ASSESSMENT OF WATER-BORNE PARASITES AMONG SCHOOL CHILDREN AND THEIR WATER SOURCES IN EFA BERI PRIMARY SCHOOL, EJERE TOWN, WEST SHAWA, OROMIA, ETHIOPIA

M.Sc. THESIS

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Assessment of Water-Borne Parasites among School Children and Their Water Sources in Efa Beri Primary School, Ejere Town, West Shawa, Oromia, Ethiopia

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Tesgera Mosissa

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APROVAL SHEET

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We herby certify that we have read and evaluated this Thesis entitled "Assessment of Water-Born Parasites among School Children and Their Water Sources in Efa Beri Primary School, Ejere Town, West Shawa, Oromia, Ethiopia" prepared under our guidance by Tesgera Mosissa. We recommend that it be submitted as fulfilling the thesis requirement.

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As a member of the board of examiners of the MSc Thesis open defense Examination, we certify that we have read and evaluated the Thesis prepared by **Tesgera Mosissa** and examined the candidate. We recommend that the thesis be accepted as fulfilling the Thesis requirements for the degree of Master of Education in biology.

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DEDICATION

I dedicated this thesis manuscript to my parents for their love, affection and unrestricted encouragement they offered me not only in accomplishing this research but also for every success in my life.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

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Place: Haramaya University, Haramaya

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BIOGRAPHICAL SKETCH

The author was born in July, 1972 at Menesibu Woreda, West Wollega, Oromia Regional State. He attended his elementary and junior education at Tenki and Mendi Elementary School, Menesibu Woreda. Then he pursued secondary school education at Mendi Secondary School.

In1990, he joined Ambo College of Agriculture and received his diploma. Then, after he worked as a teacher for ten years at Gurawa and Kombolcha Secondary Schools, he joined Haramaya University in 2001 and received his Bachelor of Education (BEd) degree in Biology in summer program. Then, he worked as a teacher at Kombolcha Secondary School and as office worker at Woreda Education Office for ten years until he joined Haramaya University for his graduate study program in biology in 2013.

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ACRONYMS AND ABBREVIATIONS

APHA	American Public Health Association
CDC	Center for Disease Control
EHNRI	Ethiopian Health and Nutrition Research Institute
ELISA	Enzyme linked immunosorbent Assay
GIP	Gastrointestinal parasite
GIT	Gastrointestinal tract
HU	Haramaya University
IF	Immunofluorscence
MRCP	Magnetic Resonance Cholangiopancreatography
RPM	Rounds per minute

SAS	Statistical analysis software
SSA	Sub Saharan African
UCEPA	United States of Environmental Protection Authority
WHO	World Health Organization

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Assessment of Water-Borne Parasites among School Children and Their Water Sources in Efa Beri Primary School, Ejere Town, West Shawa, Oromia Region, Ethiopia

ABSTRACT

The present study was conducted to assess the common water-borne parasites among school children and their water sources in Efa Beri Primary School, Ejere Town, West

Shawa Zone of Oromia Region, Ethiopia from March-April, 2017. A total of 384 fresh stool samples of school-children were examined using direct wet mount, formol-ether concentration and Modified Ziehl-Neelsen methods and 72 water samples from different sources were examined to detect the presence of water-borne parasites. The resulst indicated that the overall prevalence of water-borne parasitic infections among students was 38.0%. The result revealed that Giardia lamblia, Entamoeba histolytica, Cryptosporidium species, Ascaris lumbricoides and Trichuris trichiura were the common water-borne parasites in the study population with prevalence of 14.3%, 8.6%, 2.1%, 10.7%, and 2.3%, respectively. The prevalence of water-borne parasitic infections was significantly associated with some of the risk factors, such as family size, presence or absence of latrine, source of drinking water, water handling practices, awareness to personal hygiene and environmental sanitation and awareness to water-borne parasitic infections (p0.05 for each). However, some other socio-demographic related factors, such as sex, age and parent's educational level were not significantly associated with water-borne parasitic infections (p0.05 for each). The parasitological analysis of water samples in this study demonstrated that 100% of the samples taken from unprotected water sources were positive with one or more water-borne parasites cysts, oocysts and/or ova. The study has revealed that water-borne parasites represent a major public health problem amongst the school children of Efa Beri primary school. Therefore, health education related to personal hygiene and environmental sanitation and cost effective water purification mechanisms were recommended to minimize the risk and exposure of water-borne parasitic infections in the stud area.

Key Words: *Efa Beri,Oromia, Parasites, Prevalence, School children, water-borne.*

1. INTRODUCTION

A wide range of water problems faces nations and individuals around the world. These problems include international and regional disputes over water, water scarcity and contamination, unsustainable use of groundwater, ecological degradation, and the threat of climate change. Development Goal Baselines, presents the first global assessment of “safely managed” drinking water and sanitation services. The overriding conclusion is that too many people still lack access, particularly in rural areas (WHO, 2016). Some 3 in 10 people worldwide, or 2.1 billion, lack access to safe, readily available water at home, and 6 in 10, or 4.5 billion, lack safely managed sanitation (WHO/ UNICEF,2017).

Although water-related diseases have largely been eliminated in wealthier nations, they remain a major concern in much of the developing countries. The poor are more susceptible to ill-health than are the well-off. They lack adequate supplies of safe water and safe methods of disposing of their wastes. Lack of water and sanitation create ideal conditions under which faecal oral diseases thrive (WHO, 2017). Water-related diseases are typically placed in four classes: water-borne, water-washed, water-based, and water-related with insect vectors. The first three are most clearly associated with lack of improved domestic water supply (Peter, 2002).

Water-borne diseases are infections caused by ingestion of water contaminated by human or animal excrement, which contain pathogenic microorganisms. A person’s health may be affected by ingestion of contaminated water directly or through food. Contact with drinking water is one way that people may contact these diseases. Other ways include contact with contaminated recreational water, farm animals, sick animals, faecal matter, or other symptomatic people. (WHO, 2016). Poverty, illiteracy, overcrowding, low health services, poor personal hygiene, environmental sanitation, and lack of clean water supplies are contributing factors that directly or indirectly affect the prevalence of water-borne diseases. The World Health Organization says diarrhoeal diseases remain a leading cause of illness and death in the developing world. Every year, about 2.2 million people die from diarrhea; 90% of these deaths are among children, mostly in developing countries (WHO, 2017).

In developing countries, raw surface water such as rivers, streams and lakes are used for multiple activities, including livestock watering, bathing, and cooking. Defecation and urination often occur near water sources as well. Forty percent of the world's 6 billion people have no acceptable means of sanitation and more than 1 billion people draw their water from unsafe sources (WHO 2016). People in developing countries may have no other options for drinking water because there is a lack of water distribution, infrastructure and lack of funding for developing water treatment systems (Christine, 2003). The lack of adequate drinking water supply in developing countries is a continually growing problem due to population increase and increased demand for clean source of water (Ahmed, 2006).

Interest in the contamination of drinking water by enteric parasitic pathogens has increased considerably and a number of parasitic infections of humans are transmitted by the water-borne route. The Sub-Saharan Africa (SSA) population suffers markedly from water-borne intestinal protozoan parasitic infections due to lack of safe and sanitary water supply and disposal system. In 18 countries in SSA, only less than a quarter of the population uses an improved sanitation facilities for drinking water supply (WHO/, 2010).

The common water-borne parasites of human beings are including protozoan and helminths species. *Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica, Cyclospora cayetanensis, Toxoplasma gondii, Isospora belli, Blastocystis hominis, Balantidium coli, and Naegleria fowleri* are the major protozoan parasites responsible for water-associated outbreaks in different parts of the world (Karanis *et al*., 2007). The most prevalent and important water-borne helminths parasitic species are *Ascaris lumbricoids*, *Trichuris trichiura* and *Enterobius vermicularis* (Raso, 2006).

Many infections caused by water-borne intestinal protozoan and nematode parasites are cosmopolitan in distribution (Crompton, 2002). According to Mehraj *et al*., (2008), *Ascaris lumbricoides* infects 1.221 million people, *Trichuris trichiura* 795 million, *Entamoeba histolytica* 500 million and *Giardia lamblia* about 200 million people worldwide. The greatest numbers of intestinal protozoa and nematode parasitic infections occur in tropical and subtropical regions of Asia, especially China, India and Southeast Asia, as well as Sub-Saharan Africa (de Silva *et al*., 2003).

In developing countries, *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium* species are the major water-borne protozoan parasites (Stevens and Adam, 2004). The features of the life cycles of *Giardia* and *Cryptosporidium* species enhance the likelihood of water-borne transmission. *Giardia* and *Cryptosporidium* are monoxenous, completing their life cycles within a single host that excretes large numbers of infective transmissive stages (*Giardia* excretes cysts and *Cryptosporidium* produces oocysts) in faeces. The infective cysts and oocysts are environmentally robust, are sufficiently small to penetrate the physical barriers of water treatment and are insensitive to many disinfectants used in the water industry (Smith and Grimason, 2003).

The life cycles of most helminths follow the same pattern (Stephenson *et al*., 1989). Adult *Ascaris lumbricoides,* lives in the entire small intestine and adult *Trichuris trichiura,* whipworms live in the large intestine, especially the caecum (Crompton, 2001). The parasites can live for several years in the human gastrointestinal tract. Human beings are regarded as the only major definitive host for these parasites, although in some cases *Ascaris lumbricoides* infection can also be acquired from pigs (Crompton, 2001).

In Ethiopia, since the large majority of the rural population obtains its water supplies from unprotected sources such as rivers, streams, ponds, wells, etc, water-borne parasitic diseases are one of the most significant public health problems. Diarrheal diseases and other common diseases related to quality of water and sanitation account for 25-50 percent of reported morbidity and mortality in Ethiopia (Debelina, 2009). Although a number of studies have been conducted on the distribution and prevalence of water-borne and intestinal parasites in different parts of Ethiopia (Tadesse Zerhun *et al.*, 2008; Mehari Girmay, 201; Walelign Mengistu, 2014; Areda Dechasa, 2014), there are still many localities where more epidemiological information is not available. Hence, this study was conducted to fill the existing gap and enable decision makers to focus on improving water quality, sanitation and hygiene around Ejere Town,West Shoa Zone of Oromia Region, Ethiopia. Although water quality data was not available in this district, the health records in clinics indicate that water-borne diarrhea was the dominant case in this study area (Ejere district health office annual report 2015/ 2016). Furthermore, there were no proper water treatment systems in the area. As a result the drinking water sources were obviously expected to be contaminated with water-borne parasites contributing to the prevalence of high diarrheal cases and other water-borne parasitic infections in the study area.

