



MEKELLE UNIVERSITY
COLLEGE OF VETERINARY SCIENCES
DEPARTMENT OF
VETERINARY CLINICAL MEDICINE AND EPIDEMIOLOGY



**COPROLOGICAL STUDY OF TREMATODE INFECTIONS AND ASSOCIATED
FACTORS IN CATTLE AND SHEEP AND SPATIAL DISTRIBUTION OF
FRESHWATER SNAIL IN SOUTH AND SOUTHEASTERN ZONE OF TIGRAY,
ETHIOPIA**

By

Girmay Gebrezgi

**A Thesis Research Submitted to the College of Veterinary Sciences of Mekelle
University in Partial Fulfillment of the Requirements for the Master Degree in Tropical
Veterinary Medicine**

January, 2025

Mekelle, Ethiopia

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LIST OF ABBREVIATIONS

BCS	Body Condition Score
CDC	Centers for Disease Control and Prevention
CI	Confidence Intervals
CSA	Central Statistics Agency
OR	Odds Ratio
P	P-value
SBTs	Snail-Borne Trematodes
SETADo	Southeastern Tigray Agriculture Development office
STADo	South Tigray Agriculture Development office

ABSTRACT

Trematode infections are a significant cause of disease and economic losses worldwide. However, limited research has been conducted in the South and Southeastern zones of Tigray, Ethiopia, regarding the local epidemiology, transmission dynamics, and management strategies. To address this gap, a cross-sectional study was conducted from December 2023 to December 2024 to investigate the prevalence of trematode infections in cattle and sheep and the spatial distribution of freshwater snails in these areas. Simple random sampling was used to select study animals, and descriptive statistics were computed using STATA version 16. In total, 1536 fecal samples (768 from cattle and 768 from sheep) were examined, alongside snail collection. Additionally, 13 laboratory-bred mice were exposed en masse to *Schistosoma* cercariae for confirmation of transmission. The study revealed that the majority (60.29%, 463/768) of cattle and 53.5% (411/768) of sheep had either single or multiple trematode infections. Among cattle, 19.01% were testing positive for fasciolosis, while 20.7% exhibited mixed infections of *Fasciola* and *Paramphistomum* species. In sheep, 21.74% were positive for fasciolosis and 18.23% for mixed infections. The South zone had the highest prevalence in both cattle and sheep. Furthermore, most of the positive rates were exhibited in adult cattle with poor body condition scores while in adult sheep with medium body condition scores. Logistic regression showed a significant association between zone and bovine fasciolosis (OR = 1.76; P = 0.000), but no significant association for ovine fasciolosis. A total of 1269 snails were collected, identified as *Biomphalaria pfeifferi*, *Bulinus truncatus*, and *Lymnaea natalensis*. Intermediate hosts for schistosomiasis collected during the survey were found to be shedding *Schistosoma* cercariae. Furthermore, en masse cercariae-exposed laboratory-bred mice confirm the active transmission of schistosomiasis in the study area. The study highlights the trematode infections and the heterogeneous distribution of snails in the study area are a significant health concern for cattle and sheep, underlining the need for targeted control measures to mitigate the public health and economic impacts in the region.

Key words: Cattle, Coprological, Sheep, Snail, Tigray, Trematode,

1. INTRODUCTION

1.1. Background

Ethiopia's landscape is remarkably diverse with a wide range of climatic conditions and agro ecological zones that support a vast animal population (Zhongming *et al.*, 2013). Because it provides draft power for agricultural production, sources of income, and products including milk, meat, hides, skins, and other animal products, this diversity provides an important part in the livelihoods of a significant portion of the community. With 70 million cattle, 42.9 million sheep, 52.5 million goats, 13.3 million donkeys, 8 million camels, and 56.9 million poultry, Ethiopia currently holds the most populated position in Africa regarding livestock concentrations. There are 4.9 million cattle, 2 million sheep, and 4.8 million goats within the Tigray region (CSA, 2020/21).

Ethiopia has a significant population of livestock, however several kinds of obstacles maintain the country from fully using these resources: water scarcity, poor livestock extension services, incorrect data, inadequate knowledge on the performance of animals and marketing, frequent droughts, animal disease outbreaks, a shortage of infrastructure, a don't have of skilled labor, nutritional deficiencies, poor husbandry practices, and inadequate disease control efforts. When Ethiopia is to maximize potential livestock resources, these problems must be addressed with concentrated (Bayou and Geda, 2018).

Gastrointestinal parasites pose a wide spread challenge in livestock and agriculture, causing significant economic losses. This issue is particularly severe in Africa, including Ethiopia, due to diverse environmental conditions supporting, a wide variety of parasite species and hosts. The prevalence and impact of these parasites are notable reducing animal productivity, growth, feed efficiency, increasing mortality and morbidity in livestock herds. Multiple factors contribute to this problem, such as diverse agro ecological zones, suboptimal farm management practices, inadequate deworming programs, limited access to effective anthelmintic treatments and Environmental conditions also play a crucial role in parasite transmission and survival (Namutosi *et al.*, 2019).

Trematodes are divided into two primary subclasses: Monogenea and Digenia. The main difference lies in their life cycle; Monogenea flukes have a direct life cycle, whereas Digenia flukes need an intermediate host to complete their life cycle. The two main subclasses for trematodes are Digenia and Monogenea. Their life cycles differ mainly due to the fact Digenia

flukes required an intermediary host in order to complete their life cycle, whereas Monogenea flukes have a direct life cycle. The trematode families Schistosomatidae, Paramphistomatidae, and Fasciolidae are among those of important veterinary significance. Livestock and other domesticated animals are among the many animal hosts infected by these families of parasitic flatworms (Urquhart *et al.*, 2003). Throughout the beginning of time, humans and animals have been infested by snail-borne trematodes (SBT), which frequently results in negative consequences. Yasin *et al.*, 2018).

Trematodes of veterinary significance can be divided into two primary groups depending on their life cycle and host interactions: Monogenean trematodes which are ectoparasites residing on the external surfaces of fish, follow a direct life cycle. They can quickly spread diseases within aquaculture environments through direct transmission between fish hosts. On the other hand, Digenean trematodes are endoparasites found within the bodies of vertebrate hosts. Their life cycle is indirect or complex, involving an intermediate snail host (Jacobs, 2015).

Digenean flatworms are several different genera of parasites that can infect a wide range of invertebrates and vertebrates, including humans. These parasites may survive in the liver, lungs, intestines, and vascular system, between other organs, in their vertebrate hosts. Within the cattle the industry, the diseases caused on by these digenea parasites produce major financial losses. The health of afflicted human populations can also be negatively impacted through these parasite diseases (Walz *et al.*, 2015). Diseases caused by (SBT) including Paramphistomosis, fasciolosis, and schistosomiasis, present major obstacles to profitable livestock farming in various regions globally, particularly in humid tropical and subtropical areas (Yassin *et al.*, 2018).

1.2. Statement of the problem

Trematode infections have a negative impact on both human and animal health, which causes major issues for rural communities. These diseases cause morbidity, mortality, reduced animal production, and large economic losses, all of which have an adverse effect on the livelihoods of affected rural communities. The lack of previously conducted coprological study in the area despite their significant effect presents a lot of knowledge gaps on the local epidemiology, transmission dynamics, and possible management strategies. The creation of evidence-based strategies for minimizing the burden of these diseases has been hampered because the absence of

study. To address this significant problem and identify solutions, this study aims to investigate trematode infections in the community in depth in order to solve this serious issue and provide.

1.3. Significance of the study

This research on the zonal prevalence of trematode infections in cattle and sheep, and distribution of freshwater snails have significant implications for public health, livestock health, and socioeconomic development. Trematode infections, such those caused by *Fasciola*, *Paramphistomum* and *Schistosoma* are a major source of decreased in animal productivity. These infections result in poor weight gain, lower milk output, and economic losses because of medical costs and mortality (Fikru *et al.*, 2006). This study helps in identifying the most vulnerable livestock groups and provides vital information for focused control measures like deworming and enhanced parasite management strategies by determining the prevalence and associated risk factors, such as geographic location, age, sex, and body condition score.

Mapping their spatial distribution helps identify high-risk transmission areas, guide control measures like habitat alteration and snail management programs, and increase knowledge of how diseases spread. With zoonotic trematodes like *Fasciola* and *Schistosoma* that can infect humans through contaminated food or water, these results are also relevant to public health, underlining the significance of integrated "One Health" approaches that address the health of humans, animals, and the environment. The study also highlights the need for better water resource management and grazing techniques in snail-prone areas, while giving evidence-based data to farmers, and veterinarians to create disease preventive and control strategies that succeed. In addition, this research advances scientific understanding by providing data on the distribution of freshwater snails which serves as intermediate hosts.

1.4. Objective

1.4.1. General objective

The main aim is to examine the prevalence of trematode infections and associated factors, as well as the spatial distribution of freshwater snails in the South and Southeastern zones of Tigray, Ethiopia.

1.4.2. Specific objectives

- Determine the prevalence of trematode infections in cattle in the study area.

- Determine the prevalence of trematode infections in sheep in the study area.
- Identify potential factors associated with trematode infection.
- Identify the genus/ species/ of freshwater snails present in the study area.

2. LITERATURE REVIEW

2.1. Etiology

The two main liver fluke species responsible for causing the parasitic disease fasciolosis are *Fasciola gigantica* and *Fasciola hepatica* (Mushtaq, 2011). *Fasciola hepatica* often referred to as the common liver fluke is typically found in temperate regions. Whereas *Fasciola gigantica* is generally found in tropical and subtropical areas primarily infects livestock like sheep and cattle but humans can also be affected. (Ayele and Hiko, 2016). Similarly, Paramphistomosis is a disease caused by flukes from the genus Paramphistomum, involves various species (Lotfy *et al.*, 2010).

Schistosomiasis is caused by the genus Schistosoma. The key species that impact sheep and cattle are found in Africa *S. bovis*, *S. mattheei* and *S. leiperi* among the many species within this genus (Bont, 1995). *S. bovis* is the most critical Schistosoma species affecting animals residing in the portal and mesenteric veins of cattle and sheep (Kifle *et al.*, 2022). Likewise, there are more than 16 identified species of *Schistosoma* that may potentially infect humans. However only five cause significant morbidity, namely *S. mansoni*, *S. haematobium*, *S. japonicum*, *S. mekongi* and *S. intercalatum* (Okpala *et al.*, 2004).

