tamrat Hailegebriel, 2017) around wetlands, especially when they make regular contact with moist soil in their school ground and are not carefully cleaned thereafter (Cook *et al*., 2009; Omalu *et al*., 2017). Hence, the present study aimed at assessing the prevalence of gastrointestinal parasitic zoonosis infections on schoolchildren located closer to Lake Zwai.

Wetlands also serve as a habitat for freshwater gastropods (Mo *et al*., 2017) for feeding, aestivating, mating and juvenile life stages, particularly in the shoreline of lakes. Freshwater snails of the Subclass Pulmonata are important gastropods in the tropic and sub-tropic regions of the world (Abe *et al.*, 2017). They serve as intermediate hosts of digenetic trematode parasites of humans, sheep (Jayawardena *et al*., 2011; Qureshi *et al.*, 2015; Mohammed *et al*., 2016; Duwa, 2017), fish and birds (Arshad *et al*., 2011). There are approximately, 350 snail species, which have medical and veterinary importance (Barkia *et al*., 2014).

The successful transmission of trematode parasites between their intermediate and definitive hosts involve free swimming stages and the attachment of infective larval stages on aquatic plants (Woodruff and Upatham, 1993b). The presence and prevalence of trematode infections transmitted by the intermediate hosts are determined by the spatial distribution of these host snails (Luka and Mbaya, 2015).

Freshwater snails are found in all freshwater bodies from small temporary ponds to lakes. Within each water body, snail distribution is intermittent and detection requires examination of different sites. Identification of snail species can provide information on the distribution of trematode diseases in a certain area (Jayawardena *et al*., 2010; Pedersen *et al*., 2014) as the larval stages of trematode parasites have unique morphological features (Brown, 1994). The types of snails that release cercariae and the number of cercariae released from each infected snail play an important role in the transmission of trematodes from the snail to the host (Brown, 1994; Tigga *et al*., 2014).

Water-related parasite abundance in intermediate host populations is likely to be highest in frequently visited wetland areas (Smith, 2001). Compared to definitive hosts of trematode parasites like humans and sheep, intermediate host snails are sessile. The different distribution of snails has been suggested to be raised from the movement and behavioral pattern of these definitive hosts (Kuris and Lafferty, 1994).

The distribution of freshwater snails varies with physical, chemical and biological characteristics (Sharif *et al*., 2010). In most areas, seasonal changes in rainfall, water level and temperature cause marked fluctuations in snail species abundance. Climatic conditions like rainfall, water temperature, DO, pH, turbidity and conductivity determines the distribution and abundance of snail (Ejotre *et al*., 2015). Therefore, it is necessary to study the relationship between snails and their environment. Hence, the physicochemical characteristics of the selected sites in wetland ecosystems should be described in order to understand the abundance and distribution of freshwater snail species found in that particular lake system. The distribution and abundance of freshwater snail species and their interaction with the physicochemical parameters of the water bodies have been studied in various tropical lakes (Sharma *et al*., 2013). With its different agro-ecological regions, Ethiopia has diverse wetlands that offer suitable habitats for freshwater snail species. Most studies so far conducted in Ethiopia on freshwater snails were mainly focused on the identification of the species (Brown, 1994; Yilma Jobre and Malone, 1998; Berhanu Erko *et al*., 2006; Brook Lemma and Hayal Desta, 2016). Some of these studies largely focused on the abundance of gastropod species as biological indicators for water quality. However, these studies did not attempt to shed some light cercariae and relate them to host relationship of trematode species. Hence, this study was designed to fill that gap.

Parasitic nematodes of sheep are controlled mainly through anthelmintic treatment (Roeber *et al*., 2013). Farmers in developing country are dependent heavily on the use of anthelmintic drugs to treat their nematode infected sheep (Chandrawathania *et al*., 2002). Different anthelmintic drugs for the control of gastrointestinal helminth parasites with different modes of actions are available in the market (Berssisa Kumsa, 2004), particularly for nematodes of sheep. In Ethiopia, the use of anthelmintic drugs, particularly Albendazole, Tetramizole and Ivermectine has been practiced for long time (Bersissa Kumsa and Abebe Wossene, 2007; Dinaol Belina *et al*., 2017). Research conducted in Ethiopia by Befekadu Urga and Teka Feyera (2015); Tewodros Getachew *et al*. (2017); and Walkite Furgasa *et al*. (2017) indicated that the frequent use of certain drugs, inappropriate use and under dosage are some of the factors that affect the efficacy of anthelmintic drugs. However, the factors responsible for the development of drug resistance in sheep in one locality may not be the factor in the other locality. Hence, this study was designed to the assess of the prevalence of anti-helminthic resistance of sheep, using the most recommended and commonly used faecal egg count reduction test (Coles *et al*., 2006) in the wetlands of Zwai area.

1.2 Objectives of the Study

1.2.1 General objective

To assess the role of Zwai wetlands as reservoirs of gastrointestinal sheep and humans diseases, and habitats of snail species that are potential disease vectors for domestic animals using the wetlands of Lake Zwai.

1.2.2 Specific objectives

- To assess the prevalence of water-related gastrointestinal helminths disease of sheep and their zoonotic implications in the wetlands of Lake Zwai.
- To show the prevalence of gastrointestinal parasite infections and the associated risk factors among schoolchildren at Bochesa Elementary School, around Lake Zwai.
- To assess the distribution of snail species in relation to the physicochemical parameters and shed cercaria from the collected snail species and relate with host relationship of trematode species along the frequently sheep-humans contact area of Lake Zwai.
- To identify the occurrence of gastrointestinal nematode parasites resistance to market available drugs such as Albendazole, Tetramizole and Ivermectin used by sheep owners in naturally infected sheep under field conditions in the study area.

1.3 Hypothesis and Research Questions

1.3.1 Hypothesis

Many higher species of animals are attracted to wetlands in search of food, shelter and water or even to cool up themselves. While doing so, they interact with each other in one way or the other. Eventually they make part of the wetland-based food chain. In due course, they do not only exchange energy (food), but also diseases.

It was therefore hypothesized that in the wetlands of Zwai there should be life cycles of parasites that use the visiting animals.

- Could these diseases be reflected in domestic animals such as sheep?
- Could some of them go as far as humans, including children?
- Could some of the snail species serve as intermediate hosts of trematode diseases of sheep?
- Could some of gastrointestinal nematode parasites of sheep develop resistance against frequently used anthelmintic drugs?

1.3.2 Research questions

Based on the above objectives, the following research questions were addressed in this dissertation.

1. What is the current distribution of snail species with the potential of being vectors of humans and domestic animal diseases at Zwai wetland?

2. What are the factors that influence the seasonal prevalence of gastrointestinal helminth parasites of sheep in Zwai wetland?

3. What is the role of the wetland located on the shores of Lake Zwai in the occurrence of gastrointestinal parasites in schoolchildren at Bochesa Elementary School?

4. How prevalent is anti-helminthic resistance and what are the factors contributing to drug resistance by helminth parasites of sheep in the wetlands of Lake Zwai?

1.4 Description of the Study Area

This study was carried out at selected grazing areas of the wetlands of Lake Zwai, which is one of the Great East African Rift Valley Lakes of Ethiopian at 8° 01' N and 38° 47' E (Fig. 1.1) and at an altitude of 1636 m above sea level (Von Damm and Edmond, 1984). The Lake is fed by Ketar and Meki Rivers that flow from the Southeastern and Northwestern highlands, respectively, and drains into Lake Abijata through the Bulbula River in the south. The climate is characterized as semi-arid to sub-humid with mean annual precipitation and temperature of 650 mm and 25°C, respectively. The south-western parts of Lake Zwai (from Seda to Woshgula) were the grazing sites for ruminant animals of this study. In these sites, the lake is used by people as a source of income, primarily fishing, but also for bathing, washing clothes and watering animals.

Zwai wetland is seasonal-type and its size and water level varies at different seasons. It is predominantly fed by surface flow as well as spring flow and groundwater seepage during the wet seasons. Given that the wetlands in this study are located along the shoreline of Lake Zwai, the study has similar climatic conditions.

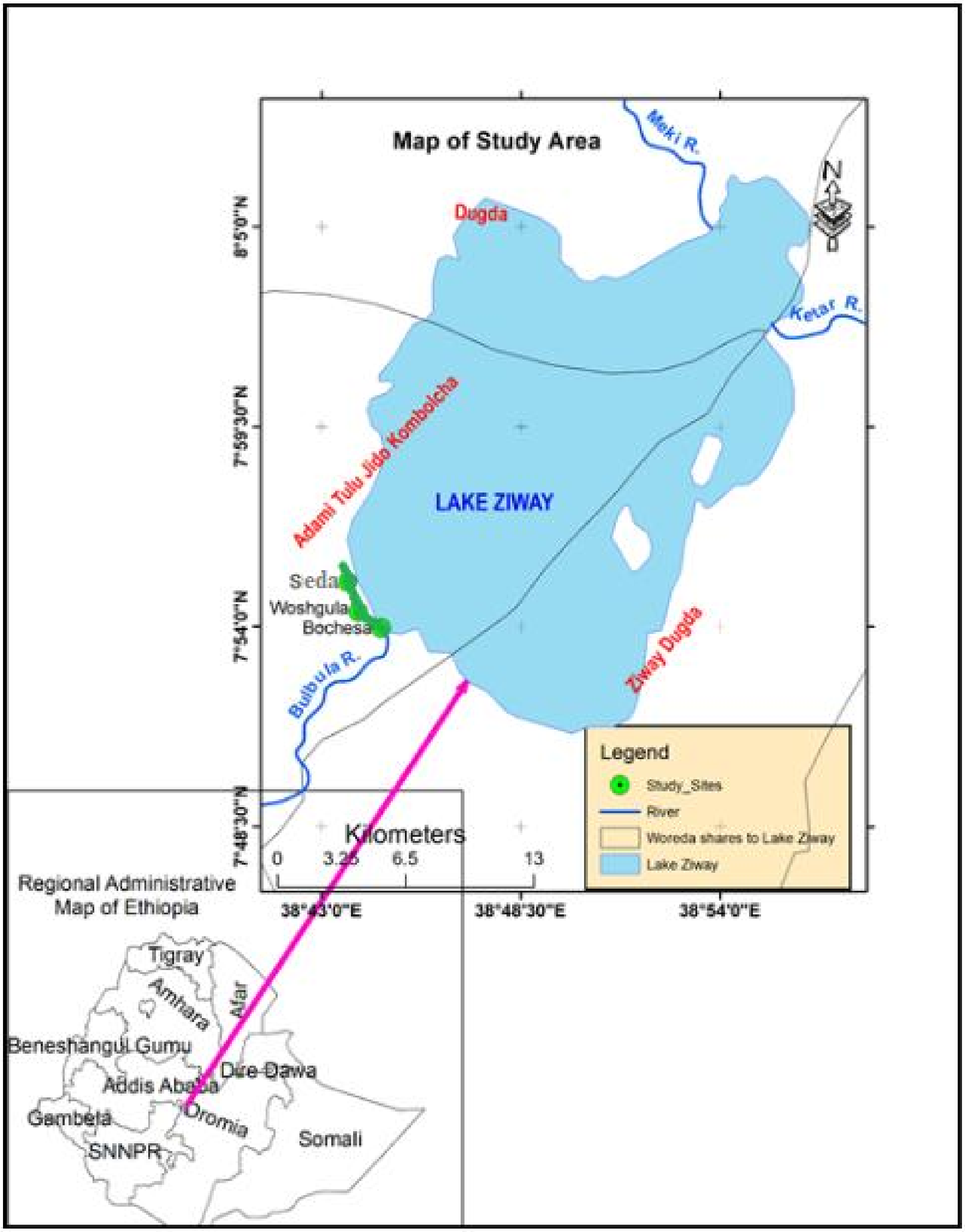

Fig. 1.1: Map of Lake Zwai with the grazing sites hereafter referred to as Zwai wetlands.

1.5 Thesis Outline

The first chapter outlines the introduction, objectives, hypothesis and research questions of the study, and description of the study area.

The second chapter deals with the literature review, mainly focused on water-related diseases, drivers of water-related diseases, interactions among water-related diseases in wetlands, impacts of climate on water-related diseases, humans behavior and water-related helminth diseases, the role of wetlands for grazing animals and water-related diseases, Zwai wetland and climate patterns and proper utilization of anthelmintic drugs.

The third chapter deals with study of Zwai wetlands for the seasonal prevalence of gastrointestinal helminth parasites of sheep. Using tracer experiment of sheep, adult worm count, faecal egg count and PCV (%) were conducted. Overall prevalence of gastrointestinal helminth parasites of sheep and the types of helminth parasite species were addresses. Climatic data were correlated with seasonal prevalence of gastrointestinal helminth parasites of sheep.

The fourth chapter deals with study of Zwai wetlands for the prevalence of gastrointestinal parasites on schoolchildren at Bochesa Elementary School around the lake. The prevalence of gastrointestinal parasites was compared with categorical variables (sex, age and grade levels). Gastrointestinal parasite co-infections were identified. Potential risk factors associated with gastrointestinal parasites were analyzed.

The fifth chapter deals with the species composition, abundance, diversity and seasonal variation of freshwater snails in relation to some physicochemical parameters of the wetlands of Lake Zwai. The possible reason for the dominance of *P. acuta* from the other freshwater snails is also discussed.

The sixth chapter deals with the assessment on the occurrence of anti-helminthic resistance and factors contributing to the development of resistance on sheep under field conditions. The test was conducted on three treatment groups of sheep using Albendazole, Tetramizole and Ivermectine, which are commonly available in the local market of Zwai Town. The faecal egg count reduction test (FECRT) was used for the evaluation of anthelmintic efficacy/resistance. The reason for the occurrence of Albendazole resistance was justified.

The seventh chapter deals more on the general conclusions and recommendations. It concludes the major findings of the thesis and recommends some controlling options of water-related gastrointestinal parasites of sheep and humans in wetland areas. It also highlights research gaps for further study.

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 Water-related Disease

In wetlands, water-related diseases are the major causes of morbidity and mortality worldwide. In developing countries like Ethiopia, water-related diseases are among one of the major public and veterinary health problems (Melake Demena *et al*., 2003). As wetlands support numerous benefits for organisms, the interactions among humans, sheep, intermediate hosts and parasites and herbages in the wetland environment continue to occur as wetlands are their meeting places. Water-related disease in both epidemic and endemic forms continue to occur in both developed and less developed countries. One of the causative agents of water-related diseases are helminths.

Helminthosis is a major cause of mortality and sub optimal productivity in sheep in traditional farming systems in sub Saharan African countries (Kusiluka and Kambarage, 1996).

The Trematode species that are parasitic in sheep belong to the subclass Digenea (Urquhart *et al*., 1996). The most important of these species in Africa are the liver flukes, *F. hepatica, F. gigantica* and *Dicrocoelium* spp., and rumen flukes (*Paramphistomum* spp.) (Soulsby, 1982; Hansen and Perry, 1994; Urquhart *et al*., 1996).

The most important Cestode parasites of sheep, in terms of public health and veterinary medicine, belong to the family Taeniidae (Radostits *et al*., 1994). These include cystic or larval stages of *Echinococcus granulosus, Taenia hydatigena,* and *T. multiceps*.

Moreover, adult cestode parasites of the genera *Moniezia*, *Avitellina*, *Thysanosoma* and *Stilesia* are also common parasites of sheep in African countries (Urquhart *et al*., 1996).

Most waterborne helminth diseases of sheep are transmitted by drinking contaminated water and / or by grazing on pastures contaminated with infective stages of the helminth parasites (Hansen and Perry, 1994; Urquhart *et al*., 1996). Freshwater environments can function as transmission foci for pathogens because of the importance of freshwater for organisms' survival, the aquatic life histories of many intermediate hosts, the concentrated aggregations of species in and around freshwater habitats and the highly altered condition of freshwater ecosystems, which can affect species interactions and disease pathology (Johnson and Paull, 2011). Waterborne helminths diseases are among the major public health problems in developing countries like Ethiopia (Melake Demena *et al*., 2003).

2.1.1 Conditions for the development of water-related helminth diseases of sheep

There are five critical conditions in the transmission of infectious agents through water: the source of the infectious agent, specific water-related modes of transmission, attributes of the organism that allow it to survive and possibly multiply and move into and within the aquatic environment, the infectious dose of the organism, and host susceptibility factors (WHO, 2014). All these conditions prevail in wetlands where wild and domestic animals converge by day or by night for grazing and fetching water to drink. For instance, the effect of infestation by gastrointestinal helminth parasites varies depending on the parasite species involved, the degree of infestation and other factors such as host species, sex, age, season and intensity of worm burden (ILCA, 1990).

2.1.1.1 Grazing behavior of the animal

The grazing behavior of some livestock helps them to escape from gastrointestinal helminth parasite infection such as goats that feed on foliage high up from the ground surface, where larval stages of helminth parasites could not find favorable refuge. In contrast, sheep, foraging on fresh and succulent wetland grasses from the ground, have high probability of collecting infective larvae that stay lodged in the dew of grasses ready to be picked by the definitive hosts such as sheep or even children that play in the wetlands or adults collecting fresh grasses for marketing and accidentally transfer the larvae into their mouths (Hutchings *et al*., 2001). This is particularly true when sheep or other organisms visit the wetlands early morning at sunrise.

Grazing animals assimilate only a small portion of the nutrients they consume and as a result substantial amounts of N, P and K are excreted into the wetland through faeces and urine (Morris and Reich, 2013). During this time, eggs of helminth parasites dropped with animal faeces to be hatched in the wetlands. The eggs of many parasites, present in faeces deposited by herbivores on the vegetation, develop into infective, third-stage larvae which migrate onto the surrounding vegetation and are ingested by herbivores (Fleurance *et al*., 2007). According to Han *et al*. (2017), most sheep are more likely to contact with the pasture in wetlands contaminated with gastrointestinal helminth eggs in faeces, which resulted in high and frequent infection. The grazing animals in wetlands being exposed to parasites and are thus constantly being re-infected when they graze in contaminated pastures (Kumar *et al*., 2013).

In Ethiopia, the main source of sheep feed is grazing on natural pasture in the wetlands. Sheep being grazers of pastures at the ground level, are regarded as museums of wide spectrum of nematode parasites that are collected from wetlands (Amin and Wani, 2012). Consequently, it has long been thought that avoidance of contaminated pastures of wetlands by ruminants such as sheep might be one defense strategy of bypassing that infective stage of parasites. Sight and smell are likely to be the principal senses involved in the recognition of fresh faeces. Several studies have shown that domestic sheep use faecal avoidance as a cue for parasite avoidance (Cooper *et al*., 2000; Hutchings *et al*., 2001), which suggests that selective foraging in relation to faeces serves a distinct anti-parasite function in these animals.

2.1.1.2 Grazing season in the wetland area

A seasonal pattern of gastrointestinal helminth parasite infections of pastures occur in the tropics where transmission is mainly restricted to the wet seasons (Radostits *et al*., 1994; Urquhart *et al*., 1996). The prevalence of gastrointestinal helminthes is influenced by climatic conditions (Nibret Moges *et al*., 2011). In wetlands, the level of helminth parasite infection on the pasture varies with the season (Ng'anga'a *et al*., 2004). Rainfall is the major factor determining the availability and transmission of gastrointestinal helminthes to sheep on pastures.

Once the rainy season start and environmental conditions become favorable for the hatching of eggs and the subsequent survival of the infective larvae, the hypobiotic larvae mature and there is a continuous cycle of infection between the host and pasture for as long as these conditions last. In Ethiopia, the level of waterborne helminths parasite infection on the pasture varies with the season (Ng'anga'a *et al*., 2004). Wetlands provide critical areas for sheep grazing, especially during the dry season (Matheson, 2004). However, high levels of infection were found among sheep during the wet season. Although the sheep on these wetlands are not allowed to graze during heavy rainfall months, farmers use a cut-and-carry system in which animals are fed vegetation from marshy areas, thereby creating the opportunity for most infective stages of the helminths parasites to be transferred to the definitive hosts such as sheep (Eyerusalem Gebeyehu *et al*., 2014).

In general, moist and warm environmental conditions are favorable for the development, survival and transmission of the larval stages of gastrointestinal helminth parasites (Hansen and Perry, 1994; Urquhart *et al*., 1996), which happens to be the primary nature of most wetlands in Ethiopia. The prevalence of gastrointestinal helminth parasites generally increase the onset of the wet season, consequently inundation of water shores in most tropical countries including Ethiopia.

During wet seasons, heavy rainfall and high relative humidity predispose grazing animals to water-related gastrointestinal helminth diseases (Almalaik *et al*., 2008; Al-shaibani *et al*., 2008; Abdelnabi *et al*., 2011; Kuchai *et al*., 2011; Kumar, 2014; Rashid *et al*., 2016). According to Kuchai *et al*. (2011), a direct relationship exists between prevalence of gastrointestinal helminth parasites with the monthly rainfall, humidity and temperature. A high rate of infection in rainy months can be attributed to suitable molarity of salt present in the wetlands which is an important factor for ecdysis of nematode parasitic larvae (Soulsby, 1982).

It has been shown that sheep defecate in wetlands than in surrounding paddocks (Collins *et al*., 2007). The faecal droppings from livestock with the availability of moisture and optimal temperature in wetlands are the conducive for the hatching of eggs. The hatched larvae must move away from the faecal mass and reach to the herbage to be ingested by grazing sheep. The movement of the larvae is assisted by a continuous film of moisture on herbage (Wheeler, 2001). The hatched parasite's eggs develop into infective larvae L_3 stage in faecal materials due to the presence of available moisture and food during the availability of rainfall (Radostits *et al*., 1994).

Wetlands provide critical areas for sheep grazing, especially during the dry season (Matheson, 2004). However, the level of infection on the grazing animals is less in the dry season as compared to the wet seasons. Although sheep are not allowed to graze on wetlands during heavy rainfall months, farmers use a cut-and-carry system in which animals are fed fresh vegetation harvested from wetlands, thereby creating an opportunity for most gastrointestinal helminth infections to spread out into the upland (Eyerusalem Gebeyehu *et al*., 2014).

2.1.1.3 Livestock husbandry practices associated with wetlands

The grazing system in practice in rural communities such as in Ethiopia has an impact on the health of animals. Traditional sheep rearing in developing countries are mainly dependent on the availability of marsh areas in the watershed of lakes and rivers for the free open-field grazing of their sheep. The limitation of grazing and watering places, which are shared by all farmers and the fact that helminths control is not coordinated are factors likely to result in environmental contamination with parasitic eggs and the subsequent development of infective stages of the parasites in any communal set up (Maposa, 2009).

In many grazing systems throughout the world, land is held communally (WHO, 2004). Communal grazing areas, particularly where grazing animals converge in wetlands, are the main sources of gastrointestinal helminth parasites. Sheep management under operation at any system has an effect on the prevalence of helminthes infection in sheep (Sangma *et al*., 2012).

The sheep management in Ethiopia is mainly based on extensive grazing of communal grazing lands including wetlands. Wetlands serving for grazing and watering places, are shared by all farmers and hence they are factors likely to result in environmental contamination with gastrointestinal helminth parasitic eggs and the subsequent development of infective stages in wetland grazing areas (Maposa, 2009). Free grazing in wetlands therefore exposes animals to water-related helminth parasites. In general, moist and warm environmental conditions are favorable for the development, survival and transmission of the pre-parasitic stages of helminths (Hansen and Perry, 1994; Urquhart *et al*., 1996).

2.1.1.4 Stocking density in wetlands

In pastoralist regions of the country, stocking density of the grazing sheep has an effect on the occurrence and transmission of gastrointestinal helminth diseases. High stocking density in communal grazing areas like wetlands and oases increase the contamination of the environment with gastrointestinal helminth eggs or larvae and thus makes the infective stages to be more accessible to susceptible animals (Yousif, 2010).

In communal grazing wetlands, overstocking is a major factor for the transmission of disease as the situation forces the animals to graze closer to faecal droppings, which result in their exposure to infective larvae of gastrointestinal nematode parasites. Research conducted in different parts of the world indicated that the prevalence of gastrointestinal nematode parasites was higher in sheep of large flock (Sangma *et al*., 2012).

Pastures are contaminated, if the infective larval stages of helminth parasites are attached onto the herbage and climatic conditions are suitable for them where they can be ingested by grazing animals (Desalegn Lidetu, 2005; Abbot *et al*., 2007; Fleurance *et al*., 2007). This situation facilitates the transmission of gastrointestinal nematode parasites from one to the other with wetlands becoming the central medium of transmission.

The concentration of livestock at watering points of wetlands particularly during the dry season may also result in massive contamination of pastures with eggs or larvae of gastrointestinal helminth parasites (Yousif, 2010). In communal grazing areas like Zwai wetland, overstocking is a major factor for the transmission of disease as the situation forces the animals to graze closer to faecal droppings, which results in the exposure of sheep to infective larvae of gastrointestinal helminth parasites. This might be due to the fact that wetlands provide potential habitat for intermediate hosts of parasites, which facilitate the transmission diseases to grazing animals (Malan *et al.*, 2009).

2.1.1.5 Ages of the grazing animal in wetlands

Numerous research conducted in different parts of the country and elsewhere indicated that young sheep are highly susceptible to gastrointestinal helminth parasites than adults (Sangma *et al*., 2012; Kakar *et al*., 2013). According to these authors, young sheep of the age of less than a year, lack strong immunity system as in the adults, where after primary infection, some level of immunity is expected to be acquired. According to Radostits *et al*. (1994), sheep over 18 month of age are less commonly affected because of immunity, resulting from previous infection.

Sheep in their first grazing season tend to have no or severely limited natural immunity to gastrointestinal nematode parasites. The availability of L_3 stage on pasture serves to infect sheep. The level of larvae on pasture and the level of immunity in the sheep will determine the level of disease seen in the sheep. Over the grazing season, the loads in the sheep tend to increase and they become the major contributors to egg contamination on pasture (Keeton, 2016).

2.1.1.6 The role of sexes of the grazing animals in wetlands

It is assumed that sex is a determinant factor influencing prevalence of gastrointestinal helminth parasites and females are more prone to infection during pregnancy and per-parturient period due to stress and decreased immune status (Urquhart *et al*., 1996; Keyyu *et al*., 2003; Fikru Regassa *et al*., 2006). The prevalence of gastrointestinal helminth parasites are higher in females than in male sheep (Sangma *et al*., 2012; Kakar *et al*., 2013).

Higher prevalence of gastrointestinal helminth infection in female sheep cannot be explained exactly but it can be assumed that the alteration in the physiological condition of females during pregnancy, lactation and parturition as well as stresses lead to reduction of immunity. Higher level of prolactin and progesterone hormones makes female individuals more susceptible to infections (Lloyd, 1983).

2.1.2 Interactions among water-related helminth diseases in wetlands

The emergence of water-related helminth disease in wetlands is a complex process involving biological, social, and ecological factors. The free-living stages of helminths parasites of grazing animals have two basic environmental requirements, namely warm temperature and availability of high moisture with fresh water of considerably low salinity (Desalegn Lidetu, 2005). Pastures are contaminated, if there are eggs and larvae present, pastures are infective, if L_3 stages of parasites are present and climatic conditions are suitable for them to move onto the herbage where they can be ingested by grazing animals (Desalegn Lidetu, 2005; Abbot *et al*., 2007). The shoreline of a lake is a common place for the completion of the life cycles of different organisms. Wetlands in the shoreline of lakes provide potential habitat for vectors or intermediate hosts of parasites, which are sources of such disease for humans and its animals (Malan *et al*., 2009). Many higher species of animals are attracted to wetlands in search of food, shelter and water or even to cool up themselves. While doing so, they interact with each other in one way or another. Eventually, they make part of the wetland-based food chain. In this area, human beings are also involved in fishing, bathing, swimming or washing clothes.

Faeces and urine from both humans and other animals are likely to be the largest source of water-related disease transmission. Under favorable conditions, some water-related pathogens can multiply in the aquatic environment. Some water-related helminths pathogens may be amplified within intermediate aquatic hosts that are commonly found in wetlands. Freshwater environments can function as transmission foci and/or reserves for pathogens because of the importance of fresh water for organism survival, the aquatic life histories of many intermediate hosts, the concentrated aggregations of species both freshwater and terrestrial in and around freshwater habitats and the highly altered condition of freshwater ecosystems, which can affect species interactions and disease pathology (Johnson and Paull, 2011).

Water-related helminth diseases are inherently ecological processes involving interactions between at least one host and one parasite in well-moistened areas. However, such interactions are often subject to influences from a suite of co-occurring species, including other pathogens, other hosts and a community of non-host species. Interactions among these species and with the abiotic environment can profoundly influence the likelihood of a disease outbreak. Disease is thus a specific state of pathology, and simple exposure to a pathogen may or may not induce disease depending on host condition, exposure dosage and factors such as local climate conditions (Johnson and Paull, 2011).

The central importance of host pathogen environment interactions is well recognized in epidemiology. These interactions create practical challenges for understanding disease causality and epidemiological patterns in natural environments. Environmental cofactors, multi-host parasite life cycles, state-dependent pathology (such as physiological stress or host age) or host–pathogen interactions that are difficult or unethical to manipulate in an experimental setting can all complicate the process of identifying the etiological agents responsible for disease (Johnson and Paull, 2011).

Wetlands contribute in diverse ways to the livelihoods of millions of people (Mccartney *et al*., 2010). Lakes and their surrounding wetlands provide important supply of water (particularly when the water is as fresh as possible) for domestic uses and livestock to the local population. The productivity of livestock increases when adequate feed and shelter are provided. However, the problem of matching livestock numbers with the available pasture can be a complex scenario in the management of livestock and grazing pastures, where land and water systems are owned communally as in Ethiopia.

2.1.3 Impacts of climate on water-related helminth diseases

Climate change and global warming are the major concern that will define livestock production systems and livestock productivity. Climate change is altering epidemiological patterns of gastrointestinal helminth infections in grazing livestock, including through effects of temperature and moisture for hatching of the eggs and the development of the larvae to its infective stage (Wang *et al*., 2018). Of all the factors influencing livestock production, climate with extended wet periods and location (near to marshy areas) are undoubtedly the most significant, while temperature is not limiting in the tropics as in Ethiopia. In fact, weather conditions such as ambient temperature and rainfall patterns have a great influence on pasture and food resources availability cycle throughout the year, and types of disease and parasite outbreaks among animal populations (Lamy *et al.,* 2012).

In wetlands that serve as communal grazing areas, the pasture quality and quantity are influenced by the seasonal changes in rainfall to attract grazing animals. Climate change is considered as a driving force for recent parasite range expansions (Fox, 2012). It will act to increase or decrease the prevalence of disease, injury or other health issues. When addressing climate change, it is important to be aware that climate change can directly or indirectly impact health. Climate has direct effects on the size of parasite populations by affecting fecundity, rates of development and survival of free-living parasites (Sutherest, 1987). Climate change has been implicated as a driving force for recent parasite range expansions that resulted in the expansion of warm and humid weather is not highlands following global warming. The climate in a certain locality is one of the major factors that determine the type and severity of parasitic infection in grazing animals (Amin and Wani, 2012). The development and survival pattern of infective larvae in the environment differs according to the climate, particularly in relation to warm conditions that speed up metabolism in the organisms and availability of fresh water. Thus, the influence of global warming may play an important role in the occurrence of parasitic disease (Maposa, 2009).

More specifically, climate has an impact on the free-living stages of helminths parasite and their intermediate host snails with the interactions between rainfall and temperature having the greatest influence on transmission efficacy (Fox, 2012). Parasite abundance and larval availability are directly affected by climate through the influence of temperature and moisture on development, dispersion and mortality of the free-living stages (Verschave, 2015). Climatic factors also influence the infective stage of helminths parasites dispersion on the herbage which increases the chance of contact between host and larvae (Urquhart *et al*., 1996). In Ethiopia, short periods of rainfall or irrigation make favorable conditions for the survival of helminths parasites larvae (Micheal Asrat, 2004). The semiarid regions of the country have conducive environment for the helminth parasites, particularly for *H. contortus* as a more rapid translation of eggs to larvae can occur in warm wet conditions (Sissay Menkir, 2007), that are commonly observed in wetlands of tropical countries such as Ethiopia.

2.1.4 Human behavior and water-related helminth diseases

The history of human settlements was and is wetland-based. According to Yilma Abebe and Geheb (2003), the Great Rift Valley of Africa has yielded fossil evidence of some of the world's earliest hominids. This area suggests that in order for humans to live here, the Ethiopian Rift must at one time have been better watered than it is presently. Wetlands provide economic and social values for humans and this indicates that wetlands and people are ultimately interdependent (Bhattarai, 2015). People in different locations interact with their environment with different pressures on ecosystems and uses of ecosystem services in wetlands. Human behavioral changes, driven by increasing population, economic and technological development, and the associated spatial expansion of agriculture, are creating more intensive interactions between humans, livestock, and wildlife (Jones *et al*., 2013). These changes have been implicated as drivers of some recent emerging disease events that had important impacts on human livelihoods and health. Even though, climate is an important driver of helminth distribution, transmission of those parasites to livestock is also controlled by human animal husbandry practices (Fox, 2012).

Many Ethiopians depend on wetland resources for their survival. People tend to establish developments and raise cattle close to local water sources, lakes and reservoirs in Africa function as reliable transmission foci for trematode diseases, which for instance are transmitted from freshwater snails to mammals (Johnson and Paull, 2011). These conditions can be exacerbated during droughts when water availability is reduced and hence people and animals converge on any available wetlands. High stocking density in wetlands increases the contamination of the environment with helminths eggs or larvae and thus makes the infective stages to be more accessible to susceptible animals (Yousif, 2010).

2.2 Zwai Wetlands and Climate Patterns

The area around Lake Zwai has a wet season from July to September, dry season from October to January, and a season of highly variable rainfall from February to June. The climate of the area around Lake Zwai has semi-arid characteristics for most of the year. Over 50% of the annual rainfall is received during the three-month wet season, rainfall distribution is variable and long dry periods are common (MoWR, 2008).

Despite this more or less regular weather pattern of the area, there is a strong influence or impact of climate change observed in the fluctuation of the wetland areas of Lake Zwai shores. Consequently, climate-related hazards have a significant impact on waterborne disease of animals and humans. Due to the climatic effect that results in rainfall variability, the water level of the lake is varying and so are the sizes of the wetlands around the lake. This area is therefore remains the convergence spot of wild and domestic animals where they come in search of water and fresh herbage, thereby exchanging waterborne diseases particularly helminth parasites.

During the dry months of the year, due to high evaporation of water in wetlands, concentration of salts increases (El-khayat *et al*., 2017) and leads the desiccation of eggs of gastrointestinal helminth parasites of livestock.

2.3 Proper Utilization of Anthelmintic Drugs

In many parts of developing countries including Ethiopia, Gastrointestinal helminth infections are very common and their control is almost entirely based on anthelmintic drugs (Fikru Regassa *et al*., 2006). To improve productivity of sheep, the control of gastrointestinal helminthes parasites relies heavily on the administration of few classes of anthelmintic drugs (Walkite Furgasa *et al*., 2017).

For the control of gastrointestinal nematode parasites of livestock, farmers prefer anthelmintic drugs as they are simple to administer, cheap and cost-effective. They kill existing parasites and reduce the production of eggs. Therefore, they can prevent diseases in infected animals and reduce the intensity of future infection in infected animals and their offspring (Stear *et al*., 2007).

Administration of correct dose of anthelmintic after assessing parasitic load and body weight of animals to prevent resistance is necessary (Kumar *et al*., 2013). In Ethiopia, the use of anthelmintic drug has been practiced for a long time and constitutes a major share of the veterinary service costs (Demelash Biffa *et al*., 2007). Three broad spectrum anthelmintic families are commonly used in Ethiopia to control gastrointestinal nematodes of sheep: benzimidazoles, imidazothiazoles and macrocyclic lactones (Silvestre *et al*., 2002). In Ethiopia, three anthelmintic drugs, specifically Albendazole, Tetramizole and Ivermectine were continuously imported and distributed throughout the country (Desie Sheferaw *et al*., 2013).

Currently, worm control strategies involving anthelmintic are under serious threat because of the development of anthelmintic resistance. There are factors for the occurrence of drug resistance in a certain area. Of these, misuse like under dosage and frequent use of anthelmintic drugs in many forms, such as illegal sales in open markets and irrational administration, are widespread in Ethiopia (Bersissa Kumsa *et al*., 2010). Thus, anthelmintic resistance has become a global problem (Coles *et al*., 1992).

CHAPTER 3.

STUDY OF ZWAI WETLANDS FOR THE SEASONAL PREVALENCE OF GASTROINTESTINAL HELMINTH PARASITES OF SHEEP, ETHIOPIA.