Therefore, the purpose of this research was to assess the possible association of prevalence of water-borne parasitic infections with water sources, and other risk factors among school children in Efa Beri Primary School, Ejere Town, West Showa Zone of Oromia Region, Ethiopia.

General Objective:

The general objective of this study was to assess the water-borne parasitic infections among school children and their drinking water sources in Efa Beri Primary School, Ejere Town, West Showa, Oromia region, Ethiopia.

Specific objectives of the study were:

1. To determine the prevalence of water-borne parasitic infections among school children in the study area.
2. To assess the prevalence of water-borne parasites in different water sources in the Study area.
3. To identify the associated risk factors for water-borne parasitic infections among

school children in the study area.

2. LITERETUR REVIEW

2.1. Water-Borne Parasitic Diseases

Water-borne parasitic diseases are most commonly transmitted by contaminated water. Various forms of water-borne diarrheal diseases are the most prominent examples and affect mainly children in developing countries (Jump *et al*., 2006). Cryptosporidiosis, giardiasis, amoebiasis, ascariasis, trichuriasis and entrobiasis are the most common water-borne parasitic diseases worldwide. The causative organisms for amoebiasis, giardiasis, cryptosporidiosis, ascariasis, trichuriasis and entrobiasis are *Entamoeba histolytica*, *Giardia lamblia, Cryptosporidium parvum, Ascaris lumbricoides, Trichuris trichiura* and *Entrobius vermicularis,* respectively (Heyworth, 1992).

The worldwide prevalence of water-born parasitic infections caused by pathogenic protozoan and helminths species is reported to be high. They result in considerable gastrointestinal morbidity, malnutrition and mortality worldwide, particularly among young children in developing countries (Feng and Xiao, 2011). It has been estimated that *Entamoeba histolytica* kills between 40,000 and 100,000 people each year; hence, is one of the deadliest parasitic infections worldwide (Stanley, 2003). The prevalence of *Giardia lamblia* has been estimated as 2–3% in the industrialized world and 20–30% in developing countries (Escobedo *et al*., 2009). *Giardia lamblia* is the most common protozoan parasite of the human small intestine (Eckmann and Gillin, 2001).

2.2. Life Cycle and Transmission of Water-Borne Parasites

Water-borne intestinal protozoa and helminths are transmitted by the fecal-oral route. Factors which increase the chance of ingesting materials contaminated with fecal material play a role in the transmission of these parasites. In general, situations involving close human-human contact and unhygienic conditions promote transmission (Chen *et al*., 2007).

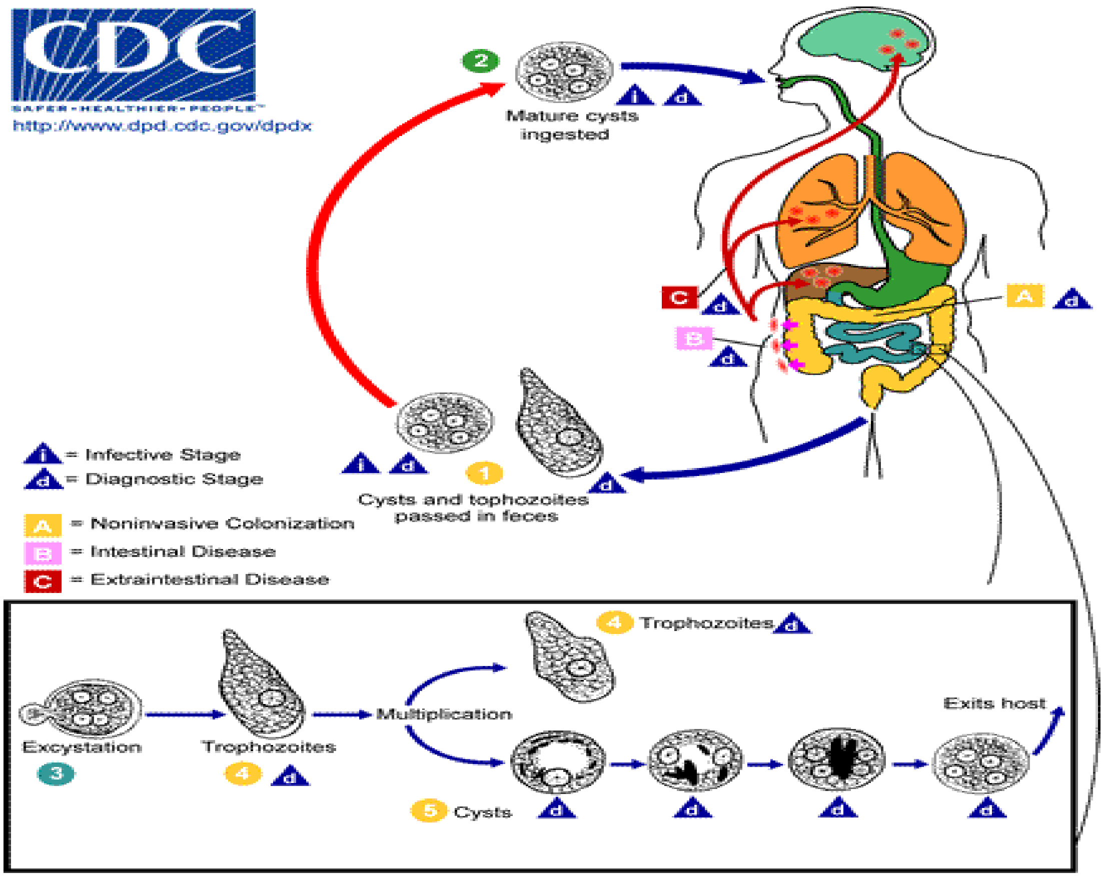

Figure1: Life cycle of *Entamoeba histolytica* (Source: http//www.dpd.cdc.gov/ dpdx, 2008)

2.2.1.Amoebiasis

Amoebiasis is caused by *Entamoeba histolytica*, the only pathogenic entamoeba species. It has two morphologic forms: trophozoite and cyst. The trophozoite form is the motile and invading stage of the parasite that usually lives as a commensally in the human large intestine where it multiplies by asexual binary fission and eventually differentiates into cyst forms. The cyst form is inactive, non motile, non-invading stage of the parasite, and it is responsible for the transmission of the disease to others through fecal-oral route (Tadesse Anteneht, *et al*.,2008).

Mature cysts in the large intestine leave the host in large numbers and remain viable and infective in a moist, cool environment for at least 12 days. In water, cysts can live for up to 30 days. Mature cysts are also resistant to chlorine levels normally used to disinfect water (Stenmark, 2009). When swallowed, cysts pass through the stomach unharmed. In the small intestine, where conditions are alkaline and as a result of nuclear division, eight motile trophozoites are produced. These motile trophozoites settled in the large intestine lumen, where they divide by binary fission and feed on host cells, bacteria and food particles. This is the first chance of the parasite making contact with the mucosa (Chatterjee *et al*., 2009).

2.2.2.Giardiasis

Giardiasis is caused by *Giardia lamblia*, which is the only pathogenic intestinal flagellate known to infect humans. It has two morphological forms, namely trophozoite and cyst. The trophozoite is actively motile and invading stage of the parasite, and lives on the villi of the small intestine. The cyst is inactive, non-motile and non-invading stage of the parasite, and responsible for the transmission of the disease to others through the fecal-oral route (Tadesse Anteneh *et al*., 2008).

The cysts possess a thin, protective wall that allows them to survive in feces for weeks or in cold water for months. Giardiasis is then contracted via ingestion of contaminated water or foods. The cysts pass through the stomach and enter the small intestine. The protective wall allows the cyst to survive the acidic conditions of the stomach until the cyst reaches the small intestine, where the conditions are alkaline.

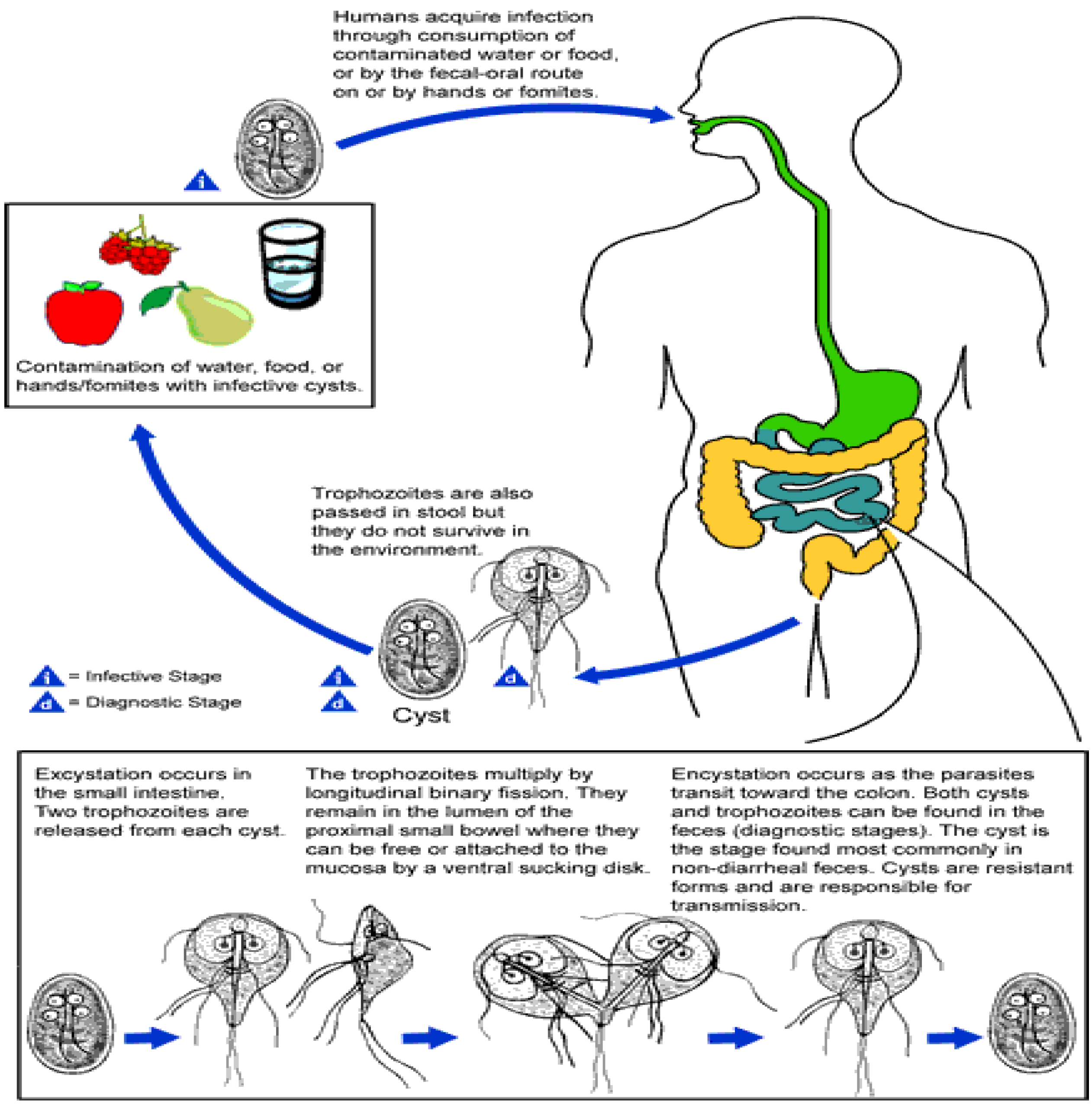
Humans acquire infection through consumption of contaminated water or food, or by the fecal-oral route on or by hands or fomites.
Contamination of water, food, or hands/fomites with infective cysts.
Trophozoites are also passed in stool but they do not survive in the environment.
= Infective Stage
= Diagnostic Stage
Cyst
Excystation occurs in the small intestine. Two trophozoites are released from each cyst.
The trophozoites multiply by longitudinal binary fission. They remain in the lumen of the proximal small bowel where they can be free or attached to the mucosa by a ventral sucking disk.
Encystation occurs as the parasites transit toward the colon. Both cysts and trophozoites can be found in the feces (diagnostic stages). The cyst is the stage found most commonly in non-diarrheal feces. Cysts are resistant forms and are responsible for transmission.

