Prevalence and Major Risk Factors of Intestinal Parasitic Infections among Under- five Aged Children Visiting the Health Center at Debre Birhan Town, Central Ethiopia

M.Sc. Thesis

By

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January, 2013

Haramaya University

Prevalence and Major Risk Factors of Intestinal Parasitic Infections among Under-five Aged Children Visiting Debre Birhan Health Center at Debre Birhan Town, Central Ethiopia

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By

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APPROVAL SHEET

SCHOOL OF GRADUATE STUDIES

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As thesis research advisor, I herby certify that I have read and evaluated this thesis by Mesgie Ayenew, entitled **Prevalence and Major Risk Factors of Intestinal Parasitic Infections among Under- Five Aged Children Visiting Debre Birhan Health Center at Debre Birhan Town, Central Ethiopia,** by Mesgie Ayenew. I recommend that it is submitted as fulfilling all the requirements.

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DEDICATION

Dedicated to my best friends and family for their love, inspiration and support.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my original work and that all sources of material have been duly acknowledged. This thesis has been submitted in Partial fulfillment of the requirements for the Degree of Master of Science in Applied Biology 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.

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LIST OF ABBRIVATIONS ACRONYMS

CDC	Center for Disease Control
CI	Confidence Interval
CSA	Central Statistical Authority
DDARD	Division of Diarrheal and Acute Respiratory Disease
DHC	Debre Birhan Health Center
DQC	Data Quality Control
HU	Haramaya University
IPPI	Intestinal Protozoan Parasitic Infection
IPI	Intestinal Parasitic Infection
IHPI	Intestinal Helminthes Parasitic Infection
MASL	Meter Above Sea Level
NCCLS	National Committee on Clinical Laboratory Standard
OR	Odd Ratio
STH	Soil Transmitted Helminthes
WHO	World Health Organization

BIOGRAPHICAL SKETCH

The author was born in Gondar, South Gondar in 1973. He attended his primary education in Agona Primary School and his secondary education in Estie Mekaneyesus Comprehensive Secondary School. He did his diploma in Biology in Kotebe College of Teachers Education in 1986 E.C. and then his Bachelor of Education degree in Bahir-Dar University in 2009. Following this, the author had been teaching Biology at Debire Birhan General Secondary School until he joined the Department of Biology, Haramaya University, for his graduate study in Applied **Biology in July, 2011**

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF TABLES IN THE APPENDIX

Prevalence and Major Risk Factors of Intestinal Parasitic Infections among Under-Five Aged Children Visiting Debre Birhan Health Center at Debre Birhan Town, Central Ethiopia

Abstract

Intestinal parasitic infections are one of the major public health problems in countries including Ethiopia. The objective of the present study was to assess the prevalence of intestinal parasitic protozoan and helminth infections and associated risk factors among less than five aged children of Debre Birhan Town, Central Ethiopia. The design of the study was a cross-sectional survey i involving a sample population of children from 0-5 years of age visiting Debre Birhan Health Center during May-August 2013. Data were gathered by means of questionnaire and lab- based experiment. A total of 384 fresh stool samples were examined using direct wet-mount, Formol-Ether concentration and Modified Ziel-Neelsen methods. Of the faecal samples examined, 176 (45.8%) were male and 208(54.2%) female children. It was found that 71 (40.3%) male and 80 (38.5%) female children were infected with intestinal parasites. Thus, the overall prevalence of infections with different types of intestinal parasites was (39.3%). The prevalence of protozoan parasites, Entamoeba histolytica, Gardia lamblia and Cryptosporidium parvum infections was 18.5%, 11.5% and 2.1%, respectively. Similarly, the prevalence of helminth parasite infections, Ascaris lumbricoides, Trichuria trichuria and Hymenolepsis nana was 7.3%, 6.3% and 2.9%, respectively. The risk factors that were most associated with intestinal parasitic infections were consumption of unwashed fruits and raw vegetables and absence of toilet facility ($p<0.05$). This suggested that personal hygiene, environmental sanitation, and health education are needed to reduce the prevalence of human intestinal parasitic infections among children in the study area.

***Key Words**: Debre Birhan, helminthes, less than five aged child, prevalence, protozoan, risk factors,*

1. INTRODUCTION

During our relatively short history on Earth, humans have acquired an amazing number of parasites, about 300 species of helminthic worms and over 70 species of protozoa. Many of these are rare and accidental parasites, but we still harbor about 90 relatively common parasite species, of which a small proportion cause some of the most important diseases in the world, inevitably, these are the ones that have received the most attention(Cox, 2002). It has been estimated that *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* infect 1,450 million, 1,300 million and 1,050 million people worldwide, respectively, while schistosomiasis affects over 200 million people (WHO, 2002). *Entamoeba histolytica* and *Giardia lamblia* are also 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 parasites cutting across all continents and regions of the world (Morales-Espinoza *et al.*, 2003; Kucik *et al.*, 2004; Okodua *et al*., 2004).

Human intestinal 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 increasing trends of intestinal protozoan and helminth infections, occur particularly in developing nations (De Silva *et al*., 2003). A high prevalence of intestinal parasitic 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 parasitic infections increase host`s susceptibility to other infections and diminish learning ability and growth in children (Karaman *et al.*, 2006).

Parasitic protozoa and helminth species are responsible for some of the most devastating and prevalent diseases of humans. Intestinal parasitic infections (IPI) 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 (De Maeyer, 1989; Quihui, 2006).

Intestinal parasitic infections are one of the major causes of morbidity and mortality throughout the world and particularly in developing countries where mortality rate due to parasitic diarrhea could be as high as 56%(WHO, 2004). Children and young adults are the most affected, particularly in regions with limited resources' and where hygienic measures are not strictly followed (Guerrant *et al.,* 2005). In Africa, diarrhea has been estimated to be responsible for 25-75% of all childhood illnesses (Kirkwood *et al*., 1991), and episodes of diarrhea led to about 14% of outpatient visits and 16% of hospital admission and accounted for an average of 35 days of illness per year in children aged less than 5 years old (Greenwood *et al*., 1987). Intestinal parasites are associated with serious clinical diseases and mortality and are known to cause malnutrition and impairment of physical development in children and affect their growth, development and learning performance. It is, thus, necessary to have a fairly-accurate picture of the situation to target intervention in affected areas. Diarrheal diseases are one of the major causes of mortality in developing countries; their control and prevention is one of the main objectives of the World Health Organization (WHO) with in Davison of Diarrheal and Acute Respiratory Disease Control Program in developing countries (WHO, 1995).

Intestinal parasitic infections are caused either by protozoa or helminthes or both and 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*, *Ascaris lumbricoides*, whip worm/ *Trichuris trichura*, dwarf tape worm / *Hymenolepis nana,* and usually infection happen via food and drinks and polluted vegetables, as in most regions of the world (Dieng *et al.*, 1999; Esfandiari *et al*., 1995).

Intestinal 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). Several studies carried out worldwide show that the prevalence of *A. lumbricoides* and *Trichuris trichiura* was very high in the patients examined, when compared with other parasites such as *Strongyloides stercoralis*, *Hymenolepis nana* and others (Fernando *et al.*, 2001; Tomaso *et al*, 2001; Crompton, 1999).

Among the conditions influencing the development of parasitic infections are poor sanitary conditions, malnutrition, poverty, lowering resistance of the host, large family size and poor personal hygiene (Olsen *et al*., 2001; Stephenson *et al*., 2000). This disease can affect child development, educational achievement, reproductive health and social and economic development (Allen *et al*., 2002; Bennett and Guyatt, 2000) and some of these parasitic infections can cause morbidity and mortality (Johnson and Boatman, 1995). Nevertheless, treatment is often neglected for economic reasons and because most patients have no symptoms (Olsen *et al*., 2001).

Despite the great development that has occurred on the quality of medical services in terms of diagnosis of parasitic diseases, treatment and control, which in turn led to a decrease evidence in the spread of these diseases in many industrialized countries and developing countries, most of parasitic diseases still consider a major challenge for health center and staff in many developing countries (Sayyari *et al.*, 2005). Due to geographical, demographic, economic and social factors, the third world countries may have the greatest contribution on the distribution and survival of parasitic diseases cause of many medical problems that have not yet been resolved (Dieng, 1999).

The prevalence of intestinal parasites among pre-school children was reported from different parts of the world by different researchers. Some of these researchers include, Awasthi and Pande, (1997), Mohammed and Yahya, (1999), Haque *et al*. (2003), Awad *et al.* (2005), Adnan and Aboud (2008), Mehraj *et al.* (2008), Nihar *et al*. (2010), Sehgal *et al. (*2010), Barbara *et al.* (2011), Thiongo *et al. (*2011), Alyousefi *et al. (*2011) and Al-Naemy *et al*. (2012).

The prevalence of IPI among pre-school children was also reported from different parts of Ethiopia at different times by a number of researchers. Some of these researchers include, Woldemichael (1990), Erko and Tedla, (1993), Roma and Worku, (1997), Birre *et al*. (1998), Assefa *et al*.(1998), Ali *et al.* (1999), Merid *et al*. (2001), Legesse and Erko, (2004), Mengistu and Berhanu (2004), Abraham (2005), Dawit (2006), Amare *et al*. (2007), Tadesse *et al*. (2009), Teklu (2009), Fayo (2010), Liza *et al*.(2010), Mengistu(2010), Nyantekyi *et al*. (2010), Asrat *et al*.(2011) and Yonas (2011).

In Ethiopia high prevalence of helminth infection is attributable to factors associated with low socio-economic status. Such factors include poor personal hygiene, environmental sanitation, low household income, overcrowding and lack of clean water supplies. For instance, Ethiopia has one of the lowest quality drinking water supply and latrine coverage (Mengistu *et al.*, 2007).

Even though, several studies have been conducted on prevalence of intestinal parasites in Ethiopia, there are still several localities in the country including the study area, Debr Birhan town, for which information about the prevalence of intestinal parasitic infections was not available. Knowledge of residents about parasite transmission, personal and environmental hygiene and sanitation, proper use of latrine, check up and treatment of children and the effect of family size on children’s infection with intestinal parasites were not studied in the present study area. Therefore, the purpose of this study is to obtain information about the prevalence of intestinal parasites and associated risk factors among less than five aged children who have visiting the Debre-Birhan Health Center, Debre Birhan town.

General objective:

- To identify the major intestinal parasite species and determine their prevalence as well as associating risk factors among under- five aged patients who have been admitted to Debre Birhan Health Center, Debre Birhan Town, North Eastern Ethiopia during May-August,2013.