3.1 Introduction

Wetlands are among the world's most productive environments in the world and they provide a wide range of ecosystem services and economic benefits (Barbier *et al*., 1997). Wetlands are now known for their exceptionally high biodiversity. They deliver a wide range of ecosystem services that contribute to human wellbeing and the survival of other animals (Niu *et al*., 2009). Wetlands that serve important natural biological functions, including food production, general habitat and nesting, spawning, rearing and resting sites for aquatic and some terrestrial animals that frequently visit wetlands for one or another benefit or benefits (May, 2001), for which wetlands are also known as "biological supermarkets" (Barbier *et al*., 1997, Clarkson *et al*., 2013 and Niu *et al*., 2009). In Ethiopia, wetlands cover nearly 2% of the total land area of the country (Tadesse Amsalu and Solomon Addisu, 2014), which happen to be in most cases grazing area for livestock, particularly sheep (Berhanu Gebremedhin *et al*., 2007 and Dawit Assefa *et al*., 2013). Sheep are the second most important species of livestock in Ethiopia, providing up to 63% of cash income and 23% of the food subsistence value obtained from livestock production and they are living banks for their owners and serve as source of immediate cash (Solomon Gizaw *et al*., 2010).

Even though sheep contribute much to the national economy and the livelihood of the rural community, their production is hampered by several constraining factors.

One of the major challenges of sheep production in Ethiopia is disease of which helminths infection ranks among the highest. Due to this reason, the current contribution of the livestock subsector in Ethiopia is below its potential); particularly, sheep production (Solomon Gizaw *et al*., 2010 and Berhanu Gebremedhin *et al*., 2007). The productivity of Ethiopian sheep is affected by diseases and specifically, Helminthosis, which is among most damaging endo-parasite infections that are responsible for economic losses through reduced productivity and increased mortality (Hansen and Perry, 1994).

In Ethiopia, Helminthosis has considerable potential to negatively affect animal performance, because sheep are kept on pasture in wetlands throughout the year and climatic conditions favor the rapid development of free-living stages of the parasites in wet grounds. Three classes of helminths responsible for helminthic disease distinguished in sheep are, namely Trematodes, Cestodes and Nematodes (Hansen and Perry, 1994; Radostits *et al*., 1994; Urquhart *et al*., 1996), which are known to be highly prevalent in wetlands containing fresh water. Gastrointestinal nematode infections are the main prevalent parasitic diseases affecting small ruminant productivity worldwide, especially in tropics and sub-tropics (Torres-Acosta and Hoste, 2008). The most common nematode parasite species of sheep are *Haemonchus* spp., *Trichostrongylus* spp., *Oesophagostomum* spp., *Bunostomum* spp., *Trichuris* spp., *Ostertagia* spp. and *Dictyocaulus* spp. that require fresh water such as found in wetlands to complete parts of their life cycles.

To increase the potential of sheep production and to ensure the optimum benefit out of them, prevention and control of water-related helminths parasite of sheep is crucial.

Hence, this study was designed with the aim of assessing the role of Zwai wetlands for the seasonal prevalence of water-related gastrointestinal helminths disease of sheep.

3.2 Material and Methods

3.2.1 Study animals

The tracer animal method using sheep was employed to assess the pattern of seasonal parasitism of helminths in sheep. Forty-six sheep were used as tracer animals to assess the load and patterns of water-related helminthic diseases of sheep over a period of two years. For this purpose, indigenous sheep of both sexes and 4-6 months of age (commonly referred to as lambs) were purchased from the local market of Zwai town which is found in close proximity from the study sites. At the research site, the experimental animals were ear-tagged and weighed. They were treated with anthelminthic drugs (Albendazole at 7 mg/Kg body weight and Ivermectine 0.2 ml) to clean them previously acquired diseases and sprayed them with an acaricide for the control of external parasites such as ticks, mites and lice that may interfere in the study.

3.2.1.1 The tracer animal method

In this study, tracer sheep were categorized into experimental tracers, on-field control tracers and housed controls (Brook Lemma *et al*., 1985; Desalegn Lidetu, 2005).

A. Experimental tracer sheep

A batch of experimental tracers consisting of 2 sheep were released at the selected wetland sites around Lake Zwai to graze on the pastures on a monthly basis and remained on pasture under the watchful eyes of a shepherd employed for this purpose.

At the end of each month, the sheep were housed under parasite-free conditions. Here, they were kept in well ventilated clean barn. They were provided with clean water and feed for three months until the parasites presumably collected in the wetlands mature in them, and finally slaughtered for post-mortem examinations (Brook Lemma *et al*., 1985) from whom their livers, lungs, abomasums, small intestines, colons and caeca were collected, contained in plastic bags to be examined fresh in less than one hour of the post-mortem examination. This process was repeated for 24 consecutive months (Appendix-I).

B. On-field control tracer sheep

Only two sheep, designated as on-field control tracer sheep (hereafter referred as control tracer sheep) were released simultaneously with the experimental tracer-sheep in the selected wetland pastures of Lake Zwai grazing sites. At the end of each month, these sheep were treated with the manufacturer's recommended dose rates of Albendazole and Ivermectine and released again with the next batch of the experimental tracer-sheep to the selected wetlands. This process was repeated every month up to the end of the study period to establish the effectiveness of the drugs and those sheep were expected to be housed at the end of the last grazing month (Brook Lemma *et al*., 1985). However, these two on-field control tracer sheep died prior to the three months before the end of study period due to suspected Anthrax. However, postmortem examinations could not be conducted on them, as veterinarians do not recommend dissection of sheep that die due to Anthrax, rather they were incinerated to avoid risk for outbreak of Anthrax.

C. Housed control sheep

Two sheep, designated as housed control were dewormed and kept housed with the first batch of experimental tracers, and remained housed until the end of the study period and slaughtered for post mortem examination with the last batch of experimental tracer sheep. The purpose of keeping these sheep indoors was to demonstrate the helminth-free status of the housing conditions (Brook Lemma *et al*., 1985; Desalegn Lidetu, 2005) and these sheep were finally slaughtered for post-mortem examination and the same organs as the experimental animals were preserved for latter examination.

3.2.2 Worm recovery during post-mortem examinations

A total of 46 gastrointestinal tracts were examined from the experimental tracer sheep. Necropsy procedures were modified from those recommended by Hansen and Perry (1994). Double ligatures were applied to separate different organs (abomasum, small intestines, caecum and colon). Each organ was carefully stripped off from fat and mesenteries, and separated at their junctions to prevent worms spilling out the organs, and placed in separate containers, which were pre-labeled with the tag numbers of the tracer sheep, opened with scissors and washed within four hours after slaughtering of the sheep as described by (Hansen and Perry 1994). Each organ was processed separately.

Following the procedures of Hansen and Perry (1994) and Urquhart *et al*. (1996), each abomasum was legated at both ends and separated from the omasum and duodenum and tied with string and finally opened longitudinally along its greater curvature and transferred to the laboratory in labeled plastic bucket. The contents of each abomasum were poured in a 2L bucket. The mucosa of abomasum wall was washed thoroughly several times under running tap water, paying attention to both sides of the abomasum folds rubbed with the thumb and the index finger to remove any adhering worms and the washings drained into the bucket. The material retained on the tray was backwashed into a bucket. More water was added to make up a total volume of 2L and the contents in the bucket were homogenized and left to settle for 15 minutes and later the supernatant was decanted through a sieve of 200µm mesh. Water was added to the remaining content in the sediment to make up the volume of 2L again and removal of the supernatant was continued. The contents were processed by repeated washing, sedimentation and decantation until the supernatant was clear enough to allow for easy worm counting. If the nematodes were too small to count, all were counted without taking subsamples. In cases where the nematodes were too numerous to count, subsample (200ml) was transferred to a labeled graduated cylinder in five steps of 40ml per step while string the mixture continuously. Small amount of this 40ml were placed in a Petri dish having parallel lines and examined under stereomicroscope of 40X magnification for worm count and total numbers calculated accordingly. The identified and counted worms were placed in pre-labeled worm collecting vials. At a later time, identification of collected worms was conducted under binocular microscope at 40X magnification to the genus and whenever possible to species levels using keys from Hansen and Perry (1994).

Similarly, the small intestines, the colons and the caeca were examined and the respective parasites were collected and kept in vials against the tag numbers of each sheep.

The liver and the gall bladder were removed. The gall bladder was then separated from the liver, emptied and washed in to a white bowl. Contents in the bowl were mixed with water and homogenized. Adult flukes were collected, counted and recorded. The bile ducts of the liver were opened with scissors and visible adult flukes were removed and placed in 70% alcohol. The liver was then cut into approximately 1cm thick slices and pressed between the fingers to expose flukes lodged in small bile ducts. The number of flukes thus recovered were counted and recorded.

The lungs were opened with scissor for examination of parasitic nodules. All suspected nodules detected in the lungs were examined in detail through dissection. The trachea was opened with fine blunt pointing scissors, searched for the presence of adult worms. The major bronchial airways were opened and closely inspected for the presence of lungworms (Sissay Menkir, 2007). The lungs were cut into pieces and immersed in physiological saline solution and placed in an incubator at 37°C for two hours. The lung pieces were latter removed and small amount of bronchial fluid obtained by squeezing the lungs were poured through sieve (Kabakci *et al*., 2007). The washings of the trachea and the bronchi were mixed with the contents of the lung. The residue on the sieve was carefully washed and marked to pre-labeled plastic bottle. All visible parasites were collected using forceps, and processed using the descriptions of Boomker *et al*. (1989) and Umur and Yukari (2005).

3.2.2.1 Identification of the parasites collected

All the collected nematodes were cleared in lacto-phenol and identified into species level using Hansen and Perry (1994) and Urquhart *et al*. (1996), and counted.

3.2.2.2 Level of infection

The level of infections was categorized as lightly, moderately and severely infected according to adult worm counts described by Hansen and Perry (1994) with some modifications (Table 3.1).

Table 3.1: Number of worm counts as indicator of levels of severity of helminth parasites in sheep.

No.	Parasites	Light	Medium	High
1	*Haemonchus contortus*	1-500	501-1,500	over 1,500
2	*Trichostrongylus axei*	1-1000	1,001-10,000	over 10,000
3	*Oesophagostomum columbianum*	1-50	51-150	over 150
4	*Bunostomum tringoincephalum*	1-20	21-50	over 50
5	*Trichuris ovis*	1-50	51-500	over 500

3.2.3 Haematocrit test

Blood samples were collected from all experimental tracer lambs for hematological analysis via jugular vein puncture with the help of disposable syringe and transferred into 10ml EDTA (ethylene di-amine tetra acetic acid) tubes and placed on ice immediately after collection until processing. All blood samples were labeled with identification number and date of collection and transported in ice boxes to laboratory. Packed cell volume (PCV) and plasma concentration (PC) were analyzed within 1-2 hours after collection (Jawasreh *et al*., 2009). The results were measured using a haematocrit reader and expressed in terms of percentage (Appendix-II).

3.2.4 Faecal egg count

3.2.4.1 Floatation technique

Using McMaster technique, the number of faecal nematode eggs was determined. Approximately, 4 grams of a faecal sample were placed into a beaker with 56 mL of salt solution as the flotation fluid and mixed thoroughly using stirrer. Thereafter, using tea strainer, a filtrate of the faecal suspension was poured into a clean beaker. Part of the top surface layer of the faecal suspension was transferred into a McMaster counting chamber using a Pasteur pipette and was allowed to stand for 5 min, after which it was examined under the compound microscope at a magnification of 100× and 400×. The eggs of nematode parasites were identified on the basis of egg color, shape, contents, and size using the keys given by Foreyt (2011), and photographic records were made using a digital camera. The eggs were counted and each count was multiplied by 50 to obtain the total number of eggs per gram of faeces as given in Hansen and Perry (1994).

3.2.4.2 Sedimentation technique

For the eggs of trematode parasites, a standard sedimentation technique recommended by Hansen and Perry (1994) was used. Approximately, 3 grams of faecal sample was weighed and placed into a beaker to which approximately 50 ml of tap water was added and mixed thoroughly using stirrer. The mixture was allowed to sediment for 5 minutes. Thereafter, the faecal suspension was filtered and removed through a tea strainer. The residual faecal material was poured into another beaker and mixed with the same amount of water as before and allowed to sediment for 5 minutes. In the third step, the filtered faecal material was poured into a test tube and mixed with 5 ml of water and allowed to sediment for 5 minutes. Using Pasture pipette, the supernatant was again carefully decanted and the sediment was stained by adding one drop of methylene blue. Finally, the stained sediment was transferred to a McMaster counting chamber for microscopic examination of *Fasciola* eggs. All the *Fasciola* eggs observed under the microscope were counted, and each count was multiplied by 50 according to Hansen and Perry (1994) to obtain the total number of eggs per gram of faeces.

3.2.5 Weather data

Climatic data were recorded every month at the meteorological station of Adami Tulu Agricultural Research Center, which is located seven kilometers away from Zwai town. Weather data collected throughout the study period (from April 2015 to March 2017) were minimum, mean and maximum monthly temperatures, total monthly rainfall and average monthly relative humidity levels.

3.2.6 Data analysis

The prevalence of infection was calculated as a percentage of n/N, where n is the number of tracer sheep infected and N is the total number of tracer sheep examined. The association of the independent factors (sex, month and season) to the continuous dependent variables, namely, number of worms recovered, number of eggs per gram of faeces and intensity of infection was calculated using One-way ANOVA (as the data fulfilled the assumption). The associations between the independent factors and the prevalence of various parasites were evaluated using the Chi-square test (χ^2). The relationship between continuous dependent variables and continuous independent variables (climatic variables) were evaluated using Pearson Correlation Coefficient. For all tests, α-values of less than 0.05 was considered for significance.

3.2.7 Ethical Clearance

To conduct research on sheep at the wetlands of Lake Zwai, permission was obtained from Adami Tulu Jido Kombolcha Woreda Agricultural Office at Zwai town.

3.3 Results

3.3.1 Climatic data

Weather data such as total monthly rainfall (mm), minimum monthly temperature (°C), maximum monthly temperature (°C) and average monthly relative humidity (%) were collected from the meteorology station of the National Meteorology Agency of Ethiopia located in close proximity from the grazing wetlands of Lake Zwai for the period from April 2015 to March 2017 (Fig. 3.1). No rainfall was recorded from October to December 2015 and from November to January 2016.

In this study, there was a trend of average monthly relative humidity (%) increase with increase in the amount of rainfall (mm) from April 2015 to July 2015 and then decrease with the reduction of the amount of rainfall (mm) up to December 2015. A rise of average relative humidity (50%) to the maximum (74%) was observed from December 2015 to July 2016 with the increase of total monthly rainfall and then decrease with the same trend except in some months. The monthly minimum temperature (°C) also shown relatively similar trend of increase from April 2015 to July 2015 with the increase of total monthly rainfall and then reduced with similar trend of reduction except in some months. The highest minimum monthly temperature (16.7°C) in June 2015 did not coincided with the highest total monthly rainfall (229.2mm), observed in June 2016; whereas the least minimum monthly temperature (6.8°C) coincided with the low value of total monthly rainfall (0mm) in January 2017. However, the trend of maximum monthly temperature did not show similar trend with the total monthly rainfall.

There were strong positive significant ($r = 0.825$; $p = 0.000$) correlations between average monthly relative humidity (%) and total monthly rainfall (mm), positive moderate significant ($r = 0.607$; $p = 0.000$) correlations between minimum monthly temperature and total monthly rainfall, and negative moderate significant ($r = -0.325$; $p = 0.027$) correlations between maximum monthly temperature and total monthly rainfall. There was also moderate negative significant ($r = -0.452$; $p = 0.002$) correlation between maximum monthly temperature with average monthly relative humidity. The maximum monthly temperature recorded in March (25ºC) coincided with a time just before the commencement of the short rainy season in the study area. Thus, the period from March to September was warm and humid and referred to as the wet season, except the period October to February, which is considered as dry season. Thus, the wet season is divided into two periods; the short rainy season (March to June) and the long rainy season (June to September), thus showing an annual bimodal rainfall pattern.

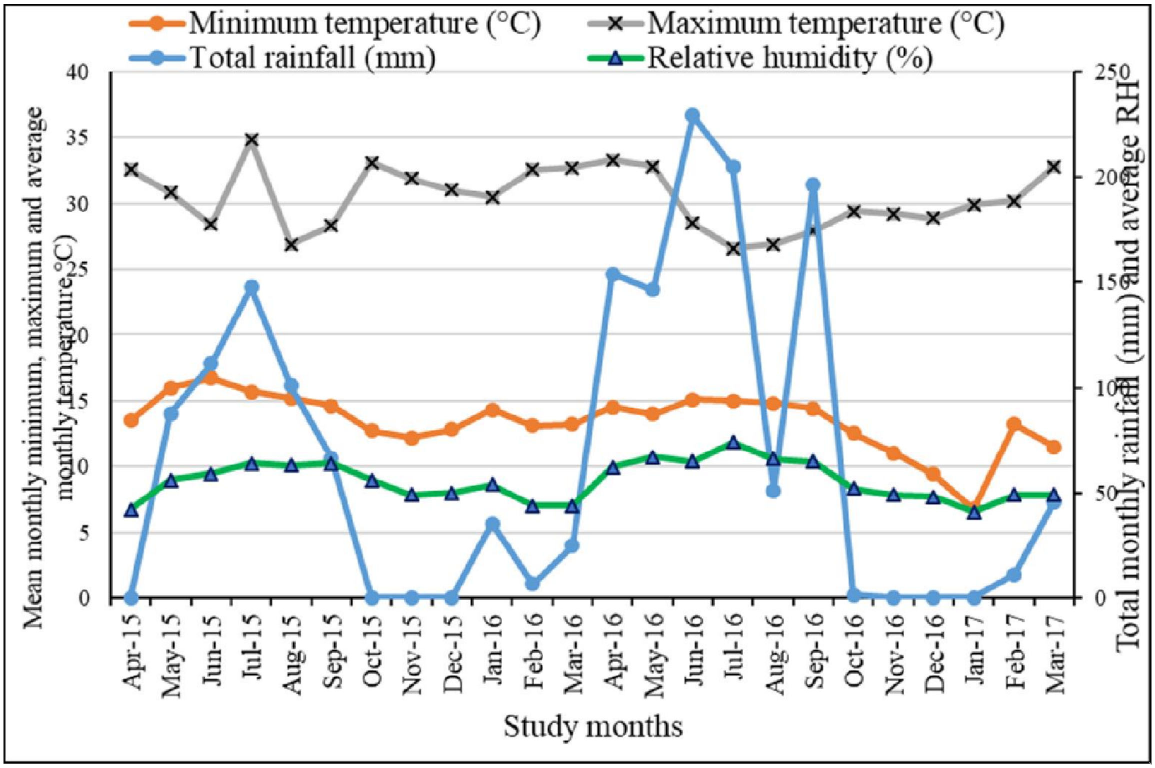

Fig. 3.1: Total monthly rainfall, minimum and maximum monthly temperature and mean monthly relative humidity of the study area from April 2015 to March 2017.

3.3.2 Prevalence of gastrointestinal helminth parasites

3.3.2.1 Overall prevalence of gastrointestinal helminth parasites

Out of 46 experimental tracer sheep examined, 45 (97.8%) were found to harbor nematode, cestode and trematode helminth parasites. Although all of these three helminth groups can contribute to the overall prevalence (97.83%) of gastrointestinal helminths parasites, it was nematodes that were the most prevalent (97.8%). A total of 10 species of parasites belonging to the three groups, namely: nematodes, cestodes and trematodes were encountered with 8, 1 and 1 species, respectively (Table 3.2).

Nematode parasites were the most prevalent, where the prevalence of *H. contortus* was (87%), *T. axei* (56.5%), *T. ovis* (69.6%), *O. columbianum* (28.3%), *B. tringoincephalum* (32.6%), *Dictyocaulus filaria* (10.9%), *Mulleries capilaris* (2.2%) and unidentified species (2.2%). The prevalence of cestode species *Moneza expanza* and trematode species *Fasciola gigantica* were 19.6 and 17.4%, respectively.

Table 3.2: Types of helminth parasites collected from the GI tract of the experimental tracer sheep during the study period.

No.	Groups of parasites	Types of helminth parasites	Prevalence (%)
1	Nematodes	*Haemonchus contortus*	87.0%
		Trichostrongylus axei	56.5%
		Trichuris ovis	69.6%
		Bunostomum tringoincephalum	28.3%
		Oesophagostomum columbianum	32.6%
		Dictyocaulus filaria	10.9%
		Mulleries capilaris	2.2%
		Unknown species	2.2%
2	Cestodes	*Moneza expanza*	19.6%
3	Trematode	*Fasciola gigantica*	17.4%

3.3.2.2 Seasonal prevalence of gastrointestinal helminth parasites

Of the 46 experimental tracer sheep examined, seasonal prevalence of gastrointestinal helminths parasites indicated that 100, 100 and 92.86% levels of infestations were observed during short rainy season, long rainy season and dry season, respectively (Fig. 3.2). However, there was no significant (χ^2 = 2.337; p = 0.311) difference between study seasons and prevalence of gastrointestinal helminth diseases.

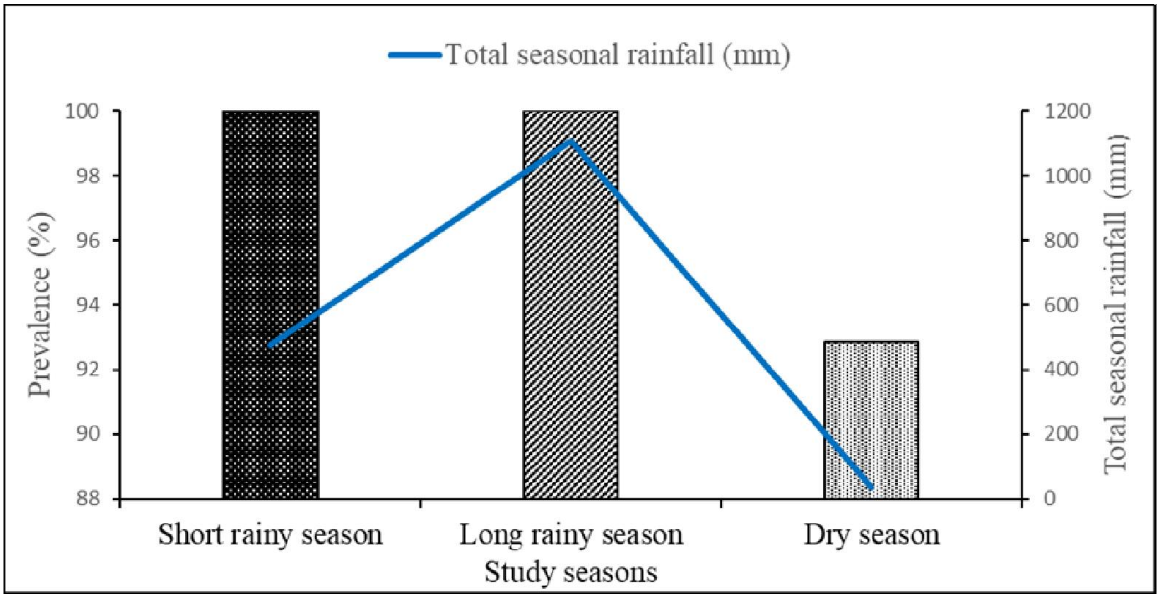

Fig. 3.2: Seasonal prevalence of gastrointestinal helminth parasites

3.3.2.3 Sex-wise prevalence of gastrointestinal helminth parasites

It was observed in this study that the overall prevalence of gastrointestinal helminth parasites in the experimental tracer sheep based on sex indicated that 23 (100%) male and 22 (95.65%) female sheep were infected with the 46th sheep remaining uninfected (Table 3.3). However, there was no significant (χ^2 = 1.022; P = 0.312) variation on the prevalence of gastrointestinal helminth parasites based on sex.

Table 3.3: Overall prevalence of gastrointestinal helminth parasites based on sex.

Sex	Frequency	Prevalence (%)	χ^2-value	P-value
Male	23	23 (100%)	1.022	0.312
Female	23	22 (95.65%)		
Total	46	45 (97.83%)		

3.3.3 Worm Burden

3.3.3.1 The seasonal relationship among monthly worm burden, rainfall and humidity

The mean ± S.E worm counts from 46 experimental tracer sheep examined during the study period (April 2015 to March 2017) indicated that there was almost a slight increase of mean ± S.E helminths count following the availability of rainfall from May to September 2015 and March to September 2016 in Zwai wetlands. The highest mean helminths count (890±20.00) was collected and recorded in the month of July 2016 following the highest monthly rainfall (229.2mm) of June 2016 in the study area and decreased thereafter during the following dry months (Fig. 3.3) as total monthly rainfall declined. The highest mean helminths count obtained in July 2016 also coincided with the highest relative humidity (74%) and least maximum monthly temperature (26.6°C) recorded in this month. Statistical analysis showed that there were positive and negative moderately significant (r = 0.388 and -0.460; p = 0.008 and 0.001) correlations between total helminths burden with total monthly rainfall and maximum monthly temperature, respectively. There were significant (F = 3.702; p = 0.001) variations of monthly mean gastrointestinal helminths count among study months.

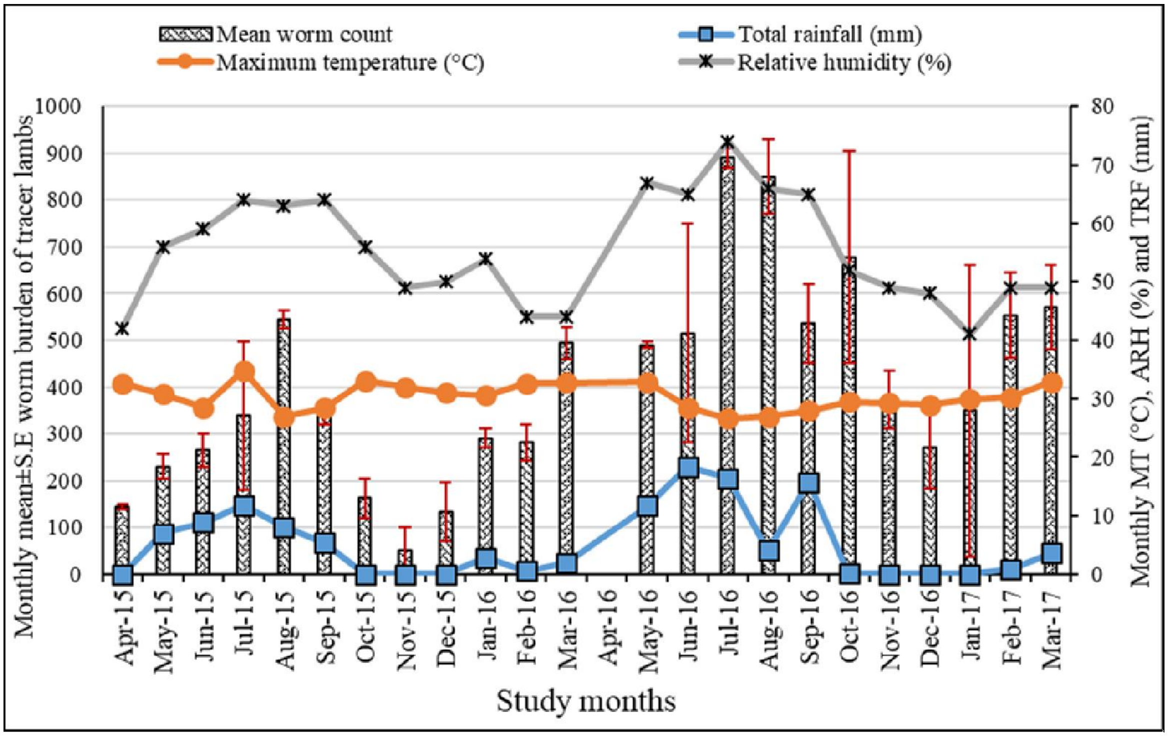

Fig. 3.3: Helminth parasites burden in the experimental tracer sheep against monthly MT (maximum temperature), ARH (average relative humidity) and TRF (total rainfall) of the study area.

3.3.3.2 The seasonal monthly relationships between each of the helminth parasites to rainfall

The helminth parasites burden showed increasing pattern with the highest mean count encountered following the rainy months and relatively lowered in dry months. However, cestode and trematodes were recorded sporadically (Fig. 3.4).

In this study, there was almost a slight increase of mean ± S.E nematode counts following the availability of rainfall from May to September 2015 and April to July 2016 in the study area. The highest mean nematode worm count (890±20 and 850±80) was recorded in the months of July and August 2016, following the highest monthly rainfall (229.2 and 204.8mm) of June and July 2016, respectively, in the study area. There was moderately (r = 0.393; p = 0.007) significant correlation between mean nematode burden with total monthly rainfall. However, mean cestode and trematode counts did not show any trend of rise with the increase of monthly total rainfall.

The highest mean ± S.E cestode and trematode worm counts were 10 and 57, respectively during May 2015 and January 2017, respectively without following the highest total monthly rainfall (229.2mm) recorded of June 2016. Thus, there were weak (r = 0.160; p = 0.287) and moderately (r = -0.283; p = 0.570) insignificant correlations between cestode and nematode burden with total monthly rainfall. Trematode (*F. gigantica*) worm counts started to show up in November 2016 and continued onwards up to February 2017, whereas the highest rainfall (229.2mm) was recorded in June 2016 and the longer rainfall (196.6mm) and continued up to September 2016.

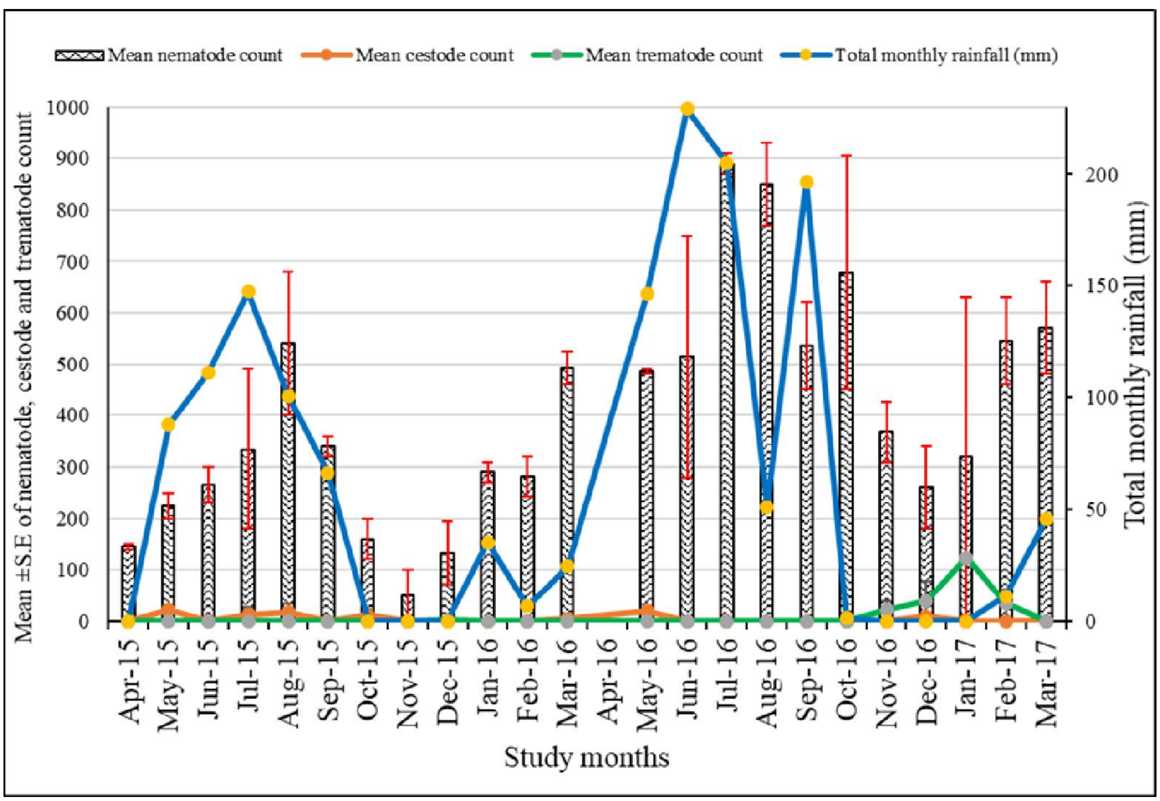

Fig. 3.4: Mean monthly nematode, cestode and trematode counts with total monthly rainfall in Zwai wetlands.

3.3.3.3 The seasonal monthly relationship between nematode parasites of sheep and rainfall

Of these 46 experimental tracer sheep examined, a mean ± S.E monthly counts of *H. contortus*, *T. axei, T. ovis, O. columbianum, B. tringoincephalum, D. filaria, M. capillaries* and one unidentified species were collected and identified. The highest (375.00±15.00 and 365.00±120.00) *H. contortus* and *T. axei* counts were observed and recorded in July 2016, following the highest amount of rainfall (229.2mm) in June 2016. There was a rise of *H. contortus* worm counts following the increase of total monthly rainfall on the study months (Fig. 3.5); whereas the remaining nematode species showed inconsistent trends with the increase of total monthly rainfall. There was moderate (r = 0.473; p = 0.001) significant correlation between *H. contortus* count with total monthly rainfall. However, the moderate correlation (r = 0.262; p = 0.079) between *T. axei* and total monthly rainfall was insignificant.

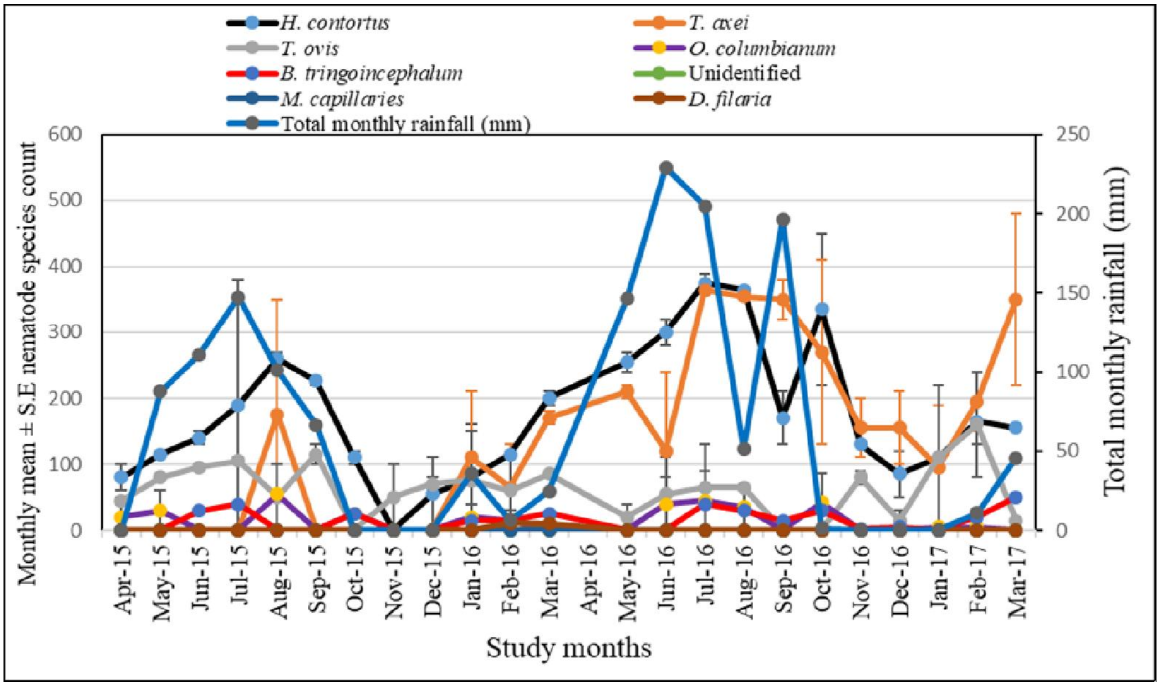

Fig. 3.5: Mean monthly nematode parasites species count with total monthly rainfall.

3.3.3.4 The seasonal relationship between mean worm burden and rainfall

Regarding mean seasonal worm counts, the highest mean ± S.E (534.63±63.91) count was observed during the long rainy season (June to August) followed by the short rainy and the dry seasons with a value of 394.57±46.25 and 288.19±59.55, respectively (Fig. 3.6). Thus, there were significant (F = 4.398; p = 0018) variations of mean gastrointestinal helminths among study seasons.

