





**ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
BIOLOGY DEPARTMENT**

**ASSOCIATION OF *Cryptosporidium parvum*, *Giardia lamblia* AND *Entamoeba histolytica/dispar* INFECTION WITH DRINKING WATER SOURCES
AMONG CHILDREN IN RURAL PART OF DIRE- DAWA,
EASTERN ETHIOPIA**

**IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE ATTAINMENT
OF THE DEGREE OF MASTER OF SCIENCE IN BIOLOGY
(BIOMEDICAL SCIENCE)**

**BY
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Association of *Cryptosporidium parvum*, *Giardia lamblia* and *Entamoeba histolytica/dispar* infection with drinking water sources among Children in rural part of Dire-Dawa, Eastern Ethiopia.

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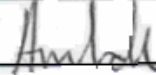
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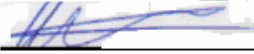
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Table of contents

Pages

Acknowledgments.....	iii
List of tables.....	iv
List of figures.....	v
List of abbreviations.....	vi
List of appendixes.....	vii
Abstract.....	viii
1. Introduction.....	1
1.1. <i>Cryptosporidium parvum</i>	3
1.2. <i>Giardia lamblia</i>	10
1.3. <i>Entamoeba histolytica/dispar</i>	15
1.4. Giardiasis, cryptosporidiosis and amebiasis in Ethiopia.....	19
2. Objective.....	24
3. Materials and Methods.....	25
3.1. The Study areas.....	25
3.2. The Study population.....	29
3.3. Stool collection and process.....	29
3.3.1. Direct wet mount method.....	29
3.3.2. Concentration method.....	29
3.3.3. Modified Ziehl-Neelsen method.....	30
3.4. Ethical clearance.....	30
3.5. Data analysis.....	30
4. Results.....	31
4.1. Comparison of the prevalence of cryptosporidiosis, giardiasis and amebiasis between sites.....	32
4.2. Prevalence of cryptosporidiosis, giardiasis and amebiasis within each .site.....	33
4.2.1. Legedini.....	33
4.2.2. Adada.....	41
4.2.3. Legebira.....	43
5. Discussion.....	45
6. Conclusions and Recommendations	53
7. References	55
8. Appendix:.....	67

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List of Tables

page

Table 1. Valid names of *Cryptosporidium species* that infect mammals and other animals3

Table 2. Prevalence of intestinal parasites among children in Legedini, Adada and Legebira Dire-
....Dawa administrative region (November 2005 – May 2006).....31

Table 3. Prevalence of giardiasis, cryptosporidiosis and amoebiasis between sites among children
using protected and unprotected water sources during dry and wet seasons sampling within
Dire-Dawa administrative region (November 2005 – May 2006).....35

Table 4. Prevalence of giardiasis, amoebiasis and cryptosporidiosis at wet and dry seasons sampling
in the three sites within Dire-Dawa administrative region (November 2005 –May 2006)....36

Table 5. Prevalence of giardiasis, amoebiasis and cryptosporidiosis between dry and wet season
sampling among children using protected and unprotected water sources in the selected sites
of Dire-Dawa administrative region (November 2005 –May 2006).37

Table 6. Prevalence of cryptosporidiosis, amoebiasis and giardiasis between children using the
protected and unprotected water sources in the three sites within Dire-Dawa administrative
regions (November 2005 –May 2006).38

Table 7. Prevalence of giardiasis, cryptosporidiosis and amoebiasis between children using protected
and unprotected water sources in dry and wet season sampling in the three sites within Dire-
Dawa Administrative region (November 2005 –May 2006)39

Table 8. Prevalence of giardiasis, cryptosporidiosis and amoebiasis among villages in Legedini within
Dire-Dawa administrative region (November 2005 – May 2006).....40

List of figures

page

Figure 1. Diagrammatic representation of life cycle of <i>Cryptosporidium</i> ...	6
Figure 2. Life cycle of <i>Giardia lamblia</i>	12
Figure 3. Life Cycle of <i>Entamoeba histolytica/dispar</i>	16
Figure 4. Monthly average rainfall (mm) in Dire-Dawa administrative region (1996-2005).....	26
Figure 5 Monthly average relative humidity in Dire-Dawa administrative region (1996-2005).....	26
Figure 6. Monthly average maximum and minimum temperatures (°c) in Dire-Dawa administrative region (1996-2005).....	27
Figure 7. Map of Ethiopia showing the location of the study areas.....	28
Figure 8. Prevalence of giardiasis and cryptosporidiosis by sex in Legedini (November 2005 – May 2006).....	40
Figure 9. Prevalence of giardiasis and cryptosporidiosis among different age groups in Legedini (November 2005 – May 2006).....	40
Figure 10. Prevalence of giardiasis and cryptosporidiosis by sex in Adada (November 2005 – May 2006).....	42
Figure 11. Prevalence of giardiasis and cryptosporidiosis among different age groups in Adada (November 2005 – May 2006).....	42
Figure 12. Prevalence of giardiasis and cryptosporidiosis by sex in Legebira (November 2005 – May 2006).....	44
Figure 13. Prevalence of giardiasis and cryptosporidiosis among different age groups in Legebira (November 2005 – May 2006).....	44

List of abbreviations

χ^2	Chi square test
°C	Degree centigrade
a.s.l	above sea level
AAU	Addis Ababa University
AIDS	Acquired Immunodeficiency Virus
CD	Cluster of Differentiation
DNA	Deoxyribo Nucleic Acid
E	East
EHNRI	Ethiopian Health and Nutrition Research Institute
ELISA	Enzyme Linked Immunosorbent Assay
ERCP	Endoscopic Retrograde Cholangiopancreatography
g	gram
Gal-GalNAc	Galactose-Nacetylglactosamine
GI	Gastro Intestinal
HAART	Highly Active Antiretroviral Therapy
HCl	Hydrochloric acid
HCS	Harrarghe Catholic Secretariat
HIV	Human Immunodeficiency Virus
IF	Immunofluorescence
IFA	Immunofluorescence Assay
IWMI	International Water Management Institute
Km	Kilometer
ml	milliliter
mm	millimeter
mm ³	millimeter cube
MUS	Multiple Use System
N	North
NaCl	Sodium chloride
PCR	Polymerase Chain Reaction
Prev.	Prevalence
PVA	Polyvinyl Alcohol
Rpm	Revolution per minute
SAF	Sodium acetate-Acetic acid-Formaldehyde
SGS	School of Graduate Studies
WHO	World Health Organization

List of appendixes

page

Appendix I: Consent form.....67

Abstract

Giardia lamblia, *Cryptosporidium parvum* and *Entamoeba histolytica/dispar* are ubiquitous protozoan parasites that affect humans, domestic animals and wildlife throughout the world and have been highlighted as significant waterborne parasitic pathogens. The present study was conducted to assess the prevalence of the three parasitic infections among children using protected and unprotected water sources in three rural sites (Legedini, Adada and Legebira) in Dire-Dawa, Eastern Ethiopia from November 2005 – May 2006. Single stool specimens were collected from a total of 1894 children under 14 years of age and processed for *C. parvum* using Modified Ziehl-Neelsen staining method. *Giardia*, amoeba and other intestinal parasites were detected using formalin-ether concentration and by direct wet mount methods. Out of 1894 children examined, 225 (11.9%), 719 (38%) and 639 (33.7%) were infected with *Cryptosporidium parvum*, *Giardia lamblia* and *Entamoeba histolytica/dispar*, respectively. The prevalence of giardiasis, cryptosporidiosis and amoebiasis during wet season sampling was significantly higher ($P < 0.05$) than the dry season in all study sites. On the other hand, no difference was observed in the prevalence of cryptosporidiosis, giardiasis and amoebiasis ($P > 0.05$) between children drinking water from protected and unprotected sources in Legedini and Legebira while in Adada significantly high prevalence ($P < 0.05$) was observed for the unprotected. The insignificant difference in prevalence between children using the two water sources indicates the presence of contamination of the drinking water at some point before consumption and also indicates the poor personal hygiene and environmental sanitation of the community. The prevalence of giardiasis, cryptosporidiosis and amoebiasis in relation to sex group showed no statistically significant difference ($P > 0.05$). On the other hand, lower age groups had a higher ($P < 0.05$) prevalence of infection with giardiasis and amoebiasis, and infections with cryptosporidiosis was not related with age ($P > 0.05$). Co-infections were also detected in 25.4% of the study subjects. In addition, other non-pathogenic intestinal parasites such as *Iodoamoeba butschilii*, *Entamoeba coli*, *Chilomasix mesnelli* and *Endolimax nana* were also detected in the study, which is an indication of fecal contamination of the drinking water source. Providing high quality drinking water may not significantly reduce the incidence of intestinal parasites other factors such as unhygienic and unsanitary situations overwhelm the beneficial effects of protected water sources. In addition untreated “protected” drinking water sources are not free of the waterborne parasitic pathogens. Therefore, health education in related to personal hygiene and environmental sanitation and cost effective water purification mechanisms such as boiling and chlorination and others will help in enhancing the health and well-being of the community particularly that of children.