Figure 2: Life cycle of *Gardia lamblia* (Source: http//www.dpd.cdc.gov/dpdx, 2008)

The alkaline environment triggers excystation. During excystation, the cyst wall ruptures at the pole opposite to the nuclei, so that flagella and other projections emerge from the rupture point. The cyst wall is then completely shed and the microbe enters the trophozoite stage of its life **(**Adam, 2001).

The trophozoite is the reproducing and motile form of *Giardia* that attaches to the intestinal wall via its ventral disc and causes the symptoms of giardiasis (Adam, 2001). In severe cases, the trophozoites can become so numerous along the intestine that they cover it as a "carpet." While the trophozoite is attached, it not only absorbs but blocks nutrients from transporting across the epithelial lining of the intestine. It inhibits the absorption of fats, carbohydrates, vitamin and folic acid. Trophozoites are rarely infective because they are not resistant to gastric acid and die rapidly outside the body. The trophozoite then undergoes encystations. Encystations take place as trophozoites pass to the posterior regions of the small intestine.

The cysts then leave the body and are transmitted from person to person by contact with infected feces directly or picked up by another host via contaminated water or food indirectly (Linnane *et al.*, 2001. Generally the cyst stage of *Giardia lamblia* causes the infection while the trophozoite causes the symptoms of giardiasis.

2.2.3. Cryptosporidium

The life cycle of *cryptosporidium parvum* is monoxenous, completed within the gastrointestinal tract of a single host (Fayer, 2000). The resistant stage that is found usually in the environment is the thick walled oocyst excreted together with feces (Fayer, 1986). Each oocyst has 4 infective sporozoites that come out from the oocyst using the suture at one side of the oocyst. The ileum is the preferable site of infection and the sporozoites penetrate epithelial cells of the ileum. *Cryptosporidium parvum* can complete its life cycle in as short as 2 days and the infection may be short lived or may be persistent for months. Excystation of the oocyst is initiated by the body temperature, interaction with stomach acid and bile salt. The released sporozoites attach to epithelial cell and become enclosed within parasitophorous vacuoles. The trophozoite stage then undergo asexual proliferation by merogony and two types of meronts are produced, Type I meronts and Type II meronts (Fayer and Ungar, 1986). Type I meronts form 8 merozoites that are released from the parasitophorous vacuole when they mature. The merozoites then enter another brush border surface epithelium where they undergo another cycle of type I merogony (multiple fission or schizogony) or else they may develop in to type II meronts. The type II meronts give rise to 4 merozoites which do not undergo further merogony but produce gamonts, the sexual reproductive stages which fuse and form the only diploid stage in the life cycle, the zygote. A resistant oocyst wall is then formed around the zygote.

The zygote undergoes asexual development (sporogony) and gives rise to sporulated oocyst that contains 4 sporozoites. Two possible auto-infective cycles occur in *Cryptosporidium parvum*. The first is by the continuous recycling of Type I meronts and the second through sporozoites rupturing from thin-walled oocyst. Experimentally infected animals have shown a prepatent period of 4 days but sometimes it could be 3 days in heavy infection .In humans when lower numbers of oocysts are probably ingested, the prepatent period is typically 4 to 6 days. The length of time in which oocysts are shed in feaces generally lasts 6 to 18 days (4 to 10 days of diarrhea) in immune competent individuals but it may be prolonged in immune compromised individuals. Some patients may discharge oocyst yet they appear asymptomatic (Fayer and Ungar, 1986).

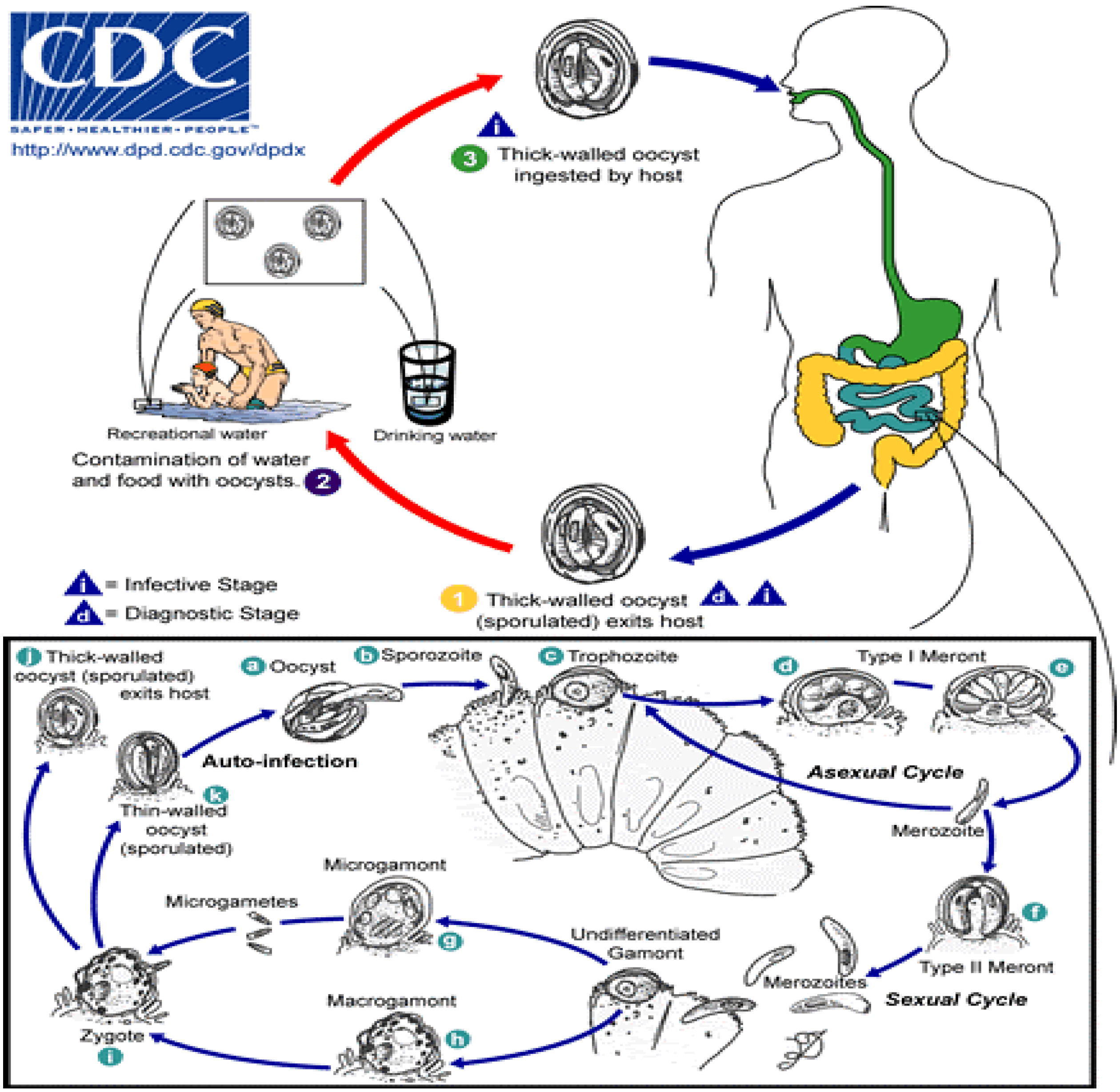
SAFER·HEALTHIER·PEOPLE™
http://www.dpd.cdc.gov/dpdx
Thick-walled oocyst ingested by host
Recreational water
Drinking water
Contamination of water and food with oocysts.
= Infective Stage
= Diagnostic Stage
Thick-walled oocyst (sporulated) exits host
Thick-walled oocyst (sporulated) exits host
Oocyst
Sporozoite
Trophozoite
Type I Meront
Auto-infection
Asexual Cycle
Thin-walled oocyst (sporulated)
Merozoite
Microgamont
Microgametes
Undifferentiated Gamont
Type II Meront
Merozoites
Sexual Cycle
Macrogamont
Zygote

Figure 3: cycle of *Cryptosporidium parvum* (Source: http//www.dpd.cdc.gov/

Dpdx, 2008

2.2.4. Helminths

The life cycles of most helminths follow the same pattern (Stephenson *et al*., 1989). Adult *ascaris* lives the entire small intestine and the adult *Trichuris (*whipworm) lives in the large intestine, especially the caecum (Crompton, 2001).

Ascaris lumbricoides, or "roundworm", infection in humans occurs when an ingested infective egg releases a larval worm that penetrates the wall of the duodenum and enters the blood stream. From here, it is carried to the liver and heart, and enters pulmonary circulation to break free in the alveoli, where it grows and molts. In 3 weeks, the larvae pass from the respiratory system to be coughed up, swallowed and thus returned to the small intestine, where they mature to adult male and female worms. Fertilization can now occur and the female produces as many as 200,000 eggs per day for a year. These fertilized eggs become infectious after 2 weeks in soil; they can persist in soil for 10 years or more (Crompton, 2001).

