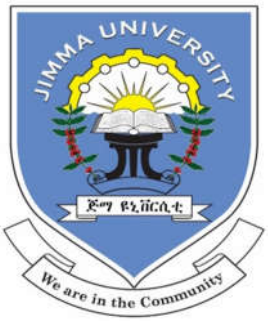

IDENTIFICATION AND PREVALENCE OF CERCARIAL INFECTION IN FRESHWATER SNAILS IN OMO-GIBE RIVER BASIN, SOUTH WEST ETHIOPIA

BY

JEMAL BEDEWI (BSc)

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Jimma, Ethiopia

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ASSURANCE OF PRINCIPAL INVESTIGATOR

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

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Abstract

Background: Cercaria is a free swimming, larval stage of trematode parasite released from the intermediate host snails and contaminate freshwater bodies. Several environmental factors influence the survival of the larva but endure a lot of challenges and can infect humans and other vulnerable organisms when they come in contact with contaminated water.

Methodolgy: Potential intermediate host snails were surveyed from freshwater bodies related to human and animal contact and examined for trematode larval infection by cercarial emergence technique for one month. The cercariae releasd by the snails were identified morphologically to genius level. Physico-chemical parameters were measured and some anthropogenic activities were assessed. To determine factors influencing the rate of snail infection factorial ANCOVA was used and Simple linear Regression analyses were used to determine the relationship between shell size and intensity of *Biomphalaria pfeifferi* infections.

Result: Seven morphologically distinguishable cercariae were released from four of five snail species. Of which the human *Schistosome* cercariae were the dominant accounting 35% of all infections. These cercariae infect 13% of the total surveyed water bodies. *Biomphalaria pfeifferi* was the most infected snail species in which 85 % of infections were observed. The intensity of infection in *Biomphalaria pfeifferi* snail species were significantly affected by its shell size ($p < 0.001$). The prevalence of cercarial infection in this study was 4%. The rate of snail infection significantly influenced by temperature, turbidity, cloth washing and swimming ($p < 0.05$).

Conclusion and Recommendation: The presence of trematode cercarial infection in water bodies can cause significant health and economic effect. The finding of this study indicates that various types of trematode parasites are prevalent in the study area. Therefore intervention should be foucused on control of the intermediate host snail to interrupt the transmission of trematodes related disease. The proper management of excreta is the primary barrier to prevent the spread of the parasite to the environment.

Key words: *B.pfeifferi*, Cercariae, trematode, *L.natalensis*, *Schistosome*.

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Acronomy

BAD	*Brivifurcate apharyngeate diastome*
BAM	*Brivifurcate apharyngeate monostome*
ANCOVA	Analysis of covariance
SNNPRHB	South Nation National Peaoples Regional Health Bearua
SPSS	Statistical package for social science

Chapter One: Introduction

1.1 Background

Snails are invertebrate organisms adapted to different sort of environmental conditions hence, grouped as marine, terrestrial and freshwater snails together forming more than 50,000 species (Johnson, 2009). Freshwater snails are group of snails dwelling in the freshwater, most of them serving as mandatory intermediate host for a number of trematode parasites (Devkota *et al*., 2008). They belong to the class Gastropoda consisting two sub-classes Prosobranchia and Pulmonata. The prosobranch were less recognized as host of important parasite but the family *Pilidae* and *Thiaridae* were found to harbor trematode cercarial parasite (Ismail *et al.*, 2011). Members of these sub-classes of freshwater snails serve as intermediate host for various trematode parasitic disease of human (Ahmed *et al.*, 2006).

Among various group of fresh water snails *Biomphalaria*, *Bulinus*, *Lymnea* and *Oncomelana* are considered medically important and commonly transmit parasitic disease to human, other mamals (Ismail *et al*., 2011) and birds (Punjab *et al*., 2011). As a result, the distribution of intermediate host snails is refractory for the occurrence of various trematode parasite infection in a specified region, since freshwater snails are vector of digenetic trematodes (Jayawardena *et al*., 2010). Human infection by the trematodes parasite relay on contact with cercariae infested water. Cercariae are the free swimming trematode's larval stage recovered from the snail and directly piercing the skin of the individual when contact with vitiated water with the parasite. Miracidium is the first stage of larva of trematodes which infect/penetrate snail host where further development and multiplication takes place and changed to primary and secondary sporocysts then to cercariae and escape to the external environment (Rowel *et al*., 2015).

Schistosomiasis is one of human parasitic disease transmited by snails. The disease is distributed various part of the globe taking the second rank next to malaria interm of causing socioeconomic and health problem (Alebie *et al*, 2014). The disease is more prevalent in Africa including Ethiopia, could be due to low socioeconomy or suitable climate for transmission. Human transmission resulted from exposure to water infested by infecteive larval stage of trmatodes calld cercaria (Mengistu *et al*., 2011).

Cercariae is free swimming, infectious larval stage of the trematode parasite, which is without cuticle emerge from intermediate host snail and contaminate water. Once released into water bodies it depends on its own stored energy reserve and susceptible to exposed environmental factors. Then the larva survives a lot of challenges and can infect humans and other susceptible organism when they come in contact with contaminated water. The disease develops in infected humans, animals and birds then the adult parasite release the parasite egg which hatch in water, hence the transmission cycle continues. These free-living cercariae vulnerable to unstable environmental factors including stressful conditions resulting from pollution and human influence The environmental factors influence transmission of parasite directly by affecting the free-living parasite and indirectly by influencing the invertebrate host snail. Temperature, oxygen concentration and pH affect the presence of parasites thus their survival and infectivity. The effect of temperature on parasite studied well, but infective stage survives different degree of temperature which is species specific and the variation from the optimum, narrows the probability of survival or infectivity. Low temperature of the surrounding increases the life of the free-living parasite whereas high temperature reduced longevity by enhancing individual development (Pietrock & Marcogliese, 2003).

Studies also suggested that factors affecting the growth of snail population will increase the survival of trematode parasite in the intermediate host. Some ecological conditions in various habitats may aid the life cycle of trematode parasite. Temperature shows positive relation with the transmission of trematode parasite. The stimulating effect of temperature enhances cercarial release from intermediate host snail and also speed up the rate of cercarial generation. Great number of cercariae emerged because of combined effect with light but some cercariae stay with snail under high temperature. Effect of hydrogen ion concentration on survival and infectivity vary in varies species of the parasite. Studies found that parasite outside their host can withstand high range of variation of pH. But the infectivity and survival of the parasite will be reduced when variation of hydrogen ion concentration increase and increase from the normal specific to the species because it brings stress on physiological properties. An increase or a decrease hydrogen ion concentration leads to a negative effect on survival, infectivity eventually on transmission outcome (Pietrock & Marcogliese, 2003).

According to (Rowel *et al*., 2015) many freshwater parasites resist slight salt concentration during outside of their host without damaging their survival. But cercariae exposed for small salt concentration results reduced infectivity. They suggested that schistosomiasis transmission is prevented by increase of salt concentration. (Pietrock & Marcogliese, 2003) found that light has a great effect on parasitic infection by resulting release of infective stage from intermediate host snails. Also suggested that extremely high light and natural ultraviolet radiation damages survival and infectivity when outside of the host. The availability of oxygen in the environment seems to decrease the survival of transmission stage because of most adult endoparasites lives anaerobic environment. (Tigga, *et a*l., 2014) reported that depth of the water body, light, temperature, age and size of the snail are some factors that affect intensity and rate of infection of trematode parasites.

The transmission stage of the trematode parasite is achieved by emerging the parasite from snail host to water environment (Pietrock & Marcogliese, 2003). Where many conditions influence the emergence of trematode parasite from the snail host, like light and temperature and Change of light immediately result the change of cercarial emergence pattern. Exposure for temperature exclusive of light cause emergence of cercariae but its effect seems less influencing than light. It is not still clear the effect of light is whether on the parasite or on the snail. Some emergence pattern related with host activity period like human schistosome has emergence pattern during the day time (Ahmed *et al*., 2006).

For transmission of trematode parasite from intermediate snail host, the number of snail that shade/release cercariae and proportion of cercariae shaded from individual infected snail (intensity of infection) play significant role. The prevalence and intensity of digenetic trematode infection affected by various biological factors of the snail and physical factors (Tigga *et al*., 2014). Cercarial incubation period differ according to parasite/host and environmental determinant especially temperature. In favorable condition this incubation lasts for 21 days. Studies suggested that snails should be incubated three to four weeks so as to release cercariae (Rowel *et al*., 2015).

Medically important fresh water snails serve as intermediate host for various trematode parasitic disease of human causing significant public health problem in tropical and subtropical countries (Ahmed *et al*., 2006).

In Ethiopia genius *Biomphalaria*, *Bulinus* and *Lymnea* are the most important intermediate host snails distributed different parts of the country consequently resulting various trematode related parasitic disease including *Schistosomiasis* and *Facsioliasis* (Alemayehu & Tomass, 2015). *Schistosomiasis* in Ethiopia transmitted by *Biomphalaria pfeifferi, Biomphalaria sudaniica, Bulinus abyssinicus* and *Bulinus affricanus*. The *Biomphalaria* is a vector of intestinal *Schistosomiasis* whereas *Bulinus* is for urinary *Schistosomiasis*. *B.pfeifferi* exists in several parts of the country, but *B.sudanica* located solely in Rift valley area, Lake Abaya, Lsake Ziway, Tikur Wuha River and Lake of Awassa. *Bulinus* species reported in Awash valley and Ethio-Sudan border (Alebie, *et al*., 2014). *Fascioliasis* caused by *Fasciola hepatica* and *Fasciola gigantica* exist in Ethiopia. It is disease of ruminant resulting major economic problem especially in areas located in the mountain region of the country (Ayana & Waktole, 2013). Controlling trematode caused parasitic disease requires understanding about infection rate and intensity among intermediate host snails, type and number of cercarial production and factors affecting snail infection. Also accurate identification of trematodes provides tremendous importance for epidemiological study, follow up and disease control. Many studies have been carried out in Ethiopia about medically important snail transmitted parasitic disease especially *Schistosomiasis*, emphasizing on disease prevalence and intensity of infection among school children. Where focusing on specific type of intermediate host snail infected with specific type of trematode. However, little information is present on total intermediate host snails and trematodes cercarial production and dynamics to plan effective prevention and control measure of intermediate host snail transmitted disease and morphological description of trematodes were also less elucidated.

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1.2. Statement of the problem

Freshwater snails transmit various medically important trematode caused parasitic diseases where *Schistosomiasis* and *Facsioliasis* are dominantly resulting public health problem in the world, which are serious in Sub Saharan Africa. *Schistosome* parasite infected around 207 million people and about 779 million are at risk to develop *Schistosomiasis* infection *(*Pedersen *et al.*, 2014). *Schistosomiasis* is the second parasitic disease next to malaria causing socioeconomic and public health burden (Alebie *et al*., 2014). The disease becomes a serious economic, physiological and psychological problem on the affected community. And it is responsible for bloody urine, enlargement of spleen and liver, reduced cognitive development, attention deficit disorder; reduce productive ability and reproductive organ anomalies among females. Also studies show that malaria, tuberculosis and HIV/ADS infections easily affect the diseased individual (Rollinson, *et al.,* 2014). *Schistosomiasis* endemic countries require praziquantel treatment for prevention and control of the disease (Rollinson, *et al*., 2014). In Sub Saharan Africa the treatment is donated from foreign countries but the treatment meets only ten percent and the rest are requiring urgent need (Mediterranean, 2010).

Infectious disease transmitted by intermediate host snails are causing significant public health problem in Ethiopia (Woldemichael *et al*., 2006). About 4 million people are affected by the *Schistosomiasis* disease and an approximately 29.89 million people are at risk of infection. Agriculture related irrigation, water resource development, migration and population displacement raises the disease incidence (Molla, 2011). The disease affects fairly large number of productive force, subsequently affecting the economy. For transmission of *Schistosomiasis* to took place, the, fresh water intermediate host, the definitive host and water contaminated with infective trematode parasite should be required (Woldemichael *et al*., 2006).

The other trematode caused parasitic disease is *Fascioliasis*. The burden caused by human *Fascioliasis* is not well reported worldwide and varies from 2.5 to 17 million (Pedersen *et al*., 2014). As most other countries in the world the prevalence in Ethiopia have not been intensively described but the prevalence of 11% to 100% was documented in rift valley and central highland respectively (Molla, 2011).

In south west Ethiopia, including Jimma, there are different water bodies like rivers, irrigation canals, dams, lakes, springs, streams and wetlands. These water bodies support the propagation

and development of intermediate host snail which play a role in the transmission of trematode parasite (Mengistu *et al*., 2011). Despite these problems, information on the prevalence of infection in total intermediate host snail, their intensity and environmental factors affecting occurrence of infected snails is not well known. Moreover, accurate data has not yet been produced on the occurrence of various trematode parasites in intermediate host snail in Southwest Ethiopia and other regions. Therefore, the objective of this study was to elucidate the prevalence and type of cercarial infection in medically important freshwater snail in Southwest Ethiopia. The finding of this study is useful in identification of disease transmission hot spot and designing effective disease prevention and control strategies.

Chapter Two: Literature review

2.1 Habitat

The trematodes intermediate snail hosts dwell in natural and man-made freshwater habitats such as rivers, lakes, streams, wetlands, ponds, irrigations and dams. The occurrence of the intermediate host snails at a particular freshwater habitat could be evidence of the occurrence of specific type of trematode disease in that geographical location. Because this intermediate host snail species transmit various trematode related parasitic disease to humans' and animals' (Choubisa and Sheikh, 2013). The transmissions of trematode parasite to the next host occur during occupational and recreational activities or other direct contact with the parasite contaminated water. The 'proportion' of snails that generate cercariae and extent of cercariae generated from each infected snails are important for transmission of trematode parasite from the intermediate host snails to mammalian host including the human being (Grimes *et al*., 2015).