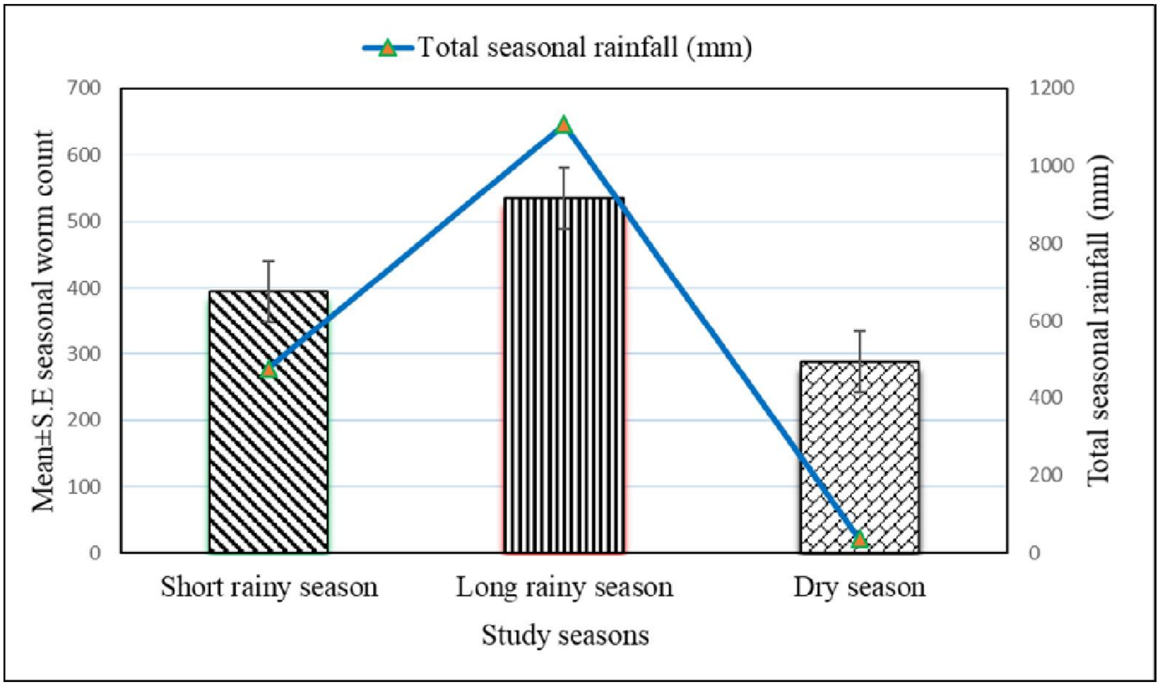

Fig. 3.6: The relationship between mean seasonal gastrointestinal helminths with total seasonal rainfall in the study area.

The seasonal mean ± S.E gastrointestinal nematode parasites count during short rainy season, long rainy season and dry season were 391.93±46.13, 533.75±63.97 and 288.25±59.40, respectively (Fig. 3.7). There were significant (F = 4.933; *p* = 0012) variations of mean gastrointestinal nematode counts among study seasons.

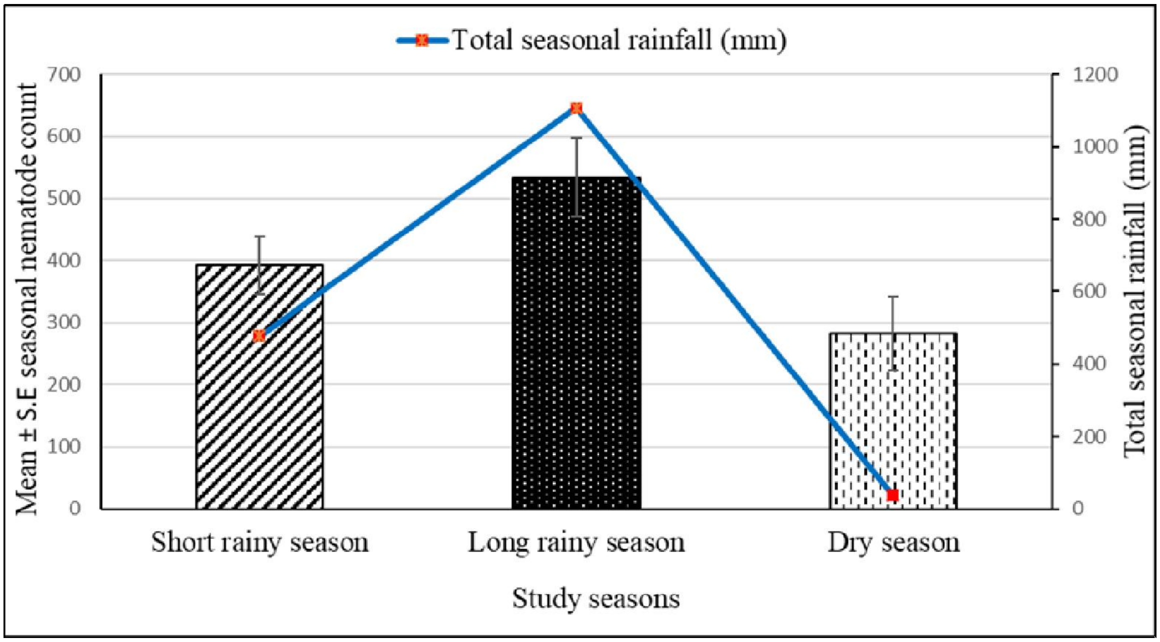

Fig. 3.7: The relationship between mean seasonal gastrointestinal nematodes with total seasonal rainfall in the study area.

The seasonal mean ± S.E gastrointestinal cestode and trematode parasite counts during short rainy season, long rainy season and dry season were 1.50±0.62 and 1.14±0.99, 0.88±0.60 and 0.00±0.00, and 0.56±0.39 and 5.38±2.56, respectively (Fig. 3.8). However, there were no significant ($F = 0.748$ and 3.115; $p = 0.479$ and 0.055) variations of mean gastrointestinal cestode and trematode counts, respectively among study seasons.

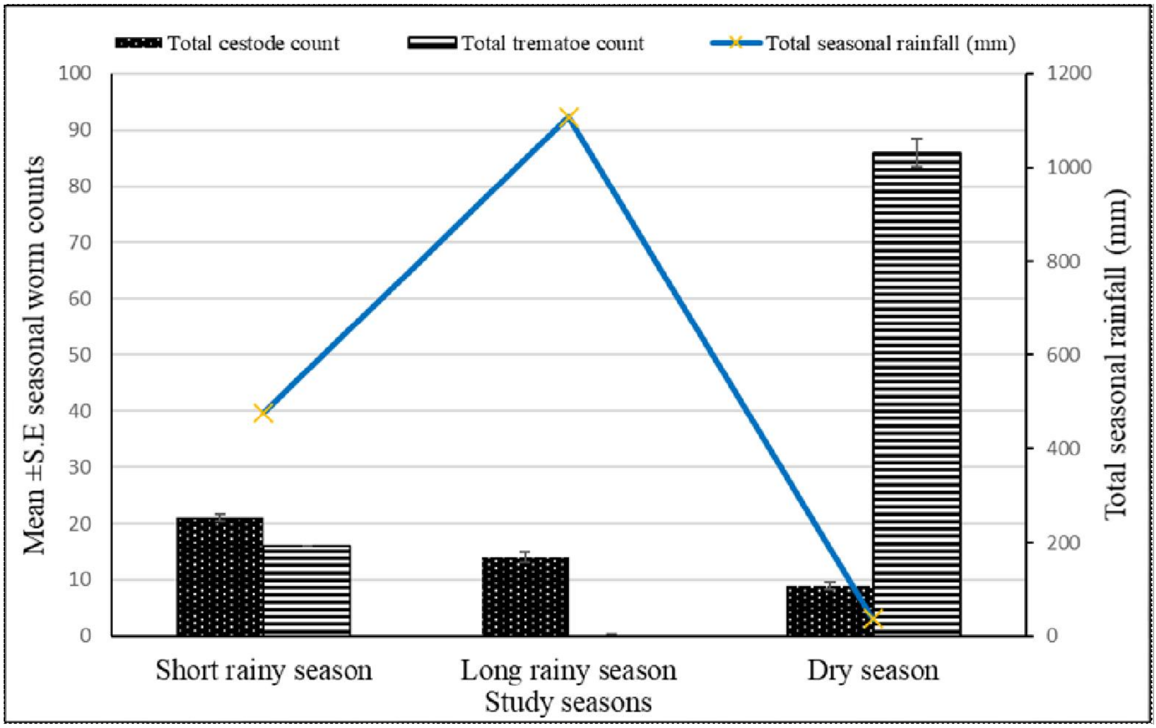

Fig. 3.8: The relationship between mean seasonal nematode, cestode and trematode counts with total seasonal rainfall in Zwai wetlands.

The mean ± S.E seasonal worm counts of *H. contortus* (155.00±15.89, 253.12±27.79 and 113.12±29.45), *T. axei* (141.43±36.48, 170.62±44.71 and 96.87±30.08)*, T. ovis* (66.43±16.59, 68.75±12.74 and 50.00±16.4)*, O. columbianum* (10.00±5.14, 21.87±8.62 and 11.56±5.80)*, B. tringoincephalum* (15.71±6.77, 19.37±7.22 and 9.69±4.22) during short rainy season, long rainy season and dry season, respectively were collected and recorded. Other gastrointestinal parasites like *D. filaria* (during short rainy season and dry season with a value of 3.00±1.69 and 0.94±0.68, respectively), *M. capillaris* (during dry season; 0.06±0.06) and unidentified spp. (during short rainy season; 0.36±0.36) were observed irregularly.

Although the mean counts of individual nematode parasites reached their highest counts during the long rainy seasons of 2016 and declined with the reduction of seasonal amounts of rainfall (Fig. 3.9), there were no significant (p > 0.005) variations of *T. axei, T. ovis, O. columbianum, B. tringoincephalum, D. filaria, M. capillaries* and unidentified spp. were observed among study seasons. Whereas in *H. contortus*, there was significant (F = 8.049; *p* = 0.001) variations of seasonal mean count among study seasons. There was moderately (r = 0.549; *p* = 0.007) significant correlation between mean *H. contortus* count with total seasonal rainfall. The correlations (r = 0.297 and 0.257) of *T. axei* and *O. columbianum* with total seasonal rainfall were moderately insignificant (*p* = 0.168 and 0.237), respectively. Whereas in *T. ovis* and *B. tringoincephalum*, their correlation (r = -0.069 and 0.146) with seasonal total rainfall were insignificant (*p* = 0.755 and 0.507), respectively.

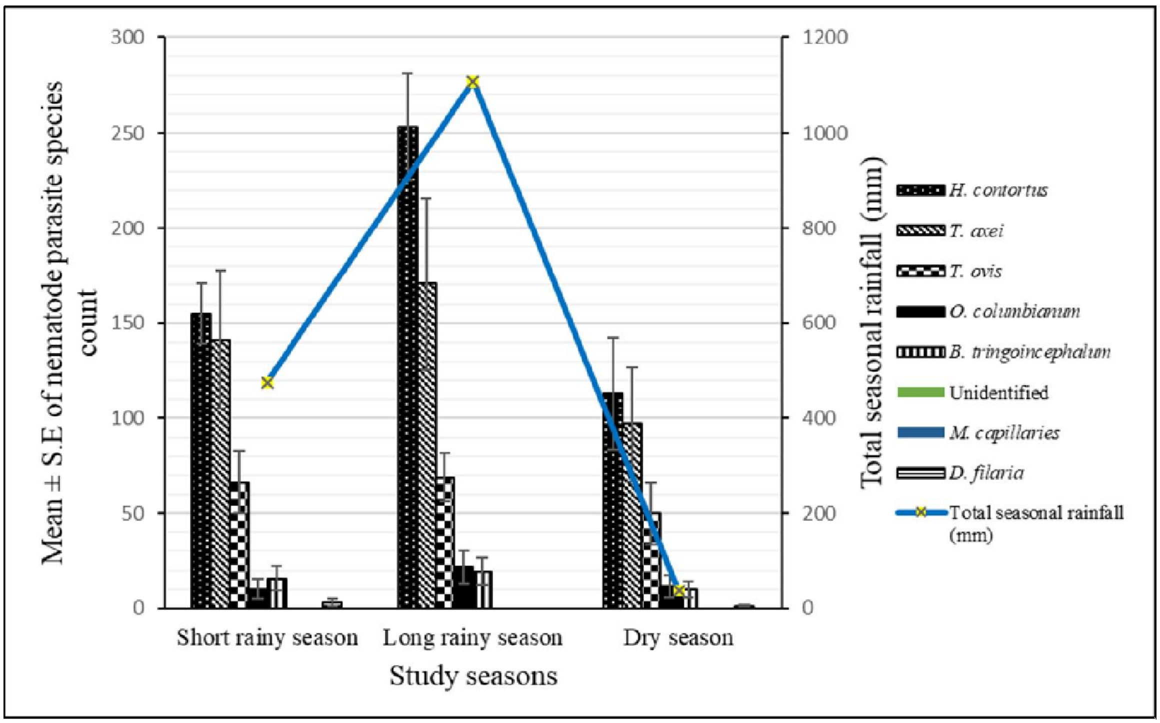

Fig. 3.9: The relationship between mean seasonal gastrointestinal nematode parasites with total seasonal rainfall in Zwai wetlands.

3.3.3.5 Levels of nematode parasite burdens

Regarding the level of infection of nematode parasite species, out of 40 and 26 experimental tracer sheep that were found infected by *H. contortus* and *T. axei,* respectively, all the sheep (100%) were found lightly affected. The level of infection for 17 sheep found infected by *B. tringoincephalum* 12 (70.59%) and 5 (29.41%) were lightly and moderately infected, respectively. Of these 21 sheep found infected by *T. ovis*, the level of infections found were light and moderate infections with a value of 6 (28.57%) and 15 (71.41%), respectively. Whereas for *O. columbianum,* of these 14 sheep, 8 (57.14%) and 6 (42.86%) were found lightly and moderately affected, respectively (Table 3.4).

Table 3.4: Level of infection based on worm counts of different nematode parasites of experimental tracer sheep.

Nematode parasites	Prevalence (%)	Range	Light	Moderate	Sever
H. contortus	40 (95.24%)	50-450	40 (100%)		
T. axei	26 (61.90%)	100-480	26 (100%)		
T. ovis	21 (50.00%)	10-240	6 (28.57%)	15 (71.43%)	
O. columbianum	14 (33.33%)	10-90	8 (57.14%)	6 (42.86%)	
B. tringoincephalum	17 (40.48%)	5-80	12 (70.59%)	5 (29.41%)	

3.3.3.6 The relationship between total worm burden and sex

Regarding sex, the mean ± S.E counts of gastrointestinal helminths were found 370.39±48.82 and 442.17±53.42 on female and male sheep, respectively. There was a trend of a slight increase of total helminth count following the availability of rainfall from May to September 2015 and March to September 2016 in Zwai wetland. The highest helminth count (930 and 910) was made in the months of August and July 2016 in female and male sheep, respectively following the highest monthly rainfall (204.8 and 229.2mm) of July and June 2016 in Zwai wetlands. However, there was no significant ($p > 0.05$) variation between female and male sheep infestaions by gastrointestinal helminth parasites (Fig. 3.10).

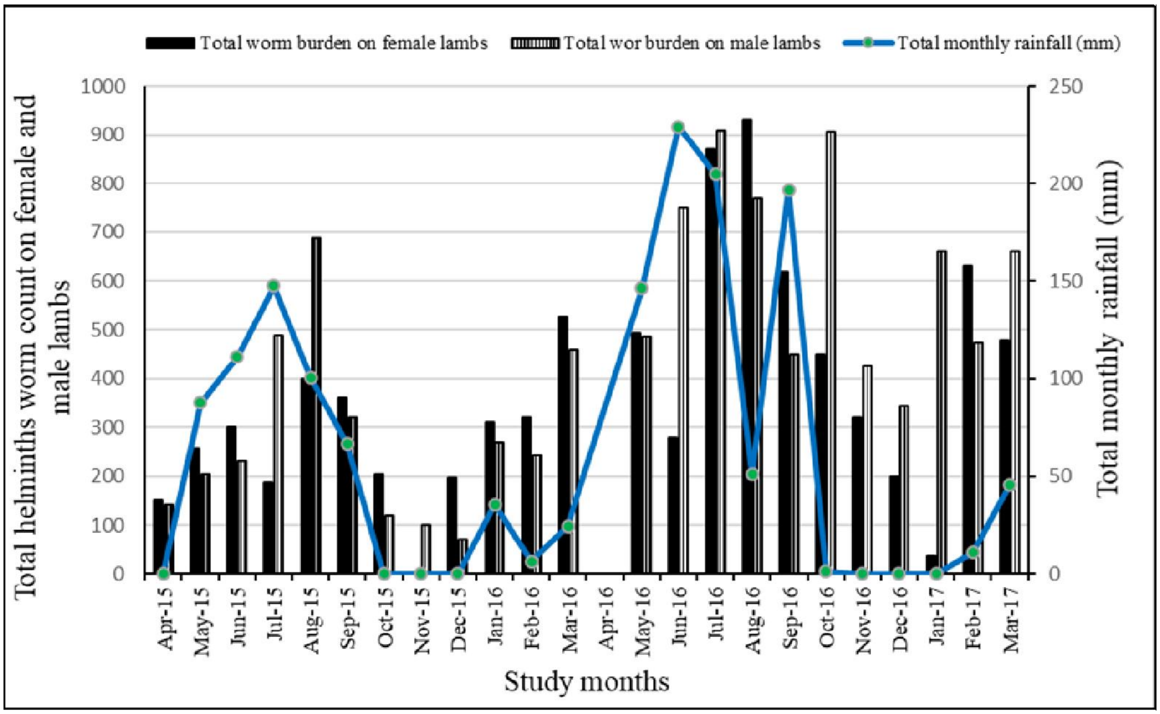

Fig. 3.10: Sex-wise monthly total worm counts with monthly total rainfall**.**

3.3.3.7 Mortality rate of tracer sheep during the study period

In this study, from a total of 50 sheep (46 tracers, 2 housed controls and 2 control grazers), a total of 4 sheep (2 control grazers and 2 experimental tracer) died during the study period before reaching their examination. Two of the control grazer sheep died in March 2017 because of suspected Anthrax, a zoonotic bacterial infection for ruminants as well as humans. Due to the early stage of the death of these animals (one week before housing for three months), the expected experimental data could not be collected. However, the cause of their death, suspected anthrax, is also associated with wetlands and warm and humid conditions (Walsh *et al*., 2018). During this period, two cows were also found dead in the same wetlands where the experimental and control sheep were grazing and the cause of their deaths was also suspected to be anthrax based on the reports of the local veterinarians of the Ministry of Agriculture. The cause for the death of one sheep was snakebite in December 2017, but the sheep died two days prior to its examination time and data was taken from it. The death for the remaining sheep was in September 2016 from a cause other than parasitism; at postmortem examination, swollen urinary bladder was observed (Fig. 3.11) and the sheep died one week prior to its examination time and the data was taken from them, too.

Fig. 3.11: Swollen urinary bladder of the tracer control sheep in the study that died due to urine obstruction.

3.3.4 Eggs per gram (EPG) counts

3.3.4.1 Faecal Strongyle nematode egg count

The EPG counts conducted on experimental tracer sheep that were grazing for designated periods in the wetlands of Zwai from April 2015 to March 2017 revealed the existence of Strongyle nematodes, *T. ovis*, *M. expanza* and *Fasciola* spp. eggs. The most prevalent eggs of gastrointestinal helminth parasites were Strongyle nematodes. Therefore, EPG count was made on Strongyle nematode parasites egg only. The reason for this was *T. ovis*, *M. expanza* and *F. gigantica* parasite burdens were less common than Strongyle nematode parasites of the experimental tracer sheep of the study area.

The mean EPG counts of male and female lambs were 3640.43±547.08 and 3641.30±529.75, Strongyle nematode parasite eggs, respectively. However, the difference in the mean EPG counts of male and female lambs was not significant (t = -0.01; p = 0.999) (Fig. 3.12).

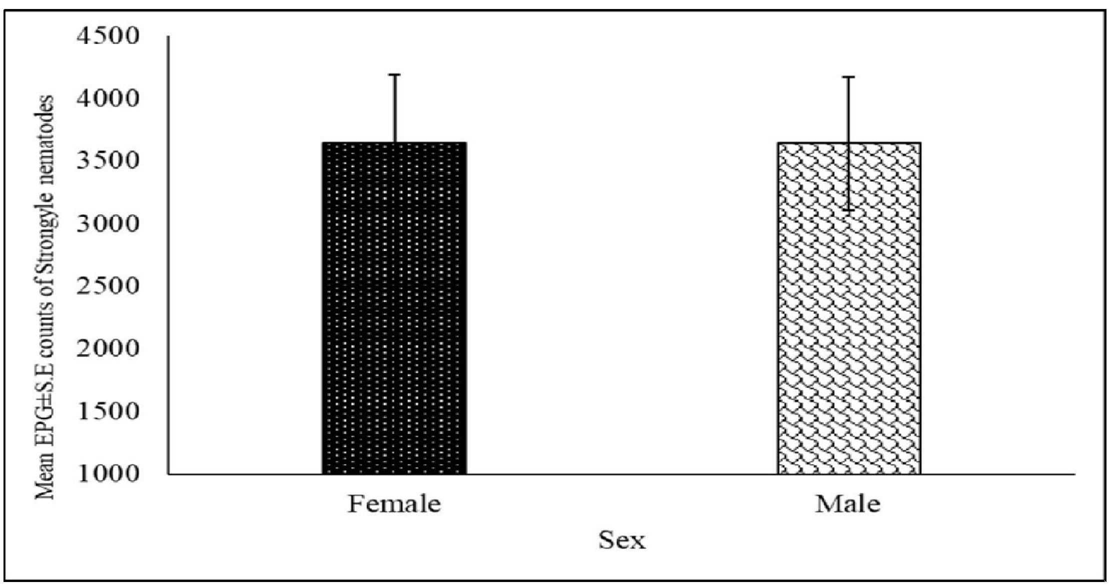

Fig. 3.12: Mean EPG counts of Strongyle nematodes based on sex.

3.3.4.1.1 The relationship between mean EPG count of Strongyle nematode and rainfall in Zwai wetlands

The mean monthly EPG counts of Strongyle nematode revealed that there were significant (F = 7.342; *p* = 0.000) variations among study months. The mean ± S.E of EPG counts of Strongyle nematode parasites during the study period (April 2015 to March 2017) indicated that there was a slight increase infestation of the wetlands with parasite eggs in Zwai wetlands with mean of ± S.E EPG count following the availability of rainfall from May to August 2015 and March to September 2016 in the study area. The total monthly rainfall (mm), and their correlation with the monthly fluctuation of EPG counts of Strongyle nematodes revealed that there was moderately positive correlation (r = 0.396; *p* = 0.006) between mean monthly EPG count of Strongyle nematodes with total monthly rainfall (mm). The highest mean EPG count (7615±615) was collected and recorded in the month of August 2016 following the highest monthly rainfall (229.2mm) of June 2016 in the study area and decreased thereafter during the following dry months (Fig. 3.13).

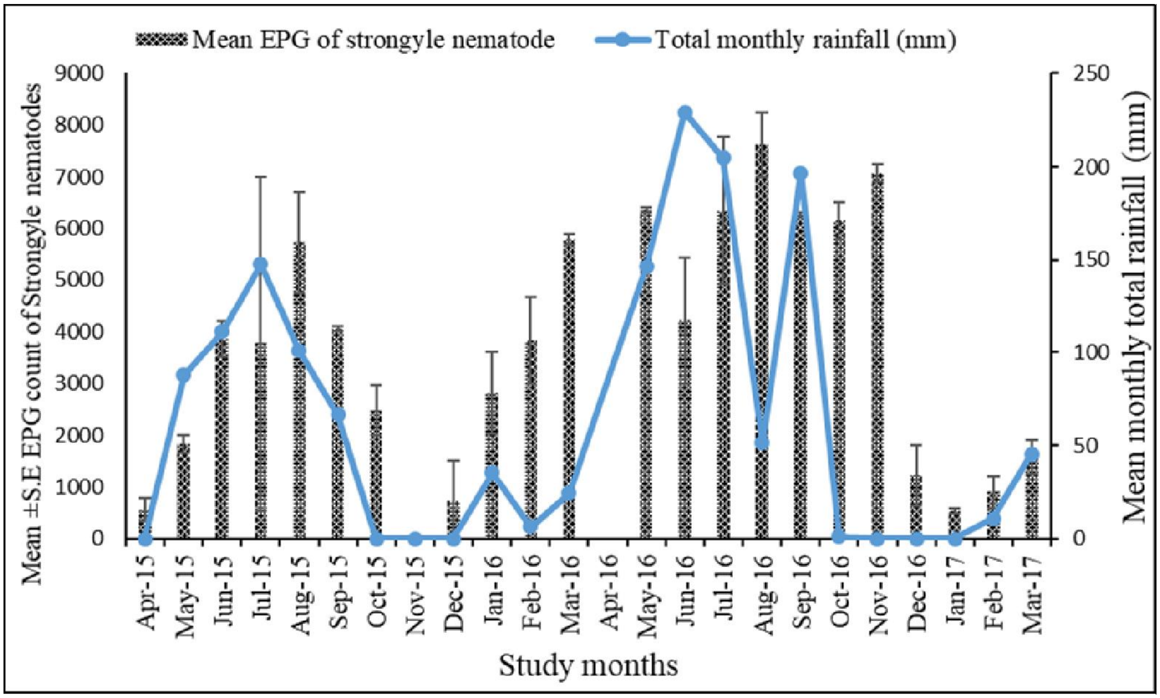

Fig. 3.13: Month wise mean EPG counts of Strongyle nematodes with monthly total rainfall.

The EPG count of Strongyle nematodes peaked in the long rainy season and declined during dry season. The mean ± S.E EPG counts of Strongyle nematode parasites in the short rainy season, long rainy season and dry season were 2980.71±615.39, 5228.75±500.18 and 2630.62±650.98, respectively, in the wetlands of Zwai. The correlation between EPG of Strongyle nematodes and mean seasonal rainfall was found positive and significant (r = -0.554, p = 0.000). There was significant (F = 5.851; p = 0.006) variation on the EPG counts of Strongyle nematode parasites among study seasons (Fig. 3.14).

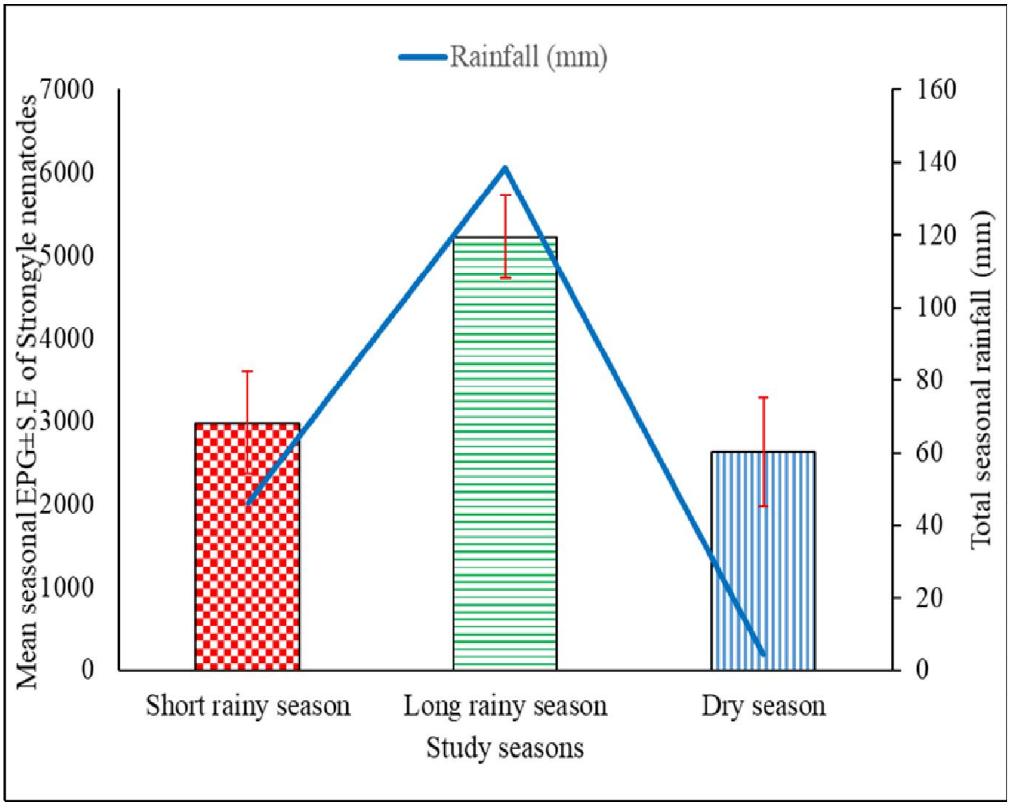

Fig. 3.14: Season wise mean EPG counts of Strongyle nematodes with total seasonal rainfall.

3.3.4.1.2 The relationship between mean EPG count and worm burden of Strongyle nematode parasites of the wetlands of Lake Zwai

The pattern of EPG counts during various months showed an increase of Strongyle nematode worm counts in the wet seasons and decreased in the subsequent dry months. There was positive and significant (r = 0.677; p = 0.000) correlation between mean EPG and mean worm counts of Strongyle nematodes (Fig. 3.15).

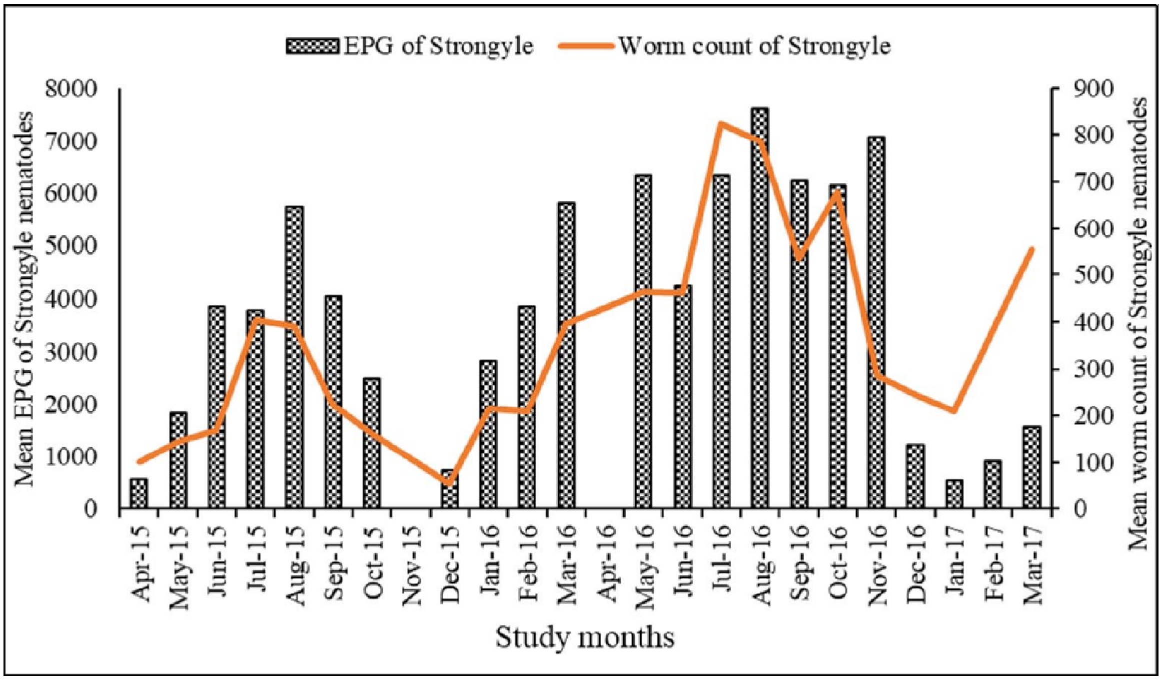

Fig. 3.15: Month wise mean EPG counts of Strongyle nematodes with mean worm counts.

3.3.4.2 Packed cell volume (PCV) (%) values of experimental tracer sheep released in Zwai wetlands

In this study, the hematological results revealed that experimental sheep had a PCV (%) range of 26 to 36.5%. The mean PCV (%) values of female and male sheep were 30.35 and 29.98%, respectively. However, there was no significant (t = 0.537; p = 0.594) difference in the PCV (%) values of male and female experimental tracer sheep (Fig. 3.16).

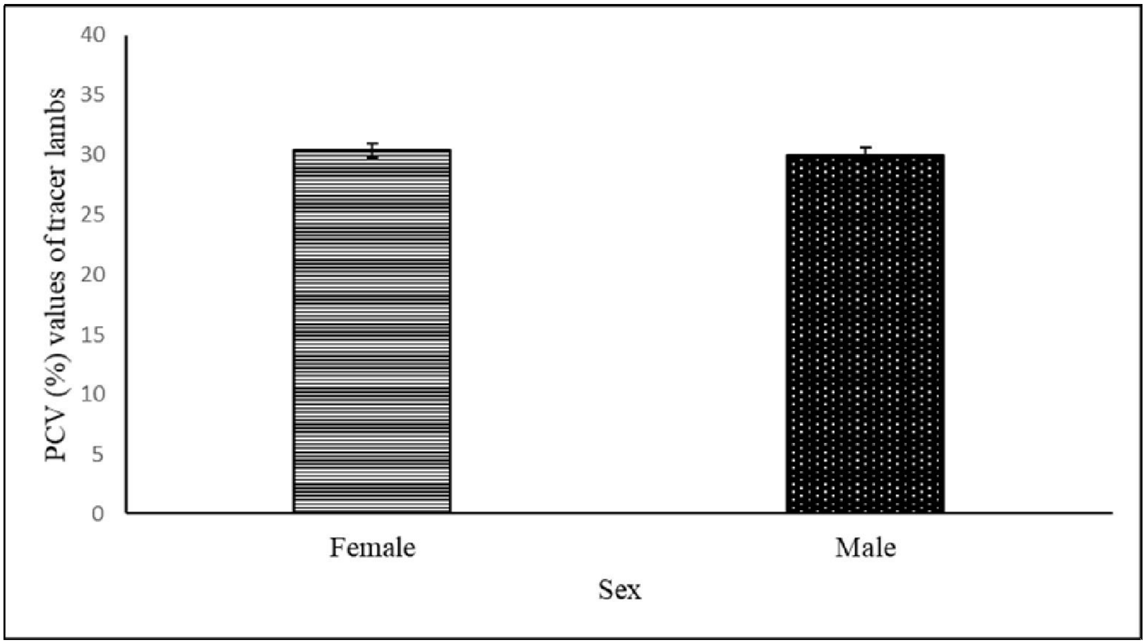

Fig. 3.16: Sex wise PCV (%) values of tracer lambs.

3.3.4.2.1 The relationship between PCV (%) and worm counts

In this study, PCV (%) value was significantly and negatively correlated with total nematode worm counts (r = -0.651, p = 0.000). As the PCV (%) decreases, there was an increase in the mean nematode worm counts (Fig. 3.17).

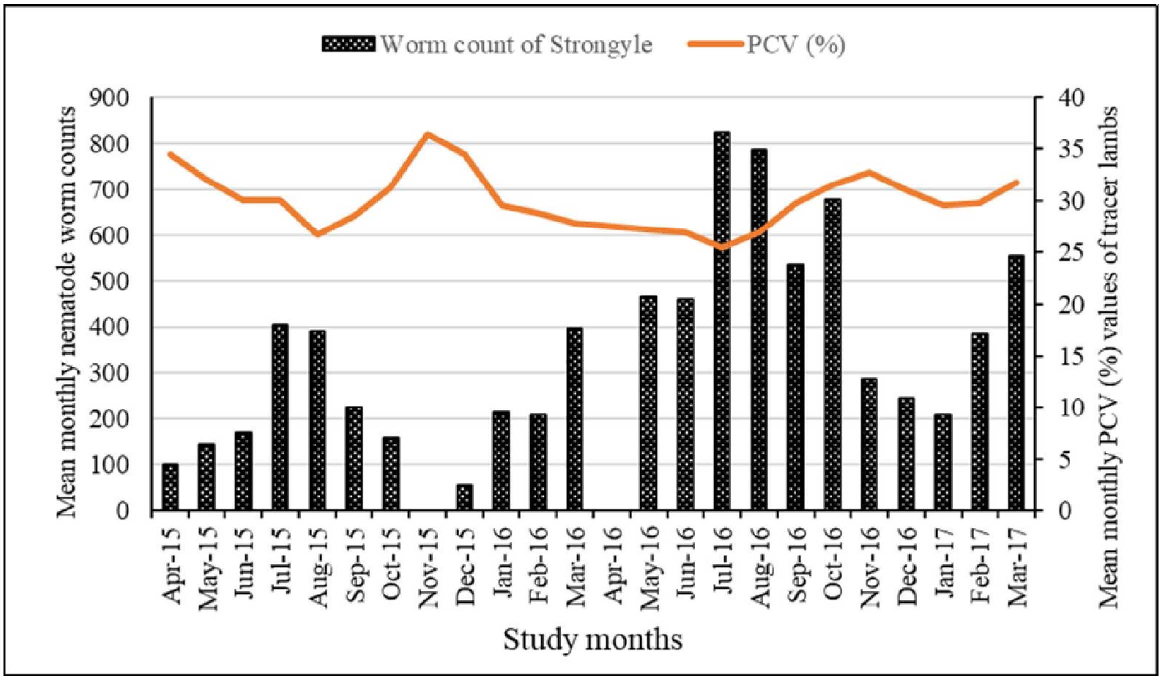

Fig. 3.17: Relationship between monthly PCV (%) values of tracer lambs with monthly nematode worm counts.