Specific objectives:

1. To identify the major intestinal parasite species among under- five aged patients visiting Debre Birhan Health Center.
2. To determine the prevalence of intestinal protozoan parasite infections among under- five aged children attending Debre Birhan Health Center.
3. To determine the prevalence of intestinal helminth parasites among under- five aged children attending Debre Birhan Health Center.
4. To assess the major risk factors that predispose under- five aged children to intestinal parasitic infections in the study area.

2. LITERATURE REVIEW

2.1. Intestinal parasitic infections

Intestinal parasitic infections (IPI's) enjoy a wide global distribution. They are estimated to affect an estimated 3.5 billion people, most of whom are children residing in developing countries (WHO, 2000). The major IPI's of global public health concern are the protozoan species: *Entamoeba histolytica* and *Giardia* species and the soil transmitted helminthes: *Ascaris lumbericoides* and *Trichuris trichiura* (WHO, 1999, 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 (Mata, 1982; Montresor *et al*., 1988). 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 IPI's (Montresor *et a*l., 1998; Crompton, 1999).

2.2. Intestinal protozoan infections

2.2.1. Amoebia infections

Amebiasis is one of intestinal protozoan disease which is caused by *Entamoeba histolytica.* It is one of the health issues in many developing countries. It is the second most common cause or death due to parasitic infection after malaria as estimated by the World Health Organization (WHO, 1997). Approximately 10% of the world population is infected with *E. histolytica/ dispar* (Gonin and Trudel, 2003), but most infection occurs due to the noninvasive species. Epidemiological studies have shown that low socioeconomic status, low standards of hygiene and sanitation, in particular those related to crowding, contamination of food and water, and inadequate disposal of faeces, are all significant risk factors for infection with *E. histolytia* (Martinez-Palomo and Espinosa-Cantellano, 1998).

Epidemiological studies have shown that low socioeconomic status and unsanitary conditions are significant independent risk factors for infection. In addition, people living in developing countries have a higher risk and earlier age of infection than do those in developed regions (Caballero-Salcedo *et al.*, 1994).

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).

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).

Prevention of amebiasis 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 amebiasis often spreads within a household, it is prudent to screen family members of an index case for intestinal *E. histolytica* infection (Petri and Singh, 1999).

All symptomatic patients with bloody stools containing motile trophozoites with ingested erythrocytes should be treated according to the severity of the disease. Therapy for invasive infection differs from therapy for noninvasive infection. According to Powell *et al*. (1966), Reed (1992), Ravdin (1995) and Pillai *et al*. (2000) and other recent publications, the drugs recommended for the treatment of noninvasive infections and invasive amebiasis was listed in a different way.There are four species of the protozoan genus *Entamoeba* which are commonly found in the human gastrointestinal tract, namely *E. coli*, *E. dispar, E. hartmanni* and *E. histolytica*. *E.histolytica* is the causes of invasive amebiasis and hence the only one with medical importance (Diamond and Clark, 1993).

Entamoeba histolytica is a protozoan parasite that causes amebic colitis and liver abscess. It exists in two forms: the motile and invasive trophozoite and an infective cyst.The trophozoites measure 10-50 micro meter in diameter and contain a single nucleus whereas, the cyst is 10-15 micro meter in diameter and contains four nuclei when matured. *E. histolytica* cysts, which are resistant to acidification, chlorination and desiccation, and capable of surviving in a moist environment for several weeks, are spread via the ingestion of facially contaminated food or water (Martinez-Palomo and Espinosa- Cantellano, 1998).

Entamoeba histolytica occurs throughout the world in humans, apes, monkeys, dogs, cats and rats. The causative agent of intestinal amebiasis is the single-celled protozoan parasite *Entamoeba histolytica*. This parasite is endemic in most tropical and subtropical areas of the world, where it causes millions of cases of dysentery and liver abscess each year (Satoskar *2009*). The trophozoite, or feeding stage inhabits the lower small intestine and colon where it multiplies by binary fission and forms characteristic four-nucleated cysts which are passed out and subsequently ingested in contaminated food or water. Sometimes the amoebae invade the mucosa and sub mucosa and may be carried via the portal vein to the liver and other parts of the body. Considerable damage may be caused in the wall of the bowel or in the liver. In most people, there is no tissue invasion and the parasite causes no harm. The symptoms following the invasion of the tissues are variable but usually include diarrhea or dysentery with the loss of blood (amoebic dysentery) (Cox, 2004).

Infection usually occurs by ingestion of water or food contaminated by faecal matter. The cyst wall is dissolved in the upper gastrointestinal tract and the organism excysts within the lumen of the small intestine. During excystation, nuclear division is followed by cytoplasmic division, giving rise to 8 uni-nucleated trophozoites. Trophozoites of *E. histolytica* are motile forms, which adhere to and invade intestinal epithelial cells which line the gastrointestinal tract. Once penetration of the intestinal mucosa is achieved, dissemination to other organs, extra-intestinal infections, usually the liver, can occur. Trophozoites which dwell in the colon multiply encyst and are passed in the stool from where further spread is possible (Martinez-Palomo and Espinosa- Cantellano, 1998; Clark *et al*., 2000).

2.2.2. Giardia infections

Giardiasis is one of intestinal protozoun disease which is caused by *Giardia intestinalis*, also called *Giardia lamblia* and *Giardia duodenalis*, and is one of the most common intestinal parasites in the world, occurring in both industrialized and developing countries with an estimated 2.8 million new cases annually. First observed by Anton Van Leuwenhoek in 1681 in a sample of his own diarrheal stool, and later described in greater detail by Vilem Lamble, *Giardia* was initially thought to be a commensal and has only been recognized as a pathogen since the mid 1900s(Stoker *et al*., 2009).

Giardia lamblia is a flagellated, binucleated microaerophilic Protozoa that inhabits the upper part of the small intestine of its host and reproduces by binary fission. This is a type of reproduction in which one cell divides into two new cells by mitosis. During a growth cycle, the components of the cell multiply so that each daughter cell is a complete copy of the parent cell. The cells then pinch off from each other, and a complete reproduction cycle occurs. This parasite has a simple direct life cycle consisting of an infective cyst and a vegetative trophozoite (Gillespie and Richard, 2001).

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

Giardia lamblia is a cosmopolitan parasite with worldwide distribution and the most common Protozoan isolated from gastrointestinal tract (CDC, 2000). The prevalence of *G.lamblia* infection varies from 2%-7% in industrialized countries to 40% in tropical/subtropical regions with poor sanitation and hygienic conditions (Scotti *et al*., 1996; Odoi *et al*.,2004).

Early symptoms include flatulence, abdominal distension, and nausea and foul-smelling bulky, explosive, and often watery, diarrhea. The stool contains excessive lipids but very rarely any blood or necrotic tissue. The more chronic stage is associated with vitamin B_{12} malabsorption, disaccharides deficiency and lactose intolerance (WHO, 2002).

The cyst of *Giardia lamblia* is elliptically shaped, range in size from 6 to 10 microns and contains two to four nuclei (Heresi and Cleary, 1997). The structure of the cyst makes the organism very resistant to environmental factors and disinfection and it is the transmittable form that causes the infection. The cysts possess a thin, protective wall that allows them to survive in feces for weeks or in cold water for months (Ortega and Adam, 1997). Giardiasis is then contracted via ingestion of contaminated water or foods. The cysts pass through the stomach and enter the small intestine. The protective wall allows the cyst to survive the acidic conditions of the stomach until the cyst reaches the small intestine, where the conditions are alkaline. The alkaline environment triggers excystation. During excystation, the cyst wall ruptures at the pole opposite to the nuclei, so that flagella and other projections emerge from the rupture point. The cyst wall is then completely shed and the microbe enters the trophozoite stage of its life **(**Ortega and Adam, 1997).

The trophozoite stage is approximately 12 - 15 microns by 6 - 8 microns. The organism has a pointed elongated median body with two symmetric nuclei and four pairs of flagella.It resembles a human face on stained preparations (Heresy and Cleary, 1997). The trophozoite is the reproducing and motile form of *Giardia* that attaches to the intestinal wall via its ventral disc and causes the symptoms of giardiasis (Ortega and Adam, 1997). In severe cases, the trophozoites can become so numerous along the intestine that they cover it as a "carpet." While the trophozoite is attached, it not only absorbs but blocks nutrients from transporting across the epithelial lining of the intestine. It inhibits the absorption of fats, carbohydrates, vitamin and folic acid. Trophozoites are rarely infective because they are not resistant to gastric acid and die rapidly outside the body. The trophozoite then undergoes encystations.

Encystations take place as trophozoites pass to the posterior regions of the small intestine. Cyst wall formation is completed within approximately 44-70 hour and appears to be initiated by the presence of bile salts in the lower small intestine. The most visible overall change during encystations is that trophozoites gradually round up and detach, lose mobility, and become refractile. Cyst formation is essential for the survival of *Giardia* outside the host intestine and for the transmission of the parasite among susceptible hosts (Adam, 1991).

The cysts then leave the body and are transmitted from person to person by contact with infected feces directly or picked up by another host via contaminated water or food indirectly (Ortega and Adam, 1997). Although infection after the ingestion of only one *Giardia* cyst is theoretically possible, the minimum number of cysts shown to infect a human under experimental conditions is ten. Generally the cyst stage of *Giardia lamblia* causes the infection while the trophozoite causes the symptoms of giardiasis (Meyer and Jarrol, 1980).

Giardia transmission occurs by the fecal-oral route, either directly, via person to person contact or indirectly, via contamination of surface water or food. The salient features of *Giardia* cysts that influence disease transmission include their stability in the environment, their immediate infectivity upon leaving the host and the small number of cysts required to cause infection (Stoker *et al*., 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).

Cysts can be found by examination of the deposit of a formol-ether concentrate of a stool preparation. The oval cysts with thick walls serve as characteristic features for these organisms. The flagella disintegrate and form a central streak which becomes visible when stained with iodine or MIF (merthiolate-iodine-formaldehyde). Cysts may be excreted intermittently; therefore it is important to examine more than one stool. Stools are usually passed 3-8 times / day and are usually pale, offensive, rather bulky and accompanied by 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(WHO, 2009; CDC, 2006).

Currently there are different groups of drugs available to treat giardiasis in stools. Based on different age group, endemicity of the parasite, pregnancy etc, the use of antimicrobial therapy varies (Gardner and Hill, 2001). The most commonly used anti *Giardia* drugs include metronidazole, Furazolidone and Paromomycin. Metronidazole is the most common drug used for the treatment of giardiasis worldwide. Unlike other drugs, it is quickly and completely absorbed and penetrates body tissues and secretions such as saliva, breast milk, semen, and vaginal secretions (Gardner and Hill, 2001).

