PREVALENCE OF INTESTINAL PROTOZOAN PARASITES AMONG PRIMARY SCHOOL CHILDREN AND DRINKING WATER SOURCES IN GERBE GURACHA TOWN, KUYU WOREDA, NORTH SHOA, OROMIA, ETHIOPIA

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PREVALENCE OF INTESTINAL PROTOZOAN PARASITES AMONG PRIMARY SCHOOL CHILDREN AND DRINKING WATER SOURCES IN GERBE GURACHA TOWN, KUYU WOREDA, NORTH SHOA, OROMIA, ETHIOPIA.

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In Partial Fulfillment of the Requirements for the Degree of Master of Science in Microbiology

By

Negase Abera

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APPROVAL SHEET

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As thesis research advisors, we herby certify that we have read and evaluated this thesis prepared, under our guidance, by **Negase Abera**, entitled **"Prevalence of Intestinal Protozoan Parasites among Primary School Children and Drinking Water Source in Gerbe Guracha Town, North Shoa,Oromia Ethiopia".** We recommend that it can be submitted as fulfilling all the thesis requirements.

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As member of the board of the examiners of the open defenses of the examination, we certify that we have read and evaluated this thesis properly which was prepared by **Negase Abera.** We recommended that the thesis can be accepted as fulfilling the Thesis requirement for the degree of Master of Science **in Microbiology**.

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DEDICATION

This thesis is dedicated to my beloved mother w/ro. Alemayou Feye and my wife Mrs. Getenesh Tola for their continual and unbound love, patience and strength that helped me to complete this work.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my original work and that all sources of material used have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for the Degree of Master of Science in Microbiology at the Haramaya University and is deposited at the University Library to be made available for borrower under the rule of the Library. I declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree. Brief quotations from this thesis are allowable without special permission provided accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or part may be granted by the major department or Dean of School of Graduate Studies in his or her judgment the proposed use of the material is in interest of scholarship.

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Date of Submission:

BIOGRAPHICAL SKETCH

The author was born in December 21, 1974 in Were JarsoWoreda, North Shoa Zone, Oromia Regional state. He started his Elementary Education at Tulu Milki Elementary School and continued his secondary education and completed at Gerbe Guracha Comprehensive High School in 1993.

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LIST OF ACRONYMS

AIDS	Aquired Immune Deficiency Syndrome
ASL	Above Sea Level
CDC	Center for Disease Control
CHC	Childrens Hygienic Conditions
CI	Confidance Interval
COF	Consistancy of Faeces
CPC	Children's Physical Conditions
EHNRT	Ethiopian Health and Nutritional Research Institute
HAART	Highly Active Antiretroviral Therapy
IPPI	Intestinal Protozoan Parasite Infection
IPP	Intestinal Protozoan Parasite
MOH	Ministry of Health
NCCLS	National Committee on Clinical Laboratory Standard
OR	Odd Ratio
PVA	Poly Vinyl Alcohol
PCR	Polymerase Chain Reaction
RPM	Rotation per Minute
SPSS	Statistical Package for Social Science
WHO	World Health Organization

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PREVALENCE OF INTESTINAL PROTOZOAN PARASITES AMONG PRIMARY SCHOOL CHILDREN AND DRINKING WATER SOURCES IN GERBE GURACHA TOWN, NORTH SHOA, OROMIA, ETHIOPIA

ABSTRACT

Intestinal protozoan parasite infections (IPPI) are one of the major public health problems in many countries including Ethiopia. The objective of the present study was to identify intestinal protozoan parasites species and to determine their prevalence of occurances among primary school children and in water samples collected from different sources (pipe, pond and river) in Gerbe Guracha townNorth shoa Ethiopia. The design of the study was a cross-sectional parasitological survey involving examinations of fresh stools of 404 sample populations drawn from Gerbe Guracha Primary School using stratified random sampling method during February-March, 2014. In addition, the study involved assessment of parasitological quality of water sources in the study area. Data were gathered by means of questionnaire survey and laboratory parasitological examination procedures. The stool samples were examined using direct wet-mount, Formol-Ether concentration and Modified Ziehl-Neelsen methods. From the total of 404 study participants, 214(52.97%) were males and 190(47.02%) were females. The result showed that 35 (16.35%) males and 33 (17.36%) females were infected with one or more intestinal protozoan parasites. The overall prevalence of IPPIs was 16.9 % .The major protozoan parasite species identified in examined primary school children were Entamoeba histolytica, Giardia lamblia and Cryptosporidium species, with their prevalence of infection 38(9.41%), 22(5.44%) and 8(1.98%) respectively. The prevalene of occurance of protozoan parasite species among 105 water samples collected in three source types (pipe, river and pond) was 11(10.47%) for Entamoeba histolytica, 16(15.23%) ,Giardia lamblia and 12(11.42%) Cryptosporidium species. In general, the study revealed that IPPIs represented major public health problems among school children of Gerbe Guracha primary school. Poor quality water sources, people's lack of awareness about IPPIs and poor environmental sanitation might significantly increase the occurance of IPPIs in the study area. Therefore, strategic integrated and community-participatory intestinal protozoan parasites infections, prevention and control programs need to be implemented in the study area.

***Key-words**: Gerbe Guracha, IPPI, Prevalence, School children.*

1. INTRODUCTION

Human beings have been exposed to diverse group of intestinal protozoan parasite. Over 60 species of protozoan parasites cause dieases on people worldwide. *Entamoeba histolytica* and *Giardia lamblia* are estimated to infect about 60 million and 200 million people worldwide, respectively (Murray *et al*., 2002). It is generally estimated that at least 2.5 billion of the estimated world's 6.9 billion people are currently infected with intestinal protozoan parasites cutting across all continents and regions of the world (Morales-Espinoza *et al.*, 2003).

Human intestinal protozoan parasites are identified as causes of morbidity and mortality throughout the world particularly in developing countries including Ethiopia. They are more prevalent throughout the tropics, especially among poor communities. Records show that increasing trends of intestinal protozoan parasitic infections in developing countries. A high prevalence of intestinal protozoan parasit infections in human are positively correlated with poverty and poor personal hygiene, lack of safe water supply and contamination of the environment by human excreta and animal wastes. Intestinal protozoan parasitic infections increase host`s susceptibility to other infections and diminish learning ability and growth esepecially in growing children (Karaman *et al.*, 2006**).** Intestinal protozoa Parasite species are responsible for some of the most devastating and prevalent diseases of humans. Intestinal protozoan parasite infections constitute a global health burden causing clinical morbidity in 450 million people; many of these were women of reproductive age and children in developing countries (Quihui, 2006)

Protozoan parasites are one of the causative agents of intestinal parasites. The main clinical manifestation of the disease caused by these parasites is diarrhea (Chacon-Cruz, 2003). The importance of parasites is due to its distribution in large population especially in children in most of the developing and poor countries, possibly because these parasites have easy way of transmission, such parasites are; *Entamoeba histolytica***,** *Giardia lamblia* and *Cryptosporidium* infection happen via feacal contamineted food and water in most regions of the world. Intestinal protozoan parasites are among the most common human infections which are distributed throughout the world with high prevalence rates in developing countries (Raza and Sami, 2008).

Among the conditions influencing the development of intestinal protozoan parasitic infections are poor sanitary conditions, lack of clean water supply lowering resistance of the host, and lack of awearance of transmission of the parasite. This disease can affect children's development, educational achievement, reproductive health and social and economic development and some of these parasitic infections can cause morbidity and mortality. Nevertheless, treatment is often neglected for economic reasons and because most patients have no symptoms (Guyatt, 2000).

Parasitic diseases are incriminated in causing more than 33% of global deaths of which intestinal parasite infections are believed to take the major share (WHO, 2004). Lack of safe drinking water and environmental sanitation are largely responsible for more than 800 million expected cases of diarrheal diseases and 4.5 million associated deaths in many developing countries every year. Morbidity and mortality due to diarrheal diseases in developing countries remain to be the main public health problems that need attention. Although there could be many other causes of diarrhea, the enteric protozoa *Cryptosporidium parvum* and *Giardia lamblia have* been recognized as important causes of both out-break-related.Sporadic diarrhea in humans of immune competent and immune compromised individuals could be the victims of diarrheal diseases due to these parasites (Esery *et al*., 1990).

Amoebiasis is an infection caused by an intestinal protozoa *Entamoeba histolytica*, is the third most common cause of death from parasitic disease (after schistomiasis and malaria). Areas of higest incidence (due to inadequate sanitation and crowding) include most developing countries in the tropics, particularly Mexico, India and nation of centeral and south America, tropical Asia, and Africa. Upon ingestion the cysts pass through the stomach and excyst in the lower portion of the small intestine, and undergo repeated rounds of binary fission . Amoebas can also metastasize to other organs and produce an extra intestinal amebiasis (Haque *et al*., 2003). The non-invasive disease is often asymptomatic, but can cause diarrhea or other gastro-intestinal symptoms such as abdominal pain or craps. This non-invasive infection can persist or progress to an invasive disease in which trophozoite penetrate the intestinal mucosa and kill the epithelial cells (Stanley, 2003). In Ethiopia, The prevalence of giardiasis ranges from 3% to 23 % (Haile *et al*., 2006). Ayale, (2006) reported a prevalence of 38% among children from eastern Ethiopia (Dire Dewa).

Reports from different parts of Ethiopia showed that there was different prevalence rate of Amoebiasis, Giardiasis and Cryptosporidiosis Amare et al; (2007) and Ayale, (2006). The prevalence of cryptosporidium infection in children with diarrhea ranged from 3.3 precent in Jimma, 5.6 percent in Addis Ababa to 9 percnt in North western Ethiopia. A number of survey and routine diagnosis in Ethiopia indicate that Amebiasis is one of the most widely distributed diseases (Gebru and Girma, 2000).

Several community-wide out breaks of Cryptosporidiosis, Giardiasis and Amoebiasis have been linked to drinking municipal water or other water sources contaminated with these parasites (Stevens and Adam, 2004). In most parts of Ethiopia, people consume unprotected water from different sources. In this respect in many villages in rural parts of Ethiopia, the population is forced to use unprotected water from river stream, irrigation channels, ponds, shallow well, water harvesting ponds, etc. In such area where people use water from different sourcs, the possibility of infection with water born disease such as Cryptosporidiosis, Giardiosis, and Amoebiasis is extremely high. Although the infection can appear at all age level, it is more common among school children. These and other intestinal protozoa infections are commonly associated to sanitary conditions and socio-economic factors. In addition there is also a marked seasonality in the onset of illness due to intestinal protozoan parasite infections (Gamboa *et al*., 2003).