Infection with *Trichuris trichiura* occurs via the oral-fecal route by the ingestion of infective eggs from contaminated food or water. These then pass through the stomach to the small intestine where they hatch. The larvae penetrate the cell of the small intestine coming to lie above the lumina to undergo four molts. The immature adults emerge and are passively transported to the large intestine where they mature and embed their thin whip-like anterior into columnar cell. The adult whipworms develop within 60-90 days after initial infection (Stephenson *et al*., 2000)

2.3. Pathogenesis and Clinical Features of Water-Borne Parasitic Infections

In Ethiopia pathogenesis of water-borne parasites such as cryptosporidiosis, giardiasis, amebiasis, ascariasis and trichuriasis is extremely high in school and non-school children with high mental and physical growth effects (Walelign Mengistu, 2014, Tadesse Tessema, 2013). Contamination of pathogenic parasites to drinking and recreational waters can result in gastrointestinal, respiratory, eye, ear, nose, throat, and skin infections (Debelina, 2009).

Symptoms of amoebiasis could be acute (frequent dysentery with necrotic mucosa and abdominal pain) and chronic (recurrent episodes of dysentery with blood and mucus in the feces). There are intervening gastrointestinal disturbances and constipation. Cysts are found in the stool. The organism may invade the liver, lung and brain where it produces abscesses that result in liver dysfunction, pneumonitis and encephalitis (WHO, 2002). The aspects of pathogenesis which have been investigated experimentally can be broadly categorized into mechanisms involving interactions with the intestinal flora, lyses of target cell by direct adherence, lytic necrosis and it looks like "flask-shaped" holes in Gastrointestinal tract(GIT) sections; lyses of target cell by release of toxins and phagocytosis of target cells (Sehgal *et al*., 2010).

The pathogenesis of diarrhea and malabsorption that can occur in giardiasis is not fully understood; diarrhea may be a result of both intestinal malabsorption and hyper secretion. The small intestine is the site of the major structural and functional abnormalities associated with giardiasis. When *Giardia* infection starts, it could results in occasional days of acute watery diarrhea with abdominal pain or patients may experience a prolonged, intermittent often debilitating disease, which is characterized by passage of foul-smelling stool associated with flatulence, abdominal distention, anorexia, malaise, weakness, weight loss, abdominal cramps, and distention can occur. Children are more liable to clinical giardiasis than adults. Immunosuppressed individuals are especially liable to massive infection with severe clinical manifestations. Symptoms may continue for long periods (Butel *et al.*, 2007).

The pathogenesis of *Cryptosporidium*-associated diarrhoea, weight loss and mortality are not well understood but recent research in animal models have provided insight into the patho-physiology of the disease and understanding of the clinical signs. Cryptosporidium *parvum* causes acute self-limiting gastroenteritis in humans and other mammals. Diarrhoea in cryptosporidiosis could result from a combination of increased intestinal secretion which may be partly mediated by entero toxin-like activity produced by *Cryptosporidium parvum* as a result of endogenous secretary mediators such as prostaglandins which alter NaCl transport primarily by stimulating the enteric nervous system and loss of absorptive epithelium due to apoptosis and villus atrophy results in malabsorption, and release of inflammatory cell mediators stimulate electrolyte secretion and diarrhoea (Fayer, 2004). During the migratory phase of *ascaris,* large number of larvae may induce allergic reactions and host sensitization resulting in asthma, coughing, and shortness of breath, fever, skin rash and eosinophilia. Mature worms in the small intestine cause clinical signs and symptoms such as abdominal pain and distention, vomiting and diarrhea (O'Lorcain and Holland, 2000).

The clinical spectrum of trichuriasis varies from asymptomatic infection to *Trichuris* dysentery syndrome (TDS), which is characterized by chronic mucousbloody diarrhea (Gillespie, 2001). Rectal bleeding and prolapse are also associated with trichuriasis. Blood loss occurs from both the feeding activities of the parasites and extensive damage to the colonic mucosa (Gillespie, 2001). Symptoms associated with moderate infection include epigastric and lower abdominal pain, vomiting, diarrhea, flatulence and weight loss. In severe infections, the worm may be observed embedded in the edematous rectal mucosa accompanied by moderate eiosinophilia. Furthermore, growth stunting associated with decreased collagen synthesis has been reported in children (Stephenson *et al.,* 2000).

2.4. Epidemiology of Water-Borne Parasitic Infections

Intestinal parasitic protozoan and helminths are the causes of water and food-borne diseases (Leclerc, 2002). These parasites are widespread in the environment, and are the causes of major diseases outbreaks that have occurred as a result of contaminated drinking water and food. Previously, it has been estimated that over one quarter of the world's population is infected with one or more helminth parasites (de Silva *et al.,* 2003). There is evidence that individuals with many intestinal parasitic infections have even heavier infections with protozoa and nematode parasitic infections (Raso *et al*., 2004).

A study estimated that *Ascaris lumbricoides* infects 1,221 million people, *Trichuris trichiura* 795 million, and about 200 million peoples were enfected by *Giardia lamblia* (Brooker *et al*., 2006).

Many reports indicated that *Entamoeba histolytica, Giardia lamblia, Cryptosporidium species, Ascaris lumbricoides* and *Trichuris trichiura* are the most prevalent water-borne protozoa and nematode parasites in Ethiopia (Endeshaw, 2004). Of 301 school children who were studied in south western Ethiopia, 68.4% harbored one or more parasites. In that study from the total of ten parasitic species identified, water-borne parasites were: *Ascaris lumbricoide* (52.2%), *Trichuris trichiura* (18.6%), *Entamoeba histolytica* (13.5%), *Giardia lamblia* (6.7%) while *Cryptosporidium species* was the least (0.3%) (Alemeshet *et al*., 2010).

2.5. Diagnosis of Water-Borne Parasitic Infections

2.5.1. Stool Examinations

Definitive diagnosis of water-borne parasites is made by identification of cysts, trophozoites and eggs of parasites in the feces. Identification is usually made by microscopic examination of direct fecal smears (Raso *et al*., 2004). Direct saline wet mount provides economical and rapid diagnosis for water-borne parasitic diseases if they are present in sufficient density in the stool samples (Ukaga *et al*., 2002). The formal-ether concentration procedure is efficient in recovering the parasites in a single or multiple stool specimens (Peters *et al*., 1980).

2.5.2. PCR Based Detection

A PCR based detection that ampilifies the 552-bp intergenic spacer (IGS) region of multicopy rRNA gene of protozoan species and 320-bp internal sequences to first PCR product has been used in diagnosis of protozoa in stool sample. The primers were found highly specific to giardia species only because no amplification was observed with DNAs from other enteric pathogens. The test could detect even less than 2pg of genomic DNA from giardia trophozoites.

2.5.3. Examination of Water Samples

Samples taken from drinking water source are used to determine if the water supply system is safe or not. The actual number and frequency of sampling must take the local conditions into account. Water sample collection, filtration, elution, centrifugation, and microscopic observation is conducted based on USEPA method for identification, determination and enumeration of parasitic cyst, oocyst and/or eggs.

2.6. Prevention and Control of Water-Borne Parasitic Infections

Human intestinal parasitic protozoan infections can be controlled through proper treatment and disposal of row swage and maintaining clear water supply including the protection of open wells, springs and rivers from contamination with swage and feces. The risk for infection can also be reduced via the adequate boiling of drinking water or treatment of water with chlorine or iodine. The principal measures that should be included in a control program consist of massive and periodic treatment of the human population to prevent environmental contamination, sanitary excreta disposal, provision of potable water and health education for the purpose of instilling personal hygiene habit in the population (Sackey *et al*., 2003).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

This study was conducted at Ejere Health Center, Ejere town, West Shawa Zone, Oromia Regional State, Ethiopia. Ejere is one of the districts in West Shawa Zone. It is bordered on the west by Dandi, on the south by Sebata Awas, on the south west by Ilu, on the north by Adia, on the North West by Meta Robi and on the east by Walmara district. The district is located at about 45km to the west of capital city of the country Addis Ababa. The area has an elevation of about 2495m above sea level with latitude and longitude of 9^0 2 'N and 32^0 24 'E respectively and annual rainfall ranging from 910-1200 mm on average. It has an estimate total population of 115026 according to the report of Central Statistical Agency (2007) with growth rate of 2.9% increase per year consisting of 58504 male and 56525 females. The dominant ethnic group living in Ejere district is Oromo people.

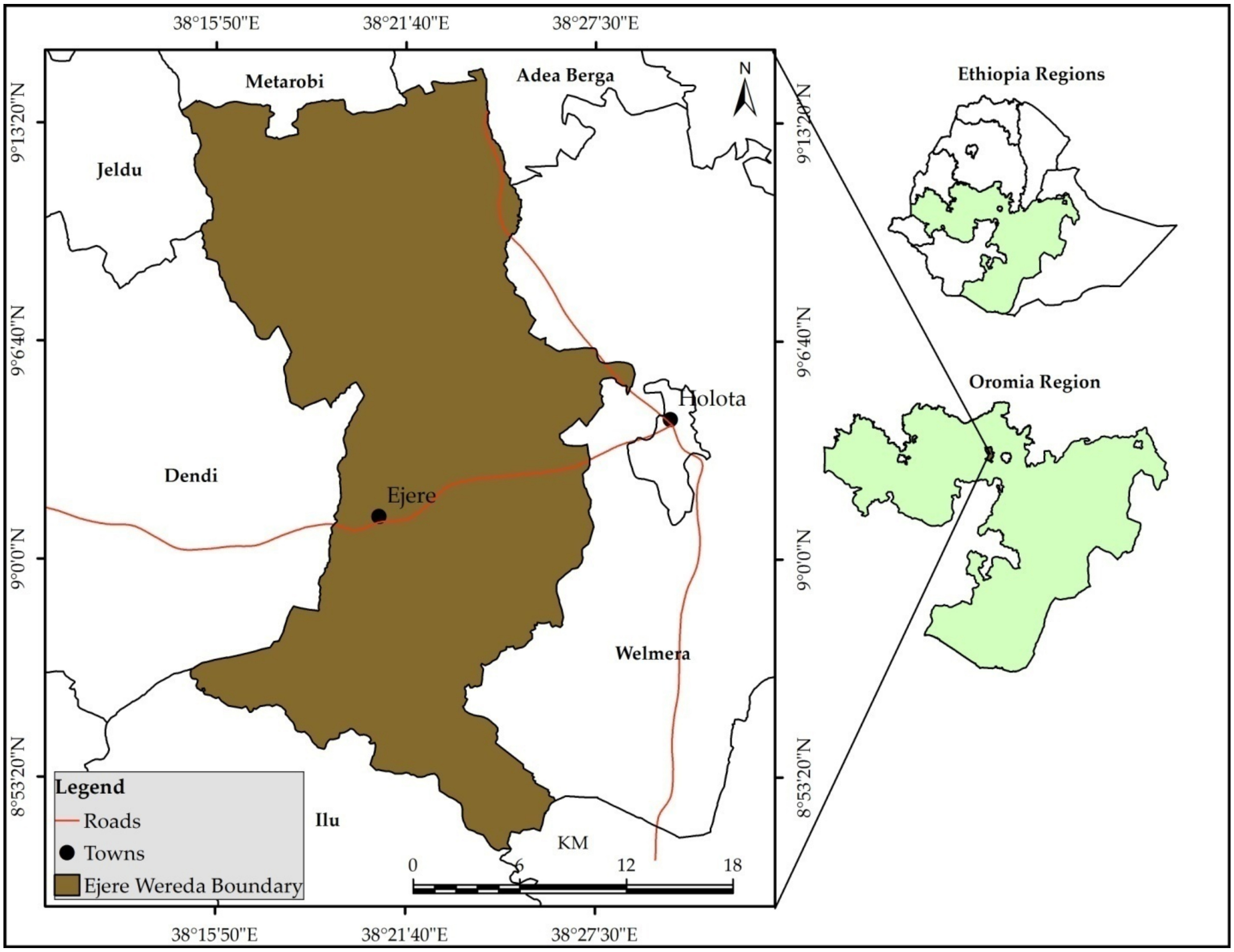

Figure 4: Map of the study area (source: Geographical Information system, 2016)