Similarly, there was an indirect relationship between PCV (%) and *H. contortus* burden on infected sheep, as the PCV (%) decreases, there is an increase in *H. contortus* worm count (Fig. 3.18). PCV (%) value was significantly and negatively correlated with *H. contortus* (r = -0.680, p = 0.000).

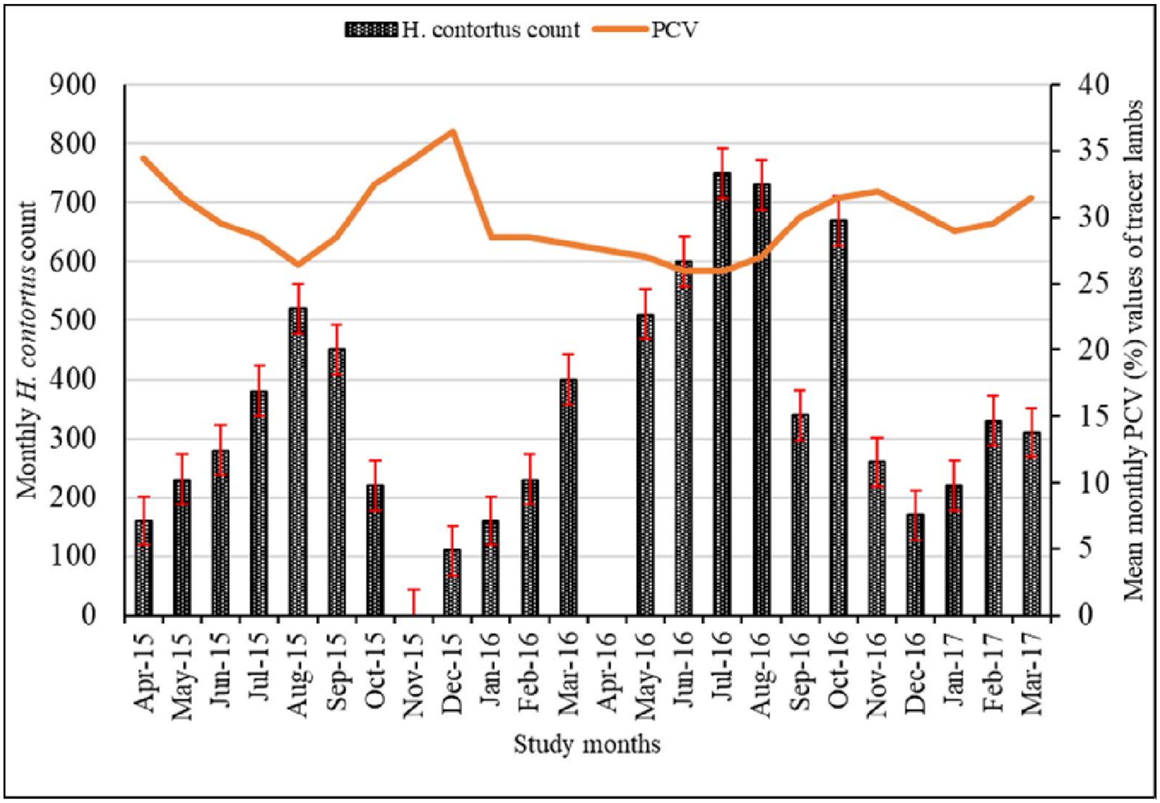

Fig. 3.18: Monthly *H. contortus* counts with PCV (%).

3.4. Discussion

3.4.1 Weather data

A significant ($p < 0.05$) correlations between total monthly rainfall and average monthly relative humidity, minimum and maximum monthly temperatures has been recorded in the study area (see also Mawonike and Mandonga, 2017). According to the authors, rainfall is at its maximum when the minimum monthly temperature and relative humidity are at high levels. On the other hand, limited rainfall expected when low levels of these parameters are realized. The higher the maximum temperature, the lower the relative humidity.

3.4.2 Prevalence of gastrointestinal helminth parasites

3.4.2.1 Overall prevalence of gastrointestinal helminth parasites

The findings of this study revealed that wetlands around the shoreline of Lake Zwai harbor a whole range of helminth parasites that require wetness and warm temperatures to complete their life cycles outside the body of their definitive and vector hosts. Of 46 tracer sheep examined through a post-mortem, 45 sheep were found infected with one or more groups of helminth parasites. The overall prevalence found in this study was 97.83%. Comparative prevalence results were reported in different areas of the country (Abebe Wossene and Esayas Gira, 2001 in Eastern parts of Ethiopia; Kassaye Aragaw and Gebrehiwot Gebreegziabher, 2014 in Hawassa; Nuraddis Ibrahim *et al*., 2014 in and around Jima Town; Samuel Kelemework *et al*., 2016 in and around Dire Dawa; Hailegebriel Bedada *et al*., 2018 in selected pastoral and agro-pastoral areas of Afar Region) and abroad (Mazid *et al.*, 2006 in Mymensingh, Bangladesh; Almalaik *et al*., 2008 in Tulus Areas of South Darfur; Abdelnabi *et al*., 2011 in Central Kordofan, Sudan; Bansal *et al*., 2015 ; Owusu *et al*., 2016 in Ghana).

In this study, infections of gastrointestinal helminth parasites were prevalent in sheep throughout the study period. The high prevalence of gastrointestinal infections in tracer sheep in the study areas was due to the availability of the infective stages of the helminth parasites in Zwai wetlands supported by the conducive environmental conditions. In addition to this, the high prevalence of this study might be due to the confined grazing space of the tracer sheep as succulent grasses are only available in the wetlands throughout the study period as well the grazing of livestock of the surrounding communities in the same wetlands, which increased the chance of infecting the pastures.

However, only one female sheep was found free from any types of gastrointestinal helminth parasites. The probable reason for this might be the selective grazing behavior of the sheep as foraging in close proximity to faeces brings a risk of parasite infection and a source of the infective stages of parasitic larvae. According to Hutchings *et al*. (2001) and Cooper *et al.* (2000), some sheep use faecal avoidance as an indication for parasite avoidance.

In this study, mixed infections of different groups of gastrointestinal helminth with the predominance of nematode parasites were observed. This study was in line with the findings of other researchers in Ethiopia (Bikila Emiru *et al*., 2013; Kasaye Aragaw and Gebrehiwot Gebreegziabher, 2014; Nuraddis Ebrahim *et al*., 2014; Temesgen Ayana and Walanso Ifa, 2015; Samuel Derso and Alemneh Shime, 2017) and elsewhere (Mazid *et al*., 2006; Torres-Acosta and Hoste, 2008; Abdelnabi *et al*., 2011; Shahnawaz *et al*., 2011; Amin and Wani, 2012; Lone *et al*., 2012). The probable reason for these findings might be the suitability of environmental conditions of un-drained pasture, moderate temperature (18-31°C) and moisture (42-75%) throughout the study period in Zwai wetlands providing optimum requirements for the development of infective helminth parasitic larvae. According to Maichomo *et al*. (2004), mixed parasitic infections in hosts is the rule rather than the exception in most grazing systems.

3.4.2.2 Seasonal prevalence of gastrointestinal helminth parasites

In this study, the prevalence obtained during the wet seasons (both long and short rainy season) was 100%, where as in the dry seasons, the value was 92.86%. However, there was no significant (χ^2 = 2.337; p = 0.311) difference between study seasons and prevalence of gastrointestinal helminth diseases. Opposite findings were reported by Mazid *et al*. (2006); Yadav *et al*. (2006); Sissay Menkir (2007); Shahnawaz *et al*. (2011); Lone *et al*. (2012); Mir *et al*. (2013); Bansal *et al*. (2015); Zvinorova *et al*. (2016). This difference in the prevalence of gastrointestinal helminth parasites may be attributed to the difference in agro-ecology and variation in management practice of animals and presence/absence of pastures contaminated by infective stages of helminth larvae in communal grazing areas elsewhere.

In these findings, absence of significant variation among seasons might be due to the presence of Lake Zwai wetland that serves as a continuous supply of fresh water to the wetlands along the shore, which again provide fresh pastures throughout the year. As a result, these free-access wetlands are frequently visited by livestock coming for grazing and drinking water get contaminated by their droppings throughout the year. Grazing of young and adult animals together with poorly drained land provide an ideal condition for the transmission of gastrointestinal helminth parasites to build up clinical infestation of the host (Gadahi *et al*., 2009). In line with these findings, Tramboo *et al*. (2015) reported that no significant association was observed between study seasons and the prevalence of gastrointestinal helminth parasites in sheep population of Kashmir valley, India.

3.4.2.3 Sexes of the animals and the prevalence of gastrointestinal helminth parasites

In this study, there was no significant variation on the prevalence of gastrointestinal helminth infection between female and male sheep. They were found to be equally susceptible to infection of gastrointestinal helminth parasites. Similar finding was reported by different authors inside the country (Temesgen Ayana and Walanso Ifa, 2015; Mohammed *et al*., 2015; Andualem Yimer and Eyerusalem Birhan, 2016; Yemisirach Yonas and Alemu Goa, 2017) and elsewhere (Mazid *et al*., 2006; Poddar *et al*., 2017). According to Bhat *et al*. (2012), differences of gastrointestinal helminth were observed around or after puberty and no difference was observed prior to puberty.

3.4.3 Worm burden

3.4.3.1 The seasonal relationship among monthly worm burden, rainfall and humidity

The mean worm counts of gastrointestinal parasites varied in different months of the year; the highest mean worm counts appeared during the months with highest rainfall and average relative humidity. In general, there was a rise of mean worm counts following the increase of total monthly rainfall and average relative humidity and declined with reduction of these climatic variables during the dry months. In this study, there was significant (r = 0.388; p = 0.008) correlation between mean helminth counts with total monthly rainfall. This result is in agreement with reports of Almalaik *et al*. (2008), Al-shaibani *et al*. (2008), Abdelnabi *et al*. (2011), Kumar (2014) and Rashid *et al*. (2016). According to those authors, rainfall and relative humidity appears to be the main factors correlated with monthly worm burden. According to Kuchai *et al*. (2011), a direct relationship exists between prevalence of gastrointestinal helminth parasites with the monthly rainfall, humidity and temperature. This might be due to the fact that heavy rainfall and high relative humidity predispose the animal to waterborne helminth gastrointestinal parasites by stimulating the development of infective stages of the parasitic helminths. In addition to this, high rates of infection in rainy months may be attributed to suitable molarity of salts present in the wetlands, which are important factors for ecdysis of parasitic larvae (Soulsby, 1982).

3.4.3.2 The seasonal monthly relationships between each of the nematode, cestode and trematode groups to rainfall

In this study, mean nematode worm count showed monthly variation with the fluctuation of total monthly rainfall and average monthly relative humidity. During rainy months and high average relative humidity, a gradual buildup of the nematode worm counts was observed with the highest burdens recorded around the peaks of the rainy months. Similar findings were reported by Al-shaibani *et al*. (2008); Abdelnabi *et al*. (2011) and Kuchai *et al*. (2011). In general, moist and warm environmental conditions are favorable for the development, survival and transmission of the larval stages of nematode parasites (Hansen and Perry, 1994; Urquhart *et al*., 1996). Being the study area is located at the moist (water front of Lake Zwai) and warm (rift valley area), high mean nematode counts have resulted; and correlated with the highest monthly rainfall and highest average relative humidity. Increasing initial soil moisture provides a water film for larvae to move in, and results in increased recovery of third stage larvae from pasture, as does increasing amounts of rainfall (Khadijah *et al*., 2013).

Mean worm counts of cestode and trematode parasites did not reveal a discernible correlation with the values of total monthly rainfall and average monthly relative humidity. In cestodes and trematodes, conducive ecological conditions may not be enough as they are also dependent on the availability of intermediate hosts as well as the interaction of those intermediate hosts with the definitive hosts. According to Soulsby

(1982), Hansen and Perry (1994) and Urquhart *et al*. (1996), helminths infections, particularly cestodes and trematodes in sheep depends on many variables including the presence of suitable intermediate hosts as well as favorable climatic and ecological conditions for them. The intermediate hosts of *Moniezia expansa* (cestode) are free-living oribatid mites. Ingestion of eggs by infected mites is accidental as they are not coprophagous (Amin and Wani, 2012). Due to this reason, a low prevalence level, as well as mean worm burden of cestodes, have resulted. Among the trematodes, the species present was *Fasciola gigantica*. This parasite is also dependent on the availability of a snail (*Lymnae natalensis*) intermediate host. The highest mean (28.5±1.5) was recorded in January 2017, following the higher rainfall (196.6mm) recorded in September of 2017, which was five months later. A similar finding was reported by Brook Lemma *et al*. (1985), who observed that the highest incidence of Fasciolosis occured 4.5 months after the beginning of heavy rainfall.

3.4.3.3 The monthly relationship between nematode parasites of sheep and rainfall

In this study, of these eight nematode parasite species, the mean counts of *H. contortus* and *T. axei* showed a similar pattern with the increase of total monthly rainfall and average monthly relative humidity. In line with these findings, Fakae and Chiejina (1993) reported that in wet months (April to October), the mean worm counts of *H. contortus* and *T. colubriformis* showed increase, and reduced then after due to the occurrence of dry months (November to March). *H. contortus* in tropics and subtropics, related to high temperature and heavy rainfall (Casey, 2014). It has intrinsically high biotic potential (Hansen and Perry, 1994) and was expected to have considerably contributed to pasture contamination.

3.4.3.4 The seasonal relationship between mean worm burden and rainfall

Regarding seasonal variations of mean ± S.E worm counts of gastrointestinal helminths, significant (F = 4.398; *p* = 0018) variations were observed with the highest mean ± S.E (534.63±63.91) and least mean ± S.E (288.19±59.55) recorded during long rainy and dry seasons, respectively. In support of these findings, Sissay Menkir (2007), reported that a gradual buildup of worm counts was observed with the highest burdens recorded during rainy seasons. According to Abdelnabi *et al*. (2011), worm burden increases with the increase of the amount of rainfall during rainy seasons. In this study, mean seasonal rainfall and average seasonal relative humidity appears to be the main factors correlated with the seasonality of the gastrointestinal helminths mean count. There were strong insignificant (r = 0.988 and 0.909; *p* = 0.098 and 0.273) correlations between total seasonal worm counts with mean seasonal rainfall and average seasonal relative humidity. This result was in agreement with the report of Almalaik *et al*. (2008) and Kumar (2014).

There was also a significant variation (F = 4.933; *p* = 0012) of mean nematode count among the study seasons. The highest mean ± S.E (533.75±63.97) nematode counts was observed during the long rainy season followed by short rainy season and dry season with a value of 391.93±46.13 and 288.25±59.40, respectively. Similar finding was reported inside the country by Sissay Menkir (2007). In general, moist and warm environmental conditions are favorable for the development, survival and transmission of the larval stages of nematode parasites (Hansen and Perry, 1994; Urquhart *et al*., 1996).

Regarding the seasonal mean counts of cestode and trematode parasites, there were no similar trends of mean worm counts with the increase in seasonal rainfall. Cestodes and trematodes were less common compared to nematodes, as they are dependent on the availability of intermediate hosts. Similar findings were reported by Abdelnabi *et al*. (2011) from Central Kordofan, Sudan.

The mean ± S.E seasonal nematode species counts during long rainy season, short rainy season and dry season were found to decrease in that order. In this study, the majority of nematode parasite species, *H. contortus, T. axei, T. ovis* and *B. tringoincephalum,* showed similar trends with increase of seasonal rainfall. In support of these findings, Abdelnabi *et al*. (2011) in Sudan reported that there was a clear seasonal influence on worm burden for most species of nematodes with the highest burdens encountered in rainy season, relatively lowered in winter and reaching the lowest levels in summer.

3.4.3.5 Level of nematode parasites burden

In this study, the intensity of infection for the majority of nematode parasite species (*H. contortus* (100%) and *T. axei* (100%), *T. ovis* (28.57%), *B. tringoincephalum* (70.59%) and *O. columbianum* (57.14%)) were lightly affected and some (*T. ovis* (71.41%), *B. tringoincephalum* (29.41%) and *O. columbianum* (42.86%)) were moderately affected. In agreement of these findings, Almalaik *et al*. (2008) reported that the intensity of the parasite infection on sheep in their study was light to moderate. The probable reason for these findings might be the availability of pasture in the wetlands of Lake Zwai throughout the study period reduce the degree of infection.

3.4.3.6 The relationship between total worm burden and sexes of the animals

In this study, the mean ± S.E counts of gastrointestinal helminths in female and male lambs were 370.39±48.82 and 442.17±53.42, respectively. However, there was no significant ($p > 0.05$) variations between female and male lambs mean worm counts. Similar findings were reported by Desalegn Lidetu (2005) and Almalaik *et al*. (2008).

3.4.4 EPG counts

3.4.4.1 Faecal Strongyle nematode egg counts

In this study, the highest Strongyle nematode EPG counts were found to be more common than *T. ovis*, *M. expanza* and *Fasciola* species. Similar findings were reported by Bersissa Kumsa *et al.* (2011) in Central Oromia. The possible reason for this might be the fact that to Strongyle nematode parasites, particularly, *H. contortus,* higher egg releasing ability than the other helminths (*T. ovis*, *M. expanza* and *Fasciola* spp.). The other possible reason for the highest EPG of Strongyle nematode might be high worm burden of those parasites in the tracer sheep, particularly in the rainy seasons. The main reason could be the existence of constant wetlands availability throughout the year coupled with warmer and humid climatic conditions in Zwai wetlands for survival of the infective stages of the Strongyle parasites.

There was no significant variation on the EPG counts of female and male lambs, indicating that both sexes are equally susceptible to Strongyle nematodes. Similar findings were reported by Odoi *et al*. (2007), Tesfaheywet Zeryehun (2012), Takele Sori *et al*. (2013) and Zelalem Mengist *et al*. (2014).

3.4.4.1.1 The relationship between mean EPG count of Strongyle nematode and rainfall in Zwai wetlands

The pattern of EPG count during various months was positively correlated with rainfall of Zwai wetlands during the period from April 2015 to March 2017. Similar findings were reported by Odoi *et al*. (2007) and Abdelnabi *et al*. (2011). According to these authors, the result of EPG count throughout the study months is positively correlated with rainfall. Rainfall that injects fresh water into the wetlands of Lake Zwai is the main determining factor to predict the level of transmission of gastrointestinal parasitic infections in grazing animals (see also Blackie, 2014) and governing the development and survival of the pre-parasitic stages in the pastures (Ng'anga'a, 2004). Hansen and Perry (1994) reported that during rainy seasons, when dew is on the pasture, the infective stages larvae migrate to the top of the herbage to be ingested by grazing sheep. Gastrointestinal helminth parasites in grazing sheep are directly related to the availability of larvae on pasture and pasture contamination (Hansen and Perry, 1994; Urquhart *et al*., 1996), as climatic conditions influence for the buildup of pasture larval contamination, which increases vulnerability of the grazing animals for parasite infections.

These results revealed that there was an increase in total seasonal EPG count of Strongyle nematodes with along with the increase in total seasonal rainfall, and the variation was found significant among study seasons. These findings were in agreement with the reports of Ng'ang'a, (2002), Desalegn Lidetu (2005), Abdelnabi *et al*. (2011), Takele Sori *et al*. (2013) and Gana *et al*. (2015). According to these authors, the EPG count of Strongyle nematode parasites were high during the rainy seasons, but begin to decline with the reduction of the amount of rainfall.

In Zwai wetlands, due to the presence of sufficient moisture in the water front of the Lake during the rainy seasons, the wetland favored the survival of the parasite in the pasture and with higher uptake probability of the infective egg/larval stages by the grazing sheep that come after succulent fresh leaves of the wetland fodders, leading to higher EPG of Strongyle nematodes. Whereas in the dry seasons, the availability of herbage in wetlands of Zwai declined due to shrinking of the wetlands and grazing beyond the carrying capacity of the wetland areas. Similar finding was also reported by Ng'anga'a *et al*. (2004).

3.4.4.1.2 The relationship between mean EPG count and worm burden of Strongyle nematode

There was positive significant correlation between EPG counts and worm burden of Strongyle nematode parasites of tracer sheep that grazed in the wetlands of Zwai. As the number of Strongyle worm count increases, there was also a subsequent increase of EPG of Strongyle nematode eggs. In line with these findings, Rinaldi *et al*. (2009) and Swarnkar and Singh (2015) reported a positive correlation of EPG and gastrointestinal Strongyle worm counts. The relationship between the number of EPG count with the worm intensity depended on the types of species involved; as adult female *H. contortus*, one of the Strongyle type nematodes release up to 10,000 eggs per day for several months (Raza *et al*., 2014) than any other types of gastrointestinal helminth parasites (Cabaret *et al*., 1998). Roberts and Swan (1981) has reported a strong correlation between EPG and the total number of *H. contortus* counts in naturally infected sheep.

3.4.4.2 PCV (%) values of experimental tracer lambs released in Zwai wetlands

The PCV (%) values recorded in this study revealed that parasite burdens of the experimental tracer sheep were in the normal range (26-36.5%) even though they were found to be infected by Strongyle nematode parasites. Supporting these findings, Rozita *et al*. (2015) reported that sheep infected by bloodsucking helminth and their PCV (%) recorded from 19-38% are considered as normal.

The PCV (%) values recorded based on sex of the tracer animals revealed that there was no significant variation between female and male experimental tracer sheep. In agreement with these findings, Tekelye Bekele *et al*. (1992) reported that sex has no effect on PCV (%) values on sheep.

3.4.4.2.1 The relationship between PCV (%) and worm counts

In this study, there was strong correlation between PCV (%) and Strongyle worm count. Similar findings were reported Yacob Hailu *et al*. (2008). This might be due to the fact that animals infected by high burden of heamoparasites like *H. contortus* may result the reduction of PCV (%) values than animals with low burden or no infections of these parasites. According to Soulsby (1982) and Yacob *et al*. (2008), *H. contortus* is known to cause PCV (%) reduction. Similarly, Hansen and Perry (1994) reported that *H. contortus* is highly pathogenic blood sucking parasite and infections with large numbers of this parasite resulted in reduction of PCV (%) in the infected grazing sheep.

CHAPTER 4.

PREVALENCE OF GASTROINTESTINAL PARASITES IN SCHOOLCHILDREN AT BOCHESA ELEMENTARY SCHOOL ADJACENT TO ZWAI WETLANDS, ETHIOPIA

4.1 Introduction

Worldwide, wetlands support large communities of people, who depend on important ecosystem services that maintain them. Within wetland ecosystems, people try to improve their livelihood by converting land cover, extracting resources and redirecting water flows (Nyandwi, 2017). Due to these human activities, useful and harmful environmental effects are induced. Properly managed wetlands are generally associated with enhanced ecosystem services and delivered improved outcomes for human health, and mismanaged wetlands provide poor ecosystem services with negative human health impacts (Finlayson and Horwitz, 2015).

For people who live in and around wetlands, their different activities will be a proximal determinant of their health (Horwitz and Finlayson, 2011). Human behavior, which results in contact between humans (particularly children) and wetlands is a risk factor for water-related gastrointestinal parasites (Dale and Connelly, 2012). In wetland areas, gastrointestinal parasites are major sources of health problems, where socioeconomic, cultural, and environmental conditions contribute towards maintaining the biological cycles of parasites and facilitating their spread. Proximity to water, combined with a humid, warm climate and sandy soil creates favorable conditions for larval development of gastrointestinal helminth parasites in the environment (Dias *et al*., 1982). Therefore, wetlands are areas of potential contributor to water-related gastrointestinal diseases when the environment is not properly managed and sanitation infrastructures are poor.

In developing countries like Ethiopia, wetland related activities like reed harvesting, fishing, irrigation and swimming are common practices done by the surrounding community; and these activities can exacerbate the transmission of water-related gastrointestinal parasites to humans. Warm climates and adequate moisture are also essential for the hatching of gastrointestinal helminth parasite eggs in the environment or development of their larvae.

Water-related diseases remain major public health problems, particularly children are the most vulnerable sector of society (Harhay *et al*., 2010) in low and middle income countries (Erismann *et al*., 2016) and cause about 1.7 million human deaths annually, and 88% is attributed to unsafe water supply, poor sanitation, and lack of hygiene (WHO, 2002).

The prevalence of zoonotic helminth infections in humans in any region is directly associated with the prevalence of infections in the animal population of that region (Ekong *et al*., 2012). Livestock are important sources of zoonotic infections to humans, due in part to the close interactions between animals and the people who keep them around wetlands. In sub-Saharan Africa; up to 250 million people are estimated to be infected with at least one or more species of intestinal nematodes (WHO, 2002).

Wetter areas are usually associated with increased transmission of soil-transmitted helminthes infections (Brooker *et al*., 2006). Transmission of gastrointestinal parasites in wetland areas increase when people engage in activities like working with bare hands, walking barefooted and using teeth as a third hand to tie reeds. For populations living around wetland areas, poverty, illiteracy, lack of access to clean water and hot and humid tropical climate are the factors associated with gastrointestinal parasitic infections (Sehgal *et al*., 2010). Improper sewage disposal and the practice of allowing untreated infected sewage to drain in fresh water lakes are responsible for the establishment of the parasites (WHO, 2002). The main determinants for human infection are lack of sanitation and inadequate hygiene (absence of hand washing with soap after defecation and before eating and walking barefoot) (Abebe Alemu *et al*., 2011). School age children are one of the groups at high-risk for intestinal parasitic infections, as they also do not pay much attention to such primary health care procedures.

Ethiopia is one of the countries where there is lack of sanitation, access to clean water and improper hygiene, which favor the survival of gastrointestinal parasites. Because of this and other reasons in Ethiopia, gastrointestinal helminthes parasite infection is prevalent because of the low level of living standards, poor environmental sanitation and ignorance of simple health promoting factors (Abebe Alemu *et al.*, 2011).

In many developing countries including Ethiopia, the most prevalent and important helminths are soil-transmitted nematodes, particularly *Ascaris lumbricoides*, *Trichuris trichuria* and hookworms (Addis Adera *et al*., 2015). Previous research regarding gastrointestinal parasites closer to the wetland areas indicted a high prevalence of these infections on schoolchildren (Mengistu Legesse and Berhanu Erko, 2004). However, research was not conducted around Lake Zwai on schoolchildren. Therefore, the objective of this study was to assess the prevalence of gastrointestinal parasite infection and associated risk factors among schoolchildren at Bochesa Elementary School, around the wetlands of Lake Zwai.

4.2 Material and Methods

4.2.1 Description of the study area

This study was conducted at Bochesa Elementary School in the southwestern direction of Lake Zwai, about 167 km south of Addis Ababa, which is the capital of Ethiopia. The school is situated at an altitude of 1636 m above sea level, which is similar to the lake. Students in the school operate under poor socio-economic status with no adequate safe water supply in their village. The majority of the inhabitants earn their livelihoods through cultivation of maize and vegetables using irrigation of the lake water and rearing of sheep and goat.

Students were instructed on how they bring stool samples of their own by providing labeled plastic containers, toilet tissue papers and applicator sticks through the assistance of their classroom teachers.

During stool collection, questionnaire was also administered for each student. The questionnaire was developed (Appendix-II) in English and translated into Afan Oromo to collect socio demographic data (gender, age, grade level and mothers' level of education), environmental factors (drinking water source, water for bathing, water for washing clothes and availability of latrine) and behavioral habits (swimming, raw meat-eating, improperly washed or raw vegetable consumption, playing with moist soil, area of defecation, nail trimming and hand washing before meals) that could be associated with high prevalence of gastrointestinal parasites in students.

4.2.2 Sample size determination

The desired sample size (n) was estimated using the statistical formula given by Daniel (1995),

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

Where, n = sample size, z = 1.96, which is z-statistic for a level of confidence, d = 0.05, which is absolute precision, and p = expected prevalence of the area.

Since the prevalence of gastrointestinal parasites is not known for the study area on schoolchildren, p-value was taken to be 50%. To obtain the sample size, a 95% confidence interval (z) and a 5% margin of error (d) was used. Therefore, the estimated sample size was 384. Among the selected 384 students, 192 were males and 192 were females in the age range from 7 to 20 years.

4.2.3 Examination of stool samples

4.2.3.1 Direct wet mount

About 5 g of fresh faecal samples were collected from each student and placed in separate labeled clean plastic stool containers. Small amount of stool (2 g) was placed on a clean microscope slide by the use of an applicator stick. A normal saline solution was added to it to emulsify the specimen, so as to enhance the clarity of the eggs and larval stages of helminth parasites, and cyst and trophozoite stages of protozoans, which may be observed under light microscope. A cover slip was then carefully placed on the prepared sample to avoid the introduction of air bubbles.

Diagnosis for positive results was based on the identification of helminthes eggs, larvae and protozoan cyst or trophozoite in the sample during microscopic examination at 100× and 400× magnifications.

4.2.3.1 Formol-ether concentration technique

Formol-ether sedimentation technique was used following standard procedure to concentrate wide range parasites from stool specimen with minimum damage to their morphology (Cheesbrough, 2006). A portion of the faecal sample (3 g) was sieved with cotton gauze and transferred to 15 ml centrifuge tube. Latter, 8 ml of 10% formalin and 3 ml of diethyl ether was added and centrifuged for 2 min at 2000 rpm. The supernatant was discarded and the remaining residues were used for smear preparation. Smear was prepared from residues transferred to microscopic slides and a cover slip was carefully used to avoid the introduction of air bubbles. Diagnosis for positive results was based on the identification of helminth eggs and protozoan cyst in the sample during microscopic examination at 100× and 400× magnifications.

The overall presence of gastrointestinal parasites was confirmed when observed by any of the methods used.

4.2.4 Data analysis

Variations in distribution patterns of positive stool samples between categorical variables (sex, age and grade level) were determined. The prevalence of infections was reported in proportions. Chi-square (χ^2) test was used to evaluate the association between categorical variables and infection prevalence.

To identify determinant factors associated with gastrointestinal helminth parasites, binary logistic regression was held, and finally the association between independent variables and dependent variables were described on the basis of odd ratio (OR) with 95 % confidence interval (CI). Crude OR was estimated by univariate regression analysis and adjusted OR was then estimated by multivariate logistic regression analysis. Values were considered statistically significant when the *p*-value was less than 0.05.

4.2.5 Ethical consideration

To conduct research on Bochesa Elementary School students, permission was obtained from Adami Tulu Jido Kombolcha Woreda Health Office at Zwai town. Finally, appropriate treatment as per the recommendations of a medical doctor was given to students who were found positive for gastrointestinal parasites by local nurses.

4.3 Results

4.3.1 Demographic data of the study participants

A total of 384, of which 192 male and 192 female students, were involved from grade 1 to 8. Students were grouped into two grade levels (grades 1 to 4 and 5 to 8) and grades 1 to 4 accounted for 63% (242) and the remaining 37% (142) were from Grades 5 to 8 categories. The age of the students who participated in the study were also grouped 7-15 years and greater than 15 years with a value of 352 (91.7%) and 32 (8.3%), respectively. Regarding the level of education for student’s parents, 18.8% (72) were illiterate and the remaining 81.2% (312) were literate, i.e., ranging from ability to read to completion of Grade 10 All of them responded positively by providing fresh faecal samples and answering the questionnaire.

4.3.2 Overall prevalence of gastrointestinal parasite

The overall prevalence of gastrointestinal parasites infection was 22.39% (95% CI: 18-26.3%). Different types of gastrointestinal parasites like cestodes, nematodes and protozoans with a value of 37 (9.64%), 40 (10.42%) and 29 (7.55%) were detected, respectively from Bochesa Elementary School students. A total of eight species (four species of nematodes, 2 species of cestodes and 2 species of protozoan parasites) were identified with different prevalence rates. *Hymenolepis nana* was the predominant parasite detected on 34 (8.85%) students of Bochesa Elementary School, and least prevalence was recorded on *Taenia* spp. with a value of 3 (0.78%) (Table 4.1).

Table 4.1: Different groups of GI parasites, species collected and their prevalence at Bochesa Elementary Schoolchildren.

GI parasites	Species obtained	Frequency (n)	Prevalence (%)
Nematodes	*A. lumbricoides*	17	4.43
	T. trichuria	8	2.08
	S. stercoralis	8	2.08
	Hookworm spp.	14	3.65
Cestode	*H. nana*	34	8.85
	Taenia spp.	3	0.78
Protozoa	*E. histolytica/dispar*	20	5.21
	G. lamblia	18	4.69

4.3.2.1 Sex-wise prevalence of gastrointestinal parasites

Regarding sex, the prevalence was high on males (14.1%) as compared to females (8.3%). There was statistically significant (χ^2 = 7.252; p = 0.005) variation on the prevalence of GIP infection between males and females (Table 4.2).

Table 4.2: Sex wise prevalence of gastrointestinal parasites at Bochesa Elementary Schoolchildren.

Sex	Frequency (n)	Prevalence (%)	χ^2-value	P-value
Males	192	54 (14.1%)	7.252	0.005
Female	192	32 (8.3%)		
Total	384	86 (22.4%)		

4.3.2.2 Age-wise prevalence of gastrointestinal parasites

The prevalence of gastrointestinal parasites on age groups of 7-14 and >15 were 20.3% and 2.1%, respectively. However, there was no statistical (p> 0.05) difference in the prevalence of gastrointestinal parasites between age groups (Table 4.3).

Table 4.3: Age wise prevalence of gastrointestinal parasites at Bochesa Elementary Schoolchildren.

Age	Frequency (n)	Prevalence (%)	χ^2-value	P-value
7-14	352	78 (20.3%)	0.136	0.428
>15	32	8 (2.1%)		
Total	384	86 (22.4%)		

4.3.2.3 Prevalence of gastrointestinal parasites based on grade category

With regard to grade category, the prevalence of gastrointestinal parasites was higher (16.7%) at Grades 1 to 4 than (5.7%) observed at Grades 5 to 8. A chi-square test revealed that was a significant (χ^2 = 5.490; p = 0.013) variation on the prevalence of GIP infection between grade categories (Table 4.4).

Table 4.4: Overall prevalence of gastrointestinal parasites based on grade category.

Grades	Frequency (n)	Prevalence	χ^2-value	*P*-value
1-4	242	64 (16.7%)	6.177	0.008
5-8	142	22 (5.7%)		
Total	384	86 (22.4%)		

4.3.2.4 Gastrointestinal parasite co-infections

Out of the 86 (22.6%) positive cases, infection with single GI parasite in an individual was more dominant (14.8%) than mixed infections. The remaining were double, triple and quadruple parasitic infections with a value of 6%, 1.3% and 0.3%, respectively (Fig. 4.1).

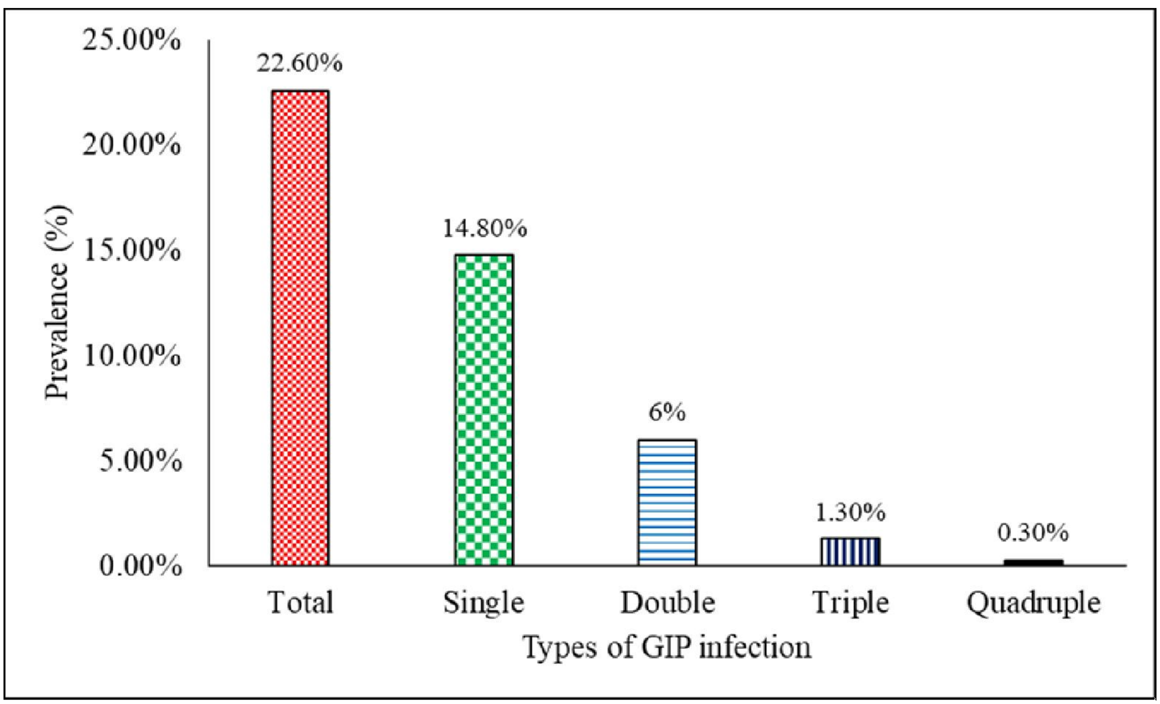

Fig. 4.1: Prevalence of single and mixed GIP infection among Bochesa Elementary Schoolchildren.