1. Introduction

Morbidity and mortality due to diarrhoeal disease in developing countries is a major public health problem. Some of the recommended ways to reduce diarrhoeal disease are providing safe drinking water, safe waste removal especially the use of facilities to dispose faeces in a sanitary way and improved hygienic standard (WHO, 1992). Cryptosporidiosis, giardiasis and amebiasis are the common cause of human diarrhoeal disease worldwide, and lead to significant morbidity and mortality in the world, particularly in developing ones. It occurs both in immunocompetent and in immunocompromised individuals. The causative organism for giardiasis is *Giardia lamblia*, for cryptosporidiosis is *Cryptosporidium parvum* and for amebiasis is *Entamoeba histolytica* (Heyworth, 1992).

Giardiasis is the most common cause of parasitic gastro-intestinal disease and it is estimated that up to two hundred million people are chronically infected with *Giardia lamblia* globally, and 500,000 new cases reported annually (WHO, 1998). The prevalence of the disease varies from 2%–5% in developed to 20%–30% in developing countries. The variation in prevalence depends on factors such as the geographical area, the urban or rural setting of the society, the age group composition and the socio-economical conditions of the study subject (Flanagan, 1992).

As it has been described by Adams (1991), *Giardia* was originally identified by Van Leeuwenhook in the 1600's. Although it was the first protozoan parasite described, its role as pathogenic organisms was not recognized until the 1970's. Prior to that time, the organism was thought to be harmless commensal of the intestine. Later on *Giardia lamblia* turned out to be one of the most common causative agents of epidemic and endemic diarrhoeal illness through out the world (Adams, 1991).

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 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; Payment, 1999). 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).

Amebiasis may have been first recognized as a deadly disease by Hippocrates (460 to 377 B.C.), who described a patient with fever and dysentery (Clark *et al.*, 2000). From that time onwards, invasive amebiasis is one of the world most prevalent and fatal infectious diseases. Around 500 million people are infected worldwide while 75,000 die of the disease annually. Behind malaria and schistosomiasis, amebiasis ranks third on the list of parasitic causes of death worldwide. The infection is common in developing countries and predominantly affects individuals with poor socioeconomic conditions, non hygienic practices, and malnutrition (Walsh, 1986).

It has been suggested that outbreaks of giardiasis, cryptosporidiosis and amebiasis in human may be attributed to fecal contamination of drinking water or recreational exposure in lakes, rivers, or swimming pools (Hojlyng *et al.*, 1987; Isaac, *et al.*, 1987; Flanagan, 1992; Serpil, *et al.*, 2005). All organisms are waterborne and contaminants of drinking water, has been the cause of epidemics of diarrhoea in both adult and children. Furthermore, *G. lamblia* is the cause of childhood diarrhoea and may be transmitted by close contact with infected

individuals. The prevalence of both *G. lamblia* and *C. parvum* is generally higher among very young children and this may be related to intimate contact with contaminated materials resulting in fecal – oral transmission of the infective stages of the parasites (Flanagan, 1992; Serpil, *et al.*, 2005).

1.1. *Cryptosporidium parvum*

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 (Table 1).

Table 1. Valid names of *Cryptosporidium species* that infect mammals and other animals (Coupe *et al.* 2005).

Species	Host Animal	Infection Site
<i>C. andersoni</i>	Cattle	Abomasum
<i>C. baileyi</i>	Chicken	Cloaca/respiratory tract/kidney
<i>C. felis</i>	Domestic cat	Intestine
<i>C. meleagridis</i>	Turkey	Intestine/respiratory tract
<i>C. muris</i>	Rodent	Stomach
<i>C. nasorum</i>	Fish	Stomach/intestine
<i>C. parvum</i>	Mammals	Intestine (predominantly)
<i>C. saurophilum</i>	Currently being researched	Currently being researched
<i>C. serpentis</i>	Snakes	Stomach
<i>C. wrairi</i>	Guinea pig	Intestine
<i>C. hominis</i>	Mammals	Intestine
<i>C. canis</i>	Mammals	Intestine
<i>C. galli</i>	Birds	Cloaca / respiratory tract

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 life cycle; 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 (figure 1). The oocyst is spherical in shape measuring 3-6 mm in diameter and it may be either thick- or thin-walled (Ramirez *et al.*, 2004). Thin-walled oocysts may excyst within the same host and start a new life cycle (autoinfection). This can lead to heavily infected intestinal epithelia and result in malabsorptive or secretory diarrhoea. Thick-walled oocysts are excreted with the faeces and it is the resistant stage found in the environment (Fayer and Ungar, 1986). Each oocyst contains 4 infective sporozoites which penetrate individual epithelial cells. These parasites are intracellular, enclosed by a thin layer of host cell cytoplasm (McDonald *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. 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 meronts. (Fayer and Ungar, 1986; O'donoghue, 1995).

“type 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 or 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 I meronts 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 autoinfective 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 in immunosuppressed individuals (Clark, 1999).

In experimentally infected animals, the prepatent period is generally 4 days but some times 3 days in heavy infections. However, in human outbreaks where lower numbers of oocysts are probably ingested, 4-6 days is a typical prepatent period. Patency, which is the length of time oocysts are shed in the feces, generally lasts 6-18 days (4-10 days of diarrhoea) in immunocompetent individuals but may be prolonged in immunosuppressed patients. Some individuals shed oocysts but appear asymptomatic (Fayer and Ungar, 1986).

Cryptosporidiosis is the most common protozoan intestinal parasite isolated worldwide in both immunocompetent 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 zoonotically from cattle and sheep. Indirectly it is spread through the environment particularly through water (Hojlyng *et al.*, 1987).

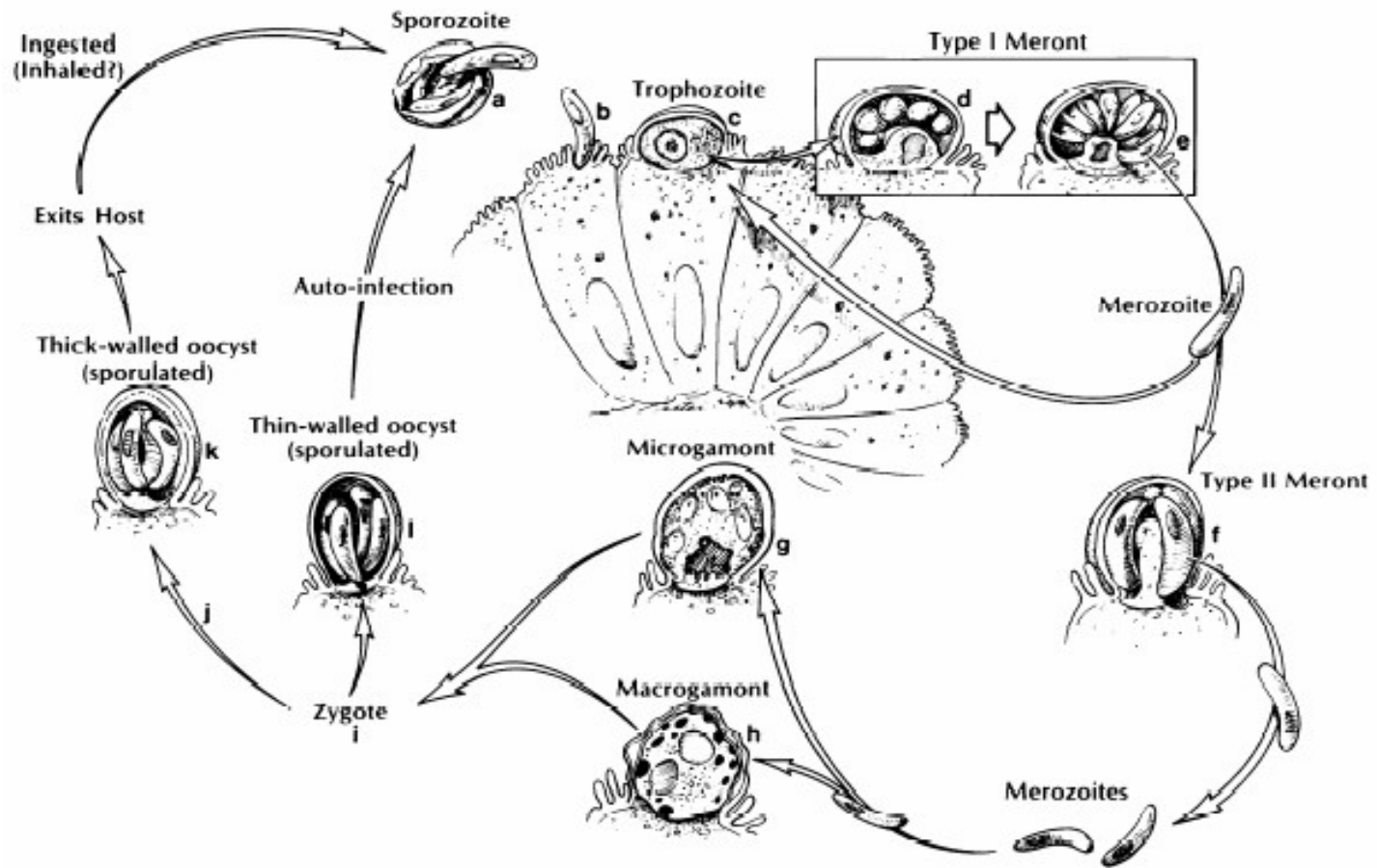


Figure 1 Diagrammatic representation of life cycle of *Cryptosporidium* (Current and Garcia, 1991).

