PREVALENCE OF INTESTINAL PROTOZOAN PARASITIC INFECTIONS AMONG KINDERGARTEN CHILDREN AND ITS ASSOCIATIONS WITH PARASITOLOGICAL QUALITY OF WATER SOURCES IN WOLISO TOWN, OROMIA REGIONAL STATE, ETHIOPIA

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Prevalence of Intestinal Protozoan Parasitic Infections among Kindergarten Children and its Associations with Parasitological Quality of Water Sources in Woliso Town, Oromia Regional State, Ethiopia

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As thesis research advisors we hereby certify that we have read and evaluated this Thesis entitled "**Prevalence of Intestinal Protozoan Parasitic Infections among Kindergarten Children and its Associations with Parasitological Quality of Water Sources in Woliso Town, Oromia Regional State, Ethiopia"** prepared under our guidance by Tsegaye Shiferaw**.** We recommend that it be submitted as fulfilling the thesis requirement.

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DEDICATION

This thesis is dedicated to my wife Nobel Siyoum, my son GenenusTsegaye and to my daughter Sonan Tsegaye for their dedicated partnership in the success of my thesis work.

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 completion of this thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

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ACRONYMS AND ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
CDC	Center for Disease Control
CSA	Central Statistical Agency
EHNRI	Ethiopian Health and Nutritional Research Institute
IPPI	Intestinal Protozoan Parasite Infection
KG	Kindergarten
NCCLS	National Committee on Clinical Laboratory Standard
PVA	Poly Vinyl Alcohol
PCR	Polymerase Chain Reaction
RPM	Rotation Per Minute
SPSS	Statistical Package for Social Sciences
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WTWSA	Woliso Town Water System Authority

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Prevalence of Intestinal Protozoan Parasitic Infections among Kindergarten Children and its Associations with Parasitological Quality of Water Sources in Woliso Town, Oromia Regional State, Ethiopia

ABSTRACT

Intestinal protozoan parasites are among the most common human infections globally. They are distributed throughout the world with high prevalence rates especially in developing countries including Ethiopia. This study was conducted to assess the prevalence of intestinal protozoan parasitic infections among kindergarten children and its associations with parasitological quality of water sources in Woliso town. The design of the study was a descriptive cross-sectional survey involving a sample population of 384 Kindergarten children at Woliso town. The children were selected from three kindergartens using stratified random sampling method. A total of 384 fresh stool samples of the study participants and 105 water samples were collected from three types of water sources and were examined using wet-mount, Formol-Ether concentration and Modified Ziehl Neelsen methods. Among 384 stool samples examined 11.5%, Entamoeba histolytica, 8.9% Giardia lambilia and 1.6% Cryptosporidium species were found in KG children. With respect to parasitological analysis, the prevalence of Entamoeba histolytica, Giardia lamblia and Cryptosporidium species in tap water were 17.1%,11.4% and 0%, respectively; and in the tap water that were kept in tanker the prevalence were 22.9%, 17.1% and 5.7%, respectively; and for borehole water were 28.6%,40% and 11.4%, respectively. There was statistically significant association between the prevalence of intestinal protozoan parasitic infection among Kindergarten children with parasitological quality of water sources (p<0.05).This suggested that environmental sanitation, water treatment, personal hygiene and awareness creation are needed to reduce the prevalence of intestinal parasitic infections among school children in Woliso town.

Key Words: *Intestinal protozoa, Kindergarten, Prevalence, Water quality, Woliso Town*

1. INTRODUCTION

Protozoa are a diverse group of eukaryotic microorganisms in which majority of them are free-living that can reside in fresh water and others are enteric protozoan parasites, which are common in the intestine of human and animals. Enteric protozoan parasites are transmitted via drinking water which is contaminated directly or indirectly by the feces of humans or other animals and have been associated with several waterborne disease outbreaks in many parts of the world (Karanis *et al*., 2007).

Human beings are exposed to diverse groups of parasites. Intestinal protozoan parasites are among the most common human infections which are distributed throughout the world with high prevalence rates in developing countries including Ethiopia (Raza and Sami, 2008).

Intestinal protozoan parasites are identified as causes of morbidity and mortality throughout the world particularly in developing countries including Ethiopia (De Silva *et al*., 2003). In addition to considerable mortality and morbidity, infection with intestinal protozoan parasites have been found to profoundly affect child's mental development, diminish learning ability, growth, physical fitness and also increase host`s susceptibility to other infectious agents (Elias *et al*., 2001; Karaman *et al.*, 2006).

Infants, toddlers and other young children are reported to be most vulnerable for the intestinal protozoan parasitic infections. One reason was that they are often associated with a greater exposure to those infectious agents by virtue of unsanitary practice associated with child development, e.g. playing in dirty places and water, sucking on dirty finger and dirty objects (Sebastiaan *et al*., 2007).

The intestinal protozoan parasites are the major public health problems in tropical regions especially among poor communities (Dianou *et al.*, 2004, Legesse and Erko, 2004). Among the conditions influencing the developments of intestinal protozoan parasitic infections are poverty, poor personal hygiene, malnutrition, poor sanitary conditions, lack of safe drinking water supply, lowering resistance of the host, and lack of awareness about transmission of the parasite and these conditions lay stage for the continuous transmission of the intestinal protozoan parasitic infections (Stephenson *et al*., 2000, Olsen *et al*., 2001).

The most common intestinal protozoan parasites are *Entamoeba histolytica, Giardia lamblia,* and *Cryptosporidium* species. The diseases caused by these intestinal protozoan parasites are known as amoebiasis, giardiasis, *and* cryptosporidiosis, respectively, and they are associated with diarrhea (Davis *et al*., 2002).

Entamoeba histolytica infection is found throughout the world, it is more common in tropical countries or other areas with poor sanitary conditions. About 10% of the world's population is infected with *Entamoeba histolytica*, and in many different countries the prevalence may approach 50% (Bethony *et al*., 2006). Amebiasis is an infection caused by an intestinal protozoan, *Entamoeba histolytica*. Most developing countries in the tropics are the highest incidence areas of this disease due to inadequate sanitation and crowding (Farhana *et al*., 2009). Humans are the only host of *E. histolytica* parasite passing virulent cysts that are transmitted chiefly by ingestion of contaminated water or food or through direct contact (Bethony *et al*., 2006; Haque *et al*., 2003).

Giardia lamblia is another major intestinal protozoan parasite found mostly in temperate and tropical countries and commonly causes diarrheal disease throughout the world. The prevalence rate of giardiasis has been found to be 2 to 7% in developed countries and 20 to 30% in developing countries (Jerlstrom-Hultqvist *et al*., 2010). The variation in prevalence depends on factors such as geographical area, the urban or rural setting of the society, the age group composition and socio- economic conditions of the study subject (Sebastiaan *et al*., 2007). Generally, children are more frequently infected by *G.lamblia* than adults particularly those who are malnourished (Mehraj *et al*., 2008). The infection is acquired through the ingestion of cysts. Factors leading to contamination of food or water with fecal material are correlated with transmission (Partovi *et al.*, 2007).

Cryptosporidium species are coccidian opportunistic intestinal protozoan parasites. Cryptosporidiosis is the most common protozoan intestinal parasite infection which is caused by *Cryptosporidium* species. It causes severe diarrhea in immune- compromised patients (Escobedo *et al*., 2009). Currently several species of *Cryptosporidium* are considered to be valid and of which *Cryptosporidium hominis* (*C. hominis*) and *C. parvum* are most commonly detected from various water samples and feces of humans (Hunter and Thompson, 2005). Cryptosporidiosis is most frequently spread by direct person to person-transmission through the fecal oral route or by sputum and vomits and zoonoticaly from cattle and sheep. Indirectly, it is spread through the environment particularly through water (Chen, 2002).

In Ethiopia, a high prevalence of intestinal protozoan parasitic infection depends on many factors. These include socio demographic variables associated with illiteracy and poverty such as reduced access to adequate sanitation, potable water and health care, as well as the prevailing climatic and environmental conditions (Walelign, 2014). For instance, Ethiopia has one of the lowest quality of drinking water supply and latrine coverage (WHO, 2010).

Woliso town is the capital of South West Shoa Zone in central Ethiopia, located in Oromia Regional State. One of the major problems encountered in the town is the extremely hazardous sanitary condition caused by raw sewerage coming out of residential houses, health and service institutions. Collected excreta and garbage are often transported in unhygienic condition and dumped on the periphery of the town without any treatment. These wastes undoubtedly will eventually gain access into the town's drinking water sources and cause serious health problems in community.