2.2. Life Cycles and Mode of Transmission of Trematode infections

The life cycles of *Fasciola* and *Paramphistomum* are comparable. In their last hosts, usually sheep and cattle, adult *Fasciola* and *Paramphistomum* worms live in the bile and rumen ducts. The eggs laid by these adult trematodes are released into the intestines and eventually eliminated in the last host's feces. Upon arriving at freshwater sources, the eggs in the feces hatch into a larval stage known as miracidium. Following infection, the miracidium develops inside the intermediate host via a number of stages, including sporocyst, rediae, and cercariae. Following separation from the snail, the cercariae enter marshy environments where they change into metacercariae and attach to aquatic or semi-aquatic plants in the vicinity. Upon ingestion by the final host (cattle or sheep), the metacercariae infect them. Once inside the final host the young flukes migrate through the body eventually reaching the bile ducts, where they mature and produce eggs (MasComa *et al.*, 2009). On the other hand the *Schistosoma* parasite undergoes a complex life cycle involving various stages and hosts. Firstly, mature worms reside within the blood vessels of the intestines or liver of cattle and sheep. Female worms produce eggs, which

are expelled through the hosts' feces. Upon hatching, the eggs release miracidium larvae. These larvae infect specific freshwater snails, where they reproduce asexually to generate cercariae larvae. Once released into the water, cercariae penetrate the skin of final hosts, such as cattle or sheep, upon contact with contaminated water. They migrate through the host's body eventually reaching the intestines or liver blood vessels, where they mature into adult worms completing the cycle (Thétiot-Laurent, 2013).

Trematodes are spread through intermediate host snails, which are crucial for their transmission. Freshwater or amphibious snail performance as vital components in the trematodes' life cycle (Habtamu *et al.*, 2013). Trematode parasites like those causing schistosomiasis and fascioliasis, can infect humans and animals in two main ways skin penetration infective larval forms such as *Schistosoma cercariae* can penetrate the intact skin of hosts when they come into contact with contaminated water and ingestion of encysted stages some trematodes form encysted larval stages called metacercariae, which attach to water vegetation or other surfaces. When hosts ingest these encysted stages usually by drinking contaminated water or eating undercooked aquatic plants the parasites can infect them (Lindbergh *et al.*, 2006).

Fasciola gigantica spreads through the intermediate snail host *Lymnaea natalensis*, while *Fasciola hepatica* is transmitted via *L. truncatula*. The regions at highest risk for infections from parasites are those with extended periods of heavy annual rainfall, high soil moisture content, and sufficient water availability. Conversely areas with shorter wet seasons and lower temperatures experience reduced risk (Soulsby, 1982). Paramphistomosis presents a significant risk of infection in low-lying grasslands with slow water flow, rice cultivation zones, flood-prone areas, and regions featuring lakes and marshes (Rolfe *et al.*, 1991).

Schistosomiasis can also occur in large permanent water bodies such as marshy ponds, pastures, and lakes. The abundance of intermediate hosts and their ability to survive in the environment are key factors in the spread of the infection. Water contamination with *Schistosoma* eggs happens when eggs hatch near water sources during drinking or if manure is used as fish feed in ponds. Cattle can become infected through skin penetration or ingestion. The type of watering facilities used for domestic livestock also plays a significant role in maintaining and transmitting the infection (Pruss *et al.*, 2004). In Ethiopia, there is a variety of aquatic habitats, including small streams in the highlands, major lakes, and irrigation canal systems. This diversity of

suitable water bodies allows snail intermediate hosts to flourish thus maintaining the transmission of schistosomiasis (Shibru *et al.*, 1989).

2.3. Morphology of the Parasites

The adult *Fasciola* (liver flukes) have a flat, leaf-shaped body with a wider front end that includes a cone-shaped protrusion and large shoulders pointing sideways. Their outer surface or tegument is covered with small spines that point backward. These spines along with the fluke's suckers serve as a mechanism for attachment helping the parasite cling to and protect itself within the host's bile duct environment (Sures, 2004). Whereas, the eggs of *Fasciola* are a fertilized ovum surrounded by a protein-based shell containing vitelline cells. These eggs are equipped with a distinctive yellow-colored lid and house immature miracidium within. Particularly, differentiating between the eggs of *Fasciola gigantica* and *Fasciola hepatica* poses a challenge as they lack visible visual distinctions under microscopic examination. (MasComa *et al.*, 2009). Paramphistomum species are small trematodes, commonly known as flukes that parasitize the fore stomachs (reticulum and rumen) of ruminants such as sheep and cattle. Adult Paramphistomum flukes are typically small, usually less than 10 mm in length, they have a conical or pear-like shape, with a more pointed front end and exhibit a reddish or pinkish color, which is more noticeable when fresh due to their blood-feeding nature (Toolan *et al.*, 2015). Their eggs are pure-shelled, barrel-shaped, gray in color, and contain mature miracidium with an operculum at one end (Lotfy *et al.*, 2010).

Adult *Schistosoma* flukes have basic ventral and oral suckers. The males are shorter and more robust than the females, who reside folded within a longitudinal groove in the male's body known as the gynecophoral canal or schist. (Radek, 2001). *Schistosoma* eggs have unique morphological characteristics. They are larger, elongated oval, thin and spindle-shaped and have a lateral or terminal spine at both ends. These eggs do not have an operculum, are dark brown in color, and contain fully developed miracidium (Urquhart *et al.*, 2003).

2.4. Epidemiology of Trematode infections

The stability of trematode epidemiology is influenced by various factors including the presence and infection rates of snail intermediate hosts, pasture contamination, climate suitability for egg hatching and larval development, frequency of interactions between hosts and their environment, climatic, and ecological conditions. Elements such as altitude, temperature, rainfall patterns, and

livestock management practices in a particular area contribute to the generally environment conducive to trematode transmission ((Materu *et al.*, 2024).

The spread of snail intermediate hosts primarily occurs due to various factors. The *Lymnaea* genus among land snails and the *Planorbis* genus among water snails have a global distribution. *Bulinus* species, important intermediate hosts for various *Schistosoma* species, are found in regions such as central, eastern, and western Africa, the Mediterranean area, and the Middle East. They include *S. bovis*, *S. mattheei*, *S. intercalatum*, and *S. japonicum*, infecting humans and causing schistosomiasis in ruminants and other hosts. *S. nasalis* is found in the nasal mucosa veins of livestock in the Indian subcontinent. The incidence of bovine trematodes has risen due to global climate changes (Fairweather, 2011).

The spread of *Fasciola* fluke species in Ethiopia is heavily influenced by altitude. *Fasciola gigantica* is more prevalent below 1200 meters, *Fasciola hepatica* is primarily found above 1800 meters. About three-quarters of Ethiopia is covered by *Fasciola hepatica*, with the exception of the desert northeastern and eastern regions, while *Fasciola gigantica* is primarily found in the western humid zone, constituting roughly one-quarter of the country. These differences in distribution are due to variations in climatic and ecological factors such as temperature, rainfall, and livestock management practices (Hotessa and Kanko, 2020). Similarly, *Paramphistomum*, the parasite known as the rumen fluke, can be found all over the world, but its highest prevalence rates are observed in subtropical and tropical regions (Rolfe *et al.*, 1994).

Schistosomiasis is mainly concentrated in subtropical and tropical regions worldwide, where warm and humid climates foster its significant endemic presence, impacting domestic livestock production. Different species of the *Schistosoma* species infect different type of livestock. Cattle and sheep are affected by *S. bovis*, horses, cattle, goats, and Indian buffalo are affected by *S. indicum*, sheep are primarily infected by *S. mattheei*, particularly in South Africa, pigs and goats are primarily infected by *S. suis*, humans and other mammals are infected by *S. japonicum* in Asia, and elephants, horses, and ruminants are infected by *S. margrebowiei* in Africa (Merawe *et al.*, 2014 In Ethiopia, schistosomiasis is common, especially in the central, eastern, northern, and southern western parts of the country (Habtamu and Woldemariam, 2011). Additionally, the two major *Schistosoma* species pathogenic to humans in Africa are *Schistosoma haematobium* and *S. mansoni*, which causes urinary and intestinal schistosomiasis, respectively (Gryseels *et al.*, 2006).

2.5. Pathogenesis

The progression of *Fasciola* infection within a definitive host comprises two distinct phases: the migratory (parenchymal) phase and the biliary phase (Mushtaq, 2011). In the migratory phase, immature flukes enclosed in cysts penetrate the intestinal wall and travel from the abdominal cavity to the liver. *F. hepatica* shows a predilection for liver tissues (Kaya *et al.*, 2007). The intestinal wall, lungs, diaphragm, subcutaneous tissue, and kidneys are also potential locations where fluke infestation can occur (MasComa *et al.*, 2009). The migratory process of flukes inflicts physical harm on tissues, causing inflammation along their path. This is followed by the biliary phase, where the parasites invade the liver's bile ducts, leading to further development and egg laying. Consequently, the affected tissues suffer from enlargement of the biliary ducts and blockage of their passages due to fluke-induced injury. Although adult flukes themselves are relatively benign, they instigate significant damage to the liver tissue, resulting in symptoms like bleeding, discoloration, swelling, increased cell growth, scarring, and cell death (Urquhart *et al.*, 2003; Taylor, 2007).

Paramphistomum infection, also known as Paramphistomosis, primarily affects the intestines during its harmful phase. The immature larvae of *Paramphistomum* species attach to and feed on the lining of the duodenum (upper part of small intestine), causing substantial damage. This activity results in several severe pathological changes, especially in cases of heavy infection edema, bleeding and ulceration (Dube and Aisien, 2010).

Schistosomiasis stands out among helminth infections in two significant ways. Firstly, the main cause of the disease's development is the eggs of the fluke, rather than the larval or adult stages, which is more common in other helminth infections. Secondly, much of the disease's impact on the body is influenced by the host's immune responses, particularly delayed-type granulomatous and hypersensitivity reactions, instead of being mainly driven by the parasitic stages themselves. Infections by *Schistosoma* are typically divided into three stages: acute, migratory, and chronic. The migratory stage occurs when cercariae move from the skin into the bloodstream. While this phase often shows no symptoms, in some individuals, it can lead to temporary dermatitis ("swimmer's itch"), and in rare instances, pneumonitis and lung injuries (Oliveira *et al.*, 2004).

The egg that reach maturity *Schistosoma* produce having a capability to enter the blood stream by their host and produce to different tissues where they release toxic substances and enzymes

that directly affect those tissues. The extremely high pathogenicity of Schistosomiasis is primarily due to the systemic involvement of adult worm eggs. (Coutinho *et al.*, 2007).

Inflammatory reactions and granuloma formation ensue around deposited eggs, potentially causing fibrosis and scarring of affected tissues (Cheever *et al.*, 2000). There are two routes where *Schistosoma* eggs can migrate: either to the liver or to the intestine near the adult worms. In cases of intestinal schistosomiasis, granulomatous inflammation surrounds eggs in the intestine causes ulceration and scarring. The severe pathology of Schistosomiasis is caused by both the intestinal and hepatic pathways of egg migration. (Friedman *et al.*, 2007).