2.2. Prevalence of infection

Freshwater Snails serve as intermediate host of different species of trematode cercariae infecting human. Knowledge about infection rate play important role in prevention and management of snail transmitting infection. So Studies conducted to observe cercariae from intermediate host snail in various parts of the world use either cercarial shading or crushing the snails which results varies snail species with varying infection rate even in similar region in different water habitat. 100 species of snail serve as intermediate host of the trematodes cercarial parasite (Mai, *et al*, 2013). Prevalence of infection is one of the determinant factors for the rate of transmission of trematode parasite to the next host (Ahmed *et al*., 2006).

According to (Jayawardena *et al*., 2010) the study done in Srilanka the prevalence of infection among intermediate host snail varied among sampling site and climatic zone. In the wet zone and intermediate zone are ranged 0-85.7% and 0-48.6% respectively. The very high infection prevalence was observing in dry zone ranging from 42-100%. *Thiara* species are the most preferred intermediate host snail with high infection prevalence. The report of (Ahmed *et al*., 2006)- in Khartoum state show that the prevalence of *Bulinus truncatus* is 14.9% where the infected snail releasing different kinds of cercariae with various prevalence rate. (Tigga, *et al*, 2014a) suggested that the prevalence of infection of *Indoplanorbis*, *Gyrulus*, *Lymnaea* and *vivipara* snail species around and in Ranchi was 7.33% where the intermediate host snails

infected by different trematode parasites. The infection rate was significantly different in different months with in various species of the intermediate host snails. In another study conducted by (Rowel *et al*., 2015) the infection rate of *Biomphalaria s*nail along Lake Albert in Uganda was 8.9% of which 15.8% was *S. mansoni* and 84.2% non-human trematode. Only 2.1% of snails were infected in Lake Victoria where, 13.9% were releasing *S.mansoni* cercariae, 85.7% were releasing non-human trematode cercariae and 0.4% was infected by multiple trematodes. *Biomphalaria stanly a*long Lake Albert and *Biomphalaria pfeifferi* along Lake Victoria are the most infected snail species that infected by both human and non-human cercarial trematode. The study by (Ahmed *et al*., 2006) in Sudan conducted on irrigation canal of east Nile locality suggested that the prevalence of *Biomphalaria pfeifferi*, *Bulinus truncatus*, *Melanoid tuberclata*, *Bulinus forskali* and *Cleoptra bulimoides* was 14.1% by cercarial shading method. The study done by (Devkota *et al*., 2008) in Central Nepal show that the prevalence of belonging the snail family *Planorbidea*, *Lymnaeidea*, *Ampoliridea*, *Bithyniidea*, *Theiridea* and *Viviparidea* was 4.3% where this low infection rate is due to death of snail caused by the parasite. In a study conducted by (Rollinson *et al*, 2014) in Tanzania the prevalence of cercaria were only 0.6% where, solely *Biomphalaria* snail release human *Schistosome* parasite from the collected *Biomphalaria* and *Bulinus* snails. According to the study although, the area is known *Schistosomiasis* transmission site, low infection rate of snail may be resulted from many different factors.

In Ethiopia, snail survey was carried out to determine the prevalence of infection in intermediate snail host in various parts of the country. According to the study conducted on Aweto, Chore and Kitto River in Jimma town to determine human intestinal schistosomiasis, 58% of *Biomphalaria* snail was infected which was observed by cercarial shading technique after the snails tested on light for 1 hour. However, the different type of cercariae was not recognized (Mengistu *et al*., 2011). Similar study conducted on Gilgel Gibe dam South Ethiopia to determine hydroelectric dam impact on *Schistosomiasis* and prevalence of *Schistosoma mansoni*. The study shows that *Biomphalaria pfeifferi* surveyed from river supplying the dam were not infected (Yami, *et al.*, 2010). In addition to this, the study conducted on Sanja River and Maho stream in Amhara region to determine transmission and infection rate of *S.mansoni* in school children. *Biomphalaria pfiefferi, Bulinus forskali* and *Lymnea natalensis* surveyed and only *Biomphalaria* pfiefferi was infected by *Schistosome* trematode with16.9% in February and 0.027% in April

showing periodic variation. The other snail species surveyed did not release any trematode parasite (Alebie *et al*., 2014).

2.3. Cercarial identification

Disease property of trematodes infection related with parasite species, hence, identification of trematode cercariae is important. Even though, classification of digenean is complex, larval property of trematodes used for classification of digenean trematodes, based on 'position and number of sucker', there are four kinds of cercarae which include "*monostome*, *amphistome*, *gastrostome* and *distome*". Based on visual appearance and relative tail size 11 monotypes are recognized: "*Pleurolorphocercous*, *Cytocercous, Microcercous*, *Furcocercous cymnophalus*, *Macrocercous*, *Leptocercous*, *Trichocercous*, cercariaea, rat-king and *Cotylocercous*." Occurrence of other body structure and eyespot also looked for classification (Jayawardena *et al*., 2010). Morphological identification of trematode cercariae to family and genus level is possible but it needs great care especially identification based on genus and species level (Zeitschrift, *et al*., 2015). Studies conducted in different parts of the world suggested that the naturally infected snails release various types of cercariae trematodes. The study conducted by (Jayawardena *et al*., 2010) in Siri Lanka claimed eight morphologically different cercariae identified from four snail species of *Thiara scabrata*, *thiara tuberculata*, *Paludomus sphearica* and *Gyraulus saigonensis* by exposing snails for artificial light which result '*Oculoplearolophocercous, Distome, Furcocercous*, *Gymnocephalous*, *Echinostomous*, *Gymnophalous*, *Xiphidiocercariae* and *Macrocercous cercariae'*. The six morphologically differentiated trematode cercariae reported by (Devkota *et al*., 2008)- in Central Nepal were '*Amphistome*, *Brivifurcate-aphareangate*, *Clinostomoide*, *Gymnocephalus*, *Longifurcate-phrengeate diastome* and *Xiphidiocercariae*'.

The report of (Ahmed *et al*., 2006)- in Khartoum state show that *Bulinus truncatus* release four kinds of cercariae by cercarial shading method namely *Schistosome cercariae* (9.5%), *Amphistome* (2.5%), *Xiphidocercariae* (2.4%) and avian cercariae (0.5%). The study conducted in Sudan on irrigation canal of east Nile locality, by (Mohammed, *et al*, 2016) indicated 20 different cercarial morphology from snail species of *Biomphalaria pfeifferi, Bulinus truncatus*, *Melanoid tuberclata, Bulinus forskal*ii and *Cleoptra bulimoides* of which 14.1% of them were infected and released trematode parasite. In this study five snail species were infected from the

seven species. The highest infection was recorded between *Bulinus truncatus*, where 46.2% of snails infected, and the snail releasing *Schistosoma haematobium* and *Xiphidiocercariae* at the same time. The *cleoptra bulimoides* releasing *Xiphidiocercariae* and *Longifurcate-pharengeat monostome cercariae vivax/LPM* simultaneously *Xiphidiocercariae* type 1 was the most prevalent type of cercariae covering 44.3% of all infections followed by *Paraplearolophocercous* and *Longifurcate-pharengeate monostome vivax* and also *Schistosoma mansoni cercariae* was the other morphological type reported. *Biomphalaria pfiefferi and Bulinus truncatus* are the preferred snail species accounting 15 types of trematode parasites in this study. The study also claimed that increased water temperature and vegetation coverage enhance the probability of snail infection. The study conducted in Ethiopia by (Mengistu et al., 2011b) in three rivers of Jimma town, *Biomphalaria* snail release *Bifurcated* tailed cercariae which was called as *Schistosome cercariae*. In this study the probability of emergence of other cercariae type were not percieved. (Alebie *et al*., 2014) also reported *Schistosome cercariae* in sanja area, Amhara region.

Digenetic trematode consists of most of familiar trematodes and includes those of major economic importance. This groups generally known as flukes and is endoparasites of all classes of vertebrates. In most of the time flukes are hermaphroditic but some memebers are exist male and females in a separate parasite. All have complicated life cycle involving two to four different hosts' and many larval stages with the first larval stage with cilia and non feding called **miracidium**. The metamorophosis of meracidia to **sporocyst** which absorb nutrient from the host. The sporocyst develop in to doughter sporocyst, rediae or in some species directly in to cercariae. **Rediae:** The embriyo in the rediae germinate in to doughter rediae or in to **cercariae.** Cercariae correspondsthe juvenile stage of vertebrate-dwelling adult. In summary the popular pattern of digenetic trematodes life cycle is egg→ miracidium → sporocyst → rediae → cercaria → metacercaria → adult. The most common observed differences in the life cycle are 1) "more than one generation of sporocyst or reiae 2) delation of either sporocyst or rediae generation and 3) delation of metacercariae" (Schmidt *et al.*, 2000). Figure 1 show some existing life cycles

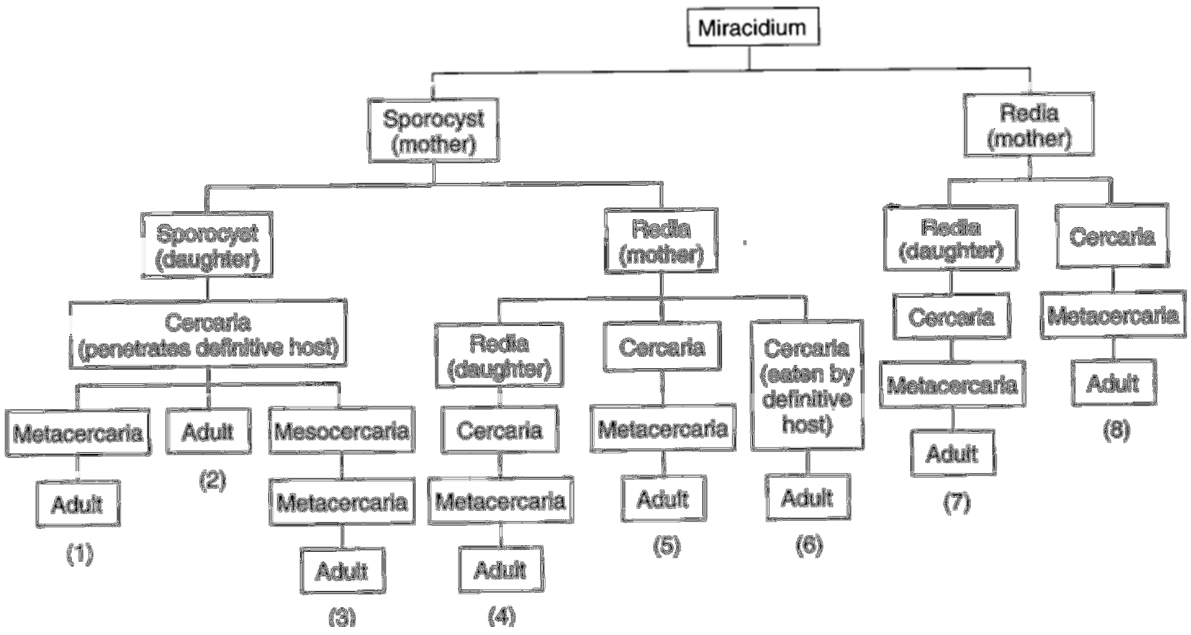


Figure 1 Some digenetic trematodes life cycles

Source: S. C. schell, how to know the trematodes, 1970, Wm.C. Brown publisher inc., Dubuque. lowa.

The emergence of digenetic trematodes cercariae from freshwater snails related with the presence of vertebrate host which is directed by external stimuli i.e. coinciding with host water activity time. This timing emergence is appropriate to achieve effective transmission since its life span is less than or equal to one day. Cercarial emergence varied with time of the day which is determined by endogenous factors in several freshwater snail species (Fingerut, *et al*., 2003). To increase transmission of the digenetic trematode parasite the daily recurrence of cercarial emergence are adapted. To justify the significance of daily recurrence several hypotheses have been considered the cercarial emergence is simultaneously occur with the presence of the next host specially for cercarial species in which the targeted host do not regularly live together with molluscan host producing cercariae is the most accommodated hypothesis.

2.4 Some ecological factors affecting infection of the snail

Investigation of snails which are vector of different trematode cercariae engage in study of ecology where the snails live, to identify ecological factors For a common snails harboring trematode cercariae in specified freshwater habitat it is certain that there is a relation between chemical ingredient, presence of snails and cercarial infection (Kakulte, 2012). The study done

by (Rowel *et al.,* 2015) in Uganda show that *S. mansoni* cercariae has indirect relation with conductivity ($p = 0.05$, CI_{95} (-0.003,-0.001) and direct relation with temperature along Lake Alberta and indirect relation with pH and direct relation with temperature along Lake Victoria. The non-human infecting cercariae directly related with temperature along both Lakes but negatively related with conductivity which is not significant. The study also claimed that depth of water affect infection of *Biomphalaria* snail where shallow water body releasing more cercariae type than deeper water along Lake Victoria. So according to this study factors influencing *Biomphalaria* infection are temperature, pH, little wave action, depth of water, conductivity and density of snail population. (Welsh & Drent, 2015) reported that temperature strongly related with the outcome and shading of cercariae from the first intermediate host. But the parasite-host interaction is not solely determined by non-living environmental factors rather the interaction between some ecological species. The increment of temperature may lead to decrement of transmission. Although increase temperature raises the production and infectivity of cercariae, increased temperature also enhances consumption rate of this free living cercariae by non-host predators thus the non-host pry results reduction of transmission of the parasite to the targeted host under climate change. Study conducted by (Widmann, 2013) claim that ecological factors affect the trematodes cercarial parasite directly through effect on the free living cercariae and indirectly by the intermediate host snail sensitivity change. According to this study increase of Cercarial production resulted from hot temperature by accelerating the local influence of the parasite, if other change could not remove it.