3.2. The Study Design

A descriptive cross-sectional survey was carried out to assess common water-borne parasitic infections among school children and their water sources in Efa Beri Primary School, Ejere Town, West Showa Zone of Oromia Region, Ethiopia. The association of water sources and some socio-demographic characteristics with the prevalence of water-borne parasites among students was also determined.

3.3. Study Population and Sampling Method

All students from grade 1 to 8 in the selected school, who were volunteers to participate, complete and signed the consent form by their parents were included in the study. The sample size (n) was determined using the following statistical formula (Naing *et al.,* 2007).

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where:

n= sample size

d= marginal error (5%)

Z= 95% confidence interval (1.96)

P= prevalence of water-borne parasitic infections.

Since the overall prevalence rate (P) of water-borne parasitic infections was not known for the study area, the maximum prevalence was taken to be 50%. For the calculation, a 95% confidence interval (Z) and a 5% margin of error (d) were used. Therefore, three hundred eighty four (384) school-children were chosen to participate in the present study (Table 1). To select the sample children, the students were first stratified according to their educational level (grade 1 to grade 8). Proportional sample numbers were then allocated for students coming from different kebeles (rural and urban). Finally, the sample children were selected using systematic random sampling technique.

Name of school	Grade	Total students population			Sample student population		
		M	F	T	M	F	T
Efa beri	1-4	494	445	939	97	88	185
	5-8	487	526	1013	96	103	199
	Total	981	971	1952	193	191	384

Table 1: Total student population and sample student population in Efa Beri primary School 2016/2017

Exclusion criteria:

Those students that have been treated for any water-borne parasitic infections for the past three months at the time of survey were excluded in the study.

3.4. Methods of Data Collection

In this study stool and water sample were collected to assess and determine the prevalence of water-borne parasitic infections among school children in the study area. In addition, questionnaire survey was conducted to assess the major socio-demographic risk factors for water-borne parasitic infections. Details of these procedures are presented below.

3.4.1. Stool Sample Collection

Each study subject was provided a labeled leak proof container, toilet paper, applicator stick and informed to put about 3 gm stool using applicator stick. Fresh stool sample was collected from each consented study subject. The stool samples were carried to Ejere Health Center on the same day of collection for laboratory examination and species identification of the parasites.

3.4.2. Water Sample Collection

A total of 72 water samples (4 samples from each source and site) were collected from unprotected (river and spring water) and protected (hand pump and pipe water) drinking water sources during March-April 2017 to determine the presence or absence of water-borne parasites. Three (3) different rivers that are found in different kebeles around Ejere town were selected for sample collection. There were three (3) collection sites every hundred (100) meters for each river. There were four (4) rounds of samplings in each site. The total water samples for the rivers were 36(3×3×4).Water samples were also collected from three (3) different springs and three (3) different hand pumps that are found in and around the town. There were four (4) rounds of samplings from each water source. The total number of water samples from springs and hand pumps were 24(2×3×4). Similarly, water samples were collected in four (4) rounds from three (3) different sites of the water pipe found in the town. The total number of samples from water pipe were 12 (3×4).

Therefore, the total numbers of water samples from all water sources (rivers, springs, hand pumps and water pipe) were 72 (36 + 24 + 12).

Samples were collected using 2L capacity white plastic container. Before sample collected, each of the containers was pre-sterilized using NaOCl (Sodium Hypo Chlorate) and distilled water based on American Public Health Association (APHA, 1999) sampling procedure. The collected samples were transported to Ambo Hospital Parasitological Laboratory by keeping the samples at 4^{0} C, using appropriate insulated coolers (Ice-box) as per the standard methods (APHA, 1999). The water samples were processed immediately after arrival within 1-6 hours of sample collection to avoid the death and growth of parasites.

3.4.3. Questionnaire Survey

Data related to socio-demographic characteristics of the study subjects; age, sex, family size, source of water and its handling practices, usage of drinking water, presence or absence of latrines in their homes, parents' education level, personal hygiene and level of awareness to water-borne parasitic infections were gathered using structured questionnaire. The questionnaire was prepared in English, and translated into the local language, Afan Oromo and/or Amharic, that is, the local language spoken by the local community. Then, the questionnaire was translated back into English. A total of 384 questionnaires were administrated in the study school.

3.5. Laboratory Parasitological Examination Procedures

Stool samples were collected and transported to Ejere Health Center and the water samples were carried to Ambo Hospital Parasitological Laboratory for examinations.

3.5.1. Direct Wet Mount Method

With a marker identification number was written at one end of the slide and a drop of physiological saline was placed in the centre of the slide. With a wooden applicator stick, a small portion of stool specimen approximately about the size of a match head was taken and added to the drop of saline and was thoroughly emulsified to make a thin uniform saline suspension (not too thick that may obscure organisms, and not too thin that may leave blank spaces). The suspension was carefully covered with a cover slip in a way as to avoid air bubbles. Then the slide was placed on the microscope stage, and the preparation was examined systematically under the low power (x10) objective so that the entire cover slip area was scanned for the parasite. The high power(x40) objective was used to see more the detailed morphology of the object for confirmation (WHO, 1991). Lugol's iodine staining was also done to observe cysts of the intestinal parasites.

3.5.2. Formol-Ether Concentration

A portion of sample was processed by formalin-ether concentration method as described by WHO (2000) at Ejere Health Center laboratory. One gram of stool specimen was added to 10 ml of 10% formalin in a small beaker and was thoroughly emulsified and brought into suspension. The suspension was strained through a double layer of wet gauze directly into a 15 ml centrifuge tube. The gauze was then discarded, and more 10% formalin was added to the suspension in the tube to bring the total volume to 10 ml. 3 ml of ether was added to the suspension in the tube, and closed with a rubber stopper and shaken vigorously for 10 seconds. With an applicator stick the plug of debris was loosen by a spiral movement and the supernatant (comprising the top 3 layers) was decanted in a single movement into a bowl containing disinfectant; allowing the last few drops of residual fluid to flow back onto the sediment. A few drops of the suspension were transferred onto a microscope slide and was covered with a cover slip. Then the prepared sample was examined using the low power (10X) objective in a systematic manner to observe the entire cover slip area. The higher magnification (40X objective) was used to observe detailed morphology of ova, cysts and trophozoites.

3.5.3. Modified Ziehl-Neelsen Method

For the detection *of Cryptosporidium species* oocyst modified Ziehl-Neelsen method was used. Two thin smears were prepared directly from fresh as well from sediments of concentrated stool and allowed to air dry. Then the slides were fixed with methanol for 5 minutes and stained with carbol fuchsine for 30 minutes. The slides then washed with tap water and decolorized with acid alcohol (1ml HCl and 99ml of 96% ethanol) for 1-3 minutes. After washing the slides with tap water, it was counter stained in methylene blue for another 1 minute. Finally the slides were washed in tap water and allowed to air dry. The slides were then observed under light microscope with X10 magnification (Endeshaw *et al.,*2004). In modified Ziehl Neelsen stained smear oocysts of *Cryptosporidium* appear small, round to oval, pink red stained bodies measuring 4–6µm, whereas, *Isospora belli* was colorless and smooth cell with ellipsoid or spindle shape, measure about 22 x 15 micrometer in diameter.

3.5.4. Microscopic Examination of Water Samples

Water samples were collected from point of sources (river, spring, hand pump and pipe water). A total of 72 water samples were collected during March-April, 2017. Microscopic water examination were conducted based on USEPA method (USEPA, 2005). This method was:

-The samples were handled aseptically in sterile glass bottles, labeled and kept in ice-box during transportation.

-Samples were transferred into 15ml centrifuge tube and were sedimented at 5000rpm on centrifuge at $40^{O}C$ for 15 minute.

-Slides were prepared from control and sedimented water.

-Then slides were stained with Methylene blue.

-The stained material for both the control and samples were observed through Olympus Bx51 fluorescent microscope for the determination of the existence of water-borne parasites.

3.6. Data Analysis

In this study the following data types were collected and analyzed. These were socio-demographic characteristics of study participants (age, sex, family size, and parents' educational level); availability of latrine, level of awareness towards water-borne parasitic infections; household's source of water and handling practices; type of water-borne parasite species found in the stool and water examinations and proportions of individuals having water-borne parasitic infections among the total examined school children. Accordingly descriptive statistics, number, percentage and frequency were used. Statistical analysis was performed with SPSS software version 20. Chi square and its associated P-value were used to verify association between infection and exposure with different factors (socio-demographic risk factors). Values considered were statistically significant when the p-value obtained was less than 0.05.

3.7. Ethical Consideration

Cooperative letter to carry out the study was obtained from Natural and Computational Science of Biology Department using an agreement letter prepared by the University of Haramaya. Ethical considerations were addressed by treating patients with positive results with the appropriate treatment and drugs. Treatments were done by clinicians working at the health center. Participants were also informed that they were free to withdraw from the study at any time and their records and specimen were examined by authorized persons, and personal information's on them were treated strictly confidential.

4. RESULTS AND DISCUSSION

Knowing the prevalence and incidence rate of water-borne parasites in a given area and identification of the major water-borne parasitic species are crucial to formulate appropriate interventions. In line with this view, the present study attempted to determine the prevalence of water-borne parasites among school children and their water sources in Efa Beri Primary School, Ejere Town, West Shawa, Oromia, Ethiopia. There were 1952 students (981 males and 971 females) enrolled during 2016/2017 academic year in Efa Beri Primary School (Table 1). The results of the study showed that there were occurrences of several water-borne parasites of public health importance among school children in the study area.