2.6. Clinical Sign of Trematode infections

In cases of fasciolosis, the severity of clinical symptoms is associated with the level of metacercariae exposure. Winter infections in dairy cows result in decreased milk quantity and quality, particularly affecting solids-not-fat content Sheep and cattle. The primary hosts display various clinical manifestations depending on the amount of metacercariae ingested (Radostits *et al.*, 2007; Mushtaq, 2011). The different clinical forms of fasciolosis are as follows including Acute Type one fasciolosis occurs when animals consume more than 5000 metacercariae, they get acute type one fasciolosis, which causes sudden deaths without symptoms. Jaundice, ascites, mucous membrane coloration, bleeding in the abdomen, and weakness are possible symptoms. (Radostits *et al.*, 2007; Kaya *et al.*, 2007). Secondly, Acute Type two fasciolosis Animals that consume 1000–5000 metacercariae may develop acute Type two fasciolosis, which can be fatal. Temporary ascites, loss of body condition, and skin discoloration are possible symptoms. The third form is Subacute fasciolosis arises when sheep and cattle consume 800-1000 metacercariae, subacute fasciolosis develops, which can result in anemia, lethargy, and even death. Weight reduction is one prominent characteristic (Radostits *et al.*, 2007). Finally, chronic fasciolosis develops when animals consume 200-800 metacercariae. Regardless of the form of the disease symptoms may progress slowly or appear asymptomatic with potential manifestations including ascites and bottle jaw. Other signs may involve emaciation, anemia, elevated eosinophil count, weight loss, poor growth, reduced milk production, chronic liver damage and fibrosis and decreased albumin levels (Kaya *et al.*, 2007). On the other hand, Symptoms due to The *Paramphistomum* parasite, commonly referred to as the rumen fluke is responsible for causing fatal Paramphistomosis, particularly in cattle and sheep. Symptoms

include severe diarrhea, anemia, lethargy, and if not treated can lead to mortality. Typical clinical signs include intestinal inflammation, bloody diarrhea, weight loss and anemia (Chaoudhary *et al.*, 2015). Immature *Paramphistomum* trematodes, termed "plug feeders," penetrate the duodenal lining and consume epithelial cells from Brunner's glands. This activity leads to significant problems for the host including anorexia, foul diarrhea, anemia, and decreased plasma protein levels. Additionally, mature *Paramphistomum* causes issues such as irregular rumenitis, disrupted rumination, inefficient nutrient utilization, poor body condition, severe diarrhea, excessive thirst, and loss of appetite. (Williams, 2012).

Cattle and sheep affected by schistosomiasis display a variety of clinical signs, including weight loss, dehydration, severe diarrhea with blood or mucus, reduced productivity, rough hair coat, pale mucous membranes, anemia, low levels of albumin in the blood (hypoalbuminemia), elevated levels of globulins in the blood (hyperglobulinemia), and a significant rise in eosinophils in the bloodstream. These symptoms typically manifest after the onset of egg excretion. Animals severely affected by the disease deteriorate rapidly and typically perish within a few months of contracting the infection. Animals with milder infections experience a chronic form of the disease characterized by delayed growth (Merck and Dhome, 2010).

In ruminants, both hepatic and intestinal forms of schistosomiasis exhibit major clinical signs such as hemorrhagic enteritis (inflammation of the intestines with bleeding), anemia, and emaciation. These symptoms become evident after the beginning of egg excretion. Animals severely affected by the disease experience a swift decline in health and usually perish within a short period (Charles and Robinson, 2004).

2.7. Diagnosis, Treatment and Control

The diagnosis of fasciolosis involves two main steps. First, a primary diagnosis is made using information about the disease's epidemiology, clinical signs, weather conditions, grazing history, and seasonal patterns of *Fasciola* transmission. Following this, a definitive diagnosis is confirmed through fecal examination in a parasitological laboratory, postmortem examination of the liver for mature flukes and serological tests to detect *Fasciola*-specific antibodies in the animal's blood (Mushtaq, 2011; Krauth *et al.*, 2015). Similarly, rumen fluke infections are often linked to young animals in the herd, particularly those grazing in areas near the habitat of the snail intermediate host. As the disease occurs during the prepatent period before fluke's reach

maturity and start releasing eggs, fecal examinations provide limited diagnostic value. The definitive way to confirm a rumen fluke diagnosis is through postmortem examination, where immature flukes can be found in the duodenal submucosa. (Urquhart *et al.*, 2003). Farther more the main method for identifying possible *Schistosoma* infections is the detection of fluke eggs in urine or fecal samples. These eggs, typically containing a single spine protruding from the shell, differ depending on the specific *Schistosoma* species responsible for the infection. In individuals infected with *S. intercalatum*, *S. mekongi*, *S. bovis*, *S. mattheei* and *S. leiperi* *S. japonicum* or *S. mansoni*, mature worms release eggs in the feces, typically found in the mesenteric venous plexus. Conversely, in cases of *S. hematobium* infection, mature fluke worms reside in the venous plexus of the lower urinary tract and release eggs in the urine (CDC, 2012).

Multiple drugs are employed to treat fasciolosis in animals with differences observed in their effectiveness, mechanism of action, cost, chemical, trade names and availability in the market. Key classes of fasciolicide drugs encompass halogenated phenols, Salicylanilides, Benzimidazole and triclabendazole. For veterinary treatment, evaluating the advantages and disadvantages of these various fasciolicide choices is essential. (Wolstenholme *et al.*, 2004). The most widely used drug for treating fasciolosis in animals is triclabendazole, usually commercialized as Fasinex, because of its exceptional efficacy against both adult and juvenile liver flukes. In many countries, it is the main medication of choice for treating fasciolosis infections in livestock and other animals. (Wolstenholme *et al.*, 2004; Dorchie, 2006).

Effective treatments for Paramphistomum infections include medications like Resorantel, oxyclozanide, levamisole, clorsulon, niclosamide, ivermectin, and bithionol (Bowman, 2008). Important *Schistosoma* species infections can be treated with praziquantel. However, because praziquantel mainly targets adult worms and depends on the development of an antibody response to achieve maximal efficacy, timing is essential (CDC, 2012). Over the years, various medications with established effectiveness against *Schistosoma* have been researched for their potential in treating *Schistosoma* infection in cattle and sheep (Tsotetsi *et al.*, 2013).

Several methods have been developed to control trematode infections. One important approach is to reduce the snail population, as snails serve as an intermediate host for the parasite. The first step involves surveying the area to assess the extent of snail habitats. Drainage is considered the most effective long-term solution, as it can permanently eliminate mud snail habitats. For smaller snail habitats, options include fencing off the area or applying molluscicide such as copper

sulfate annually (Urquhart *et al.*, 2003; Taylor *et al.*, 2007). However, in nomadic management systems, continuous implementation of these measures may not be feasible. In such cases other methods can be used. One option is to reduce the population of snail intermediate hosts through chemical or biological interventions in areas where transmission occurs, aiming to disrupt the parasite's life cycle. Another approach involves eradicating or controlling the snail population using mechanical fences or snail traps to reduce transmission and effectively manage the disease (Merck and Dhome, 2010). Moreover, the plant *Phytolacca dodecandra* referred to as "endod" in Ethiopia or Africa soapberry acts as a natural molluscicide serving to manage snail populations. Endod native to Ethiopia and other parts of East Africa, has traditionally been employed for various purposes, such as a natural soap, fish poison, and molluscicide (Shibru *et al.*, 1989).

2.8. Habitat of Freshwater Snails

Freshwater snails live in various aquatic environments globally. These habitats can be grouped into several types, each offering distinct conditions that support different snail species. Common habitats for freshwater snails include river, streams, lakes, ponds, marshes, swamps, springs, ditches, canals, temporary flooded areas, freshwater marshes, bogs, caves and underground waters (Strong *et al.*, 2008). They have the ability to flourish in a wide range of freshwater habitats. The majority of land-based snail species prefer moist environments with elements such as aquatic vegetation, wood, rocks, stones, soft sediment, and vegetated areas like bushes. Among freshwater snails, certain genera have distinct habitat preferences. For example, *Lymnaea* snails commonly inhabit swampy regions and irrigation canals, while *Bulinus* and *Planorbis* species are typically found along river edges and near dams. (Salew and Munshea, 2018).

In aquatic environments, freshwater snails are essential to the ecosystem as both prey and consumers. They are an important source of food for many other aquatic species, mostly consuming plants, algae, and debris. However, certain species particularly those within the Planorbidae family can pose health risks as intermediate hosts for severe parasitic infections like schistosomiasis, fascioliasis, and Paramphistomosis. These snails can spread the parasites to people or animals when they contract the larval stages of the infection. These snail-borne parasitic infections are most common in parts of Asia, Africa, and the Americas (Alebie *et al.*, 2014; Woldegerima *et al.*, 2019). Snails have the ability to lay up to 1,000 eggs in their lifetime,

snails have an exceptionally prolific reproductive technique. When environmental conditions are suitable, snail populations can grow quickly due to their high fertility, though the exact number of eggs laid may vary depending on the species (Mereta *et al.*, 2019).

3. MATERIALS AND METHODS

3.1. Study Area

The investigation was carried out in Tigray's south and southeastern area as depicted in Figure 1. The southeast area is situated about 783 kilometers north of Addis Ababa, Ethiopia's capital. The geographic coordinates were 13.24° north latitude and 39.53° east longitude. The height above sea level ranges from 1400 to 2800 meters. The surrounding area receives 400 to 700 mm of rain annually. The temperature ranges from 14°C to 28°C yearly (SETADo, 2023). The same as South Tigray is about 663 kilometers from Addis Ababa and 120 kilometers south of Mekelle. With an elevation of 1800–3954 meters above sea level, its geographic coordinates are 12.96° north latitude and 39.52° east longitude respectively. The minimum annual temperature is 14.5°C, while the maximum is 27.5°C and the surrounding area receives 630 to 1380 mm of rain annually (STADo, 2023).