The conceptual frame work in the figure 1 below show the effect of some factors on the prevalence of snail infection and effect of *B.pfeifferi* shell size on the intensity of infection.

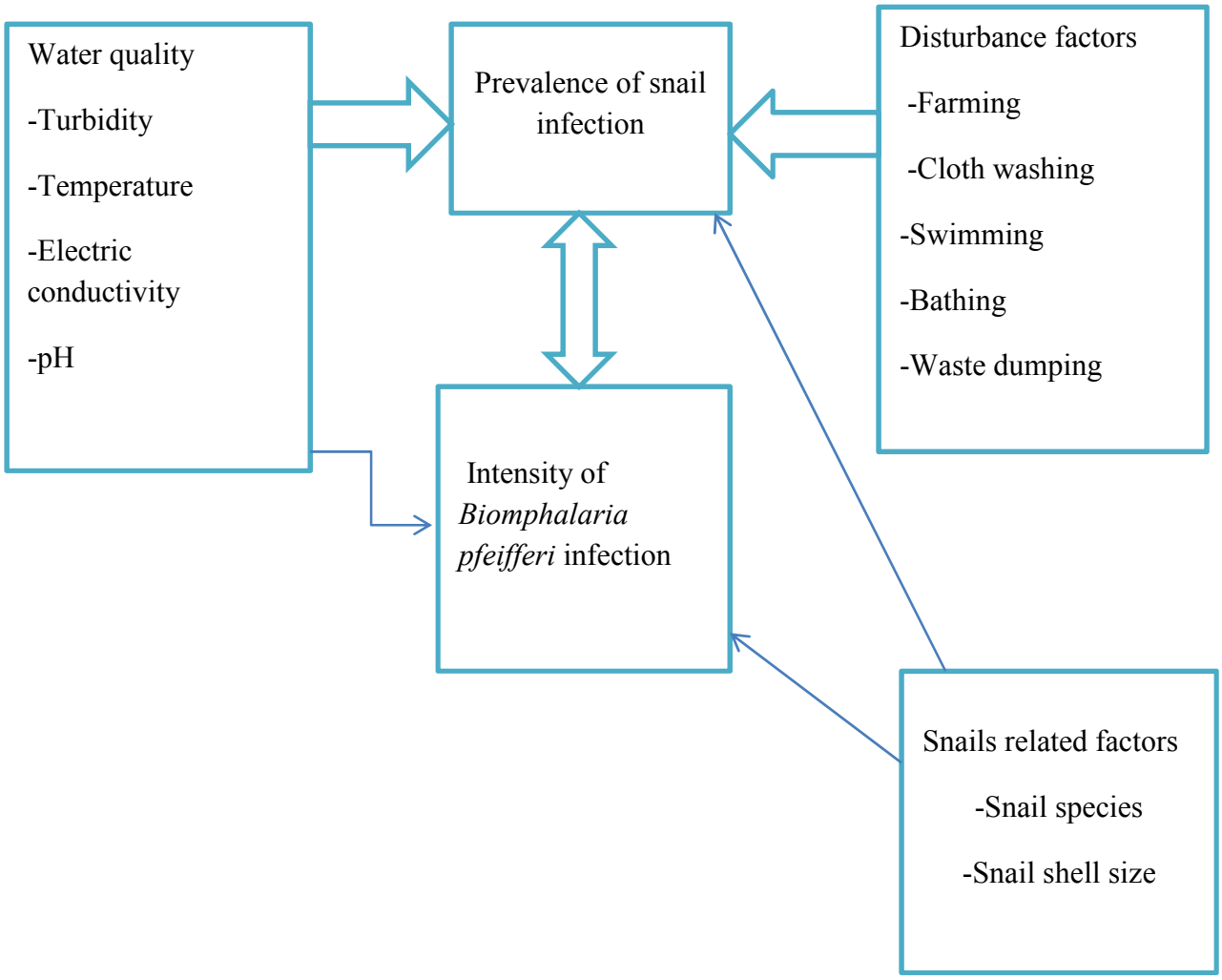


Figure 2 Schematic presentation of conceptual frame work for identification and prevalence of cercarial infection in freshwater snail in Omo-Gibe river basin-May, 2016

2.5 Significance of the study

Activities like irrigation, dam construction for hydroelectric power and water conservation for various purposes are governmental concerns. But provide habitat for fresh water intermediate snail host. Human behavior like fishing, swimming, poor human excreta disposal, use of water bodies for domestic purpose require frequent and longtime exposure of water bodies which may infested by snail intermediate host subsequently pose to trematode infection. Thus occupational, recreational and domestic water contact practice has direct relevance to medically important fresh water snail in Ethiopia. This indicates infection rate, identification of cercarial trematode and factors influencing snail infection are relevant to plan effective prevention and control method. Therefore, it is clear that the need to determine the prevalence and identification of cercarial infection in medically important freshwater snail.

This study attempt to investigate prevalence of trematode cercarial infection. The identification of trematodes gives valuable data for implementing body based on guiding disease transmission hotspot area and types of trematodes parasitic disease. The study also provides the real picture of parasite harboring fresh-water snail species that may indicate the magnitude of the problem caused by the snail species; hence the government body as well as the individual community member will help designing better prevention and control program for the problem caused by intermediate snail hosts. Furthermore, the study will lay possible base line information for further study and investigation especially in Ethiopia.

Chapter Three: Objective

3.1 General objective

- To determine the prevalence and type of cercarial infection in freshwater snails in Omo-Gibe river basin, South West Ethiopia.

3.2 Specific Objectives

- To determine the prevalence of cercarial infection in intermediate host snails inhabiting Omo-Gibe river basin.
- To identify the type of trematodes cercariae infecting the intermediate host snails.
- To determine factors affecting the cercarial infection rate of freshwater snails.
- To evaluate the effect of *B.pfeifferi* snail shell size on cercarial infection intensity.

Chapter Four: Methods and Materials

4.1 Study area

The study was conducted in Omo-Gibe river basin South West Ethiopia from March to May 2016. The area is situated between latitude of $4^0$25'51.611" - $9^0$22'28.047" N and longitude of $33^0$0'24.434-$38^0$24'42.242" E. The Omo-Gibe river basin is estimated to cover 79561.2km$^{2.}$ ASTER DEM imagery shows the elevation of the river basin found between 500 and 3000 m.a.s.l, with 57% of the total area below 1500 m.a.s.l. Based on DEM estimates the water shade has four Agro-ecological zone: Kola (38%), Weyna Dega (37%), Bereha (19%) and Dega (6%). The Omo basin is the second largest river system accounting for 14% of Ethiopia's annual run off volume (Awulachew *et al*., 2007). The whole character of the basin is topography dependent, rising as it does from 500 m.a.s.l around Lake Rudolph to over 3000 m.a.s.l around Bako in North. Soils, climate, drainage, rainfall, vegetation, agriculture and human activity differ accordingly and the Master plan study is covering all the resources and potential, hydropower, fisheries, minerals, wild life, demography and transport, forestry linked with the complex web of traditional social and agricultural practices.

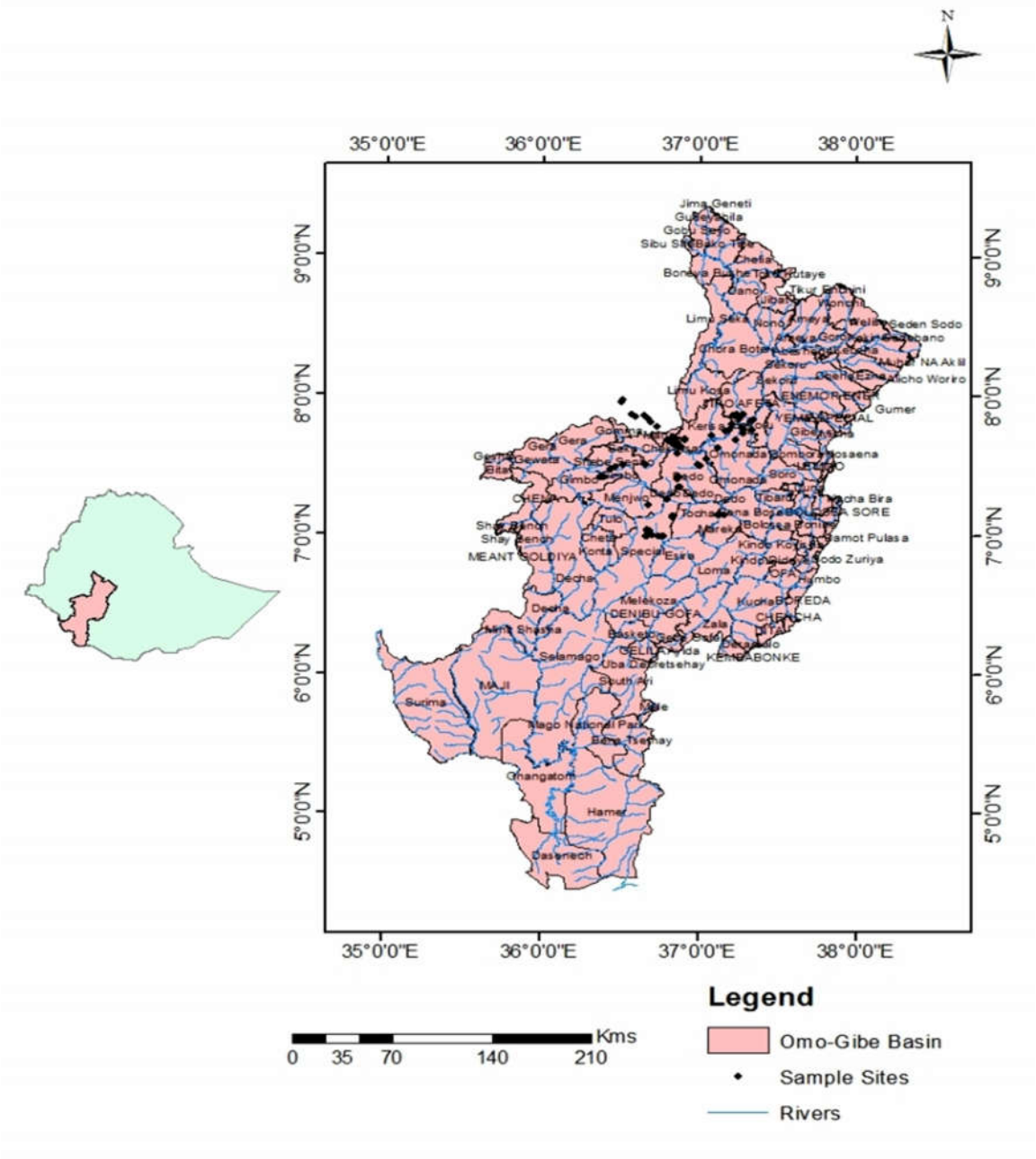


Figure 3 Map of Omo-Gibe river basin-May, 2016

4.2 Study design and period

A cross sectional study was conducted between March to May 2016.

4.3 Sampling locations and frquency

A total of 130 sites of 69 water bodies were sampled once during dry season: This includes fifty-five Rivers, two lakes, one irrigation canal, six wetlands, four streams, and 1 dam. Habitates were selected based on land use type, altitude and accessibility (Mohammed *et al*., 2016).

4.4 Data collection

4.4.1 Snail collection

Snail sampling conducted by trained person searching all probable habitat including under stone and log crevices, on the leaves and surface of leave debris for 60 minutes for each sampling site using handled metallic 2mm mesh size scoop aided by iron frame (Kariuki *et a*l., 2004) by passing scoop through water surface and vegetation on a bank and depth of the water body (Hussien *et al*., 2011). Using the wire mesh dragging the surface of water bodies mid part, the border and near to the bottom, shaking vegetation towards the scoop net and a tray to detach the snail from the substrate. Also the snail separated from attached aquatic plant and substrates by forceps and picking by gloved hands. After collection from each site the recovered sample inserted in pre labeled plastic bucket without lid - (Barkia *et al*., 2014), which were filled to its half with water and natural vegetation from each sampling site- (Punjab *et al*., 2011). The non-targeted organism returned to their habitat. After the whole sampling finished the content was taken to Jimma University laboratory of Environmental Health, then snails washed with water to remove algae, mud and other remains of substances from the shell of the snail to perceive the real shell color of the species (Afshan, *et al*, 2013).

In the laboratory the recovered individual snails of each species enumerated for that specified site of each water body (Hussien *et al*, 2011) and identified morphologically using (Mandahl-Barth, 1962) key and (WHO, 2010) snail identification guide then maintained in plastic container containing tape water. The water added in the plastic container was kept minimum for two days to evaporated the chlorine gas (Jayawardena *et al*., 2010). The holding capacity of the plastic container were according to the number of snails collected from each sampling site were, ranged from 2 liters to 10 liters. The size and direction of the opening, number of shell coiling, shape of

the shell and direction of twisting of the shell was taken as morphologic characteristics used for identification of snail species (Mandahl-Barth, 1962).