Providing safe drinking water is critical if *Giardia* transmission is to be controlled. The traditional methods employed to insure water safety are protection of the watershed, fl occulation and sedimentation (using compounds such as alum to bridge contaminating organisms into clumps that can then be removed by sedimentation), chemical disinfection usually with chlorine, and filtration (WHO, 200).

2.2.3. Cryptosporidium infections

Cryptosporidiosis is the most common protozoan intestinal parasite infection which is caused by *Cryptosporidium parvum*. It has been isolated worldwide in both immune competent and immunocompromised humans and has been reported from 3 days to 95 years old. It is responsible for both epidemic as well as endemic levels of intestinal disease (Rose, 1997). Infection is most frequently spread by direct person-to person-transmission through the fecal oral route or by sputum and vomits and zoonoticaly from cattle and sheep. Indirectly it is spread through the environment particularly through water (Hojlyng *et al*., 1987).

Cryptosporidium parvum (C. parvum) was first described in 1907 by Ernest Edward Tyzzer (Tyzzer, 1907). His finding was not regarded as important at that time. Later its importance increased in 1971, when *Cryptosporidium* was found to be associated with diarrhoea in cows. Although the first case of human cryptosporidiosis was reported in 1976 (Meisel *et al.,* 1976), more awareness of the organism really came to the fore in the 1980s, due to its association with HIV infection (James, 1988). During the 1990s *Cryptosporidium* became one of the most important pathogenic contaminants found in drinking water. This is mostly attributed to its low infective dose and high resistance to the common water disinfectant such as chlorine, and against environmental factors such as low temperature (Fayer *et al*., 1998). In Nordic countries, recent data reveal that, the parasite was detected in surface water sources, in rivers and lakes and can pose a potential biothreat for drinking water supplies (Robertson and Gjerde, 2001).

Cryptosporidiosis is a leading cause of diarrhoea, particularly persistent diarrhoea, among children in developing countries (Griffith, 1998). Recent epidemiologic studies indicate that cryptosporidiosis may also present as an acute, self-limited diarrhoeal disease in immunocompetent individuals and may account for 1%-10% of diarrhoeal disease worldwide (Xian-Ming and LaRusso, 1999). It is also found in 6% of all patients with acquired immunodeficiency syndrome (AIDS) and in 21% of AIDS patients with diarrhoea (Chen *et al.*, 2003).

Cryptosporidium is a coccidian parasite and one of the many genera of phylum Protozoa. Currently there are thirteen species of *Cryptosporidium* categorized based on differences in host specificity, oocyst morphology and site of infection, and most of them infect only one or a few groups of animals. The genus *Cryptosporidium* comprises parasites that grow and reproduce within epithelial cells of the digestive organs and the respiratory tract of vertebrates.

It has a monoxenous lifecycle; all stages of development (asexual and sexual) occurring in one host (O' donoghue, 1995).

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 oocystsare excreted with the faeces and it is the resistant stage found in the environment (Fayer and Ungar, 1986).

These parasites are intracellular, enclosed by a thin layer of host cell cytoplasm (Mc Donald *et al.,* 1990). 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.

According to Fayer and Ungar (1986) and O' donoghue(1995),the freed sporozoites attach to epithelial cells and become enclosed within parasitophorous vacuoles. The trophozoites then undergo asexual proliferation by merogony and form two types of merontstype I meronts form 8 merozoites that are liberated from the parasitophorous vacuole when mature; the merozoites then invade other epithelial cells where they undergo another cycle of type I merogony (Multiple fission or Schizogony) or develop into type II meronts. Type II meronts form 4 merozoites, which do not undergo further merogony but produce sexual reproductive stages (called gamonts). Sexual reproduction occurs by gametogony and both microgamete (male) and macrogametocytes (female) are formed. Macrogametocytes are then fertilized by mature microgametes, thus forming a zygote. A resistant oocyst wall is then formed around the zygote (the only diploid stage in the life cycle). The resultant zygotes undergo further asexual development (sporogony) and form sporulated oocysts containing 4 sporozoites. Most oocysts are thick-walled and are excreted from the host in faecal material lor perhaps via respiratory secretions. *C. parvum* appears to have two autoinfective cycles: the first by continuous recycling of Type I meronts and the second through sporozoites rupturing from thin-walled oocysts. The presence of these autoinfective oocysts and recycling type Imeronts are believed to be the means by which persistent chronic infections may develop in hosts without further exposure to exogenous oocysts.

Development of *Cryptosporidium* occurs more rapidly, and each generation can develop and mature in as little as 2 days. Due to the fastness of the life cycle, and the auto infective cycles, enormous numbers of organisms can colonize the intestinal tract in several days. As a result, the ileum soon becomes crowded and secondary sites such as the duodenum and large intestine are often infected. *C. parvum* lacks tissue specificity and has been found infecting the biliary tract, the respiratory system, middle ear, pancreas and the stomach particularly inimmunosuppressed individuals (Clark, 1999).

2.2.4. Protozoan infection in Ethiopia

According to the Ministry of Health (1997), nearly 80% of the rural and 20% of urban population have no access to safe water. Three-fourth of the health problems of children in Ethiopia are communicable diseases arising from the environment, especially water and sanitation. A lot of mortality in less than five years is due to diarrhea in which water related diseases occupy a high proportion.

McConnel and Armstrong (1976) reported an overall giardiasis prevalence of about 11.4% in a study conducted on the central plateau of Ethiopia. Seyoum *et al*. (1981) have also reported varying degree of prevalence rate in different communities. According to Birrie and Erko (1995) based on a countrywide survey of giardiasis, the overall prevalence among school children and residents were 8.9% and 3.1%, respectively and that of the non- school children were 4.4%.

A number of survey and routine diagnosis in Ethiopia indicate that amebiasis is one of the most widely distributed diseases (Kloos and Tesfayohanis, 1993)**.** In a countrywide survey of amebiasis in 97 communities, the overall prevalence of *Entamoeba histolytica* infections, as measured by rate of cyst-passers, in schoolchildren and non-school communities were 15.0% and 3.5%, respectively (Erko *et al*., 1995). In another survey of 50 communities of the central plateau of Northern Ethiopia, the parasite was reported in 94% of the communities, with prevalence rate ranging from 3% to 55% (McConnel and Armstrong, 1976).

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

2.3. Intestinal Helminthic Disease

Helminthes are parasitic worms, which infect humans (Maizels and Yazdanbakhsh, 2003), and mainly found in two phyla, Platyhelminths and Nematoda (Hotez *et al*., 2008). Helminth infections affect over one quarter of the world's population (Bethony *et al.*, 2006; Brooker *et al*., 2006). It is quite conceivable that one billion humans are infected with both ascariasis which is caused by *Ascaris lumbricoides* and trichuriasis whose causative agent is *Trichuris trichiura (*Cox, 2004).

2.3.1. Ascaris Disease

Ascariasis is the most common human helminthic infection. It is a soil-transmitted infection which is caused by *Ascaris lumbricoides*. Current estimates indicate that more than 1.4 billion people are infected worldwide. In the United States, there are an estimated 4 million people infected, primarily in the southeastern states and among immigrants. The etiologic agent, *Ascaris lumbricoides,* an intestinal roundworm, is the largest nematode to infect humans. The female worms are larger than the males. Important factors associated with an increased prevalence of disease include socio-economic status, defecation practices and cultural differences relating to personal and food hygiene as well as housing and sewage systems.
Most infections are subclinical; more severe complications occur in children who tend to suffer from the highest worm burdens (Stoker *et al*., 2009).

Ascaris lumbricoides is the largest and the most common helminthes parasitizing the human intestine and currently infects about 1 billion people worldwide (CDC, 2006). It is estimated that 25% of the world population harbors the parasite. Hand to mouth transmission is most common; it is found in association with poor personal hygiene, poor sanitation, and in places where human feces are used as fertilizer. Consumers of uncooked vegetables and fruits grown in or near soil fertilized with sewage are most at risk for acquiring infection. Water is rarely implicated as a source of *Ascaris* (Bogitsh *et al*., 2005).

The infection occurs by ingestion of food contaminated with infective eggs which hatch in the upper small intestine. The larvae (250 x 15 micrometers) penetrate the intestinal wall and enter the venules or lymphatics. The larvae pass through the liver, heart and lung to reach alveoli in 1 to 7 days during which period they grow to 1.5 cm. They migrate up the bronchi, ascend the trachea to the glottis, and pass down the oesophagus to the small intestine where they mature in 2 to 3 months (WHO, 2002).

A female may live in the intestine for 12 to 18 months and has a capacity of producing 25 million eggs at an average in daily output of 200,000. The eggs are excreted in feces, and under suitable conditions (21 to 30 degrees C, moist, aerated environment) infective larvae are formed within the egg. The eggs are resistant to chemical disinfectant and survive for months in sewage, but are killed by heat (40 degrees C for 15 hours). The infection is man to man. Auto infection can occur (WHO, 1997).

Symptoms are related to the worm burden. Ten to twenty worms may go unnoticed except in a routine stool examination. The commonest complaint is vague abdominal pain. In more severe cases, the patient may experience listlessness, weight loss, anorexia, distended abdomen, intermittent loose stool and occasional vomiting. During the pulmonary stage, there may be a brief period of cough, wheezing, dyspnea and sub-sternal discomfort. Most symptoms are due to the physical presence of the worm (Girum, 2005).

The adults of *A. lumbricoides* may be expelled through the anus, mouth or nose. It is important to distinguish the adult worms from earthworms which are segmented and are often collected as a contaminant from toilets. The microscopic examination of stool deposits after concentration reveals the characteristic bile stained ova. Eggs may be difficult to identify if an excess of iodine is added to the wet preparation as they retain the stain thus resembling debris. Ova may also become decorticated. In most symptomatic cases identification is easy due to the vast number of eggs, which can be found within a few seconds of starting to scan the slide (WHO, 2009).The diagnosis of ascariasis is usually made via stool microscopy. The Microscopi**c** characteristic of eggs may be seen on direct examination of feces or following concentration techniques. However, eggs do not appear in the stool for at least 40 days after infection; thus, the main drawback of relying upon eggs in feces as the sole diagnostic marker for Ascaris infection is that an early diagnosis cannot be made, including during the phase of respiratory symptoms. In addition, no eggs will be present in stool if the infection is due to male worms only. Sometimes an adult worm is passed, usually per rectum. If an Ascaris worm is found in the feces, a stool specimen can be checked for eggs to document whether or not additional worms are present prior to instituting therapy (WHO, 2009). A number of drugs can be used in the treatment of ascariasis. These include: pyrantel pamoate, mebendazole, albendazole, ivermectin, piperazine citrate, and levamizole.