Single parasitic infection was higher in males than in females with a value of 36 (9.4%) and 21 (5.5%), respectively and the variation was statistically ($\chi^2 = 4.635$; $p = 0.022$) significant. Regarding grade category, the prevalence of single parasite infection was higher on 1-4 grade than 5-8 with a value of 40 (10.4%) and 17 (4.4%), respectively. However, the variation was statistically ($p > 0.05$) insignificant. Similarly, the prevalence of single parasitic infection among ages 7 to 14 ages was higher than the ages >15 with a value of 50 (13%) and 7 (1.8%), respectively, and the variation was not statistically ($p > 0.05$) significant.

Among double parasitic infections, *H. nana* and *E. histolytica dispar, H. nana* and *A. lumbricoides*, *H. nana* and *G. lamblia*, *A. lumbricoides* and *E. histolytica*, *E. histolytica/dispar* and *G. lamblia*, *H. nana* and *S. stercoralis*, *A. lumbricoides* and *G. lamblia*, Hookworm and *E. histolytica/dispar,* Hookworm and *A. lumbricoides*, Hookworm and *S. stercoralis*, and Hookworm and *H. nana* were recorded with a value of 4 (1.04%), 3 (0.78%), 3 (0.78%), 3 (0.78%), 3 (0.78%), 2 (0.52%), 1 (0.26%), 1 (0.26%), 1 (0.26%), 1 (0.26%) and 1 (0.26%), respectively.

Double parasitic infection on males and females were 13 (3.4%) and 10 (2.6%), respectively; however, there was no statistically significant ($p > 0.05$) variation. Regarding grade category, the prevalence of double parasite infection was higher among 1 to 4 grade students than among 5 to 8 grade students with a value of 19 (4.9%) and 4 (1%), respectively; and statistically significant ($\chi^2 = 4.028$; $p = 0.033$) variation was observed on the prevalence of GIP between grade category. Whereas, the prevalence of double parasitic infection on 7 to 14 ages was higher than the age >15 with a value of 23 (6%) and 0 (0%), respectively, and the variation was statistically ($p > 0.05$) insignificant.

Triple infection was observed among *H. nana*, *E. histolytica/dispar* and *G. lamblia* with a value of 3 (0.78%) and Hookworm, *E. histolytica/dispar* and *G. lamblia* with a value of 2 (0.52%). Whereas, quadruple parasitic infection was observed on one (0.26%) individual only among *H. nana*, *S stercoralis*, *E. histolytica/dispar* and *G. lamblia*.

The prevalence of triple parasitic infection on male and female students were 1.04% (4) and 0.26% (1), respectively, and the variation was not statistically ($p > 0.05$) significant. Regarding grade category also, the prevalence of triple parasite infection was higher among 1 to 4 Grades group than 5 to 8 Grades group with a value of 4 (1.04%) and 1 (0.26%), respectively; however, the variation was statistically ($p > 0.05$) insignificant. Similarly, the prevalence of triple parasitic infection among 7 to 14 ages was higher than among ages >15 years with a value of 4 (1.04%) and 1 (0.26%), respectively. However, the variation was statistically ($p > 0.05$) insignificant (Table 4.5).

Table 4.5: The types of mixed infections, species associated and their prevalence of GIP on Bochesa Elementary School students.

Mixed infection	Frequency	Species associated	Prevalence (%)
Double	4	*H. nana* and *E. histolytica/dispar*	1.04%
	3	*H. nana* and *A. lumbricoides*	0.78%
	3	*H. nana* and *G. lamblia*	0.78%
	3	*A. lumbricoides* and *E. histolytica/dispar*	0.78%
	3	*E. histolytica/dispar* and *G. lamblia*	0.78%
	2	*H. nana* and *S. stercoralis*	0.52%
	1	*A. lumbricoides* and *G. lamblia*	0.26%
	1	*H. nana* and Hookworm	0.26%
	1	*A. lumbricoides* and Hookworm	0.26%
	1	Hookworm and *E. histolytica/dispar*	0.26%
	1	Hookworm and *S. stercorais*	0.26%
Triple	3	*H. nana*, *E. histolytica/dispar* and *G. lamblia*	0.78%
	2	Hookworm, *E. histolytica/dispar* and *G. lamblia*	0.52%
Quadruple	1	*H. nana*, *S stercoralis*, *E. histolytica/dispar* and *G. lamblia*	0.26%

4.3.2.5 Potential risk factors associated with gastrointestinal parasites

From the results of binary logistic regression analyses, the socio demographic and behavioral determinant variables like sex, grade category, fishing habit, raw meat-eating habit, playing with moist soil, washing hands before meal, availability of latrine, place of defecation, finger nail trimming, eating improperly washed vegetables and shoe wearing habits showed statistically significant association ($p < 0.05$) with prevalence of gastrointestinal parasites.

Whereas the other factors like age, parent's education, source of water for drinking, source of water for bathing, swimming habit and source of water for washing clothes did not show significant ($P > 0.05$) association with gastrointestinal parasites infection.

All variables that were significantly associated with gastrointestinal parasites from univariate analysis of binary logistic regression were rechecked in multivariate analysis. After adjustment, shoe wearing, water for bathing, fingernail trimming and latrine availability were found insignificantly associated with the prevalence of gastrointestinal parasites ($P > 0.05$).

Whereas the other variables remained significantly associated with gastrointestinal parasite infection, these include sex, grade category, fishing habit, raw/unwashed vegetable eating, raw meat/fish eating, habit of playing with moist soil, hand-washing habit before meal and place of defecation (Table 4.6).

Table 4.6: Binary logistic regression analysis for factors potentially associated with gastro intestinal parasite infection among Bochesa Elementary Schoolchildren.

Risk factors	Positive (%)	Negative (%)	Adjusted OR (95% CI)
Sex			
Male	51	141	7.779* (1.948-31.222)
Female	32	160	
Grade category			
1-4	61	181	0.307* (0.110-0.855)
5-8	22	120	
Fishing habit			
Yes	53	117	3.027* (1.117-8.206)
No	30	184	
Raw meat-eating habit			
Yes	63	165	4.710* (1.626-13.643)
No	20	136	
Playing with moist soil			
Yes	36	34	8.571* (3.884-18.913)
No	47	267	
Water source for bathing			
Lake water	79	255	2.125 (0.720-6.274)
Ground water	4	46	
Raw vegetable eating			
Yes	47	88	2.800* (1.126-6.961)
No	36	213	
Washing hands before meal			
Sometimes	27	13	4.961* (1.773-13.879)
Yes	56	288	
Finger nail trimming			
Yes	32	200	
No	51	101	1.869 (0.801-4.361)

Risk factors	Positive (%)	Negative (%)	Adjusted OR (95% CI)
Latrine availability			
Yes	47	255	
No	36	46	1.734 (0.815-3.692)
Area of defecation			
Latrine	9	151	
Open defecation	74	150	6.118* (2.414-15.510)
Shoe wearing habit			
Yes	27	190	2.109 (0.852-5.220)
No	56	111	

*Significant association

As the above table shows, being males increases the risk of gastrointestinal parasite infection 7.79 folds than being females (AOR: 7.799: 95% CI: 1.948-31.222).

Students who were from 5-8 grades category decreases the risk of gastrointestinal parasite infection by 69.3% than among students from 1-4 grades category (AOR: 0.307: CI 95%: 0.110-0.855).

Students who had fishing habit were 3.027 times more likely to be infected by gastrointestinal parasites than their counter parts (AOR: 3.027: CI 95%: 1.117-8.206).

Students who had raw meat-eating habit were 4.710 times more likely to be infected by gastrointestinal parasites than students who did not eat raw meat (AOR: 4.710: CI 95%: 1.626-13.643). Similarly, students who had raw vegetable eating habit were 2.8 times more likely to be infected by gastrointestinal parasites than their counter parts (AOR: 2.800: CI 95%: 1.126-6.961).

Students who had the habit of playing with moist soil increased their risk of infection by gastrointestinal parasites by 8.571 times those who do not play with moist soil (AOR: 8.571: CI 95%: 3.884-18.913). Similarly, students who had sometimes hand washing habit before meals increased by 4.961 folds the risk of gastrointestinal parasites than students who washed their hands before meals (AOR: 4.961: CI 95%: 1.773-13.879). Students who practiced open field defecation increased their risk of infection by gastrointestinal parasite by 6.118 times than students who used latrine (AOR: 6.118: CI 95%: 2.414-15.510).

4.3.3 Prevalence of gastrointestinal helminth infections among schoolchildren

The overall prevalence with one or more gastrointestinal helminthes parasite was 19.8% (95% CI: 15.6-24.1%) among children of Bochesa School. Six species of gastrointestinal helminthes parasites were identified with variable prevalence. The study revealed that *H. nana* was the most prevalent gastrointestinal helminth parasite with a prevalence of 8.85% (34/384) and Taenia spp. was the least with the prevalent with of 0.78% (3/384). *A. lumbricoides*, *T. trichuria*, Hookworm and *S. stercoralis* were recorded in the prevalence of 4.43%, 1.82%, 3.65% and 2.08%, respectively.

There was significant (χ^2 = 9.449; p = 0.002) difference in the prevalence of gastrointestinal helminthes parasites between male and female. The prevalence was higher in males 50 (13%) than in females 26 (6.8%). Regarding grade category, the prevalence of gastrointestinal helminthes parasites from 1 to 4 and 5 to 8 students were 57 (14.8%) and 19 (4.9%), respectively and there was significant (χ^2 = 5.835; p = 0.010) variation in the prevalence of gastrointestinal helminthes parasites between grade category of students. The prevalence of gastrointestinal helminthes parasites was slightly higher (18.5%) in students from 7-14 age categories than among ages above 15 years' categories (1.3%). However, no statistically significant (*p*>0.05) variation was observed in age wise prevalence of gastrointestinal helminthes parasites (Table 4.7).

Table 4.7: Prevalence of gastrointestinal helminthes parasites based on sex, grade category and age groups.

Sex	Frequency	Prevalence (%)	χ^2-value	*P*-value
Male	192	50 (13%)	9.449	0.002
Female	192	26 (6.8%)		
Total	384	76 (19.8%)		
Grade category				
1-4	242	57 (14.8%)	5.835	0.010
5-8	142	19 (4.9%)		
Total	384	76 (19.8%)		
Age				
7-14	352	71 (18.5%)	0.382	0.363
>15	32	5 (1.3%)		
Total	384	76 (19.8%)		

The prevalence of *A. lumbricoides* was higher on males (2.6%) than females (1.8%), however, there was no significant (p > 0.05) difference between the genders. The prevalence of *A. lumbricoides* was higher among 7 to 14 age groups (4.4%) than >15 age (0%) groups. However, there was no significant (p > 0.05) difference between the age groups. The prevalence of *A. lumbricoides* was also higher in the 1 to 4 grades category (3.9%) than in the 5 to 8 (0.5%) grade students. The difference in the prevalence of A. lumbricoides, among the different age categories was found to be significant (χ^2 = 4.855' p = 0.020).

Except the hookworms (χ^2 = 10.675, p = 0.001), the remaining helminth parasite species such as *A. lumbricoides*, *T. trichuria*, *S. stercoralis, H. nana* and Taenia spp. showed no significant (p > 0.05) difference in prevalence between the genders. However, among the grade categories of students, *A. lumbricoides* and *S. stercoralis* showed significant (χ^2 = 4.853 and 4.794, p = 0.020 and 0.024, respectively) difference. The remaining helminth parasites such as *T. trichuria*, hookworm, *H. nana* and Taenia spp. showed no significant (p > 0.05) variation between grade categories of students. Across the students' age groups, *A. lumbricoides*, *T. trichuria*, hookworm, *S. stercoralis* and *H. nana* showed no significant (p > 0.05) difference in prevalence. However, *Taenia* spp. showed significant (χ^2 = 13.469, p = 0.019) difference between age groups.

4.3.3.1 Relationship between gastrointestinal helminthes species with some selected risk factors

Shoe wearing habit was significantly (χ^2 = 10.932 and 14.409; p = 0.001 and 0.000) associated with *A. lumbricoides* and hookworm infections, respectively among the study subjects. Hand washing habit was also significantly (χ^2 = 6.878, 16.343 and 13.718; p = 0.023, 0.001 and 0.005) associated with *A. lumbricoides,* hookworm and *S. stercoralis* infections, respectively among study subjects. In addition, playing with moist soil was significantly (χ^2 = 10.932, 14.409 and 5.383; p = 0.001, 0.000 and 0.042) associated with *A. lumbricoides,* hookworm and *S. stercoralis* infections, respectively among the study subjects.

Availability of latrine was significantly (χ^2 = 15.946; p = 0.000) associated with hookworm infections among the study subjects. Similarly, defecation area was significantly (χ^2 = 12.705 and 5.836; p = 0.000 and 0.013) associated with *A. lumbricoides* and *T. trichuria* infections, respectively among the study subjects.

4.3.3.2 Potential risk factors associated with gastrointestinal helminth infections

From the results of Binary Logistic Regression Analyses, the socio demographic determinant variables like sex, grade category, fishing, raw meat-eating, shoe wearing, playing with moist soil, washing hands before meals, availability of latrine, place of defecation, finger nail trimming and eating with improperly washed vegetables showed statistically significant association (p < 0.05) with the prevalence of gastrointestinal helminth parasites.

Whereas the other factors like age, parent's education, source of water for drinking, sources of water for bathing, sources of water for washing cloths and swimming habits did not show significant (p > 0.05) association with gastrointestinal helminth parasite infections.

All variables that were significantly associated with gastrointestinal helminth infections from univariate analysis of binary logistic regression were rechecked in multivariate analysis. After adjustment, grade category, habit of washing hands before meal, raw/unwashed vegetable eating, fingernail trimming habit and latrine availability were found insignificantly associated with the prevalence of gastrointestinal helminth parasites (p > 0.05).

The other variables remained significantly associated with gastrointestinal helminth parasite infections (Table 4.8).

Table 4.8: Binary Logistic Regression Analysis for factors potentially associated with gastrointestinal helminth parasite infections among Bochesa Elementary School.

Risk factors	Positive (%)	Negative (%)	AOR (95% CI)
Sex			
Male	50	142	5.262* (1.413-19.599)
Female	26	166	
Grade category			
1-4	57	185	0.406 (0.150-1.098)
5-8	19	123	
Fishing habit			
Yes	52	118	3.721* (1.407-9.841)
No	24	190	
Raw meat-eating habit			
Yes	60	168	3.494* (1.228-9.939)
No	16	140	
Playing with moist soil			
Yes	34	37	5.213* (2.499-10.876)
No	42	271	
Raw/unwashed vegetable eating			
Yes	44	91	1.873 (0.799-4.389)
No	32	217	
Washing hands before meal			
Yes	53	291	
Sometimes	23	17	2.334 (0.932-5.842)
Finger nail trimming			
Yes	30	202	

Risk factors	Positive (%)	Negative (%)	AOR (95% CI)
No	46	106	1.212 (0.516-2.846)
Latrine availability			
Yes	43	259	
No	33	49	1.782 (0.868-3.657)
Area of defecation			
Latrine	10	150	
Open defecation	66	158	4.128* (1.702-10.017)
Shoe wearing habit			
Yes	23	194	
No	53	114	2.919* (1.214-7.006)

*Significant association

As the above table shows, males were 5.262 times more likely to be infected by gastrointestinal helminth parasites than female students (COR: 5.262: 95% CI: 1.413-19.599). Students who had fishing habits were 3.721 times more likely to be infected by gastrointestinal helminths parasites than those who had no fishing habits (COR: 3.721: 95% CI: 1.407-9.841). Students having raw meat-eating habit were 3.494 times more likely to be infected by gastrointestinal helminth parasites than those who have not eaten raw meat-eating habits (COR: 3.494: 95% CI: 1.228-9.939). Students who had a habit of playing with moist soil were 5.213 times more likely to be infected by gastrointestinal helminth parasites than their counter parts (COR: 5.213: CI: 2.499-10.876). In addition, students who did not wear shoes regularly were 2.919 times more likely to be infected by gastrointestinal helminthes parasites than those who wore shoes regularly (COR: 2.919: 95% CI: 1.214-7.006). In addition, students who practiced open-field defecation were 4.128 times more likely to be infected by gastrointestinal helminth parasites than those who used latrines (COR: 4.128: 95% CI: 1.702-10.017).

4.3.4 Prevalence of Gastrointestinal protozoan infection

The overall prevalence of gastrointestinal protozoan parasites was 7.55% (95% CI: 4.9-10.4%). Two species of intestinal protozoan parasites were identified with variable prevalence. The study revealed that the prevalence of *E. histolytica/dispar* was slightly higher (5.21%) than the prevalence of *G. lamblia* (4.69%). However, 9 (2.34%) students were found infected by both *E. histolytica/dispar* and *G. lamblia* (Fig. 4.2).

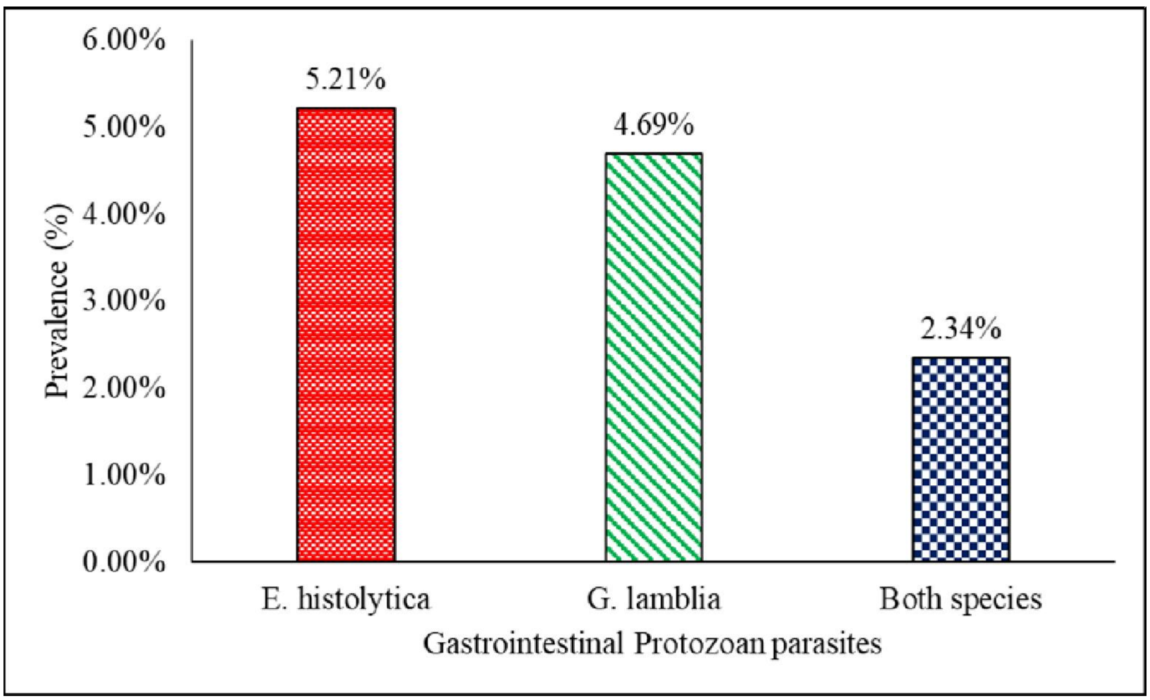

Fig. 4.2: Prevalence of gastrointestinal protozoan parasite species on Bochesa Elementary Schoolchildren.

The prevalence of gastrointestinal protozoan parasites was slightly higher in males (4.4%), 1-4 grade category (5.5%) and 7-14 age group (6.8%) than females (3.1%), 5-8 grade category (2.1%) and >15 age groups (0.8%), respectively. However, there were no statistical significant (p > 0.05) difference in gastrointestinal protozoan parasites infection of different sexes, grade categories and age groups (Table 4.9).

Table 4.9: Prevalence of gastrointestinal protozoan parasites based on sex, grade category and age groups.

Sex	Frequency	Prevalence (%)	χ^2-value	*P*-value
Male	192	17 (4.4%)	0.932	0.220
Female	192	12 (3.1%)		
Total	384	29 (7.55%)		
Grade category				
1-4	242	21 (5.5%)	1.188	0.188
5-8	142	8 (2.1%)		
Total	384	29 (7.55%)		
Age				
7-14	352	26 (6.8%)	0.166	0.443
>15	32	3 (0.8%)		
Total	384	29 (7.55%)		

4.3.4.1 Potential risk factors associated with gastrointestinal protozoan infection

From the results of binary logistic regression analyses, the socio demographic and behavioral determinant variables like level of parent's education, playing with moist soil, hand washing habit before meal, eating improperly washed vegetables, and place of defecation, finger nail trimming habit showed statistically significant association ($p<$ 0.05) with the prevalence of gastrointestinal protozoan parasites. However, other factors such as sex, age, grade category, swimming habit, source of water for drinking and source of water for bathing did not show a significant (p > 0.05) association with gastrointestinal protozoan parasites infection.

All variables that were significantly associated with gastrointestinal helminthes infection from univariate analysis of binary logistic regression were rechecked in multivariate analysis. After adjustment, availability of latrine, eating improperly washed vegetables and fingernail trimming habit were found insignificantly associated with the prevalence of gastrointestinal protozoan parasites (p > 0.05).

Other variables that remained significantly associated with gastrointestinal protozoan parasite infection; include parent's education, habit of playing with moist soil, habit of washing hands before meal and place of defecation (Table 4.10).

Table 4.10: Binary logistic regression analysis for factors potentially associated with gastro intestinal protozoan parasite infection among Bochesa Elementary Schoolchildren.

Risk factors	Positive (%)	Negative (%)	Adjusted OR (95% CI)
Parents education			
Illiterate	10	62	3.262*(1.071-9.937)
Literate	19	293	
Playing with moist soil			
Yes	20	51	7.025* (2.626-18.793)
No	9	304	
Washing hands before meal			
Some times	16	24	6.120* (2.126-17.619)
Yes	13	331	
Raw vegetable eating			
Yes	18	117	1.930 (0.594-6.269)

No	11	238	
Latrine availability			
No	16	66	2.496 (0.855-7.291)
Yes	13	289	
Place of defecation			
Open defecation	28	196	9.165* (1.152-72.934)
Latrine	1	159	
Finger nail trimming			
No	20	132	1.877 (0.591-5.967)
Yes	9	223	

*Significant association

As the above table shows, students who came from illiterate parents were 3.262 times more likely to be infected by gastrointestinal protozoan parasites than those from literate families (AOR 3.262: 95% CI: 1.071-9.937). Similarly, students who played with moist soil were 7.025 times more likely to be infected with gastrointestinal protozoan parasites than those who did not play with moist soil (AOR: 7.025: 95% CI: 2.626-18.793). Students who washed sometimes their hands before meal were 6.120 times more likely to be infected with gastrointestinal protozoan parasites than those who washed their hands regularly before meal (AOR: 6.120: 95% CI: 2.126-17.619).

4.4 Discussion

4.4.1 Overall prevalence of gastrointestinal parasites

The present study revealed the occurrence of eight species of gastrointestinal parasite among the Bochesa Elementary School students. The overall prevalence of gastrointestinal parasites observed in this study was 22.39%, which is compares closely with the prevalence values of 26.2% for schoolchildren in Southeastern Ethiopia (Begna

Tulu *et al*., 2014) and a value of 22.2% in rural areas of Angola (de Alegria *et al*., 2017). However, much higher prevalence values have been reported for schoolchildren from other parts of the country. Examples of very high prevalence values are as follows: 83.8% recorded in rural areas closer to South East Lake Langano by Mengistu Legesse and Berhanu Erko (2004); 79.8% recorded in North Gondar by Asrat Ayalew *et al*. (2011); 81% in Chencha Town, Southern Ethiopia by Ashenafi Abossie and Mohammed Seid (2014); 61.7% recorded in East Gojam, Ethiopia by Berhanu Elfu (2016); 58.8% recorded in Mekane Selam, Borena by Yimam Ali (2016) and 65.5% recorded in Bahir Dar Town by Tamirat Hailegebriel (2017). Higher prevalence values recorded in other countries include: 44.6% recorded in Southeastern Anatolian Region of Turkey by Doni *et al*. (2015) and 79% recorded in Tanta, Egypt by El-sehry *et al*. (2017).

The lower prevalence of GIP infection in this study is probably because in this school, students had received treatment for worms three months prior to the study (personal communication with the school principal and the manager of Zwai Health center); thus, this may contribute to the observed low prevalence of gastrointestinal parasite infection in the area. The other factors for the variation of prevalence values might be attributed to the variation in environmental and living conditions of the study participants. However, even though school based treatment was given to Bochesa Elementary school students, prior to three months at the time of data collection, the current result indicates the occurrence of gastrointestinal parasites in the area. According to Ashenafi Abossie and Mohammed Seid (2014), the hot and humid weather with wet soil are favorable for the occurrence of the infective stages of gastrointestinal parasites.

4.4.1.1 Prevalence of gastrointestinal helminth parasites

In this study, *H. nana* (8.85%) was the dominant gastrointestinal helminth parasite, followed by *A. lumbricoides* (4.43%) and Hookworm (3.65%). Similar findings were reported by Aschalew Gelaw *et al*. (2013) and Ephrem Tefera *et al*. (2015) as *H. nana* is the dominant gastrointestinal parasite in Gondar University community school and Babile Town elementary school students, respectively. The observed prevalence (8.85%) of *H. nana* in this study was in agreement with different researchers inside the country (9.6% by Mengistu Endris *et al*. (2010) in Azezo Town; 6.7% by Admasu Haile *et al*. (2017) in Guragie Zone; 11.46% by Ashenafi Teklemariam *et al*. (2014) in Enderta Woreda) and other countries: 6.5% by Erismann *et al.* (2016) in Burkina Faso.

This result was also relatively higher when compared to other studies among schoolchildren in different areas of the country (4.2% by Desta Haftu *et al*. (2014) in Arba Minch Town; 1% by Eleni Kidane *et al*. (2014) in Wukro Town; 4.4% by Begna Tulu *et al*. (2014) in Southeastern Ethiopia; 4.5% by Mengistu Wale *et al*. (2014) in Lumame Town; 4.7% by Tamirat Hailegebriel (2017) in Bahir Dar Town; 1.9% by Tilahun Workneh *et al*. (2014) in Debre Elias Town; 0.5% by Tilahun Alelign *et al*. (2015) in Durbete, 1.20% by Gebremicheal Gebretsadik (2016) in Homesha District of Benishangul Gumz; 4.6% by Meriem Abdi *et al*. (2017) in Zegie Peninsula, Bahir Dar Zuria) and other countries (2.05% by Lone *et al*. (2011) in India; 1% by El-sehry *et al*. (2017). This different might be due to the variation in environmental and living conditions of the study participants.

The prevalence 4.4% of *A. lumbricoides* obtained in this study was in agreement with the study conducted in Southeast Lake Langano (Mengistu Legesse and Berhanu Erko, 2004), in Jiga Town (Yonas Yimam *et al*., 2016), in Gondar (Aschalew Gelaw *et al*., 2013), in Southeastern Ethiopia (Begna Tulu *et al*., 2014) and in Wukro Town (Eleni Kidane *et al*., 2014). This result was also lower than other reports in Ethiopia by Endris *et al*. (2010) in Azezo Town, Asrat Ayalew *et al*. (2011) in North Gondar, Alamneh Abera and Endalkachew Nibret (2014) in Tilili Town, Ashenafi Abossie and Mohammed Seid (2014) in Chencha Town, Desta Haftu *et* a*l*. (2014) in Arba Minch Town, Fikru Gashaw *et al*. (2015) in Maksegnit and Enfraz Towns, Mengistu Wale *et al*. (2014) in Lumame Town, Yimam Ali (2016) in Mekane Selam, Admasu Haile *et al*. (2017) in Guragie Zone, Merem Abdi *et al*. (2017) in Zegie Peninsula, and elsewhere in Kashmir India by Lone *et al*. (2011). Lower prevalence value of *A. lumbricoides* were also reported inside the country by Tilahun Workneh *et al*. (2014) in Debre Elias Town, and Gebremichel Gebretsadik (2016) in Homesha District, Benishangul Gumz, and elsewhere in African countries by Sam (2012) in Ghana and de Alegria *et al*. (2017) in Angola. According to Zerihun Tadesse *et al*. (2008), the national estimated average prevalence of *A. lumbricoides* is 37%, which was far higher than the current result of this study. The probable reason for the lower prevalence of this study might be due to the school-based deworming activity held on Bochesa elementary school three months prior to the study time. According to Yalewayker Tegegne *et al*. (2018), recent deworming programs in schoolchildren for helminthiasis are being undertaken twice a year all over Ethiopia and this results a reduction of new spread and burden of parasitic worms.

According to Scott (2008), the control program of *A. lumbricoides* most frequently focus on de-worming through school-based system, as school aged children tend to have the highest intensity of Ascariasis infection. For instance, in Republic of Korea, the nationwide schoolchildren deworming activity held twice a year between 1971 and 2004 reduced the national prevalence of *A. lumbricoides* from 54.9% to 0.03% (Hong *et al*., 2006). The other possible reason for this variation might be the difference of the study period as this study was conducted at the end of short rainy season (May 2016).

The prevalence of Hookworm, *T. trichuria, S. stercoralis* and Teania spp. were 3.6%, 2.1%, 2.1% and 0.8%, respectively obtained in this study was lower compared with a study conducted in the Southeast of Lake Langano by Mengistu Legesse and Berhanu Erko (2004) with a value of 60.2%, 14.7%, 5.8% and 13.9%, respectively. The prevalence of hookworm (3.6%) obtained in this study was also lower compared with other reports conducted around wetlands inside the country by Mulat Alamir *et al*. (2013) in Bahir Dar, Tamirat Hailegebriel (2017) in Bahir Dar, and Merem Abdi *et al*. (2017) in Zegie Peninsula (Lake Tana), and abroad by Ross *et al*. (2017) in Philippines. The prevalence 2.1% of *T. trichuria* obtained in this study was lower compared with other reports inside the country by Merem Abdi *et al*. (2017) in Zegie Peninsula, and abroad by Ross *et al*. (2017) in Philippines. Similarly, the prevalence 2.1% of *S. stercoralis* was lower than other report in the country by Tamirat Hailegebriel (2017) in Bahir Dar. The prevalence 0.8% of Taenia spp. in this study was also lower with a report of Mulat Alamir *et al*. (2013) in Bahir Dar. Habit of eating raw meat in Ethiopia plays significant role in the transmission of Taenia spp. throughout the country. According to

Ayalew Jejaw *et al*. (2014), dietary habit of consuming raw meat as "kitfo" and "kurt" in Ethiopia contribute for the occurrence of Taeniasis.

The national average prevalence of *A. lumbricoides*, Hookworm and *T. tricuria*are estimated to be 37%, 16% and 30%, respectively (Zerihun Tadesse *et al*., 2008), which are by far higher than the current prevalence of the above-mentioned parasites in this study. The probable reason for the lower prevalence of this study might be due to the school-based deworming activity held on Bochesa elementary school three months prior to the study time.

The occurrence of gastrointestinal helminth parasitic infections among students of Bochesa Elementary School found in Zwai wetlands is an indication of faecal contamination of soil, improper utilization of latrine and poor personal hygiene in the area. Similar finding was reported by Girum Tefera (2014) in Zwai Town on pregnant woman.

4.4.1.2 Prevalence of gastrointestinal protozoan parasites

The prevalence of *E. histolytica/dispar* and *G. lamblia* found in this study was 5.2 and 4.7%, respectively. These results were relatively lower than the study conducted in Ethiopia by Asrat Ayalew *et al*. (2011) in North Gondar, Mulat Alamir *et al*. (2013) in Bahir Dar, Ashenafi Abossie and Mohammed Seid (2014) in Chencha Town, Southeastern Ethiopia, Desta Haftu *et al*. (2014) in Arba Minch Town, Eleni Kidane *et al*. (2014) in Wukro Town, Tamirat Hailegebriel (2017) in Bahir Dar, Gebremicheal Gebretsadik (2016) in Homesha District, Benishangul Gumz, Yimam Ali (2016) in

Mekane Selam by Admasu Haile *et al.* (2017) in Gurage Zone, and elsewhere in Burkina Faso by Erismann *et al*. (2016).

The low occurrence of the parasitic protozoan infections in the present study might be due to the difference in the study periods. This study was conducted in the dry season of the year; but outbreaks are more common during the summer season (Macpherson, 2005). According to Macpherson (2005), outbreaks of protozoan parasitic zoonosis are more common during the summer which may be due either to increased contamination of water supplies or greater numbers of susceptible persons using swimming pools. In addition to this, extension health workers may play their role on the prevention of gastrointestinal protozoan parasites in the area.

4.4.2 Sex-wise prevalence of gastrointestinal parasites

The prevalence of gastrointestinal parasites was higher on males than female and significant ($p < 0.05$) variation was observed between male and female. In line with these findings, different researchers reported inside the country by Mengistu Endris *et al*. (2010) in Azezo Town, Begna Tulu *et al*. (2014) in Southeastern Ethiopia, Ephrem Tefera *et al*. (2015) in Babile Town, and Admasu Haile *et al*. (2017) in Guragie Zone, and abroad by Golia *et al*. (2014) in Bangalore reported that males were highly affected by gastrointestinal parasites than females. The probable reason for this variation might be due to more involvement of males in outdoor activities such as irrigation, fishing and reed harvesting, and swimming more frequently than females because of social and religious restrictions. These activities sometimes carried out bare-footed and this situation predisposes males to different gastrointestinal parasitic infections. Similar finding was also reported outside the country by Albonico *et al*. (1997) and Baral *et al*. (2017).

4.4.3 Age-wise prevalence of gastrointestinal parasites

The prevalence of gastrointestinal parasites was higher on 7-14 age groups than >15; however, the variation was statistically insignificant ($p > 0.05$) between age groups. In agreement with these findings, other reports inside the country by Worku Meles *et al*. (2015) in Lumame Town, Gebremicheal Gebretsadik (2016) in Homesha District, Benishangul Zone and Melesse Birmeka *et al*. (2017) and abroad by Golia *et al*. (2014) in Bangalore reported that the prevalence of gastrointestinal parasite infection was higher on lower age groups. The probable reason for this difference might be due to frequent contact of lower aged students with contaminated soil in the wetland of Lake Zwai. Similarly, Norris *et al*. (2012) reported that as the age of children increases, they can protect themselves from intestinal parasitic infection due to maturity. Nyantekyi *et al*., (2010) in Wondo Genet, Southern Ethiopia reported that younger children are exposed to gastrointestinal parasitic infections due to less developed immune systems and habitually play in faecal contaminated soil. In addition to this, the habit of inserting soil contaminated fingers to their mouth and less awareness of hand washing practice before eating may increase the chance of acquiring gatrointestinal parasites.

4.4.4 Prevalence of gastrointestinal parasites based on grade category

The prevalence of gastrointestinal parasites was higher on 1-4 grade categories than 5-8 and the variation was statistically significant ($p< 0.05$) between grade categories. In support of these findings, Aschalew Gelaw *et al*. (2013) in Gondar, Worku Meles *et al*.

(2015) in Lumame Town and Gebremicheal Gebretsadik (2016) in Homesha Ditrict, Benishangul Gumuz reported higher prevalence of gastrointestinal parasites on lower grade category than upper grade category. The probable reason for this variation might be due to awareness created to keep personal hygiene in 5-8 grades than 1-4 grades. Personal hygiene greatly reduces the burden of intestinal parasites. This is due to the reason that proper personal hygiene breaks the chain of intestinal parasite transmission.

4.4.5 Gastrointestinal parasite co-infections

The present study revealed that the existence of parasitic co-infection in the wetland of Lake Zwai on Bochesa Elementary schoolchildren. In line with these findings, parasitic co-infections were reported inside the country by Mengistu Legesse and Berhanu Erko (2004) in Southeast of Lake Langano, Mengistu Endris *et al*. (2010) in Azezo Town, Asrat Ayalew *et al*. (2011) in North Gondar, Yonas Yimam (2016) in Jiga Town, Ashenafi Abossie and Mohammed Seid (2014) in Chencha Town, Begna Tulu *et al*. (2014) in Southeastern Ethiopia, Tamirat Hailegebriel (2017) in Bahir Dar, Worku Meles *et al*. (2015) in Lumame Town, Gebremicheal Gebretsadik (2016) in Homesha District, Benishangul Gumz. In sub-Saharan Africa, multiple infections are frequent in school age children (Norris *et al*., 2012).