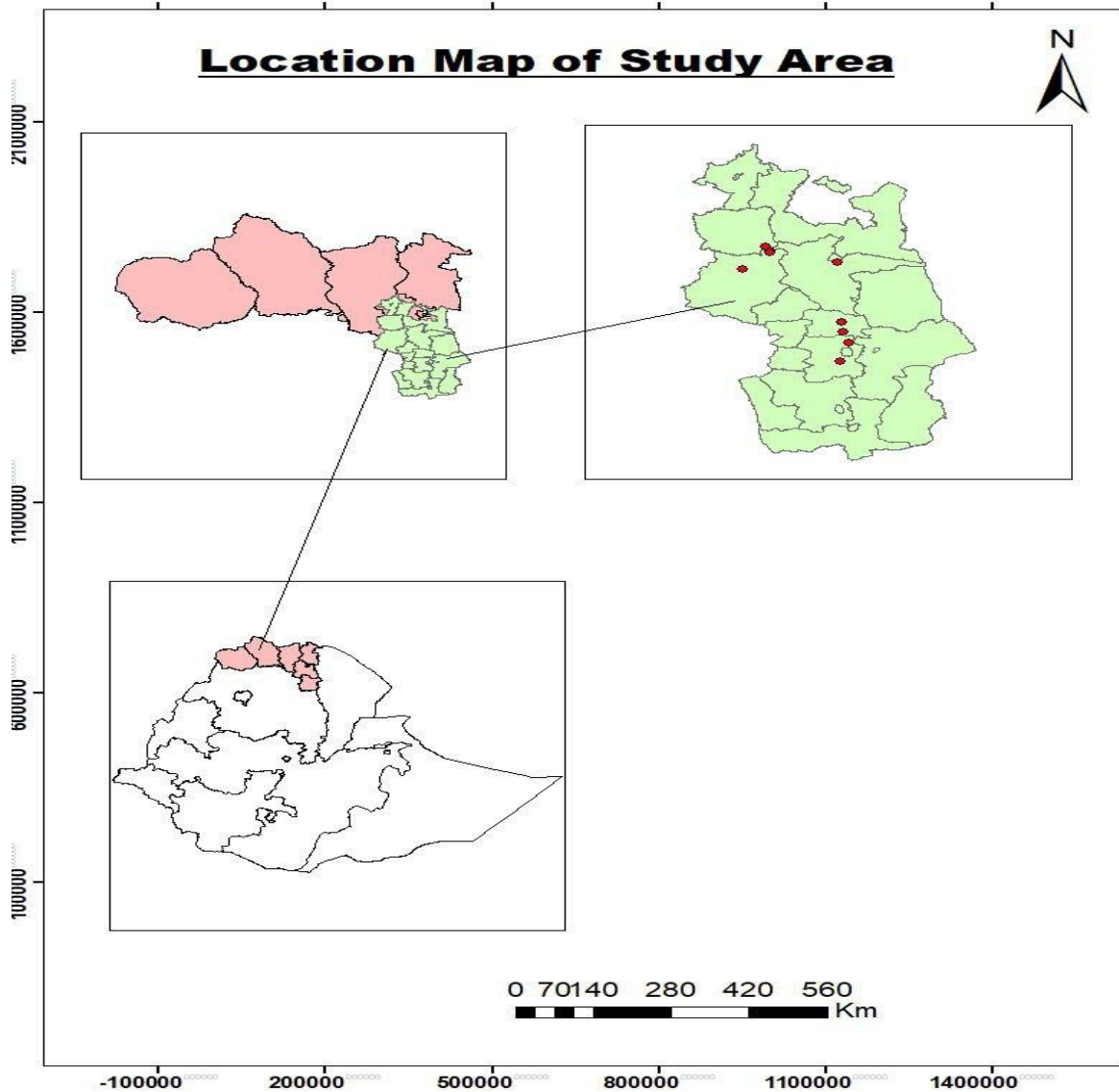


Figure 1. Map of study area

(Shapefiles for Ethiopia were retrieved from [https:// Africa open data.org/data set/ Ethiopia-shape files](https://Africaopen.org/data-set/Ethiopia-shape-files) and the program ArcMap 10.1 of ArcGIS was used to create the map)

3.2. Study Population and Study subjects

The study population were those cattle and sheep found in the study area. The study subjects are those animals managed under an extensive production system, grazing near to swampy areas, water lodges, and small dams in the study areas. Animals of both sexes, any breed and aged over six months were selected for the study.

3.3. Study Design

Across sectional study was employed from December 2023 to December 2024 to investigate the prevalence of trematode infections and its associated factors in cattle and sheep and spatial distribution of freshwater snails in two selected zones of Tigray region of Ethiopia.

3.4. Sampling Technique and Sample Size Determination

Multistage sampling technique was employed to select study sites. Accordingly, two zones were selected purposively as indicated in figure 2. The districts and kebeles (local administrative units) included in the study areas were purposefully selected based on factors such as prevailing livestock populations, potential areas for snail population, grazing and watering spots, human/animal water contact sites, and ecological zones (highland, midland, & lowland). Animals were selected for the study using a simple random sampling method which used to graze in the potential predisposing factors for the occurrence of the infection.

The sample size was determined using Thrusfield formula (Thrusfield, 2010) considering a 50% estimated prevalence in cattle and sheep, a 95% confidence interval, and a 5% required absolute precision.

$$n = \frac{(1.96)^2 * P_{exp}(1 - P_{exp})}{d^2}$$

Where, n= the required sample size

P exp = expected prevalence (P = 50%)

d = desired absolute precision (5%)

Z = 1.96 for a 95% confidence interval

Accordingly, the calculated sample size was 384 for cattle and 384 for sheep, since the sampling technique used was multi-stage and then the total sample was multiplied by 2 (design effect). Thus, a total sample size of 1536 (768 from cattle and 768 from sheep) were selected from the study area.

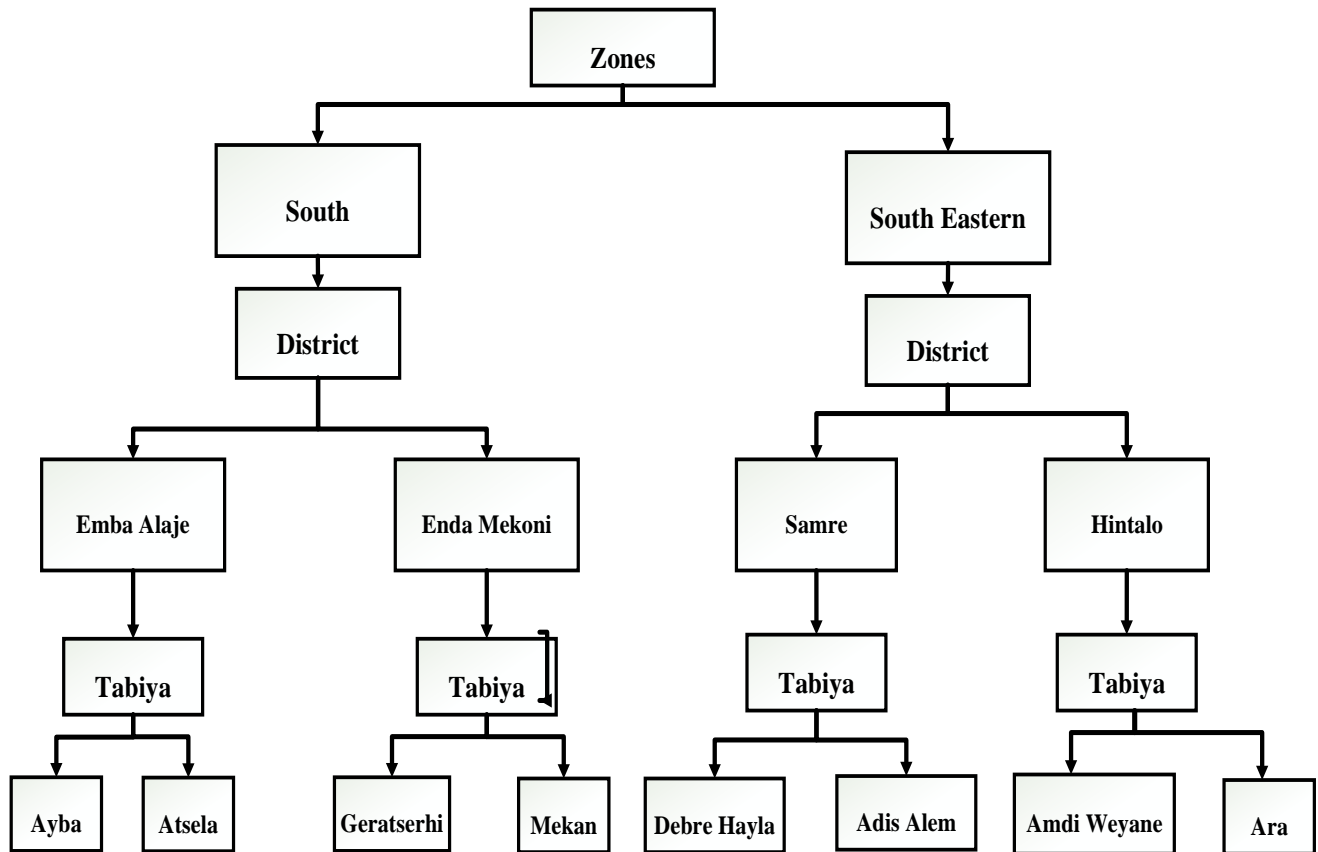


Figure 2. Sampling frame for fecal sample and snail collection

3.5. Data Collection

3.5.1. Fecal sample collection and examination

Before sample collection, essential data such as breed, sex, body condition score, and age were recorded for each individual animal. Age of animals were determined by asking the owner and tooth eruption as described by Food Safety Inspection Service (2013). Additionally, by analyzing physical elements such as the vertebral column, ribs, spines, tail, the skull, and hip bone, the cattle's bodily condition was categorized into three categories poor (scores 1 to 3), medium (scores 4 to 6), and good (greater than 6) (Genzebu *et al.*, 2012). Similarly, structural components that include the ribs, spines, brisket, eye muscle, the lower part of the flank, and loin were examined in order to classify the body condition of sheep into three categories: poor (scores 1 to 2), medium (scores 3), and good more than 4 (Tadesse and Gebremariam, 2010).

During the initial data collection fecal samples were directly collected from the rectum of randomly chosen cattle and sheep. This was performed using sterile disposable plastic gloves with two fingers utilized for collection. Each sample was clearly labeled with details including animal identification, site of collection, date, breed, age, sex, and body condition. Samples were carefully packaged and sent in cool boxes or preserved in universal bottles filled with 10% formalin to prevent egg hatching and development during the samples transportation (Antonia, 2010). The samples were transported to the parasitological laboratory at Mekelle University College of Veterinary Science (MU-CVS).

The samples were either examined immediately or stored at 4°C until processing. The sedimentation technique was then utilized to detect and count trematode eggs. For each case, 3g of feces were measured and placed into container 1. Next 42 ml of tap water were added to the same container and mixed thoroughly using a glass rod. The fecal suspension was filtered using a mesh size of 60µm diameter into container 2, and then transferred to a centrifuge tube. The tube was centrifuged at 1500 rpm for five minutes. In the absence of a centrifuge, the mixture was allowed to sediment undisturbed for 20 to 30 minutes, followed by careful resuspension in 5 ml of water and sedimentation for an additional five minutes. The supernatant was then carefully discarded. The sediment was stained with one drop of 1% methylene blue and the sediment was placed on a slide covered with a coverslip. Finally, the slide was examined under low power microscope magnification to identify the presence or absence of trematode eggs (Shimizu and Robyn, 2007). This staining technique resulted in distinct colors: Paramphistomum eggs appeared gray, while Fasciola eggs appeared yellowish and Schistosoma egg dark brown. To quantify the egg counts, a differential egg per gram (EPG) was calculated for each parasite. This involved dividing the specific parasite egg count found in a 3-gram fecal sample by 3 (Van Wyk and Mayhew, 2013).