Infection with *C. parvum* have been reported among children, parents of infected children those who drink unfiltered, untreated water and people who drink from shallow, unprotected wells, swimmers who swallow water while swimming in pools, lakes, rivers, ponds and streams. It can also be considered as an occupational and environmental hazard, among individuals who are involved in farming practices such as lambing and calving, veterinarians who come in contact with farm animals, people living in densely populated urban areas, etc are most likely infected by *Cryptosporidium parvum* (O'Donoghue, 1995; Rose, 1997; Fayer *et al.*, 1998).

Cryptosporidium parvum oocysts may remain viable for several months, especially under moist conditions (Fayer *et al.*, 1998). From prevalence studies, oocyst excretion rates are known to vary between 1%-3% in industrialized countries and 10% in less industrialized nations. Worldwide, it is estimated that 0.6-4.3% of humans are infected with *Cryptosporidium parvum* (Geldreich, 1989). *C. Parvum* infection was reported in 10%-15% of children with diarrhoea and 30%-50% of AIDS patients with chronic diarrhoea in the developing world (Sanchez-Mejorada, 1994). Infection mainly occurs in the intestine in both immunocompetent and immunocompromised individuals but biliary cryptosporidiosis has only been reported in HIV-infected patients. In contrast, when samples from asymptomatic individuals were examined, prevalence ranged from 0 to 2 % in developed countries compared with 0 to 9.8 % in developing countries (O'Donoghue, 1995).

The pathogenic mechanisms by which *Cryptosporidium* causes diarrhoea, malabsorption and wasting are poorly understood. Whatever these mechanisms may be, the initial host-parasite interactions of attachment and invasion are the critical primary events in pathogenesis. In general, Goodgame (1996) indicates that, epithelial cells are damaged as result of *Cryptosporidium parvum* infections in two ways. This involves (a) cell death as a direct result of parasite invasion, multiplication, and extrusion and (b) cell damage that could occur through T cell-mediated inflammation, producing villus atrophy and hyperplasia of the crypt.

Although symptoms of cryptosporidiosis differ greatly between immunocompetent and immunocompromised individuals, the most common clinical manifestation of cryptosporidiosis is profuse and watery diarrhoea, often containing mucus but rarely blood or leucocytes and the symptoms includes abdominal cramps, low grade fever, nausea and vomiting (Fayer and Ungar, 1986). In immunocompetent patients, the disease is an acute self-limiting diarrhoeal illness lasting approximately 1- 2 weeks. In immunocompromised individuals, the disease is much more severe and symptoms include watery diarrhoea with stool frequency of up to 10 times a day with a mean volume of one liter (Juraneck, 1995).

Traditionally, cryptosporidiosis is diagnosed by microscopic observation of developmental stages of the organism in an intestinal biopsy specimen. This day, most Cryptosporidial infections are diagnosed by microscopic examination of the host faecal material for the presence of *C. parvum* oocysts. However, a variety of diagnostic options and staining procedures are available for the detection of *Cryptosporidium* in stool samples, modified Ziehl-Neelsen acid-fast staining techniques are the techniques of choice for many diagnostic laboratories. (Clark, 1999).

Immunologically, anti- *Cryptosporidial* IgM, IgG and IgA can be detected by Enzyme Linked Immunosorbent Assay (ELISA) or by Immunofluorescence Assay (IFA), but neither of these assays can give a direct diagnosis of *Cryptosporidium* infection (Heyworth, 1992). Nowadays, new genetic methods of detecting *Cryptosporidium* infection have been developed using polymerase chain reaction (PCR) (Leav *et al.*, 2003).

In the absence of effective and specific therapy against infection with *Cryptosporidium parvum*, preventive measures are of great importance. Identifications of the most common routes of transmission and a better understanding of the species risk factors for exposure that lead to infection would greatly facilitate development of a more targeted prevention strategy (Fayer, 1994). Since most infections of *Cryptosporidium* are initiated through ingestion of oocysts, control of this stage limits the spread of the disease.

Strategies for prevention of *Cryptosporidial* infections are those usually recommended for avoiding any pathogen transmitted by the fecal oral route (NSTC, 1995).

One of the most biologically intriguing, and clinically frustrating, features of cryptosporidiosis is its resistance to antimicrobial drugs. Unlike many of its relatives (*Toxoplasma gondii*, *Eimeria*, and *Plasmodium*), there is no effective anti microbial treatment available for cryptosporidiosis in man and animals, probably because it establishes a unique compartment (parasitophorous vacuole) within the host cell, which is morphologically different from other related parasites. This vacuole may shelter the parasite from antimicrobial drugs (Griffiths., 1998).

Treatment options of cryptosporidiosis depend largely on the immune status of the host, (Griffiths, 1998). Since the disease is self-limiting, in immunocompetent individuals there is no need of specific therapy; however, supportive care with oral fluids and electrolyte replacement due to diarrhoea is beneficial in alleviating the dehydration. In immunocompromised hosts, particularly AIDS patients with CD4 cell counts below 200/mm³, cryptosporidiosis can be life-threatening and must be treated properly. In people with AIDS, the ideal treatment involves partial restoration of immune function with HAART (Highly Active Anti Retroviral Therapy). Several case reports have demonstrated that the resolution of *Cryptosporidial* diarrhoea coincident with a rise in CD4 cell count upon initiation of antiretroviral therapy (Carr *et al.*, 1998; Xian-Ming and LaRusso, 1999).

In AIDS patients in addition to HAART therapy, a number of antibiotics such as paromomycin, nitazoxanide, azithromycin that have partial efficacy against cryptosporidiosis are available on trial (Fichtenbaum *et al.*, 1993; Xian-Ming and LaRusso, 1999). Of these Paromomycin is the only agent so far that has been found to have efficacy in animals and humans in the treatment of intestinal cryptosporidiosis (Xian-Ming and LaRusso, 1999). Recently, Nitazoxanide became an effective antibiotic against cryptosporidiosis in immunocompetent and probably in immunocompromised patients (Xian-Ming and LaRusso, 1999).

1.2. *Giardia lamblia*

Giardia lamblia (also known as *Giardia duodenalis* or *G. intestinalis*) is a unicellular, flagellated intestinal protozoan parasite of humans isolated worldwide and is ranked among the top 10 parasites of man (Wolfe 1992; Farthing, 1997). The organism has been found in more than 40 animal species (Meyer, 1994). Nowadays there are five species of *Giardia* known to infect different animal species: *Giardia lamblia* in mammals including man, rodents, reptiles and possibly birds; *Giardia muris* in rodents, birds and reptiles, *G. agilis* in amphibians (Filice, 1952); *G. ardae* in the great blue heron (Erlandsen *et al.*, 1990); and *G. psittaci* in the budgerigar (Erlandsen and Bemrick, 1987).

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 (Figure 2).

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 encystation.

Encystation 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 encystation 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 lamblia is the most common Protozoan intestinal parasite isolated worldwide as causative agents of diarrhoea. Epidemiological studies suggest that the parasite is responsible for about 5% of acute diarrhoea and 20% of chronic diarrhoeal illness in the

world (Thompson *et al.*, 1993). The incidence of diarrhoea associated with *Giardia* is generally higher in developing countries in Africa, Asia, South and Central America where access to clean water and basic sanitation is lacking. The prevalence for *Giardia lamblia* in developed countries is around 2-5% but in developing countries may be up to 20-30% (Thielman and Guerrant, 1998). Nearly all children in developing countries will acquire *Giardia* at some point in their childhood. In developed countries such as Western Europe and the United States, *giardia* infection is associated with ingestion of contaminated water, person-to-person contact, recent foreign travel, and recreational swimming (NATHNAC, 2004)