4.1. Socio-Demographic Characteristics of the Study Subjects

The socio-demographic characteristics of the school children are summarized and presented in Table 2. A sample population of 384 school children were participated in the present study, of these, 193(50.3%) were males and 191(49.7%) were females. The minimum and maximum age of the study subjects were 6 and 18 years old, respectively. More than 87.5% of these students were in the age groups of 6-14 years old (Table 2).

As shown in Table 2, 46.9% and 53.1% of the respondents said that they have family size of ≤4 and ≥5 persons, respectively. With regard to their parents' educational level 45.6%, 31.7%, 13.8% and 8.9% study participants said that they were illiterate, primary education, secondary education and above 12 grade, respectively. With regared to their source of water 25.5%, 27.9%, 20.1% and 26.6% study participants said that they have pipe water, river, spring and hand pump for domestic uses respectively. 46.4 of the study participants reported the presence of latrines in close vicinity of their homes. The remaining 53.6 did not have latrines at their homes. 55.7% of the respondents were did not have awareness, while 44.3% were had awareness to personal hygiene and environmental sanitation. The majority of participants (62.8 were did not have awareness, while (37.2 were had awareness to water-borne intestinal parasites.

Table 2. Some socio- demographic characteristics of study participants in Efa Beri Primary School, Ejere Town, West Shawa Zone of Oromia Region during March-April, 2017

Characters		Frequency	Percent (%)
Sex:	Male	193	50.3
	Female	191	49.7
Age group:	6-10	161	41.9
	11-14	175	
	15-18	48	45.6
			12.5
Family size:	≤4 persons	180	46.9
	≥ 5 persons	204	53.1
Parents education level:			
	Illiterate	175	45.6
	Primary education	122	31.7
	Secondary education	53	13.8
	Above 12 grade	34	8.9
Source of water :			
	Pipe water	98	25.5
	River		27.9

Spring		20.1
Hand pump	107	26.6
Use of water: by:	77	
Boiling	102	14.6
Directly		65.6
Filtering	56	3.1
Chemical treating	252	16.6
Availability of latrine:	12	
Present	64	46.4
Absent		53.6
Do you wash properly after toilet:	178	
	206	24.2
Always		32.3
Some times	93	43.5
	124	
Never	167	
Awareness to environmental sanitation and Personal hygiene:		
	214	55.7
No		44.3
Yes	170	
Awareness to water-borne parasitic infections:		62.8
		37.2
No	241	
Yes	143	

4.2. Prevalence of Water-Borne Parasitic Infections among School Children

The overall prevalence of water-borne parasitic infections among all age groups of the study participants was 38% (Table 3). The observed prevalence of water-borne parasitic infections (38%) was lower compared to the report of some other similar studies which was 72.9% in Gonder, Azezo (Endries *et al.*,2010), 83% in Jimma (Mengistu, 2007) and 47 in Goranda village, Merhabete (Demisew Gulilat, 2014). On the other hand, the prevalence of infections with water-borne parasites observed in this study was higher than the study conducted in Babile, which was 27.2% (Taddesse, 2005). The differences in findings of various studies can be explained by variations in geography, environmental sanitation, inadequate medical care, socio-economic conditions, drinking water sources, personal hygienic conditions of the study subjects, and health care as well as prevailing climatic and environmental conditions under consideration (WHO, 1996).

The prevalence of water-borne parasitic infections in this study area was 38.8% and 37.1% in males and females, respectively (Table 3). In this study, water-borne parasitic infections among males were higher than that of females children but the difference was not statistically significant (P>0.05). Similarly, the study conducted in school children at Babile town, eastern Ethiopia showed that the prevalence of intestinal protozoan parasites among males (28.8%) was higher than that of females (24.3%) (Taddesse, 2005). In contrast to this, a study conducted by Shakya in Dhankuta and Sunsari, Nepal showed that the prevalence of intestinal parasite infections was slightly lower in males (65%) than females (66%) (Shakya, 2003). This indicated that the gender may or may not play the role in parasitic infections, depending up on the region and other environmental or behavioral factors.

The prevalence of water-borne parasitic infection in age group 6-10 years old was 43.6% and 41% in males and females, respectively (Table 3). While, for the age group 11-14 years old the prevalence of water-borne parasitic infections in male and female pupils was 35.7% and 36.3%, respectively. The prevalence of water-borne parasitic infections for the age group of 15-18 years was 34.6% for males whereas it was 27.3% for females. Generally, in each age group of the study participants there was no statistically significant differences (P>0.05 for each) in prevalence of water-borne parasitic infections between males and females children (Table 3).

As the result shown in Table 3 in both sexes, the prevalence of water-borne parasitic infections decreased as the age of children increased. Accordingly, high prevalence of water-borne parasite infections was observed in age group of 6-10 years old than older children (i.e., age groups 11-14 and 15-18 years old). A similar study in Brazil also reported that high prevalence of *Entamoeba histolytica* infection and other intestinal parasites in the age group 5-10 years old children than older children (Fleming, 2006). The possible reason for the higher prevalence of water-borne parasitic infections in the younger age group (6-10 years old) in the present study may be due to low immunity in younger children, low level of life skills such as practice of washing hands and other personal hygiene measures in this age group. In addition to this, the younger children were more exposed to over crowed living conditions (school, play grounds, nurseries etc). Higher prevalence of parasitic infections among school children may be due to the poor sanitary conditions in the schools (Oguntibeju, 2006).

Table 3: Prevalence of water-borne parasitic infections by age and sex among school children of Efa Beri Primary School, in Ejere Town, from March-April, 2017.

Age group (in years)	Male		Female		Both Sexes		χ^2	P-value
	No. Exam	No. Pos (%)	No. Exam.	No. Pos (%)	No. Exam.	No. Pos (%)		

6-10	83	36(43.6)	78	32(41)	161	68(42.2)	0.277	0.601
11-14	84	30(35.7)	91	33(36.3)	175	63(36)	0.534	0.872
15-18	26	9(34.6)	22	6(27.3)	48	15(31.3)	2.110	0.146
Total	193	75(38.8)	191	71(37.1)	384	146 (38.0)	0.831	0.773

No. Exam. = Number of examined children.

No. pos. = Number of students positive for water-borne parasitic infections.

4.3. Major Water-Borne Parasites Species Identified in Examined School Children

In the present study, microscopic stool sample examinations were done using direct, formol-ether concentration and Modified Ziehl-Neelsen techniques. The results showed that infections with various water-borne parasite species were common among the study school children. As shown in Table 4, five common water-borne parasite species were identified in examined stools of school children. The detected parasite species and their prevalence arranged in their order of dominance were *Giardia lamblia* (14.3%), *Ascaris lumbricoids* (10.7), *Entamoeba histolytica* (8.6%), *Trichuris trichiura* (2.3) and *Cryptosporidium species* (2.1%) (Table 4).

The overall prevalence of *Gardia lamblia* infection among the study participants in the present study was 14.3% (Table 4). It was higher than report of previous study, 8.6% (Endeshaw *et al*., 2004) and slightly higher than the study conducted in Côte d'Ivoire, 13.9 (Quihui *et al*. 2010). However, it was lower than 19.8% (Mehari, 2013) and the one done in Dire Dawa, Eastern Ethiopia, which was 38% (Ayalew, 2006). In current study, the prevalence of *Gardia lamblia* (14.3%) was higher than the prevalence of infection with *Ascaris lumbricoids*(10.7), *Entamoeba histolytica* (8.6%), *Trichuris trichiura* (2.3) and *Cryptosporidium species* (2.1%). The higher prevalence of *Gardia lamblia* infection might be attributed to the fact that most children in the rural areas were exposed to low level of environmental sanitation, high degree of food and water contamination with human excreta and lack of awareness in simple health promotion practices such as personal hygiene and food hygiene (Endaeshaw, 2005).

The overall prevalence of *Entamoeba histolytica* infection among the study participants in the present study was 8.6%. It was lower than report of previous study from Alemketema town, Central Ethiopia (Fetlework, 2010) which was 16.7% and the one done in Dire Dawa, Eastern Ethiopia, which was 38% (Ayalew, 2006). The prevalence of *Entamoeba histolytica* detected in the present study (8.6%) was higher than the study conducted on school children in Ginnir town, Bale zone which was 4.5% (Tadess 2013). However, it was a little bit similar to that reported by Areda (2014) (9.1%) from the study done on school children in Grawa town, Estern Ethiopia.

In the present study, the overall prevalence of infection of school children with *Cryptosporidium species* was 2.1%. It was lower than the one done in Tigray, Dura village (10.9%) (Mehari, 2013) and East Harerge, Grawa town (3.4%) (Areda, 2014). Variation in prevalence of *Cryptosporidium species* among school children might be due to low level of socio-economy (poor living condition), exposure of food and water with *Cryptosporidium species* oocysts, immunity status of the study subjects and poor personal and environmental sanitation practice among the study subjects (Gupta *et al.,* 2008). The prevalence of Cryptosporidium *species* infection observed in children would not only be the result of contaminated water and food but their contact with domestic animals could also contribute to the prevalence as the community was mainly involved in rearing cattle (Goh *et al*., 2004).

The overall prevalence of *Ascaris lumbricoides* in the present study was 10.7% (Table 4). Its prevalence (10.7%) was in agreement with previous report from Ginnir town, Bale zone (10.4%) by Tadesse (2013), but it was higher than the one reported from Lake Langano, (6.2%) by Mengistu and Berhanu (2004), and from Babile town (3.9%) by Tadesse (2005). Conversely, the result of the present study was lower than the prevalence reported in northwest Ethiopia, Chilga district, (42.9%) by Leykun (2001) and different parts of Ethiopia, (37%) by Gezahegn (2008). This variation in the prevalence of *Ascaris lumbricoides* infection most probably was an indication of the variations in the local environments with regard to water and food contamination, soil type, temperature, etc., that determine the transmission of the parasite. In addition, environmental sanitation and difference in exposure to infection probably play an important role in affecting prevalence rate of *ascariasis* (Gezahegn, 2008).

In the present study, the prevalence of *Trichuris trichiura* infection (2.3%) was slightly similar to the study reported in Mojo, (2.6%) by Alemnesh (2013) and Arba Minch, (2.8%) by Walelign (2014). However, it was lower than the study conducted in Lake Langano (14.7%) by Mengistu and Berhanu (2004), northwest Ethiopia, Chilga district, (14.8%) by Leykun (2001) and different parts of Ethiopia (30%) by Gezahegn, (2008). The observed differences in the prevalence of *Trichuris trichiura* infection in the present study from its reported prevalence might be due to differences in diverse environmental conditions of the study sites as epidemiology of parasite is highly affected by environmental sanitation and personal hygiene, surface temperature, altitude, soil type and rainfall (Brooker *et al*., 2003). The observed differences could also be explained by the fact that the prevalence and distribution of water-borne helminth parasite infections varies by place and with age in Ethiopia as reported by Yeshambel *et al.,* (2010).