Epidemiological surveys have indicated that the most important source for human infection is contaminated drinking and recreational water, food, household animals and infected people in our country high prevalence of intestinal protozoan parasit infection is attributable to factors associated with low socio-economic status. Such factors include poor personal hygiene, environmental sanitation, low household income, low level of education, unproper sanitation of dining utensil, resident areas of parents, toilet facility and lack of clean water supplies. For instance, our country has one of the lowest quality drinking water supply and latrine coverages (Mengistu *et al.,* 2007). Eventhough, several studies have been conducted on prevalence of intestinal protozoan parasites in Ethiopia, there are still several localities in the country including the study area, Gerba Guracha Twon, for which epidemiological information about the prevalence of intestinal Protozoan parasite infections among school children and drinking water source was not available. Knowledge of society about parasite transmission, way of control, personal and environmental hygiene, proper sanitation, use of latrine, water quality and habit of eating unwashed firut and vegetables.

Knowlage of rural resident about the parasite, cleaning of dining utensil on societies infection with intestinal protozoan parasites did not studied in the present study area. Therefore, the purpose of this study was to obtain information about the prevalence or distribution of Intestinal protozoan parasites among school children and drinking water source in Gerbe GurachaTwon, kuyu *woreda*. Generaly the present study was conducted to find out water quality analysis and stool examination to determine the prevalence of intestinal protozoan parasite among school children and drinking water source in kuyu wereda Gerbe Guracha Twon.

General Objective was:

- To determine the prevalence of intestinal protozoan parasits infections among primary school children and in drinking water sources of Gerba Guracha town, Kuyu Woreda, North Shoa, Oromia ,Ethiopia From February-March 2014

Specific Objectives were:

- To determine the prevalance of intestinal protozoan parasites infections among school Children in the study area.
- To identify intestinal protozoan parasites species among primary school children in the study area.
- To detect protozoan parasite species and determine their prevalences of occurances in drinking water sources of the study area.

2. LITERATURE REVIEW

2.1 Human Intestinal Protozoan Parasitic Infection

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 a totally parasitic existence. The enteric protozoa that cause human illness are usually transmitted by the consumption of food and drink, or through environmental contamination and poor hygiene. Some of these can cause substantial illness, and have economic consequences (Buzby and Roberts, 1997).

Intestinal protozoal diseases are caused by unicellular microorganisms which invade the wall of the intestine such as Amebiasis, Giardiasis, and Cryptosporidiosis. Numerous protozoa inhabit the gastro-intestinal tract of humans. The majority of intestinal protozoa is 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 immunocompromised 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 (Marshall *et al*, 1997).

2.2. Life Cycle of Intestinal Protozoan Parasites 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*, but is not pathogenic. 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).

E. histolytica is reported to be responsible for approximately 50 million cases of invasive amoebiasis and upwards 100,000 deaths/year (WHO, 1997a). Thus it is ranked second next to malaria as the cause of mortality due to intestinal protozoan parasites infection; the parasite normally inhabits the large intestine but is also capable of invading other organs such as the liver, brain and spleen (Petri and Singh, 1999). The majority of amoebic infections are reported to occur in Central America, South America, Africa and Asia. These are often associated with poor water and food hygiene and sanitation practices. In the study of Tikur Anbessa Hospital shown that *E. histolytica* trophozoite was the most commonly reported parasite, which was seen in 13.6% of the patients over the five years study period (Petri and Singh, 1999).

The life cycle of *Entamoeba histolytica* as showed 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 amoeba pores and phospholipidases, causing ulceration of the mucous membrane (called flask shaped ulcers), and sometimes large vessels may be eroded and severe intestinal hemorrhage result (Petri and Singh,1999).

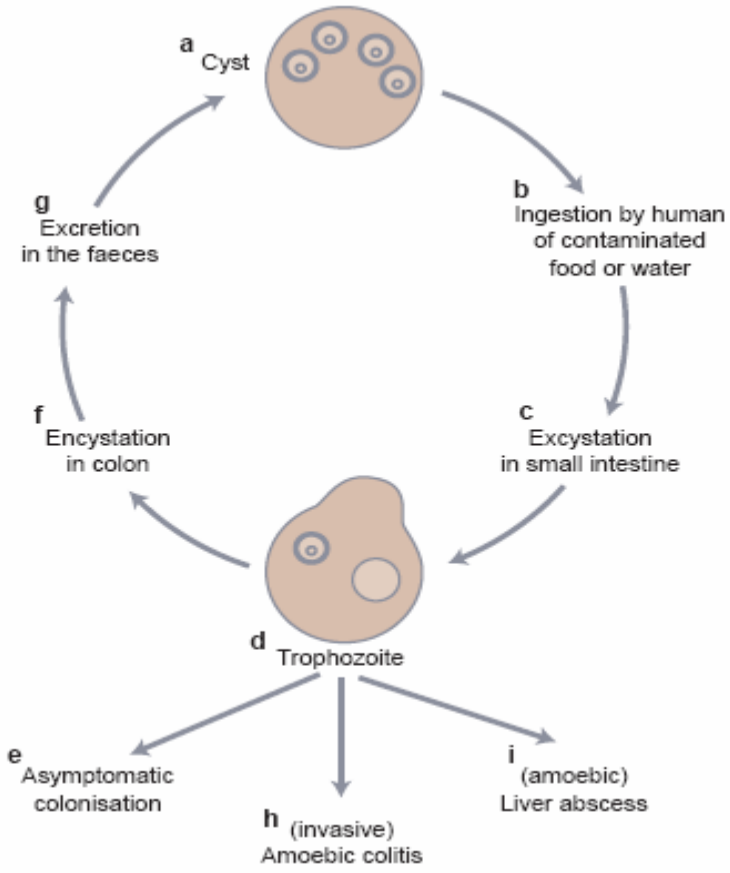

Figure 1. Life cycle of *Entamoeba histolytica* Source**:** http://www.dpd.cdc.gov/dpdx**)**

The life cycle of *Cryptosporidium parvum* commence 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 (Fayer and Ungar, 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* 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 has also occurred with just a single oocyst (Ramirez *et al*., 2004).

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; O'donoghue, 1995). 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 parvum*. The first is by the continuous recycling of Type I meronts and the second through sporozoites rupturing from thin-walled oocyst.

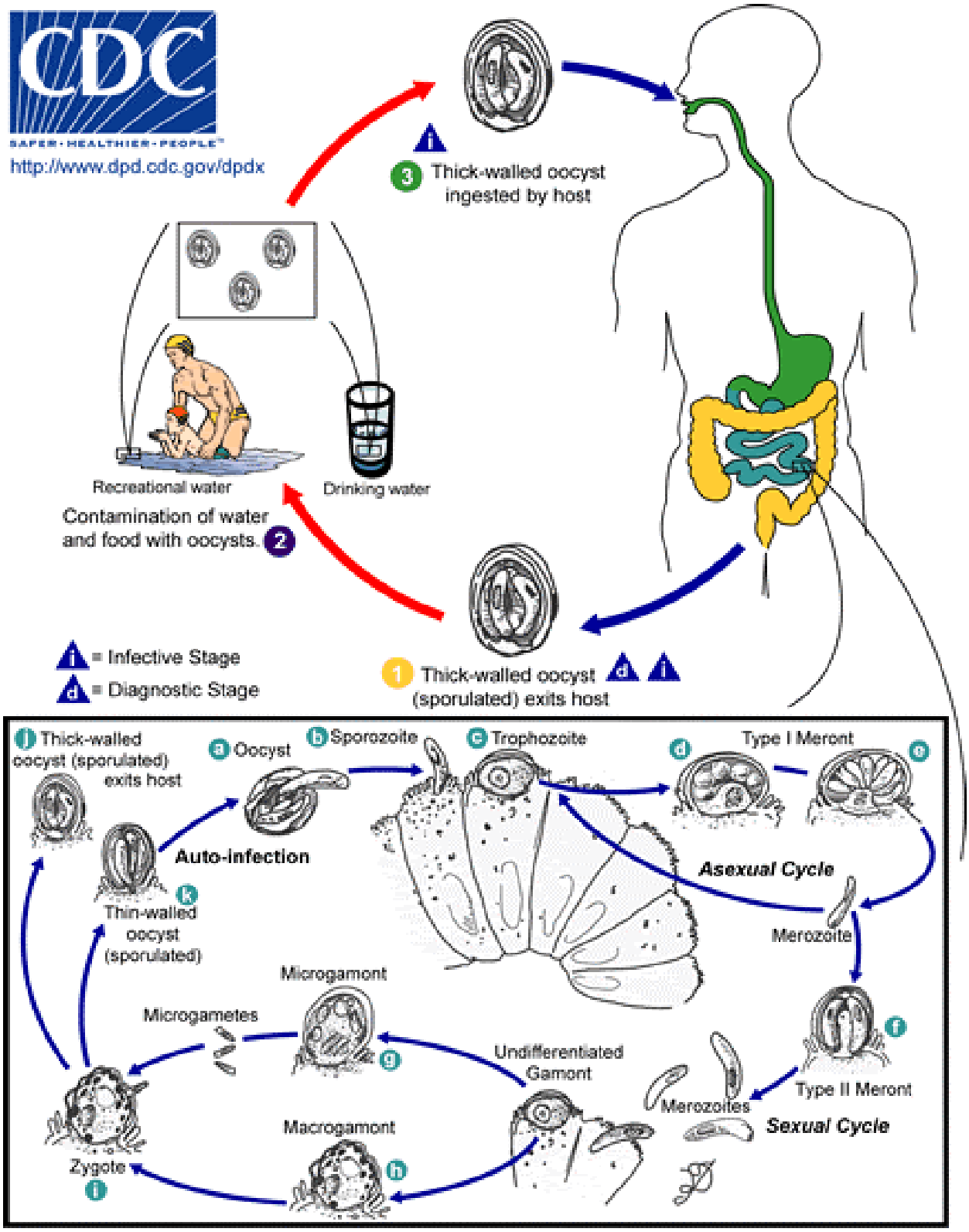

Figure 2. Life cycle of *Cryptosporidium* species (Current and Garcia, 1991).

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. 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^{C} (Meyer and Jarrol, 1980).

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 thine protective wall to protect it until it reaches the alkaline environment, the small intestine (Ortega and Adam, 1997). This alkaline environment initiates excystment of the cyst (Erlandsen and Mayer, 1984). 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 (Erlandsen and Mayer, 1984).