3.5.2. Malacological Study

Intermediate snail hosts were surveyed in various habitats, including leaf-filled surface depressions, log-mud interfaces, stone crevices, emergent or submerged vegetation, and at points of human water contact in streams, and marshy areas within the study area. Snails were collected manually while wearing gloves. To maintain the natural habitat conditions during transportation, snails were carefully placed into an open plastic bags along with fresh water and grass/algae.

All the freshwater snail samples were taken to MU-CVS parasitology laboratory. Snails were identified using morphological characteristics for genus and species with the aid of the identification keys of (Upatham *et al.*, 1983; Brown, 1994). This examination included inspecting various aspects of the snail shells, such as size, number of whorls, apex shape, suture patterns, spire structure, columella features, shell shape, coil direction, surface textures, colors, and patterns (Mandahl -Barth, 1962).

Medically important snails were examined for trematode infection by shedding method (individual snails were placed in vials/Petri dishes containing aged water and exposed them to natural or electric light for 30-40 minutes) as described by(Sri-aroon *et al.* 2005). Emerged cercariae were collected and identified to the genus level under stereomicroscope.

13 laboratory-bred mice, obtained from College of Health Sciences, were exposed in mass to schistosomes cercariae for 40 minutes in a beaker. Then the mice were transferred to their cage and maintained in animal house at CVS. Adult *S. mansoni* parasites were harvested from these mice after 6 weeks of laboratory maintenance (Duval and Dewitt, 1967). Definite identification of the schistosomes was made using eggs and adult worm morphology from feces of mice and by dissecting the mice, respectively.

3.6. Data Analysis

The collected data was entered into a Microsoft Excel 2007 spreadsheet and analyzed by using STATA statistical software version 16. The data was cleaned, coded, recoded, and checked for statistical assumptions. Descriptive statistics was employed to summarize the data and reported as frequency and percentage in table form. Cross tabulation was conducted to determine the prevalence of trematode infection (Fasciolosis, Paramphistomosis and Schistosomiasis) across age, sex and topographic differences. Logistic regression was conducted to determine the degree of association between prevalence of trematode infection and various risk factors and the result was expressed in terms of Odds Ratio (OR) and 95% confidence Interval (CI). For all analysis a P-value < 0.05 was used as a cut-off point for significance difference.

3.7. Ethical Considerations

Before fecal sample collection verbal consent was obtained from the local community leaders and animal health departments of the selected sample collecting sites. The farmers were also informed of the significance of the study. Additionally, it is guaranteed that the findings of the

study will be shared with farmers through community service at the conclusion of the study so they can understand the impact of the diseases. Furthermore, for animal (mice) experimentation, institutional ethical clearance will be obtained from Animal Ethics and Experimentation committee (AEEC) of Mekelle University (AEEC No: AEEC 31/2024).

4. RESULTS

4.1. Demographic Characteristics of the Study Subjects

A total of 1536 animals (768 cattle and 768 sheep) were included from Southeastern and South zones of the regions in the current study (Table 1). Majority (65.8%) of the study subjects were adults while 45% of them with medium body condition score. The cattle and sheep demographic data reveals a balanced distribution between the South Eastern and South Zones. The distribution across districts shows that Samre had the highest sample collected with 214 cattle (27.8%), followed closely by Emba Alaje with 200 cattle (26.0%), Enda Mokoni with 184 cattle (23.9%). Whereas, Emba Alaje had the highest count with 200 sheep (26.04%), followed by Samre and Hintalo, each with 192 sheep (25.00%). Sex distribution was skewed towards females in both of the animals.

Table 1: Summary of demographic characteristics of the study subjects

Variables	Cattle		Sheep	
	Frequency	Percent	Frequenc	Percent
Zone				
South eastern	384	50	384	50
South	384	50	384	50
District				
Samre	214	27.8	192	25.00
Hintalo	170	22.1	192	25.00
Emba alaje	200	26.0	200	26.04
Enda mokoni	184	23.9	184	23.96
Tabia				
Debre hayla	107	13.9	84	10.93
Adis alem	107	13.9	108	14.06
Amdiweyane	104	13.5	95	12.36
Ara	66	8.5	97	12.63
Ayba	112	14.5	114	14.84
Atsela	88	11.4	86	11.19
Gratserhi	90	11.7	94	12.23
Mekan	94	12.2	90	11.71
Sex				
Male	315	41.0	304	39.58
Female	453	58.9	464	60.42
Age				
Young	262	34.1	262	34.11
Adult	506	65.8	506	65.89
BCS				
Poor	221	28.7	220	28.65
Medium	347	45.1	353	45.96
Good	200	26.0	195	25.39

4.2. Prevalence of Trematode infection in Cattle

Majority (60.29%, 463/768) of the study cattle had either single or multiple trematode infections (fasciolosis, Paramphistomosis and schistosomiasis). Considering, single infection, majority (19.01%) of them were positive for fasciolosis while 20.7% of them had a mixed infection of by both *Fasciola* and *Paramphistomum* species (Table 2).

Table 2: Prevalence of Trematode infection in cattle based on fecal examination by parasite type

Trematode infection	Frequency	Percent
Fasciola species	146	19.01
Paramphistomum species	130	16.93
Schistosoma species	19	2.47
Fasciola species + Paramphistomum species	159	20.7
Fasciola species + Schistosoma species	5	0.65
Fasciola species + Paramphistomum species + Schistosoma species	4	0.52
Total number of cattle infected by trematode	463	60.28

At the zone level, the South zone had higher positive rates with 47.1%, 42.4%, and 3.4% for *Fasciola*, *Paramphistomum*, and *Schistosoma* species, respectively. While, Emba alaje (49.5%) and Ayba (57.14%) had the highest number of positive animals for *Fasciola* egg comparing at district and tabia level, respectively. On the other hand, *Schistosoma* egg was only detected from tabia Adisalem (Samre district) and tabia Ayba (Emba alaje district) with a positive rate of 14.02% (15/107) and 11.61% (13/112), respectively. Furthermore, most of the positive rates were exhibited in adult animals with poor body condition scores (Table 3).

Table 3: Prevalence of Trematodes infection in cattle based on the fecal examination by demographic type

Variable	Total Examined	Fasciolosis		Paramphistomosis		Schistosomiasis	
		No. of Positive	%	No. Positive	%	No. Positive	%
Zone							
South eastern	384	129	33.59	130	33.85	15	3.91
South	384	181	47.14	163	42.45	13	3.38
District							
Samre	214	75	35.05	80	37.38	15	7.01
Hintalo	170	54	31.76	50	29.41	0	0.00
Emba alaje	200	99	49.50	88	44.00	13	6.50
Enda mokoni	184	82	44.56	75	40.76	0	0.00
Tabia							
Debre hayla	107	39	36.45	41	38.3	0	0
Adis alem	107	36	33.6	39	36.4	15	14.02
Amdiweyane	104	31	29.8	31	29.8	0	0
Ara	66	23	34.85	19	28.8	0	0
Ayba	112	64	57.14	56	50	13	11.61
Atsela	88	35	39.8	32	36.4	0	0
Gratserhi	90	38	42.2	37	41	0	0
Mekan	94	44	46.8	38	40.4	0	0
Sex							
Male	315	124	39.37	126	40	14	4.4
Female	453	186	41.1	167	36.9	14	3.1
Age							
Young	262	107	40.8	97	37	5	2
Adult	506	203	40.1	196	38.7	23	4.5
BCS							
Poor	221	105	47.5	91	41.2	9	4.1
Medium	347	149	42.9	149	43	10	2.9
Good	200	56	28	53	26.5	9	4.5

Logistic regression analysis revealed that cattle in the southern zone are 76% more likely to be infected with fasciolosis compared to cattle from South eastern and the difference were statistically significant (OR = 1.76; 95% CI = 1.316, 2.359; P = 0.000). Similarly, it has been observed also that cattle from south zone were 44% more likely to be infected by paramphistomosis as compared to cattle from south eastern and the difference were statistically significant (OR=1.44, 95% CI:1.075, 1.930, P=0.014) (Table 4).

Cattle with poor body condition were 2.32 times more likely to have fasciolosis as compared to cattle with good body condition (OR= 2.32; 95% CI: 1.550, 3.493). The difference in prevalence of fasciolosis between the body condition scores were found to be statistically significant

($P < 0.05$). likewise, Cattle with poor body condition were 94% higher to be have Paramphistomosis as compare to cattle with good body condition (OR= 1.94; 95% CI: 1.284, 2.933) and the difference were statistically significant ($P < 0.05$). However, there was no significant difference in prevalence of Schistosomiasis among the body condition scores ($P > 0.05$). Moreover, there were not significant difference in prevalence of fasciolosis, Paramphistomosis and Schistosomiasis between different sexes and age groups (Table 4).