2.3.2. Trichuris Disease

Trichuriasis or whip worm infection is caused by *Trichuris trichiura*. The infection is estimated to affect around 1049 million persons worldwide. Of these, 114 million are children of preschool age and 233 million are school age (Chan, 1997). Humans are the primary host for infections caused by *Trichuris trichiura* but the species has been detected in some none – human primates (Horii and Usuii, 1995). *Trichuris trichiura* infection is endemic in tropical and subtropical countries, but few sporadic cases have occurred in nonendemic areas, mainly as a result of immigration. It is rare or nonexistent in arid, very hot, or very cold regions (Bogish *et al*., 2005). It is estimated that over 40.1 million African school-aged children are infected with *T. trichiura* (Brooker *et al*., 2006).

According to CDC and prevention(2010 and 2011), Trichuriasis is transmitted when the infective eggs of the whipworm are unintentionally ingested, usually through consuming soil that has been contaminated with human feces via dirt covered food or hands. The spread of human whipworm eggs usually occurs in areas where outside defecation takes place or human feces is used as fertilizer.

Life cycle eggs require a warm, moist environment with plenty of oxygen to ensure embryonation, but once they have embryonated they are extremely resistant to environmental conditions. Adult worms are found in the cecum and upper part of the colon of man. In heavy infection they can be found in the colon and the terminal ileum. They attach to the mucosa by the anterior end or by embedding the anterior portion of the body in the superficial tissues, obtaining nutrition from the host tissues. Once fertilized the female worms lay several thousands of eggs, which are unsegmented at the oviposition and are passed out in the feces. Once they have been passed out they require an embryonation period in the soil which may last from two weeks to several months, after which they become infective. When embryonated eggs are swallowed by human host's larvae are released into the upper duodenum. They then attach themselves to the villi lower down the small intestine or invade the intestinal walls. After a few days the juveniles migrate slowly down towards the cecum attaching them to the mucosa, reaching their final attachment site simultaneously. The larvae reach maturity within three weeks to a month after infection, during which they undergo four molts. There is no lung migration and the time from ingestion of infective eggs to the development of adult worms is about three months. Infection is achieved by swallowing soil that contains embryonated eggs. Therefore, children are most commonly seen to possess the infections, as they are more likely to swallow soil (Shibru, 1986).

Frequently, infection with *Trichuris* is asymptomatic or results only in peripheria. Clinical disease most often occurs in children, as it is this population that tends to be most heavily-infected and presents as *Trichuris* colitis. In fact, this is the most common and major disease entity associated with infection. Acutely, some patients will develop *Trichuris* dysentery syndrome, characterized by abdominal pain and diarrhea with blood and mucus. With severe dysentery, children develop weight loss and become emaciated. Anemia is common and results from both mucosal bleeding secondary to capillary damage and chronic inflammation. The anemia of trichuriasis is not as severe as that seen with hookworm. *Trichuris* infection of the rectum can lead to mucosal swelling. In that case, tenesmus is common and if prolonged can lead to rectal prolapse, especially in children. Adult worms can be seen on the prolapsed mucosa. Chronic trichuriasis often mimics inflammatory bowel disease. Physical symptoms include chronic malnutrition, short stature and finger clubbing. These symptoms are often alleviated with appropriate anthelminthic treatment. Rapid growth spurts have been reported in children following deworming with an anthelminthic agent. Deficits in the cognitive and intellectual development of children have also been reported in association with trichuriasis (WHO, 2009; Stoker *et al*., 2009).

The adult worms of *T. trichiura* are rarely seen in the feces. The microscopic examination of stool deposits after an iodine stained and formol-ether concentration method reveals the characteristic barrel shaped ova. In symptomatic infections numerous numbers of eggs can be seen due to the prolific nature of the female worms, even in light infections many eggs can be seen in the smear (WHO, 2009).

2.3.3. Hymenolepis Disease

Hymenolepis nana / the dwarf tapeworm, and is the smallest tapeworm to infect humans. This cestode belongs to a large family known as Hymenolepididae. The diagnostic features of this family are: scolex armed with one circlet of five hooks; one to three large testes and sacciform uterus. In addition to the *H. nana*, three other species, *H. diminuta, H. microstoma* and *H. citelli* have been used extensively for studies on cestodes. *Hymenolepis nana* has a cosmopolitan distribution and is thought to be the most common tapeworm throughout the world. The infection is more frequently seen in children although adults are also infected, causing *hymenolepiasis* (WHO, 2004). The life cycle of *H. nana* is the only tapeworm that can be transmitted directly from person to person. Eggs of *H. nana* are immediately infective when passed with the stool and cannot survive more than 10 days in the external environment. When eggs are ingested by an arthropod intermediate host (various species of beetles and fleas may serve as intermediate hosts), they develop into cysticercoids, which can infect humans or rodents upon ingestion and develop into adults in the small intestine (WHO, 2009).

A morphologically identical variant, *H. nana* var. *fraterna*, infects rodents and uses arthropods as intermediate hosts. When eggs are ingested (in contaminated food or water or from hands contaminated with feces), the oncospheres contained in the eggs are released. The oncospheres penetrate the intestinal villus and develop into cysticercoid larvae. Upon rupture of the villus, the cysticercoids return to the intestinal lumen, evaginate their scoleces, attach to the intestinal mucosa and develop into adults that reside in the ileal portion of the small intestine producing gravid proglottids. Eggs are passed in the stool when released from proglottids through its genital atrium or when proglottids disintegrate in the small intestine. There is a high likelihood of internal autoinfection, without passage through the external environment. The life span of adult worms is 4 to 6 weeks, but internal autoinfection allows the infection to persist for years (Ash and Orihel, 2003; Bogitsh *et al*., 2005; Heelan, 2004.

Diagnosis is based on recovery and identification of the characteristic ova in a formol-ether concentrate of feces. Adult worms and proglottids are rarely seen in stool samples (WHO, 2009). The recommended drugs for the treatment of *Hymelepis nana* are Praziquantel or niclosamide are the drugs most frequently used to treat *H. nana* infection. Developing *H. nana* cysticercoids are not as susceptible to drug therapy as adult tapeworms. Therefore either a higher dose of anti parasitic or prolonged duration of anti- parasitic to eliminate emerging tapeworms is required to successfully treat infection (King, 2005).

2.3.4. Hook worm Disease

It is one of the common human intestinal parasitic infections. The two major species of hook worm to infect humans are Necator americanus and Ancylostoma duodnale. Eggs of both species are identical and morphological identification can be done on the basis of the adults' morphology (Katz *et al*.1989; Pawloski *et al*. 1999). Hook worms are estimated to infect 151 million people worldwide and cause mortality in another 65,000 (Montresor *et al*. 1998). No animal reservoirs have been identified for hook worms (Katz *et al.* 1989; Neva and Brown, 1994). Mature hook worms typically inhabit in the jejunum where they attach to the intestinal mucosa with their ventral teeth (A.duodenale) or cutting plates (N. americanus) (Katz *et al*.

1998; Neva and Brown 1994). The worms drive their nourishment by feeding on the villous tissue and sucking blood at the point of attachment (Katz *et al.*1989; Gilgen and Mascine – Taylor, 2000). Average blood losses of 0.1 -0.2 ml of blood per day have been reported for A. duodenale and 0.02ml per day for N. americanus (Roche and Layrisse, 1966).

In their life cycle, adult female passes about 28,000 eggs per day. Both the eggs and larvae are very sensitive to exposure to the environment and do not survive for more than a month in the soil. Human infection is acquired through penetration of the skin by the infective third stage filari form of larvae (Neva and Brown, 1994). Penetration usually occurs in bare feet with skin abrasion, areas on and between the tose, or through a hair follicle (Neva and Brown 1994). The larvae then enter the circulation and are carried to the lungs. During their fourth stage of development; the larvae break out of the alveoli and migrate up the bronchi and trachea to the pharynx where the larvae are involuntary swallowed and pass down to the small intestine (Katz *et al*., 1989; crompton, 2000).

Clinical signs and symptoms of hook worm include pneumonia in heavily infected children during larval migration and epigastric pain and iron deficient anemia during the intestinal phase of the infection (White *et al.*, 1986; Max well *et al*., 1987; crompton 2000).

2.3.5. Helminthes Infection in Ethiopia

In Ethiopia, a number of surveys have shown that helminths are prevalent in varying magnitudes (*Shibru* and *Leykun*, 1985; Berhanu and Girmay, 2003). In the study of geohelminthiasis in Wondo Genet, Southern Ethiopia, the prevalence of infection for *A. lumbricoides* was 83.4%, while it was 86.4% for *T. trichiura* among schoolchildren (Berhanu and Girmay, 2003). In the epidemiological study of STHs, South Gondar, Ethiopia, 49% of the examined children had one or more types of helminthes infection, of which 32.5%, 13.3% and 2.4% were single, double and triple infections, respectively. About 28.4%, 8.3% and 12.1% of the children had moderate infections of ascariasis, trichuriasis and hookworms, respectively (Leykun, 2000).

In the study of intestinal helminthes infections in Lake Zway Islands in the Southern Ethiopia, 287 out of 506 (56.7 %) were found to harbor one or more species of intestinal helminthes. The study revealed higher prevalence of *T. trichiura* (46.2%), low prevalence of *A. lumbricoides* (4.1%) and almost total absence of hookworm (less than 1%) (Tesfa- Michael and Teklemariam, 1983).

According to Mengistu and Berhanu (2004) prevalence of intestinal parasites among children were hookworm was the highest (60.2%), followed by *Schistosoma mansoni* (21.2%), *Trichuris trichuria* (14.7%), *Taenia* spp. (13.9%), *Entamoeba histolytica/dispar* (12.7%), *Ascaris lumbricoides* (6.2%), *Giardia duodenalis* (6.2%) and *Strongyloides stercoralis* (5.8%), in that order.