Snails from each site maintained by providing fresh lettuce and spinach kept in circular plastic container containing aged tape water which was changed daily and the container located at a room temperature. During changing of aged tape water the plastic container washed repeatedly to remove debris and feces of the snail. Also the snails rinsed with water and the dead snails removed from the container if present (Mohammed *et al*., 2016). After the snails acclimatized for one day, exposed to natural light for four hours to initiate cercarial shedding (Tigga *et al*., 2014). For shedding, snails were placed in Petri-dish and examined repeatedly for emerged cercariae by naked eye occasionally using hand lens and dissecting microscope. For cercariae positive petri-dish re-examination of the petridish and the cercariae for identification using microscope. The infected snails inserted in a separate beaker which is also separated according to the surveyed site. The snails that did not shade cercariae maintained in a plastic container in the laboratory and re-exposed to light once a week for a month (Mohammed *et a*l., 2016).

4.4.2 Examination of snails for prevalence

The surveyed snails were segregated in to species and grouped basd on sites. Each species was then placed in to aged tape water filled lower layer of a glass petri dish then exposed to natural light. In a non-sunny day, petri dish containing the surveyed snail located near 100 Watt artificial light (Tigga *et al*., 2014) and examined for cercarial release. The snails tested after one day of survey and weekly for one month because diagnosis is only possible after the larva of the parasite complete its development in the intermediate host snail for pre-patent infection. The Petri-dish plate height was 2.5cm and diameter of 9.5cm and the snails located individually and in group of twenty and exposed for direct natural light or artificial light during cloudy day for four hours to stimulate cercarial release. Then the water sample was checked frequently for the presence of trematode cercariae (Ahmed *et al*., 2006). If any Petri dish confirmed to contains trematode parasite, the snails of the petri dish in group subsequently transferred to individual Petri dish to identify the snail that release the parasite and the number of snail shedding cercarie recorded (Opisa, *et al.,* 2011). The individual petri-dish released cercariae observed using dissecting microscope for position and swimming property. Then the water was taken using dropper pipette, stained with iodine solution to look the morphology of the trematode parasite

using compound microscope (Devkota *et al*., 2008). Through the compound microscope the photo of cercariae was taken by smart phone fitting the eye lens of microscope then cercariae were identified to genus level based on (Zeitschrift *et al*., 2015) key. Then the infected snails transferred to another beaker, labeled separately for each collected site and preserved with 97% alcohol for latter investigation. The recovered cercariae stained with two to three drop of iodine solution and counted totally using dissecting microscope after 15 minute of staining. After exposure for four hours the snails with no cercariae shedding was returned to maintaining container and re-examined weekly (Grimes *et al*., 2015). All the information was recorded on the prepared data sheet. The infection rate or the prevalence of snail infection explained by using the number of infected snails divided by the total number of snails examined (Jayawardena *et al*., 2010).

4.4.3 Observation and Identification of cercariae

Water in each petri-dish plate of infected snail was examined for cercariae and isolated. Before staining the drop of infected water, determination of position of resting and swimming property of the cercariae was tried using dissecting microscope. Then the drop of water transferred on to slide and stained with one to two drop of iodine solution and inspected under compound microscope for identification. To foster the contamination, the dropper was rinsed with clean water repeatedly. For double infection, the presence of more than one identified cercarial morphology from the specified snail was checked by staining the drop of water (from petri-dish containing infected snail) more than one time using glass slide and a petri-dish. Also individual who has related professional practice was consulted. Eventually, the remaining water specimen containing cercariae were preserved with 10% formalin using plastic beaker for further identification (Tigga *et al*., 2014). The main identification characteristics used includes 'tegument, body sucker, cercarial tail and general anatomical appearance' (Zeitschrift *et al.*, 2015).

4.4.4 Intensity and size of infected *B.pfeifferi* snails

The *B.pfeifferi* snails were exposed for light twice. First to test the presence of cercariae in the intermediate host snail, secondly to measure the intensity of each infected snail. After checking the snail released cercariae, immediately the snail transferred in to another petri-dish having 20ml holding capacity. The infected snail was exposed to light in Petri-dish containing 10ml of

chlorine free water for 1 hour. Then the total numer of *B.pfeifferi* a Petri-dish counted totally. All petri-dishes holding the infected snail had the same size and contains similar amount of chlorine free water in it for each exposure. The snail in the Petri-dish removed after 1h of exposure, the water in it examined using dissecting microscope after stained with iodine solution without transferring in to another beaker. Then the total number of specimen in the Petri-dish counted, repeating three times in order to estimate the specimen number correctly. The counting and intensity was determined for each infected *B.pfeifferi* snail species. The intensity of infection was obtained as, the average count of cercariae released per 1hour duration. To know the shell size of infected *B.pfeifferi* snail species, the shell was measured three times to estimatethe correct size using tape meter then the average of nearest 0.1mm was taken as the size (Graham, 2003). The diameter was taken as their shell size (measure from the margin of the external lip of the snail to the side parallel to it) (Mandahl-Barth, 1962).

4.4.5 Environmental data

In situ measurement was carried out for water temperature, dissolved oxygen, conductivity and pH using multi-probe meter (HQ30d single input multi-parameter digital meter Hach). Turbidity was measured using turbidity meter. Water depth (cm) was measured using calibrated metallic meter. The water sample was taken from each snail sampling site before the start of scooping and hand picking snail by using a plastic beaker. The plastic beaker rinsed repeatedly with water from the site then dipped and filled with water and measured using multi-probe meter. Observation of the general ecology of the sampling site was undertaken, like washing, bathing and swimming, waste dumping, grazing, farming and the presence of settlement area (Rowel *et al*., 2015). The observation was for presence absence and if all the activities and grazing observed with in one hundred meter distance of sampling were recorded as present. From the observation in the study indicated that more than 70% of snail collected sites were manipulated by human and animal activities like washing, farming, grazing, settlment and bathing. Swimming and waste dumping occur less frequently relative to the other human activities this could raise the transmission of trematode cercariae thus, human water use and contact behavior is the determainig factor for the transmission of trematode cercarial parasite to huan as well as to infect the intermediate host snail (Grimes *et al*., 2015).

4.5 Variables

Independent variables

- Habitat condition
- Water quality
- Disterbance factors
- Intermediate host snail related factors

Dependent variables

- Prevalence of cercarial infection
- Intensity of *B.pfeifferi* infection

4.6 Data processing and analysis

Data on data sheets were transferred in to Microsoft excel. Excel data transferred to SPSS.Then the data was compiled and analyzed by both descriptive and inferential statistics. Prevalence of infection was estimated and illustrated by table and graphs using descriptive statistics. In generalized linear model (GLM), ANCOVA was carried out by using SPSS version 20 to determine factors affecting the prevalence of snail infection. To evaluate the effect of *B.pfeifferi* snail shell size on intensity of infection Simple Linear Regression was applied. For both ANCOVA and Linear Regression the assumptions were considered. The p value and 95% confidence interval were calculated for explanatory factors. $P \leq 0.05$ was taken as significant. Snail count, morphological characteristics of snails and cercariae were expressed by descriptive statistics. The prevalence of cercarial infection calculated as the number of individual infected snails divided by the total number of screened snails for cercarial shading and multiplying by 100 to present in percentage (Tigga *et al*., 2014).

4.7 Data quality

Before commencement of the study equipment and material tested for reliability and accuracy by carrying out pilot study. Sampling equipment was rinsed with tap water to avoid contamination. To prevent personal bias related professional was participated and consulted for identification of snail species and cercarial type using compound microscope. Data collection protocol was used during data collection and laboratory work and data sheet was used during laboratory activity. The data collection protocol was cross checked for its completeness after the survey.

4.8 Ethical consideration

Permission letter was obtained from JU collage of health Sciences Department of Environmental Health Science and Technology for conducting the study. From the administration of tourism office of SNNPR permission paper was obtained for surveying Chebera-churchura national park. Throughout the whole survey in the national park, rules and regulation of the park were strictly respected. The non-targeted organisms were returned to the water considering the impact on the aquatic life. Access to private land owner was obtained through verbal agreement.

4.9 Limitation of the study

This study was based on dry season data, may not represent the wet season. It was a one time crossectional study.

4.10 Definition of terms

Cercariae: larval stage of worms of a class trematodes parasite which is infective and free swimming.

Parasite: A unicellular or multi cellular organism that obtain some or all of basic nutritional need through depending on other living organisms and can have effect on the host.

Intermediate host: The organism in which the parasite survives during developmental period only or carries disease causing parasite.

Host: The living organism in or on which the parasite lives and results harm.

Trematodes: The non-segmented parasitic worms causing disease in humans and animals.

4.11 Dissemination of the study

The finding of this study will be submitted to Jimma University, collage of Health Science, Department of Environmental Health Science and Technology, Oromiya Regional Health bureau and SNNPR Health bureau. The finding also disseminated to stakeholders, who have a stake in *Schistosomiasis* prevention and control. Eventually effort will be made to present in different workshops and for publication in international journal.

Chapter Five: Result

5.1. Intermediate Snail Host

A total of 3,044 snails belonging *Planorbidea* and *Lymnaeidea* families and five species were collected from 69 freshwater bodies of Omo-Gibe river basin Southwest Ethiopia. In a river habitat all types of snail species collected in the study was recorded and 76% of the total gathered snails were addressed by the river. Following the river habitat relatively the diversified snail species were notice in wetland habitat covering 10% of the entire snail number. The least number of snails collected was in Lake habitats. No snails were collected from dam (Table 1).

Table 1 Distribution of snail species in different types of water bodies in Omo-Gibe river basin-May, 2016

Habitat type	Number of habitats	Number of sites	Snail positive sites	% Positive sites	Relative abundance	% Relative abundance
Lake	2	10	4	40	55	2
Wetland	6	24	14	58	298	10
Dam	1	2	-	0	-	0
Irrigation ditch	1	2	2	100	104	3
Stream	4	4	2	50	271	9
River	55	88	31	35	2316	76

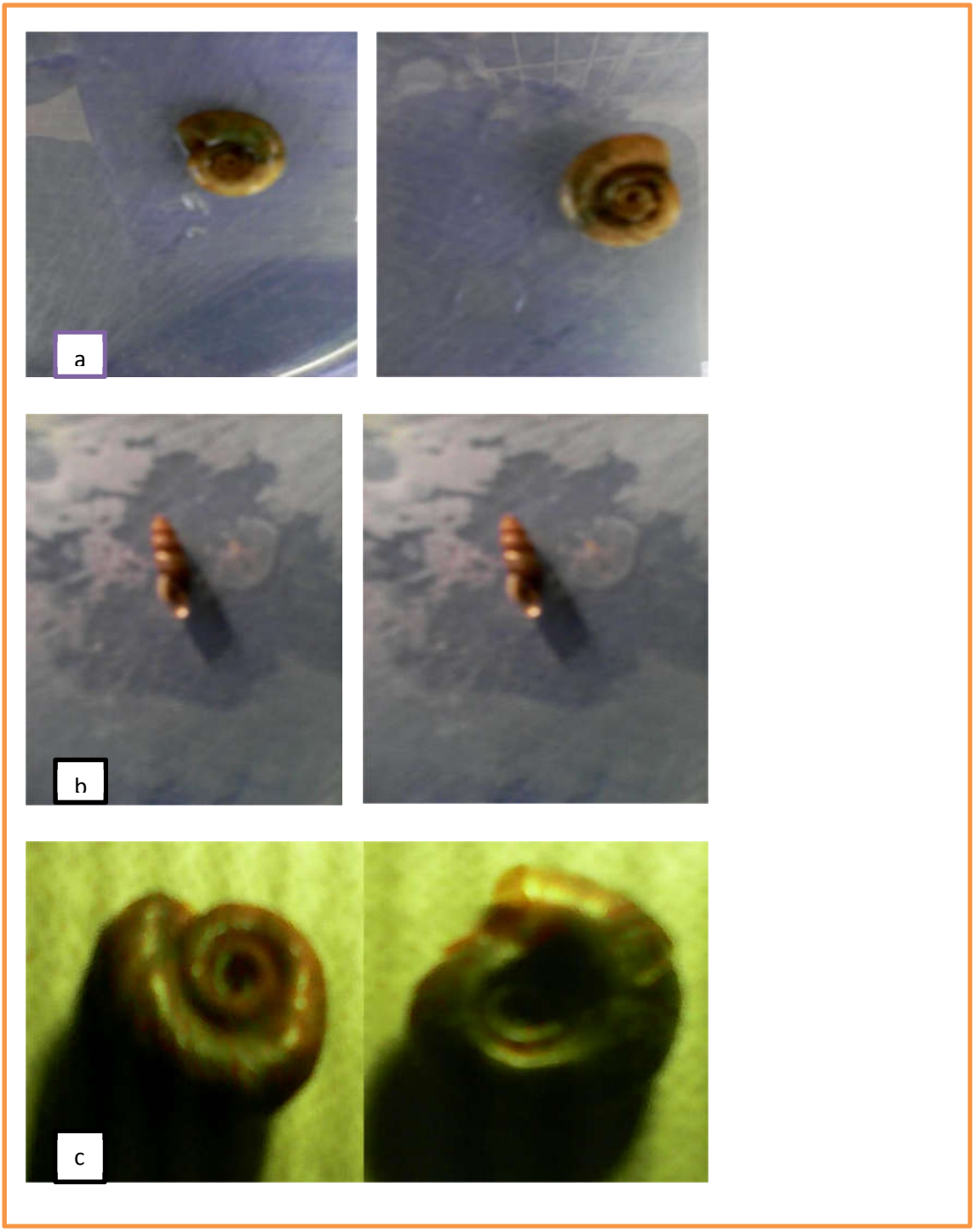
Among the freshwater snails *B.pfeifferi*, *L.natalensis* and *B.globosus* were found in 39%, 29% and 17% of sampling sites respectively. In addition *B.pfeifferi* the intermediate host of *S.mansoni* has the highest abundance; contributed to 66% of total snail population. *B.sudanica* species were found only in a single study site i.e in Merry River along Chebera-churchura national park. *B.forskalii* the intermediate host of *S.haematobium* was found in five sites: Merry river in Amaya, in Asendabo (Yedi) river, in Jimma town (Haro and Bore wetland and Dololo river) (Table 2).