Table 4: Logistic regression of Trematodes infection in cattle based on the fecal examination

Variable	Total Examined	Fasciolosis			Paramphistomosis			Schistosomiasis		
		No. of Positive (%)	OR (95%CI)	P-value	No. of Positive (%)	OR (95%CI)	P-value	No. of Positive (%)	OR (95%CI)	P-value
Zone										
South eastern	384	129 (33.6)			130(33.9)			15(3.9)	1.160(0.544, 2.472)	0.70
South	384	181(47.14)	1.76(1.316, 2.359)	0.000	163(42.45)	1.441(1.075, 1.930)	0.014	13(3.4)		
District										
Samre	214	75(35.05)	1.15(0.755, 1.778)	0.499	80(37.4)	1.432(0.931, 2.204)	0.102	15(7)		
Hintalo	170	54(31.8)	Ref		50(29.4)	Ref		0		
Emba alaje	200	99(49.5)	2.10(1.375, 2.222)	0.001	88(44)	1.885(1.223, 2.905)	0.004	13(6.5)		
Enda mokoni	184	82(44.6)	1.72(1.118, 2.666)	0.014	75(40.8)	1.651(1.061, 2.569)	0.026	0		
Tabia										
Debre hayla	107	39(36.45)	1.35(0.755, 1.778)	0.306	41(38.3)	1.536(0.794, 2.973)	0.202	0		
Adis alem	107	36(33.6)	1.19(0.667, 2.134)	0.55	39(36.4)	1.418(0.731, 2.751)	0.301	15(14)		
Amdiweyane	104	31(29.8)	Ref		31(29.8)	1.050(0.532, 2.070)	0.887	0		
Ara	66	23(34.85)	1.25(0.652, 2.432)	0.492	19(28.8)	Ref		0		
Ayba	112	64(57.14)	3.13(1.788, 5.511)	0.000	56(50)	2.473(1.292, 4.733)	0.006	13(11.6)		
Atsela	88	35(39.8)	1.55(0.854, 2.830)	0.149	32(36.4)	1.413(0.710, 2.810)	0.324	0		
Gratserhi	90	38(42.2)	1.72(0.951, 3.113)	0.073	37(41)	1.726(0.876, 3.403)	0.114	0		
Mekan	94	44(46.8)	2.07(1.155, 3.714)	0.014	38(40.4)	1.678(0.855, 3.291)	0.132	0		
Sex										
Male	315	124(39.37)	Ref		126(40)	1.141(0.849, 1.534)	0.379	14(4.4)	1.458(0.685, 3.103)	0.327
Female	453	186(41.1)	1.07(0.800, 1.439)	0.638	167(36.9)	Ref		14(3.1)		
Age										
Young	262	107(40.8)	1.03(0.760, 1.396)	0.847	97(37)	Ref		5(2)		
Adult	506	203(40.1)	Ref		196(38.7)	1.754(0.790, 1.463)	0.643	23(4.5)	2.447(0.919, 6.514)	0.073
BCS										
Poor	221	105(47.5)	2.32(1.550, 3.493)	0.000	91(41.2)	1.941(1.284, 2.933)	0.002	9(4.1)	1.430(0.571, 3.578)	0.444
medium	347	149(42.9)	1.93(1.330, 2.814)	0.001	149(43)	2.087(1.428, 3.049)	0.000	10(2.9)	1.587(0.634, 3.976)	0.323
Good	200	56(28)	Ref		53(26.5)	Ref		9(4.5)	Ref	

4.3. Prevalence of Trematode infection in Sheep

An overall, 53.5% (411/768) of the study sheep had either single or multiple trematode infections (fasciolosis, Paramphistomosis and schistosomiasis). Considering, single infection, 21.74% of them were positive for fasciolosis while 18.23% of them had a mixed infection of *Fasciola* and *Paramphistomum* species (Table 5).

Table 5: Prevalence of Trematode infection in sheep based on fecal examination by parasite type

Trematode Genus	Total number of positive	Percent
Fasciola species	167	21.74
Paramphistomum species	103	13.41
Schistosoma species	1	0.13
Fasciola species and Paramphistomum species	140	18.23
Total number of sheep infected with trematode	411	53.52

At the zone level, the South zone had higher positive rates with 42.19%, 36.46%, and 0.26% for *Fasciola*, *Paramphistomum*, and *Schistosoma* species, respectively. While, Emba alaje (44.50%) followed by Samre (40.63%) had the highest number of positive animals for *Fasciola* egg comparing at district and tabia level, respectively. On the other hand, most of the positive rates were exhibited in adult sheep with medium body condition scores (Table 6).

Table 6: Prevalence of Trematodes infection in sheep based on the fecal examination by demographic type

Variable	Total Examine	Fasciolosis		Paramphistomosis		Schistosomiasis		
		No. positive	%	No. positive	%	No. positive	%	
Zone								
South eastern	384	145	37.76	103	26.82	0	0.00	
South	384	162	42.19	140	36.46	1	0.26	
District								
Samre	192	78	40.63	49	25.5	0	0	
Hintalo	192	67	34.89	54	28.1	0	0	
Emba alaje	200	89	44.50	76	38.0	1	0.5	
Enda mokoni	184	73	39.67	64	34.7	0	0	
Tabia								
Debre hayla	84	26	30.9	22	26.19	0	0	
Adis alem	108	52	48.1	27	25	0	0	
Amdiweyane	95	37	38.9	31	32.6	0	0	
Ara	97	30	30.9	23	23.7	0	0	
Ayba	114	61	53.5	46	40.3	0	0	
Atsela	86	28	32.5	30	34.8	1	1.16	
Gratserhi	94	37	39.3	29	30.8	0	0	
Mekan	90	36	40	35	38.8	0	0	
Sex								
Male	304	123	40.5	97	31.9	0	0	
Female	464	184	39.7	146	31.4	1	0.2	
Age								
Young	262	96	36.8	75	28.6	0	0	
Adult	506	211	41.7	168	33.2	1	0.1	
BCS								
Poor	220	89	40.4	77	35	1	0.4	
Medium	353	147	41.6	115	32.5	0	0	
Good	195	71	36.4	51	26.1	0	0	

In the present study higher proportion of fasciolosis (42.1%), paramphistomosis (36.4%) and schistosomiasis (0.2%) infestation were found among sheep from Southern zone. Sheep from south zone were 20% (OR =1.20; 95% CI: 0.900, 1.606) higher to be infected with fasciolosis as compared to sheep from Southeastern zone. However, the difference was not statistically significant ($P>0.05$). Similarly, sheep from south zone were 56% (OR = 1.56; 95% CI: 1.151, 2.127) higher to be infected with paramphistomosis as compared to sheep from Southeastern zone and the difference were found to be statistically significant ($P<0.05$). Moreover, there was no significant difference observed in prevalence of fasciolosis, paramphistomosis and schistosomiasis between different sexes and age groups ($P>0.05$) (Table 7).

Table 7: Logistic regression of trematodes infection in sheep based on the fecal examination

Variable	Total Examined	Fasciolosis			Paramphistomosis			Schistosomiasis		
		No. of Positive (%)	OR (95%CI)	P-value	No. of Positive (%)	OR (95%CI)	P-value	No. of Positive (%)	OR (95%CI)	P-value
Zone										
South eastern	384	145(37.7)	Ref		103(26.8)	Ref		0		
South	384	162(42.1)	1.202(0.900, 1.606)	0.211	140(36.4)	1.56(1.151, 2.127)	0.00	1(0.2)	1	-
District										
Samre	192	78(40.6)	1.27(0.844, 1.930)	0.247	49(25.5)	Ref		0		
Hintalo	192	67(34.8)	Ref		54(28.1)	1.14(0.726, 1.794)	0.56	0	1	
Emba alaje	200	89(44.5)	1.49(0.995, 2.247)	0.053	76(38.0)	1.78(1.161, 2.755)	0.00	1(0.5)	1	-
Enda mokoni	184	73(39.6)	1.22(0.807, 1.864)	0.338	64(34.7)	1.55(0.998, 2.426)	0.05	0	1	
Tabia										
Debre hayla	84	26(30.9)	1.00(0.532, 1.883)	0.997	22(26.1)	1.064(0.554, 2.045)	0.851	0		
Adis alem	108	52(48.1)	2.07(1.169, 3.676)	0.013	27(25)	Ref		0	1	
Amdiweyane	95	37(38.9)	1.42(0.784, 2.586)	0.245	31(32.6)	1.45(0.788, 2.677)	0.231	0	1	-
Ara	97	30(30.9)	Ref		23(23.7)	0.932(0.492, 1.767)	0.830	0	1	
Ayba	114	61(53.5)	2.57(1.458, 4.529)	0.001	46(40.3)	2.029(1.412, 3.603)	0.016	0	1	
Atsela	86	28(32.5)	1.07(0.577, 2.001)	0.813	30(34.8)	1.607(0.863, 2.992)	0.135	1(1.1)	1	
Gratserhi	94	37(39.3)	1.44(0.797, 2.634)	0.223	29(30.8)	1.338(0.721, 2.481)	0.355	0	1	
Mekan	90	36(40)	1.48(0.814, 2.720)	0.196	35(38.8)	1.909(1.039, 3.505)	0.037	0	1	
Sex										
Male	304	123(40.5)	1.02(0.769, 1.389)	0.824	97(31.9)	1.02(0.748, 1.392)	0.89	0		
Female	464	184(39.7)	Ref		146(31.4)	Ref		1(0.2)	1	-
Age										
Young	262	96(36.6)	Ref		75(28.6)	Ref				
Adult	506	211(41.6)	1.23(0.909, 1.681)	0.175	168(33.2)	1.23(0.89, 1.716)	0.197	1(0.1)	1	-
BCS										
Poor	220	89(40.4)	1.18(0.797, 1.764)	0.398	77(35)	1.52(0.996, 2.220)	0.05	1(0.4)	1	-
medium	353	147(41.6)	1.24(0.869, 1.786)	0.231	115(32.5)	1.36(0.924, 2.013)	0.11	0	1	
Good	195	71(36.4)	Ref		51(26.1)			0		

4.4. Malacological Findings

In the study area a comprehensive survey was conducted to identify the species of freshwater snails present. A total of 1,269 individual snails were collected from various study sites, including damp and slow moving water (Table 8). On the basis of shell morphology field survey showed that presence of three snail species of the snails collected were putatively identified as *Biomphalaria pfeifferi*, *Bulinus truncatus*, and *Lymnaea natalensis*. *L. natalensis* was the most abundant species with 584 individuals representing 46.0% of the total snail population. This species was found in a variety of sampling sites indicating its wide distribution and adaptability within the study area. While *B. pfeifferi* and *B. truncatus* accounted for 35.6% and 18.4% of the total snails making it the second and third common species with 452 and 233, respectively individuals collected. Of the total collected snails, most of (71.9%) were live.

Table 8: Spatial distribution of freshwater snail species by district

Genus	Species	Total snails collected	Status of snails		Proportion and status of snail species by district							
			Live	Dead	Samre		Hintalo		Emba alaje		Enda mokoni	
					Live	Dead	Live	Dead	Live	Dead	Live	Dead
Lymnaea	<i>L. natalensis</i>	584 (46.0)	425(72.8)	159(27.2)	90(15.4)	19(3.2)	52(8.9)	44(7.5)	130(22.2)	51(8.7)	155(26.5)	43(7.3)
Bulinus	<i>B. truncatus</i>	233(18.4)	173(74.3)	60(25.7)	61(26.1)	8(3.4)	36(15.4)	21(9)	37(15.8)	19(8.1)	32(13.7)	19(8.1)
Biomphalaria	<i>B. pfeifferi</i>	452(35.6)	314(69.5)	138(30.5)	230(50.8)	64(14.1)	29(6.4)	42(9.2)	39(8.6)	25(5.5)	16(3.5)	7(1.5)
	Total	1269	912(71.9)	357(28.1)	335(26.3)	91(20.1)	117(9.2)	107(8.4)	206(16.2)	95(7.4)	203(15.9)	69(5.4)

All of snail species were placed in vials/Petri dishes/beakers and exposed to sun light for shedding. Some of the snails which served as intermediate host for schistosomiasis collected during the survey were found to be shedding *Schistosoma* cercariae. Infected *B. truncatus* and *L. natalensis* snails were absent. Morphological identification of shaded *S. mansoni* cercariae was performed by looking the typical elongated head and biforked tail. Further verification of identification was made from in mass cercariae exposed laboratory bred mice after six weeks of laboratory maintenance using eggs (miracidium) and adult worm morphology from feces of mice and by dissecting the mice, respectively. On the other hand, these results confirm the snail dynamics in such a way that there was an active transmission of schistosomiasis in the study area.