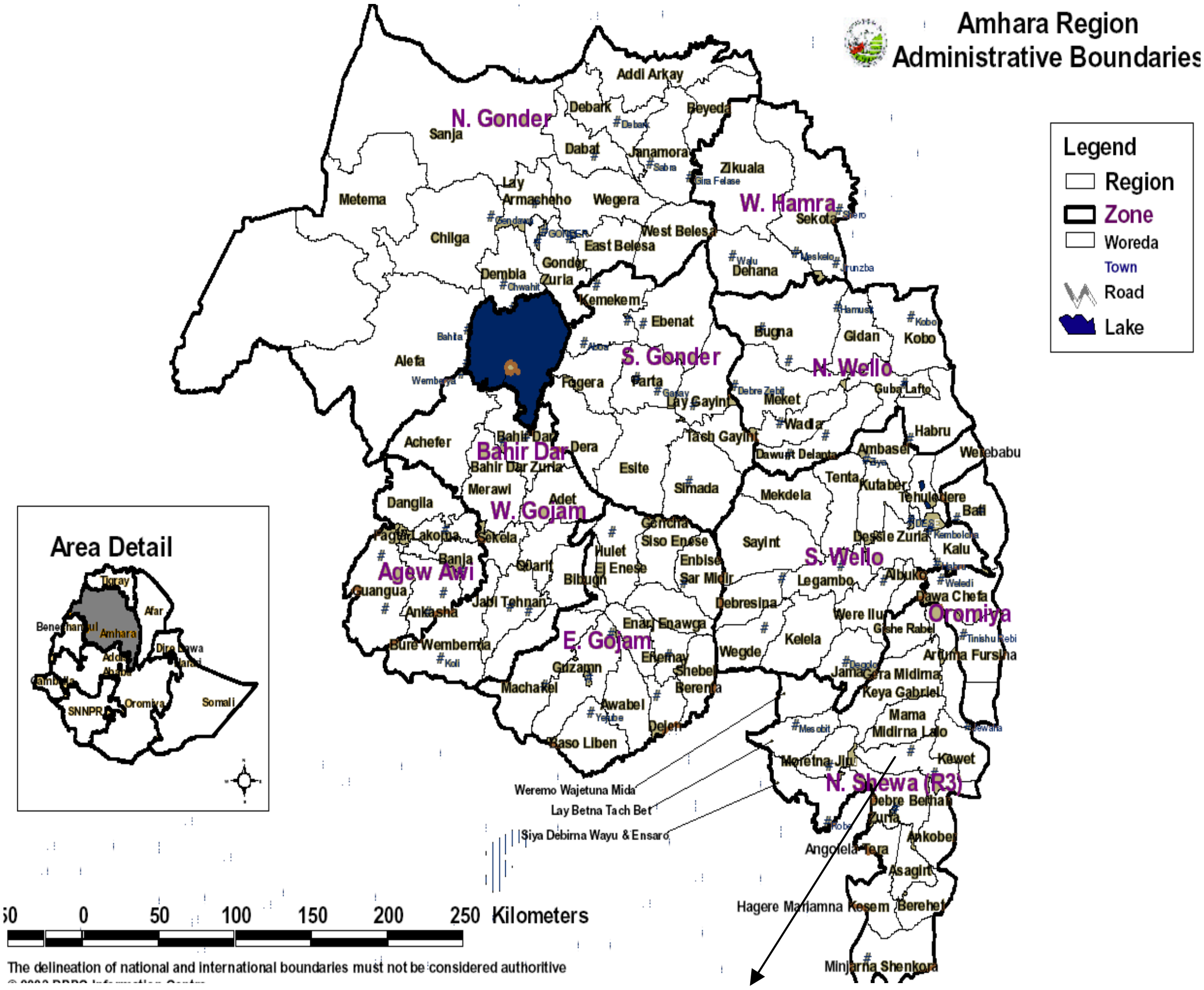
Intestinal parasitic infections cause serious public health problem in Ethiopia. In this country, number of surveys has shown that STHs are prevalent in varying magnitudes (Tedla and Jemaneh, 1985; Erko and Medhin, 2003), reported that the prevalence and distribution of helminthes infection varies by place and with age in Ethiopia. World Health Organization's also indicated that the prevalence of helminthes infections in Ethiopia ranges from 31-57.8% (WHO, 2004). As in other developing countries, high prevalence of STH infection is attributable to factors associated with low socio-economic status. Such factors include poor personal hygiene and environmental sanitation, with low household income, overcrowding and lack of clean water supplies for most parts of the country. For instance, Ethiopia has one of the lowest quality drinking water supply and latrine coverage in the world. As comparison Ethiopia had only 12% latrine coverage while Kenya had 87% (Kumie and Ali, 2005 as cited by Mengistu *et al.*, 2007).

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in Debre Birhan Health Center at Debre Birhan town capital of North Shoa Administrative zone, (Figure 1). Debre Birhan is the largest town of North Shoa zone in the Amhara Regional State which is located at the central highlands of Ethiopia and situated at a distance of 130 kms away from Addis Ababa in the North East direction. The geographical location of Debre Birhan town is 09^{0} 01’232’’ North latitude, 038^{0} 48’177’’ East longitude and at an altitude of 2780 m.a.s.l. Debre Brihan town consists of nine Kebeles with a total population of 94,829 people, of which 46,582 are males and 48,247 are females (CSA, 2008).

The rainfall pattern of the study area is bimodal in distribution. The climate is characterized by a long rainy season extending from July to September, a short rainy season that extends from February to March. According to data obtained from Debre Birhan Research Center, the average annual rainfall from 2008 to 2010 was 79.8 mm, while the maximum total monthly rainfall was in the range of 357.4 – 435.1 mm in July and 273.2 – 397.7 mm in August. The average monthly minimum air temperature ranges from 3.1°C in November to 9.3°C in August. While the average monthly maximum air temperature ranges from 18.1°C in August to 23.7°C in January.



Debre Birhan town

Figure 1. Map of study area

Source: Amhara Reginal State Agricultural and Natural Resource Bureau, (2013).

3.2. The study design

The study design was a cross-sectional survey on intestinal parasitic infections and major risk factors among less than five aged children who have visited Debre Birhan Health Center. It was conducted from May to August, 2013.

3.3. The study participants

All less than five years old children who were suspected for any intestinal parasitic infections and admitted in Debre Birhan Health Center during May-August 2013 were included in the present study.

3.4. Exclusion criteria

All less than five aged children who started anti parasitic drugs and also completed treatment before three days were not included in the study. Furthermore, those patients from the surrounding *Woreda* and school aged children were excluded in this study.

3.5. Sample size determination and Sampling techniques

The sample size was estimated using the following statistical formula (Daniel, 1999):-

$n= Z^2 P (1-P)/d^2$

Where: n= sample required

Z= 95% confidence interval (1.96)

D= margin of error (5%)

P= prevalence rate.

Since the overall prevalence rate (p) of intestinal parasites is not known for the study area, prevalence was taken to be 50%. Hence, the required sample size was 384.

3.6. Method of Data Collection

3. 6. 1. Clinical examination

The study participants were examined physically for variables such as the presence or absence of clean clothing, hygienic conditions, edema, and for any other body abnormalities by the physician and investigator. The observations were recorded in the recording format.

3. 6. 2. Stool Sample collection

Each fecal sample specimen was consisting of 10-20g of fresh stool which was obtained with the cooperation of the family of less than five years old children. The collected samples were kept in a plastic container and transported to the laboratory for examination within one hour of delivery. At the time of sampling; date of sampling, age, sex, presence or absence of intestinal parasitic infections, and code number was recorded for each child on the record format.

3. 6.3. Questionnaire Survey

A pre-tested standardized questionnaire based on common risk factors was developed. The questionnaire was constructed in English and then translated into Amharic. Then, the children's families were interviewed in their mother tongue, Amharic. This standardized questionnaire was used together with relevant general information on demographic and socioeconomic data of the children and their parents in the study area. The questionnaire was administered and observations on physical situations of each child attending at health center were recorded by the investigator and an oriented health assistant.

3.7. Laboratory Parasitological Procedures

3.7.1. Direct Wet Mount Method

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

3.7.2. Formol-Ether Concentration Method

Using an applicator stick, approximately 2g or pea sized fecal materials were placed in a centrifuge tube containing10 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 ether/ethyl acetate. The tube was closed with a stopper and it was shacked vigorously to mix. The stopper was removed and it was 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 was a plug of debris and the third was a clear layer of formalin and the fourth was 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 ova and cysts and trophozoites (Lindo *et al.,* 1998).

3.7.3. Modified Ziel-Neelsen Method

In this method a thin smear was prepared directly from fresh as well from sediments of concentrated stool and allowed to air dry. Then the slides were fixed with methanol for 5 minutes and stained with carbol fuchsine for 30 minutes. Then the slides were 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, then observed under light microscope with 1000× magnification (Asefa *et al.*, 1996). Each slide was observed for 10 minutes to decide whether it is negative or positive.

3.8. Data analysis

The data was computerized using Excel 2007, cleaned and checked against original document before analysis. All statistical analyses were performed using SPSS for windows version 20 statistical package. The prevalence of intestinal parasites was determined by Pearson chi-square (x2) test verifying the relationship between independent factors and the outcome variables. Odds ratios (OR) was used to determine association of independent variables with the intestinal parasitic infections. The 95% CI was used to show the accuracy of data analysis. Probabilities less than 5% ($P < 0.05$) was considered statistically significant.

3.9. Data Quality Control (DQC)

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

3.10. Ethical Considerations

The Amhara Regional State Health Bureau and The head of the North Shoa Zone Health Department both were given their written consent for the study. All children tested positive for intestinal parasitic inflections were treated with a single dose of drug prescribed by the physician.

4. RESULTS AND DISCUSSION

4.1. Prevalence of Intestinal Parasitic Infections among Under-Five Aged Children

The prevalence of intestinal protozoan and helminth parasite infections among children aged less than five years old is summarized in Table 1. The prevalence of intestinal protozoan parasite infections (IPPI) and intestinal helminth parasite infections(IHPPI) among children less than five years old of both sex and all age groups was 27.9(107/384) and 11.4% (44/384), respectively; with 39.3% (151/384) overall ratio. Of these, the prevalence of IPPI and IHPI among male children was 25.6% (45/176) and 14.8% (26/176), respectively; with 40.3% (71/176) overall prevalence. Similarly, the prevalence of IPPI and IHPI among female children was 29.8% (62/208) and 8.7% (18/208), respectively; with 38.5% (80/208) overall prevalence. Although there was no statistically significant difference, the prevalence of IPPI in female children under-five years was greater than that of males. To the contrary the prevalence of IHPI in male children under-five years was greater than that of females. This might be male children have a chance to play out of their homes and increased the chance of infection with STH.

As it is well known in children, intestinal parasitic infections, particularly IPPl and STH infections are the cause of common health problems in tropical countries. Younger children are predisposed to heavy infections with intestinal parasites since their immune systems are not yet fully developed, and they also habitually play in faecally contaminated soil. In addition to considerable mortality and morbidity, infection with intestinal protozoan helminths parasites have been found to profoundly affect a child's mental development, growth and physical fitness while also predisposing children to other infectious agents (karaman *et al*.,2006).

Table 1. Prevalence of Intestinal Protozoan and Helminth Parasitic Infections among Study Participants (n=384) in Debre Birhan Health Center, Debre Birhan Town, during May- August, 2013.