Even though, several studies have been conducted on prevalence and associated risk factors of intestinal protozoan parasites in some towns of Ethiopia, there has been no previous study conducted in this study area regarding the prevalence of intestinal protozoan parasitic infections among kindergarten children and the associations with parasitological quality of water sources.

This study was conducted to determine the prevalence of intestinal protozoan parasites of KG children and to assess the parasitological quality of drinking water sources in the study area.

General Objective was:

To assess the prevalence of intestinal protozoan parasitic infections among kindergarten children and its associations with parasitological quality of the water sources in Woliso town.

Specific Objectives were:

1) To determine the prevalence of intestinal protozoan parasitic infections among kindergarten children in the study area.
2) To identify the common intestinal protozoan parasite species among kindergarten children in the study area.
3) To assess the parasitological quality of water sources utilized by the study population.
4) To associate the intestinal protozoan infections of kindergarten children with parasitological quality of the water sources they use.

2. LITERATURE REVIEW

2.1. Human Intestinal Protozoan Parasitic Infections

Protozoa are a diverse group of organisms that have evolved to occupy a variety of ecological niches. There are over 30 phyla of protozoa; most of these have evolved totally for parasitic existence. The enteric protozoa that cause human illnesses are usually transmitted by the consumption of food and drink, or through environmental contamination and poor hygiene. Some of these can cause substantial illnesses, and have economic consequences.

Intestinal protozoan diseases are caused by unicellular microorganisms and invade the wall of the intestine such as Amebiasis, Giardiasis, and Cryptosporidiosis. Numerous protozoa inhabit the gastro-intestinal tract of humans. The majorities of intestinal protozoa parasites are non-pathogenic commensals, or only result in mild disease. Some of these organisms can cause severe disease under certain circumstances. *Apicomplexa* and *microsporidia* species, which normally do not evoke severe disease, can cause severe and life-threatening diarrhea in AIDS patients and other immune compromised individuals (Adamu *et al*, 2006).

Intestinal protozoan parasite infections are a significant problem with more than 58 million cases in children each year. Pathogenic intestinal protozoa are especially important in the developing world where they may cause death. Most intestinal protozoan parasites are spread by faecal–oral contact or contamination of water or food. Poor sanitation and poverty are contributory factors in many low income countries. Symptoms of intestinal protozoan parasite infections include diarrhea, abdominal pain, and nausea, vomiting and weight loss.

2.2. Life Cycle of Intestinal Protozoan Parasitic Infections

Several members of the genus *Entamoeba* infect humans. Among these only *E. histolytica* is considered pathogenic and the disease it causes is called amoebiasis or amebic dysentery. E. dispar is morphologically identical to E.histolytica. The two species are found throughout the world, but like many other intestinal protozoa, they are more common in tropical countries or other areas with poor sanitary conditions. It is estimated that up to 10% of the world's population may be infected with either *E. histolytica* or *E. dispar* and in many tropical countries the prevalence may approach 50%. It is also estimated that about 100,000 deaths and 50 million cases of amoebiasis occur per year in the world and humans are the only host of *E. histolytica* and there are no animal reservoirs (Haque *et al.*, 2003).

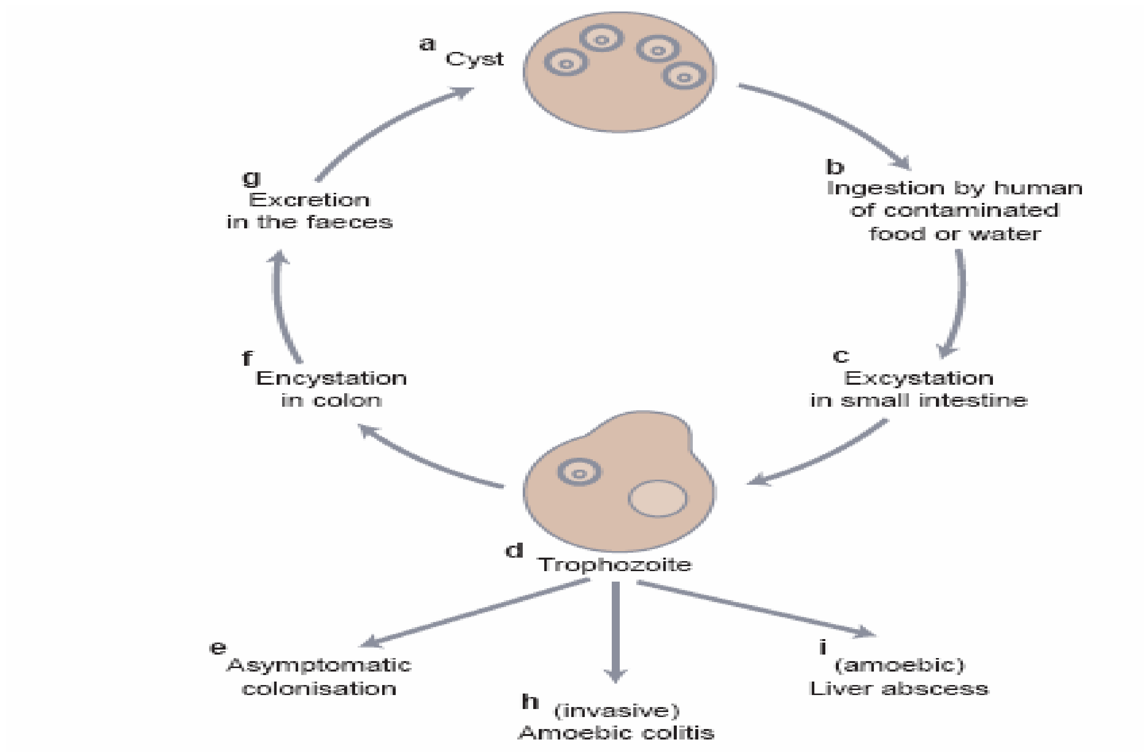

Figure 1. Life cycle of *Entamoeba histolytica (Source*:-http://www.dpd.cdc.gov/dpdx*)*.

The life cycle of *Entamoeba histolytica* as showen in Figure-1 includes the infective cyst and the invasive trophozoite forms. Infection is acquired by ingestion of infectious cyst through water or undercooked food contaminated by human feaces. After ingestion of the cyst, which is resistant to gastric acids and enzymes, excystation occurs in the ileoceacal area of the intestine to form trophozoites. The trophozoites are larger in size and actively motile organisms. According to the bind-lyse-eat model, the trophozoites bind to the large intestine and invade the wall releasing phospholipases, causing ulceration of the mucous membrane (called flask shaped ulcers), and sometimes large vessels may be eroded and severe intestinal hemorrhage result (Haque *et al.*, 2003).

The life cycle of *Cryptosporidium* as shown in Figure 2 commences after ingestion of the infective stage, the oocyst, by a susceptible host. The oocyst is spherical in shape measuring 3-6μm in diameter and it may be either thick or thin walled the resistant stage that is found usually in the environment is the thick walled oocyst excreted together with feces or it is resistant stage found in the environment. Thin-walled oocysts may excyst within the same host and start a new life cycle (autoinfection). This can lead to heavily infected intestinal epithelia and result in malabsorptive or secretory diarrhoea. 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 (Hijjawi *et al*., 2001).

Cryptosporidium resides on the luminal surface of epithelial cells and it is used to be thought to reside extracellularly. However, ultra structural observations have revealed that it is intracellular but extracytoplasmic, enclosed by a thin layer of host cell cytoplasm. Infection could possibly occur with ingestion of as few as 30 oocysts; some infection have also occurred with just a single oocyst (Ramirez *et al*., 2004).

Cryptosporidium 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 undergoes asexual proliferation by merogony and two types of meronts are produced, Type I meronts and Type II meronts. 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 into 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*. The first is by the continuous recycling of Type I meronts and the second through sporozoites rupturing from thin-walled oocyst (Akiyoshi *et al.*, 2003).