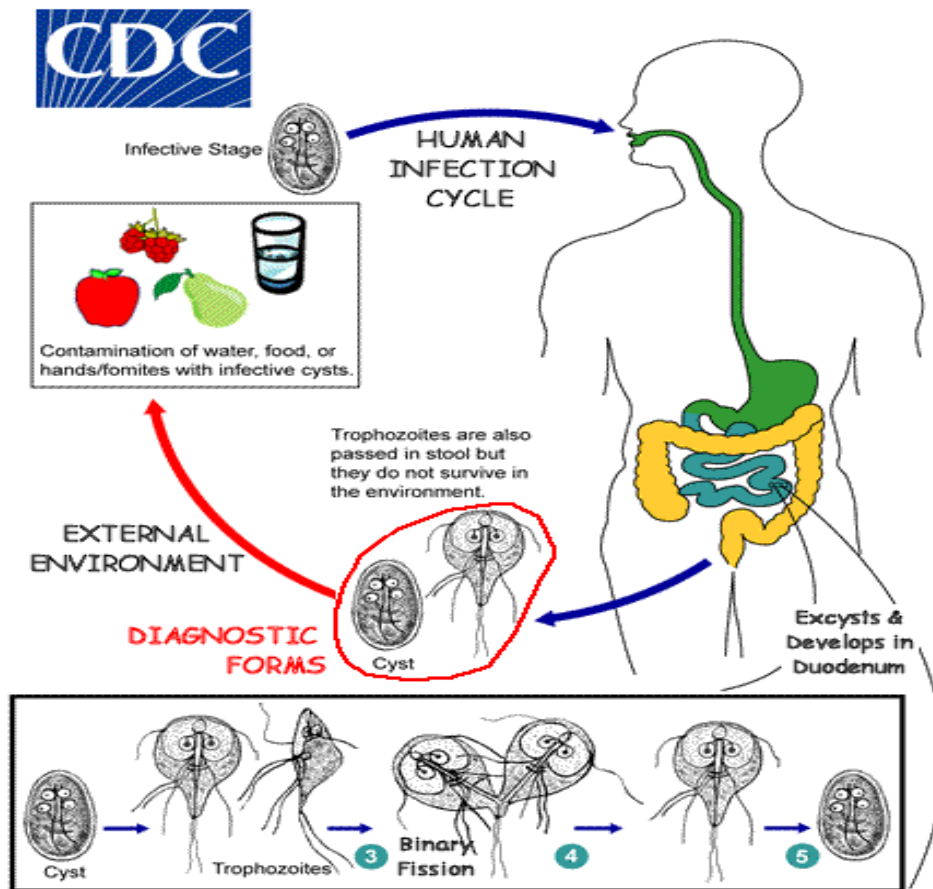


Figure 2. Life cycle of *Giardia lamblia*

Source: - <http://www.dpd.cdc.gov/dpdx>

Humans are the principal reservoir of infection, but *Giardia lamblia* can infect dogs, cats, beavers, and other animals. These animals can contaminate water with feces containing

cysts that are infectious for humans. The transmission of *Giardia* to humans is dependent upon the ingestion of cysts excreted in the feces of infected persons or animals. The principal mode of transmission to humans appears to be person-to person, although indirect transmission from contaminated water and food, originating from humans and animals has been described. Although Animal sources of *Giardia* are common, except for *G. lamblia*, the ability of *Giardia* species found in non-human sources to cause human illness is unclear (Thompson, 1994).

There are additional sources of water contamination, including domestic and wild animals (Steiner *et al.*, 1997). Infected cattles have been suspected to be an important source of human giardiasis (Warburton *et al.*, 1994). Concentrations of *Giardia* cysts in water have been found to be significantly associated with the prevalence of the *Giardia* in animals (Ongerth *et al.*, 1995). Moreover, significantly higher concentrations of *giardia* cysts have been reported in watersheds accessible to cattle for drinking than in those with no cattle access (Ong *et al.*, 1996).

Untreated surface water is a recognized vehicle of *Giardia* transmission (Sykora *et al.*, 1988). Since concentrations of chlorine used in drinking water treatment do not kill *Giardia* cysts, municipal water or inadequately treated sewage has been linked to outbreaks of giardiasis. Well water could also be a source for *Giardia* (Payment, 1999).

Food that may have been washed in contaminated water, or prepared by an infected person can transmit the disease (Petersen *et al.*, 1988; Mintz *et al.*, 1993). Although *Giardia* does not reproduce in food, the small infective dose (< 10 cysts) suggests that it could be easily transmitted by fecally contaminated food (Petersen *et al.*, 1988).

As in any parasitic infections, host parasite interaction is the initial steps in the pathogenesis of giardiasis. In this interaction, first the *Giardia* trophozoites attach to the cell surface of villi by means of a disk on their posterior or ventral surface. Lectin, a protein on the trophozoite lining, recognizes specific receptors on the intestinal cell and may be partly responsible for the tight attachment between the parasite and the villi. Following attachment of trophozoites, there will be major structural and functional

abnormalities in the small intestine. Some of these abnormalities includes mucosal damage as a result of mechanical obstruction or blockage of the intestine by a large number of parasites, the release of cytopathic substances such as thiol proteinases and lectins from *Giardia* trophozoites, the stimulation of a host immune response with release of cytokines and mucosal inflammation and deconjugation of bile salts (Heyworth, 1992; Djamiatun and Faubert, 1998).

Although symptomatic infection causes a broad spectrum of clinical manifestations, *Giardia* results in asymptomatic carrier state in a majority of cases. The asymptomatic infections are most common in children and people with prior exposure to a source of infection (Ortega and Adam, 1997). When the disease occurs, it can result in occasional days of acute watery diarrhoea with abdominal pain, or patients may experience a protracted, intermittent, often debilitating disease, which is characterized by passage of foul-smelling stools associated with flatulence, abdominal distention, and anorexia.

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 or by zinc sulfate flotation, and trichrome-stained polyvinyl alcohol (PVA)-preserved stools (Broke, 1977).

Immunofluorescence (IF) and enzyme-linked immunosorbent assay (ELISA) assays have been developed for detection of *Giardia* antigens in the stools. Both assays are based on the use of *Giardia*-specific polyclonal or monoclonal antibodies. Both are much easier and require less experience than microscopy besides it permit large numbers of stool samples to be tested rapidly and may reduce technician's time and bias among observers (Addiss *et al.*, 1991).

There are effective treatments against *Giardia*, still preventive measures are of great importance. As in most diarrhoea-causing agents, disease outbreaks can also be prevented by: testing of purified and unpurified water to check for the presence of cysts of the

parasites, boiling water intended for consumption, thoroughly washing hands before handling food, maintaining good personal cleanliness, properly disposing of fecal material and information dissemination through print media to educate the public regarding the dangers of giardiasis (Backer, 2000).

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). In developed countries, unlike the developing countries, all patients who have *G lamblia* in stools should be treated (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). Of the common anti-*Giardia* therapeutics, Furazolidone is the only one available in a liquid suspension and is an important therapeutic agent worldwide and it has been widely used in pediatric populations (Lerman and Walker, 1982). Paromomycin has been proposed as a treatment for *G. lamblia* in resistant infections and during pregnancy (Kreutner *et al.*, 1981).

1.3. *Entamoeba histolytica/dispar*

Entamoeba histolytica is an enteric protozoan parasite, belongs to the pseudopod forming protozoan with the sub phylum Sarcodina (Levinne *et al.*, 1980). 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 where as, 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 faecally contaminated food or water (Martinez-Palomo and Espinosa-Cantellano, 1998).

Infection usually occurs by ingestion of water or food contaminated by faecal matter (figure 3). 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).

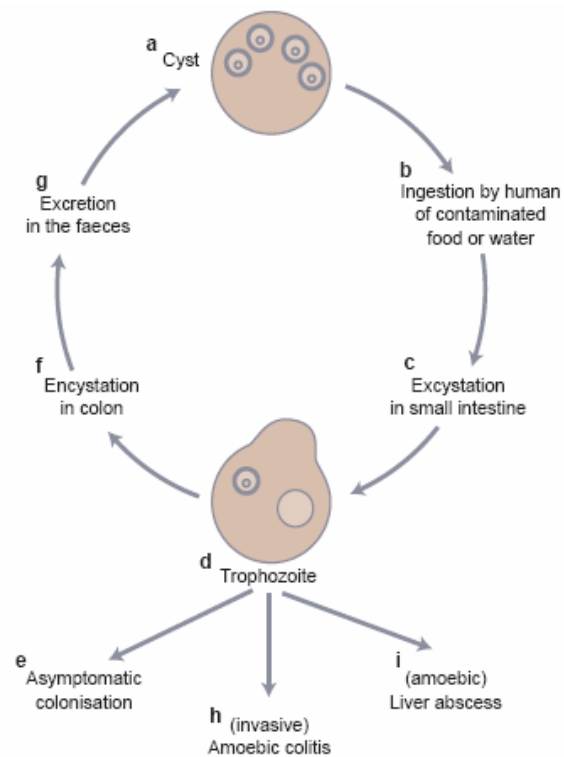


Figure 3. Life Cycle of *Entamoeba histolytica/dispar*.

Source: Expert Reviews in Molecular Medicine ©1999 Cambridge University Press

Amebiasis is one of the health issues in many developing countries. It is the second most common cause of 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. histolytica* (Martinez-Palomo and Espinosa-Cantellano, 1998).

The prevalence of amebiasis varies with the population of individuals affected, differing between countries and between areas with different socioeconomic conditions. Sometimes up to 50% of the population is affected in regions with poor sanitary conditions. 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. For example, in Mexico, 11% of the tested population aged 5 to 9 years was infected with amoeba (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).

Haque, *et al.* (2003) described amoebic colitis results when the trophozoite penetrates the intestinal mucous layer. Invasion is mediated by the killing of epithelial cells, neutrophils, and lymphocytes by trophozoites amoebapore, a 5-kD pore-forming protein. During chronic infection, *E. histolytica* cysteine proteinases degrade secretory IgA and

serum IgG, possibly protecting amebae from opsonization. Finally, amebae appear to suppress both the macrophage and antigen presenting cell.