The prevalence of parasitic co-infection in this study was 7.55%, which was agreed with other reports inside the country by Abayneh Unasho (2013) in Southern Ethiopia and Melesse Birmeka *et al*. (2017) in Guragie Zone. The possible reason for the existence of parasitic co-infection in the study area might be associated with Zwai wetland, which is found in front of their resident as well as their school, and the behavioral factors of students like frequent contact for swimming, bathing, fishing and irrigation purpose in the Lake. As Begna Tulu *et al*. (2014), in areas where different species of gastrointestinal parasites found, parasitic co-infections frequently encountered.

In this study, single parasitic infection was more common than parasitic co-infection. In line with these findings, Aschalew Gelaw *et al*. (2013) in Gondar, Ashenafi Abossie and Mohammed Seid (2014) in Chencha Town, Southern Ethiopia, Begna Tulu *et al*. (2014) in Southeastern Ethiopia, Merem Abdi *et al*. (2017) in Zegie Peninsula, and Tamirat Hailegebriel (2017) in Bahir Dar reported high prevalence of single parasite infection than parasitic co-infections.

Contrasting to these findings, Asrat Ayalew *et al*. (2011) in North Gondar reported low prevalence of single parasite infection than multiple parasitic infections. These discrepancies in the prevalence of single and multiple parasitic infections in different areas could be attributed to the differences in sample size, study population, socioeconomic and educational status of the community, environmental and personal hygiene, frequent contact of the study subjects with moist soil in wetlands, geographical variation, source of households' water supply, habit of walking bare-footed and diagnostic techniques in detections of various parasites.

More frequency of parasitic co-infection was observed between *H. nana* and *E. histolytica/dispar*. Whereas studies from other areas of the country showed that *A. lumbricoides* and *T. trichuria* by Ashenafi Abossie and Mohammed Seid (2014) in Chencha Town, Southern Ethiopia, Hookworm and *A. lumbricoides* by Mulusew Andualem (2014) in Motta Town, *E. histolytica/dispar* and *G. lamblia* by Gebremicheal

Gebretsadik (2016) in Homesha District, Benishangul Gumz. This difference might be due to the mode of transmission of the parasites as well as the behavioral activity of the study subjects that exposed them for different parasitic infections.

Single, double and triple parasitic infection in this study was higher on males than females. This might be due to the more involvement of males in outdoor activities than females. Similar finding was also reported outside the country by Baral *et al*. (2017) as males are highly vulnerable for gastrointestinal parasites than female.

Regarding grade category and age groups, single, double and triple parasitic infections were higher on 1-4 grade category and 7-14 age groups than 5-8 grade category and >15 age groups. The possible reason for this result might be schoolchildren in lower age groups and 1-4 grade categories might often spend more of their leisure time outdoors and are more often in contact with moist contaminated soil and eat indiscriminately with unwashed hands. Whereas the decreased parasitic infection rate in >15 age groups and 5-8 grade categories might be attributed to the better hygienic practices compared to their counter parts. Similar finding was reported by Golia *et al*. (2014) in Bangalore.

4.4.6 Potential risk factors associated with gastrointestinal parasites

The presence of Lake Zwai, which is closely located around Bochesa Elementary School, may provide suitable habitat for the sustainability of gastrointestinal parasites and may lead to increased risks for school-aged children, particularly through increased water contact exposure for bathing, fishing, swimming, cloth washing, and even irrigation activities due to its accessibility. According to Brooker *et al*. (2006), areas with adequate moisture and warm temperature are important determinant factor for the larval development of parasites in the soil.

The present study attempted to show the possible association of gastrointestinal parasitic infections with potential risk factors among Bochesa Elementary schoolchildren. A lot of researches have been conducted inside the country regarding the identification of potential risk factors associated with gastrointestinal parasites on schoolchildren (Asrat Ayalew *et al*., 2011 in North Gondar; Abebe Alemu *et al*., 2011 in Zarema Town; Aschalew Gelaw *et al*., 2013 in Gondar Town; Ashenafi Abossie and Mohammed Seid, 2014 in Chencha Town, Southern Ethiopia; Desta Haftu *et al*., 2014 in Arba Minch Town; Ephrem Tefera *et al*., 2015 in Babile Town, Gebremicheal Gebretsadik, 2016 in Homesha District, Benishangul Gumz; Admasu Haile *et al*., 2017 in Guragie Zone, Southern Ethiopia).

In this study, significant sex related difference in infection prevalence of gastrointestinal parasites was observed. The likelihood of acquiring gastrointestinal parasites on males was 7.79 times higher than being female. In line with these findings, Begna Tulu *et al*. (2014) in Southern Ethiopia, Ephrem Tefera *et al*., 2015 in Babile Town and Esmael Besufikad *et al*. (2017) in Tepi Town reported that males were more likely to be infected by gastrointestinal parasites than female students; and Doni *et al*. (2015) in Anatolian Region of Turkey reported that males were more likely to be infected by gastrointestinal parasites than females. Contrasting to this idea, Ephrem Tefera *et al*. (2015) in Babile Town, Southern Ethiopia and Admasu Haile *et al.* (2017) in Guragie Zone, South Ethiopia reported that female students were more likely to be infected by gastrointestinal parasites than male students.

The difference in the prevalence of gastrointestinal parasites on males than females might be a reflection of different behavior between male and female. The logic behind might be males engaged in irrigation activities more than their counterpart in the study area.

According to the additional analysis of gastrointestinal helminthes parasitic infection, males were 5.262 times more likely to be infected by parasitic infection than their counter parts. In accordance with these findings, Tadesse Dejenie and Beyene Petros (2009); Ashenafi Teklemariam *et al*. (2014) reported that males were more likely to be affected by gastrointestinal helminthes parasites than female. The probable reason for this gender variation might be due to more involvement of males on outdoor activities on wetland like agricultural and fishing areas at the lake borders and irrigation canals are common defecation places by males during working times, therefore transmission of gastrointestinal parasites would be facilitated.

Regarding gastrointestinal protozoan parasites also, students having fishing habits were 3.03 times more likely to be infected by gastrointestinal parasites than students who had no fishing habits. In lake bordering areas, occupational activities like fishing exposes the inhabitants for gastrointestinal parasites (Adenowo *et al*., 2015). Additional analysis on gastrointestinal helminthes infections indicated that students who had fishing habits were 3.721 times more likely to be infected by helminthes infections than students who had no fishing habit. In line with these findings, Adenowo *et al*. (2015) reported that frequent contact with infected water is a vital factor for the transmission of water-related gastrointestinal parasitic diseases.

Students having raw-meat eating habit were 4.71 times more likely to be infected by gastrointestinal parasites than students who had no raw-meat eating habit. Recently, the custom of eating raw-meat has grown worldwide and has led to the emergence of parasitic zoonoses in ethnic groups where eating raw-meat was not previously common (Macpherson, 2005). Therefore, raw-meat eating is a risk factor for gastrointestinal parasites.

Students who had raw/undercooked vegetable-eating habit were 2.80 times more likely to acquire gastrointestinal parasites than students who had no raw/undercooked vegetable-eating habit. In line with these findings, Asrat Ayalew *et al*. (2011) in North Gondar and Berhanu Elfu (2016) in Bahir Dar reported that students who had a habit of ingesting raw/undercooked vegetables were more likely to acquire gastrointestinal parasites than those who had no eating raw/undercooked vegetable habits. The probable reason for this might be due to the fact that raw vegetables are good culture media for gastrointestinal parasites (Asrat Ayalew *et al*., 2011); as a result, eating raw vegetable increases the chance of acquiring infection.

Students of lower grade are 4 times more likely to develop intestinal helminthes infection than students of higher grade (Ephrem Tefera *et al*., 2015). This could be speculated by increase in awareness; as grade increases awareness increases so that students of higher grade will have less exposure to intestinal helminthes infection than students of lower grade.

According to Asrat Ayalew *et al*. (2011), Aschalew Gelaw *et al*. (2013), Alamneh Abera and Endalkachew Nibret (2014), Desta Haftu *et al*. (2014), Tilahun Alelign *et al*. (2015)

and Gebremicheal Gebretsadik (2016), Admasu Haile *et al*. (2017), the likelihood of acquiring gastrointestinal parasite infections among students who do not practice hand washing habits were higher than among those who had good hand washing practice.

In addition to this, students who had no clean finger nails were 3.3 times more likely to acquire gastrointestinal parasites than their counter parts (Mulat Alamir *et al*., 2013; Alamneh Abera and Endalkachew Nibret, 2014). Children who had dirty materials in their fingers were 2.6 times more likely to acquire intestinal parasites infection (Desta Haftu *et al*., 2014; Esmael Besufikad *et al*., 2017). Sah (2016) in Eastern Region of Nepal. Similar findings were also reported that poor hand washing and hygiene of nails is comfortable environment for faecal-oral transmission of intestinal parasites. Thus, lack of personal hygiene might increase the probability of acquiring gastrointestinal parasite infection.

Students who had no habit of wearing shoes were 2.7 time more likely to acquire gastrointestinal parasitic infections as compared to those who wore shoes (Mulat Alamir *et al*., 2013). Abebe Alemu *et al*. (2011) in Zarema Town, Alamneh Abera and Endalkachew Nibret (2014) in Tilili Town, Tilahun Alelign *et al*. (2015) in Durebete Town; Berhanu Elfu (2016), reported that gastrointestinal parasitic infections for students who did not wear shoe increased by 29%. Similar finding was reported by Sah (2016) in Eastern Region of Nepal.

Students who played with moist soil were 8.57 times more likely to be infected by gastrointestinal parasites than students who had no such moist soil playing habit. Personal hygiene greatly reduces the burden of intestinal parasites. According to Bayeh

Abera *et al*. (2013), moist soil creates an environment conducive for a high prevalence of intestinal parasitic diseases. This might be due to the fact that proper personal hygiene breaks the chain of intestinal parasite transmission (Berhanu Elfu, 2016).

Availability of latrine greatly reduces the burden of intestinal parasites. This might be due to the fact that latrine dumps all the intestinal parasites away from the susceptible host (Berhanu Elfu, 2016). Similar reports were also reported by Alamneh Abera and Endalkachew Nibret (2014) in Tilili Town, Eleni Kidane *et al*. (2014) in Wukro Town, and Admasu Haile *et al*. (2017) in Guragie Zone.

After having come so far with the study of gastrointestinal infections of schoolchildren who have some contacts with wetland environments such around Lake Zwai, it is important to reflect if there are any diseases of zoonotic nature between livestock and humans, here represented by schoolchildren.

The results obtained clearly show that the diseases observed in schoolchildren and the animals used in the study, do not have much relations that warrant diseases of zoonotic nature. Although in some literature one may find people infected with *Fasciola* spp. (Acosta-Ferreira *et al*., 1979; Mas-Coma *et al*., 1999; Qureshi *et al*., 2015), for instance, these are considered as professional hazards where infected people work on wetland grasses infected with cercariae of *Fasciola*. Even such cases and the case of *Trichura* spp. in both the experimental sheep and schoolchildren do not represent the presence of zoonotic diseases. These appeared to be parallel or independent infections that occurred between the infective stages of the parasites and the sheep or the schoolchildren independently encountering the infections.

CHAPTER 5.

ASSESSMENT ON THE ABUNDANCE OF MOLLUSK SPECIES AND PREVALENCE OF SNAILS INFECTED WITH TREMATODE CERCARIA IN THE WETLANDS OF LAKE ZWAI

5.1 Introduction

There are many different animals that depend on wetlands in freshwater ecosystems, and without the habitat that wetlands provide, they would not be able to survive. Some of these species are freshwater mollusks. Freshwater mollusk communities are important in terms of biodiversity, ecosystem health and serve as important links in the food web. They play significant roles in public and veterinary health status (Mohammed *et al*., 2016). Mollusks like gastropods and bivalves are almost dependent on the availability of water for their survival. Gastropods are the largest, extremely diverse taxa that includes over 40, 000 species of which 5,000 are freshwater snails found in wetlands like lakes, ponds and streams worldwide (Galan *et al*., 2015). Lakes are known to be home to many snails (Azugo, 2007). Some species of snails found in different lake habitats with either no vegetation, floating or submerge vegetation and in habitats with emergent and sub emergent vegetation (Lydig, 2009) in lakes.

Snails have a wide range of importance to humans both economically and medically. Many snails act as an intermediate host to spread parasitic diseases in humans and his domestic animals (Brown, 1994; Qureshi *et al*., 2015). Snail transmitted diseases constitute an integral part of parasitic diseases transmissible to humans as many fresh water snails serve as intermediate host in the transmission of trematode parasites (Luka and Mbaya, 2015). Some trematode parasitic diseases move back and forth through freshwater bodies between man and snails in the course of changing their life cycle in those hosts.

Mollusks inhabits permanent water bodies across a large range of African fresh waters (Toyosi *et al*., 2007). For instance, a study conducted by Yirenya-Tawiah *et al*., (2011) indicted that five different species of intermediate host snails for the disease Schistosomiasis were found in Kpong water body of Ghana. Nkwengulila and Kigadye (2009) reported that around 12 species of snails were collected along the Ruvu River flood plain of Tanzania. In Ethiopia, according to Habtamu Mitiku *et al*. (2010), the presence of two snail species, *Biomphalaria sudanica* and *Lymnaea sp.* in Tikur Wuha River at Hawassa were found in a snail survey. Study conducted by Amare Lema (2005) in Lake Zwai also indicated the presence of freshwater snails like *Anisus natalensis, Biomphlaria sudanica, Balinus forsical, B. truncates,* and *Lymnae natalensis* and *Melanoides tuberculata*. The presence of *Melanoides tuberculata* in Lake Zwai was also reported by Brown (1994); Brook Lemma and Hayal Desta (2016).

Most regions of the world have specific snail hosts responsible for the transmission of trematodes infection (Mohammed *et al*., 2016). Snail species are the intermediate host of the majority group of helminth diseases (trematode) such as schistosomiasis, fascioliasis, heterophyisiasis and swimmers itch (Cercarial dermatitis) in the world, including Ethiopia (Farahnak *et al*., 2005). Some of the freshwater snails mostly serve as intermediate hosts to the trematode parasites, namely the Genus Fasciola and Schistosoma of humans and animals (Brown, 1994).

Most studies so far conducted in Ethiopia about the diversity of snail species were targeted on listing the type of species found in the study areas. There were no trials to shed cercaria from the collected snails and relate the type of animal disease they bring about in their definitive hosts. This study therefore, attempted to assess the distribution of snail species in relation to the physicochemical parameters, and shed cercaria from the collected snail species and relate them with host relationship of trematode species along the watershed of Lake Zwai.

5.2 Material and Methods

5.2.1 Sampling design

5.2.1.1 Mollusk species survey

Screening for the presence of Mollusks was held in the selected sites of the shorelines of Lake Zwai at Bochesa, Seda and Woshgula sites, where there is frequent interaction among livestock, humans and snails. Collection was held on seasonal basis. In Ethiopia, three seasons exist: (1) the main rainy season (June to September, called "Kiremt"); (2) the short rainy season (March to May, called "Belg"); and (3) the dry season (October to February, known as "Bega"). At each site, sampling was conducted twice per season: the beginning of dry season (October and November) and the beginning of short rainy season (March and April) (Appendix-III). In the sampling area, the prospected sites in the wetlands of Lake Zwai along the shoreline, where livestock graze and in some cases water was available at depths of 15 to 170 cm, and the sampling period lasted 15 min per site covering a randomly selected area of $1m^2$ (Barkia *et al*., 2014). At each sampling point, a $1m^2$ quadrant was laid and all snails within the quadrat visible in the naked eye were collected using gloved hand picking method to avoid infection with infective cercariae (Oguoma *et al*., 2010 and Barkia *et al*., 2014).

Alive collected snails were transported to Adami Tulu Agricultural Research Center laboratory and washed with clean water to remove the debris to identify the species using the key established by the Danish Bilharzia Laboratory (Kristensen, 1987) and Harrold and Guralnick (2010).

5.2.1.2 Shedding and identification of cercariae

In the laboratory, to harvest cercariae, natural emergence method was used by placing individuals of identified snails per 30 ml of water collected from the wetlands they were collected from after filtering it with 55 µm mesh net to avoid contaminations by larvae (Luka and Mbaya, 2015). Tap water supplied in the laboratory was not used to avoid any salinity or other chemical interferences that may kill the live larvae that were shading out of the snails. Then after, the labeled plastic bottles having individual snails with 25 ml water from the wetlands were placed in an incubator at a temperature of 25^{o}C for 6 hours to stimulate shedding of cercariae (Frandsen and Christensen, 1984). After 6 hours, the water in each of the bottle was separately decanted into the Petri dish to determine the presence of shedding cercaria under dissecting microscope (Ngoen-klan *et al*., 2010). Snails that did not shed cercariae in the first exposure were re-exposed to artificial light and rechecked for cercaria shedding by changing the water to avoid buildup of toxicity arising from the snail's metabolic wastes (Luka and Mbaya, 2015) and to avoid pH change in the container (Jayawardena *et al*., 2010). Collected cercariae were observed carefully and in the absence of fixatives, photos were taken directly from the microscope by attaching the Android cell phone for identification purposes. Identification of emerging cercariae was made to the genus level using tail morphology, swimming behavior and the general anatomical appearance (Frandsen and Christensen, 1984).

Prevalence of snails infected by cercariae was determined by percentage taking the number of snails that released cercariae, divided by the total number of snails collected from each site. The number of cercariae released per snail was counted using a Sedgwick Rafter Cell (Jayawardena *et al*., 2010).

5.2.1.3 Physicochemical parameters

The physicochemical parameters were determined according to standard methods using onsite examination. The parameters include, Temperature (°C), pH, DO (mg/l), Conductivity and Turbidity, which were measured *in situ* using a portable combined meter (Model YSI QUATRO (10102030)). In one sampling date, physicochemical parameters were measured three times and the average values were taken (Appendix-IV).

5.2.2 Data analysis

Mean values of physicochemical parameters were compared among sampling sites (Bochesa, Woshgula, and Seda) using One Way ANOVA using SPSS Version 21. The abundance of mollusk species among sampling sites was compared using Kruskal Wallis Test, as the data failed to fulfill the assumption of One Way ANOVA.

To see the main factors of species distribution along the physicochemical parameters, Redundancy analysis (RDA) was made using CANOCO 4.5 software followed by the Pearson Correlation Coefficient. To do this, DCA (Detrended Correspondence Analysis) was made to check the response of the study sites to the physicochemical parameters. As the gradient length (Appendix-I) of the physicochemical data found less than 2 during DCA analysis, RDA was used (Leps and Smilauer, 1999). Whereas for the relationship between study sites and mollusk species data, PCA was used.

Pearson correlation coefficient was performed to determine if there were any correlation between the physicochemical variables and the number of individuals using SPSS of version 21.

Diversity Indices were applied using Past software (Girum Tamire, 2014) to measure the diversity of snail species and relative abundance of each species among sampling sites.

The prevalence of snails infected by cercariae was compared among snail species, study sites, study months and seasons using Pearson Chi-square (χ^2) test. P-values less than 0.05 were considered for statistical significance.

5.3 Results

5.3.1 Diversity of mollusk species

In this study, a total of 1331 Mollusks from two class: Gastropoda and Bivalvia contributing 1270 and 107, respectively were collected. Class Gastropoda included four species of snails namely *Physa acuta* (Draparnaud, 1805)*, Lymnea natalensis* (Krauss, 1848), *Melanoides tuberculata* (Muller, 1774) and Planorbidae were collected and identified, whereas class Bivalvia comprised of two species, namely *Corbicula* *fluminalis* (Muller, 1774) and *Unio terminalis* (Bourgignat, 1852) were collected and identified.

Distribution of mollusks in relation to the study sites is presented in Fig. 5.1. Snail counts differed among study sites. In the collected snail species of Class Gastropoda, the highest species richness was obtained at Bochesa (4 species), followed by Seda and Woshgula, 3 species each; where as in Class Bivalvia, all the three sites involved the two species; namely *C. fluminalis* and *U. terminalis*.

The most abundant Mollusk species was *P. acuta*, constituting 84.02 % of the entire sample. The remaining species such as *U. terminalis*, *L. natalensis*, *C. fluminalis*, Planorbidae, and *M. tuberculata* covered 15.98%. However, no single live snail species of Planorbidae were collected in the study sites.

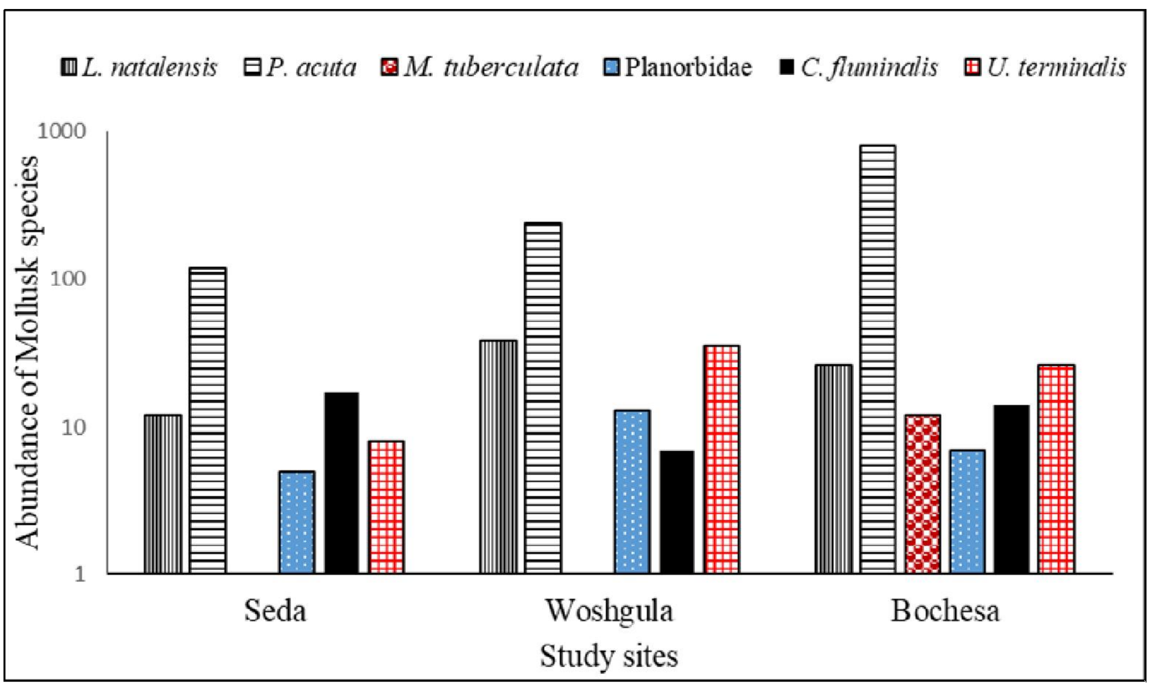

Fig. 5.1: Mollusks distribution in relation to the study sites.

5.3.2 Relationship between study sites and abundance of mollusk species

In this study, as the Kruskal Wallis Test showed, the mean rank values of *M. tuberculata* at Bochesa, Seda, and Woshgula were 12.5, 8 and 8, respectively. There was significant ($\chi2 = 6.733$; $p = 0.035$) variation of the abundance of *M. tuberculata* among the study sites. The mean rank values of *P. acuta, L. natalensis,* Planorbidae, *C. fluminalis* and *U. terminalis* at Bochesa, Seda, and Woshgula were (12.17, 6.83 and 9.5), (7, 9.5 and 12), (9.5, 9.33 and 9.67), (8.42, 10.58 and 9.5) and (9.25, 8.92 and 10.33), respectively. However, there were no significant ($p > 0.05$) variations of the abundances of *P. acuta, L. natalensis,* Planorbidae*, U. terminalis,* and *C. fluminalis* among the study sites.

Ordination of the study sites in relation to different snail species represented by a biplot diagram from the PCA is shown in Fig. 5.2. The percentage variance on the PCA axis-1 and 2 explain 99.46 and 0.54%, respectively of variability in the mollusk species.

These two axes explained together 100% of the total variation. Axis-1 divided the study sites into two groups: one group composed of Bochesa in the positive part whereas Seda and Woshgula in the negative part. This axis mainly represented a significant and positive correlation of *M. tuberculata, P. acuta*, *L. natalensis*, *U. terminalis*, and *C. fluminalis*. The results of PCA showed that Bochesa was associated with *M. tuberculata*, *P. acuta* and *C. fluminalis*.

Axis two also divides the study sites into two groups: one group comprised of Woshgula in the positive part whereas Seda and Bochesa in the negative part. This axis mainly represented a significant and positive correlation with Planorbidae, *U. terminalis* and *L. natalensis*.

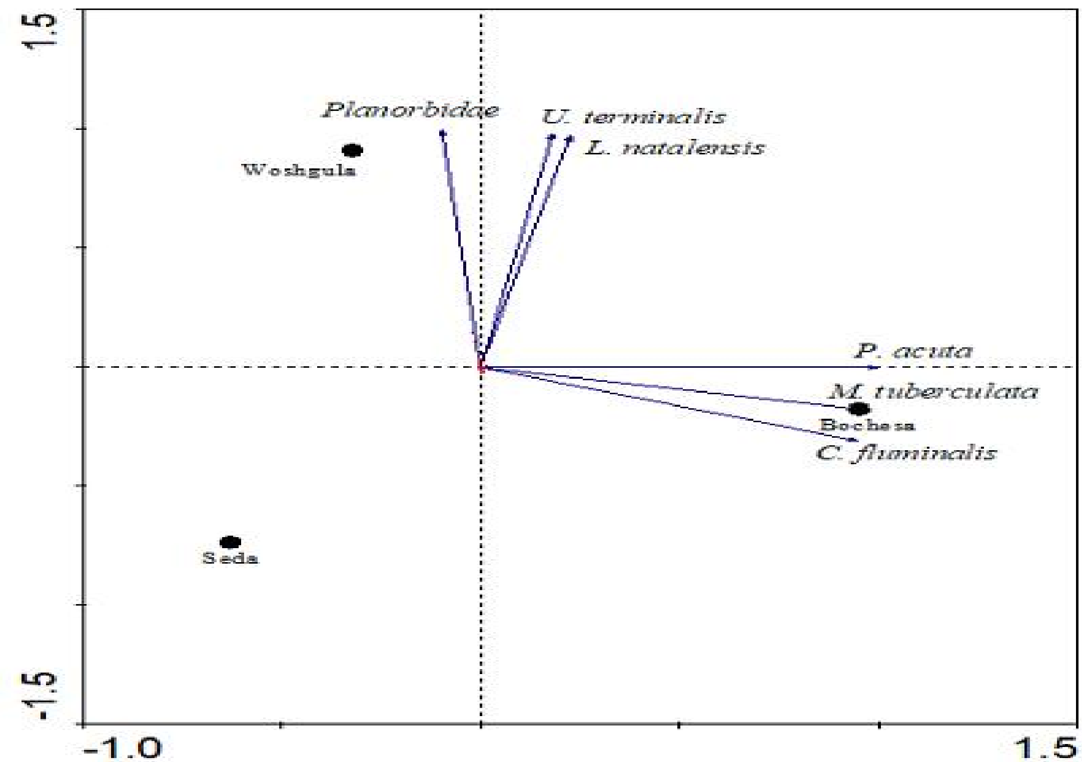

Fig. 5.2: A Principal Components Analysis (PCA) ordination bi-plot of mollusk species recorded from three sampling sites along the shoreline of Lake Zwai (variance explained: PCA-1 = 99.80%, PCA-2 = 0.20%).

5.3.3 Shedding of cercariae in the collected snail species

The findings of shedding cercariae from collected live snail species is presented in Table 5.1. Overall, regardless of the species, 86 (6.91%) of the snails were found to be shedding various species of larval trematodes. In this study, two species of Bivalves namely; *U. terminalis* and *C. fluminalis* were collected. However, these were of lesser interest to this study and were not investigated further on the shedding of trematode parasites.

Out of 1245 snails examined, 86 (6.91%) were found infected by different trematode cercariae. The prevalence of infection in some snail species is high. Of these cercariae infected snail species, *P. acuta* was the most prevalence (74.42%) of infection, followed by *L. natalensis* and *M. tuberculata* with prevalence of 20.93 and 4.65%, respectively. There was significant (χ^2 = 49.703; p = 0.000) variation in the prevalence of cercaria infection among snail species (Table 5.1).

Table 5.1: Prevalence of snails infected by cercariae based on different species.

Species	Frequency	Prevalence (%)	χ^2-value	*P*-value
P. acuta	1157	64 (74.42%)	14.342	0.001
L. natalensis	76	18 (20.93%)		
M. tuberculata	12	4 (4.65%)		
Total	1245	86 (100%)		

It was observed in this study that the prevalence of snails infected by cercariae varied over the study sites. The result indicated that, 44 (51.16%), 37 (43.02%) and 5 (5.81%) were observed at Bochesa, Woshgula and Seda, respectively. There was significant (χ^2 = 14.342; p = 0.001) variation in the prevalence of cercaria infection among study sites (Table 5.2).

Table 5.2: Prevalence of snails infected by cercariae based on study sites.

Sites	Frequency	Prevalence (%)	χ^2-value	*P*-value
Bochesa	476	44 (51.16%)	14.342	0.001
Woshgula	507	37 (43.02%)		
Seda	262	5 (5.81%)		
Total	1245	86 (100%)		

The monthly variation in the prevalence of infection showed that highest 35 (41.86%) was observed during March-2016, followed by April-2016, November-2016, November-2015, October-2015 and October-2016 with a prevalence of 24 (27.91%), 15 (17.44%), 8 (9.30%), 3 (3.49%) and 0 (0%), respectively. There was significant (χ^2 = 32.116; p = 0.000) variation in the prevalence of cercaria infection among study months (Table 5.3).

Table 5.3: Prevalence of snails infected by cercariae based on study months.

Study months	Frequency	Prevalence (%)	χ^2-value	*P*-value
October 2015	45	3 (3.49%)	32.116	0.000
November 2015	77	8 (9.30%)		
March 2016	617	36 (41.86%)		
April 2016	424	24 (27.91%)		
October 2016	18	0		
November 2016	64	15 (17.44%)		
Total	1245	86 (100%)		

The seasonal variation in the prevalence of infection showed that the highest value 60 (69.77%) was observed during short rainy season than in the dry season with a value of 26 (30.23%). There was significant (χ^2 = 12.929; p = 0.001) variation in the prevalence of cercaria infection between the seasons (Table 5.4).

Table 5.4: Prevalence of snails infected by cercariae based on season.

Season	Collected snails	Prevalence (%)	χ^2-value	*P*-value
Dry season	204	26 (30.23%)	12.929	0.001
Short rainy season	1041	60 (69.77%)		
Total	1245	86 (100%)		

Five different morphological types of cercariae were released namely; furcocerous, gymnocephalous, monostomous, echinostome and heterophydae type cercariae; some of which might represent more than one species (Fig. 5.3). These cercariae belonged to five trematode families; Schistosomatidae, Fasciolidae, Notocotylidae, Echinostomatidae and Heterophyidae (Table 5.5).

Table 5.5: Types of cercariae encountered from the collected snail species.

Snail species	Collected snails	Prevalence (%)	Types of cercariae
P. acuta	1157	64	Furcocerous cercariae
L. natalensis	76	18	Gymnocepahalous cercariae
			Monostome cercariae
			Echinostome cercariae
M. tuberculate	12	4	Heterophydae cercariae
Total	1245	86	

Fig. 5.3: Types of cercariae that emerged from the collected snail species.

5.3.4 Physicochemical parameters in the study sites

The results of mean temperature, *p*H, DO, conductivity and turbidity of the study sites are presented in Table 5.6. The highest (30.1, 29.2 and 27ºC) and lowest (20.6, 19.7 and 20.9ºC) water temperatures were observed at Bochesa, Woshgula and Seda, respectively. However, there was no statistically significant variations of temperature among study sites (one-way ANOVA, F = 0.154, p = 0.859). Regarding pH ranges from the highest to the lowest by the sampled wetlands were recorded as 8.67, 8.38 and 8.02 and the lowest 7.76, 7.45 and 8.02 at Bochesa, Woshgula and Seda, respectively. However, there was no statistically significant variations of pH among study sites (one-way ANOVA, F = 1.383, p = 0.281). The highest (9.55, 9.17 and 8.26) and least (3.97, 3.34 and 5.05) DO values were observed at Bochesa, Woshgula and Seda, respectively. However, there was no statistically significant variations of DO among study sites (one-way ANOVA, F = 2.971, p = 0.082). The highest (621, 974 and 579µS/cm) and least (108.5, 154.4 and 105.8µS/cm) electrical conductivity values were observed at Bochesa, Woshgula and Seda, respectively. However, there was no statistically significant variations of conductivity among study sites (One-way ANOVA, F = 0.330, p = 0.724). Comparing to the sampling sites, the highest (299, 247 and 295) and lowest (89, 155.33 and 139.67) turbidity values were recorded at Bochesa, Woshgula and Seda, respectively. However, there was no statistically significant variations of turbidity among study sites (one-way ANOVA, F = 1.373, p = 0.283).

Table 5.6: Mean ± Standard deviation values of physicochemical parameters in the sampling sites of the shoreline of Lake Zwai.

Parameters	Bochesa	Woshgula	Seda
Temperature (ºC)	24.573±3.487	24.290±4.432	23.488±2.315
*p*H	8.348±0.400	7.937±0.459	8.425±0.217
Dissolved Oxygen (mg/l)	7.045±2.406	5.182±2.720	7.003±1.245
Conductivity (ohms/cm)	405.417±235.451	497.095±302.689	384.933±218.175
Turbidity	211.722±70.401	197.605±50.701	251.662±76.162

Ordination of the study sites in relation to physicochemical parameters represented by a bi-plot diagram from the PCA is shown in Fig. 5.4. The percentage variance on the PCA axis-1 and axis-2 showed 99.80 and 0.20%, respectively of variability in the physicochemical parameters.

These two axes explained together 100% of the total variation. Axis-1 divided the study sites into two groups: one group composed of Bochesa in the positive part and another group composed of Woshgula and Seda in the negative part. This axis mainly represented a positive gradient of temperature, pH and DO. However, only the temperature was strongly correlated. On the other hand, conductivity and turbidity were negatively correlated.

Axis-2 also divides the study sites into two groups: one group composed of Woshgula in the positive part and the other group composed of Bochesa and Seda in the negative part. This axis mainly represented significant and positive gradients with conductivity and temperature. Whereas, pH, turbidity and DO were explained in the negative direction.

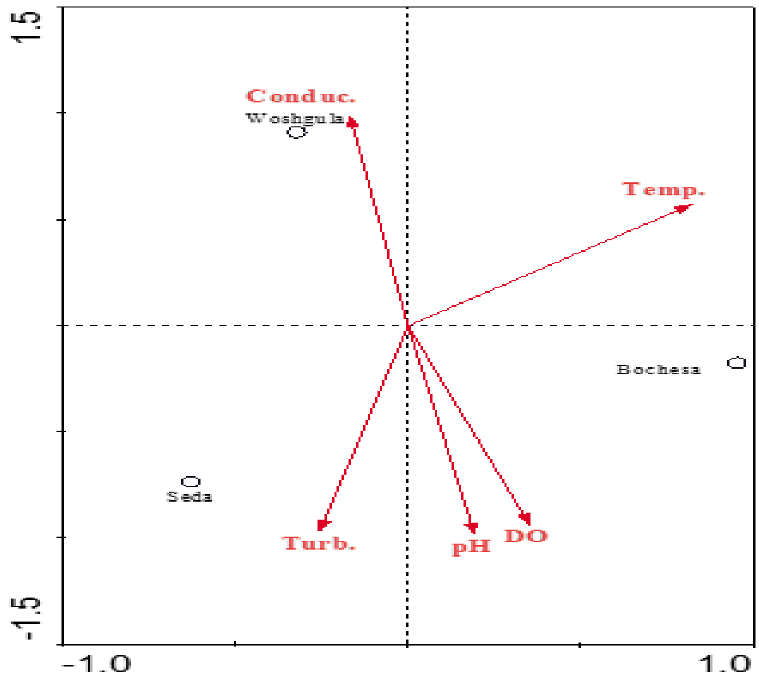

Fig. 5.4: A Principal Components Analysis (PCA) ordination bi-plot of the physicochemical parameters recorded from three sampling sites along the shoreline of Lake Zwai (variance explained: PCA-1 = 99.80%, PCA-2 = 0.20%).

5.3.5 Relationship between mollusk species and physicochemical parameters

Ordination of the mollusk species in relation to physicochemical parameters represented by a bi-plot diagram from the RDA is shown in Fig. 5.5. The percentage variance on the RDA axis-1 and 2 explain 99.80 and 0.20%, respectively of variability in the snail species.