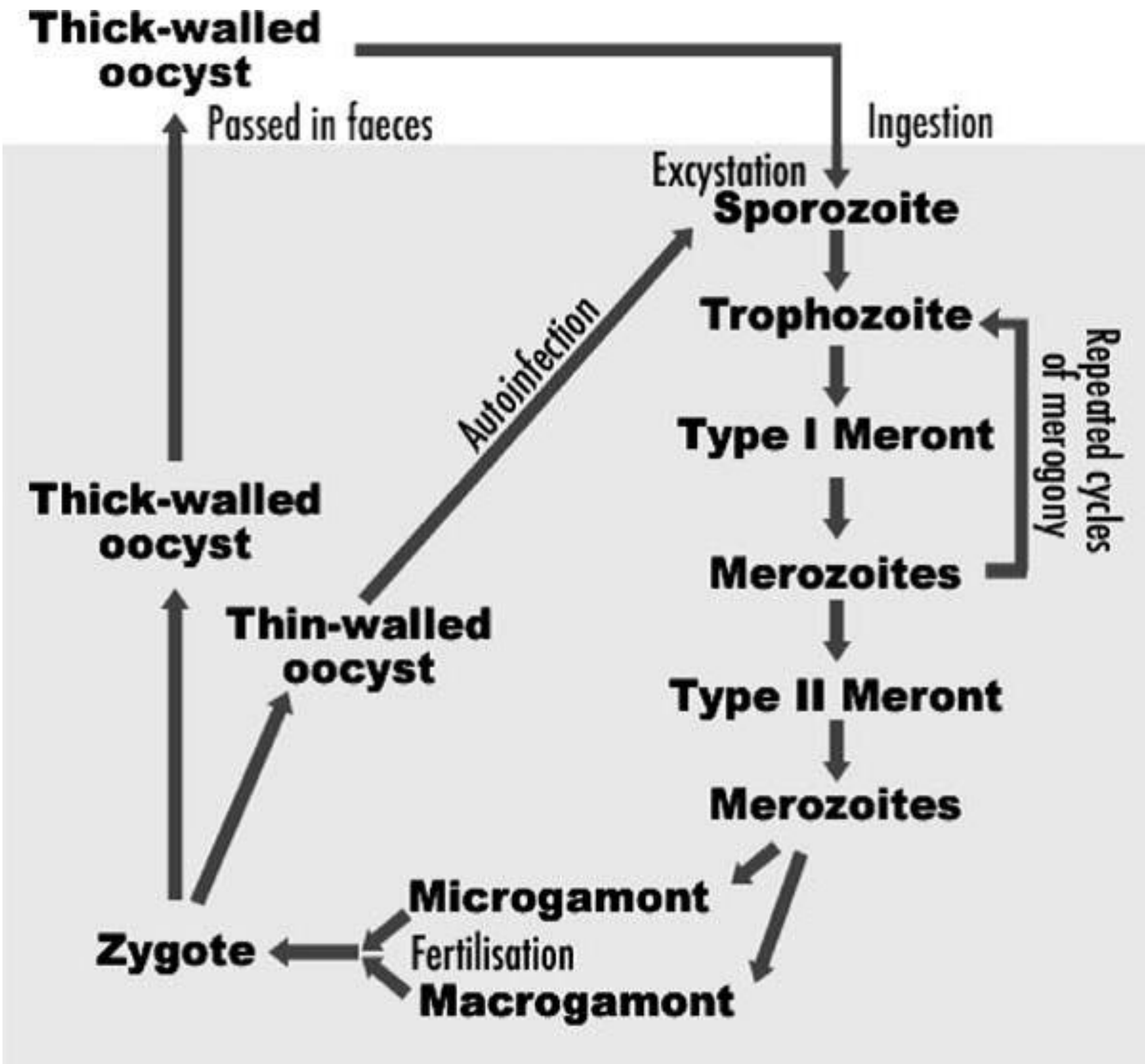

Figure 2. Life cycle of *Cryptosporidium parvum* .

The parasite *Giardia lamblia* reproduces by binary fission which is a type of reproduction in which one cell divides into two new cells by mitosis as shown in Figure3. During the growth cycle the components of the *Giardia* cell multiply so that each daughter cell would be a complete copy of the original parent cell. The newly formed cells then pinch off from each other; in so doing a complete reproduction cycle would occur the infective stage of *Giardia lamblia*, the cyst, is elliptical in shape and its size ranges from 6 to 10 microns and contains two to four nuclei. The cyst possesses a structure that enables it to be resistant to most environmental factors and disinfection and make it successful in being the infective stage of the parasite. The cyst has a thin and protective wall that allows it to survive in feces for weeks or for about 3 months in water at $40^{o}C$ (Jerlstrom-Hultqvist *et al*., 2010).

Giardiasis could be contracted through drinking contaminated waters or ingestion of contaminated food stuffs. The cyst passes through the stomach and enters the small intestine. The acidic environment of the stomach could not harm the cyst because it has a thin protective wall to protect it until it reaches the alkaline environment, the small intestine .This alkaline environment initiates excystment of the cyst (Erlandsen and Mayer, 1990). During excystation, the cyst wall raptures at the pole opposite to the nuclei so that the flagella and other projections emerge from the rupture point. The cyst wall is then completely shed and the parasite will enter into its trophozoite stage.

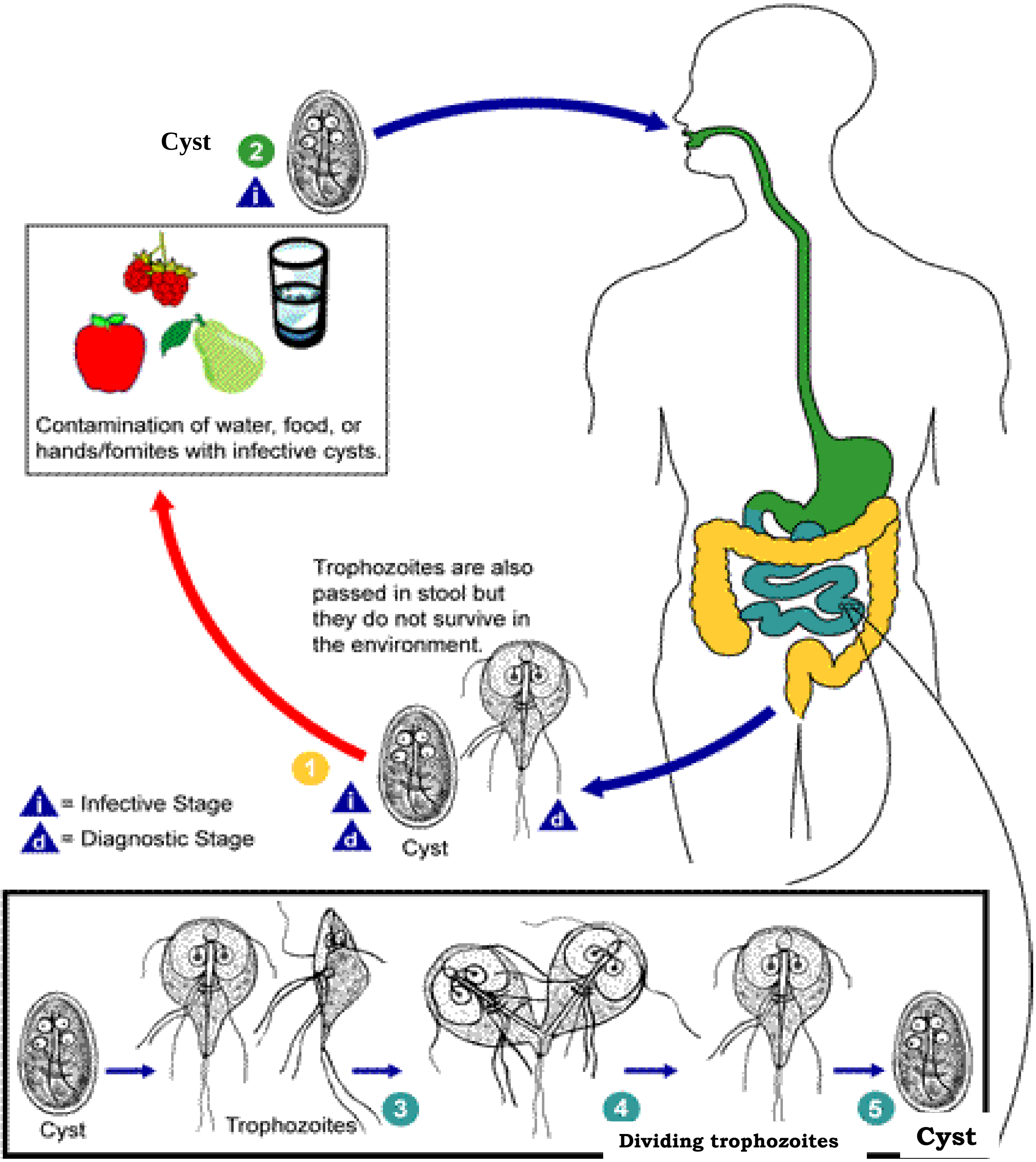

Figure 3. Life cycle of *Giardia lamblia* (source:-http//www.dpd.cdc.gov/dpdx)(last modified:12/05/2008).