Age group (years)and sexes	№.of examined	IPPI	IHPI	PHPI	X^2	P-value
		№. Posit (%)	№.Posit (%)	№. Posit (%)		
0-1						
Males	16	2(12.5)	0.0	2(12.5)	.016	
Females	18	1(5.6)	1(5.6)	2(11.1%)		1.000
1-2						
Males	10	1(10.0)	2(20.0)	3(30.0)	4.168	.078
Females	12	0.0	0.0	0.0		
2-3						
Males	9	5(55.6)	0.0	5(55.6)	.000	1.000
Females	9	4(44.4)	1(11.1)	5(55.6)		
3-4						
Males	16	5(31.3)	2(12.5)	7(43.8)	.083	1.000
Females	23	9(39.1)	0.0	9(39.1)		
4-5						
Males	125	32(25.6)	22(17.6)	54(43.2)	.011	1.000
Females	146	48(32.9)	16(11)	64(43.8)		
All age group						
Males	176	45(25.6)	26(14.8)	71(40.3)		
Female	208	62(29.8)	18(8.7)	80(38.5)		
Total	384	107(27.9)	44(11.4)	151(39.3)		

IPPI= Intestinal Protozoan Parasitic Infections, IHPI= Intestinal Helminth Parasitic Infections, PHPI= Protozoan and Helminth Parasitic Infections

As shown in Table 1, it was found that children`s age ranged between 0-1 was 4 (11.8%), 1-2:3 (13.6%), 2- 3:10 (55.6%), 3- 4:16 (41%), 4-5:118 (43.5%) were infected with human intestinal parasites. The low rate of infections was observed in aged less than 2 years old and high rate of infections was for the children aged from 4-5 years old followed by 3- 4 years old. Children`s in 4-5 years old were highly infected with both protozoa and helminth infections and, indicating a risk for acquiring parasite infections. The prevalence of intestinal parasite infections and species diversity in the study site revealed that increment with age group as the age increased; particularly it was highest in age group from 4-5 years old (43.5%) Table1. This might be due to the common childhood behaviors of eating soil, neglecting to wash hands after defecation, using of river or spring water or non-purified water and improper cleaning of child dinning utensils. The prevalence of intestinal parasites in this study were not statistically significant ($p>0.05$). On the other hand, others: Dawit , 2006 in Dire- Dawa, Eastern Ethiopia, Adnan I,AL-Hindi, 2008 in Gasa, Palestine, Teklu (2009) in Addis Ababa disagreed and reported that the prevalence of human protozoan parasites among the age group were statistically significant ($P < 0.05$). The reason for the fact that children in this age group may not know the hygienic conditions, such as eating different foods from a variety of sources may be outside the home which gives a greater chance of infection (Rice *et al*., 2003).

Table 2.The Prevalence of Human Intestinal Parasite Infections among Different Kebele's in Debre Birhan Town.

Kebeles	№.of examined	IPPI	IHPI	PHPI
		№.Posit (%)	№.Posit (%)	№. Posit (%)
01	40	11(27.5)	3(7.5)	14(3.6)
02	24	6(2.5)	0	6(1.6)
03	28	3(10.7)	5(17.9)	8(2.6)
04	53	21(39.6)	8(15.1)	29(54.7)
05	43	10(23.3)	4(9.3)	14(3.6)
06	30	8(26.7)	2(6.7)	10(2.6)
07	45	13(28.9)	6(13.3)	19(42.2)
08	40	11(27.5)	4(10)	15(3.6)
09	81	24(29.6)	12(14.8)	36(44.4)
Total	384	107(27.9)	44(11.6)	151(39.3)

As shown from Table 2, the highest prevalence of intestinal parasite infections was identified in *kebele* 04, 54.7% (29/53) came next in *kebele* 09,44.4% (36/81) and *kebele* 07,42.2% (19/45), and the lowest, 1.6% (6/24) in *kebele* 02, 2.6% (8/ 28) in *kebele* 03, and 2.6% (10/ 30) in *kebele* 06. This might be due to contaminated water and raw vegetables, because these *kebeles*: *(kebele* 09, 04 and 07) shares Beressa river commonly for washing their clothes, bathing their body and eating vegetables cultivated by the river through irrigation system. Hence, Beressa river could be contaminated by the wastes that flow from Debre Birhan Blanket Factory, Debre Birhan University, Debre Birhan Regional prisoner's station and private release of wastes and open defecation.

4.2. Common Intestinal Parasite Species Identified from Examined Children Aged Less than Five years old

In this study the result of prevalence of intestinal protozoan and helminth parasite species among less than five aged children are summarized and presented in Table 3. The prevalence of intestinal protozoan parasite species was the highest as compared to the prevalence of intestinal helmenth parasite species, i.e., E. histolytica,18.5% (71/384) , G.lamblia,11.5% (44/384) except *C. parvum* which was 2% (7/384), where as helminth parasite species was A.lumbercoides,7.8% (28/384) , followed by *T. trichuria* 7.3% (24/384) and H.nana 6.3% (11/384). The overall prevalence in the study site (39.5%) was a bit higher compared to the studies of Malta and Roberto (2009), reported 37% in Brazil/São Paulo State, Eyasu *et al*. (2010) 34.6% in Benishangul-Gumuz, Girum, (2005) 27.2% in Babile town, eastern Ethiopia and Al-Hindi and Aboud (2008) 16.6% in Gaza, Palestine**.** The variation of overall prevalence might be due to less personal hygiene and environmental sanitation, consumption of spring or river water and untreated pipe water, and eating of uncooked vegetables and unwashed fruits.

Table 3. Common Prevalence of Intestinal Protozoan and Helminth Parasite Species Identified from Examined Children (n=384) aged less than five years in Debre Birhan Health Center, Debre Birhan Town,during May-August,2013

Agegroup and sex	№.of examined	PPS			HPS			MPS
		Eh/d	Gl	Cp	Al	Tt	Hn	№.of pos (%)
		№. Pos. (%)	№. Pos. (%)	№. Pos. (%)	№. Pos. (%)	№. Pos. (%)	№. Pos. (%)	
0-1								
Males	16	2(12.5)	0	0	0	0	0	
Females	18	1(5.6)	0	0	1(5.6)	0	0	
1-2								
Males	10	1(10)	0	0	2(20.0)	0	0	
Females	12	0	0	0	0	0	0	
2-3								
Males	9	4(44.4)	1(11)	0	0	0	0	
Females	9	2(22.2)	1(11)	0	0	0	1(11.1)	
3-4								
Males	16	3(18.8)	1(6.2)	0	2(12.5)	0	1(6.2)	0
Females	23	5(21)	4(17.4)	0	0	0	0	2(8.7)
4-5								
Males	125	16(12.8)	17(13.6)	4(3.2)	10(8.0)	9(7.2)	7(5.6)	4(3.2)
Females	146	37(25)	20(13.7)	3(2.1)	13(8.9)	15(5.5)	2(1.4)	4(2.7)
Allovergroup								
Males	176	26(14.8)	19(10.8)	4(2.3)	14(8)	9(5)	8(4.5)	4(2.3)
Females	208	45(21.6)	25(12)	3(1.9)	14(5.1)	15(7.2)	3(1.4)	6(2.9)
Total	384	71(18.5)	44(11.5)	7(2)	28(7.3)	24(6.3)	11(2.9)	10(2.7)

PPS= protozoan parasitic species, HPS=helmimth parasitic species, MPS =multiple parasitic species, Eh/d= Entamoeba histolytica/disper, Gl= Giardia lamblia, Cp Cryptospridium parvum, Al=Ascaris lumbricoides, Tt trichuris trichuria, Hn= Hymenolepis nana

The predominant protozoan parasites were *Entamoeba histolytica* 71 (18.5%) and *G.lamblia* 44 (11.5%) (Table 3). This prevalence rate was in agreement with previous studies reported in the prevalence of *Entamoeba histolytica* infection by Malta and Roberto, 2009 (22%) in Brazil/São Paulo State, Dawit, 2006(23.7%) in Dire- Dawa, Eastern Ethiopia. On the other hand it was much higher to the report written by Awad and Suzan, 2005*(60.5*%) in *Basrah* city. To the contrary it was lower with studies reported by Eyasu *et al*., 2010 (6.3%) in Benishangul-Gumuz, Adish *et al*., 1999(13.6%) in northern Ethiopia, Liza *et al.*, 2010 (0.35%) in Shesha Kekele, Wondo Genet, Southern Ethiopia and the prevalence of *G.lamblia* infection reported by Awad and Suzan, 2005 (4%) in Basrah city, Al-Hindi and Aboud , 2008 (0. 3%) in Gaza, Palestine, Teklu, 2009 (3.8%) in North Shewa Zone, Oromia Region and Mengistu, 2010 (6.0%) inTach Gayint District, South Gondar Zone.

4.3. Parents`/Caretaker`s Socio-Demographic Characteristics and Household Factors Associating with Intestinal Parasitic Infections of Children Less than Five Years Old

The prevalence of common human intestinal protozoan and helminthic parasites was investigated in accordance with risk factors such as place of residence, family educational level, marital status, sources of driking water, availability of toilet facility, family occupation, wash before and after meal, eating unwashed and uncooked vegetables,hand wash after toilet, and training or knowhow about environmental hygiene and personal sanitation. As indicated in table 4, parents`/caretaker`s marital status and household factors associated with intestinal parasitic infections of children of less than five years old in Debre Birhan town during May-August 2013, was statistically significant ($P < 0.05$). In marital status children who came from divorced family were highly infected with intestinal parasitic species 60% (9/15) and similarly in education level the greater prevalence was recorded with children who had illiterate families 60.0% (45/75) come next only with primary education 59.1% (94/159).

Table 4. Relationship of Parents/Caretakers` Socio-Demographic Characteristics and Household Factors Associated with Intestinal Parasitic Infections of Children Under-Five years old in Debre Birhan Health Center during May-August, 2013.

Character	**Number**	**Intestinal parasitic infections**		**OR (95% CI)**	X^2	**P-value**
		№. Pos. (%)	№. Neg. (%)			
Water treatment						
By boiling	15	11(73.3)	14(48.3)			
By chemicals	341	207(60.7)	137(39.3)			
As it is	28	15(51.7)	13(46.4)	1.674(.493-5.684)	1.60	.449
Education Level						
illiterate	75	45(60.0)	30(40.0)			
Primary	159	94(59.1)	65(40.9)			
Secondary and above	150	94(62.7)	56(37.3)	.966(.708-1.316)	.425	.809
Marital Status						
Married	306	103(37.7)	203(66.3)			
Single	56	31(55.4)	25(44.6)			
Divorsed	15	9(60)	6(40)			
Widowed	7	1(14.3)	6(85.7)		14.394	0.02
Occupation						
Unemployed	162	97(59.9)	65(40.1)			
Civil Servant	53	33(62.3)	20(37.75)			
Private	169(44.0)	103(60.9)	66(39.1)	.997(.836-1.190)	1.210	.876
House condition						
Poor	170	122(71.8)	48(28.2)			
Good	214	29(13.6)	185(86.4)	.062(.037-.103)	1.346	.00

The result from Table 4 showed that the prevalence of human intestinal parasite infections in accordance to children`s family occupation and family education level were unemployed, civil servant, private, illiterate, primary education, secondary and above; 59.9% (97/162), 62.3% (33/53), 60.9% (103/169), 60% (45/75), 59.1% (94/159) and 62.7% (94/150), respectively. Although the association between the prevalence of human parasites infection and children family occupation and education level were statistically insignificant ($p > 0.05$), it was found that higher parasitic infection among children who had unemployed and private worker families. The results of the present study was not statistically agreed ($p<0.05$) with the study reported by Quna M, (1994) in Portugal. As Quna, Parents` educational level and family occupation was found to be highly associated with the prevalence of intestinal infection among children.