The wide spectrum of intestinal infection ranges from asymptomatic to transient intestinal inflammation. In up to 90% of *E. histolytica* infections, the symptoms are absent or very mild (Jackson *et al.*, 1985). Although people can be asymptotically colonized with *E. histolytica*, they should be treated. Otherwise, the cyst carriers may be dangerous environmentally or may develop amoebic colitis (dysentery) after a period of months (Gathiram and Jackson, 1987). It was thought that signs and symptoms of invasive amebiasis develop in approximately 10% of the infected population. Symptoms commonly attributed to *E. histolytica* colitis or dysentery is abdominal pain or tenderness and diarrhea (watery, bloody, or mucous).

Extraintestinal amebiasis is brought about by spread of trophozoites and can infect the liver, brain, lung, skin and rarely genitourinary structures but amoebic liver abscess is by far the most common complication. Amebic liver abscess is associated with fever, abdominal pain and weight loss in most patients. It occurs more commonly in adults than in children (Adams and MacLeod, 1977).

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).

Antigen detection ELISAs and PCR are the two methods that can distinguish accurately between infection with *E. histolytica* and *E. dispar*. They are replacing microscopy for both clinical and research purposes. ELISA is among the most popular methods used in

diagnostic laboratories throughout the world (Gonzalez-Ruiz *et al.*, 1994). PCR method is another molecular approach for the diagnosis of intestinal amebiasis. Since it is costly, it may not yet be well suited for use in developing countries where amebiasis is endemic because of the specialized skills and equipment that it requires (Haque *et al.*, 1998).

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. Noninvasive infections may be treated with paromomycin whereas invasive amebiasis (e.g., colitis, liver abscess) should be treated particularly with metronidazole (Powell *et al.*, 1966).

1.4. Giardiasis, cryptosporidiosis and amebiasis in Ethiopia

Over 60% of the communicable diseases in Ethiopia are due to poor environmental health conditions arising from unsafe and inadequate water supply and poor hygienic and sanitation practices (Abebe, 1986). 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 under five years is due to diarrhea in which water related diseases occupy a high proportion.

As a result of low level standards of living, poor environmental sanitations and ignorance of simple health promoting factors, intestinal parasitism is very high. Even though the prevalence of individual parasites varies in different parts of the country, *Ascaris lumbricoides* is the most prevalent intestinal parasites (Tedla and Ayele, 1986). In a study

conducted in South Western Ethiopia, the prevalence of Giardiasis was 13.7% though the rate is much lower than *Ascaris lumbricoides* (Ali *et al.*, 1999).

Intestinal parasites including *Giardia*, *Cryptosporidium* and amoeba are widely distributed in the country (McConnel and Armstrong, 1976). Reports from different parts of Ethiopia showed different prevalence rate of cryptosporidiosis, giardiasis and amebiasis. The prevalence of *Cryptosporidium* infection in children with diarrhoea ranged from 3.3 percent in Jimma, 5.6 percent in Addis Ababa to 9 percent in North-western Ethiopia (Mersha and Tiruneh, 1992; Assefa *et al.*, 1996; Gebru and Girma, 2000). In Ethiopia the prevalence of cryptosporidiosis in HIV / AIDS patients reached up to 25.9% (Fisseha, *et al.*, 1998, Endeshaw *et al.*, 2004).

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). The prevalence of amebiasis as high as 55% was reported in a survey conducted among Saysay population, in Blue Nile gorge (Torrey, 1965). 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).

Recent report indicate that the prevalence of *Cryptosporidium parvum* and *Giardia lamblia* among diarrhoea patients referred to EHNRI (Ethiopian Health and Nutrition Research Institute) were 20.8% and 8.6% respectively (Endeshaw *et al.*, 2004).

Water and health in Ethiopia

The WHO statistics show that about 80 percent of all diseases in the developing countries are related to unsafe water supply and inadequate sanitation, resulting in high infant mortality, low life expectancy and poor quality of life. Thus, access to safe drinking water and sanitation has proven to be essential to good health. Combined water supply, sanitation, hygienic practices and health/hygiene education are associated with greater health benefits and reduce diarrhoea incidence by 35-50 percent (Raza, 2003).

In Ethiopia, water supply and sanitation situation is inadequate. Most of the populations in urban and rural areas do not have access to safe and adequate water supplies and sanitation facilities. Regarding food, water and personal hygiene, only few households show sufficient understanding of environmental sanitation or hygienic practices. As a result, three-fourths of the health problems in Ethiopia are due to communicable diseases attributable to unsafe/inadequate water supply, and unhygienic/unsanitary waste management, particularly excreta (UN-WATER/WWAP- National Water Development Report for Ethiopia, 2004).

Diarrhoeal diseases caused by improper management of water and sanitation are among the major causes of infant and child morbidity and mortality. Water and sanitation programs have a direct bearing on the prevalence of diarrhoeal diseases in the population. Water and sanitation projects, which are properly designed and implemented, have the potential of reducing diarrhoea-caused deaths by 55 percent. The combination of safe water supply, sanitation facilities and hygienic practices has demonstrated a potential in contributing to a remarkable reduction in mortality (UN-WATER/WWAP- National Water Development Report for Ethiopia, 2004).

Although significant water resources are available in the country, the status of water supply coverage is very low. The communities have poor access to supplies of safe and adequate water particularly in the rural areas. It was estimated that only 32 percent of the total population, 80 per cent of urban population and 24 per cent of the rural population have reasonable access to adequate water supply. According to the specification of NPA

(National Program of Action for Women and Children), adequate water supply is defined as 20 liters per capita per day made available within a range of one to two km. from the dwelling. Estimates for average per capita per day water consumption vary between 10 and 20 liters per day in some areas and is as low as 3-4 liters per capita per day in most rural areas of Ethiopia. Women and children particularly girls have to fetch water, often walking for 3-8 km. from their dwellings (UN-WATER/WWAP- National Water Development Report for Ethiopia, 2004).

The shortage of sufficient quantities of clean water crucially weaken the ability of most rural populations to engage in appropriate personal, food and environmental hygienic practices which could greatly assist in reducing infectious diseases. The inaccessibility of protected, improved water supplies to about 80 percent of the rural population and 20 percent of the urban dwellers clearly indicates that the health and well-being of the population in general and that of women and children (make up nearly 75 percent of the population) in particular, is at great risk to multitude of water-borne or water related disease. Because of the lack of an effective monitoring and surveillance system and countrywide baseline survey, limited information on disease prevalence reported indicates that water-borne or water-related diseases are among the major causes of sickness and death. Among the major water related diseases, diarrhoea alone is accountable for 46 per cent of under-five child mortality. Women and children particularly girls are the main water carriers and having regular contacts with contaminated water. They are the segment of the population most vulnerable to water related diseases. Control or prevention of infectious, water related diseases will mainly depend on safe disposal of all human wastes, provision of safe and adequate water, community health/hygiene education and safe socioeconomic development project undertakings (UN-WATER/WWAP- National Water Development Report for Ethiopia, 2004).

Statement of the problem

Several community-wide outbreaks of cryptosporidiosis, giardiasis and amebiasis have been linked to drinking municipal water or other water sources contaminated with these

parasites (Stevens and Adam, 2004). In most parts of Ethiopia, people consume unprotected water from different sources. In this respect in many villages in rural parts of Ethiopia, the population is forced to use unprotected water from river stream, irrigation canals, ponds, shallow well, water harvesting pond, etc. In such area where people use water from different sources, the possibility of infection with water born disease such as cryptosporidiosis, giardiasis and amebiasis is extremely high. Although the infection can appear at all age level, it is more common among young children (Current and Garcia, 1991). These and other intestinal protozoa infections are commonly associated to climatic factors, sanitary conditions and socioeconomic factors. In addition, there is also a marked seasonality in the onset of illness due to intestinal protozoan infections (Soriano *et al.*, 2001; Gamboa *et al.*, 2003).

Although a number of studies have been conducted on the distribution and prevalence of intestinal parasites in different parts of Ethiopia (Mcconnel and Armstrong, 1976; Seyoum *et al.*, 1981; Tedla and Ayele, 1986), there are still several localities like the present study sites for which epidemiological information is not available especially among children.

Previous water quality analysis in Legedini Dire-Dawa indicated the presence of *C. parvum*, *G. lamblia* and other bacteria such as *E. coli* in different water sources. In this water quality analysis, the frequency of *Giardia* cyst and *Cryptosporidium* oocyst in open wells and ponds (Selela and Hado Sere villages) was higher than the closed tap and spring system (Ajo and Kora villages) (Scheelbeek, 2005). The present study was conducted in light of the findings of water quality analysis, to determine the prevalence of the two parasites among children using those water sources in Legedini. In Legebira and Adada, the water source and the sanitary conditions the community practices were comparable to that of Legedini. In addition, in all the present study sites the laboratory technicians report more of the intestinal protozoan than Helminths. Beside no assessment of Cryptosporidiosis prevalence among children was made before the present study in those study areas.