2.3. Water-Borne Intestinal Protozoan Parasites

Water-borne diseases are caused by pathogenic microorganisms that most commonly transmit by contaminated water infection commonly results during bathing, washing, drinking, in preparation of food and consumption of food. Various forms of water born diarrheal disease are the most prominent examples (Jump *et al*., 2006). Water-borne intestinal protozoan parasites including organism such as *Amoeba*, *Cryptosporidium* and *Giardia* have become a challenge to human health worldwide. These protozoan have several common characteristics biologically. Their major habitat is intestinal epithelial cells. In addition, they produce infectious spores that are excreted from the hosts in their stools and *Giardia* produces cyst (Akiyoshi *et al.*, 2003).

Cryptosporidium is a coccidian parasite and one of the many genera of phylum Protozoa. Currently there are thirteen species of *Cryptosporidium* categorized based on differences in host specificity, oocyst morphology and site of infection, and most of them infect only one or a few groups of animals. The genus *Cryptosporidium* comprises parasites that grow and reproduce within epithelial cells of the digestive organs and the respiratory tract of vertebrates. It has a monoxenous lifecycle; all stages of development (asexual and sexual) occurring in one host (Hijjawi *et al*., 2001).

These parasites are enclosed by a thin layer of host cell cytoplasm. Once the oocyst is ingested, the host body temperature, the interaction with stomach acid and bile salts triggers excystation and releasing infective sporozoites in the gastrointestinal tract (Li *et al*., 2005). After oocyst excystation in the intestinal lumen, sporozoites penetrate the host cell and develop into trophozoites within parasitophorous vacuoles located in the microvillous region of the mucosal epithelium.

2.4. Pathogenesis and Clinical Manifestation of Human Intestinal Protozoan Parasitic Infections

Intestinal protozoan parasite infection can result in Gastro intestinal disease in humans. As a result of infection of the parasite more or less similar clinical signs and symptoms can be observed. For example, infections with *E. histolytica* have no symptoms in many individuals, and most can clear their infections without any signs of disease.

For unexplainable reason, however, 4-10 % of asymptomatic individuals infected with *E. histolytica* develop disease over a year. In other words, different studies indicate that in upto 90 % of *E. histolytica* infections, the symptoms are absent or very mild.

There is a wide spectrum of clinical presentations of *E. histolytica* infections. Symptomatic amebiasis is primarily an intestinal disease, and when it becomes extra intestinal, it usually involves the liver. Pathogenesis of amebiasis is believed to be a multi step, multi factorial process. Though a large number of studies have attempted to unravel the factors/molecules responsible for the pathogenesis of amebiasis, the processes involved in pathogenesis are poorly understood. The aspects of pathogenesis which have been investigated experimentally can be broadly categorized into mechanisms involving (i) interactions with the intestinal flora, (ii) lysis of target cell by direct adherence, (iii) lysis of target cell by release of toxins and (iv) phagocytosis of target cells (Sehgal *et al*.,2010).

Symptoms of Amebiasis 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).

G. lamblia is usually weakly pathogenic for humans. Cysts may be found in large numbers in the stools of entirely asymptomatic persons. In some persons, however, large numbers of parasites attached to the bowel wall may cause irritation and low-grade inflammation of the duodenal or jejunal mucosa, with consequent acute or chronic diarrhea associated with crypt hypertrophy, villous atrophy or flattening, and epithelial cell damage. The stools may be watery, semisolid, greasy, bulky, and foul-smelling at various times during the course of the infection. Malaise, weakness, weight loss, abdominal cramps, distention, and flatulence can occur. Children are more liable to clinical Giardiasis than adults. Immuno suppressed individuals are especially liable to massive infection with severe clinical manifestations. Symptoms may continue for long periods (Butel and Stephen, 2007).

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. Light microscopy may demonstrate no abnormalities, mild or moderate partial villous atrophy, or subtotal villous atrophy in severe cases. An increase in crypt depth may be seen, and microvilli shortening or disruption may occur. Deficiencies in epithelial brush border enzymes, such as lactase, may develop (Buret, 2011).

As in any parasitic infections, host parasite interaction is the initial steps in the pathogenesis of giardiasis. In this interaction, first the *Giardia* trophozoites attach to the cell surface of villi by means of a disk on their posterior or ventral surface. Lectin, a protein on the trophozoite lining, recognizes specific receptors on the intestinal cell and may be partly responsible for the tight attachment between the parasite and the villi following attachment of trophozoites, there will be major structural and functional abnormalities in the small intestine. Some of these abnormalities include mucosal damage as a result of mechanical obstruction or blockage of the intestine by a large number of parasites, the release of cytopathic substances such as thiol proteinases water intended for consumption, thoroughly washing hands before handling food, maintaining good personal cleanliness, properly disposing of fecal material and information dissemination through print media to educate the public regarding the dangers of giardiasis (Backer, 2000).

The pathogenesis of *Cryptosporidium are* associated with diarrhea, 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.

2.5. Epidemiology and Transmission of Intestinal Protozoan Parasitic Infections

2.5.1. Global epidemiology of intestinal protozoan parasites infections

Intestinal protozoan parasitic infections enjoy a wide global distribution. They are estimated to affect 3.5 billion people and most of them are children and young residing in developing countries.

Intestinal protozoan parasitic infections are endemic worldwide and have been described as constituting the greatest single worldwide cause of illness and disease. Poverty, illiteracy, poor hygiene, lack of access to potable water and hot and humid tropical climate are the factors associated with intestinal parasitic infections. Intestinal protozoan parasitic infections (IPPI) constitute a global health burden causing clinical morbidity in 450 million people, many of these women of reproductive age and children in developing countries (Quihui *et al*.,2006).

The high occurrence of amoebiasis is often related to poverty, poor living conditions and hygiene, and inadequate sanitation and water supply. *Entamoeba histolytica* has been recovered worldwide and is more prevalent in the tropics and sub-tropics than in colder climates. However, in poor sanitary conditions in temperate and colder climates, infection rates have been found to equal that seen in the tropics. In a related study in Ardabil Iran, a total of 10 species were identified with *Giardia lamblia* (14%), *Blastocystis hominis* (10%) and *Entamoeba coli* (4.1%) being the most common parasites (Aksoy *et al*., 2005).

Giardia lamblia also has a worldwide distribution with an incidence rate of between 11% and 30% and it is now considered to be the most common intestinal parasite of man and the leading cause of diarrhea due to protozoan infections in humans (Beltran *et al*., 2004). The intensity of infection is a major determinant of morbidity and approximately reflected in the number of characteristic cysts passed out in faeces (Kongs *et al*., 2001). In Africa, Asia and Latin America about 200 million cases have been estimated to occur annually.

Cryptosporidium is known to cause diarrheal diseases in immune competent people and shown to be especially common among persons with AIDS or other forms of immunodeficiency. The application of PCR assays to identify *Cryptosporidium* from stool samples has shown that *C. hominis* and *C. parvum* are the major causes of human cryptosporidiosis (Cacciò *et al*., 2005).

2.5.2. Epidemiology of intestinal protozoan parasitic infections in Ethiopia

In Ethiopia surveys across all regions of the country show giadiasis prevalence to be around 10% in the 1970s and early 1980s and it is more common in children than in adults.

According to 1996 Federal Ministry of Health of Ethiopia reported that more than half a million out patients visited hospital/clinic due to intestinal protozoan parasitic infections. However, this might be an underestimate, as most of the health institutions lack appropriate diagnostic methods to detect parasites with small detection limits (WHO, 2009).

In addition, some of the diagnostic methods for specific intestinal parasites, especially for the newly emerging opportunistic intestinal parasites, were not available to most health institutions. Among the common intestinal protozoan parasites *Giardia lamblia* and *Entamoeba histolytica* are widely distributed in Ethiopia (WHO, 2009).