5. DISCUSSION

This study is the first to evaluate the prevalence of snail-borne trematode infections in live cattle and sheep within the selected area. Trematode parasitism poses a major challenge to ruminant productivity worldwide, resulting in reduced productivity and significant economic losses due to slower growth, diminished milk production, and increased risk of secondary infections and Considered a significant parasitic problem for cattle, sheep, and other ruminants across various global regions, including Africa, where they particularly impact livestock health and agricultural productivity (Dey *et al.*, 2022).

Infections by trematodes are prevalent in areas where their intermediate snail hosts are thriving. Identifying how these diseases spread and setting control measures into place to reduce exposure to these hosts are essential to minimizing the risk of obtaining them, particularly among cattle and sheep. The study identified that local sheep and cattle breeds were most frequently affected, most likely as a result of their widespread adoption in extensive farming systems (Megersa *et al.*, 2024).

A higher percentage of female cattle 58.9% and female sheep 60.4% were sampled compared to their male counterparts 41% and 39.5%, respectively. This is because farmers typically keep more female animals for herd expansion and milk production (Majekodunmi *et al.*, 2014).

Trematode infections were found in sheep and cattle in the southeastern and south zones of the current study, with overall prevalence rates of 53.5% and 60.2%, respectively, according to coprological analysis. The prevalence is in agreement with results from other Ethiopian regions, such as Bahir Dar in the northwest, where 61.0% were identified. (Aragaw and Tilahun, 2019). Similarly, high prevalence rates of trematode infections have been documented worldwide, including 69% in Rwanda (Tumusiime *et al.*, 2023), 64.6% in north-central Nigeria (Elelu *et al.*, 2016), 65.7% in Tanzania (Materu *et al.*, 2024), and 44.3% in Ukraine (che-Kruchynenko *et al.*, 2021). This indicate that these trematode infections are a common problem for livestock worldwide (Materu *et al.*, 2024).

More over the higher prevalence of trematode infections in cattle and sheep observed in this study may be attributed to the study area's significant presence of permanent, slow-moving water bodies and damp environments. This contrasts with some other studies conducted near permanent water sources and irrigation activities. The prevalence of Trematode infection in a

particular area is greatly influenced by the availability of appropriate habitats for aquatic snails, which act as intermediate hosts for trematodes (Megersa *et al.*, 2024).

The prevalence of single trematode infections in cattle and sheep was also highlighted by the current study; Fasciola were the most widespread, followed by Paramphistomes and Schistosoma. Remarkably, among the animals investigated, the frequency of mixed infections was similar to that of single infections. The biological properties like temperature, complex life cycle and ecological interaction of the parasites and their intermediate hosts in the research area may be associated to these variations in fluke infection rates (Dube *et al.*, 2023).

Fasciola was the most common of the trematode genera found, infecting 21.7% of sheep and 19% of cattle. These prevalence levels are similar to those found in earlier research that found coprological prevalence rates in three agro-ecological zones of 21% and 24.5% in cattle and sheep, respectively (Megersa *et al.*, 2024) and 20.92% in sheep were reported in Ejere District, West Shewa respectively (Adugna *et al.*, 2023). This similarity could be linked to the favorable ecological conditions in the study area that support the intermediate hosts of Fasciola (Sisay and Nibret, 2013).

The prevalence of fasciolosis in cattle and sheep in the current study area was lower compared to findings from other regions in Ethiopia. Specifically, previous studies reported coprological conducted in Jimma, eastern Ethiopia, which found prevalence rate 35% (Kebede and Wakgari, 2016), 25.48% in Gechi District (Husen *et al.*, 2017), and 35% in Jimma, Western Ethiopia (Kebede *et al.*, 2016). The prevalence of *Fasciola* infections is known to vary over time, largely influenced by changes in rainfall patterns and amounts. Snail vectors for *Fasciola* spp. thrive at optimal temperatures between 10°C and 16°C, which are essential for the development of the parasites within their intermediate hosts. Additionally, sufficient moisture, achieved when rainfall exceeds evaporation and fields are saturated, is critical for snail reproduction and the development of larval stages. These conditions also facilitate the maturation of fluke eggs, the search for snails by miracidium, and the dispersal of cercariae (Ardo and Aliyara, 2014).

The second most prevalent Trematode infection in sheep and cattle, as determined by the current study is Paramphistomosis which has prevalence rates of 13.4% and 16.9%, respectively. As reported by (Kebede *et al.* 2023), these rates are greater than those from Southern Ethiopia's Wolaita Zone 5.2% and Ethiopia's three agro-ecological zones 11.7% (Megersa *et al.*, 2024).

However, they are lower compared to reports from other parts of the world, such as Iraq 34.6% (Hajipour *et al.*, 2021) and Malaysia 46.9% (Che-Kamaruddin and Isa, 2023).

The prevalence of Paramphistomosis in this study was also lower than previously reported figures from South Wollo, which showed a rate of 39.1% (Wondmnew *et al.*, 2019). These differences could be attributed to variations in livestock management practices, the presence or absence of swampy areas, the impact of deworming programs, and differences in study periods (Pokharel and Sharma, 2023).

In our study, the prevalence of *Schistosoma* infection in cattle 2.4% and sheep 0.1% was lower compared to previous reports from various regions. For instance, higher prevalence rates were reported in northwest Ethiopia, with 24.6% in cattle and 2.3% in sheep (Yirsaw and Zewdu, 2016), and in Gozamen District, with 26.3% and 25.9% (Adane and Regasa, 2023). Fogera Wereda had 16.28% (Wudeneh, 2018), Tis Abay had 13.02% (Kifle *et al.*, 2022), Debre Tabor town had 7.6% (Tsega and Derso, 2015), and other Ethiopian regions, including South Wollo and the Oromia Zones of the Amhara Region, reported 16.7% (Molla *et al.*, 2022). Worldwide, investigations have shown the prevalence of *Schistosoma* in cattle in Côte d'Ivoire 2.4% (Kouadio *et al.*, 2020) and 4.52% in India (Bulbul *et al.*, 2022).

A number of variables, including as variations among study locations, meteorological and ecological circumstances, and animal husbandry practices, may be important for the reported variation in prevalence across studies. In addition, although *Schistosoma* eggs might become remain in tissues, they may not always be identified in fecal samples, which would reduce the detection rates (Takaki *et al.*, 2021).

The prevalence of concurrent trematode infections in cattle was identified to be 20.7% for *Fasciola* and *Paramphistomum*, 0.6% for *Fasciola* and *Schistosoma*, and 0.5% for *Fasciola*, *Paramphistomum*, and *Schistosoma* mixed in the current study. Only concurrent infections of *Paramphistomum* and *Fasciola* were identified in sheep, with an 18.2% frequency. These results contrast with other studies; in Wolaita Zone, only 8.33% of cattle had concurrent infections of *Fasciola* and *Paramphistomum* (Kebede *et al.*, 2023), whereas studies in Bangladesh and Malaysia reported higher rates of 39.5% and 11%, respectively (Khadijah *et al.*, 2017). The favorable conditions in nature that support both the parasites and their intermediate hosts in the research area may be the reason of the higher coinfection rates found in this investigation (Megersa *et al.*, 2024).

Based to the sex-specific examination of the infection rates, female cattle and sheep had a greater prevalence than male animals, with rates of 58.9% and 60.4% for females and 41.0% and 39.5% for males, respectively. This increased susceptibility in females can be attributed to several factors. During lactation, cows are particularly vulnerable to trematode infections due to the potential depletion of immune resources caused by high milk production. Additionally, most people traditional feeding practices, where lactating cows are often given grasses collected from areas near rivers and marshes, during the dry season, to attain a high milk yield from cows (Alim *et al.*, 2021).

This indicates that sex does not significantly affect the infection rate, as both male and female animals appear to be equally susceptible to the disease. The similar exposure levels to pasturelands and watering points for both sexes likely contribute to this equal susceptibility. Additionally, the management system, while males may have longer periods of outdoor exposure, females are often kept indoors during the early stages of lactation. This management practice might influence the overall infection rates (Dey *et al.*, 2022).

This study also examined the prevalence of concurrent trematode infections in cattle and sheep by age group. In cattle, infection rates were consistent across different ages, with adult cattle showing a rate of 40.1% and young cattle at 40.8%. On the other hand, the infection rate in sheep was lower in young sheep (36.6%) and greater in mature sheep (41.6%). Infected sheep were almost 1.23 times more likely to be adults than younger ones (OR: 1.23; 95% CI: 0.909, 1.681). This increased prevalence in adult animal can be attributed to the chronic nature of the disease, as adults often harbor parasites for extended periods without obvious symptoms. Additionally, adults are more frequently exposed to pastures contaminated with metacercariae and typically graze for longer durations, which contributes to their higher infection rates compared to younger (Kebede *et al.*, 2023).

According to the study, the prevalence of snail-borne trematode infections was higher in cattle and sheep with low and medium body condition scores than in those with good body condition scores. Infections such fasciolosis, Paramphistomosis, and schistosomiasis were more common in animals suffering from malnourished. They are probably at a higher risk because of their decreased immune systems and poor health, which leave them more susceptible to these parasites. Animals in good physical condition, on the other hand, had decreased infection rates,

suggesting that improved diet and general health increase resistance to these diseases (Dey *et al.*, 2022).

In the current investigation we collected snails belonging to three genera were identified, a result consistent with a previous study (Megersa *et al.*, 2024). The study areas exhibited a higher abundance of *Lymnaea*, followed by *Biomphalaria* and *Bulinus* species. The variation in species distribution across districts suggests that factors such as water availability, temperature, vegetation, and possibly anthropogenic activities may play a role in shaping snail habitats. Additionally, the dominance of *Lymnaea natalensis* raises concerns about the potential for trematode transmission in areas where this species thrives, as it is a known intermediate host for parasites like *Fasciola*, which affect livestock and humans.