2. Objective

General objective:

To assess the prevalence of *Giardia lamblia*, *Cryptosporidium parvum* and *Entamoeba histolytica/dispar* infection among children using different water sources in three rural communities in Dire-Dawa, Eastern Ethiopia (Legedini, Adada and Legebira).

Specific objectives:

1. To determine the wet and dry season sampling variations in prevalence of cryptosporidiosis, giardiasis and amebiasis among children in Dire-Dawa (Legedini, Legebira and Adada).
2. To compare the prevalence of cryptosporidiosis, giardiasis and amebiasis among children according to the use of different water sources between the three sites.
3. To determine the prevalence of cryptosporidiosis, giardiasis and amebiasis among children at different age groups and sex.

3. Materials and Methods

3.1. The Study areas

Dire-Dawa is located in Eastern parts of Ethiopia, which is 508 km away from Addis Ababa. The present study was conducted between November 2005 and April 2006 in three selected areas of Dire-Dawa administrative region: Legedini, Adada and Legebira (Figure 7). All the three areas receive an average monthly rainfall of 55.71mm (Figure 4) and have bimodal pattern; the big rains occurring from July to September, and the small rains from March to April. The monthly average maximum and minimum temperatures are 32.4°C and 19.1°C, respectively (Figure 5) and the mean annual relative humidity is 48.2% (Figure 6) (source from the National Meteorological Services Agency). All the study areas are mountainous and are not suitable for farming activities though the people use it for farming.

Though Farmers in this study area are engaged in crop- livestock mixed agriculture, they are not food self sufficient and most of the time they are dependent on donation from government and other donor organizations. The major crops cultivated by the farmers are maize and sorghum. The livestock owned by the people are mainly camels, cows, donkeys, oxen, goats and sheep.

In each of the study sites some village uses water from protected sources such as spring, boreholes, deep and shallow protected well, hand-dug wells, and others use from unprotected water sources such as surface water, river, seepage, unprotected well. The common problems of the three study sites are inadequacy of clean drinking water, lack of water for agricultural and household activities and insufficient sanitary facilities. As a result, waterborne and hygiene related diseases occur frequently.

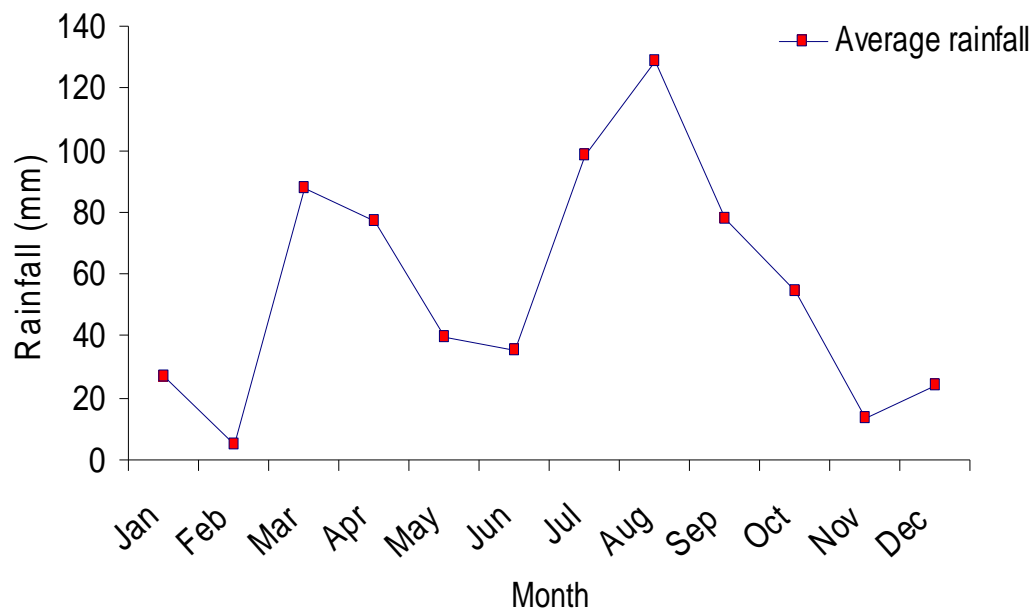


Figure 4. Monthly average rainfall (mm) in Dire-Dawa administrative region (1996-2005)

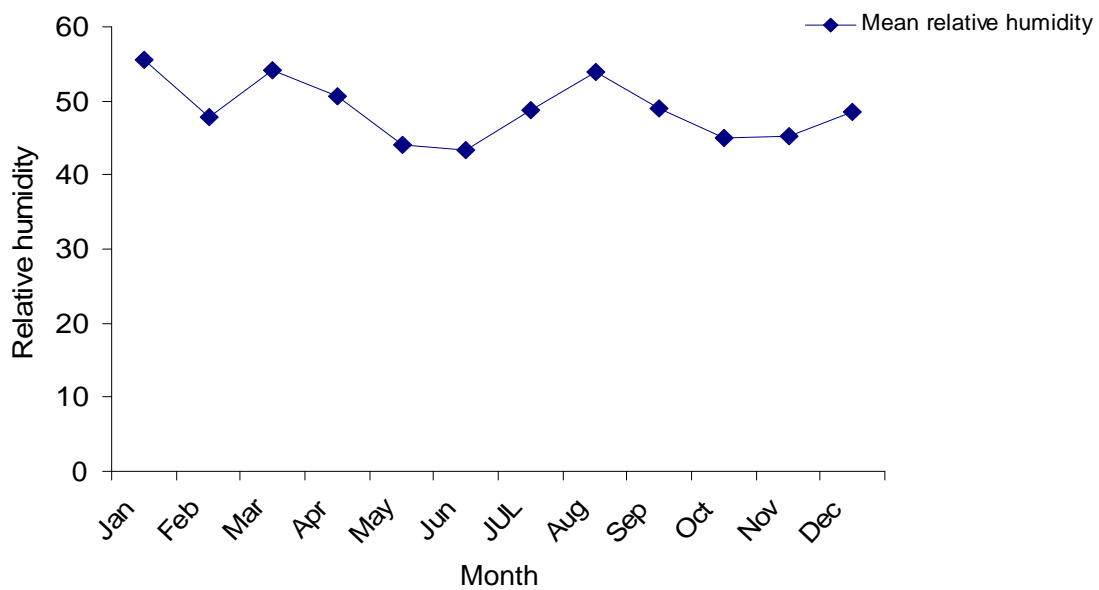


Figure 5. Monthly average relative humidity in Dire-Dawa administrative region (1996-2005)

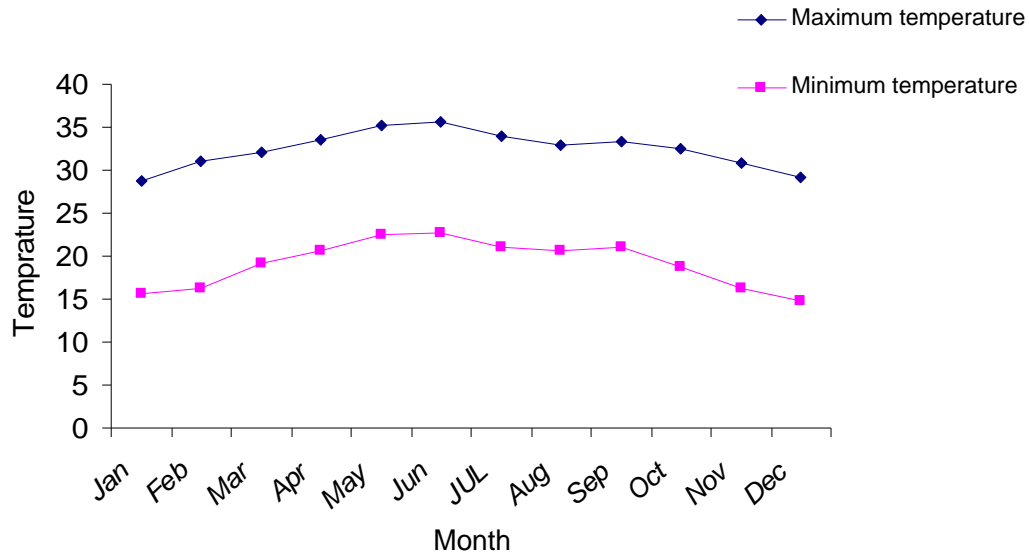


Figure 6. Monthly average maximum and minimum temperatures (°C) in Dire-Dawa administrative region (1996-2005)

Legedini

Legedini is located 28 km east of Dire-Dawa city, at 09°37'57.3" N latitude and 042°02'44"E longitude and an altitude of 1100-1600m a.s.l. The area has 9 villages with a total population of 4500-5000. Of these villages Ajo, Halo, Iddo and Iddo Bolo, Kora, Konya uses water from a protected source and *Selela*, Hado Sere, Kore Chafe and Lallo from unprotected source. All of the inhabitants are Muslim Gorgora.