Cryptosporidium is now becoming a common opportunistic intestinal parasite in Ethiopia even though it is not diagnosed routinely. Reports from different parts of the country showed different prevalence rates of cryptosporidiosis. Recently a study conducted in Lege Dini, rural area in Dire-Dawa, showed the prevalence of cryptosporidiosis to be 12.2 % (Ayalew, 2008).

Another report indicated that the prevalence of *Cryptosporidium* among diarrheal patients was 20.6 %) and the prevalence of *Giardia lamblia* among diarrhea patients referred to EHNRI (Ethiopian Health and Nutrition Research Institute) was 8.6% (Endeshaw *et al*., 2004).

2.5.3. Factors affecting epidemiology and transmission of human intestinal protozoan parasitic infections

Each environmental change of natural phenomena or through human intervention alters the ecological balance. Deforestation and ensuring changes in land use, human settlement, commercial development, construction of roads, water control systems (dams, canal, irrigation system) and climate change have been accompanied by global increases in morbidity and mortality from a number of emergent parasitic diseases hence changes in types and amounts of bodies of water, temperature, pH, movement, and changes in climatic condition affects prevalence and risk factor of intestinal protozoan parasite infections.

Intestinal protozoa are transmitted by the fecal-oral route, water-borne and exhibit life cycles consisting of a cyst stage and a trophozoite stage. The cysts consist of a resistant wall and are excreted in the feces. The cyst wall functions to protect the organism from desiccation in the external environment.

Unhygienic conditions promote transmission of most protozoa (Gascón *et al*., 2000). The result of inter-related social, economic, cultural, historical, and political factors control strategies involving improved drinking water supplies, excreta disposal, sewage management, sanitation, and education have been related with reduced prevalence of intestinal parasitism. Programmes of nutrition, immunization, family planning, and de-worming have been shown to effectively promote health by influencing the knowledge, perceptions, and behaviour of mothers toward intestinal parasitic infections. (Wamani *et al*., 2004)

2.6. Diagnosis of Human Intestinal Protozoan Parasitic Infections

Intestinal protozoan parasites are widely prevalent causing considerable medical and public health problem in developing countries. Malabsorption, diarrhea, blood loss, impaired work capacity, and retarded growth can be associated with these intestinal infections. Some infections occur focally in school and preschool age children (Kanmarnee *et al*., 2004).

Diagnosis of *E. histolytica* has relied on microscopic examination of protozoan morphology, but examinations by this method are unable to differentiate among protozoa with similar morphological features. A common way to distinguish *E. dispar* from *E. histolytica* microscopically is erythrophagocytosis. Classical microscopy does not allow of the invasive protozoon (*E. histolytica*) to be distinguished from the non invasive one (*E. dispar*) unless erythrophagocytosis is seen during microscopic examination. This classical feature has long been considered the definitive diagnostic criterion for *E. histolytica*. However in some cases *E. dispar* is also observed to ingest RBCs .Laboratory diagnosis is made by finding the characteristic cysts in iodine stained, formol-ether concentration method or by detecting the characteristic trophozoites in a wet preparation or a permanent stained preparation (Haque *et al.,* 1995).

Where amebic dysentery is suspected, the laboratory should be informed that a "hot stool" is being supplied so that it can be examined within twenty minutes of being passed. On cooling the ameba stops moving which then becomes very difficult to identify. Direct microscopy should be done by mixing a small amount of the specimen in 0.9% sodium chloride solution (WHO, 2009). This permits detection of motile trophozoites of *Entamoeba histolytica* and can also provide information on the content of the stool (i.e., the presence of leucocytes and red blood cells). On search e.g. primarily for cysts, not for Ameba, several stool samples are required to be examined, by direct microscopy and a sensitive concentration technique. Three negative stool samples are required before it can be accepted that there is no amebic infection. Microscopic examination of an amebic abscess aspirate (e.g. in the liver or lungs), may reveal hematophagous trophozoites. It must be examined immediately by mixing a drop of warm saline with some aspirated pus on a microscope slide (WHO, 2009)

Diagnosis of *Giardia* infections has been carried out using microscopic identification of cysts or trophozoites in either single or multiple stool specimens. The standard methods used to increase the sensitivity of *Giardia* detection includes iodine-stained wet smears, trichrome- stained cyst concentrates prepared by Formalin ethyl acetate centrifugation and trichrome-stained polyvinyl alcohol (PVA)-preserved stools. Much flatus trophozoites are found by examination of saline wet preparations of fresh, diarrheic stool, duodenal or jejunal aspirate or in a permanently stained fecal preparation (CDC, 2006; WHO, 2009).

Detection of *Cryptosporidium* oocysts has been performed using: histological sections of small intestine using staining techniques to identify the oocysts in the feces. Oocyst antigen detection via immuno fluorescence, enzyme linked and agglutination immuno-assays polymerase chain reaction (PCR) amplification of *Cryptosporidium* specific DNA targets. Serological diagnosis of *Cryptosporidium* specific antibodies has also been applied to detect wide range of time span post infection and also can be used as a marker for epidemiological surveys (Gardner and Hill, 2001).

2.7. Prevention and Control of Intestinal Protozoan Parasites Infections

There are different mechanisms for the prevalent of intestinal protozoan parasites. The variation in prevalence depends on factors such as the geographical area, the urban or rural setting of the society, the age group composition and the socio-economic conditions of the study subject (Sehgal *et al*., 2010).

Prevention of intestinal protozoan parasites at present requires interruption of the fecal-oral spread of the infectious cyst stage of the parasite. Because cysts are resistant to chlorine or iodine, in developing countries and water must be boiled before to drink and raw vegetables must be washed with soap and then soaked in vinegar for 15 min before they can be eaten. Since protozoan infection often spreads within a household, it is prudent to screen family members of an index case for intestinal *G.lamblia, C.species* and *E. histolytica* infection (Backer, 2000). Safe disposal of human and animal wastes, improved personal and environmental hygiene, proper use of latrine, early detection and treatment drinking water and immunization pre-school and school children are the major mechanisms of prevention and control of water-borne protozoan parasitic infections (Melake *et al*., 2003).

2.7.1. Health Education

Health education and promotion of healthy behaviors can play a key role in reducing the incidence of human intestinal protozoan parasitic infections. However, the effectiveness of those activities in reducing transmission of infection varies according to different reports. In some cases, health education can decrease costs, increase levels of knowledge, and decrease re-infection rates. Health education efforts can build trust and engage communities in aspects that are crucial to the success of public health initiatives (Lansdown *et al.,* 2002).

2.7.2. Improved Sanitation

The most important community control measure is reduction of the source of infection through the sanitary disposal of human feces. It is important to treat all infected persons, even if they are asymptomatic, in order to reduce the possibility of contaminating the environment. The only way to completely prevent parasites from food and water is by cooking. Food prepared by individuals infected with parasites who have not thoroughly washed their hands after using the bathroom may pose a risk. Not all water borne intestinal protozoan parasites are killed by chlorine; therefore, those organisms can exist in the water supply. Complete elimination can only be achieved by boiling (for a few minutes), filtering with a one micron filter, or drinking distilled water. 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).

2.7.3. Treatments

Now a days, different groups of drugs are available that control intestinal protozoan parasites infections. Based on different age groups, endemicity of the parasite and use of antimicrobial therapy vary. The most common anti giardial drug is metronidazole,Tinidazole and etc (Gardener and Hill, 2001). Unlike other drugs, metronidazole is quickly and completely absorbed and penetrates body tissues and secretions such as saliva, breast milk, semen and vaginal secretions (Gardner and Hill, 2001).

3. MATERIALS AND METHODS

3.1. Descriptions of the Study Area

Woliso is one of the towns found in Oromia Regional State of Ethiopia, which was established in 1926. It is located at a distance of 114 kilometers from Addis Ababa and 225 kilometers away from Jimma town along Addis Ababa–Jimma main road.

Geographically, the town is located 8.31^0 60" north latitude and 37.58^0 60" east longitudes. According to new master plan of the town, the total area of Woliso town is about 2225.25 hectares. The elevation of the town ranges from 1900 to 2000 meters above sea level. The mean temperature of the town is 22.5^0C and the mean annual rainfall is 1200mm.

One of Woliso town water reservoirs is located in the centre of the town on the top of Abohill (Gara) behind Abo Church, the second is located in Ejersa village and the third water reservoir in Obi Koji village at distance of 5 km to the north direction of Woliso town. The Woliso water system supplies to the community by gravity-fed water system. It has 200,000m^3 per day service reservoir that directly supply disinfected water to the community. Total population that gets water services from the town is about 64,316 (Source WTWS Authority 2015 annual report).