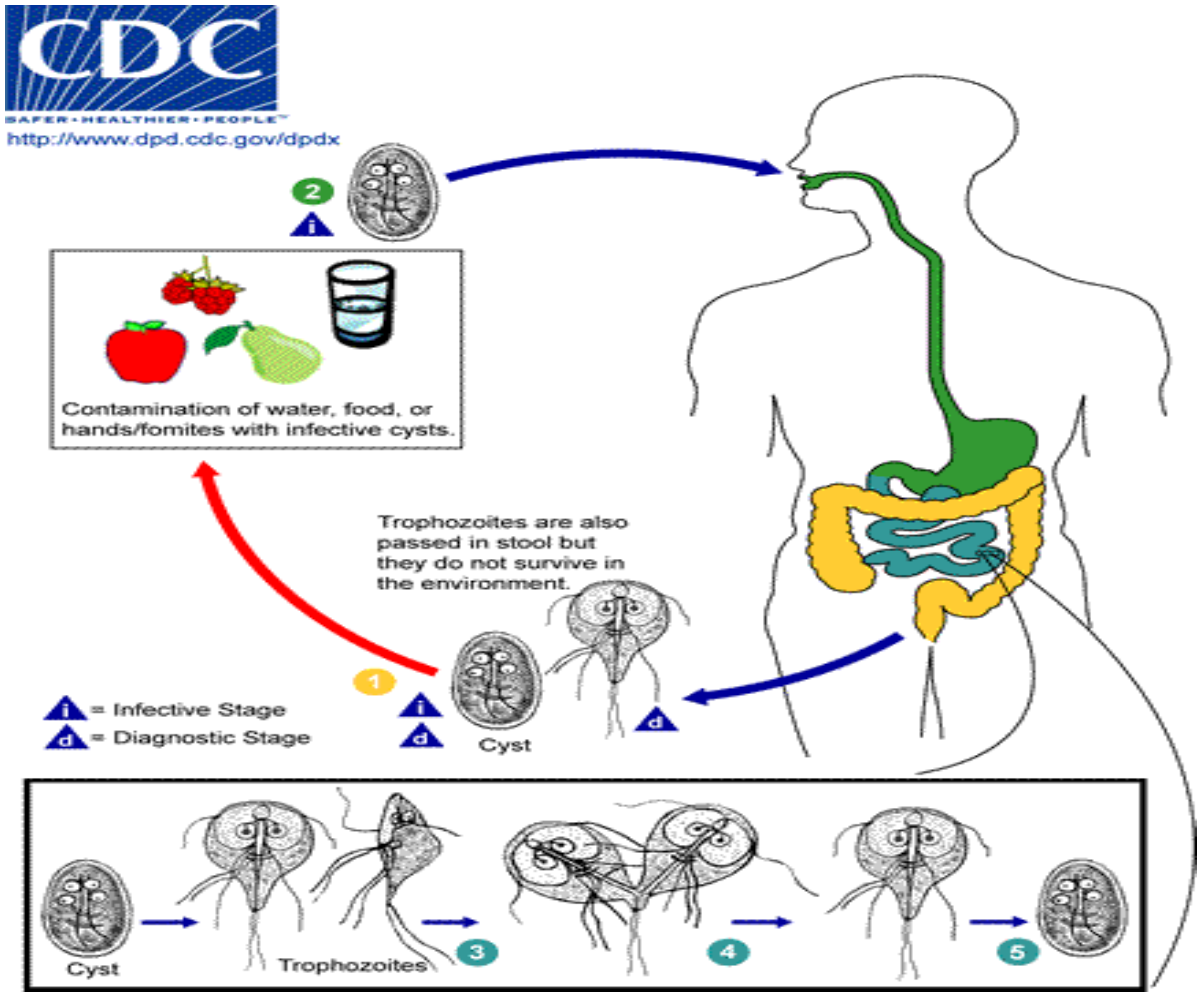

Figure 3: Life cycle of *Giardia lamblia* (Source: - http://www.dpd.cdc.gov/dpdx) (Last modified: 12/05/2008).

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 include such as *Amoeba, Cryptosporidium* and *Giardia* have become a challenge to human health worldwide. These protozoans have several common characteristics biologically. Their major habitat is intestinal epithelial cells, and they are all intracellular parasites. In addition, they produce infectious spores that are excreted from the hosts in their stools and *Giardia* produces cyst (Akiyoshi *et al.*, 2003).

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 hypersecretion. 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).

Pathogenesis of *Amoebiasis* is believed to be a multi step, multifactorial process. Though a large number of studies have attempted to unravel the factors/molecules responsible for the pathogenesis of *Amoebiasis*, the processes involved in pathogenesis are poorly understood. The aspects of pathogenesis which have been investigated experimentally can be broadly categorized into mechanisms involving interactions with the intestinal flora, lysis of target cell by direct adherence, lysis of target cell by release of toxins and phagocytosis of target cells (Sehgal *et al*., 2010).

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, 2004).

The life cycle of *C. parvum* begins following ingestion of the oocyst by a susceptible host. The oocyst is spherical in shape measuring 3-6 mm in diameter and it may be either thick- or thin-walled. 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. Thick-walled oocysts are excreted with the faeces and it is the resistant stage found in the environment (Fayer *et al*., 2000).

These parasites are intracellular, 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. Trophozoites undergo asexual division (merogony) to form merozoites. After release from type I meronts, merozoites enter adjacent host cells and multiply to form additional type I meronts, or to form type II meronts. Type II meronts do not recycle but enter host cells to commence the sexual phase of the life cycle with the formation of microgamonts and macrogamonts. Most (approximately 80%) of the zygotes formed after fertilization develop into environmentally resistant, thick-walled oocysts that undergo sporogony to form sporulated oocysts containing four sporozoites. A smaller percentage of zygotes (approximately 20%) form thin-walled oocysts surrounding the four sporozoites that represent the auto infective life cycle forms that can maintain the parasite within the host without repeated oral exposure to the thick-walled oocysts present in the environment.The presence of these autoinfective oocysts and recycling type I meronts are believed to be the means by which persistent chronic infections may develop in hosts without further exposure to exogenous oocysts (Carey *et al*., 2004).

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

Intestinal protozoan parasite infection can resalt in Gasterointestinal desease in humans. As a result of infection of the parasite more or less similar clinical sign and symptom can be observed. For

example Infections with *E. histolytica* have no symptoms in many individuals, and most canclear their infection without any signs of disease (Ravdin & Petri, 1995). 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* infection Symptomatic amebiasis is primarily an intestinal disease, and when it becomes extraintestinal, it usually involves the liver. Pathogenesis of amebiasis is believed to be a multi step, multifactorial 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*., 1996).

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 be 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 and Stephen, 2007). The pathogenesis of *Cryptosporidium are* associated with 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. The complicated life cycle, the variety of parasitic forms within the host, the different *Cryptosporidium* species.

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).

2.5. Epidimology and Transmision of Intestinal Protozoan Parasitic Infections

2.5.1. Global epidemiology of intestinal protozoan parasites infection

Intestinal protozoan parasitic infections enjoy a wide global distribution. They are estimated to affect 3.5 billion people.most of who are children and young residing in developing countries the major intestinal Protozoan parasit of global public health concern of protozoan species are*: Entamoeba histolytica, Giardia lamblia and Cryptosporidium* species (WHO, 2000). The majority of infections are associated with poverty conditions such as reduce access to safe drinking water, housing and inadequate access to health care. They also are affected by poor family and community hygiene and sanitation practices and prevailing climatic and environmental conditions (Jemeneh, 2001). These conditions lay stage of for the continuous transmission of the Intestinal parasitic infection

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. Parasitic Intestinal protozoa and helminths are responsible for some of the most devastating and prevalent diseases of humans. 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).

Intestinal protozoan parasitic infections are among the most common infections in the world and are responsible for considerable morbidity and mortality (Kongs *et al*., 2001). The epidemiology of intestinal parasitic infections shows that these parasites are found in every age group and in both sexes. However, the incidence is high in some areas and in some age groups. Human intestinal parasitic infections have a worldwide distribution, with the greatest incidence and intensity occurring in developing countries (Mccarthy *et al*; 2004). Invasive amoebiasis is prevalent in certain areas of the world including West and South-east Africa, China, and Mexico. The high occurrence of these parasites is often related to poverty, poor living conditions and hygiene, and inadequate sanitation and water supply. In Turkey it was noted that the prevalence of pathogenic parasites was high among people who had no toilets in their houses. In Tehran Province, the highest infection rate (41.5%) was related to protozoan parasites, *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%. In the United States of America, it is now considered to be the most common intestinal parasite of man and the leading cause of diarrhoea due to protozoan infections in humans It is also the most frequently reported intestinal parasite in Peru (Beltran et al.,2004). Intestinal infections in general affect more than two-thirds of the human population and mostly children .The intensity of infection is a major determinant of morbidity and approximately reflected in the number of characteristic cysts passed out in faeces (kongsw *et al*., 2001). Giardiasis is one of the most common parasitic infections having a worldwidedistribution and occurring both in developed & developing nations. In Africa, Asia and Latin America about 200 million cases have been estimated to occur annually. In Ethiopia surveys across all regions of the country show giadiasis prevalence to bearound 10% in the 1970s and early 1980s and it is more common in children than inadults. Cryptosporidium is known to cause diarrheal diseases in immunocompetent people and

shown to be especially common among persons with AIDS or other forms of immunodeficiency the application of PCR assays to identify *Cryptosporidium* species from stool samples has shown that *C. hominis* and *C. parvum* are the major causes of human cryptosporidiosis (Cacciò, 2005). Interestingly, the prevalence of these species varies in different regions of the world.

2.5.2. Epidemiology of intestinal protozoan parasitic infections in Ethiopia

Acording 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. 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 (McConnel and Armstrong, 1976). *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 *et al.*, 2008).

Another report indicated that the prevalence of *Cryptosporidium* among diarrhoeal patients referred to Ethiopian Health and Nutrition Research Institute (EHNRI) was 20.6 % (Endeshaw *et al*., 2004). The prevalence of *Cryptosporidium* infection alone in children with diarrhoea ranged from 3.3 % in Jimma, 5.6 % in Addis Ababa, and 9 % in North-western Ethiopia (Mersha and Tiruneh, 1992 and Assefa *et al*., 1996). Another study with special emphasis on opportunistic parasitic infections among paediatric diarrhoeal patients in visiting hospitals in Addis Ababa, showed that the rate of *Cryptosporidium* spp. infection among these patients to be 8.1% (Gebru and Girma, 2000).