Adada

Adada is located 18km east of Dire-Dawa city. Geographically the area is located at 09°32'53.6"N latitude and 41°56'23.7"E longitude and an altitude of 1506m a.s.l. The area has 15 villages with a total population of 14,000. Only Awale, Gebro, Afuretu Kebele, Hamessa and Gudora use unprotected water sources and the rest Huri, Negeye, Berento, Elimo, Bajje, Adami, Kulu, Dema and Chore use water from protected sources. All of the inhabitants are Muslim Oromo.

Legebira

Geographically Legebira is located at $09^{\circ}31'23.4''\text{N}$ latitude and $41^{\circ}57'16.5''\text{E}$ longitude with an altitude of 1646m a.s.l. which is at 15km east of Dire-Dawa city. The area has 6 villages with a total population of 2500-3500. Four villages Bira, Horro, Ware and Rebena use protected water sources and Shenno and Abdure use from unprotected water sources. All of the inhabitants are Muslim Oromo.

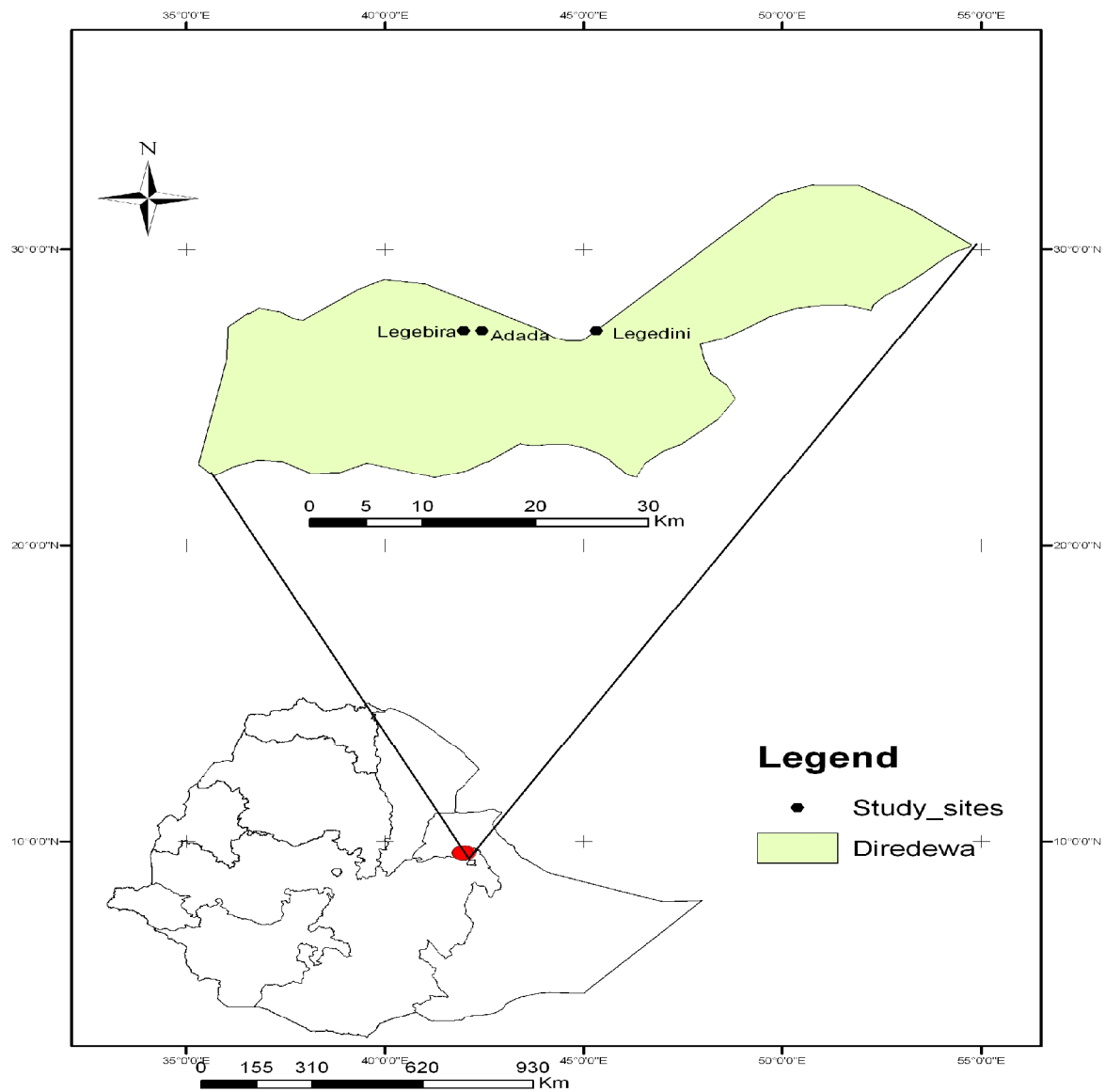


Figure 7. Map of Ethiopia showing the location of the study areas

3.2. The Study population

A total of 1894 children, 655 from Legedini, 628 from Adada and 611 from Legebira were examined at two different seasons (dry and wet). All children are below 14 years old and were selected from different educational background and from different water sources.

In the estimation of the sample size, previous works were considered for the study and were calculated by EPI-Info software Version 6.04. A simple random sampling method was employed in the selection of households in both protected and unprotected water sources.

3.3. Stool collection and process

A single fresh stool was collected with a labeled cup from consulted study subject i.e. 950 during dry season (November 2005) and 944 stool sample during wet season (May 2006). A portion of stool was preserved with SAF (15g sodium acetate, 20ml glacial acetic acid, 40ml formalin and 925ml distilled water) in a proportion of 1 g of stool in 3 ml of SAF. The remaining part was processed using the following methods (Ortega and Adam, 1997).

3.3.1. Direct wet mount method

The direct wet mount with saline (0.85% NaCl solution) was prepared in the field and observed for motile intestinal parasites and trophozoites under light microscope at 10X and 40x magnification. Lugol's iodine staining was also done to observe cysts of the intestinal parasites.

3.3.2. Concentration method

A portion of preserved stool samples were processed by formalin-ether concentration method at EHNRI as described by Ritchie (1948) with some modification. The preserved stool sample was sieved with cotton gauze and transferred to 15ml centrifuge tube. Then 8ml of 10% formalin and 3ml of diethyl ether was added and centrifuges for 2 minutes at

2000rpm. The supernatant was decanted and the residues were transferred to microscope slides and observed under light microscope at 100X and 400 X magnifications for the presence of cysts and ova of the parasites.

3.3.3. Modified Ziehl-Neelsen method

For the detection of *Cryptosporidium parvum* and *Isospora belli* oocysts, Ziehl-Neelsen method was used. 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 fuchsin for 30 minutes. The slides then washed with tap water and decolorized with acid alcohol (1ml HCl and 99ml of 96% ethanol) for 1-3 minutes. After washing the slides with tap water, it was counter stained in methylene blue for another 1 minute. Finally the slides were washed in tap water and allowed to air dry. The slides were then observed under light microscope with X1000 magnification (Garcia *et al.*, 1993; Asefa *et al.*, 1996; Endeshaw *et al.*, 2004). Each slide was observed for 10 minutes to decide whether it is negative or positive.

3.4. Ethical clearance

First the study was reviewed and approved by the ethical committee of biology department, Addis Ababa University. Ethical considerations were addressed by treating positive intestinal protozoa and helminths using the standard drug. Those individuals with positive results were treated with the required treatment and the drugs were administered by the sites health officer. The questionnaires concerning the prevalence study were filled during sample collection. Written consent was sought from parents or guardians of the selected study children. Beside parents or caregivers of children were asked to fill the questionnaire and assist those children during sample collection.

3.5. Data analysis

Statistical analyses were performed with SPSS Software version 13. The data were analyzed by use of Chi square test (χ^2). Values were considered to be statistically significant when the p-value obtained was less than 0.05.

4. Results

In the present study, microscopic stool sample examination by direct, formol ether concentration and Modified Ziehl-Neelsen techniques showed that infections with various intestinal helminths and protozoan parasites were common in the study areas among children. The prevalence of infection with different intestinal helminths and protozoan parasites is shown in Table 2.

Table 2. Prevalence of intestinal parasites among children in Legedini, Adada and Legebira Dire-Dawa administrative region (November 2005 – May 2006).