According to population census of Central Statistic Agency (CSA, 2007) report, the population size of Woliso town was 37, 868. and This number was projected to reach about **49,947.892** in the year 2016/17 out of which Male accounts for **24,187** and female accounts for **25,760**.

There are 6 primary schools, two high schools, 10 kindergartens, 3 governmental clinics and one hospital in Woliso town.

3.2. The Study Design

A descriptive cross-sectional survey was carried out among kindergarten children to determine the prevalence of intestinal protozoan parasitic infection. In addition, water samples from different sources were examined to determine their prevalence in the study areas. Laboratory examination of stool and water samples were carried out using Wet Mount, Formol Ether Concentration and Modified Zeihl-Nelsen method. A survey was conducted from December to January, 2016/17 in Woliso KG and in different water sources in the study area.

3.3. The Study Population

The total population of pre-school children enrolled in the three study kindergartens (Abenu KG, Medresa KG and 04 Community KG) during 2016/17 academic year in Woliso town was 821. This was considered as the study population for the present study.

3.4. Sample Size Determination and Sampling Techniques

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

z= 1.96 (score corresponds to 95% confidence interval)

d= 0.05 (margin of error)

p= 0.5 (prevalence value)

n= 384

Since the overall prevalence rate (P) of intestinal protozoan parasites was not known in 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, 384 pre-schoolers were chosen to participate in the present study area.

From Woliso Kindergarten, three Kindergartens (04 kebele community KG, Abenu KG and Medresa KG) were chosen purposively due to their demographic characteristics.

To select the sample children, the students were first stratified according to their KG level (KG 1 to 3). Then proportional sample number was allocated for each KG (Abenu KG 186, Medresa KG 120 and 04 community KG 78 children) and for each level/section proportional sample also allocated. For example sample number children that were selected from Kg1 of Abenu, Medresa and 04 Community were 61,66 and 59 respectively. The proportional samples allocated for Kg2 of Abenu,Medresa and 04 Community were 47,37and 36 respectively and from KG3 of Abenu, Medresa and 04 community were 26,28 and 24 respectively. Finally, the sample children were selected using systematic random sampling technique by using class rosters as the sample frame.

3.5. Method of Data Collection

In this study stool and water samples were collected. The stool samples were collected from the sample study population and water samples were collected from three different sources (direct tap water, tap water kept in tanker and borehole water).

3.5. 1. Stool Sample Collection

Each study participant was provided a leak proof container and informed to give about 3grams of stool using applicator stick. A stool sample was collected from 384 children from three KGs, of Woliso town and transported to Woliso Health Center laboratory for examination within five (5) to six (6) hours after collection. At the time of sampling; name,date of sampling, age, sex, and code number were recorded for each child on the record format. Data collectors and assistants were involved in the data collection.

3.5.2. Water Sample Collection

A total of 105 water samples were collected from three water sources (direct tap water, tap water kept in tanker and borehole water) from seven sampling sites. These water sources were chosen purposively due to the geographical/ topography of the study area and the sites were selected using systematic random sampling techniques by lottery system.

All water samples were collected based on APHA (1999) sampling procedure. The collected water samples were transported to Microbiology and Parasitology laboratory of Woliso Health Center by keeping the samples at $4^{o}C$ using appropriate insulated coolers (Ice box) according to standard methods (APHA, 1999). A water samples were processed in laboratory immediately within 2-6 hours of sample collection to avoid the death and growth of parasites of sample water collected from each source.

3. 6. Laboratory Parasitological Examination Procedures

Laboratory examinations of stool samples were undertaken at laboratory of Woliso Health Center and water samples were undertaken at laboratory of St.Lukas Hospital, respectively, by using standard procedures.

3.6.1. Wet Mount Method

With a marker, the identification number of each study participant 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 placed into the drop of saline and it was thoroughly emulsified to make a thin uniform saline suspension (i.e not too thick that faecal debris may obscure organisms, and also not too thin that blank spaces may be present).

The suspension was carefully covered with cover slip in a way to avoid air bubbles. Then the slide was placed on the microscope stage, and the specimen was examined systematically under the low power so that the entire cover slip area was scanned for the presence of parasite stages (trophozoite/cysts/oocyst).

3.6.2. Modified Zeihl-Neelsen Method

For the detection of *Cryptosporidium* oocysts, modified Ziehl-Neelsen method was used. Two thin smears was prepared directly from fresh stool 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 were washed with tap water and decolorized with acid and alcohol 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 with tap water and allowed to air dry. Then the slides were observed under light microscope with high objective power at 100X magnification (Endeshaw *et al.*, 2004).

3.6.3. Formol-Ether Concentration Method

Using an applicator stick, approximately 2 grams of faecal materials were placed in a centrifuge tube containing 10 milliliter of formalin. After emulsifying the feces in the formalin, it was filtered through the nylon filter into the test tube. The filtrate was washed to discard any lumpy residue with a normal saline solution. Then the filtrate was washed again, by transferring into a test tube containing 7 milliliter of ethyl acetate.

The tube was closed with a stopper and it was shacked vigorously to mix. The stopper was removed and it centrifuged for 2 minutes. The tube was rested in stand for five minutes. Four layers became visible with the top layer of ether, second were a plug of debris and the third were a clear layer of formalin and the fourth had the sediment. The plug of debris from the side of the test tube was removed with the cotton swab and poured off the liquid leaving a small amount of formalin for suspension of the sediment.
Then after, the sediment was removed with a pipette and a drop of fluid was added on the slide for examination under a cover slip. Some drop of iodine solution was added on the second glass slide. A 10x and 40x objectives were used to examine the whole of the deposit for cysts, oocyst and trophozoites.

3.6.4. Microscopic Examination of Water Samples

Water sample was collected from seven different areas. Each sample (21 samples) from different location were collected from three different water sources (7 samples from tap water kept in tanker,7 from direct tap water and 7 from bore-hole water) using white plastic container. There were five round of sampling. Before samples were collected, each of the containers was pre-sterilized using Sodium Hypochlorite and distilled water.

A total of 105 water samples were collected during December –February 2016/17. The sample was handled in sterile glass bottles, labeled and kept in ice-box during transportation to St.Lukas Hospital. Parasitological water sample analysis,
Centrifugation and microscopic observation were conducted based on USEPA method by Wet Mount Method, Modified Zeihl-neelsen Method and Formol-Ether Concentration method (USEPA, 2005). Samples were transferred into centrifuge tube and were sediment at 5000rpm on centrifuge at $40^{o}C$ for 15 minute. Then slides containing sample

were stained and observed through fluorescent microscope for the existence of cysts of *Entamoeba* and *Giardia* and oocyst of *Cryptosporidium*. The *Cryptosporidium* Oocyst was identified as oval to round shape.

3.7. Data Analysis

Prevalence of intestinal protozoan parasitic infections was analyzed using SPSS, window Version 20. A statistically significant difference in frequencies were tested using chi-square analyses. Values were considered to be statistically significant when the p-value obtained was less than or equal to 0.05 and if not values were considered to be insignificant.

3.8. Data Quality Control

To ensure quality control, all the laboratory procedures including collection and handling of stool and water samples were carried out in accordance with standard protocols (WHO, 1991). To ensure general safety, disposable gloves were worn and universal bio-safety precautions (NCCLS, 2002) were followed at all times.

3.9. Ethical Consideration

Data collection was conducted in study area after obtaining informed consent from the concerned office (i.e Woliso Town Water Service Authority (WTWSA) , Woliso Town Education Bureau and Woliso Woreda Health Office, all have given their written consent for the study. All KG children tested and become positive for intestinal protozoan parasite inflections were treated with a single dose of drug prescribed by the physician.

4. RESULTS AND DISCUSSION

4.1. Age and Sex Distribution of the Studied Kindergarten Children

A total of 384 kindergarten children participated in the present study. Of these, 202 were males and 182 were females. There were 134 KG1 Children which had age range 3-4 years, 131 KG2 Children which had age range 5-6 years and 119 KG3 Children which had age >6 and among these the number of males KG1, KG2 and KG3 children involved in present study were 54 , 100 and 48 respectively. Similarly, the number of females KG1, KG2 and KG3 children involved in the present study were 80, 31,and 71 respectively.