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

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 in countries (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 noninvasive 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 (Haque *et al.,* 1995). 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. 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

stop moving which then become very difficult to identify. Direct microscopy should be done by mixing a small amount of the specimen in 0.9% sodium chloride solution. This permits detection of motile trophozoites of *Entamoeba spp* 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 (Broke, 1977). 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 (Meisel *et al*., 1976); staining techniques to identify the oocysts in the feces oocyst antigen detection via immunofluorescence, enzyme linked and agglutination immuno-assays (Petry, 2000); 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 (Petry, 2000).

2.7. Control and Prevention of Intestinal Protozoan Parasites Infections

There are different mechanisms to prevalent 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 (Flanagan, 1992).

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 water must be boiled before it is safe 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.parvum* 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 behaviours can play a key role in reducing the incidence of human intestinal 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 (WHO, 1999). 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

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

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study area was Kuyu Woreda, Gerbe Guracha town, North Shoa Zone which is located 156Km away from the capital city of Ethiopia, Addis Ababa and 42km from Fitche North Shoa Adminstive Zone. Gerbe Guracha town covers a total area of 3000 sq.Km and has 2 *kebeles* with total population of 26,334. There are three school in the town & one Hospital and two health center. Each *kebele* has at least one health extension worker who is assigned to provide home-to-home health service to the community

The altitude ranges 1900m - 2650m ASL at the highest peak. The annual minimum and maximum temperature range from 6 to 24 degree centigrade, respectively. The mean annual temperature is 15 degree centigrade. The rainfall is bimodal pattern occurring during mid February- April (small rains) and June-August (main rainy season).The mean annual rainfall is about 1800 mm. Most of the area is known to have fertile soil, which is suitable for agricultural activities. The livelihood of the communities is based on mixed farming (cultivation of crop and raring animals), Merchant and Employers. The area experiences "*daga*" (90%) *&* *temprate* (10%) Climates (GGTICO, 2014). The town is divided into six *sub-kebeles* with total households of 11,340. Most of the dwellers of Gerbe Guracha town are merchants, urban farmers and employers. There are two primary schools, one Secondary and compressive secondary school in Gerbe Guracha town. The present study was conducted in Gerbe Guracha Number three primary schools.

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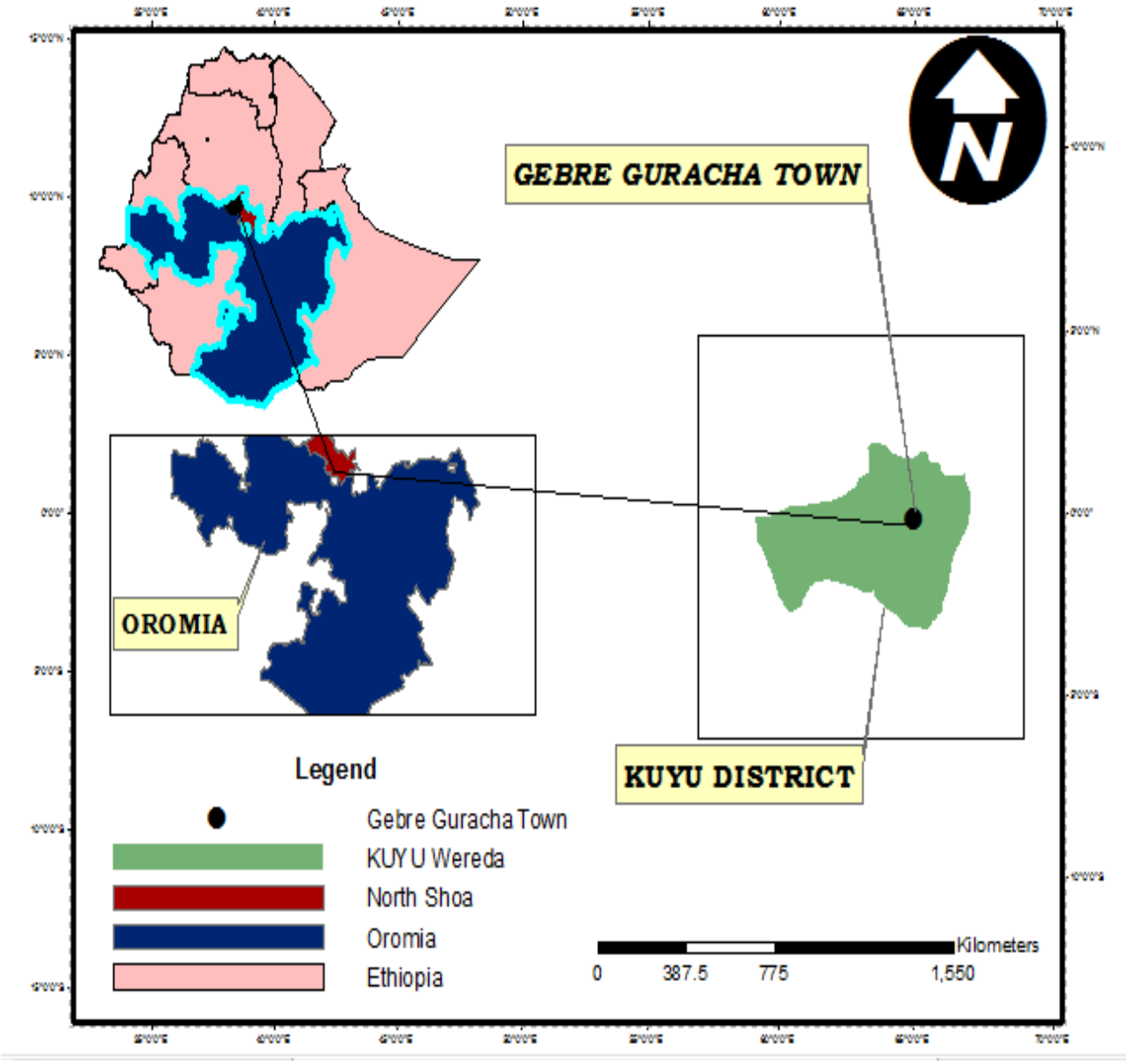

1mm=1000km

▶ Figure Map of study areas

3.2. The Study Design

A descriptive cross-sectional survey was carried out among primary school children to determine the prevalence of intestinal protozoan parasites infection. In addition water sample from different source were examined to determine their prevalence in the study areas. Laboratory examination of stool sample was carried out using direct wet mount, formol ether concentration and modified-zeihl-Nelsen method. In addition structural and pre-tested questionnaire were used to collect data regarding socio demographic characters, enviromenental related facters, sanitary indicaters, water source, latirin facility and habit of eating unwashed friut and vegetables and resident areas. Respondant were obtained from 404 study population of school childrens. Survey was conducted from February to March, 2014 in Gerbe Guracha Number three primary schools and in different water sources in the study area.

3.3. The Study Population

A total of 404 study participants of volunary primary school childern of different sex and age groups were visited in Gerbe Guracha number three primary school of Gerbe Guracha town, North shoa, Oromia region, Ethiopia.

3.4. Sample Size Determination and Sampling Methods

Students from Gerbe Guracha number three primary school children, who were volunteers to participate and complete and signed the consent form by their parents were included in the present 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

p = 0.5 (prevalence value)

Z=1.96 (score corresponds to 95% confidence interval.)

d= 0.05 (margin of error)

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, Four hundrerd four (404) school-children were chosen randomily to participate in the present study. To select the sample children, the students were first stratified according to their educational level (grade 1 to 8). Proportional sample number was then allocated for each grade and each section. 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 questionnaire survey was conducted to assess the prevalence of intestinal protozoan parasite and in drinking water among primary school children in the study area. In addition, stool and water sample were collected to assess and determine the prevalence of intestinal protozoan parasites.

3.5.1 Clinical examination

Each study participants was examined by health profession, physically and clinical condition was recorded onpreperd aproprates format developing for their purpose. Wheather, they have or not clean water and clothing, hygienic condition and environmental sanitation source of water, place of toilet for any body abnormality by the investigator.

3.5.2. Questionnaire survey

The questionnaire was constructed in English and then translated into local language (Afan Oromo) for parents or care takers are interviewed by raising standardized question in their mother tongue. A total of 404 respondant were involved in filing the question. Validity and reliability of the questionair was evaluated by one of the laboratory techinologisty of kuyu Hospital.This standardized questionnaire was used to gather the relevant general information on demographic and socio-economic data of the school children in the study area. The questionnaire was administered for each parent of primary school childern of Gerba Guracha number three primary school in Gerbe Guracha twon.

3.5. 3. Stool sample collection

Fecal sample specimen of 2-3gm of fresh stool was collected from primary school children then after kept in a plastic container and transported to the laboratory for examination within five (5) to six (6) hour after collection then examined by the use of microscopy. At the time of sampling; date of sampling, age, sex, presence or absence of intestinal protozoan parasite infections and code number was recorded for each childern on the record format. In addition data was collected from primary school children. Two data collectors and 5 assistants (health workers of hospital and community member of respective town) were involved in the data collection. Sample of stool were collected from feaces of 404 children individuals from Gerba Gurracha number three primary school children, in Gerbe Guracha town. The staining techniques and the stool film examination for intestinal protozoan parasite like *Giardia lamblia*, *Cryptosporidium species* and *Entamoemba histolytic*a were conducted in Gerba Gurracha town.

3.5.4. Water sample collection

Atotal of 105 water sample was taken from three water source of seven sites within two hour interval for sixty days. In order to asses water borne intestinal protozoan parasite in the study area water sample of different source such as river, pond and hand pump were used for parasitological quality analysis. A total of 105 water samples (7 samples from each source were taken for five rounds from unprotected (Gerbe Guracha River, and pond) and protected (hand pump) drinking water sources to determine the presence or absence of cysts and oocysts of intestinal protozoan parasites.

All water samples were collected based on APHA (1999) sampling procedure. The collected water samples were transported to Kuyu Hospital Microbiology and Parasitology laboratory by keeping the samples at 4^{o}C, using appropriate insulated coolers (Ice box) according to Standard methods (APHA, 1999). All water samples were processed immediately after arrival within 2-6 hours of sample collection to avoid the death and growth of parasites of sample water collected from each source in different sampling location in Gerbe Guracha town. About 240 ml of water samples were collected from three types of water sources in different sampling site 7 from river, pond and hand pump water that makes a total of water samples- 105.

3. 6. Laboratory Parasitological Examination Procedures

About 90% laboratory examinations of stool samples and water sample were under taken at microbiology and parasitology laboratory of Kuyu Hospital.