These two axes explained together 100% of the total variation. Axis-1 divided the mollusk species into two groups: one group composed of *P. acuta*, *M. tuberculata*, *C.*

fluminalis, *L. natalensis* and *U. terminalis* in the positive part, and the other group composed only Planorbidae in the negative part.

The RDA ordination of mollusk species-physicochemical parameter association indicated that temperature, DO and *p*H were positively correlated with the first axis. However, only the temperature was strongly correlated (r = 0.8207). Whereas; turbidity and conductivity in the first axis were negatively correlated with values of r = -0.1671 and -0.2551, respectively.

Axis two also explained conductivity and temperature in the positive part with values of 0.9859 and 0.5713, respectively. Whereas; pH, turbidity and DO in axis two were strongly negatively correlated with values of r = -0.9813, -0.9669 and -0.9360, respectively (Table 5.7).

Table 5.7: Results of RDA of mollusk species with physicochemical parameters including eigenvalues and cumulative percentage

Axes	1	2
Eigenvalues	0.998	0.002
Cumulative percentage variance of species environment	99.8%	100%
Temperature	**0.8207**	0.5713
DO	0.3521	**-0.936**
pH	0.1925	**-0.9813**
Conductivity	-0.1671	**0.9859**
Turbidity	-0.2551	**-0.9669**

Strong correlations are marked bold

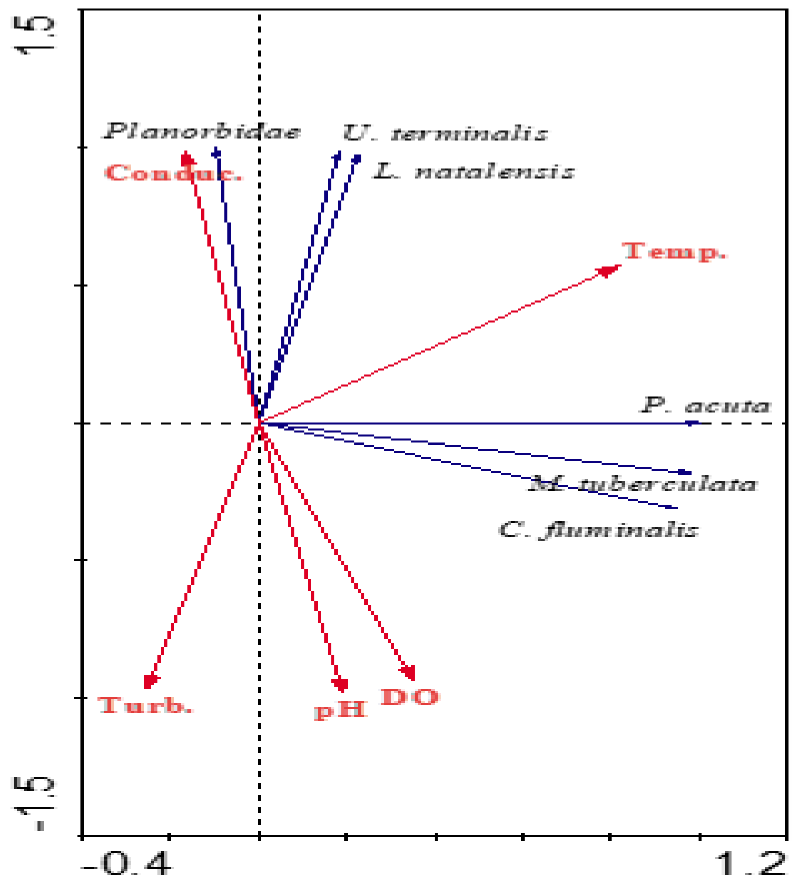

Fig. 5.5: A Redundance Components Analysis (RDA) ordination bi-plot of the mollusk species to the physicochemical parameters recorded from three sampling sites along the shoreline of Lake Zwai (variance explained: Axis-1 = 99.80%, Axis-2 = 0.20%).

5.3.6 Diversity indices of mollusk species

To see the diversity of mollusk species, four diversity indices (Table 5.8) were used such as Dominance, Shannon index, Evenness and Equitability index. The results of the diversity indices indicated that the Dominance (D) value raged from 70.77% at Bochesa to 72.04% at Seda. The Shannon-Wiener value was higher (0. 6882) at Bochesa than

Woshgula (0.6223) and Seda (0.6321), even though the variation is very narrow. Evenness and Equitability indices were higher at Seda than Bochesa and Woshgula.

Table 5.8: Diversity indices at three study sites of mollusk species in the study sites of the shoreline of Lake Zwai.

0	Bochesa	Woshgula	Seda
Taxa (S)	6	5	5
Individuals	**876**	333	160
Dominance (D)	0.7077	0.7155	**0.7204**
Shannon-Wiener diversity (H)	**0.6882**	0.6223	0.6321
Evenness (e^H/S)	0.3317	0.3727	**0.3763**
Equitability (J)	0.3841	0.3867	**0.3928**

5.4 Discussion

5.4.1 Diversity of mollusk species in Zwai wetlands

This study revealed that the existence of two classes of freshwater mollusks, namely, Gastropoda and Bivalvia are prevalent in the studied Zwai wetlands. In this study, predominance of gastropods was observed over bivalves which agrees with the findings of Toyosi *et al*. (2007) at Lake Alau in Nigeria and Sharma *et al*. (2013) in Gho-Manhasan Stream, India.

Regarding Gastropods, this study confirmed the presence of four species of freshwater snails that serve as intermediate hosts of some trematode species. The availability of Gastropods in this Lake was also reported by Amare Lema (2005). The presence of different species of gastropods (Snails) indicate the availability of trematode disease in humans and animals. Planorbidae and *L. natalensis* are the intermediate host to *Schistosoma mansoni* and *Fasciola gigantica*, respectively in many parts of Africa

(Brown, 1994) including Ethiopia (Yilma Jobre and Malone, 1998). The snail, Planorbidae collected in this study were not alive, unlike the findings of Berhanu Erko *et al*. (2006) who collected live snails during their study in Zwai wetlands.

In this study, the dominant snail species was *P. acuta*. In support of these findings, Brown (1994) stated that this snail is remarkably well tolerant to pollution and can be found in both stagnant and flowing waters. This might be due to the high dispersal ability of *P. acuta* over other snails. Similar findings were reported by Barkia *et al*. (2014) in Morocco, Yves *et al*. (2003) in Cote D'Ivore. According to Yves *et al*. (2003), most freshwater habitats are prone to the invasion of *P. acuta*.

Regarding Bivalves, this study also confirmed the existence of two species; namely *U. terminalis* and *C. fluminalis*. According to Sousa *et al*. (2008), benthic communities in lotic and lentic habitat in different regions of the world are a common habitat for *C. fluminalis*, also mentioned that, currently, high abundance of *C. fluminalis* is observed in freshwater lakes around the world. Of these collected 21 bivalves in this study, 17 (%) were *U. terminalis* and the remaining 4 (%) were *C. fluminalis*. In line with these findings, the dominance of *U. terminalis* was also reported by Aravind *et al*. (2005) in the Western Ghats.

5.4.2 Relationship between study sites and abundance of mollusk species

It is difficult to compare the result of these findings with previous studies, as there is scarcity of information on mollusk in Lake Zwai wetlands. There was significant variation on the abundance of *M. tuberculata* among the study sites. The abundance of *M. tuberculata* was found restricted to the Bochesa wetland.

The abundance of *M. tuberculata* was higher at Bochesa than Seda and Woshgula. This might be due to the flowing nature of the water in that particular site; as Bochesa is the place where the outlet flowing River Bulbula is found. As to Supian and Ikhwanuddin (2002); Giovanelli *et al*. (2005); (Galan *et al*., 2015), *M. tuberculata* is exclusively found in lotic habitats. According to these authors, the structural features of the *M. tuberculata* shells are designed in response to flowing water. At Bochesa, the shore-line is covered by grasses, which are serving as a habitat for *M. tuberculata*. As to Brown (1994), epiphytic algae attached to aquatic plants are the main source of food for *M. tuberculata*. Aquatic plants are also used for oviposition as well as hiding themselves from predators (Abe *et al*., 2017).

In this study, majority of mollusk species abundance in the wetlands around Lake Zwai shows little or no pattern. For instance, the abundances of *P. acuta*, *L. natalensis,* Planorbidae*, U. terminalis* and *C. fluminalis* showed no significant ($p > 0.05$) variation among the study sites. This might be due to the similarities of factors that affect the abundance of these mollusk species among the study sites. The similarity of the physicochemical values recoded in this study might contribute the absence of significant variations on these mollusk species. The interactions between the physical and chemical properties of water determine a significant role in the abundance of aquatic life (Mustapha and Omotosho, 2005) like mollusk species.

5.4.3 Shedding of cercariae in the collected snail species

The prevalence of snails infected by cercariae was higher at Bochesa than Woshgula and Seda. Higher cercariae immergence in this site might be a result of snail-trematode compatibility. This considerable rate of infestation of snail vectors could be enhanced by a higher population density of the snails at Bochesa (see also Rowel *et al*. 2015). This might be due to the availability of more irrigation canals at Bochesa suitable for the survival of snail species that serve as intermediate hosts for different digenean trematodes as compared to the other sites.

Regarding the monthly variation, the prevalence of snails infected by cercariae was higher during March 2016 and April 2016. This might be due to the increase in the availability of water and rise of air temperature in these months favoring the survival of snail species, hatching of trematode eggs and searching of miracidia to locate their intermediate hosts as compared to other months (see also Islam *et al*. 2015).

The seasonal variation of in the prevalence of snails infected by cercariae was higher during short rainy season (March to April) than during the dry season (October to November). In support of these findings, Yadav *et al*. (2006) and Tigga *et al*. (2014) reported that the number of snails shedding cercariae was highest during the rainy season. The probable reason for low prevalence of snails infected in the current study during dry seasons was mainly attributed to shrinkage of the wetlands with increase in the salinity of the water in the Zwai wetlands. Tenalem Ayenew and Dagnachew Legesse (2007) estimated a net annual loss of 74 million m^3 water from Lake Zwai due to evaporation. Therefore, evaporation is a cause for increase in salinity. Hart *et al*. (1991) reported that pulmonated snails are among the most sensitive of freshwater snails to salinity.

It is evident from the results that there was direct relationship between the prevalence of snails and the infection rate of trematodes. Seasonal variation may be directly correlated with the range of trematodes miracidial stage available on the study site to locate their intermediate hosts. Seasonal changes in the feeding preferences of the host play an important role in the seasonal cycle of the parasites. Seasonal changes in the infection rates with larval trematodes have been reported by researchers in other area (Arshad *et al*., 2011).

In this study, five types of larval trematodes that collected from three live snail species, namely, *Physa acuta*, *Lymnea natalensis* and *Melanoides tuberculata* were furcocerous, gymnocephalous, monostomous, echinostomes and heterophid cercariae types. The overall prevalence of the identified larval trematodes in the snails was 6.91%, indicating the suitability of the environment for helminth diseases by way of snail vectors in Zwai wetlands. Similarly, Islam *et al*. (2012) reported that 5.3% of the collected snails were found infected by different types of trematode cercariae in Mymensigh Sadar, Bangladesh. Recently, Tigga *et al*. (2014) also reported that the overall prevalence of snail's intermediate host infected with different cercariae was 7.33% in India. Contrasting to these findings, higher 16% prevalence of snail's intermediate host infected with cercariae was reported by Jayawardena *et al*. (2010) in Sri Lanka, which appears to be possible if the wet seasons are longer coupled with warm and humid environments.

Multiple infections by larval trematodes, namely, gymnocephalous, monostomous and echinostomous cercareae were also observed on *L. natalensis.* In line with this result, Loker *et al*. (1981) and Qureshi *et al*. (2015) reported that *L. natalensis* was found to serve as vector for six and four different trematode species in Tanzania and Pakistan, respectively. Jayawardena *et al*. (2010) in Sri Lanka reported one snail with mixed infections. Soldánová *et al*. (2010) in Germany also supported these findings that at least 71 species of trematodes are involved in infestations of Lymnaid snails.

Single infections of snails were observed in this study on *P. acuta* and *M. tuberculata*. For instance, Frandsen and Christensen (1984), Farahnak *et al*. (2005b) and Krailas *et al*. (2014) reported that various species of larval trematodes were obtained from *M. tuberculata*. Devkota *et al*. (2011) also reported that double species of larval trematode from *M. tuberculata*. Regarding *P. acuta*, contrasting to these findings, Gérard *et al*. (2007) and Mohammed *et al*. (2016) reported that no infection was found during their study. The absence of double or multiple infections in this study on *P. acuta* and *M. tuberculata* might be attributed to inter-species antagonism. Snails with multiple infections face higher chances of mortality compared to those with single infections (Sousa, 1992; Mohammed *et al*., 2016).

In this study, echinostomous cercariae emerged from *M. tuberculata*. Similarly, Farahnak *et al*. (2005b) and Krailas *et al*. (2014) reported that *M. tuberculata* was found infected by echinostomous cercariae in Iran and Thailand, respectively.

5.4.4 Physicochemical parameters in the study sites

In this study, physicochemical parameters were compared among study sites (Bochesa, Woshgula and Seda). However, there were no significant variations of the physicochemical parameters ($p > 0.05$) among the sites. This might be due to the close proximity of the study sites with each other.

Based on PCA, conductivity showed negative correlation with Axis-1 indicating decreasing turbidity gradient. Temperature, DO and pH showed negative correlation with Axis-2.

5.4.5 Relationship between mollusk species with physicochemical parameters

Water temperature depicted a positive correlation with mollusks in the present study. The positive correlation of temperature observed with mollusk species in this study agreed with Toyosi *et al*. (2007) and Sharma *et al*. (2013). This might be because of temperature influences the distribution of mollusk species and the rate of trematode development in the snail hosts, and probably influences the distribution of trematode animal diseases. It is known that temperature has a pronounced effect on the rate of chemical and biological processes in water; no other single factors affect development and growth of aquatic organism as much as water temperature (Babalola and Agbebi, 2013). Similar findings were reported by Toyosi *et al*. (2007) and Garg *et al*. (2009). This is likely to be because rise in temperature within the observed range favors the growth of mollusks. However, in Planorbidae, the correlation was negative.

DO in this study showed negative correlation with *L. natalensis, U. terminalis, P. acuta* and *C. fluminalis*. In line with these findings, Ikpeze and Obiwelu (2016) and Abe *et al*. (2017) reported that DO was negatively correlated with all snail species collected in their study. Contrasting to these findings, Toyosi *et al*. (2007) found that DO was positively correlated with all mollusk species collected during their study. However, in the present study, DO was positively correlated with *M. tuberculata* and Planorbidae contrasting to Abe *et al*. (2017). As Yirenya-Tawiah *et al*. (2011) and Marie *et al*.

(2015), the highest ratio of snail species was collected at DO ranges of 0.4-16mg/l and 0.6-8mg/l, respectively. The range of DO in this study was 2.01-9.55mg/l. However, the variation for the positive and negative correlation might be due to the different oxygen requirements of snail species.

pH depicted a negative correlation with *U. terminalis, L. natalensis, P. acuta* and *C. fluminalis*; where as positive correlation with *M. tuberculata* and Planorbidae in the present study. Similar findings were reported by Zukowski and Walker (2009). Contrasting to these findings, Toyosi *et al*. (2007) and Abe *et al*. (2017) reported that pH was positively correlated with all snail species collected in their study. A pH range of 6.2-7.2 in this study is favorable for most intermediate snail hosts, indicating that snails tolerate a wide range of pH (5.8-9.2) to exist in water bodies. The concentration of hydrogen ions is rarely a factor conditioning the presence and distribution of the snails. Investigations have shown that the pH can fluctuate in the same habitat. The pH is of importance, but not as a limiting factor.

The turbidity of the water in the wetlands showed negative correlation with all mollusk species collected in the study area; except Planorbidae. In line with these findings, Ngassam *et al*. (2014) in Cameroon reported that the number of collected snail species seemed to decrease with increasing turbidity. Previous studies conducted by Adamneh Dagne *et al*. (2008) and Girum Tamire (2014) in Lake Zwai revealed that turbidity increases of Lake Zwai over recent years. The probable reason for this might be due to increased anthropogenic activities that mainly resulted in the degradation of the lake watershed (Girum Tamire, 2014) as the study sites are closer to the shoreline of the lake. Intensive agricultural practices and low coverage of vegetation of the surrounding also contributed the increase of turbidity and decline of water transparency of Lake Zwai and its surrounding wetlands.

Conductivity depicted positive correlation with *L. natalensis, U. terminalis, P. acuta* and *C. fluminalis*; whereas negative correlation with Planorbidae and *M. tuberculata*. These findings were partially agreeable with the findings of Toyosi *et al*. (2007). Contrasting to the present findings, Abe *et al*. (2017) reported that conductivity was negatively correlated with all snail species collected during their study. The range of electrical conductivity in this study was 105.8-974µs/cm. In line with these findings, Marie *et al*. (2015), reported that the highest snail species were collected under electrical conductivity range of 501-1500µS/cm. According to these authors, increase in electrical conductivity, reduced the abundance of snail species.

5.4.6 Diversity indices of mollusk species

Biodiversity in aquatic environments depends largely on the water quality. Some species are more tolerant to pollution than others, and the abundance of sensitive species can be used as an indication of possible pollutants in the water. Community structure index is a measurement for biological structure, which incorporates two distinct aspects; richness (the number of taxa) and evenness (the distribution of individuals among taxa). The result obtained in this study revealed that in all sampling sites (Bochesa, Seda and Woshgula), the values of Shannon-Wiener (H) indices recorded were <1, which were very low. As to Sharifnia *et al*. (2012), Shannon-Weiner (H) values less <1 indicating high pollution in these sites. The presence of Gastropods and Bivalves in these site also indicated being they are stressor tolerant (having tolerant value of 7 for both classes)

(Bouchard, 2004), they are adapted and surviving in all sampling sites. However, the Shannon-Weiner and other values of indices revealed the similarities of compared sites due to their proximity and anthropogenic activities held at the wetlands of Lake Zwai along the shoreline.

CHAPTER 6.

ASSESSMENT ON THE OCCURRENCE OF ANTI-HELMINTHIC RESISTANCE AND FACTORS CONTRIBUTING TO THE DEVELOPMENT OF SUCH RESISTANCE OF HELMINTHS IN SHEEP OF THE WETLANDS OF LAKE ZWAI

6.1 Introduction

Gastrointestinal helminth parasites remain one of the most prevalent infections in grazing livestock worldwide (Pena-Espinoza, 2018). They are responsible for both direct and indirect major losses in livestock productivity around wetland areas. Of these, gastrointestinal nematode infections are one of the most prevalent and the principal health problems affecting small ruminant's worldwide (Farooq, 2009; Yitayew Demessie *et al*., 2016). To improve productivity of sheep, the control of gastrointestinal helminth parasites depend heavily on the administration of a few classes of anthelmintic drugs (De Graef *et al*., 2013; Walkite Furgasa *et al*., 2017).

Gastrointestinal helminth infections are very common in many parts of Ethiopia and their control is almost entirely based on anthelmintic drugs (Fikru Regassa *et al*., 2006). Anthelmintic drugs are simple to administration, cheap and cost-effective methods of controlling nematodes. They kill existing parasites and reduce the production of eggs. Therefore, they can prevent disease in infected animals and reduce the intensity of future infections in animals and their offspring (Stear *et al*., 2006).

In Ethiopia, the use of anthelmintic drugs has been practiced for a long time and constitutes a major share of the veterinary service costs (Demelash Biffa *et al*., 2007).

Three broad spectrum anthelmintic families of drugs are commonly used in Ethiopia to control gastrointestinal nematodes of sheep, namely, benzimidazoles, imidazothiazoles and macrocyclic lactones (Silvestre *et al*., 2002). In Ethiopia, three anthelmintic drugs, specifically Albendazole, Tetramizole and Ivermectine were continuously imported by the Ministry of Agriculture and distributed throughout the country (Dese Sheferaw *et al*. 2013).

In the past 25 years, no new classes of anthelmintic have been developed for use in animals, and given the limited economic potential of small ruminant production (Fleming *et al*., 2006). Almost predictably, the more these chemicals are used, the sooner drug resistance will emerge (Lyons *et al*., 2011). As a result, anthelmintic resistance is becoming a serious problem worldwide, particularly in developing countries where there is no rational use and the various anthelmintic drugs (Urquhart *et al*., 1996).

Now-a-days, worm control strategies involving anthelmintic drugs are under serious threat because of the development of anthelmintic drug resistance. The increasing incidence of resistance by the gastrointestinal parasites to available anthelmintic drugs is challenging the ability of producers to maintain high levels of productivity in the sheep industry (Vlassov *et al*., 2001). The primary factor to promote anthelmintic drugs resistance is related to the frequent and continuous use of the same anthelmintic drugs (Bastos *et al*., 2017). Thus, anthelmintic drug resistance has become a global problem (Coles *et al*., 1992).

There are many reports on anthelmintic drug resistance from several parts of the world (Coles *et al*., 1992). It becomes a serious problem in countries with small ruminant's industry, due to the frequent use of the same drug for extended years. In most regions of Africa, the development of anthelmintic drugs resistance could be expected to be slow, because of limited availability and infrequent use of anthelmintic drugs by most small-scale farmers (Sissay Menkir, 2007).

Resistance to anthelmintic drugs has particularly become a major problem in sheep infected with gastrointestinal nematode parasites (Várady *et al*., 2011). In recent years, anthelmintic drug resistance in sheep is becoming a wide spread threat in some African countries (Ayalew Niguse *et al*., 2014). Different researchers have also been reported on anthelmintic drug resistance in Africa; for instance, in Cameroon (Ndamukong and Sewell, 1992), Tanzania (Ngomuo *et al*., 1990), Zimbabwe (Boersema and Pandey, 1997), South Africa (van Wyk *et al*., 1997), Mozambique (Atanasio *et al*., 2002) and Ethiopia (Befekadu Urga and Teka Feyera, 2015).

The use of anthelmintic drug has been practiced for a long time in Ethiopia, and constitutes a considerable share of the costs spent by the country in the control of helminth diseases (Demelash Biffa *et al*., 2006). In the absence of other options, helminth parasites control will continue to rely on anthelmintic drugs. The appearance of drug resistance is therefore a practical problem in many countries of Africa and other continents due to their frequent use.

There are factors for the occurrence of drug resistance in a certain area. Of these, misuse like under dosage and frequent use of anthelmintic drugs in many forms, such as illegal sales in open markets and irrational administration, are widespread in Ethiopia (Bersissa Kumsa *et al*., 2010).

According to Lyons *et al*. (2011), The main factor affecting helminth parasites to develop resistance is frequent exposure to anthelmintic chemical compounds. Almost certainly, the more these chemicals are used, drug resistance will occur.

In Ethiopia, there was a rumor by the Regional Animal Health Officers and animal owners regarding the effectiveness of available anthelmintic drugs, especially Albendazole (Dese Sheferaw *et al*., 2013). Bersissa Kumsa and Girma Abebe (2009) also reported the existence of anthelmintic drug resistance in a goat farm in Hawassa; and on the other side, Kassahun Asmare *et al*. (2005) reported the absence of anthelmintic drug resistance in sheep in Southern Ethiopia. Therefore, the objective of this study was to assess the occurrence of gastrointestinal nematode parasites drug resistance to easily available drugs such as Albendazole, Tetramizole and Ivermectin by naturally infected sheep under field conditions in the study area.

6.2 Material and Methods

6.2.1 Study animals

Only sheep that had not been treated for at least the last two months were included in the anthelmintic drug resistance test (Chaparro, 2016; Walkite Furgasa *et al*., 2017). Twenty-four sheep of the same sex (all males) were used in this experiment; as the same sex is recommended (see also Thomaz-Soccol *et al*., 2004).

Those sheep were selected according to their pretreatment faecal egg count results (above 150 EPG), individually identified and randomly allocated into three treatments and a control groups with six sheep in each group (Klauck *et al*., 2014). One untreated group was used as control reference. Each sheep in every group was identified by numbered ear tags. All anthelmintic drugs were purchased from private veterinary pharmacies in Zwai Town. Each sheep was weighed by measuring balance for dose determination.

6.2.2 Sample collection and EPG counting

On the first day after the initial FEC, sheep were randomly distributed into treatment and control groups of six sheep each (Klauck *et al*., 2014), considering the initial EPG and their weight. Four sheep with the highest EPG counts were placed randomly, one in each group. Thus, all groups began the test with a very similar average number of EPG counts and the weight of individual sheep was recorded in each group.

Drug resistance test was conducted on three treatment groups of sheep using Albendazole, Tetramizole and Ivermectine, which are commonly available in the local market of Zwai Town. Each sheep in the treatment groups were dosed according to individual weight by measuring balance and treated with one of the three anthelmintic with the calculated dose based on their actual weight: Albendazole (3.8mg/kg), Ivermectin (0.2ml/kg) (Silvestre *et al*., 2002), which is half dose (Coles *et al*., 2006) and Tetramizole (10mg/kg) according to the manufacturer's recommendation (Chaparro, 2016); whereas, sheep in the 4th group remained untreated until the end of the study.

Albendazole and Tetramizole were administered orally; whereas, Ivermectine was administered subcutaneously (Table 6.1).

Table 6.1: Description of anthelmintic drugs used in the FECRT.

Generic name	Trade Name	Manufacturer	Dose mg/kg	Administration
Albendazole	Albenda-QK 300MG	Chengdu QiankunVet. Pharm. co. Ltd., China	3.8 mg/kg	Orally
Tetramizole	Doxamin	Daehan, Korea	10 mg/kg	Orally
Ivermectine	Ivermic	ShenyangSunvictor Pharmaceutical Co., Ltd., China	0.2 mg/kg	Subcutaneously

Faecal samples were taken in the morning from the rectum of the experimental sheep on days 0, 7 and 14 after treatment (Getachew Terefe *et al*., 2013) for nematode egg counts using McMaster Technique (Hansen and Perry, 1994). Counting of eggs took place immediately after collection of faecal samples. Fifty-six ml of saturated salt solution was added to four grams of faeces in a beaker. The faecal suspension was filtered into another beaker, and the sub samples were examined under compound microscope at 100 magnifications after both grooves of McMaster counting chamber filled with the suspension and allowed to stand for 5 minutes. The number of nematode eggs in both grooves of the McMaster chamber was multiplied by 50 to get EPG.

The faecal egg count reduction test (FECRT) was used for the evaluation of anthelmintic efficacy/resistance (Appendix-VI). The resistance of the drugs was tested according to the World Association for the Advancement of Veterinary Parasitology (WAAVP) recommendations for the detection of anthelmintic resistance (Coles *et al*., 1992). The anthelmintic drug efficiency in the FECRT was calculated by the RESO 2.0 program, where the mean arithmetic of EPG for the control group and the group treated by calculating the confidence interval of the mean was used (Klauck *et al*., 2014). Resistance is considered to be present if the percentage reduction in egg count is less than 95% or the 95% confidence level is less than 90% (Sissay Menkir, 2007). If only one of the two criteria is met, resistance is suspected (Coles *et al*., 1992).

6.2.3 Questionnaire survey

The desired sample size for the questionnaire survey was estimated based on the formula given by Kothari (2004).

$$n = \frac{Z^2 * p * q * N}{e^2 (N-1) + Z^2 * p * q}$$

Where, n = sample size, z = 1.96, which is z-statistic for a level of confidence, p = 0.03, which is the estimate within 3% of the true value, q = 0.07, which is 1-P, $e^2 = (0.02)^2$, and N = 517, which is the number of farmers who owned sheep at the selected four *kebele*'s (Batu, Bochesa, Dodicha and Adami Tulu) (personal communication with the responsible body at Adami Tulu Jido Kombolcha District). Therefore, the calculated sample size of sheep owners in four administrative Kebeles (Batu, Bochesa, Dodicha and Adami Tulu) found closer to the wetlands of Lake Zwai and River Bulbula was 100. The questionnaire was developed in English (Appendix-VII) and translated into Afan Oromo to obtain information on the pattern of anthelmintic drugs use including details of the estimated number of doses used per year, the estimated frequency of anthelmintic drugs used annually and the names of the drugs that they frequently used, and to make recommendations for the implementation of drugs using based on the real practice of participant farmers in the area.

6.2.4. Data analysis

After faecal analysis, data was analyzed using SPSS software, version 21. The effectiveness of the different anthelmintic drugs was evaluated by computing the mean faecal egg count reduction for each treatment group. The data on FECRT was analyzed through RESO program (Martin and Wursthorn, 1991). Anthelmintic resistance tests were carried out according to the methods of Coles *et al*. (1992) and RESO computer techniques were used for the detailed calculation of FECRT.

According to Coles *et al*. (1992), resistance is suspected when the percentage reduction in egg count is less than 95% or the lower limit for its 95% confidence interval is equal or below 90%.

The questionnaire survey was analyzed by using descriptive statistics to compute frequency of responses and percentage to summarize the data. Probability (P) value less than 0.05 was used to determine the level of significance.

6.3. Results

6.3.1 Demographic data of participant farmers

A total of 100 (69 males and 31 females) farmers were involved from four villages; namely Bochesa, Adami Tulu, Dodicha and Batu with a values of 25 for each. Based on their educational status, participant farmers were grouped into four levels (illiterate, 1 to 4 grades completed, 5 to 8 grades completed and grade 9 and above completed) with frequency of 12, 31, 44 and 13% of the total 100 participants, respectively. All of them voluntarily agreed to participate in the implementation of the questionnaire. The mean ± SE size of sheep ownership for the study participants was 9.36 ± 1.82.

6.3.2 Questionnaire survey

The questionnaire survey regarding to the grazing areas of sheep indicated that majority (57%) of the respondents replied that sheep grazed in the wetlands of Lake Zwai; whereas the remaining 26 and 17% responded that sheep grazed anywhere, including the wetlands and even in the backyards of the owner's residences. Concerning grazing practices, the majority (96%) of the respondents confirmed that their sheep grazed mixed with other livestock; whereas the remaining 4% said that their sheep grazed without mixing with other animals.

Regarding the seasonal occurrence of sheep disease, most (70%) participants responded that their sheep got sick all year round; whereas the remaining 17 and 13% replied that their sheep got sick during the wet seasons and dry season, respectively.

Most farmers (97%) treated their sheep using anthelmintic drugs, and three (3%) of the respondents indicated that they do not administer any treatment to their sheep at all. Regarding the involvement of their sheep treatment, the majority of the farmers (67%) treated their sheep by their own; whereas the remaining (33%) went to veterinarians.

Most farmers (83%) responded that sheep got treatment when they get sick; whereas the remaining 1 and 16% replied that their sheep got treatment once per year and more than three times per year, respectively. Regarding the choice of anthelmintic drugs, the majority of the participant farmers (95%) mentioned that Albendazole; whereas the remaining 5% have selected Tetramizole. The main criterion for the selection a specific anthelmintic drug by the majority of the participant farmers (52%) was its color; whereas the remaining 25, 18 and 5% selected the drug they bought due to its lower cost, recommendation by the veterinary workers and recommendation by their colleague farmers, respectively.

Concerning the place where drugs were purchased, 82, 11 and 7% of the respondents replied that they purchased the drugs from government owned veterinary clinics, open market/from other farmers and private drug vendors, respectively.

Most farmers (82%) had the knowledge on the dose of the drugs they used to treat their sheep and the remaining 18% did not know the dose of the drug. Majority of the respondents (66%) indicated that dose determination was based on visual judgment; whereas the remaining 34% replied that determination was based on prescription orders issued by veterinarians. Regarding drug rotation practice of the participant farmers, the majority (97%) revealed that they did not rotate drugs; whereas the remaining 3% said that they did rotate drugs.

6.3.3 Faecal egg count reduction test

Following the treatment with different types of drugs on Day 14, the efficacies of anthelmintic drugs like Albendazole, Tetramizole and Ivermectine were evaluated by using the faecal egg count reduction percentage test. The mean faecal egg counted on day 14 for the positive control group was 950, while the three treatment groups that received Albendazole, Tetramizole and Ivermectine were 17, 17 and 8, egg per gram counts, respectively following the standard McMaster egg counting procedure. The faecal egg count reduction test revealed that Albendazole, Tetramizole and Ivermectine were found with a reduction values of 98% (100-86%), 98% (100-93%), and 99% (100-93%) (Table 6.2).

Table 6.2: Results of the faecal egg count reduction test after treatment of sheep with Albendazole, Tetramizole and Ivermectine.

Measurements	Treatment groups				
	Pre-Test	Control	Albendazole	Tetramizole	Ivermectine
Number of sheep	6	6	6	6	6
Arth. Mean	950	950	17	17	8
% Reduction in EPG			98	98	99
95% Upper CL			100	100	100
95% Lower CL			86	93	93
Interpretation			Low resistance	Susceptible	Susceptible

6.4. Discussion

6.4.1 Questionnaire survey

In this study, the result of the questionnaire survey revealed that most farmers released their sheep to graze in Zwai wetlands where the above experiments were conducted, as. wetland areas provide valuable forage sources for livestock in general and particularly for sheep (Kauffman and Krueger, 1984). Regarding the grazing practice, majority of the participant farmers replied that sheep grazed mixed with other grazing animals. As Zwai wetlands are common places for all grazing animals in the area, all animals grazed together.

The majority of the participant farmers (70%) revealed that sheep diseases occur throughout the year in the study area. The reason for this might be the availability of Zwai wetlands, where moisture with fresh water and warm climate are always available for creating suitable condition for the occurrence of gastrointestinal helminth parasites that can diseased the grazing livestock. In line with these findings, Tramboo *et al*. (2015) reported that the prevalence of gastrointestinal helminth parasites in sheep population is common throughout the year of Kashmir valley wetland areas, India.

In this study, the results of the questionnaire survey revealed that most farmers treated their sheep using anthelmintic drugs. Similar findings were reported in different areas of the country (Desalegn Lidetu, 2005; Shimelis Dagnachew *et al*, 2011; Takele Sori *et al*., 2013; Ayalew Niguse *et al*., 2014; Befekadu Urga and Teka Feyera, 2015; Dinaol Belina *et al*., 2017; Zewdu Seyoum *et al*., 2017 and abroad (Klauck *et al*., 2014). This might be due to the ease of access to the drugs as they are cheap, simple to administer and are cost effective (Stear *et al*., 2006). The other probable reason for the dependent of farmers on anthelmintic drugs for the control of gastrointestinal helminth parasites might be absence of other effective controlling strategies for sheep in Ethiopia (Bersissa Kumsa et *al*., 2010). In this study, the majority of the farmers administered anthelmintic drugs by themselves. Similar findings were reported by Befekadu Urga and Teka Feyera (2015) and Dinaol Belina *et al*. (2017).

Regarding the timing of drug administration, most farmers (83%) revealed that sheep got treatment when they get a sign of illness like loss of appetite or weakness. Similar findings were reported by other researchers such as Bersissa Kumsa and Ajebu Nurfeta (2008); Achenef Melaku *et al*. (2013); and Ayalew Niguse *et al*., (2014). Zewdu

Seyoum *et al*. (2017) reported that some farmers conduct routine treatment regimens by following bi-annual treatments before and after the wet seasons, irrespective of any disease symptoms. Most of the respondents revealed that Albendazole was the most commonly used anthelmintic drug in the study area to de-worm their sheep followed by Tetramizole. Similar findings were reported by Bersissa Kumsa and Ajebu Nurfeta (2008) from Hawassa; Achenef Melaku *et al*. (2013) from North Gondar; Getachew Terefe *et al*. (2013) from Bedelle District; by Ayalew Niguse *et al*. (2014) in and around Jigjiga; and by Zewdu Seyoum *et al*. (2017) from Dabat District. According to Ayalew Niguse *et al*. (2014), Albendazole is the most widely used anthelmintic in Ethiopia. Similar findings were also reported outside the country by Silvestre *et al*. (2002); Dyary (2018).

The main criterion for the selection of anthelmintic drug by farmers for use to treat their animals was based on its color, where the green colored Albendazole (Fig. 6.1) was considered the most effective in cleaning their animals from parasitic helminth worms. This choice of the drugs by their color is associated with the avoidance of learning the difficult technical names of the drugs and the need to differentiate the effective of the drugs, to farmers understanding, by their colors (see also Getachew Terefe *et al*. 2013). The second level of drug choice is related to the price affordability (Ayalew Niguse *et al*. 2014). According to the work of Zewdu Seyoum *et al*. (2017), the majority of participant farmers (84%) selected anthelmintic drugs for their animals based on the prescription of veterinarians at their respective branch offices of the Ministry of Agriculture or regional administration, followed by the color of the drugs.

Fig. 6.1: Albendazole, which is the most widely used by farmers around Zwai wetlands.