Table 4: Major water-borne parasite species identified from examined school children in Efa Beri Primary School, West Shawa Zone of Oromia Region, from March-April, 2017

Age group (in age and Sex)	**N<u>o</u> exam**	**Parasites species identified**					
		Gl	Eh	Csp	Al	Tt	All
		No pos. (%)	No pos. (%)	No pos. (%)	No pos. (%)	No pos. (%)	No pos. (%)
6-10							36(43.4)
Male	83	15(18.1)	8(9.6)	2(2.4)	9(10.8)	2(2.4)	32(41.0)
Female	78	13(16.7)	6(7.7)	2(2.6)	7(9.0)		
						4(5.1)	
11-14							
Male	84	11(13.1)	4(4.8)	3(3.6)	12(14.3)	0(0)	30(35.7)
Female	91	12(13.2)	9(9.9)	1(1.1)	10(11.0)		33(36.3)
						1(1.1)	
15-18							
Male	26	3(11.5)	3(11.5)	0(0)	2(7.7)	1(3.8)	9(34.6)
Female	22	1(4.5)	3(13.6)	0(0)	1(4.5)		6(27.3)
						9(34.6)	
						1(4.5)	
						6(27.3)	
All age group							
Male	193	29(15)	15(7.7)	5(2.6)	23(11.9)	3(1.5)	75(38.8)
Female		26(13.6)	18(9.4)	3(1.6)	18(9.4)		71(37.1)
						75(38.8)	

						6(3.1)	
	191						
						71(37.1)	
Total	384	55(14.3)	33(8.6)	8(2.1)	41(10.7)	9(2.3)	146(38.0)

Key: Al=*Ascaris lumbricoides,* Csp=*Cryptosporidium* species*, Eh*=*Entamoeba histolytica,* Gl= *Giardia lamblia,* N0 exam = Number of examined, No pos = Number of positive individuals, Tt= Trichuris trichiura

4.4. Association of Water-Borne Parasitic Infections with Socio-Demographic Characteristics of School Children

This study has also analyzed correlation between socio-demographic factors of the School children and the prevalence of water-borne parasitic infections. The prevalence of water-borne parasitic infections in the study pupils was diagnosed in relation with the proportion of different socio-demographic factors and summarized and presented in Table 5.

The prevalence rate of water-borne parasitic infections in terms of family size was found to be 28.8% and 46.1% for family size of 4 and 5, respectively. Family size of students were one of the risk factors for the prevalence of water-borne parasitic infections in the present study (*P*=0.001) (Table 5). Similar study done by Sierra (2011) in Southwest Nigeria, there was statistically significant association between family size and rate of water-borne parasitic infections (*P*=0.003).

As the result shown in Table 5, among the study participants who were positive for water-borne parasitic infections, the parents of 46.3% participants were reported to be illiterate, 34.4% participants whose parents were completed the primary education, 28.3% participants whose parents were completed secondary education and 23.5% participants whose parents had above 12 were found to be positive for one of water-borne parasitic infections. The result showed that there was no statistical significance association between the parents' level of education and water-borne parasitic infections among the study participants in the present study area (P=0.193). However, as the level of education increased the rate of infection by water-borne parasites decreased. This finding indicated that the overall improvement of hygienic conditions and sanitation had increased with educational level. Another study had shown such relation between increase in educational level and lower incidence of intestinal parasite infection (Ostan *et al*., 2007).

In the present study, 178 of the students' households had latrines in their homes, out of these 31.5% were found to be positive for water-borne parasitic infections (Table 5). The households of the remaining 206 students did not have latrines in their homes; of these 43.7% were found to be positive for water-borne parasitic infections. There was statistically significant association between prevalence of water-borne parasitic infections and latrine availabilities (P=0.016) (Table 5). This might be due to the fact that presence of latrine enables to keep their environmental sanitation and personal hygiene. As a result, the probability of food and water sources contamination, which are the route for water-borne parasites transmission, becomes reduced. Under poor hygienic condition, feces and urine often enter water body due to lack of proper latrine, this enhances transmission probabilities of water-borne parasites like protozoan and helminths through indiscriminate defecation habits (Wadood *et al*., 2005).

Significant association was found between water-borne parasitic infections and awareness of participants to personal hygiene and environmental sanitation practices
(P=0.034) (Table 5). Study participants who had poor awareness to personal hygiene practices and environmental sanitation was more likely to acquire water-borne parasite infections (43%) compared to those who had good awareness to personal hygiene practices and environmental sanitation (31.8%). This may due to children with poor awareness do not take care of their personal hygiene and environmental sanitation. Usually children play in contaminated outdoor environments, in and around disposal sites (which can certainly cause serious health problems), face problems of absence of latrine, using of contaminated water and lack of basic life skills, such as washing hands before and after meals (Abu Mourad, 2004). The people in developing countries live in conditions that are highly conducive to the acquisition of water-borne parasitic infections. Poor hygiene, crowded household conditions, dietary habits, education level of the community and deficient sanitation mark their day-to-day life (Culha *et al*., 2007).

The association of water-borne parasitic infections and awareness to water-borne parasitic infections of the study participants were significant (p=0.027) (Table5). A higher prevalence of water-borne parasitic infections was found among children who had poor level of awareness about water-borne parasitic infections (41.9%) compared to those who had good level of awareness (31.5%) in the present study. This was because children's low level of knowledge may dispose them to parasitic infections through feco-oral transmission, unwashed hand after toilet and other poor hygiene practices. This finding was in line with studies conducted by United Nations Children's Fund (UNICEF) and the Ethiopian Ministry of Health found that study participants in rural Ethiopia had poor status regarding knowledge, attitude and practice of hygiene (Kumie and Ali, 2005). Approximately 60% of school children surveyed did not know about possible transmission of parasitic infection through human wastes (Kumie and Ali, 2005).

From the respondents who used river, spring, hand pump and pipe water for drinking water source, 50.5%, 44.2%, 33.3% and 24.5% were positive for water-borne parasitic infections, respectively (Table 5). The prevalence of water-borne parasitic infections was high in unprotected water sources (river and spring) than the protected sources (hand pump and pipe water) with significance variation (p=0.024). This was because poor environmental sanitation might lead to repeated contamination of rivers and springs with water-borne parasites. The association between the protected (pipe water and hand pump) and unprotected (river and spring) source of water for different purposes and prevalence of human protozoan parasites with school children was statistically significant (p = 0.00) (Areda, 2014). Analysis on protozoa and nematode infections indicated that unprotected water sources was found to be a risk factor and has a significant relationship than protected water sources (Tadesse, 2013).

Regarding the water handling practice, the result in Table 5 showed that the prevalence of water-borne parasitic infections was higher in those who used water directly as it is from the source (48%) than the others who used different water handling practices, such as boiling (5.4%), using chemical treating (26.6%) and filtering (41.7%) with significant variation (p-value=0.000). The water sources that study participants get directly from the source might be full of water-borne parasites and it can infect the school children directly. Filtration and chemical treating were also not the safest way of treating drinking water as boiling to make it save. Filtration might be done simply by using cloth and it might have large pore size. Thus, water-borne parasites can pass through pore and infect the school children. Cysts or oocysts have high resistance to the common water disinfectants such as chlorine (Fayer and Xiao, 2008). *Giardia* cysts are highly resistant to environmental conditions, being able to survive in cold mountain streams, stomach acid, chlorine and even in UV-treated waste water (Li *et al*., 2005).

Generally, the association of prevalence of water-borne parasitic infections and some risk factors such as family size, availability of latrine, awareness to environmental sanitation and personal hygiene, source of water for drinking and awareness to water-borne parasitic infections of the study participants were statistically significant. In the present study, there were high parasitic infections, but there were not statistically significant associations between some socio-demographic factors such as age and sex of participants, parents' educational level and hand wash after toilet.

Table 5: Association of water-borne parasitic infections with socio-demographic characteristics among school children in Efa Beri Primary School from March – April 2017

Character	No Study subjects	Prevalence of water borne parasites: No of + (%)	No of - (%)	X^2	P-value
Family size:4	180	52(28.8)	128(71.1)	11.521	0.001
5	204	94(46.1)	110(53.9)		
Parents educational level:					
Illiterate	175	81(46.3)	94(53.7)	4.763	0.193
Primary education	122	42(34.4)	80(65.6)		
Secondary education	53	15(28.3)	38(71.7)		
Above 12 grade	34	8(23.5)	26(76.5)		
Availability of latrine:					
Present	178	56(31.5)	122(68.5)	5.843	0.016
Absent	206	90(43.7)	116(56.3)		
Awareness to environmental and personal hygiene: No	214	92(43.0)	122(57.0)	4.489	0.034
Yes	170	54(31.8)	116(68.2)		
Awareness to water-borne parasitic infections: No	241	101(41.9)	140(58.1)	5.307	0.027
Yes	143	45(31.5)	98(68.5)		
Source of water for drinking:					
River	107	54(50.5)	53(49.5)	9.642	0.024
Spring	77	34(44.2)	43(55.8)		
Hand pump	102	34(33.3)	68(66.6)		
Pipe water	98	24(24.5)	74(75.5)		
Water source usage practice by:					
Directly	252	121(48)	131(52)	11.437	0.000
Boiling	56	3(5.4)	53(94.6)		
Filtering	12	5(41.7)	7(58.3)		
Chemical treating	64	17(26.6)	47(73.4)		

Key: No +ve =number of positive students, No –ve =number of negative students

4.5. Parasitological Quality of Drinking Water Sources in and around Ejere Town, West Shewa Zone of Oromia Region

In the present study, prevalence of water-borne parasites in different water sources (that the school children were used for drinking) was analyzed, summarized and presented in Table 6. According to this analysis, the prevalence of *Giardia lamblia* in water samples taken from river, spring, hand pump and pipe water were 58.3%, 58.3%, 25% and 8.3%, respectively. The prevalence of *Entamoeba histolytica* was 41.7%, 25%, 8.3% and 8.3% in samples taken from river, spring, hand pump and pipe water, respectively. The prevalence of *Cryptosporidium species* in water samples taken from river, spring, hand pump and pipe water were 5.6%, 16.7%, 0% and 0%, respectively. The prevalence of *Ascaris lumbricoides* was 44.4%, 33.3%, 8.3% and 16.7% in samples taken from river, spring, hand pump and pipe water, respectively. Similarly, the prevalence of *Trichuris trichiura* in water samples taken from the same water sources was 2.8%, 16.7%, 0% and 0%, respectively.