3.6.1. Direct microscopy or Wet mount method

A direct wet mount with normal saline (0.85% NaCl solution) was prepared at study site laboratory and observed for the presence of motile intestinal protozoan parasites like trophozoites, cyst and oocyst under low objective power at 10X and 40X magnification. Lugol's iodine staining was also used to observe cysts of protozoan parasites (WHO, 1991).

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 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 high objective power at 1000X 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.

3.6.3. Formol-ether concentration method

Using an applicator stick, approximately 2gm faecal materials were placed in a centrifuge tube containing 10 ml of 10% 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 after, the filtrate was washed again, by transferring into a test tube containing 7 ml of ethyl acetate. The tube was closed with a stopper and it was shacked vigorously to mix. The stopper was removed and it centrifuged at 1500 rpm for 2 minutes. The

tube was rested in stand for five minutes. Four layers became visible with the top layer consist of ether, second were a plug of debris and the third would be a clear layer of formalin and the fourth would have been 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. Then, 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 was used to examine the whole of the deposit for cysts, oocyst and trophozoites (Lindo *et al.,* 1998).

3.6.4. Microscopic examination of water samples

Water sample was collected from point of sources (7 from Gerbe Guracha river, 7 from pond and 7 from hand pump) using 2L capacity white plastic container. There are five round of sampling. Before sample collected each of the containers was pre-sterilized using NaOCl (Sodium HypoChlorate) and distilled water. A total of 105 water samples were collected during February to March, 20014. The sample was handled in sterile glass bottles, labeled and kept in ice-box during transportation to microbiology and parastology laboratory at Kuyu Hospital. Samples were transferred into 15ml centrifuge tube sediments at 5000 RPM on centrifuge at 40^{o}C for 15 minute. Parasitological water sample analysis, centrifugation, and microscopic observation were conducted based on USEPA method (USEPA, 2005).

3.7. Data Analysis

Prevalence of intestinal protozoan parasitic infections was analyzed using SPSS, window Version 20. A statistically significant difference in frequencies was tested using chi-square analyses. All statistics was set to the significance level of P value $\leq$0.05 to indicate statistical significant differences where as p-value greater than 0.05 was insignificant.

In this study the following data types were collected and analyzed. These parents' educational level, availability of latrine, level of awareness towards water-borne protozoan parasitic infections; clinical sign and symptoms; household sanitation, environmental and personal hygiene's, family resident,way of eating unwashed friut and vegetables, family occupation,age ,sex, source of water and handling practices; type of intestinal protozoan parasites species found in the stool and water

sample of individual having intestinal protozoan parasitic infections among the total examined school children.

3.8. Data Quality Control

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

3.9. Ethical Consideration

The head of the North Shoa Zone Health Department and Kuyu *Woreda* Health office both have gaven their written consent for the study. All children tested positive for intestinal protozoan parasite inflections were treated with a single dose of drug prescribed by the physician.

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4. RESULTS AND DISCUSSION

4.1. Prevalence of Intestinal Protozoan Parasite Infections in School Children

A total of 404 Pimary School children were participated in the present study, of these, 214(52.9%) were males and 190(47.1%) were females (Table 1). Majority of study participants 319(78.9%) live in urban while the rest 85(21.1%) live in surrounding rural place of the study area .The minimum and maximum age of the study subjects were 7 and 16 years old respectively.

The prevalence of intestinal protozoan parasites in Gerbe Guracha number three Primary School children was showed in (Table 1). Out of 404 stool samples collected 68(16.9%) were found positive for at least one intestinal protozoan parasite species. Cysts of protozoan parasites of *Entamoeba histolytica* and *Giardia lamblia* and oocysts of *Cryptosporidium* species were found in some of the stool samples collected from Gerbe Guracha number three primary school children and. The prevalence of *Entamoeba histolytica* was higher among school children coming from surrounding rural area than from Gerbe Guracha twon ($P < 0.05$).

The prevalence of intestinal protozoan parasites infection among age group of 7-9 was 20.4% for males and 23.6% for females. For the age group of 10-12 years old, 15.4% was for males and 15.6% was for females. The prevalence of IPPIs for the age group of 13 and above was 8.8% and 6.4% for males and females, respectively. High prevalence of protozoan parasites infection was examined between the age group of 7-9 years old in both sexes (Table 1).

In general, the prevalence of IPPIs in primary school children at Gerbe Guracha number three primary schools are summarized and presented in Table 1. Among (16.9%) positive study participants, 8.2% were males and 8.7% were females. The result of the present study was much more less than studies conducted by Ayalew (2006) in Dire Dawa villages, who reported 25-45% prevalence of intestinal protozoan infection in school children. Although female were slightly higher in prevalence than male in the present study, there were no statistically significant differences in the prevalence of intestinal protozoan parasites infections regarding to sex among all age groups. Some how the sex of children has influence on the prevalence of *Cryptosporidium* species, *Giardia lamblia, Entamoeba histolytica* and other IPPIs (Table 1). This was due to the fact

that intestinal protozoan parasites infections were acquired by oro- feacal consumption of food and drinking water contaminated with infective cysts or oocyst. In the present study there were a few possible variations in prevalence of intestinal protozoan parasites infections between males and females. This is may be due to the fact that females were more often involved in food processing and handling activities than males and hence water and food contamination which is one of the most common modes of transmission (WHO, 1987)

In this study age was the risk factor for the prevalence of intestinal protozoan parasites infection (x^2=7.18, p =0.05). 21.8% of the positive participants were between the age group of 7-9, 15.5%, 7.8% were between age group of 10-12 and ≥13 old, respectively (Table 1). The higher prevalence of intestinal protozoan parasites infections in the study area was seen in the age groups of between 7-9 years old, indicated that these age groups were at higher risk for acquiring protozoan parasites infections. The possible reason for the higher prevalence in this age group of the present study was children have weak immune system, poor hygiene and lack of awareness to these protozoan parasites. Higher prevalence of protozoan parasites infections among school children may occur due to the poor sanitary conditions in the schools and at your home (Oguntibeju, 2006).

The studies conducted in different parts of Ethiopia indicate that 7-9 age groups have significant association with prevalence of intestinal protozoan parasites. Prevalence of IPPIs like cryptosporidiosis and giardiasis among asymptomatic children less than 14 years old was higher than adult children (Assefa *et al*., 1996). In endemic region, the highest infection rates have been seen in earlier age, for example in Mexico, 11% of the tested population aged 5 to 9 years was infected with *E. histolytica* (Caballero-Salcedo, *et.al.*, 1994). A similar study in Brazil also reported that high prevalence of *E. histolytica* other intestinal parasites was between the age group of 5-10 years (Fleming, 2006). This is because, younger people have lower resistance to intestinal protozoan infections as compared to adults since many of the defense systems are not fully developed in children. In addition to this, children are more exposed to over crowded conditions (schools, nurseries, playgrounds etc).

Table 1 Prevalence of intestinal protozoan parasites infections among school children in Gerbe Guracha Number three Primary School, North Shoa, Ethiopia, during February-March, 2014

Age group &sex	Male		Female		Both sex		X^2	p-value
	Number of examined	No of Positive (%)	Number of examined	No of Positive (%)	No of examinend	No of Positive (%)		
7-9	98	20(20.40)	76	18(23.66)	174	38(21.8)	1.031	0.794
10-12	71	11(15.49)	83	13(15.66)	154	24(15.5)	12.726	0.005
≥13	45	4 (8.88)	31	2(6.41)	76	6(7.89)	0.871	0.832
Total	214	35(16.35)	190	33(17.36)	404	68(16.9)	7.818	0.050

4.2. Major Intestinal Protozoan Parasites Species Identified from School children

As the result was summarized and presented in Table 2, three major species of intestinal protozoan parasites were identified in examined stools of Gerbe Guracha number three primary School children and significant association was observed between males and females children within the age group 10-12 years old ($P<0.05$). The predominant protozoan parasites species identified in the study were *Entamoeba histolytica* (9.40%), *Giardia lamblia* (5.44%) and *Cryptosporidium* species (1.985%). The result was higher than the prevalence of amoebiasis and giardiasis reported in South west Ethiopia as 3.1% and 3.6%, respectively (Amare *et al*., 2007). The higher prevalence of *Entamoeba histolytica* infection in current study might be attributed to the fact that most children in the rural communities 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 (Endeshaw, 2005).

The overall prevalence of intestinal protozoan parasite infections was (16.9 %) in the present study, which was lower than the prevalence of intestinal protozoan parasite infections of school children reported in different parts of Ethiopia. For example, Ayalew (2006) reported 38% prevalence of amoebiasis among school children from eastern Ethiopia (Dire-Dawa).

According to the study conducted by Yeneneh (1994), the prevalence of intestinal protozoan parasite infections among residents of four villages in South west Ethiopia was 82.7%. Similar studies done by Legesse Mengistu and Erko Birhanu (2004) on the school children in Lake Langano showed that the prevalence of intestinal protozoan parasites infections was 60.2%. Another study conducted by Legesse and Erko (2004), among school children around Lake Langano also reported that the prevalence of intestinal protozoan parasites species was 83.8%. The result of the present study was also lower than another school-based study done in Jimma by Haile and Mola (1994), who reported 68.4% prevalence for intestinal protozoan parasite infections.The differences in findings of the various studies can be explained by variations in geography, environmental sanitation, inadequate medical care, socio-economic conditions, drinking water source, hygienic conditions of the study subjects and health care as well as prevailing climatic and environmental considerations (WHO, 1996).

Table 2 Prevalence of major intestinal protozoan parasites species identified from examinend stool sample of school children, Gerbe Guracha number three primary school, Gerbe Guracha Town, by age and sex North shoa, Ethiopia, during February-March, 2014

Age group in sex &year	N0, of Examined	IPP			X^2	P-Value
		E/h	Gl	Cps		
		No, of positive (%)	No,of Positive (%)	No, of positive (%)		
7-9	174					
Male	98	12(12.24)	6(6.12)	2(2.04)		
female	76	9(11.84)	7(9.24)	2(2.63)	1.031	0.794
10-12	154					
Male	71	5(7.040)	4(5.62)	2(2.81)	12.726	0.005
female	83	8(9.63)	2(2.41)	1(1.20)		
13&above	76					
Male	45	1(2.22)	2(4.40)	1(2.22)	0.871	0.832
female	31	3(9.67)	1(3.23)	-		
Total	404	38(9.40)	22(5.44)	8(1.98)	7.818	0.050

E/h=*Entamoeba histolytica*, GL=-*Giardia lamblia*, CPs=*Cryptosporidium* and IPP-Intestinal protozoan parasite

As shown in Table 2, the overall prevalence of *Entamoeba histolytica, Giardia lamblia* and *Cryptosporidium* species was 38(9.40%), 22(5.44%) 8(1.98%), respectively. This was within the range of national wide prevalence of Amoebiasis and Giardiasis which ranges from 3-55% and 3-23 %, respectively. As shown in table 2, the prevalence of intestinal protozoan parasite of age wise distribution of Gerbe Guracha number three Primary School children indicate that 7-9 years old had higher prevalence rate than 10-12 and $\geq$ 13 years old. The distributions of intestinal protozoan parasite for 7-9 years, 10-12 and 13 and above years old were 38(21.83%), 24 (15.58%) and 6(7.89%), respectively. The result of table 2 showed that the infection rate of *Entamoeba histolytica was* higher in relation to *Garidia lamlia* and *Cryptosporidium* species.