4.2. Prevalence of Intestinal Protozoan Infections of Kindergarten Children

Result of prevalence of intestinal protozoan parasitic infections among examined kindergarten children are summarized and presented in Table 1.

Table 1. Prevalence of Intestinal Protozoan Parasitic Infections of Kindergarten Children of Woliso town.

	Sex		Abenu Kindergarten			Medresa Kindergarten			04 Community Kindergarten			All KGs			Total
			3-4yrs	5-6yrs	>6yrs	3-4yrs	5-6yrs	>6yrs	3-4yrs	5-6yrs	>6yrs	3-4yrs	5-6yrs	>6yrs	
IPP Infection	Male	No. exam	20	48	28	24	29	14	10	23	6	54	100	48	202
		No pos (%)	4(20)	10(20.8)	6(21.4)	8(33.3)	5(17.2)	4(28.6)	2(20.0)	5(21.7)	1(16.7)	14(25.9)	20(20)	11(22.9)	45(22.3)
	Female	No. exam	41	18	31	23	8	22	16	5	18	80	31	71	182
		No pos (%)	8(19.5)	5(27.5)	8(25.8)	7(30.4)	3(37.5)	2(9.1)	3(18.8)	1(20.0)	2(11.1)	18(22.5)	9(29.0)	12(16.9)	39(21.4)
	Both Sex	No. exam	61	66	59	47	37	36	26	28	24	134	131	119	384
		No. pos (%)	12(19.7)	15(22.7)	14(23.7)	15(31.9)	8(21.6)	6(16.7)	5(19.2)	6(21.4)	3(12.5)	32(23.9)	29(22.1)	23(19.3)	84(21.9)
	χ^2											0.21	1.12	0.67	0.04
	P-value											0.65	0.29	0.42	0.84

Key: IPP = Intestinal protozoan parasite, No_Exam = Number of examined, No.pos= number of positive, $\chi 2$=Chi-square

The prevalence of intestinal protozoan parasitic infections for all KG-1 was 23.9% and had age range 3-4 years and the prevalence of intestinal protozoan parasitic infections for all KG-2 was 22.1% and had age range 5-6 years and while for all KG3 was 19.3% and had age range >6 years. The overall prevalence of intestinal protozoan parasitic infections among all kindergartens of the KG children in the study area was 21.9%. In this study there was difference in prevalence of intestinal protozoan parasitic infections statistically not significant difference between age groups of KG1(3-4years), KG2 (5-6 years) and KG3 (>6years) ($p=0.84$, $\chi^2=0.04$).

The observed result shows that there was relatively higher prevalence of intestinal protozoan parasitic infections among age group 3-4 years / KG1 (23.9%) and among age group 5-6 years / KG2 (22.1%) than age groups of > 6years / KG3 (19.3%) children. This might be due to the fact that this pre-school children age groups werein higher risk in terms of acquiring parasitic infections. The reason could be their immunity strength compared to KG3 (3-4years) due to their age, relatively have no enough awareness how to take care of their personal hygiene and not well skilled to wash hands after defecation, playing with and drinking non-purified water as compared to KG3 children (Melake *et* al., 2003). Due to not well develop of immune system, young children are highly affected by water-borne protozoan infections in compared with any other age group

Generally there were higher prevalence of intestinal protozoan parasitic infections among Pre-school children of KG levels. This might be due to the poor sanitary conditions of their Kindergarten,or due to not well develop of immune system, children are highly affected by water-borne protozoan infections in compared with any other age group or children do not take care of their personal hygiene. For instance, they play in contaminated outdoor environments, in and around waste disposal sites, drink untreated water/long days stayed water which can certainly cause serious health problems. They also face difficulties in proper use of toilet and lack of basic life skills, such as washing hands after toilet, washing hands before and after meals.

4.3. Major Intestinal Protozoan Parasite Species Identified from Examined Kinderg arten Children by Age and Sex

The results for the prevalence of major intestinal protozoan parasite species among Kindergarten children are summarized and presented in Table 2

Table 2. A Prevalence of Major Intestinal Protozoan Parasite Species Identified from Examined Kindergarten Children by Age and Sex.

KG	Age and Sex		No. Exam	Detected Intestinal Protozoan Parasite Species		
				Eh	Gl	Csp
				No.pos (%)	No.pos (%)	No.pos (%)
Abenu	3-4	Male	20	3 (15.0)	1 (5.0)	0
		Female	41	4 (9.8)	3 (7.3)	1
	5-6	Male	48	5 (10.4)	5 (10.4)	0
		Female	18	1 (5.6)	4 (22.2)	0
	> 6	Male	28	3 (10.7)	2 (7.1)	1 (3.6)
		Female	31	4 (12.9)	3 (9.7)	1 (3.2)
Medresa	3-4	Male	24	5 (20.8)	3 (12.5)	0
		Female	23	4 (17.4)	3 (13.0)	0
	5-6	Male	29	2 (6.9)	2 (6.9)	1 (3.4)
		Female	8	2 (25.0)	1 (12.5)	0
	> 6	Male	14	2 (14.3)	1 (7.1)	1 (7.1)
		Female	22	1 (4.5)	1 (4.5)	0
Woliso 04 community	3-4	Male	10	1 (10.0)	1 (10.0)	0
		Female	16	2 (12.5)	1 (6.3)	0
	5-6	Male	23	3 (13.0)	2 (4.3)	0
		Female	5	0	0	1 (20)
	> 6	Male	6	1(16.7)	0	0
		Female	18	1 (5.6)	1 (5.6)	0
All KGs	3-4	Male	54	9 (16.7)	5 (9.3)	0
		Female	80	10 (12.5)	7 (8.8)	1 (1.3)
	χ^2			0.99	0.02	1.49
	P-value			0.75	0.89	0.22
	5-6	Male	100	10 (10.0)	9 (9.0)	1 (1)
		Female	31	3 (9.7)	5 (16.1)	1 (3.2)
	χ^2			1.04	0.68	0.63
	P-value			0.31	0.41	0.43
	> 6	Male	48	6 (12.5)	3 (6.3)	2 (4.2)
		Female	71	6 (8.5)	5 (7.0)	1 (1.4)
	χ^2			0.08	0.94	0.89
	P-value			0.78	0.33	0.43
Total		Male	202	25 (12.4)	17 (8.4)	3 (1.5)
		Female	182	19 (10.4)	17 (9.3)	3 (1.6)
			384	44(11.4)	34(8.9)	6 (1.6)

Key: KG = Kindergarten, No_Exam = number of examined, No.pos= number of positive, Eh = *Entamoeba histolytica*, Gl= *Giardia lamblia*, Csp= *Cryptosporidium* species, χ^2=Chi-square

The major intestinal protozoan parasite species that were detected in the present study area including their prevalence were *Entamoeba histolytica*, 11.5% (44/384) of these 12.4%, 10.4% in males and females respectively, *Giardia lamblia*, 8.9% (34/384) of these 8.4%, 9.3% in males and females respectively and *Cryptosporidium* species, 1.6% (6/384) of these 1.5%, 1.6% in males and females, respectively as shown in table 2.

There was higher prevalence of *E.histolytica* infection in study area. The reason might be attributed to the fact that most children were exposed to low level of environmental sanitation while they play, or high degree of water contamination with wastes and lack of awareness on practices such as water using and handling practices and food hygiene (Endaeshaw, 2005). The other reason might be the kindergarten children's infected through other oro-fecal routes such as contaminated food or lack of hygiene.

The overall prevalence infection with major intestinal protozoan parasite species in the study area was (21.9%). Knowing the prevalence of intestinal protozoan parasite in a given place and identification of the major intestinal protozoan parasite species are crucial to formulate appropriate interventions. Health education and promotion of healthy behaviors can play a key role in reducing the incidence of human intestinal protozoan parasitic infections. However, the effectiveness of those activities in reducing transmission of infection varies according to different reports. In some cases, health education can decrease costs, increase levels of knowledge and decrease re-infection rates. Health education efforts can build trust and engage communities in aspects that are crucial to the success of public health initiatives (Lansdown *et al.,* 2002).

4.4. Parasitological Quality of Water Sources Utilized by the Study Population

Peoples of the study area gain water from different water sources for the purpose of drinking, washing and other utilization. These sources include direct tap water, tap water kept in tanker and borehole water and presented and summarized in table 3.

The prevalence of *Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium* species in a samples taken from direct tap water was 17.1%, 11.4% and 0%, respectively. The prevalence of *Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium* species in tap water that was kept in tanker was 22.9%, 17.1% and 5.7%, respectively. The prevalence of *Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium* species in water samples taken from borehole was 28.6%, 40% and 11.4%, respectively.