Table 4 also showed that the prevalence of human intestinal parasite infections in relation to method of water treatment was 4(26.7%) boiling, 134(39.3%) a purifying chemicals and 13(46.4%) no treatment or as it is. As the result shown in table 4, relatively high prevalence of intestinal parasitic infections was observed in untreated water users (46.4%). But the association between the prevalence of human parasites infection in relation to method of water treatment was statistically insignificant ($p > 0.05$). This was not in agreement with previous study reported by Olusegun *et al*. (2011). According to the Olusgun study, the association between the prevalence of human parasites infection and method of water treatment was statistically significant ($p<0.0001$).

The results from Table 4 revealed that the prevalence of intestinal parasite infections in relation to house conditions and marital status were highly significant (p-value = 0.00, 0.02) respectively. This indicated that the prevalence of parasite infections was highly related to housing conditions and marital status. As shown from the Table 4 children who came from the Divorced families were highly infected 9(60%) with intestinal parasites. This might be the Divorced families did not bother about their housing sanitation and personal hygiene’s including their children.

4.4. Parents`/Caretakers' Level of Knowledge and Management Practices of Intestinal Parasitic infection of Children Less than Five Years Old

Result of assessment paternal knowledge and management practices of intestinal parasitic infection of children less than five years old is summarized and presented in table 5. The result revealed the existence of significant association between prevalence of intestinal parasitic infection among under-five years old children and maternal /caretakers awareness or knowledge about the symptoms of the disease ($P = 0.002$). In the study, mothers /caretakers limited knowledge about clinical manifestations and mode of transmission of intestinal parasitic infections as well as impact of infections on their children.

The association between eating of unwashed fruits and raw vegetables and intestinal parasite infections was found statistically significant ($p<0.05$). A similar approach was reported by Mohamed *et al.* (2009) in Nasiriyah. According to this research report, the rates of prevalence of human intestinal parasite infections in regions was high mainly due to direct contact and a long time staying of children in dirty areas with dust and mud and eating of unwashed fruits and raw vegetables there.

Table 5 Association of Parents'/Caretakers' Level of Knowledge and Management Practices with Intestinal Parasitic Infections of their Children Less than Five Years Old in Debre Brihan Town during May-August 2013

Character	Number of respondents	IPI		OR(95% CI)	X^2	P-value
		№. Neg. (%)	№. Pos. (%)			
Toilet facility						
Private	218(56.8)	131(60.1%)	87(39.9)	1.189(.794-1.781)	10.177	0.006
Public	157(40.9)	101(64.3%)	56(35.7)			
Wherever	9(2.3)	01(11.1%)	8(88.9)			
Residence						
Rural	28(7.3)	15(53.6)	13(46.4)	.730(.337-1. 582)	.639	.424
Town	356(92.7)	218(61.2)	138(38.85)			
HWM						
Always	320	195(60.9)	125(39.1)	.538(.231-1.250)	.055	.815
Sometimes	64	38(59.45)	26(40.6%)			
EUWFV						
Sometimes	256	136(53.1)	120(46.9)	2.349(1.391-3.968)	8.636	.013
Never	28	15(53.6)	13(46.4)			
KnSIPI						
yes	59	34(57.6)	25 (42.4)	414 (.235- . 727)	9.789	0.002
No	325	117(36)	208(64)			
Water source						
Spring/river	29(7.6)	15(51.7)	14(48.3)	1.674(.493-5.684)	1.600	.449
pipe	355(92.4)	218(61.4)	137(38.6)	.000	1.054	.305

EUWFV=Eating of unwashed fruits and vegetables, HWM= Hand wash before meal,

KnSIPI = knowhow about the symptoms of intestinal parasitic infection, As shown in Table 5, the prevalence of human intestinal parasite infections in rural areas was 13(46.4%), and in urban area 138 (38.85%) with no significant difference (p- value >0.05). The current result was not approached with the study reported by Mohamed *et al.* (2009) conducted outside the country in the Sudan in Kassala Town, the proportion of rural infection 22.2% was higher than the urban infection 12.9% and the prevalence of human intestinal parasite infections significantly (p< 0.05) associated with urban and rural residence. This might be improvement in sanitary conditions, environmental factors, timing and seasonal differences in the design of the survey work and personal hygiene (Albonico *et al*., 1998).

As shown from Table 5, the prevalence of human intestinal parasite infections in children at the study site in relation to personal hygiene and the source of household water was 14 (48.3%) from spring or river water and 137 (38.6%) from pipe water. The association between the source of household water and prevalence of human parasites with under- five aged children in Debre Birhan town was statistically insignificant (p > 0.05). To the contrary, the result was not approached (p < 0.05) with studies conducted by Dawit, (2006) in Adada, Dire-Dawa Ethiopia, Fentaw (2010), Girum (2005), in Babile town. This might be due to environmental condition, timing and seasonal differences in the design of the survey work and personal hygiene (Albonico *et al*., 1999). Table 5 showed that the prevalence of human intestinal parasite infections in under-five aged children in relation to toilet facilities, 87(39.9%), private, 56 (35.7%) and public, 8(88.9%) wherever. The association between the prevalence of parasitic infections and toilet facilities in the research site was statistically significant (p < 0.05). This result was similar to the studies reported by Girum (2005) and Olusegun *et al*. (2011). Their study report indicated that toilet facilities are highly risky to the prevalence of human intestinal parasite infections.

4.5. Associated Morbidity Related Factors for Intestinal Parasitic Infections of Children Less than Five Years Old in Debre Birhan Town

The study participants were examined physically for variables such as children hygienic condition, children physical condition, stool type, nausea , abdominal discomfort, vomiting and other abnormalities, by the physician and investigator (Table 6). The result of prevalence of intestinal protozoan and helminth parasite infections are summarized in Table 6. The prevalence of intestinal protozoan parasite infections of both sex and all age group and clinical manifestation like poor children hygienic conditions, Physical condition was highly significant ($P = 0.00$) (Table 5).

Table 6. Observed Clinical Signs and Symptoms among Examined Children (N= 384) Of Less Than Five Years Old and Its Relationship with Intestinal Parasitic Infections, during May- August, 2013

Clinical Manifestations	Number	IPI		OR (CI)	X^2	P-value
		№. Pos.(%)	№. Neg.(%)			
CHC						
Poor	116	97(83.6)	19(16.4)	.049 (.028- .088)	1.367	.000
Good	267	54(20.1)	214(79.9)			
CPC						
Poor	92	56(60.9)	36(39.1)	.310 (.191- .504)	23.541	.000
Good	292	95(32.5)	197(67.5)			
Stool Type						
Diarrheic	75	43(57.3)	32(42.7)	.400 (.239- .668)	12.671	.000
Non- Diarrheic	309	108(35)	201(65)			
Nausea						
Yes	59	34(57.6)	25 (42.4)	.414 (.235- . 72)	9.789	.002
No	325	117(36)	208(64)			
Loss of Appetite						
Yes	82	46 (56.1)	36 (43.9)	.417 (.254 - .685)	12.296	.000
No	302	105(34.8)	197(65.2%)			
Abdominal discomfort						
Yes	132	126(95.5)	6(4.5)	.004(.002-.012	2.694	.000
No	252	25(10)	227(891)			

CHC= child health condition, CPC= child physical condition

The prevalence of human intestinal parasite infections in children at the study site in relation to personal hygiene and health and physical conditions was highly associated with the prevalence of intestinal parasitic infections and revealed greater significant ($p < 0.05$). The prevalence of human intestinal parasite infections in children at the study site in relation to clinical manifestations like abdominal pain, 92.2% (126/132), poor child health condition 83.6% (97/116), loss of appetite 56.1% (46/82) was greatly related to IPI.

In this study, mothers'/caretakers’ management practices were also assessed and the result showed that most of them responded that feeding a child with uncooked vegetables was found significantly associated with intestinal parasite infections.

This might be due to factors like climatic conditions, poor sanitation, unsafe drinking water, and lack of toilet facilities are the main contributors to the high prevalence of intestinal parasitic infection in the study site. Further, lack of awareness about mode of transmission of parasitic infections increases the risk of infection.

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

The objective of the present study was to identify thecommon human intestinal parasites species and determine the prevalence of human intestinal infections as well as associable risk factors with IPI.

The design of the study was a cross-sectional parasitological survey involving a sample population of less than five aged children in Debre Birhan Health Center at Debre Birhan town during May to August 2013. A total of 384 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 and helminthic parasites were common in the study area. The overall prevalence of infections with different types of intestinal parasites was 151 (39.3%).

The predominant parasites involved in this study were *Entamoeba histolytica,* 71 (18.5%), *lamblia,* 11.5% *Criptosporidium SPP,* 2.1%, *lumbricoides*, 7.3% *and nana,* 2.9%.

The rates of prevalence of human intestinal parasite infections in Debre Birhan town mainly due to direct contact and a long time for children in the unclean areas with dust and mud and unwashed fruits and uncooked or unwashed raw vegetables before eating. The prevalence of human intestinal parasite infections with under-five aged children in relation to children and their family toilet condition was 87(39.9%) Private, 56 (35.7%) and Public 8(88.9%) open defecation ($p < 0.05$). This revealed that the association between the prevalence of IPI and toilet facility was statistically significant ($p< 0.05$).

5.2. Conclusion

The common intestinal parasite species diagnosed among under-five aged children of Debre Birhan town were *histolytica*, *lamblia*, *parvum ,lumbricoides*, *trichiura and nana*. The finding reported in the present study was that intestinal parasitic protozoan and helminthes infections represent a health problem among under-five aged children of Debre Birhan town.

Most intestinal parasitic infections represent a child health threat because of their contaminated water and foods born transmission. *E. histolytica, G.lamblia*, *A.lumbricoides* and *T. trichiura* were found as a dominant species of intestinal protozoans and helminth parasites diagnosed in the stool samples of the children and have a higher prevalence in the age group of 0-5 years old.