4.3 Protozoan Parasite Species Identified in Different Drinking Water Source of Gerbe Guracha Town

Parasitological analysis of water sources showed that unprotected water sources (Gerbe Guracha river and pond) were found to be positive for cyst of *Giardia lamblia*, *Entamoeba histolytica/dispar* and oocyst of *Cryptosporidium* ranging from (37.14%) to (71.42%) from the sample taken, wher as from protected (hand pump) only 2.85% cyst of *Giardia* was found. Like wise the distribution of oocyst *Cryptosporidium* was found to vary among the different water sources. From the unprotected pond sample water taken, 11.42% was positive, from Gerbe Guracha River 22.85% were *Cryptosporidium* oocyst positive, no oocyst was found in hand pump. These result indicates there was a significant different in prevalence of Amoeba cyst, *Cryptosporidium* oocyst and *Giardia lamblia* cyst between protected Gerbe Guracha River, handpump and unprotected pond water source. In addition *Entamoeba histolytica/dispar/* was found 22.85%, 8.57% and 0.0% in Gerbe Guracha River, pond and hand pump, respectively. (Table 3). Like that of *Entamoeba histolytica* and *Cryptosporidium* species, *Giardia lamblia* were found 25.71%, 17.14% and 2.85% in Gerbe Guracha River, pond and hand pump, respectively.

There was no much variation on oocyst and cyst count among the different water samples except the sample from hand pump, but highest count was recorded from Gerbe Guracha River. Gerbe Guracha River was more exposed to feaces defecation and parasitological contamination and hand pump was less exposed to contamination with parasites and treated with chemicals. 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 lamblia* and other protozoan parasites are not common in protected water source than unprotected water source such as pond and Rivers (Parry *et al*., 2004).

Table 3. Intestinal protozoan parasites identified in drinking water source, North shoa, Gerbe Guracha Twon, water source (N-105), during February-March, 2014

water source	Number	IPP. In drinking water source			Total number of positive (%)
		E/h (%)	G/l (%)	C/s (%)	
Gerbe Guracha river	35	8(22.85)	9(25.71)	8(22.85)	25(71.42)
Pond water	35	3(8.57)	6(17.14)	4(11.42)	13(37.14)
Pipe water	35	-	1(2.85)	-	1(2.85)
Total sample	105	11(10.47)	16(15.23)	12(11.42)	39(37.14)

KEY-IPP=Intestinal protozoan parasite, E/H=*Entamoeba histolytica*, G/L=*Giardia lamlia* and C/S=*Crpytosporidium* species

Table 3 showed that among 105 water samples collected from Gerbe Guracha River, pond and hand pump, 25(71.42%), 13(37.13%) and 1(2.85%) intestinal protozoan parasite was identified in the study site,respectively. The study shown that the prevalence of intestinal protozoan parasite in Gerbe Guracha River was very high in compard to water source of pond and hand pump. The over all prevalence of intestinal protozoan parasite in dirinking water source of Gerbe Guracha town was 39(37.14%).

The parasitological analysis of this study demonstrated that, a sample taken from Gerbe Guracha River water source contain about 71.42% of protozoan parasite like *Entamoeba histolytica* cyst, *Cryptosporidium* oocyst, *Giardia lamblia* cyst and the least percent was detected from hand pump. For the concentration value of giardiasis and cryptosporidiosis, there was a significance difference between unprotected water source (pond and river) and protected water sources. Similar study was conducted in Addis Ababa drinking water sources demonstrates that, there was significance difference in concentration of *Giardia lamblia* and *Cryptosporidium* species between protected and unprotected water (Nigus *et al*., 2008). A study conducted by Atnafu (2010) in quality (Addis Ababa) shows that 33.3%-55.6% protozoan parasites were found in the sample taken from raw surface water and public tab (Atnafu, 2010).

unlike with the present study, the research conducted in South Africa revealed that protozoan parasites like *Giardia lamblia* and *Cryptosporidium* species were detected in all (100%) water samples collected from different water source such as River and pond (Sigudu *et al*., 2008). A study conducted by Karanis shows that 81.81% of *Giardia lamblia* and *Cryptosporidium* species were detected in samples from River (Karanis *et al.*, 2005). A study conducted by LeChevalier *et al.* (1995) on pond the average concentration *Giardia lamblia* and *Gryptosporidium* were within 0.4-6.3 and 0.3-9.8 respectively.The present study was much higher than the prevous finding by (LeChevalier *et al.* 1995).

The differences distribution of protozoan parasites in different drinking water source in the study area may be resulted due to public and domestic animal contaminations and lack of good awareness to intestinal protozoan parasites, lack of adequate water treatment and unhygienic practices around the water source. Protection of water sources and treatment of water supplies have greatly reduced the parasitological and microbial load of water source (WHO, 2003).

4.4. Relationship of Intestinal Protozoan Parasite Infections of School Children with Water Source and Handling Practices

Out of 404 participants of the study that used protected water source, 15.2% were found to be positive for intestinal protozoan parasites and from 56 study participants that use unprotected water like pond and River, 26.7% were found to be positive for IPPIs. In this study using of protected and unprotected water sources had significant variation in the prevalence of protozoan parasites (p=0.047). This finding was in agreement with what was reported by Ayalew (2006) that describes the prevalence of cryptosporidiosis, giardiasis and amebiasis between children using protected and unprotected water sources showed significant variations in Adada village (Dire-Dawa). The possible reasons for this variation might be the infections was restricted to some water sources, some households probably use treatment such as filtration, or boiling before consumption and understanding the poor quality of unprotected drinking water, however some household do not so. 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). From the respondents who use hand pump, pond and River for drinking water source, 2.8%, 37.1% and 71.4% were positive for IPPIs, respectively.

The prevalence of protozoan parasites was high in unprotected water source (River and pond) than the protected (hand pump) with showing of significance variation. The high activities of domestic animals and humans in the water sources might lead to repeated contamination of River with oocysts and cysts of protozoan parasites. A similar study was conducted in British showed that large number of intestinal protozoan parasites including *Cryptosporidium* species, *Giardia lamblia* and *Entamoeb*a *histolytica/dispar* were detected in drinking water samples from unprotected water sources where human activities was high. On the other hand, in protected water with no minimal human activities, the prevalence of cryptosporidiosis, Giardiasis, Amebiasis and other intestinal protozoan parasites were reduced (Isaac-Renton *et al*., 1999). To reducing parasite burden, keeping waste disposal from entering drinking water source and keeping personal hygiene and environmental sanitations.

Two types of water usage practices were obtained from the questionnaire distributed during stool sample collection directly and chemical treated water. Out of the 404 study subjects who said they use water directly/as it is/, 16.9 % were found positive for intestinal protozoan parasites and 0.00% for chemical treated respectively (Table 4). In this case the prevalence of intestinal protozoan parasites was high in the study areas, which use water directly without any chemical and physical treatment, no prevalence was observed in these who use by chemical treatment of water.

In general, in present study water-borne intestinal protozoan parasite infections was common among children in Gerbe Guracha town, this was due to water source and its handling as well as usage practice was improper, those showed significance variation in prevalence to the infection. Worldwide, in all endemic regions, the development of water resource plays an important role in the spread of protozoan and helminthes. Practices like using and playing in different water sources especially children's in school age continues to be important risk factor for contracting protozoan parasitic infection (Lo *et al*., 1988).

Table 4. Relationship of water source, handling and usage practices with prevalence of intestinal protozoan parasites among school children in Gerbe Guracha Town, during February-March, 2014

Characters	Number of examined	IPP.				X^2	P.value
		NO, of positive (%)	E/H Positive (%)	G/L Positive (%)	C/Ss Positive (%)		
water source							
River	35	25(71.4)	8(22.8)	9(25.7)	8(22.8)		
Pond	35	13(37.1)	3(8.5)	6(17.1)	4(11.4)	35.241	0.000
Hand pump	35	1(2.8)	-	1(2.8)	-		
Watersource&usage							
As it is	402	68(16.9)	38(9.4)	22(5.4)	8(1.9)	9.932	0.002
Chemical treated	2	-	-	-	-		
W/S/H/P							
Unprotected	56	15(26.7)	4(7.1)	6(10.7)	5(8.9)	3.923	0.047
protected	348	53(15.2)	34(9.7)	16(4.5)	3(0.8)		

Key-E/H=*Entamoeba histolytica*, G/L= *Giardia lamblia* and C/S=*Cryptosporidium* species
W/S/H/P=Watersource handling and practice

4.5. Association of Intestinal Protozoan Parasite Infections with Socio-Demographic Characteristics of School Childrens

The result of correlation between socio-demographic factors of the school-children and the prevalence of major IPPIs are presented in Table 5. The over all prevalence of each intestinal protozoan parasite among school children was diagnosed in the study pupils in relation with the proportion of different socio-demographic factors are summarized and presented in (Table 5).16.3% and 17.3% of positive participants male and female, respectively. Sex of students were the one of the factors for the prevalence of intestinal protozoan parasitic infections in the present

study (p=0.768) (Table 5). As the result showed in (Table 5) among the study participants who were positive for one or more IPPIs, the parents of 23.8% participants were reported to be illiterate, 15.04% participants parent had primary eduction 14.2% participants parents had secondary education and above were found to be positive for one or two of IPPIs. The result showed that there was statistical significance association between the parents level of education and intestinal protozoan parasite infection in the present study school children (p=0.049). 283(70.04%) of the student's households had latrines in close vicinity in their homes, 27(9.5%) were found to be positive for intestinal protozoan parasitic infection, the remaining 121(29.9%) of the student's households did not have latrines in their homes, 41(33.8%) were found to be positive for protozoan parasites infections.