Measurement of infection rates in field populations of snails is one of the basic tools for studies on the epidemiology of trematode infections to estimate the transmission potential of an area (Sturrock, 2001). In the present study, malacological survey at human water contact sites, slow moving water, small dams and other potential lodging sites showed the occurrence of three snail species namely *B. pfeifferi*, *B. truncatus*, and *L. natalensis*. Most of the snails collected during the survey were found to be live. Some of the snails collected during the survey were found to be shedding schistosome cercariae. Previous reports from Jimma town showed a 50% rate of infection in the intermediate host (*Biomphalaria species*) (Abebe *et al.*, 1998) and 58% from the same place (Mengistu *et al.*, 2011). While quite lower natural prevalence of shedding schistosomes cercariae was recorded with 5.42% in *B. pfeifferi* in Waja-Timuga, District of Alamata, northern Ethiopia (Abebe *et al.*, 2014) and infection rate of 0.5% - 6% in Lake Zway (Erko *et al.*, 2006). An explanation for the variability of these results could be due to difference in efficiency of parasite in flux from vertebrate to snail host, which is determined by factors including seasonal variation, snail density, prevalence and intensity of *Schistosoma* infection and deposition rate of *Schistosoma* eggs in water bodies.

Collection of schistosomes infected *B. pfeifferi*, and the establishment of lab infection in mice showed that transmission of intestinal schistosomiasis is taking place in the area which is in line with previous reports from Waja-Timuga, District of Alamata, northern Ethiopia (Abebe *et al.*, 2014).

6. CONCLUSION AND RECOMMENDATIONS

The present study revealed that trematode infections are moderately prevalent in the study area, indicating a significant health concern for cattle and sheep. This prevalence may adversely affect economic outcomes from these animals, potentially hindering the growth of cattle and sheep production in the region. The study identified age and body condition score as risk factors for trematode infections in both cattle and sheep. Additionally, female cattle and sheep showed higher prevalence rates of Trematode infection compared to males. The occurrence of Trematode infection is closely linked to the primary feed resources in the area, which are predominantly natural pastures and seasonally waterlogged grazing lands. The lack of clean piped water for animals further increases their exposure to trematode infections. In addition, the local environment is suitable for the development and distribution of intermediate hosts. Furthermore, the heterogeneous distribution of snails, particularly *B. pfeifferi*, which could have more public health concern, has important implications for potential mass treatment programs in the study areas. The following recommendations have been given forward considering the implications of the above mentioned conclusions.

- Implementing strategic deworming programs against trematode infection.
- Awareness creation against grazing of animals in the potential environments which favor for the habitat of intermediate hosts.
- Targeted snail control and environmental management strategies should be applied at district-specific, and addressing the ecological conditions that favor the proliferation of these species.
- Complement coprological examinations with additional diagnostic methods, such as postmortem and immunological testing should be conducted to improve the accuracy of trematode detection.
- Further investigation on the temporal variation of intermediate hosts should be implemented.

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8. ANNEXES

Annex I: Data Collection Sheet for Individual Samples from the research area.

Sir No	Sample code	Site of Sample collection		Date of sample collection	Tabia	Breed	Species	sex	Age	BCS	Results		Species						
		anus	Ground								+ve	_ve	F	P	SCH	F&P	F&SCH	F&SCH&P	
1	01																		
2	02																		
3	03																		
4	04																		
5	05																		
6	06																		
7	07																		
8	08																		
9	09																		
10	010																		
11	011																		
12	012																		
13	013																		
14	014																		
15	015																		

Annex II: Sources of infection sites of the study areas











Annex III: distribution of snails

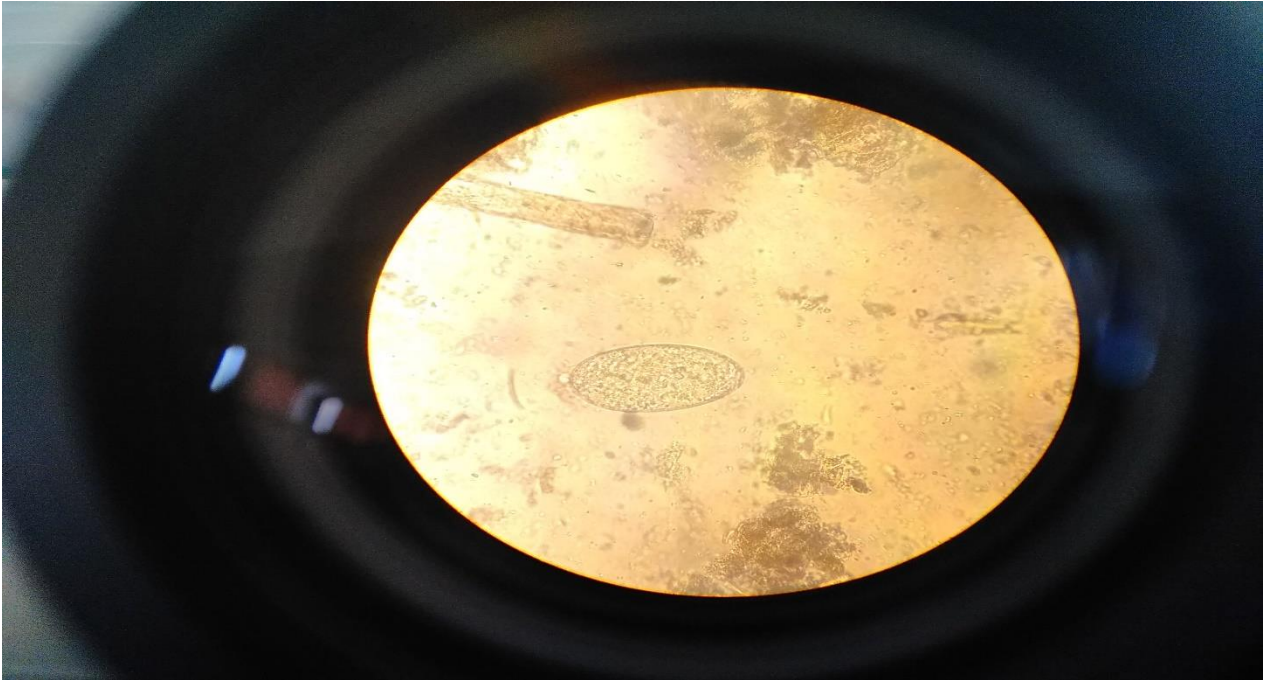


Annex IV: Samples submitted to the laboratory

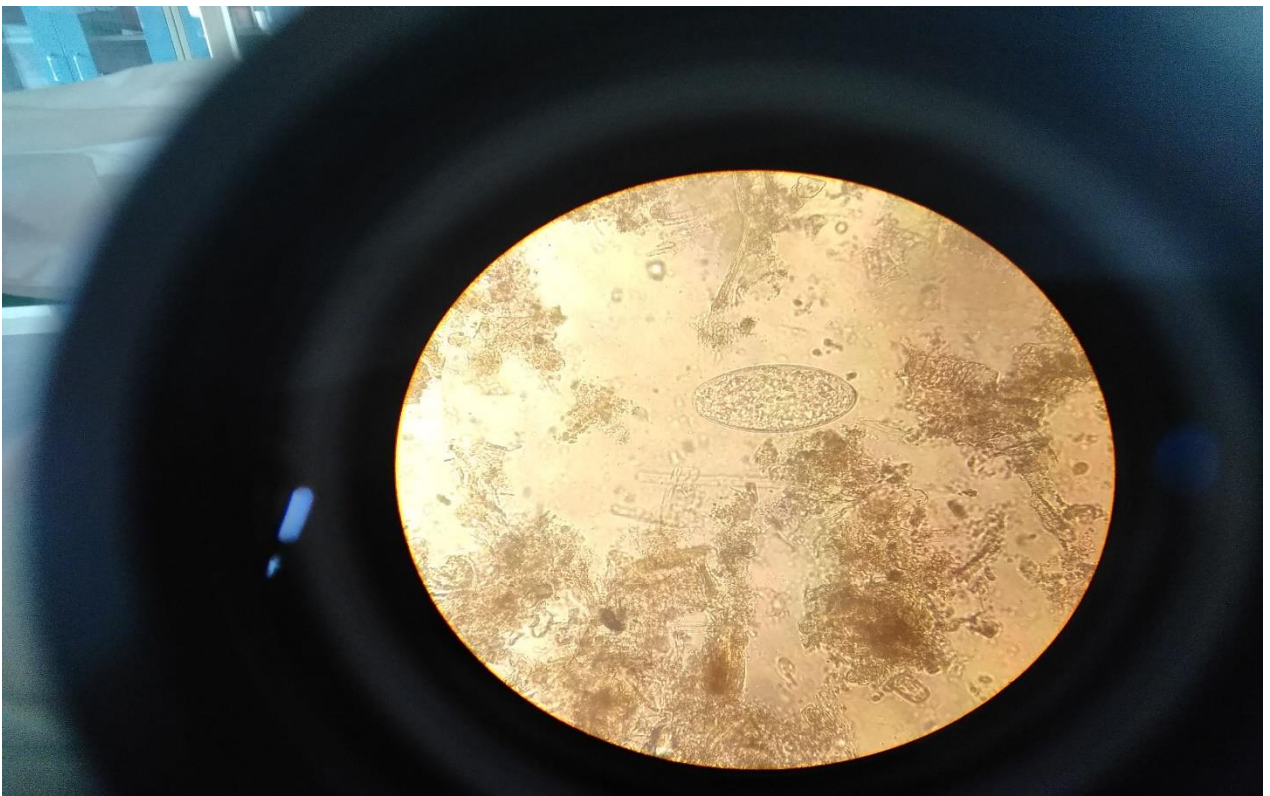


Annex V: Results of the coprological study





Coprological Result 10X



Coprological Result 40X







Mekelle University
Animal Ethics and Experimentation Committee (AEEC) Clearance Letter

Date: November 23, 2024

To: Girmay Gebrezgi, Principal Investigator (PI)

Mekelle

Subject: Notification of AEEC decision on your research proposal

AEEC No: AEEC 31/2024

Protocol (Title of the study): Coprological Study of Trematode Infections and Associated Factors in Cattle and Sheep and Spatial Distribution of Freshwater Snail in South and Southeastern Zone of Tigray, Ethiopia

Dear PI,

The aforementioned research proposal which was submitted by you for animal ethics and use clearance letter has been reviewed by the Animal Ethics and Experimentation Committee (AEEC).

The AEEC has discussed and examined the research proposal in detail from animal ethics and use principles and values perspective.

Finally, the AEEC has (approved, approved with modification or withhold approval) your research proposal.

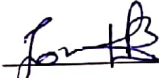
This animal ethics clearance letter is valid for only one year (23/11/2024 – 23/11/2025).

Furthermore, any other correspondence and inquiries concerning your research proposal with committee must include the AEEC No., the name of the PI and the proposal title.

Best Regards,

AEEC Chairperson


Name: Nigus Abebe (PhD)

Signature: 

Date: 23/11/2024

AEEC Secretary

Name: Enquebaber Kassaye (PhD)

Signature: 

Date: 23/11/2024