Association was observed between prevalence of human intestinal infection and children eating unwashed fruits and uncooked vegetables and their family toilet condition. Providing of washed fruits and cooked vegetables, proper toilet condition, well protected and treated drinking water, proper education on hygienic and environmental sanitation would help in reducing the prevalence of intestinal parasites infection and need more medical attention to avoid the intestinal parasites consequences.

5. 3. Recommendations

The findings of the present study showed that human intestinal parasite infections were prevalent among under- five aged children. The prevalence of parasite infections in the study area indicated that much work remains to be done in improving the health of the children from intestinal parasitic infections. This calls for the initiation of control measures including treatment of infected children, improvement of personal hygiene and environmental sanitation, and provision of clean water and foods. The impact of each measure would be maximized through the health education program directed to families and their children in particular, and to communities level in general.

Generally, health authorities should make concerned efforts to ensure the prevention of these parasitic protozoan and Helminthes from infecting children. For the control of these parasitic infections, a half-yearly repeated anti parasitic treatment is recommended for children as well as the community to reduce re-infection. This is because if one is de-wormed at intervals, there is a possibility of killing most parasitic helminthes and protozoan parasite cysts in the intestine before they cause heavy infestation leading to severe health consequences.

Therefore, to reduce the prevalence of intestinal soil-transmitted helminth and protozoan infections, increase the knowledge and awareness about the causes and prevention mechanism of intestinal parasitic infections through:

- Providing training and creating awareness to children families about personal hygiene, environmental sanitation, proper waste disposal, tranismition and preventions of human intestinal parasite infections.
- Establishing and maintenances of a network for the treatment of intestinal parasitic infections and provision of health education program in family and community level.
- Further studies should be required in the study area such as, a longitudinal study on the prevalence of intestinal parasite infections and associated risk factors.

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7. APPENDICES

Appendix 1. Laboratory data collecting format

Code	Sex	Age	Date/Month/Year	Presence/Absence	Parasites identified in the stool examination						Single infection	Double infections	Multiple infection	Others	Remark
					E. hictolytica	G.lamblia	C. parvu	A.lumbricoides	T.trichura	H.nana					

Key:

1. E. histolitica = Entamoeba histolitica
2. G. lamblia = Giardia lamblia
3. C. parvum = Cryptosporidium parvum
4. A. lumbricoides = Ascaris lumbricoides
5. T. trichura = Trichuris trichura
6. H. nana = Hymenolepis nana

Presence(Y)

Absence(X)

Appendices 2 Observed Clinical Signs and Symptoms recording format

Children's hygienic condition		**Remark**
Poor		
Good		
Children's physical condition		
Poor		
Good		
Stool Type		
Diarrheic		
No diarrheic		
Nausea		
Yes		
No		
Abdominal discomfort		
Yes		
NO		
Loss of Appetite		
Yes		
No		
Vomiting		
Yes		
No		

X= for agreement **Y= for disagreement**

Appendix 3. Amharic version of the Questionnaire

<u>ቃለ መጠየቅ</u>

እድሚያቸው ለትምህርት ያልደረሱ ህጻ ናት ወላጆች መረጃ መስጫ በጎ ፈቃደኛነት ማረጋገጫ ቅጽ

<u>ክፍል አንድ፡</u> እድሚያቸው ለትምህርትያልደረሱ_ህጻናት ወላጆች መረጃ አሰጣጥ መግቢያ

<u>ጤና ይሰጥልኝ !!</u>

በቅድሚያ ለቃለ መጠይቁ ፈቃደኛ በመሆንዎ እጅግ አድረጌ አመሰግናለሁ!!

የእኔ ስም -- ይባላል፡፡ በሀረማያ ዩኒቨርሲቲ በተፈጥሮና ኮምፒቴሽናል ሳይንስ ኮሌጅ በባሎጅ ትምህርት ክፍል ለሁለተኛ ዲግሪ መመረቂያ ጽሁፍ ተሳታፊ ነኝ፡፡

የዚህ ጥናታዊ ጽሁፍ ዓለማ የርሰዎንና የቤተሰብዎን የጤና ሁኔታ ለማሻሻል የሚረዳ ይሆናል ተብሎ ስለሚታሰብ እና ለጥናቱም ስኬታማነት የርሰዎ ሙሉ ፈቃደኛነትና ትክክለኛውን መረጃ መስጠት ጠቀሜታው የጎላ በመሆኑ ትብብረዎን እጠይቃለሁ፡፡

በመጠይቁ ቅጽ ፎርም ላይ ስም፣ ልዩ አድራሻ፣ ማንነት እንዲሁም የሰጡት መረጃ የርስዎ ስለመሆኑ በምንም አይነት ሁኔታ አይገለጽም፡፡ በዚህ ጥናተዊ ጽሁፍ ለመሳተፍ የርሰዎን ሙሉ ፈቃደኝነት ስጠይቅ ያለምንም አስገዳጅነት ሲሆን ፈቃደኛ ካልሆኑ ላለመተባበር መብተዎ የተጠበቀ ነው፡፡

<u>ክፍል ሁለት፡</u> እድሚያቸው ለትምህርት ያልደረሱ ህጻናት ወላጆች የበጎ ፈቃደኝነት መረጃ መሰብሰቢያ ቅጽ

ከላይ በተሰጠኝ ማብራሪየ መሰረት እውነተኛ መረጃ ለመስጠት፤ ተሰማምቻለሁ----------- አልተስማማሁም-----------

መጠይቁ የተደረገበት ቀን-------------------------------- መጠይቁ የተጠናቀቅበት ሰዓት----------------------------

መጠይቁን ያደረገውና ያጠናቀረው ሰው ስም --- ፊርማ------------------

መጠይቅ

ጥናቱ የተደረገበት አካባቢ --------------------------------------

የታካሚው ህጻን መለያ ቁጥር-------------------------------------

የህጻኑ/ ኗ እና የቤተሰብ ሁኔታ

1. የህጻኑ/ ኗ ጾታ፤ ወ--------- ሴ-------------

2. ዕድሜ ፡-

ሀ/ 0-1 ዓመት ለ/ 1-2 ዓመት ሐ/ 4.2-3 ዓመት መ/ 3-4 ዓመት ሠ/ 4-5 ዓመት

3. የቤተሰብ መኖሪያ አድራሻ ሀ/ከተማ ለ/ገጠር

4.የቤተሰብ ስራ: ሀ/ የመንግስት ሰራተኛ ለ/ ግብረና ሐ/ ንግድየግል ሠ/ ስራዓጥ

5. የቤተሰብ የትምህርትደረጃ: ሀ/ ያልተማሩ ለ/ የመጀመሪያትምህርትየተማሩ ሐ/ሁለተኛደረጃየተማሩናከዚያበላይየተማሩ

6. ልጅዎ ለምግብነት የሚጠቀምባቸው ቁሳቁሶች በሚገባ ንጽህናቸውን የጠበቁ ናቸው? ሀ/ እስማማለሁ ለ/ አልስማማም

7. ልጅዎ ከተጸዳዳ በኋላ በሚገባ የታጠባል? ሀ/ ሁልጊዜ ለ/ አልፎአልፎ ሐ/የለም

8. በአግባቡ ያለታጠቡና ያልበሰሉ ፍራፍሬና አትክልቶችን ይመግባሉ? ሀ/ ሁልጊዜ ለ/ አልፎአልፎ ሐ/የለም

9. የመጸዳጃ ቤትዎ ሁኔታ: ሀ/ የግል ለ/ የጋራ ሐ/ ሜዳላይ

10. የሚገለገሉበት የውሀ ምንጭ: ሀ/የቧንቧ ውሀ ለ/የምንጭ ወይም የወንዝ ውሀ

11. የሚጠቀሙበትን ውሃ በእንዴት አይነት ሁኔታ ይወስዳሉ ወይም ለለጆችዎ የሰጣሉ? ሀ/ በማፍላት ለ/ የውሃ ማጣሪያ በመጨመር ሐ/ እንዳለ

12. የልጅዎ ምግብ፡ ሀ/ሁልጊዜትኩስ ለ/ አልፎአለፎ ትኩስ ሐ/ ብዙጊዜ የዋለ

13. የልጅዎ የመጫዎቻ ቦታ: ሀ/ ንጽኅናውን የጠበቀ ለ/ ንጸህናውን ያልጠበቀ

14. የልጅዎ ጥፍር ሲያድግ በዎቅቱ ይቆርጣሉ? ሀ/ ሁልጊዜ ለ/አልፎአልፎ ሐ/የለም

15. ከዚህ በፊት ስለግልና አካባቢ ንጽህና ትምህርት አግኝተው ያውቃሉ? ሀ/አዎ   ለ/ የለም

16. በተራ ቁጥር መልሰዎ **"አዎ** ከሆነ ትምህርቱን ከየት አገኙት? ሀ/ ከጎረቤት ለ/ ከጤና ኤክስቴሽን ባለሙያ   ሐ/ከጤና ጣቢያ ከሆስፒታል   መ/ ከቤተሰብ   ሠ/ ከመገናኛብዙሃን

Appendix 4. English version of the questionnaire

An ensuring format of parents whose children are not old enough to begin schooling.

Part one: A format of gathering data for parents whose children are not old enough to begin schooling.

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 child (patient) --

Details about a child and family

1. child`s sex: M ------------ F-------------
2. Age : A) 0-1 years
   B) 1- 2 years
   C) 2- 3 years
   D) 3- 4 years
   E) 4-5 years

3. family`s dwelling areas; A) urban B) rural

4. Job of family A) government worker B) farmer C) merchant D) private worker

   E) unemployed

5. family`s educational status; A) illiterate B) literate of primary education C) literate

   of secondary education and above

6. Are dinning utensils which your child uses kept clean A) agree B) disagree

7. Does your child wash or do you wash after toilet? A) Always B) sometimes

   C) Never

8. Do you eat unwashed fruits and vegetables? A) Always B) sometimes C) never

9. Your toilet; A) private B) public C) open defection

10. Your sources of water; A) pipe B) spring or river

11. Water consumption; A) by boiling B) adding purifying chemicals C) as it is

12. Your child meal is; A) always fresh B) sometimes fresh C) not fresh

13. Your child playing ground is A) clean B) not clean

14. Do you cut your child nail when grown? A) Always B) sometimes

C) Never

15. Did you get informations and training about personal and environmental hygiene and sanitation respectively before? A) Yes B) No

16. If your answer for question is “yes “where did you get? A) From families B) health extension personnel’s C) health center / hospital`s professionals D) media E) not at all