There was statistically significant association between prevalence of IPPIs and latrine availabilities i.e. p=0.000 (Table 5). This might be due to presence of latrine enable to keep their environmental sanitation and personal hygiene. As a result the probability of food and water source contamination which are the route for protozoan parasites transmission becomes reduced. Under poor hygienic condition, faeces and urine often enter water body due to lack of proper latrine, this enhances transmission probabilities intestinal parasites like protozoan and helmenths through indiscriminate defecation habits (Wadood *et al*., 2005

About 85(21.03%) participants of the study who live rural resident, 32.9% were found to be positive with IPPIs and about 319(78.9%) urban resident, 40(12.5%) of them were positive for IPPIs. Among 85 and 319 study participants who were rural and urban about 32.9% and 12.5%, were found positive to one or more IPPI(s), respectively (Table 5). Awareness of parent and children of rural resident about intestinal protozoan parasitic infections were important to prevent protozoan parasites infections ($p \leq 0.05$). This may due to lack of awarance of children of rural resident about the parasite. 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)

Generally, the prevalence of IPPIs and some factors such latrine, sex, age, educational level, residant of parent and source of water are the major facter for intestinal protozoan parasites

infections are statistically significant in the present study ($p \leq 0.05$). Therefore, school-based hygiene education is vital in order to decrease the rate of transmissible diseases. Children are more receptive to learning and are very likely to adopt healthy behaviors at younger age. They can also be agents of change by spreading what they have learned in school to their family and community members. Enhanced comprehensive Knowledge (awareness) about these issues should be used to improve low-cost but highly effective programs that will meaningfully attenuate the burden of transmissible diseases among school children in rural settings (Lopez-Quintero *et al.*, 2009)

Table 5 Socio-demographic factors associated with intestinal protozoan parasites infections among school children of Gerbe Guracha number three Primary School, Gerbe Guracha Town, North Shoa, Oromia, Ethiopia, during February-March, 2014

Characteristics	Numbers	IPPI No of positive. (%)	OR(95%CI)	X^2	P-value
Sex					
male	214	35(16.35)	0.925(0.549-1.558)	0.87	0.768
female	190	33(17.36)			
Age					
7-9	174	38(21.83)	0.00(0.00-0.007	52.27	0.000
10-12	154	24(15.58)			
≥13	76	6(7.89)			
Residence					
Rural	85	28(32.94)	3.426(1.956-6.003)	19.957	0.000
Urban	319	40(12.53)			
Jobs/ocupation/					
Private	376	68(18.05)	0.014(0.002-0.023)	6.089	0.012
Civil servant	28	0(00)			
Educational level					
Illitrate	88	21(23.86)	0.054(0.032-0.077)	7.428	0.049
Primary	246	37(15.04)			
≥9	70	10(14.28)			
Wash after toilet					
Always	61	4(6.55)	0.035(0.017-0.052)	5.418	0.020
Some times	343	64(18.65)			
Eat unwashed friut &vegetable			1.274(0.274-2.183)	0.782	0.377
Always	236	43(18.22)			
Sometimes	168	25(14.88)			
Use of toilet				35.882	
Open field	121	41(33.84)	4.859(2.813-8.395)		0.000
Private	283	27(9.54)			
Water source					
Hand pump	348	53(15.22)	0.510(0.260-1.002)	3.932	0.047
Spring &pond	56	15(26.78)			

IPPI=Intesinal protozoan parasite infection

Table 5 showed that the prevalence of intestinal protozoan parasite infections in accordance to childrens: resident area (rural and urban), in regard to educational level (illitrate, primary education and secondary education and above), in relation to toilet usage (open field and private close

vicinity) and in regard to water source(protected had pump and unprotected spring/pond) and occupation(private and civil servant),eating un washed friute and vegetables(always fresh,some times fresh) 32.9%, 12.5%, 23.8%, 15.04%, 14.2%, 33.8%, 9.4%, 26.7%, 15.2%,18.05%,00% ,18.22%,14.89% respectively. The association between the prevalence of IPPIs and children habit of eating unwashed firut and vegetable and jobs of a family/ occupation/ were statistically insignificant ($p > 0.05$). It was found that higher prevalence of protozoan parasites infections among school children who had desifect in open field, rural resident and illitrate families were stastically significant ($p \leq 0.05$). Most of the results of the present study was statistically agreed with ($p<0.05$) with the study reported by Quna (1994) in Portugal. As indicated in the above table residance of the family, habit of washing after toilet, place of toilet and educational states of the family are highly associated with the prevalence of IPPIs among primary school children.

Table 5 also showed that the prevalence of human intestinal protozoan parasite infections in relation to place of using toilet was 41(33.84%) open field, 27(9.54%) private. As the result shown in table 5, relatively high prevalence of intestinal protozoan parasites infections was observed in rular dwellers in relation to urban 28(32.94%) and 40(12.535%), respectively. The association between the prevalence of human intestinal protozoan parasites infections in relation to resident area was statistically significant ($p \leq 0.05$). In addition to the above in regard to source of water using unprotected water 15(26.78%) compared to pipe/hand pump/ water 53(15.22%). This was in agreement with previous study reported by Olusegun *et al*. (2011). According to the Olusgun study, the association between the prevalence of intestinal protozoan parasitic infections and method of source of water was statistically significant ($p<0.05$).

The results from Table 5 revealed that the prevalence of intestinal protozoan parasite infections in relation families residance area, place of use of toilet and habit of washing after toilet were highly significant (p-value =0.00, 0.000- 0.020), respectively. In this study, mothers'/caretakers' management practices were also assesed and the result showed that most of them responded that using toilet in open field resident of a child lack of habit of washing after toilet was found significantly associated with intestinal protozoan parasite infections. This might be due to factors like poor sanitation in the field, lack of awareness of rural families about the parasite use of unsafe drinking water and lack of toilet facilities are the main contributors to the high prevalence of intestinal protozoan parasitic infection in the study area.

Table 6 House hold and hygienic condition that related with school children intestinal protozoan parasite infections in Gerbe Guracha primary number three primary School, Kuyu woreda, North shoa, Ethiopia during February- March, 2014

Characters	Numbers	IPPIs			
		No, of positive (%)	OR (95%CI)	X^2	p.value
Children meal					
Always fresh	41	6(14.6)	0.832	0.157	0.692
Sometimes fresh	363	62(17.1)			
W/C					
Add chemical	2	-	6.091	9.932	0.002
As it is	402	68(16.9)			
D/U/C					
Yes	270	36(13.3)	0.490	7.117	0.008
No	134	32(23.8)			
P/H/E/S					
YES	359	60(16.7)	0.629	1.190	0.027
No	45	8(17.7)			

KEY=W/C—water consumption, D/U/C=Dining utensil clean and P/E/H=Personal hygiene &Environimental sanitation

The prevalence of intestinal Protozoan parasite infections in accordance to children`s meals (always fresh,some times fresh), in regard to water consumption(use by adding chemical, as it is), inregard to dining utensil kept clean(yes or No) and personal and environmental sanitation,6(14.63%), 62(17.07%), 0(00%), 68(16.915%), 36(13.33%) 32(23.87%) and 60(16.71%), 8(17.77%) were positive for one or two intestinal protozoan parasite, respectively (Table 6). The associations between the prevalence of human intestinal protozoan parasites infection in relation to childrens meals are statistically insignificant ($p > 0.05$). It was found that higher prevalence of intestinal protozoan parasites infections among children lack of kepping dining utensil kept clean and personal hygiene and envaronimental sanitation. As indicated in table

6 most of the rest of the characters of the present study was statistically significant ($p<0.05$). The prevalence of intestinal protozoan parasite infections in relation to method of using dining utensil yes or No 13.35% and 23.87% was found to be positive for intestinal protozoan parasites infections respectively. Relatively high prevalence of intestinal protozoan parasites infections was observed in those who had lack of keeping dining utensil cleans 32(23.87%). The prevalence of human intestinal protozoan parasites infections in relation to method of water consumption, use of dining utensil kept clean and knowledge of personal hygiene and environmental sanitation were statistically significant where ($p \leq 0.05$). (Table 6).

Table 7. Observed Clinical Signs and Symptoms among Examined (N= 404) of primary school children of Gerbe Guracha number three and Its relationship with Intestinal protozoan Parasite Infections, during Februrary-March,2014

Clinical manifestation	Numbers of examined	IPPIs	OR (95%CI)	X^2	P-value
		No, of positive (%)			
CHC					
Poor	168	40(23.80)	2.419(1.416)	10.853	0.001
Good	236	28(11.86			
CPC					
poor	133	33(24.81)	2.3(1.350-3.920)	9.703	0.002
Good	271	35(12.91)			
COF					
Watery	18	13(72.22)			
Softy	36	8(22.22	0.00(0.00-0.007)	4.094	0.000
Pasty	0	0(00)			
Normal	350	47(13.42)			
Nausea					
Yes	52	13(25.00)	1.725(0.866-3.435)	2.453	0.117
No	352	55(15.62)			
AD					0.000
Yes	76	24(31.57)	2.979(1.670-5.313)	14.543	
No	328	44(13.41)			
Flatulance					
Yes	65	10(15.38)	0.881(0.424-1.829)	0.116	0.734
No	339	58(17.10			
Loss of appitite					
Yes					
No	66	9(13.63)	0.017(0.005-0.030)	10.725	0.013
	338	59(17.45)			

KEY-CHC-Children hygienic condition, CPC- Children physical condition, COF- consistancy of faeces &AD=Abdominal discomfort

As showed in (Table7), hygienic condition, physical condition, consistency of feces, abdominal discomfort, and loss of appetite were statisticaly significant ($P<0.05$). Although Nausea and filatulance had its own impact, it was statisticaly insignificant ($P>0.05$). Children who had watery diarrhea (72.22%), abdominal discomfort (31.57%), poor hygienic (23.8%) and poor physical condition (24.8%) were positive for intestinal protozoan parasite. Where as children who had normal faeces (13.42%), no had abdominal discomfort (13.4%), good hygienic condition (11.8%), good physical condition (12.9%) were positive for at least one intestinal protozoan parasite. Hand washing after desfecation is one of the most effective ways to prevent intestinal protozoan parasite infections (Curtis *et al*, 2009). The high prevalence of intestinal protozoan parasite in poor personal hygienic children may be due to their lack of awearance or poor knowledge about parasite transmission (Lopez *et al*, 2009).