Table 2 Abundace of freshwater snails among water bodies in Omo-Gibe River basin- May, 2016

Water body	Snail species					
	B.pfeifferi	*B.sudanica*	*B.globosus*	*B.forskalii*	*L.natalensis*	Total
Chebera irrigation	29	0	0	0	75	104
Shoshuma river	4	0	0	0	25	29
Merry river	11	7		10	165	193
Nech-wuha stream	0	0	0	0	9	9
Kerebela lake	0	0	30	0	25	55
Seto-semero stream	222	0	0	0	36	258
Awetu wetland	0	0	3	0	33	36
Kitto-furdisa wetland	55	0	16	0	105	176
Langebo river	1330	0	16	0	28	1374
Kebela river	140	0	42	0	36	218
Arer river	5	0	0	0	1	6
Chilelo river	17	0	7	0	0	24
Bore wetland	0	0	1	7	3	11
Lotte river	59	0	0	0	36	95
Yedi river	0	0	0	104	0	104

Dololo river	1	0	0	1	33	35
Chore river	0	0	2	0	21	23
Boye wetland	0	0	0	0	20	20
Chefea river	1	0	0	0	11	12
Haro wetland	0	0	1	11	2	14
Shakta river	0	0	0	0	12	12
Bodiro river	4	0	0	0	1	5
Gibea-boy river	2	0	28	0	1	31
Shonkore river	7	0	0	0	0	7
Gulfa river	0	0	0	0	11	11
Chefea-abadega river	62	0	0	0	0	62
Kitto wetland	18	0	2	0	21	41
Umech river	43	0	0	0	21	64
Relative abundance	2010	7	148	133	747	3044
% Relative abundance	66	0.3	4.9	4.3	24.5	100
Frequency of occurrence (%)	30	0.77	13.1	3.85	22.3	53

All snails collected from the study site were identified as *Lymnea natalensis*, *Biomphalaria pfeifferi, Biomphalaria sudanica, Bulinus globosus* and *Bulinus forskalii* snail species (Fig 2). *L.natalensis, B.pfeifferi and B.globosus* were common at the study area.



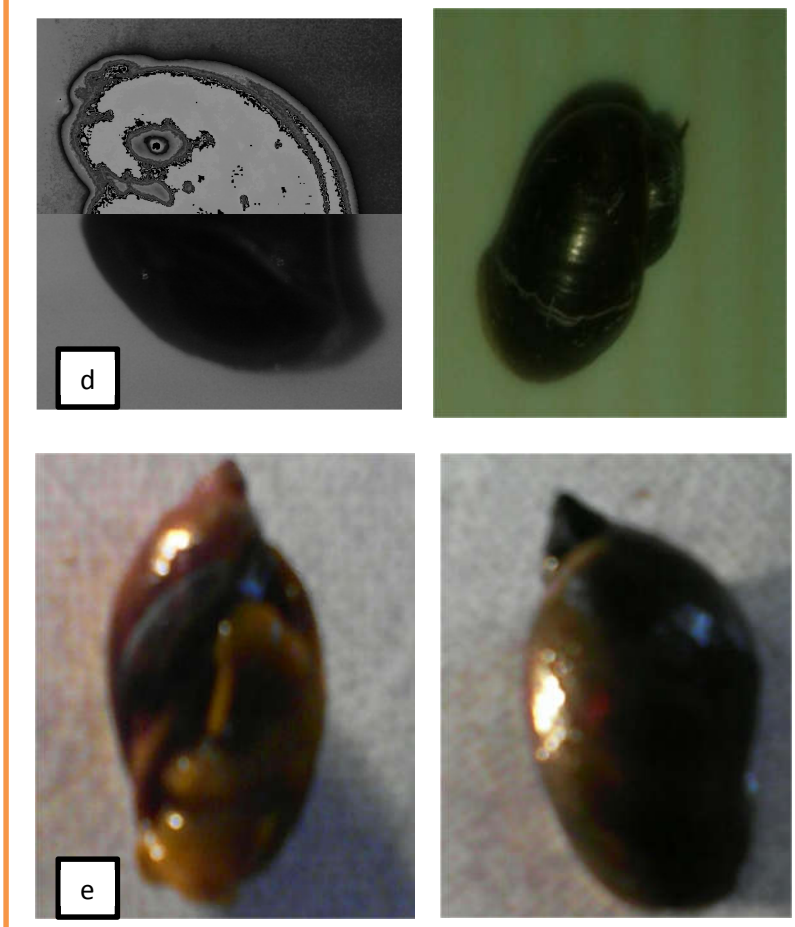


Figure 4 Morphologically identified freshwater snails of Omo-Gibe river basin-May, 2016

The types are a, *B.sudanica* b, *B.forskalii* c, *B.pfeifferi* d, *B.globosus* e, *L.natalensis*

L.natalensis species were the frequently observed snail next to *B.pfeifferi* snails from the study area. The least observed snail species from the collected were *B.sudanica* snails only found in river. All the five snail species were found abundantly in river habitat. Next to the river wetland were preferred by four snail species. Only *L.natalensis* and *B.globosus* were collected in Lake Habitat (Fig 5). These relative high existed snails in different water bodies may enhance the transmision of trematode parasite to definitive host including in humans.

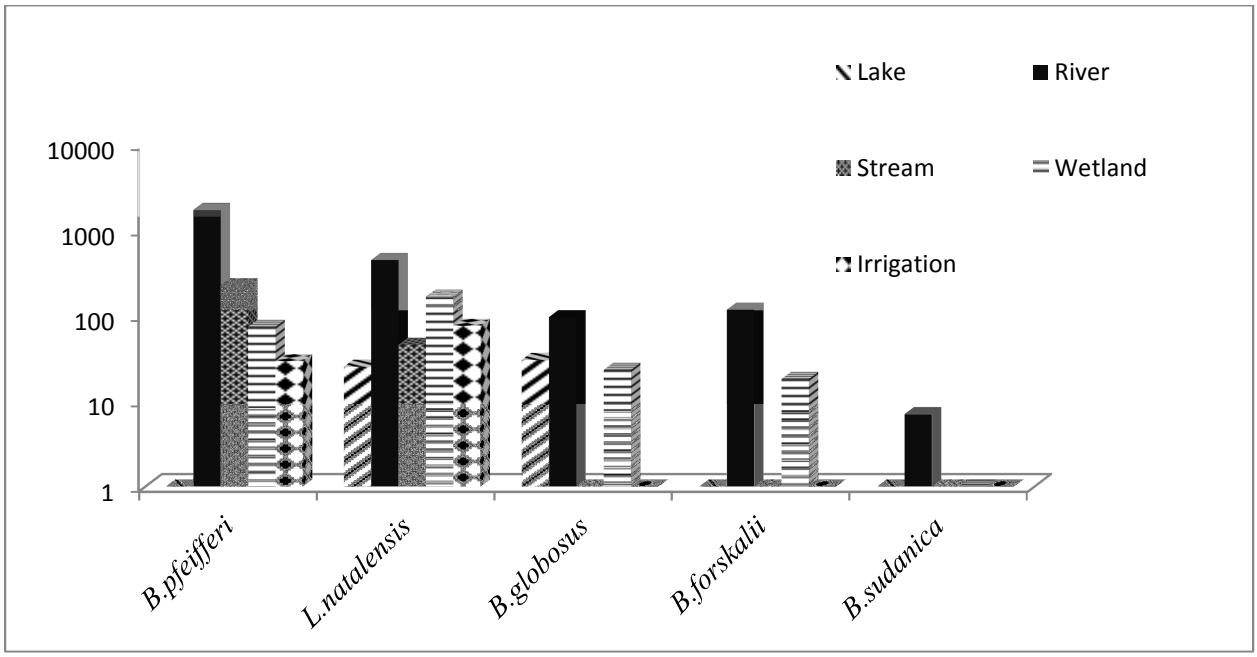


Figure 5 Relative abundance of medically important freshwater snails in different habitat type in Omo-Gibe river basin-May, 2016

5.2 Types and Prevalence of Cercarial Infection of Freshwater Snails

A total of 30 sampling sites (23%) were infected by cercariae of trematode parasites. Of 3,044 snails collected, 109 of them released one or more types of cercariae. Four of five snail species surveyed: *B.pfeiferi, B.globosus*, *B.forskalii* and *L.natalensis* were shed cercariae. No infections were registered in *B.sudanica* species. The cercarial type considered were *Echinostome*, *BAD*, *BAM*, *Xiphidiocercaria*, *Amphistome* cercariae, *Metacercariae* and three un-identified type of cercariae from which two were furcocercous group of cercariae. The cercariae were classified in to individual groups based on morphologically differentiable structure according to (Zeitschrift *et al*., 2015).

5.2.1 Types of cercarial infections

A total of seven different types of cercariae observed from this study

Echinostome cercariae were shedded by *B.pfeifferi, B.globosus* and *L.natalensis. Brivifurcate apharyngeate diastome cercariae* and *Brivifurcate apharyngeate monostome cercariae* were also shedded by *B.pfeifferi. Xiphidiocercariae* were released by *L.natalensis. Amphistome cercariae* were shedded by *B.pfeifferi* and *B.forskalii. Metacercariae* were also shedded by *B.pfeifferi.* Detailed description of the different cercariae types are given in Annex 1.

5.2.2 Prevalence of Cercarial infection in Freshwater snails

The prevalence of cercariae infection was varied from 0.04 to 1.15% giving the total prevalence 4.06%. The highest cercarial infection was recorded from Seto-semero stream in Jimma town which accounted 28.5% of overall infection in the study area (Table 3). This water body also provide habitat for diverse cercarial *fauna* (five types of cercariae). The types of cercariae harvested from snails of this water body were *Echinostome*, *BAD*, *Amphistome*, *Metacercariae* and un-identified single tail cercariae. *Amphistome, Echinostome* and *BAD* cercariae were the most commonly encountered cercarial type in this stream. The highest infection rate was followed by Langebo River (17.5%) in Dimtu town and Umech River (10 %) in Gojeb town along the main road of Bonga town. *BAD* cercariae were dominant in Umech River but in Langebo River it was the second dominant next to *Echinostome* cercariae.

In the current study *BAD* cercariae was the most common registered type of cercariae which covered 35.14% of all recovered cercarial type where all the identified *BAD* cercariae were human *Schistosome* cercaria (Zeitschrift, *et al.,* 2015). The most frequently observed *BAD* were recorded from Chilelu River (near to Deneba town) which covered 23 % of the whole reported *BAD.* This may indicate the area is highly affected by the transmison of *Schistosome* parasite relative to other water body in this study. The over all prevalence of infection in the area was 0.4%. Although this number is small the burden caused by the parasite might be large. The prevalence of other infected water bodies shown in (Table 2). No infection was recorded from Shoshuma, Dololo, Chefea, Chore, Shakta, Bodiro, Gibe-boi, Shenkore, Shata and Gulfa rivers; and also Haro and Boye wetlands were negative for the infection. Almost 54% of infection was observed in rivers followe by stream (29.3%) and wetlands (15.7%). The least infection was observed in Lakes (0.98%).

Table 3 Cercarial infection of freshwater bodies in Omo-Gibe River basin-May, 2016

Water body	**Infected snail spp**	**Number of infected snail**	**Types of cercariae**								**Infection rate %**
			Ech	*Bad*	*Xip*	*Amp*	*Met*	*Bam*	Un-idenf	Un-idsing	
Merry river	*L.natalensis*	2	2	-	-	-	-	-	-	-	0.07
Kerebela lake	*L.natalensis*	1	-	-	-	-	-	-	1	-	0.04
Nech-wuha stream	*L.natalensis*	1	1	-	-	-	-	-	-	-	0.04
Langebo river	*B.pfeifferi*	19	12	7	-	-	-	-	1	-	0.71
Kebela river	*B.pfeifferi* *B.globosus*	6	4	1	-	-	-	1	1	-	0.22
Seto-semero stream	*B.pfeifferi*	31	6	5	-	17	2	-	-	1	1.15
Awetu wetland	*L.natalensis*	1	-	-	1	-	-	-	-	-	0.04
Kitto-furdisa wetland	*B.pfeifferi* *B.globosus* *Lnatalensis*	5	3	2	-	-	-	-	-	-	0.19
Bore wetland	*B.forskalii*	1	-	-	-	1	-	-	-	-	0.04
Kitto-	*B.pfeifferi*	10	3	1	1	2	3	-	-	-	0.37

wetland	*L.natalensis*										
Lotte river	*B.pfeifferi* *L.natalensis*	3	2	-	1	-	-	-	-	-	0.11
Yedi river	*B.forskalii*	1	-	-	-	1	-	-	-	-	0.04
Chilelu river	*B.pfeifferi*	10	1	9	-	-	-	-	-	-	0.37
Arer river	*B.pfeifferi*	2	1	1	-	-	-	-	-	-	0.07
Chefea-abadega river	*B.pfeifferi*	5	-	5	-	-	-	-	-	-	0.19
Umech river	*B.pfeifferi*	11	3	8	-	-	-	-	-	-	0.41
Total		109	38	39	3	21	5	1	3	1	4.06

Figure 6 Morphology of *BAD* (human *Schistosome cercariae*) in Omo-Gibe river basin-May, 2016

In this study *B.pfeifferi* was the most common infected snail species accounting (85%) of the total infected snails, followed by *L.natalensis* (10%) and *B.globosus* (3%). *B.forskalii* was the least infected snail species covering 2% (Table 3). *B.pfeifferi* snails were susceptible for various cercarial infections. All the identified cercariae in this study area except *Xiphidiocercariae* were recovered from this snail species. Over all ninety three *B.pfeifferi* snails were infected. These show that *B.pfeifferi* snails were the most susceptible snail species in the study area.