Regarding the place where drugs are purchased, majority (82%) of the respondents replied that they bought them from nearby government owned veterinary clinics followed by purchasing them from anonymous dealers who sell drugs illegally and who could even sell expired drugs to illiterate farmers that lack the appropriate awareness of drugs and their proper applications. In line with these findings, Getachew Terefe *et al*. (2013) and Zewdu Seyoum *et al*. (2017) reported that majority (72, 70 and 68%, respectively) of the respondents purchased from nearby governmental veterinary clinics.

In this study, the majority of the participant farmers knew the dose of the drug they used. However, determination of the body weights of their sheep to decide on the proportional amount of dosage of the treatment any drug is done by estimation of the body weights of the animals. Such estimation that does not employ actual measurements using balance invites over or most of the time under dosage to save the drugs for longer and more animals invites the possibility of developing drug resistance by the parasites (Achenef Melaku *et al*. (2013), Zanzani *et al*. (2014), Befikadu Urga and Teka Feyera (2015), Tsegaye Teklemariam *et al*. (2017), Atanasio-Nhacumbe *et al*. (2017) and Bersissa Kumsa and Abebe Wossene (2007), Zanzani *et al*. (2014) and Dereje Bahiru *et al*. (2017).

One of the simplest methods of offsetting drug resistance by parasites is rotating the use of different kinds of drugs over time. However, in this study this was not the case where the majority (97%) of the respondents replied that they had no experience of anthelmintic drug rotation. In support of these findings, Getachew Terefe *et al*. (2013) reported that 94% of the respondents had no knowledge on drug rotation. The possible reason for the absence of anthelmintic drugs rotation experience by the respondent farmers might be the possibility of always going for the cheapest and most easily available drug and lack of awareness on the importance of drug rotation to offset the development of anthelmintic drug resistant by helminth parasites.

6.4.2 Faecal egg count reduction test on the efficacy of some anthelminthic drugs

Evaluation of the current situation of anthelmintic resistance on locally available three brand drugs: namely Albendazole, Tetramizole and Ivermectine were held. In this study, the percentages of faecal egg count reduction test after the application of Albendazole, Tetramizole and Ivermectine as per the manufacturers' instruction resulted in mean percentages of 98% (86-100%), 98% (93-100%), and 99% (93-100%), respectively, which indicates that low resistance is observed in Albendazole and susceptible in the remaining two drugs, which are very effective in parasite load or de-activation in the animals.

In this study, the efficacy of Albendazole was lower compared to Tetramizole and Ivermectine. In support of these findings, Tsegaye Teklemariam *et al*. (2017) reported the lower efficacy of Albendazole compared with Ivermectine and Tetramizole in Gimbo District, Kaffa Zone.

The lower (86%) confidence limit for Albendazole was within the range of resistance for gastrointestinal helminth parasites. In line with these findings, Kassahun Asmare *et al*. (2005) in Southern Ethiopia; Desie Sheferaw *et al*. (2013) in Dale District, Southern Ethiopia; Takele Sori *et al*. (2013) in Western Oromia; Tewodros Getachew *et al.* (2016) in Arekka Research Center, Southern Ethiopia; Walkite Furgasa *et al*. (2017) in Haramaya University farm reported that the presence of Albendazole resistance for gastrointestinal nematode parasites of sheep. The possible reason for the appearance of Albendazole resistance gastrointestinal helminth parasites of sheep in the study area might be the frequent use of one class of anthelmintic associated with miss estimation of sheep body weight to determine the dose rate leads some nematode parasites to develop capacity to survive against the particular drug (see also Befekadu Urga and Teka Feyera, 2015).

In the absence of calibration apparatus, visual estimation of body weight to determine the dose of the drug by farmers may result under dosage of the drug. According to Tewodros Getachew *et al*. (2016) in Areka Research Center, Wolaita Zone and Walkite

Furgasa *et al*. (2017) in Haramaya University farm, continuous under dosages treatments of sheep by farmers may result lower efficacy of the drug and the buildup of drug resistance. This idea was strengthened by Papadopoulos *et al*. (2000). According to Papadopoulos *et al*. (2000), utilization of limited group of drugs for a long period may favors the development of resistance. The other possible reason for the presence of Albendazole resistance in the study area might be frequently using of the same communal grazing area (Zwai wetland), which pre-dispose sheep for re-infection and contamination of the pasture by surviving resistant parasite strains (see also Walkite Furgasa *et al*., 2017). Contrasting to these findings, Dereje Bahiru *et al*. (2017) in Sebeta, Central Ethiopia reported the effectiveness of Albendazole against the treatment of gastrointestinal nematode parasites. The probable reason for this variation in the efficacy of Albendazole at different localities might be due to the occurrence of resistant nematode strains in the communal grazing areas (wetlands), dosing errors as a result of miss estimation of body weight and presumably low quality brands (Bersissa Kumsa *et al*., 2010). The other possible reason might be the frequency of using Albendazole to treat their sheep in different areas.

As regard to Tetramizole, the lower (93%) confidence limit obtained in this study revealed that this drug was found in the range of susceptible, which confirming very good efficacy of the drug for gastrointestinal helminth parasites of sheep in the area. Supporting to these findings, the effectiveness of Tetramizole was reported by Kassahun Asmare *et al*. (2005) in Southern Ethiopia; Bersissa Kumsa and Ajebu Nurfeta (2008) in Hawassa; Dese Sheferaw and Amenu Asha (2010) in Wolaita, Southern Ethiopia; Getachew Terefe *et al*. (2013) in Bedelle District, Oromia Region; Ayalew Niguse *et al*.

(2014) in and around Jigjiga, Eastern Ethiopia; Dereje Bahiru *et al*. (2017) in Sebeta, Central Ethiopia, who reported the effectiveness of Tetramizole. The possible reason for the effectiveness of Tetramizole in the study area might be sheep owners were dewormed only severely infected sheep as alternative to Albendazole from the flock.

The faecal egg count reduction value of Ivermectine obtained in this study was 99% with the lower (93%) confidence limit, which confirming very good efficacy of the drug for gastrointestinal helminth parasites of sheep in the area. The effectiveness of Ivermectine obtained in this study was in accordance with other reports inside the country by Dereje Bahiru *et al.* (2017) in Southern Ethiopia; Bersissa Kumsa and Ajebu Nurfeta (2008) in Hawassa; Dese heferaw and Amenu Asha (2010) in Wolaita, Southern Ethiopia; Dese Sheferaw *et al*. (2013) in Dale District, Southern Ethiopia; Getachew Terefe *et al*. (2013) in Bedelle District, Oromia Region; Takele Sori *et al*. (2013) in Western Oromia; Ayalew Niguse *et al*. (2014) in and around Jigjiga, Eastern Ethiopia; Jemal Ahmed *et al*. (2017) in Haramaya District, Eastern Ethiopia; Tsegaye Teklemariam *et al*. (2017) in Gimbo District, Southwestern Ethiopia; Zewdu Seyoum *et al*. (2017) in Dabat District, Northwest Ethiopia who reported the effectiveness of Ivermectine. Contrasting to these findings, resistance to Ivermectine was reported inside the country by Dereje Bahiru *et al*. (2017) in Sebeta, Central Ethiopia; Walkite Furgasa *et al*. (2017) in Haramaya University farm and Tewodros Getachew *et al*. (2016) in Areka Research Center, Southern Ethiopia. The probable reason for this difference might be the improperly utilization and the frequent usage of Ivermectine in the area. According to Papadopoulos *et al*. (2000), utilization of limited group of drugs for a long period may favor the development of resistance for that particular drug. However, according to Walkite Furgasa *et al*. (2017), Ivermectine resistance is not common in gastrointestinal nematodes of sheep in Ethiopia. This is because, Ivermectine is not preferred by farmers as Albendazole is the most commonly used anthelmintic drug by livestock owners to deworm their sheep in Ethiopia (Ayalew Niguse *et al*., 2014).

CHAPTER 7.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

It was concluded that Zwai wetlands that provide fresh herbage to livestock throughout the year and attractive playing grounds to schoolchildren of a nearby school, provide conducive environments mostly to water-related gastro-intestinal parasites of sheep and to a lesser extent to humans (schoolchildren), indicating that Zwai wetlands serve as a reservoir of animal disease (both humans and sheep) like gastrointestinal parasites.

In all cases of the definitive hosts (sheep and humans), their performance in life is severely affected, if in rare cases deaths do not occur. Sheep population grazing at Zwai wetlands suffer from gastrointestinal helminth parasites, occurring during all seasons of a year. A total of 10 species of gastrointestinal helminth parasites from three groups, namely: nematodes, cestodes, and trematode were obtained in the tracer experimental sheep grazed at the wetlands of Lake Zwai. The overall prevalence of gastrointestinal helminth parasites in Zwai wetland indicated gastrointestinal helminth disease to be an important health problem due to its high prevalence and occurrence of mixed parasitism. There was domination of nematode parasites over cestode and trematode. In general, the high nematode burden observed in Zwai wetland was significant for its pathogenic potential as a cause for clinical disease in sheep.

The study confirmed that wet seasons are favorable for the survival and transmission of gastrointestinal helminth parasites of sheep in Zwai wetland, as new fresh water of low salinity enter the wetlands creating conducive environment for parasite eggs to hatch and fresh herbage grows to attract sheep to graze. The total worm burdens observed on sheep fluctuated with the change of season of the year. The present study revealed that the worm burdens increased in short rainy season peaked in the long rainy season and declined towards dry season. The worm burden increase during the rainy season was very evident and was almost in parallel with the increase in rainfall. In this study, total rainfall and average relative humidity appeared as the main factors affecting the monthly and seasonal variations of worm counts throughout the study periods. The presence of snail species serving as the intermediate hosts of some gastrointestinal trematode parasites is an indicator for the occurrence of *F. gigantica* in the experimental tracer sheep.

It is concluded that there is a continuous infestation of gastrointestinal helminth parasites throughout the study period as domestic animals (for purposes of drinking water and feeding on succulent vegetation) and humans (for various social purposes) visit Zwai wetlands throughout the year and hence that a number of generations of nematodes, trematode and cestodes may be acquired each year. Hence, significant economic losses in production of sheep are expected to occur in Zwai wetlands.

The study conducted on schoolchildren revealed that water-related gastrointestinal parasites were prevalent on children who spent some of their time in contact directly with water at the wetlands of Lake Zwai or indirectly by frequent contacts with animals, exposure to contaminated soils or unsafe feeding behaviors. As the weather condition of Zwai wetland is hot and humid coupled with wet soil is a suitable environment for the occurrence of infective stages of gastrointestinal parasites. In addition to the water contact, domestic animals might be an important source of some gastrointestinal zoonotic infections to children in the wetland-bordering inhabitants, due in part to the close interactions with animals.

The overall prevalence of gastrointestinal parasites on Bochesa elementary schoolchildren highlight that the environment is conducive to water-related disease. The predominant parasite detected on schoolchildren was *H. nana*.

Some activities, which frequently practiced by schoolchildren at the wetland of Lake Zwai like fishing habits, the habit of playing with moist soil and habit of defecation around the lake are the risk factors that play an important role in the occurrence of gastrointestinal parasites on schoolchildren.

It has been clearly shown that the disease observed in the experimental sheep and the schoolchildren do not have zoonotic nature as it was originally anticipated.

In the shoreline of Zwai wetland, six species of mollusks were obtained with the domination of Gastropoda over Bivalvia. The invasive snail species; *P. acuta* was the most abundant mollusk in Zwai wetland. The presence of this snail in Zwai wetland indicated the presence of habitat change in the shoreline of the lake. This study demonstrated that transmission of trematodes and the prevalence of infection in the snail hosts are confirmed. Five types of cercariae emerged from the collected snail species from Zwai wetlands. Snails like *P. acuta, L. natalensis,* and *M. tuberculata* are serving as intermediate hosts of different trematode parasites of humans, sheep, birds, fish and amphibians. The physicochemical parameters measured in Zwai wetlands also influenced directly on the trematode parasites.

This study revealed that anthelmintic drugs are commonly applied to control gastrointestinal helminth parasites of sheep but improperly utilized in the area. Three groups of anthelmintic drugs, namely Albendazole, Tetramizole, and Ivermectin were used with their order of frequency.

The most commonly used drugs by sheep owners in the area were Albendazole. Albendazole has shown a sign of resistance to gastrointestinal helminth parasites of sheep in the area; whereas the remaining Tetramizole, and Ivermectine found susceptible to gastrointestinal helminth parasites of sheep in the study area.

This study revealed that failure to base any dosage on the correct of animals' body weight to dose the drugs coupled with the frequent use of the same drug for long years are the main factors for the development of anthelmintic drug resistance by some nematode parasites that affect the grazing sheep at the wetland of Lake Zwai.

The absence of regulation by responsible bodies on drug marketing and professionally unsupervised drug use by sheep owners increase the risk of anthelmintic resistance development in the study area.

7.2 Recommendations

Based on the above conclusions, it was therefore recommended that:

- Visits of sheep and humans to the wetlands should be restricted particularly at peak infection months by putting in place alternative methods of collecting safe feeds and water for both the animals and humans from the wetlands during these peak infection periods.
- Safe wetland playing grounds for schoolchildren should be identified.

- Safe waste handling methods should be introduced into the local population and sheep owners. Inflow of animal wastes with parasite eggs should be diverted for treatment before joining to the lake, such as buffering the shoreline of Zwai wetlands with vegetation cover to reduce the direct inflow of infected animal wastes into the wetlands.
- Effective control strategies against gastrointestinal helminth parasites of sheep and schoolchildren around Zwai wetlands should be practiced through an integrated regular de-worming program. In other words, regimes of prophylactic treatments should be introduced for both sheep and humans to reduce infections during peak infection periods.
- Herbages for animals should be collected and treated before feeding to animals to terminate the lifecycle of gastrointestinal helminth parasites by regulating the wetland to be grazed for a certain period, particularly during rainy season.
- Rotational grazing of the different portion of the wetland by sheep should be applied to skew the convergence of infective parasite stages and grazing animals at fresh succulent vegetation.
- Shedding of cercariae on freshwater bivalves should be made to identify the type of trematode parasites.
- Teachers should create awareness in their students about the mode of transmission of gastrointestinal parasites to reduce exposure of their students.
- Awareness creation to sheep owners should be made by veterinary and agricultural extension workers about drug rotation, proper estimation of sheep

body weight for delivering an accurate dose of drugs and selective treatment of affected sheep.

- Sustainable wetland management should be applied to obtain optimum services from the wetlands and to alleviate negative health impacts on humans and sheep through continued research in collaboration with the local community and other stakeholders.

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APPENDIXES

Appendix-I

Data collecting sheet for gastrointestinal helminth parasites on experimental tracer sheep

Tag No	Grazing Months	McMaster for EPG		Worms collected during post-mortem examination
		Species found	EPG	
T1-F	April 2015	*H. contortus*	350	100 from abomasum
		T. ovis	250	50 from large intestine
T2-M	April 2015	*H. contortus*	800	60 from abomasum
		O. columbianum		40 from large intestine
		T. ovis	350	40 from large intestine
T3-F	May 2015	*H. contortus*	1650	110 from abomasum
		O. columbianum		60 from large intestine
		M. expanza	100	6 from small intestine
		T. ovis	400	80 from large intestine
T4-M	May 2015	*H. contortus*	2000	120 from abomasum
		T. ovis	300	80 from large intestine
		M. expanza	90	4 from small intestine
T5-F	June 2015	*H. contortus*	4200	150 from abomasum
		B. tringoincephalum		60 from small intestine
		T. ovis	500	90 from large intestine
T6-M	June 2015	*H. contortus*	3500	130 from abomasum
		T. ovis	450	100 from large intestine
T7-F	July 2015	*T. ovis*	500	100 from large intestine

		M. expanza	200	6 from small intestine
		B. tringoincephalum	550	80 from small intestine
T8-M	July 2015	*H. contortus*	7000	380 from abomasum
		T. axei		350 from abomasum
		T. ovis	450	170 from large intestine
T9-F	August 2015	*H. contortus*	4750	250 from abomasum
		O. columbianum		50 from large intestine
		T. ovis	400	100 from large intestine
T10-M	August 2015	*H. contortus*	6710	270 from abomasum
		T. axei		150 from abomasum
		O. columbianum		60 from large intestine
		M. expanza	200	8 from small intestine
T11-F	September 2015	*H. contortus*	4100	230 from abomasum
		T. ovis	650	130 from large intestine
T12-M	September 2015	*H. contortus*	4000	220 from abomasum
		T. ovis	600	100 from large intestine
T13-F	October 2015	*H. contortus*	2960	120 from abomasum
		B. tringoincephalum		50 from small intestine
		O. columbianum		30 from large intestine
		M. expanza	150	5 from small intestine
T14-M	October 2015	*H. contortus*	2000	100 from abomasum
		O. columbianum		20 from large intestine
T15-F	November 2015			

T16-M	November 2015	*T. ovis*	450	100 from large intestine
T17-F	December 2015	*H. contortus*	1500	110 from abomasum
		T. ovis	450	80 from large intestine
		D. filaria		5 from lung
		F. gigantica	0	1 from liver
T18-M	December 2015	*T. ovis*	300	60 from large intestine
		D. filaria		10 from lung
T19-F	January 2016	*H. contortus*	3600	160 from abomasum
		T. ovis	320	150 from large intestine
T20-M	January 2016	*T. axei*	2030	200 from abomasum
		O. columbianum		40 from large intestine
		B. tringoincephalum		30 from small intestine
		D. filaria		10 from lung
T21-F	February 2016	*H. contortus*	4680	120 from abomasum
		T. axei		130 from abomasum
		O. columbianum		30 from large intestine
		B. tringoincephalum		30 from small intestine
		D. filaria		10 from lung
T22-M	February 2016	*H. contortus*	3000	110 from abomasum
		T. ovis	260	120 from large intestine
		D. filaria		12 from lung
T23-F	March 2016	*H. contortus*	5900	210 from abomasum
		T. axei		180 from abomasum

		B. tringoincephalum		50 from small intestine
		T. ovis	280	80 from large intestine
		Unidentified		5 from small intestine
		M. expanza	150	2 from small intestine
T24-F	March 2016	*H. contortus*	5700	190 from abomasum
		T. axei		160 from abomasum
		T. ovis	260	90 from large intestine
		D. filaria		20 from lung
T25-F	May 2016	*H. contortus*	6300	270 from abomasum
		T. axei		220 from abomasum
		M. expanza	150	3 from small intestine
T26-M	May 2016	*H. contortus*	6400	240 from abomasum
		T. axei		200 from abomasum
		T. ovis	280	40 from large intestine
		M. expanza	160	6 from small intestine
T27-F	June 2016	*H. contortus*	3000	280 from abomasum
T28-M	June 2016	*H. contortus*	5450	320 from abomasum
		T. axei		240 from abomasum
		O. columbianum		80 from large intestine
		T. ovis	300	110 from large intestine
T29-F	July 2016	*H. contortus*	7760	390 from abomasum
		T. axei		370 from abomasum
		B. tringoincephalum		20 from small intestine
		O. columbianum		90 from large intestine

T30-M	July 2016	*H. contortus*	4910	360 from abomasum
		T. axei		360 from abomasum
		B. tringoincephalum		60 from small intestine
		T. ovis	280	130 from large intestine
T31-F	August 2016	*H. contortus*	8230	370 from abomasum
		T. axei		360 from abomasum
		B. tringoincephalum		60 from small intestine
		O. columbianum		70 from large intestine
		T. ovis	540	70 from large intestine
T32-M	August 2016	*H. contortus*	7000	360 from abomasum
		T. axei		350 from abomasum
		T. ovis	500	60 from large intestine
T33-F	September 2016	*H. contortus*	6200	210 from abomasum
		T. axei		380 from abomasum
		B. tringoincephalum		30 from small intestine
T34-M	September 2016	*H. contortus*	6300	130 from abomasum
		T. axei		320 from abomasum
T35-F	October 2016	*H. contortus*	6500	220 from abomasum
		T. axei		130 from small intestine
		B. tringoincephalum		15 from small intestine
		O. columbianum		85 from large intestine
T36-M	October 2016	*H. contortus*	5800	450 from abomasum
		T. axei		410 from abomasum
		B. tringoincephalum		45 from small intestine

		M. capillaris		1 from lung
T37-F	November 2016	*H. contortus*	7250	110 from abomasum
		T. axei		110 from abomasum
		T. ovis	750	90 from large intestine
		F. gigantica	150	9 from liver
T38-M	November 2016	*H. contortus*	6900	150 from abomasum
		T. axei		200 from abomasum
		B. tringoincephalum		5 from small intestine
		T. ovis	700	70 from large intestine
		F. gigantica	0	1 from liver
T39-F	December 2016	*H. contortus*	1800	50 from abomasum
		T. axei		100 from abomasum
		T. ovis	450	30 from large intestine
		F. gigantica	400	18 from liver
T40-M	December 2016	*H. contortus*	5450	120 from abomasum
		T. axei		210 from abomasum
		B. tringoincephalum		10 from small intestine
		M. expanza		4 from small intestine
T41-F	January 2017	*O. columbianum*	200	10 from large intestine
		F. gigantica	800	27 from liver
T42-M	January 2017	*H. contortus*	1700	220 from abomasum
		T. axei		190 from abomasum
		T. ovis	350	220 from large intestine
		F. gigantica	1100	30 from liver

T43-F	February 2017	*H. contortus*	1400	200 from abomasum
		T. axei		150 from abomasum
		B. tringoincephalum		40 from small intestine
		T. ovis	200	240 from large intestine
		F. gigantica	0	2 from liver
T44-M	February 2017	*H. contortus*	1200	130 from abomasum
		T. axei		240 from abomasum
		O. columbianum		10 from large intestine
		T. ovis	150	80 from large intestine
		F. gigantica	200	14 from liver
T45-F	March 2017	*H. contortus*	550	160 from abomasum
		T. axei		220 from abomasum
		B. tringoincephalum		80 from small intestine
		T. ovis	90	20 from large intestine
T46-M	March 2017	*H. contortus*	600	150 from abomasum
		T. axei		480 from abomasum
		B. tringoincephalum		20 from small intestine
		T. ovis	110	10 from large intestine

Appendix-II

Data collecting sheet for Haematocrit values of tracer experimental sheep

No	ID Number	PCV		Plasma		Buffy	
01	T1-F	1st	0.34	1st	0.65	1st	0.1
		2nd	0.35	2nd	0.64	2nd	0.1
		Mean		Mean		Mean	
02	T2-M	1st	0.35	1st	0.64	1st	0.1
		2nd	0.34	2nd	0.65	2nd	0.1
		Mean		Mean		Mean	
03	T3-F	1st	0.32	1st	0.67	1st	0.1
		2nd	0.33	2nd	0.66	2nd	0.1
		Mean		Mean		Mean	
04	T4-M	1st	0.31	1st	0.68	1st	0.1
		2nd	0.32	2nd	0.67	2nd	0.1
		Mean		Mean		Mean	
05	T5-F	1st	0.31	1st	0.68	1st	0.1
		2nd	0.30	2nd	0.69	2nd	0.1
		Mean		Mean		Mean	
06	T6-M	1st	0.30	1st	0.69	1st	0.1
		2nd	0.29	2nd	0.70	2nd	0.1
		Mean		Mean		Mean	
07	T7-F	1st	0.32	1st	0.67	1st	0.1
		2nd	0.31	2nd	0.68	2nd	0.1

		Mean		Mean		Mean	
08	T8-M	1st	0.28	1st	0.67	1st	0.1
		2nd	0.29	2nd	0.68	2nd	0.1
09	T9-F	1st	0.27	1st	0.68	1st	0.1
		2nd	0.27	2nd	0.68	2nd	0.1
		Mean		Mean		Mean	
10	T10-M	1st	0.27	1st	0.66	1st	0.1
		2nd	0.26	2nd	0.67	2nd	0.1
		Mean		Mean		Mean	
11	T11-F	1st	0.28	1st	0.71	1st	0.1
		2nd	0.29	2nd	0.70	2nd	0.1
		Mean		Mean		Mean	
12	T12-M	1st	0.29	1st	0.70	1st	0.1
		2nd	0.28	2nd	0.71	2nd	0.1
		Mean		Mean		Mean	
13	T13-F	1st	0.30	1st	0.69	1st	0.1
		2nd	0.30	2nd	0.69	2nd	0.1
		Mean		Mean		Mean	
14	T14-M	1st	0.33	1st	0.66	1st	0.1
		2nd	0.32	2nd	0.67	2nd	0.1
		Mean		Mean		Mean	
15	T15-F	1st	0.39	1st	0.60	1st	0.1
		2nd	0.38	2nd	0.61	2nd	0.1

		Mean		Mean		Mean	
16	T16-M	1st	0.35	1st	0.64	1st	0.1
		2nd	0.34	2nd	0.765	2nd	0.1
		Mean		Mean		Mean	
17	T17-F	1st	0.32	1st	0.67	1st	0.1
		2nd	0.33	2nd	0.66	2nd	0.1
		Mean		Mean		Mean	
18	T18-M	1st	0.36	1st	0.63	1st	0.1
		2nd	0.37	2nd	0.62	2nd	0.1
		Mean		Mean		Mean	
19	T19-F	1st	0.31	1st	0.68	1st	0.1
		2nd	0.30	2nd	0.69	2nd	0.1
		Mean		Mean		Mean	
20	T20-M	1st	0.28	1st	0.71	1st	0.1
		2nd	0.29	2nd	0.70	2nd	0.1
		Mean		Mean		Mean	
21	T21-F	1st	0.29	1st	0.70	1st	0.1
		2nd	0.29	2nd	0.70	2nd	0.1
		Mean		Mean		Mean	
22	T22-M	1st	0.28	1st	0.71	1st	0.1
		2nd	0.29	2nd	0.70	2nd	0.1
		Mean		Mean		Mean	
23	T23-F	1st	0.27	1st	0.72	1st	0.1
		2nd	0.28	2nd	0.71	2nd	0.1

		Mean		Mean		Mean	
24	T24-M	1st	0.28	1st	0.71	1st	0.1
		2nd	0.28	2nd	0.71	2nd	0.1
		Mean		Mean		Mean	
25	T25-F	1st	0.28	1st	0.71	1st	0.1
		2nd	0.27	2nd	0.72	2nd	0.1
		Mean		Mean		Mean	
26	T26-M	1st	0.27	1st	0.72	1st	0.1
		2nd	0.27	2nd	0.72	2nd	0.1
27	T27-F	1st	0.28	1st	0.71	1st	0.1
		2nd	0.28	2nd	0.71	2nd	0.1
		Mean		Mean		Mean	
28	T28-M	1st	0.26	1st	0.73	1st	0.1
		2nd	0.26	2nd	0.73	2nd	0.1
		Mean		Mean		Mean	
29	T29-F	1st	0.25	1st	0.74	1st	0.1
		2nd	0.25	2nd	0.74	2nd	0.1
		Mean		Mean		Mean	
30	T30-M	1st	0.26	1st	0.73	1st	0.1
		2nd	0.26	2nd	0.73	2nd	0.1
		Mean		Mean		Mean	
31	T31-F	1st	0.27	1st	0.72	1st	0.1
		2nd	0.27	2nd	0.72	2nd	0.1

		Mean					
32	T-32-M	1st	0.27	1st	0.72	1st	0.1
		2nd	0.27	2nd	0.72	2nd	0.1
		Mean					
33	T-33-F	1st	0.29	1st	0.70	1st	0.1
		2nd	0.30	2nd	0.69	2nd	0.1
		Mean					
34	T-34-M	1st	0.30	1st	0.69	1st	0.1
		2nd	0.30	2nd	0.69	2nd	0.1
		Mean		Mean		Mean	
35	T-35-F	1st	0.31	1st	0.68	1st	0.1
		2nd	0.32	2nd	0.67	2nd	0.1
		Mean		Mean		Mean	
36	T-36-M	1st	0.31	1st	0.68	1st	0.1
		2nd	0.32	2nd	0.67	2nd	0.1
		Mean		Mean		Mean	
37	T-37-F	1st	0.33	1st	0.66	1st	0.1
		2nd	0.34	2nd	0.65	2nd	0.1
		Mean		Mean		Mean	
38	T-38-M	1st	0.32	1st	0.67	1st	0.1
		2nd	0.32	2nd	0.67	2nd	0.1
		Mean		Mean		Mean	
39	T-39-F	1st	0.32	1st	0.67	1st	0.1
		2nd	0.31	2nd	0.68	2nd	0.1

		Mean		Mean		Mean	
40	T-40-M	1st	0.30	1st	0.69	1st	0.1
		2nd	0.31	2nd	0.68	2nd	0.1
		Mean		Mean		Mean	
41	T-41-F	1st	0.30	1st	0.69	1st	0.1
		2nd	0.30	2nd	0.69	2nd	0.1
		Mean		Mean		Mean	
42	T-42-M	1st	0.29	1st	0.70	1st	0.1
		2nd	0.29	2nd	0.70	2nd	0.1
		Mean		Mean		Mean	
43	T-43-F	1st	0.30	1st	0.69	1st	0.1
		2nd	0.30	2nd	0.69	2nd	0.1
		Mean		Mean		Mean	
44	T-44-M	1st	0.29	1st	0.70	1st	0.1
		2nd	0.30	2nd	0.69	2nd	0.1
		Mean		Mean		Mean	
45	T-45-F	1st	0.32	1st	0.67	1st	0.1
		2nd	0.32	2nd	0.67	2nd	0.1
		Mean		Mean		Mean	
46	T-46-M	1st	0.31	1st	0.68	1st	0.1
		2nd	0.32	2nd	0.67	2nd	0.1
		Mean		Mean		Mean	

Appendix-III

Questions prepared for Bochesa Elementary School Students

Dear students, the purpose of this questionnaire is to get supplementary data for PhD dissertation in the field of zoological science, fisheries and aquatic science stream to study the role of Zwai wetland for the prevalence of gastrointestinal parasites on schoolchildren. Your response will serve for research purpose only, and your privacy will be kept confidential. Therefore, your genuine response will serve for the successful accomplishment of my research as well as for the prevention and controlling mechanisms of water-related gastrointestinal parasites of children taken by stallholders.

Students, who are the age of under 18, please sign your parents

Parents name ______________________________ Signature ________________

❖ To answer the following questions, you are requested to circle the letter that can represent your feeling.

1. Sex of the respondent.

   A. Female B. Male

2. Grade category of the respondent.

   A 1-4 B. 5-8

3. What is your parent's educational status?

   A. Illiterate B. Literate

4. Age category of the respondent's?

   A. 7-15 B. Above 15

5. Do you have swimming habit?

   A. Yes B. No

6. Do you have fishing habit?

   A. Yes B. No

7. Which one is your source of water for drinking?

A. Lake water B. Water pipe C. Groundwater

8. Which one is your source of water for bathing?

A. Lake water B. Water pipe and Groundwater

9. Do you have raw fish/meat eating habit?

A. Yes B. No

10. Do you have a habit of playing with moist soil?

A. Yes B. No

11. Do you have a shoe wearing habit?

A. Yes B. No

12. Do you have a habit of washing hands before meal?

A. Yes B. Sometimes C. No

13. Do you have nail trimming habit?

A. Yes B. No

14. Do you have latrine in your home?

A. Yes B. No

15. Where is your common place of defecation?

A. Latrine B. Open defecation

16. Do you have a habit of eating improperly washed vegetables?

A. Yes B. No

Appendix-IV

Data collecting sheet for different mollusk species at different sites of Zwai wetlands

Study Months	Sites	Mollusk species collected					
		L. natalensis	*P. acuta*	*M. tuberculata*	Planorbidae	*C. fluminalis*	*U. terminalis*
October 2015	Bochesa	0	0	4	0	14	0
November 2015	Bochesa	0	0	1	7	0	0
March 2016	Bochesa	0	238	7	0	0	26
April 2016	Bochesa	0	119	0	0	0	0
October 2016	Bochesa	2	215	0	0	0	0
November 2016	Bochesa	24	219	0	0	0	0
October 2015	Seda	0	0	0	0	4	0
November 2015	Seda	4	0	0	0	0	0
March 2016	Seda	0	52	0	0	3	17
April 2016	Seda	3	66	0	0	1	0
October 2016	Seda	2	0	0	0	0	0
November 2016	Seda	3	0	0	5	0	0
October 2015	Woshgula	3	43	0	0	0	0
November 2015	Woshgula	2	24	0	13	0	0
March 2016	Woshgula	0	0	0	0	4	21
April 2016	Woshgula	3	25	0	0	3	14
October 2016	Woshgula	5	17	0	0	0	0
November 2016	Woshgula	25	139	0	0	0	0

Appendix-V

The average physicochemical values collected at different sites of Zwai wetlands

Study Months	Sites	Physicochemical parameters				
		Temp. (°C)	DO	pH	Conduct.	Turbid.
October 2015	Bochesa	25.6	9.55	8.57	478	256
November 2015	Bochesa	21.72	9	8.72	493	214
March 2016	Bochesa	30.1	7.38	8.67	621	200
April 2016	Bochesa	23.1	8.16	8.42	617.5	89
October 2016	Bochesa	26.33	4.21	7.76	108.5	299
November 2016	Bochesa	20.6	3.97	7.95	114.5	212.33
October 2015	Seda	21.1	7.99	8.4	484	295
November 2015	Seda	23.9	8.26	8.55	488	228
March 2016	Seda	23.2	6.6	8.58	579	213.3
April 2016	Seda	27	6.23	8.02	544.5	143.67
October 2016	Seda	24.83	5.08	8.4	105.8	367
November 2016	Seda	20.9	7.86	8.6	108.3	263
October 2015	Woshgula	19.7	7.37	8.38	481	247
November 2015	Woshgula	20.97	9.17	8.62	487.7	241.3
March 2016	Woshgula	29.2	2.01	7.78	974	242
April 2016	Woshgula	29.2	5.64	7.62	674	155.33
October 2016	Woshgula	26.17	3.56	7.45	154.4	139.67
November 2016	Woshgula	20.5	3.34	7.77	211.47	60.33

Appendix-VI

Data collecting sheet for EPG counts for drug resistance test.

Tag No	Pre-treatment	Post-treatment			
	EPG value	Types of treatment	Day 7 EPG value	Day 14 EPG value	Mean
M-62	800	Albendazole	200	0	
M-23	600		100	0	
M-75	900		200	0	
M-21	1200		800	100	
M-67	1200		200	0	
M-54	900		100	0	
M-14	950	Tetramizole	300	0	
M-65	500		100	0	
M-60	1000		400	50	
M-58	1150		200	0	
M-63	1300		100	50	
M-55	1000		100	0	
M-17	800	Ivermectine	100	50	
M-16	700		900	0	
M-52	950		200	0	
M-61	1100		400	0	
M-56	1100		100	0	
M-11	950		100	0	
M-12	1150	Control	700	850	900
M-68	700		650	750	700
M-13	1200		800	1000	1000
M-51	1300		1200	1100	1200
M-15	1200		1000	1100	1100
M-22	700		800	900	800

Appendix-VII

Questions prepared for farmers

Dear farmers, the purpose of this questionnaire is to get supplementary data for PhD dissertation to assess the occurrence of anthelminthic resistance and factors contributing to the development of resistance on sheep. Your response will serve for research purpose only, and your privacy will be kept confidential. Therefore, your genuine response will serve for the successful accomplishment of my research as well as to know the status of locally available drugs for the control of gastrointestinal nematode parasites.

- To answer the following questions, listen the questions and react accordingly that can represent your feeling.

1. Gender of the respondent.

   A. Female B. Male

2. Educational status of the respondent.

   A. Illiterate B. 1-4 completed

   C. 5-8 completed D. Grade 9 and above completed

3. The locality of your Kebele ___________________________

4. How many sheep do you have?

   A. Lambs __________ B. Adult __________ C. None ________

5. Where do your sheep graze?

   A. Back yard B. Wetland grazing sites near to the Lake

   C. Anywhere the animals get feed

6. Which health problems have encounter with your sheep?

   A. External infectious diseases B. Helminthes diseases

7. During which season your sheep show signs of illness?

A. During wet season B. During dry season C. All year round

8. Under what condition you treat your sheep?

A. When get sick B. Once per year C. More than three times per year

9. Did you have sick sheep last season?

A. Yes B. No

10. Did you treat your sheep by anti-helminths drug?

A. Yes B. No

11. Who takes the treatment of your sick sheep?

A. Self B. Veterinarians

12. Which anthelmintic drug is your first choice?

A. Albendazole B. Tetramizole C. Ivermectine

13. What is the main criteria to select anthelmintic drugs?

A. Color B. Cost

C. Recommendation from veterinarians D. Recommended by farmers

14. While you are treating your sheep, from where do you purchase the drugs?

A. Governmental veterinary clinics B. Open market

C. Private drug vendors

15. Do you know the dose of the drug to treat your sheep?

A. Yes B. No

16. How do you estimate the weight of your sheep to dose the drugs??

A. Visual estimation B. Prescription ordered by veterinarians

17. Do you have a habit of drug rotation to treat your sheep?

A. Yes B. No

Thank you for your cooperation!!!