The prevalence of protozoa and helminths parasites were higher in unprotected water sources (river and spring) than protected water sources (hand pump and pipe water) (Table 6). The proportions of protozoa and helminthes parasites were high in river and spring water because, it was more exposed to contamination factors and it may contaminated during washing of clothes, washing of different materials, some people dispose waste materials to and around the river, some people wash their body at river and animal and human feces can contaminate them. Because improper waste disposal and poor sanitations are the major factors that contribute for the pollution of drinking water. The present finding had agreement with study conducted by Atnafu (2010) in Addis Ababa, explained that cysts and oocysts of protozoan parasites per samples of water in public tap is lower than source that taken from unprotected water sample. Because of protected water like tap or hand pump are not exposed to defecation and other contamination factors, *Cryptosporidium*, *Giardia* and other protozoan parasites are not common in protected water source than unprotected water source such as pond and rivers (Parry *et al*., 2004).

The result of this finding is one indication for why those school children who used unprotected water sources (river and spring) for drinking were more infected by water-borne parasites than those school children who used protected water sources (hand pump and pipe water) for drinking(Table 5).

Table 6: Major water borne parasitic species identified from examined water sources

Water sources	No of samples examined	Occurrence of parasites				
		Gl	Eh	Csp	Al	Tt
		No (%)	No (%)	No (%)	No (%)	No (%)
Rivers	36	21(58.3)	15(41.7)	2(5.6)	16(44.4)	1(2.8)
Springs	12	7(58.3)	3(25)	2(16.7)	4(33.3)	2(16.7)
Hand pumps	12	3(25)	1(8.3)	0(0)	1(8.3)	0(0)
Pipe water	12	1(8.3)	1(8.3)	0(0)	2(16.7)	0(0)
Total	72	32(44.4)	20(27.7)	5(7)	23(32)	5(7)

Key: Al=*Ascaris lumbricoides,* Csp=*Cryptosporidium* species*, Eh=Entamoeba histolytica,* Gl= *Giardia lamblia,* Tt= Trichuris trichiura

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary

The main objective of this study was to assess the major water-borne parasites among school children and their water sources in Efa Beri Primary School, Ejere Town, West Shawa Zone of Oromia Region, Ethiopia. The design of the study was descriptive cross-sectional survey of the major water-borne parasites from school children and their drinking water sources including their prevalence in Efa Beri primary school from March-April, 2017.

A total of 384 stool samples were collected from children of Efa Beri Primary School and examined using direct wet mount, Formol-Ether concentration and Modified Zeihl-Neelsen technique. After screening of 384 stool samples, the overall prevalence of water-borne parasitic infection was found to be 38.0 % (146 of 384 children). The predominant water-borne parasite detected in this study was *Giardia lamblia*, which was observed in 55(14.3%) of the students and *Ascaris lumbricoides* was the second predominant found in 41(10.7%) of the study subjects and followed by *Entamoeba histolytica* in 33(8.6%), *Trichuris triciura* in 9(2.3%) and *Cryptosporidium* species in 8 (2.1%) of the study subjects.

Socio-demographic characteristics of study subjects like family size, availability of latrine, awareness to environmental sanitation and personal hygiene, source of water for drinking and water source handling practice were major risk factors which are significantly associated with the prevalence of water-borne parasites (p=0.001, p=0.016, p=0.034, p=0.024 and p=0.000, respectively). Although there were high water-borne parasitic infections, some socio-demographic characters like age, sex, and parents education level were not significantly associated with the prevalence of water-borne parasitic infections in the present study.

The result of water sources analysis indicates that the prevalence of water-borne parasites were higher in the samples taken from unprotected water sources (river and spring) than protected water sources (hand pump and pipe water).

5.2. Conclusions

The major water-borne parasites diagnosed in the school children and drinking water sources in the present study were *Giardia lamblia, Entamoeba histolytica, Cryptosporidium* species*, Ascaris lumbricoids and Trichuris triciura.* Most water-borne parasites infections represent a public health threat because of their water-borne transmissions. An increasing prevalence of water-borne parasitic infections in this study was associated with factors such as large family size, absence of toilet, poor personal hygiene and environmental sanitation, poor handling practice of water source, less awareness to water-borne parasites and poor quality of water for consumption. High concentration of water-borne parasites in water samples taken from unprotected water samples than protected water samples in the present study indicates that, the requirement of water development project in the study area. This plays an important role in reducing illness caused as a result of water-borne parasites. Creating awareness and health education particularly with health sector should be conducted side by side with water project development.

5.3. Recommendations

High proportion of water-borne parasites in the present study indicates that more efforts are expected to be done in improving the health of the community from water-borne parasitic infections. This invites for the initiation of control measures to water-borne parasitic infections including health education concerning water-borne parasites, treatment of infected individuals, improvement of sanitation and provision of clean water necessary for school children and community. So the decision makers should mobilize the community, particularly to school children to improve health situations through;

- Creating good awareness and health education to personal hygiene and environmental sanitation in their local languages, such as hand washing after using the toilet and before handling food, proper handling of drinking water source, proper defecation of feaces etc.
- Provision of adequate and safe water supply.
- Cost effective water purification mechanisms such as boiling and chlorination.
- Moreover, in-depth studies should be made on socio-demographic factors like, latrine usage, family size, education level and water usage practices to better evaluate the epidemiology of parasitic infection in the study area.

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7. APPENDICES

Appendix I: Questionniare to be completed by Children's parents (care takers) (English version).

Kindly give response to the questionnaire taking into consideration that this data will be employed only for scientific research; abiding by top confidentiality and privacy. It is your special response that will help to conduct the study aimed to study the Prevalence of water-borne parasites among school children and drinking water sources and its associated risk factors at Efa Beri primary school, Ejere Town, West Shawa, Oromia, Ethiopia.

Date___________________

1. Sex A/Male B/Female
2. Age___________
3. What is your family size? A) ≤4 B) ≥ 5
4. Parents educational status?
   A. Illiterate B. Primary C. Secondary D. Above 12
5. From where do you get your water for drinking, washing and other utilization?
   A. pipe water B. river C. spring D. hand pump
6. How do you use water from the source?
   A. by boiling B. directly C. by filtering D. by treating with Chemical
7. Do you have your own latrine? A. present B. absent
8. If no to question No 8, where do you defecate and dispose the feces?
   A. Near the river B. Away from the river
9. Do your parents wash or do you wash properly after toilet? A) Always B) Sometimes

   C) Never
10. Do you have awareness to environmental sanitation and personal hygiene?
   A/ No B/ Yes

11. Do you have awareness to water-born parasitic infections? A/ No B/Yes

Appendix II. (Afan Oromo Version) Gaaffannoo Afaanii Kan maatii (guddistuu)

Ijoollee baratoota Waliin Taasifamu

Gaaffiiin armaan gaditti dhihaate qorannoo ijoollee baratootaa mana barumsa Ifa Barii,magaalaa Ejeree, Lixa Shawaa, Oromiyaa, Etoopiyaa keessatti argaman irratti waa'ee argamiisa maxxantuu bishaaniin daddarbanii daa'imman mana barnootaa fi madda bishaan dhugaatii akkasumas wantoota sababa ta'an qorachuuf qofa waan ta'eef isinis isa kana hubattanii deebii sirrii ta'e akka naaf laattan kabajaan isin gaafadha.

Guyyaa___________

1. Saala A. dhiira B. dubara

2. Umurii___________

3. Baa'inni maatii keessanii meeqa A. ≤ 4 B. ≥ 5

4. Sadarkaa barnoota maatii? A. Hin baranne B. sad.1ffaa C. sad.2ffaa D. kutaa 12 ol

5. Maddi bishaan dhugaatii, dhiqannaa fi kanneen biro eesaa argattu?

A. Bishaan ujummoo B. Laga C. Burqaa D. Paampii harkaa

6. Bishaan naannoo keessan irraa argattan akkamitti itti fayyadamtu?

A. Danfisuun B. Kallatumaan C. Dhinbibun D. Keemikaalan qulqulleessun (wuhagar)

7. Mana fincaanii ni qabduu? A. Eeyyee B. Lakkii .

8. Yoo mana fincaanii hin qabdan ta'e eessatti bobbaa teessu? A. Laga cinatti

C. Laga irra siqne

9. Mana fincaanii booda harka ni dhiqatuu? A. Yeroo mara B. Yeroo tokko tokko

C. Hin dhiqannu

10. Hubannoon waa'ee qulqullina naannoo fi dhuunfaa irratti qabddan maal fakkaata?

A. xiqqaa dha B.Gahaa dha

11. Hubannoon waa'ee dhukuboota bishaaniin daddarbanii irratti qabddan maal fakkaata? A. xiqqaa dha B. Gahaa dha

Appendix III. Laboratory Data Collecting Format

Code	Sex	Age	Type of water-borne parasites identified	Remark

WAAJJIRA EEGUMSA FAYYAA
AANAA EJEREE

EJERE WOREDA HEALTH OFFICE
የኤጀሬ ወረዳ ጤና ጥበቃ ጽ/ቤት

Ref.no/ቁጥር /Lakk X-1/18
Date/ቀን/Guyyaa 11/07/09

Obbo Tasgaraa Moosisaatiif

Ejeree

Dhimmi isaa:- Qorannoo fi qo'annoo akka gaggeessituuf hayyamni(Ethical clearance)**siif kennamuu isaa si beeksisuu ta'a**

Akkuma armaan olitti ibsamuuf yaalametti **iyyannoo dhuunfaa** gaafa **30/06/09** barreeffamaan nu gaafatteen mata duree "Assessment of Water-Borne parasites among School children and their water sources in Efa Beri primary school,Ejere Town, West showa, Oromia,Ethiopia" jedhu irratti Qorannoo fi qo'annoo akka gaggeessituuf hayyamni(Ethical clearance) nuu gaafattee jirta propozaalli kees Universiitii Haramayyaattii fudhatama argachuu isaa raga dhiyaate irraa waan hubanneef qorannoo fi qu'annoo kana akka gaggeessituu siif hayyamamuu isaa si beeksifna.

Nagaa Waajjin !

Ajjamaa Nagaasaa Gabbisaa
Itt. Gaafatamaa Waajjira Eegumsa Fayyaa Aanaa Ejeree

G/G

- *Waj. Keenyaaf*
- *Univarsiitii Haromayyaatiif*

  Haromayyaa
- *M/B/Ifa Barii sad1^{ffaa}tiif*

  Ejeree

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EJEREE