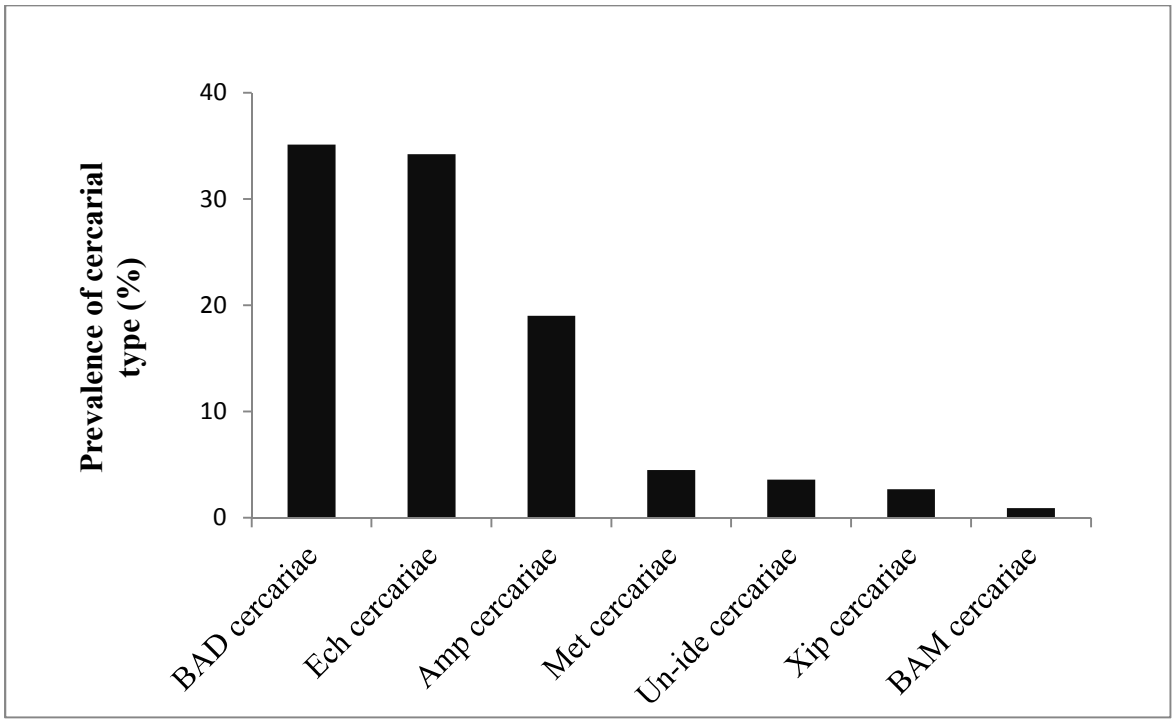


Figure 7 Proportions of cercarial infection in freshwater snails in Omo-Gibe river basin-May, 2016

Echinostome cercaria*e* were the frequently observed type of cercariae which covered *34% of* infection next to *BAD* (*35%).* Then *Amphistome, Metacercariae and Xiphidiocercariae* were followed accounting 19%, 4.5% and 2.7% of infections respectively. The un-identified cercariae accounted (3.6%) which were morphologically distinict and composed of furcocercous and single tail cercariae types. *BAM* cercariae hold the least (1%) (Figure 7) The *Echinostome* cercariae infect *B.pfeifferi*, *B.globosus* and *L.natalensis* snail species and that of *Amphistome* cercariae infection were found in *B.pfeifferi* and *B.forskalii* snail species. The rest identified cercariae types were recorded in only one snail species. Two *B.pfeifferi* snail species were

infected by two cercariae at the same time: *Amphistome* and un-identified single tail cercariae in one of the snail and in the other *BAD* and un-identified furcocercous cercariae (Table 3).

Table 4 The prevalence of trematode cercariae infections registered in four different snail species collected from Omo-Gibe river basin-May, 2016

Snail species	**Total no of snails collected**	**Total no of snails examined**	**Total no of snails infected**	**No of snails infected by cercariae**								**Infection rate %**
				Ech	Bad	Bam	Amp	Xip	Met	Unfu	Unsi	
B. pfeifferi	2010	1729	93	28	39	1	19	-	5	2	1	3.46
B.sudanica	7	7	-	-	-	-	-	-	-	-	-	-
B. globosus	148	148	3	3	-	-	-	-	-	-	-	0.1
B. forskalii	133	133	2	-	-	-	2	-	-	-	-	0.07
L. natalensis	747	667	11	7	-	-	-	3	-	1	-	0,41
Total	3044	2684	109	38	39	1	21	3	5	3	1	4.06

5.3 Factors affecting prevalence of snail infection

Univarate analyisis of variance had been conducted to determine the effect of factors on the prevalence of snail infection. In this analysis the factorial ANCOVA F-test, tests the hypothesis that the mean score of the explanatory factors are equal. The out come of the final univarate analysis showed that temperature, turbidity, waste dumping, washing and swimming were associated with the prevalence of snail infection. Prevalence of infection positively associated with water temperature F(1,53) = 11.076; p=0.02 CI 95%, (0.026,0.103) and turbidity F(1,53) =7.306, p =0.009 CI (0.001,0.006) and had negative association with washing F(1,53) =9.822 p = 0.03; 95% CI (-0.907,-0.199), and swimming F (1,53) = 4.302, p= 0.043 ; 95% CI(-0.49,-0.008).

Table 5 The arthimatic mean of physicochemical factors and depth of water along Omo-Gibe river basin-May, 2016

	Water temperature (^{0}C)	DO%	DO(mg/l)	pH	EC(µS/cm)	Turbidity (NTU)	Water depth(m)
N	52	52	52	52	52	52	52
Mean	24.7023	57.7827	3.9398	7.1787	188.63	60.4144	.3832
Minimum	20.34	33.00	2.36	5.26	42	1.50	.06
Maximum	30.22	96.40	5.85	9.26	423	545.00	1.30

Table 6 Levene's Test of Equality of Error Variance for dependent variable prevalence of infection (%) in Omo-Gibe river basin-May, 2016

F	df1	df2	Sig.
2.119	97	7	.147

Tests the null hypothesis that the error variance of the dependent variable is equal across groups as we can see the test is not significant (p = 0.147), thus we can accept the null hypothesis error

variance are homogenous. So this did not violate the assumption and we can preceed the next test.

As shown in the (Table 7) Dissolved oxygen, pH, EC, water depth, habitate type, snail species, and types of cercariae released, animal grazing, farming, bathing and settlement had no significant influence on the prevalence of snail infection.

Table 7 Test between subject effects using ANCOVA for prevalence of infection (%) in Omo-Gibe river basin-May, 2016

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncen. Parameter	Observed Power[b]
Corrected Model	3.786[a]	51	.074	8.254	.000	.888	420.935	1.000
Intercept	.038	1	.038	4.171	.046	.073	4.171	.518
Temperature	.100	1	.100	11.076	.002	.173	11.076	.904
DO	.024	1	.024	2.641	.110	.047	2.641	.358
PH	.000	1	.000	.046	.831	.001	.046	.055
EC	.001	1	.001	.097	.757	.002	.097	.061
Turbidity	.066	1	.066	7.306	.009	.121	7.306	.756
Waterdepthm	.007	1	.007	.792	.377	.015	.792	.141
SSpecies	.009	4	.002	.254	.906	.019	1.017	.101
Habitat	.000	0	.	.	.	.000	.000	.
Sites	.848	22	.039	4.284	.000	.640	94.240	1.000
TypeCS	.044	7	.006	.705	.668	.085	4.936	.274
Grazing	.017	1	.017	1.836	.181	.033	1.836	.265
Farming	.021	1	.021	2.312	.134	.042	2.312	.321
wastedumping	.040	1	.040	4.497	.039	.078	4.497	.549
Washing	.088	1	.088	9.822	.003	.156	9.822	.868
Bathing	.006	1	.006	.703	.406	.013	.703	.130
Swimming	.039	1	.039	4.302	.043	.075	4.302	.531
Settlement	.005	1	.005	.572	.453	.011	.572	.115
Carwashing	.067	1	.067	7.425	.099	.123	7.425	.763
Error	.477	53	.009					
Total	5.811	105						
Corrected Total	4.263	104						

a. R Squared = .888 (Adjusted R Squared = .781)

b. Computed using alpha = .05

5.4 Evaluation of the effect of *B.pfeifferi* snail shell size on infection intensity

Simple Linear regression analyses were conducted to investigate the relationship between the size of *B.pfeifferi* snail shell and its intensity of infection. First the correlation was identified for the size and intensity variables using correlation analyses which resulted (r = 0.747). This indicated that the two variables were correlated (Table 9).

Table 8 Discriptive stastistics using Simple Linear Regression in Omo-Gibe river basin-May, 2016

	Mean	Std. Deviation	N
Intensity of infection in *B.pfeifferi*	39.27	75.718	52
Average size of infected *B.pfeifferi* (mm)	3.63	5.354	52

Table 9 Model summary using Simple Linear regression in Omo-Gibe river basin-May, 2016

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.747	.558	.549	50.860

a, Predictors: (Constant), Average size of infected *B.pfeifferi*

b, Dependant Variable : Intensity of nfection in *B.pfeifferi*

The linear regression F-test has the null hypothesis that there is no linear relationship between the shell size of *B.pfeifferi* snail and its intensity of infection ($R^2 = 0$). With F= 63.036 and 51 degree of freedom, the test is highly significant ($p < 0.001$), thus we can assume that there is a linear relation ship between the variables in this model (Table 9).

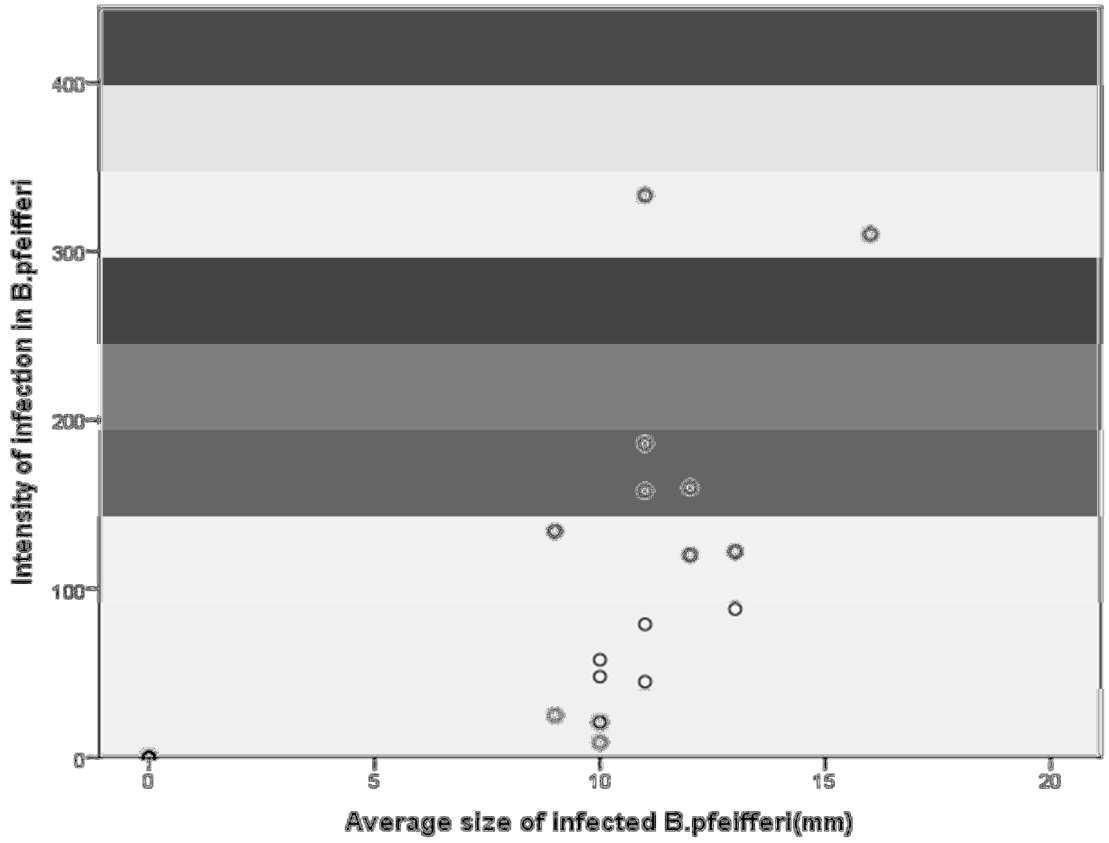


Figure 8 Intensity of infection in *B.pfeifferi* snail (The average number of cercariae released/h by *B.pfeifferi* species) in Omo-Gibe river basin-May, 2016

Table 10 F-test using Simple Linear Regression in Omo-Gibe river basin-May, 2016

Model	Sum of Squares	Df	Mean Square	F	Sig
Regression	163055.921	1	163055.921	63.036	P < 0.0001
Residual	129336.310	50	2586.726		
Total	292392.231	51			

a, Dependent variable : Intensity of infection in *B.pfeifferi*

b, Prridictor (Constant) : The average size of infeted *B.pfeifferi*

The intercept and the significance of all regression coefficient observed in the model (Table 10). Simple linear regression analysis estimates the linear regression function to be Y=.886+10.561*X. This means that an increase one unit of x (shell size of *B.pfeifferi* snail) results increase 10.561 unit y (intensity of infection). The test of significance of linear regression analysis tests the null hypothesis that the estimated coefficient is zero. But the t-test in this study indicated that the variable is highly significant ($p < 0.001$) and thus we could say they are significantly different from zero.