The overall prevalence of intestinal protozoan parasite taken from 105 samples was 22.9%, 22.9% and 5.7% for *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidiu m* species, respectively. Higher prevalence of water-borne intestinal protozoan parasites were found in borehole water than tap water kept in tanker and direct tap water. Borehole water and water kept in tanker relatively had high prevalence of water-borne intestinal protozoan parasite than direct tap water.

The proportions of intestinal protozoan parasites were highest in borehole water because this water sources might be contaminated by different waste materials during washing of clothes, washing different material such as home utensils near borehole water, and animal and human feces might be contaminate it at rainy seasons by means of floods.

The reasons for the contamination of tap water kept in tanker and direct tap water might be lack of cleaning the tanker regularly, improper waste disposal and poor sanitation which was the major factors that contribute for the pollution of drinking water sources and pipe line that crosses through sewage lines also exposes the treated water for contamination. The other possible explanation could be due to the unhygienic practice of children immersing their contaminated hands into stored water in the house (Jensen *et al*., 2004).

Water quality and quantity used by people of the study area was not sufficient for drinking and as well as for different purpose. This condition leads the Kindergarten children expose to different intestinal protozoan parasite infection.

Table 3. Prevalence of Intestinal Protozoan Parasite Species Detected in Examined Water Samples.

Water Sources	Number of Sample Examined	IPP Species			Total number of positive (%)
		Eh (%)	Gl (%)	Csp (%)	
Direct tap water	35	6(17.1)	4(11.4)	-	10(28.6)
Tap water kept in tanker	35	8(22.9)	6(17.1)	2(5.7)	16(45.7)
Borehole water	35	10(28.6)	14(40)	4(11.4)	28(80)
Total	105	24(22.9)	24(22.9)	6(5.7)	54(51.4)

Key- IPP=Intestinal protozoan parasite

4.5. Association of the Intestinal Protozoan Parasitic Infections of Kindergarten Children with Parasitological Quality of Water Sources they Use.

An association of the intestinal protozoan parasitic infections of kindergarten children with parasitological quality of water source utilized by kindergarten children of Woliso town was summarized in table 4.

Table 3. Association of the Intestinal Protozoan Parasitic Infections of Kindergarten Children with Parasitological Quality of Water they use.

Water sources	No sample examined for IPP	IPP's	χ^2	P.value
		Entamoeba histolytica Positive (%)		
Direct tap water	35	6(17.1)	4.08	0.44
Tap water kept in tanker	35	8(22.9)	5.55	0.02
Borehole water	35	18(51.4)	13.9	0.00
		Giardia lamblia Positive (%)		
Direct tap water	35	4 (11.4)	2.66	0.10
Tap water kept in tanker	35	6 (17.1)	4.08	0.04
Borehole water	35	16 (45.6)	12.09	0.00
		Cryptosporidium species Positive (%)		
Direct tap water	35	0	0	0
Tap water kept in tanker	35	2 (5.7)	1.31	0.25
Borehole water	35	4 (11.4)	2.66	0.10
Total	105	64(60.9)	29.48	0.00

Key- IPP's =Intestinal protozoan parasites

The data were analyzed using chi square to see association of intestinal protozoan parasites of Kindergarten children with parasitological quality of different water sources (direct tap water, tap water kept in tanker and borehole water) they use.

As shown above in table 5 associations of *Entamoeba histolytica*, with borehole water, tap water kept in tanker and direct tap water were χ^2=13.9; p=0.00, χ^2=5.55; p=0.02 and

χ^2=4.08, p=0.44 respectively. An association of *Giardia lamblia* with borehole water, tap water kept in tanker and direct tap water were χ^2=12.09; p=0.00, χ^2=4.08; p=0.04 and χ^2=2.66; p=0.10 respectively and association for *C. species* with borehole water, and tap water kept in tanker were χ^2=2.66; p=0.10, χ^2=1.31; p=0.25. The result showed that there were a statistically significant association between major intestinal protozoan parasites infection with parasitological quality of different water sources(tap water, tap water kept in tanker and borehole water) utilized by population of the study area ($p<0.05$).

This might be due to poor environmental sanitation and water handling practices. As a result the probability of water sources contamination with different wastes those can be the route for intestinal protozoan parasites transmission becomes increased. Due to lack of proper environmental sanitation, such as liquid discharged from homes, urine and solid discharge may enter water body and this enhances association probabilities for intestinal protozoan parasites with different drinking water sources.

Therefore these different water sources utilized by people of the study area might be the major cause for the intestinal protozoan parasites infection of kindergarten children of Woliso town.

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary

The main objective of this study was to assess the prevalence of intestinal protozoan parasite infections among kindergarten children and its association with parasitological quality of their water sources in Woliso town. The design of the study was a cross-sectional parasitological survey involving sample populations of 384 pre-school children were selected using stratified random sampling techniques from KG1to KG3 in Woliso town.

A total of 384 stool samples were collected from children of three Kindergarten and 105 water sample were collected and examined using direct wet mount, Formal-Ether concentration and Modified Zeihl-Neelsen techniques by laboratory technician at Woliso health center and St. Lukas Hospital. The result showed that the prevalence of intestinal protozoan parasite infection were common among kindergarten children and in different water source of the study areas.

After screening of 384 stool samples, the overall prevalence of intestinal protozoan parasitic infection was found to be 21.9% (84/384). The predominant protozoan parasite detected in this study was *Entamoeba histolytica*, which was observed in 44 (11.5%) of kindergarten children, *Giardia lamblia* was the second predominant found in 34 (8.9%) of the study participants and less frequently observed parasite species was *Cryptosporidium* species 6 (1.6%) among the study participants.

The prevalence of *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium* species from different water samples (direct tap water, tap water kept in tanker and borehole water) were 32(30.5%), 26(24.8%) and 6(5.7%), respectively.

The result revealed significant association ($p< 0.05$) between the prevalence of intestinal protozoan parasite infections and parasitological quality of water sources utilized by the study areas.

5.2. Conclusions

In general, the findings of the present study show that intestinal protozoan parasites infection was prevalent and one of health problem among kindergarten children of Woliso town. A common human intestinal protozoan parasite species identified among kindergarten children in Woliso town and in different water sources of Woliso town were *Entamoeba histolytica, Giardia lamblia and Cryptospordium* species and they were highly associated with parasitological quality of different water sources utilized by people in study area.

Water quality and quantity used by people of the study area was not full enough for drinking, washing clothes and for different purpose. This problem can create conditions that Kindergarten child to be exposed to different intestinal protozoan parasite infection.

Providing well treated drinking water, giving proper education on way of treating borehole water, hygienic and environmental sanitation for people of the study area and providing always washed fruits and vegetables also could help in reducing the prevalence of intestinal protozoan parasites infection.

5.3. Recommendations

Based on the finding of the present study, to reduce the prevalence of intestinal protozoan infections, among Kindergarten children the following recommendations can be made.

- Strategies to control intestinal protozoan parasitic infections through sanitization should be implemented targeting school age children.
- Regular drinking water quality assessment from the source, reservoirs distribution system and pipes should be employed to ensure that the water is safe for human use.
- The role of *Entamoeba histolytica, Giardia lamblia and Cryptosporidium* Species as an etiology of diarrhea in the area needs to be confirmed by using more precise techniques.
- Moreover, in-depth studies should be made on socio-economic factors like, hand washing practice, family size, education level and water handling habit to better evaluate the epidemiology of intestinal protozoan parasitic infection in the study area.

- Strategies to control the predominant intestinal protozoan parasitic infections (*Entamoeba histolytica* and *Giardia lamblia*) through Mass Drug Administration (MDA) and sensitization should be implemented targeting preschool age children.

- Further studies are also recommended using different methods on wider ranges and school of the population in the area regarding the prevalence of intestinal protozoan parasite infections and association with parasitological quality of water sources.

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7. APPENDIX

Appendix Table 1: Laboratory data collecting format for stool sample

No_	Name	Code	Sex	Age	KG Level	Type of IPP in stool			Infection			Remark
						Eh	Gl	Cp	Single	Double	Multiple	

Appendix Table 2: Laboratory data collecting format for different water sources

No_	Name of Water sources	Code	Presence of water borne IPP's			Remark
			Eh	Gl	Cp	