As showed in (Table 7) the chance of having one or two intestinal protozoan parasite is highest for those who had watery diarrhea (72.22%), incompard to softy (22.2%), pasty (0.00%) and normal (13.42%) respectively. As indicated in table 7 nausea and flatulance were stastically insignificant p-value (≥ 0.05). Were as hygienic condition of children, physical condition, consistency of feaces, abdominal discomfort and loss of appetite were stasticacally significant ($P \leq 0.05$).

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

The objective of the present study was to determine the prevalence of intestinal protozoan parasites infections among primary school children and in drinking water sources of the study area. The design of the study was a cross-sectional parasitological survey involving a sample participant of primary school children in Gerbe Guracha Town during Febrary –March, 2014. A total of 404 stool samples were collected and examined by Direct Wet Mount, Formol-Ether concentration and Modified Ziehl-Neelsen Method and showed that the prevalence of infections with various human intestinal protozoan parasites were common in the study area. The overall prevalence of infections with different types of intestinal protozoan parasites was 68(16.9%).

The predominant parasites involved in this study were *Entamoeba histolytica* 38(9.40%), *Giardia lamblia*, 22(5.44%) and *Criptosporidium* species *8(1.98%).* In addition, the prevalence of intestinal protozoan parasite in drinking water source were 11(10.47%) *Entamoebahistolytica*, 16(15.23%) *Giardia lamlia* &12(11.42%) *Cryptosporidium* species. The sum total prevalence of intestinal Protozoan parasite in drinking water source of the study site was 39 (37.14%). The prevalence of IPPIs in Gerbe Guracha town was mainly due to lack of toilet facilities especially in rural resident, unclean dining utensil, lack of clean water source, low level of educational status of the family and personal hygiene and Environmetal sanitation.

The prevalence of human IPPIs within primary school children regarding toilet facilities was 41(33.84%) open defecation, 27(9.54%) closed vicinity, rural dweller 28(32.94%), urban 40(12.53%), water source spring water 15(26.78%), pipe water 53(15.22%) and educational level of the family illitrate 21(23.86%) ($P < 0.05$), primary education 15.4% and secondary and above 14.2%. This showed that the association between the prevalence of intestinal Protozoan parasite infection in regard to resident areas of parents, water source, toilet facility and hygienic condition of dining utensil was statistically significant ($p< 0.05$).Whereas the rate of prevalence of intestinal protozoan parasite in drinking water source of the study site was 25(71.42%) in Gerbe Guracha river, 13(37.14%) in pond water and 1(2.85%) in hand pump water.

5.2. Conclusions

The prevalence rate of *Entoamoeba histolytica* 38(9.41%), *Giardia lamblia* 22(5.44%) and *Cryptosporidium* species 8(1.98%) were detected in the present study. The higher prevalence was due to lack of of aweranse of the rural resident about the parasite, family toilet condition, use of spring water for drinking, unproper sanitation of dining utensil and low level of family education. In drinking water sources, the prevalence rate of *Entoamoeba histolytica* 11(10.47%), *Giardia lambilia* 16(15.23%) and Cryptosporidium 12(11.42%). The over all prevalence of IPPs in stool sample collected was 68(16.9%), where as in drinking water sources the prevalence of Giardiasis was 16(15.25%), Amoebiasis 11(10.47%) and Cryptosporidiasis 12(11.42%). The sum total prevalence of IPPs in drinking water source was 39(37.14%). Generally the prevalence of IPPIs in different drinking water source was higher in relation to fresh stool sample collected from primary school shildren.

5.3. Recommendations

Based on the findings of the present study, about the prevalence of IPPIs among school children, the following recommendations are made;

- There is a need to provide a well protected and treated drinking water to the community.
- Strategies to control IPPIs through sanitization should be implemented targeting school age children.
- Further analysis of the drinking water sources for the presence of cysts and Oocysts should be done.
- Woreda health sector should collaborate with school health program for delivering health education to increase the knowledge and attitude of primary school children about personal hygiene, environmental sanitation, toilet facilities, proper waste disposal, transmition and preventions of human IPPIs.

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7. APPENDICES

7.1.AppendixIV.English Version of the Questionnaire

An ensuring format of parentsof primary school children.

Part one: A format of gathering data for primary school children parents.

My respectful greetings go to you here.

I would like to thank you so much for your voluntariness to complete this Questionnaire.

My name is---------- I am a post graduate student in Haramaya University, Natural and Computation Sciences Collage, Biology Department and marking on a Thesis for masters' degree partial fulfillment.

The objective of this study is intended to improve your and your family health. Hence, I honestly need your voluntariness and genuine information for it's of successful results.

Your name, special address, personality and information's you have provided are not indicated with what so ever ways explicitly.

Despite your willingness, the researcher assures you have a right not to cooperated if you don`t want to do so.

Part two: An ensuring format for Volunteer parents.

1. I agree to participate in the study based on the above explanation; ----------
2. I disagree to participate in the study based on the above explanation; ----------

Date of the questionnaire held ----------, starting time----------

Ending time ----------

Researcher`s name----------, Signature----------

Questionnaire

I. Research Site ----------
II. Code number of the childrens ----------
Details about a child and family

1. children`s sex: M ---------- F----------
2. Age : A) 7-9 years
   B) 10-12 years
   C) 13& above years
3. family`s dwelling areas; A) urban B) rural

4. Job of family A) government worker B) farmer and other private worker
5. family`s educational status; A. primary education; B.secondary and above
   C.Illtrate
6. Are dinning utensils which your child uses kept clean A) Yes B) NO
7. Do your children wash their hand after toilet? A) Always B) sometimes
8. Do your children eat unwashed fruits and vegetables? A) Always B) sometimes
9. What about Your toilet condition; A) private B) Openfield
10. Your sources of water; A) pipe B) spring
11. Water consumption; A) adding purifying chemicals B) as it is
12. Your children meal is; A) always fresh B) sometimes fresh
13. Do you cut your children nail when grown? A) Always B) sometimes
14. Did you get informations and training about personal and environmental hygiene and sanitation before? A) Yes B) No

15. If your answer for questionnNo 14 is "yes"where did you get? A) Fromfamilies
    B) health extension personnel'sand health center / hospital`s professionals

7.2. Appendix V Gaaffannoo Afaan Oromo

Gaaffannoo

Maatii barattoota sadarka tokkoofaa fedhiin oddeffannoo nu laatan Kutaa tokko;-seensa baratota sadarka tokkoofa oddeeffannon itin kenamu.

Akkam jirtan

Duran durse gaaffannoo keenyaf Eeyyemama ta',u keessanif baayy,ee isin galateefana.

Maqaan koo----------jedhama

Yenversity Haramayati kollejii saayinsii Uumama Mumme Baayoloojiin hirmata kitaba Eebaa digirii lammaffatti.

Kaayyoon qo, aanno fi qoranno kana faayyuma keessani fi maatiikeessan ni foyyessa jedhame waan yaddamuf, galma ga, insa qo, anno kanatiif fedhin isinqabdaniif fi oddeeffannonsirrii isin nu laatan baayy,ee barbaachisaa waan ta,ef fedhiin akka nutti himtan kabajan isin gaafan.

Formii Gaaffannoo kana irratti, Iddo adda, Eenyuma nama raga kenne fi ragan kan isa ta,u mirkannesu hin ibsu.

Waraqaa Qo, annoo kana irratti akka nu hirmatan yeroon isin gaafadhu fedhii irratti kan hunda,e malee dirqama hin qabu.

Kutaa lamaa

Formiioddeeffaannon maatii barattota sadarkaa tokkoffaa fedhii irratti hunda, ee ittin gurramu.

Akkuma armaan olittii ibsi naa godhametti odeeffannoo kennuf –waligaleera-----walihin galee.

Gaffannoon kuni guyyaa ittii geggeeffamee -------gaaffannoon kun guyyaa itti xumurame----

Gaaffannoo kan qopheessee fi kan rawwate ----------Mallatto----------

Gaaffannoo

Nannoo qo'annoon itti gageeffame----------

Koodii barattoota yaalamani----------

Haala barattoota sadarkaa 1ffaa fi maatii isaan

1. Saala barattoota Dhi----------Dha----------
2. Umuri

   A.Waggaa 7-9 B.Waggaa 10-12 CWaggaa13 and above

3. Iddoo jireenyaa maatii barattoota A.Badiyaa B.Magalaa
4. Hojii maatii A. Qonnaan bulaafihojeta biro B.Hojjettaa motummaa
5. Sadarkaa barumsa maatii A. Barumsaa sadarka tokkoffa Kan xumuree B. Sadarkaa 2ffaa fi isaa ol kan baratee.C. Kan hin barane
6. Ijoolleen keessan meeshaan nyaataf itti fayyadaman haala gariin Kan qulquula'ee

   A.Eyyeen B.Miti

7. Muucan keessan ergaa man fincaani seenan baha an booda ni dhiqatuu?

   A.Yeroo hundaa B.Darbee darbee

8. Kuuduraa fi fuduraa hin bilchanee fi hin dhiqamnee ni nyaatu?

A..Darbee darbee B. Yeroo hundaa

9. Haalii mana fincaan keessani A.Bakkeetti B.Kan dhunfaa C.kan walii

10. Madda bishaan itti fayyadamtaan A.Kan kuree B.Kan burqaa

11. Bishaan itti fayadamtaan akkamitti fudhataan (ijoollee keessani latuu) ?

A.Danfisuun B.Akkumaa jiruutti

12. Nyaani ijoolleen keessan nyaatan A.Yeroo hundaa ho'aa B.Darbee darbee ho'aa

13. Haalii ijoolleen keessan qeensaa osoo hin dheerresiin murataan?

A.Yeroo hundaa B.Darbee darbee

14. Kana duraa wa'ee qulqulinaa dhuunfa fi naannoo irratti barumsa argataani beektu?

A.Eeyyee B.Miti

15. Gaaffilee 14 irratti deebin keessan eyyee yoo ta'e barumsa kan eessaa argataan?

73. Appendix VI Laboratory Data Collecting Format

Code	Student Name	Sex	Age	Presence/Absence of parasite	IPP.Identified in stool sample			Single infection	Double infection	Others	Remarks
					Entoamoeba histolytica	Giardia lamblia	Crptosporidium parvium				

Key-IPP=Intestinal protozoan parasite, Presence=X and Absence=A

7.4. Appendix VII Observed Clinical Signs and Symptom Recording Format

Children hygienic condition Poor Good		Remark
Phsical condition of children Poor Good		
Consistancy of feces Watery Softy Pasty Normal		
Nausea Yes No		
Abdominal discomfort Yes No		
Filatulance Yes No		
Loss of appetite Yes No		

KEY=poor—p, Good-g, Yes-y and No-n