Table 11 Regression coefficients using Simple Linear Regression in Omo-Gibe river basin-May, 2016

Models	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% CI for B	
	B	Std. Error	Beta			upper	lower
Constant	.886	8.551		.104	.918	-16.289	18.061
Average size of infected *B.pfeifferi* (mm)	10.561	1.330	.747	7.939	.000	7.889	13.232

a. Dependent Variable : Intensity of infection in *B.pfeifferi*

The estimated regression model was intensity of *B.pfeifferi* infection = 0.886+10.561*shell size of infected *B.pfeiferi* snail with an adjested R^2 of 54.9%. The standard error of the estimate was 50.860. Besides the positive relationship it can be concluded that for every additional infected shell size of *B.pfeifferi* snail the intensity of infection would raise approxmatly by 10.561 units.

Chapter Six: Discussion

In the current study Omo-Gibe river basin considered to be an important habitat for five feshwater snail species. *B.pfeifferi* were the dominant species which covered (66%) of the total collected snails. Mohammed *et al*., 2016 also reported *B.pfeifferi* was the most abundant species composing 48.6% of the total collected samples. Such local distribution of intermediate host snails have been observed by (Alebie *et al*., 2014) in Amhara region Ethiopia, reported *B.forskalii*, *B.pfeifferi* and *L.natalensis* in their epidemiological study on *S.mansoni* infection. They reported abundance variation in *B.pfeifferi* species in Maho Stream and Sanja River was due to the difference in water flow velocity and vegetation coverage of the water bodies.

However the present study indicated that the distribution and relative snail abundance of particular snail species observed in Omo-Gibe river basin (Table 1) could be due to association with substratum rich organic matter in the area which provides support for aquatic plants giving protection, egg laying site and food for snail population. The variation was also due to the natural responsive adjustment which is different for different species (Isaac, 2009). Another possibility could be the depth of water bodies which had indirect relation with oxygen content that lakes in this study were relatively deeper than other water bodies (Rowel *et al*., 2015). Next to *B.pfeifferi*, *L.natalensis* which is the common intermediate host of liver fluke was dominant in the study area (Ayana & Waktole, 2013).

The abundance of appropriate snail intermediate host with intensive human activities in an area enhance the risk of transmission of trematode parasitic disease (Asma *et al*., 2015). High frequency of anthropogenic activities was observed from the study site. These human and animal water contact behavior play a significant role in the transmission of trematode parasite. Where, its impact could be perceived in two angles. The occupational or recreational activities in and near water bodies related to contaminating the water with infected feaces or urine by defeacating in or near water bank which could enter in to water with flood and other agents. The human aquire this trematode parasite when they come in contact with infected water with cercariae of trematdes which released by intermediate host snail or by ingesting uncooked or partially cooked water vegetation, fiish, edible mollusk (for foreign countries) and others. The intermediate host snails were infected by miracidium emanated from egg of infected human or animal feaces. Infected domestic and wild animals contaminate the water by excrating their feaces in to the

water or carring infected animal or human feaces with their hoof to the water. And the animals aquire the parasite when grazing the water vegetation This human and animal infected water contact activites could be indication of transmission of trematode parasite in humans and animals (Grimes *et al.*, 2015).

The present report corresponds on the diversity of cercariae trematode parasites of the freshwater snails that recorded seven morphologically different types of trematode cercariae. This finding is higher when we compared to (Ahmed *et al*., 2006) in Sudan observed four types of cercariae and lower when we compared to (Mohammed *et al*., 2016) in Sudan suggested twenty different types of cercariae but similar with those of (Jayawardena *et al*., 2010), the study done in Sri Lanka regarding the number of cercariae recovered which reported eight morphologically different type of cercaraie. Of the four snail speies, *B.pfeifferi* was the highly infected snail species covering 85% of all infections. (Alebie *et al*., 2014) reported only infection of *B.pfeifferi* from the collected three snail species. This could be due to large number of *B.pfeifferi* in the area may cause the increament of *B.pfeifferi* infection (Isaac, 2009). The study revealed that some snail species are capable of acting as primary host for a number of trematode species. Noticeable heterogeneous group of cercariae were observed from *B.pfeifferi* snail species. Six cercarial types were identified in a single *B.pfeifferi* species. *Echinostome, Brivifurcate apharyngeate diastome*, *Brivifurcate apharyngeate monostome, Amphistome*, *Metacercariae* and un-identified cercariae were recovered from *B.pfeifferi* species in the current study. Two individual *B.pfeifferi* snails infected by two cercarial types in the same time in a single snail species This finding is in line with (Mohammed *et al*., 2016) in Sudan, he and his colleagues found nine types of cercariae in a single snail species and three snails infected by two types of cercariae at the same time. Based on these report we predicted that *B.pfeifferi* has high capability in propagating snail born disease like *Schistosomiasis*, *Echinostomiasis, Amphistomiasis* in humans and animals and also *BAM* cercariae cause disease in birds (Devkota *et al*., 2008).

L.natalensis was collected from most of the sampled area covering (25%) of the total collected snails. This species infected by three types of cercariae. *Echinostome*, *xiphidiocercariae* and un-identified furcocercous cercariae where as *B.globosus* species infected by only *Echinostome* cercariae and the *B.forskalii* species infected by *Amphistome* cercariae. This difference might be due to the genetic difference in resistance of some snail species for various trematode parasites

(Jamjoom & Banaja, 2007). And also this could be the implication of the presence of trematode parasite in the intermediate host snails by necessity increased by circumstances affecting the the growth and reproduction of the snail population (Jayawardena *et al*., 2010).

The *BAD* cercsariae were dominant cercarial types covering (35%) of the total infection (here all are identified as human *Schistosome* cercariae). This finding is lower when compared with (Mengistu et al., 2011b) in Jimma town Ethiopia that 58% schistosome cercariae were reported from *Biomphalaria* snail species. In their study all bifurcated cercariae released were perceived as *Schistosome* cercariae. The finding is higher when compared with (Rowel *et al*., 2015) in Uganda that 15.6% of infection was caused by *S.mansoni* and also higher when compared with (Mohammed *et al*., 2016) in Sudan where they reported only 1.6% of snails were infected by *S.mansoni*. But similar with (Ahmed *et al*., 2006) in Sudan that reported highest infection (63.7%) was resulted by *Schistosome* cercariae. The reason for high rate of *Schistosome* cercariae infection than other cercarial type in this study could be due to human high water contact behavior observed during data collection and might be due to most of *Biomphalaria pfeifferi* snail species infected by *Schistosome* miracidium (Alebie *et al*., 2014). *BAD* (*Schistosome*) crcariae followed by *Echinostome* (34.23%), *Amphistome* (19%) and *Metacercariae* (4.5%) and also the remaining covered by the other three types of cercaraie *Xiphidiocercariae* (2.7%), un-identified furcocercous cercariae (3.6%) and *BAM* cercariae (0.9%). The genus *Echinostome* cercariae are in family *Echinostomatidae* which are parasites of intestine causing ehinostomiasis in human and oral, respiratory and duodenal livestock disease. The disease result diarrhea, deficiency of red blood cells, loss of appitite, abdominal discomfort, intestinal and duodenal ulcer and dyspepsia in human (Graczyk & Fried, 1998). *Amphistome* cercariae (*Paramphistomatidae* family) can cause *Amphistomiasis* or *Paramphistomiasis* in humans and domestic animals more commonly cattle and sheep. But most paramphistomes are responsible in livestock animals and wild mamals (Pfukenyi, *et al*., 2005). The *Armatae xiphidiocercariae* recovered were 'plagiorchidae family which is intestinal parasites in all group of vertebrates.' Released by only by *L.natalensis* snail species in the study area (Zeitschrift *et al*., 2015). *Metacercariae* is relatively the passive stage of trematode which encyst in the intermediate host or external substrate because *Metacercariae* are trophically transmited to definitive host (Cnidaria *et al*., 2005). So the finding of large number of snails released non-

human *Schistosome* also require further study so as this may linked with infections of domestic animals probably transmissions to humans of zoonotic parasitic disease.

The magnitude and frequency of transmission of trematode parasite to the definitive host from the intermediate snail host is influenced by rate of infection in snails and number of trematode cercariae released by an individual infected snail (Jayawardena *et al*., 2010). Determination of infection prevalence or rate of infection by observing the number of infected snails collected from a field is essential to understand and judge the epidemiology and existing transmission posiblity of trematode related disease (Alebie *et al*., 2014). The prevalenc of infection in intermediate host sails observed (4.06%) in the present study was low compared to the previous study done in the area by (Mengistu *et al*., 2011) that reported (58%) of the snails shadde cercariae in the study done to determine human intestinal *Schistosomiasis* and 8.9% in Uganda by (Rowel *et al*., 2015). This low prevalence may be because of fast flowing of streams and rivers foster the contamination of parasite from feces of humans and animals with the snails. Since rushing water takes the feacal substance far away from the intermediate host snail then the probability of infection of the snails become narrow (Jayawardena *et al*., 2010). The other reason could be due to lacke of suitability of snail host for parasite and it also might be because of the death of snails thus Parasaitized snails may be less likely to survive after infection which reduces the number of infected snails found in the area (Alebie *et al*., 2014).

Some environmental conditions promotes the growth and reproduction of freshwater snails this indirectly influence the trematode parasite or different environmental situation directly influence the trematode parasite differently hence, the prevalence of infection in the intermediate host vary (Jayawardena *et al*., 2010). In this study the prevalence of infection significantly and positively influenced by temperature ($p = 0.02$, CI_{95} 0.026-0.103). This may be due to the tempreture of the area recorded (20.34-30.22 ^{0}C) which could encourage the proliferation of the trematode parasite in the snail host (Tigga *et al*., 2014). Conductivity and pH had no significant relation with prevalence of snail infection. This observation agree with (Rowel *et al*., 2015) that reported *Biomphalaria* infection of cercariae which did not infect human being positively related with temperature and not significantly related with conductivity, total dissolved salt and pH along Lake Albert and Lake Vectoria. Cloth washing significantly and negatively influence prevalence of snail infection ($p = 0.03$, CI_{95} (-0.907,-0.199)). The finding contrasts with the previous study

of (Alebie *et al*., 2014) which analized the influence of several anthropogenic parameters and supported that washing cloth, swimming and bathing in a river was positively and significantly affect *S.mansoni* infection rate in school children. This variation is might be because of infection rate among human being and among snail host were different or could be because of soap used for cloth washing in the current study results poisonous effect on cercariae, miracidia, and on some freshwater snails. This implies soap used during water contact attributed to reduction of the number of miracidia reaching to penetrate the snail host that influence the development and survival of intramolluscan stage of cercariae or kill some infected snails which could decrease the rate of infection in snails (Grimes *et al*., 2015). The shedding of cercariae by intermediate host snails also significantly affected by turbidity ($p = 0.009$, CI_{95} (0.001, 0.006) of the water bodies. This finding is similar with the study done by (Mohammed *et al*., 2016) that reported turbidity associated with snail infection. Otheer factors such as dissolved oxygen, water depth, snail species, type of cercariae, grazing, farming, bathing and settlement were not significantly related with snail infection rate.

The intensity of *B.pfeifferi* infection significantly ($p < 0.001$) influenced by its shell size. This could be due to in large size of snails provide more surface area for penetration of large number of miracidia in to the snail and have more space to harbored large number of cercariae. Probablity for this relation could be due to large sized snails exposed for longer duration thus exposed for accumulation of multiple infections because field snail size is indicator of its age. This results are in agreement with (Graham, 2003).

Chapter Seven: Conclusion and Recommendation

7.1 Conclusion

Freshwater snails widely distributed in different water bodies in Omo-Gibe river basin. Five snail species were identified of which four of them were infected by one or more type of trematode cercariae. No infection was identified in *Biomphalaria sudanica.* A total of seven morphotypes of trematode cercariae were observed which infect sixteen water bodies in different localities of the study area including water bodies in Chebera-churchura national park. This indicates besides *Schistosome* cercariae other larval trematodes are prevalent. Especially, *Echinostom and Amphistome* carring the risk of *Echinostomiasis* and *Amphistomiasis/Paramphistomiasis* in human and animals. The trematode cercariae recovered from the park were non-*Schistosome* cercarial parasite. The human schistosome cercariae were dominantly observed (35%) cercarial type infecting nine water bodies in six localities. The highest infection rate was recorded in Jimma town Seto-semero stream (28%) followed by Dimtu town in Langebo river (17.5%). The total prevalence of infection was 4% where the highst prevalence of infection was recorded in *B.pfeifferi* snail species. And also the *B.pfeifferi* was the most common intermediate host snail species for most of trematode cercariae, harboring six cercarial types. Water temperature, turbidity, cloth washing and swimming were the most important factors affecting cercarial infection of snails ($p<0.05$). Intensity of *B.pfeifferi* infection positively related with the size of its shell thus increase shell size of *B.pfeifferi* snail could increase the intensity of infection.

7.2 Recommendation

Based on the finding the following recommendations are forwarded

- Information education communication should be established for local community on the prevention and transmission way of intermediate host snail transmited disease
- Provision of safe and adequate water supply to prevent human contact with water infested by trematode cercariae
- Reducing environmental contamination by improving local water sanitation and hygiene
- Schistosomiasis control measure should focus on these area
- Avoidance of contact with trematode infested water foci
- Snail control measures should be applied especially *B.pfeifferi* species

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Annex 1

Types and Descriptions of Cercariae

5.2.1.1 *Echinostome cercariae*

Snail host: *B.pfeifferi, B.globosus* and *L.natalensis*

The body is oval in shape consist of a band that fits around the oral sucker and with single long tail. The ventral sucker is situated at the center of the body and larger than the oral sucker included in the 'family echinostomatidea' (Fig.7a).

5.2.1.2 *Brivifurcate apharyngeate diastome cercariae*

Snail host: *B.pfeifferi*

'Tail is brivifurcate possessing oral and ventral sucker on the body, yielded by the family spirochiidae and *Schistosomatidae* which are blood parasite of reptiles, mammals and birds.' Had no an eye like marking on the body. The tail turned towards the rear which were perceived in some stained cercariae and non-stained cercariae (when observed in dissecting microscope). This specimen is the same to human *Schistosome* cercariae (Fig. 7b).

5.2.1.3 *Brivifurcate apharyngeate monostome cercariae*

Snail host: *B.pfeifferi*

'Tail brivifurcate' with only oral sucker which produced by the family *clinostomatidae* (parasites of birds)' (Fig. 7c)

5.2.1.4 *Xiphidiocercariae*

Snail host: *L.natalensis*

Short, un-branched and layered tail with pear shaped body. The ventral sucker located at the middle of the body and somewhat larger than oral sucker and the oral sucker is appeared with 'stylet'. They are in the' family *plagiorchidae*' (Fig.7f).

5.2.1.5. *Amphistome cercariae*

Snail host: *B.pfeifferi* and *B.forskalii*

The short circularly inclined body with elongated simple tail. The conspicuous ventral sucker situated at the rear of the body. An eye like marking present near to the oral sucker and the cercariae attach on the non-animate materials or other organism outside of the intermediate host snail. They were grouped in *Paramphistomatidae* family (Fig.7e).

5.2.1.6. Metacercariae

Snail host: *B.pfeifferi*

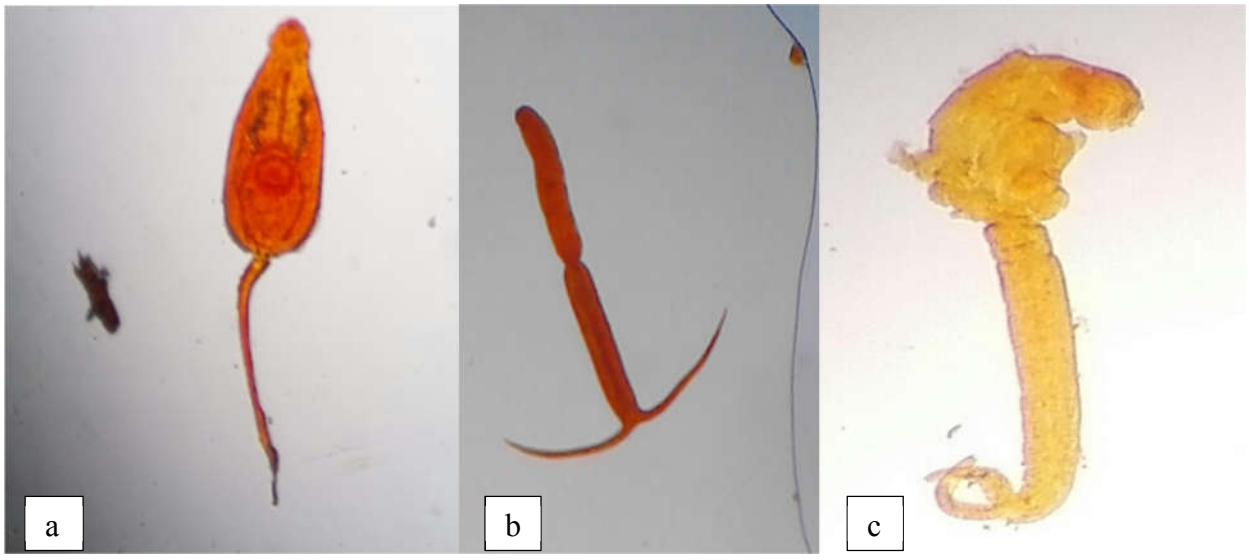
The circular non tailed body which encyst immediately on the petri-dish (Fig. 7d)

5.2.1.7. Un-identifiedcercariae

Snail host: *B.pfeifferi*

These are because their morphology was not distinctive to group in to their genus level. The un-identified cercarie in this study had three distinct morphology which were recovered from three different snails from different water body

Two were *furcocercous* type, the first one with circular body and had ventral sucker larger than oral sucker. The other furco types were with appendage like tail and there were observable distinction between head and body parts. The third one was with segmented body with single tail (Fig.7g-i).



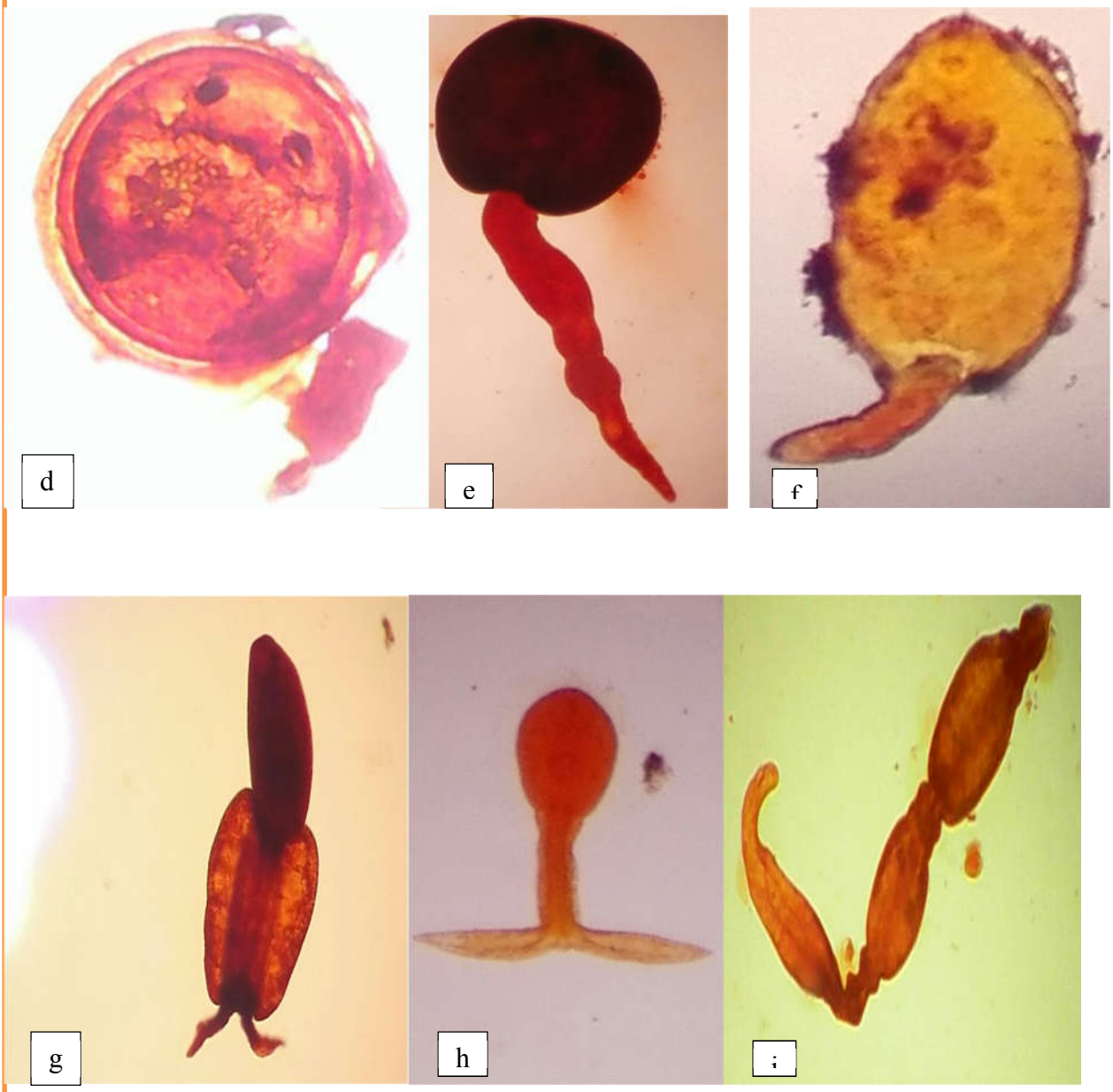


Figure 9 Morphotypes of trematode cercariae recovered from freshwater snails of Omo-Gibe river basin-May, 2016. The types are: a. *Echinostome* cercariae b. *BAD* cercariae c. *BAM* cercariae d. *Metacercariae* e. *Amphistomecercariae* f. *Xiphidiocercariae* g & h.

Annex 2

Field Assessment Form

1. DD/MM/YYY……Habitat type……
2. Site Code……..Name of Stream……..
3. Altitude(m)……Coordinates……,……..
4. Previous days rainfall history……..

Physico-Chemical Parameters

5. Water temperature (^{o}C)…….
6. DO (mg/l)……%.....ECμS/cm)……..pH……...
7. Water depth (m)…….
8. Turbidity…….. ……..

Notes and/or sketch of the site

9. Disturbance

Disturbance		**present**	**Absent**	**Remark**
Habitat alteration	Grazing			
	Vegetation removal			
Land Use	Farming/Cultivation			
	Settlment			
	Waste dumping			
Hydrological modification	Swimming			
	Cloth washing			
	Bathing			

10. Any additional comments

Annex 3. DATA RECORDING SHEET (LABORATORY)

1. DD/MM/YY (of collection)----------Time--
2. Location----------
3. Name of fresh water----------sampling station----------
4. Number of snails collected----------

5. Snail species identified Number of snails in each species
   a)---------- ----------
   b) ---------- ----------
   c) ---------- ----------
   d) ---------- ----------
   e) ---------- ----------
   f) ---------- ----------
   g) ---------- ----------

6. **Shedding trial round**----------
7. DD/MM/YY (of shedding)----------Time(from----------to----------
8. Source and quality of light----------
9. Number of snails exposed to light ----------
10. Number of snails that shed cercaria ----------
11. Average size of snails shedding cercaria
    A) Biomphalaria sp.----------B) Bulinus sp.----------C) Lymnaea sp.------

12. Cercaria shedding record

Snail species of shedding Type of cercaria shedded Av. No /ml water

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13. Percentage of snail spps shedding cercaria----------
14. Percentage of cercaria spps. Recovered----------

Annex 4. Habitat type, Laboratory work and snail collection

Figure 10 Human and animal water contact behavior in Omo-Gibe river basin-May, 2016

Figure 11 Maintainance of freshwater snails from Omo-Gibe river basin in laboratory –May 2016

Figure 12 Identification of cercarial trematodes in laboratory-May, 2016

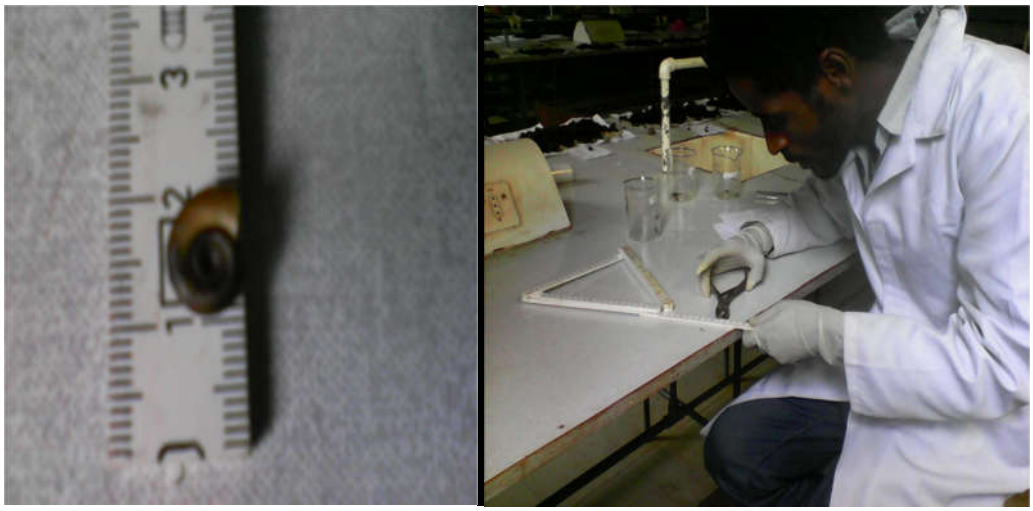

Figure 13 Measurment of infected snail shell in the laboratory-May, 2016

Figure 14 Cloth washing in Omo-Gibe river basin-May, 2016

Figure 15 Snail collection using hand scoop and D-fram kicknet in Omo-Gibe river basin-May, 2016