Parasite identified	Legedini No. observed (%) N=655	Adada No. observed (%) N=628	Legebira No. observed (%) N=611	Total No. observed (%) N= 1864
Helminthes				
<i>Ascaris lumbricoides</i>	10 (1.5)	2 (0.3)	9 (1.5)	21 (1.1)
<i>Schistosoma mansoni</i>	3 (0.5)	10 (1.6)	6 (1)	19 (1)
<i>Enterobious vermicularis</i>	1 (0.2)	7 (1.1)	3(0.5)	11 (0.6)
<i>Strongyloides stercoralis</i>	5 (0.8)	1 (0.2)	2 (0.3)	8 (0.4)
<i>Hymenolepis nana</i>	–	19 (3)	20 (3.3)	39 (2.1)
<i>Hymenolepis. diminota</i>	1 (0.2)	–	–	1 (0.1)
<i>Taenia species</i>	–	1 (0.2)	2 (0.3)	3 (0.2)
Protozoa				
<i>Giardia lamblia</i>	231 (35.3)	260 (41.4)	228 (37.3)	719 (38)
<i>Cryptosporidium parvum</i>	80 (12.2)	67 (10.7)	78 (12.8)	225 (11.9)
<i>Entamoeba histolytica/dispar</i>	199 (30.4)	232 (36.9)	208 (34)	639 (33.7)
<i>Blastocystis hominis</i>	109 (16.6)	56 (8.9)	68 (11.1)	233 (12.3)
<i>Isospora belli</i>	1 (0.2)	–	–	1 (0.1)
<i>Iodoamoeba butschilii</i>	42 (6.4)	62 (9.9)	77 (12.6)	181 (9.6)
<i>Entamoeba coli</i>	45 (6.9)	39 (6.2)	40 (6.5)	124 (6.5)
<i>Chilomasix mesnelli</i>	23 (3.5)	34 (5.4)	38 (6.2)	95 (5)
<i>Endolimax nana</i>	47 (7.2)	41 (6.5)	41 (6.7)	129 (6.8)

4.1. Comparison of the prevalence of cryptosporidiosis, giardiasis and amebiasis between sites

The dry season sampling infection prevalence of cryptosporidiosis in children using the protected water sources of Legedini, Adada and Legebira was 2.8%, 2% and 1%, respectively, showed no significant difference ($P>0.05$), and the prevalence for the wet season sampling for the same were 18.8%, 10.6% and 23.1% respectively and showed no significant difference ($P>0.05$). Similarly, the dry season sampling infection prevalence of cryptosporidiosis in children using unprotected water sources of Legedini, Adada and Legebira was 4.1%, 5.5% and 2.7%, respectively. The prevalence of the wet season sampling was 23.6%, 22.1% and 25.8%, respectively. In both cases, the difference was not statistically significant ($P>0.05$) (Table 3).

The prevalence of giardiasis during the dry season sampling in children using the protected water sources of Legedini, Adada and Legebira was 25.8%, 27.3% and 31.2%, whereas the wet season sampling infection prevalence rate for the same were 39.8%, 41.5% and 45.1% ,respectively. In both cases no statistical significant difference ($P>0.05$) was observed. Similarly the prevalence of giardiasis during the dry season sampling in Legedini, Adada and Legebira was 32.4%, 39.4% and 27.3%, respectively in children using unprotected water sources and the wet season prevalence was 43.9%, 54.2% and 45%, respectively. The results showed that there was no significant difference ($P>0.05$) in infection prevalence of giardiasis among children using unprotected water sources between the study sites both in dry and wet season sampling (Table 3).

The dry season sampling prevalence of amebiasis among children using protected water sources of Legedini, Adada and Legebira was 21.3%, 23.3% and 25.6%, respectively. Similarly the prevalence of amebiasis among children using unprotected water sources during the dry season was 25.7%, 33.3% and 25.5% in Legedini, Adada and Legebira respectively. In both cases no statistical significant difference ($P>0.05$) was observed. During the wet season sampling the prevalence of amebiasis among children using protected water sources was 33.1% in Legedini, 35% in Adada and 43.4% in Legebira. Similarly the wet season prevalence among children using unprotected water sources was

42.6%, 52.1% and 41.7% in legedini, Adada and Legebira respectively. This result showed no statistical significant difference ($P > 0.05$) between the three sites (Table 3).

In the present study, single parasite infection had the highest prevalence followed by double and triple. Among the double parasitic infections *G. lamblia* and *E. histolytica/dispar* comprised the highest prevalence. Similarly in the three parasitic infections, *G. lamblia*, *C. parvum* and *E. histolytica/dispar* consisted of the highest prevalence.

4.2. Prevalence of cryptosporidiosis, giardiasis and amebiasis within each site

4.2.1. Legedini

Out of the 655 fecal samples examined (326 during the dry and 329 during the wet season), 12.2%, 35.3% and 30.4% were found positive for cryptosporidiosis, giardiasis and amebiasis respectively. The prevalence of cryptosporidiosis in dry (3.4%) and wet season sampling (21%); giardiasis in dry (28.8%) and in wet season (41.6%) sampling and amebiasis in dry (23.3%) and in wet season (37.4%) sampling showed highly significant difference ($P < 0.01$) (Table 4).

The prevalence of cryptosporidiosis between wet (18.8%) and dry (2.8%) season sampling among children using protected water sources showed highly significant difference ($P < 0.01$). Similarly there was a highly significant difference in infection prevalence of cryptosporidiosis between wet (23.6%) and dry (4.1%) season sampling in children using unprotected water sources. Among children using protected water source 25.8% (during dry) and 39.9 (during the wet season) for giardiasis and 21.3% in dry and 33.1% in wet season sampling for Amebiasis) and unprotected water source (32.4% in dry and 43.3% in wet season sampling for giardiasis and 25.7% in dry and 42.6% in wet season sampling for amebiasis), there was a statistical significant difference ($P < 0.05$) in infection prevalence between dry and wet season sampling (Table 5).

Generally the prevalence of cryptosporidiosis among children using protected water sources was 10.9% and in unprotected water sources was 13.9%. Similarly for the children using protected and unprotected water sources, the infection prevalence was 32.9% and 38.2% respectively for giardiasis and 27.3% and 34.1% respectively for amebiasis. In all cases no statistical significant difference ($P>0.05$) was observed (Table 6).

Analysis of the prevalence of cryptosporidiosis finding in relation to water sources revealed that no statistical significant difference ($P>0.05$) between children using protected (2.8%) and unprotected (4.1%) water sources during the dry season sampling. Similarly there was no statistical significant difference ($P>0.05$) in infection prevalence of cryptosporidiosis between children using protected (18.8%) and unprotected (23.6%) water sources during the wet season sampling. The dry season sampling prevalence between children using protected and unprotected water sources was 25.8% and 32.4%, respectively for giardiasis and 21.3% and 25.7%, respectively for amebiasis. In addition, the wet season sampling picture in children was 39.8% for protected and 43.9% for unprotected water sources for giardiasis and 33.1% for protected and 42.6% for unprotected for amebiasis. In all cases the difference were not statistically significant ($P>0.05$) (Table 7).

In addition the prevalence of the three parasites in the respective villages of Legedini showed higher total prevalence in Sellela and Hado Sere villages which have protected water sources and lower total prevalence in Ajo and Kora villages (unprotected water sources) (Table 8).

There was no statistical difference ($P>0.05$) in prevalence for cryptosporidiosis by sex as well as by age groups. On the contrary, there was a significant difference ($P<0.05$) in prevalence of giardiasis and amebiasis within age groups but not ($P>0.05$) within sex groups (Figure 8. and Figure 9).

Table 3. Prevalence of giardiasis, cryptosporidiosis and amebiasis between sites among children using protected and unprotected water sources during dry and wet seasons sampling within Dire-Dawa administrative region (November 2005 – May 2006).

Sampling Season	water source	Study sites	No. of examined children	Parasite identified								
				<i>Giardia lamblia</i>			<i>Cryptosporidium parvum</i>			<i>Entamoeba histolytica/dispar</i>		
				No. of positive	Prev (%)	P-value	No. of positive	Prev (%)	P-value	No. of positive	Prev (%)	P-value
dry	protected	Legedini	178	46	25.8	0.497 ^{NS}	5	2.8	0.437 ^{NS}	38	21.3	0.618 ^{NS}
		Adada	150	41	27.3		3	2		35	23.3	
		Legebira	199	62	31.2		2	1		51	25.6	
	unprotected	Legedini	148	48	32.4	0.104 ^{NS}	6	4.1	0.541 ^{NS}	38	25.7	0.227 ^{NS}
		Adada	165	65	39.4		9	5.5		55	33.3	
		Legebira	110	30	27.3		3	2.7		28	25.5	
wet	protected	Legedini	181	72	39.8	0.586 ^{NS}	34	18.8	0.021 [*]	60	33.1	0.105 ^{NS}
		Adada	123	51	41.5		13	10.6		43	35	
		Legebira	182	82	45.1		42	23.1		79	43.4	
	unprotected	Legedini	148	65	43.9	0.116 ^{NS}	35	23.6	0.753 ^{NS}	63	42.6	0.109 ^{NS}
		Adada	190	103	54.2		42	22.1		99	52.1	
		Legebira	120	54	45		31	25.8		50	41.7	

* represents significant difference (P< 0.05)

NS represents non significant difference (P>0.05)

P- Value comparing prevalence between the three study sites in dry and wet seasons sampling of children using protected and unprotected water sources

Table 4. Prevalence of giardiasis, amebiasis and cryptosporidiosis at wet and dry seasons sampling in the three sites within Dire-Dawa administrative region (November 2005 –May 2006).

Study sites	Sampling Season	No. of examined children	Parasite identified								
			<i>Giardia lamblia</i>			<i>Cryptosporidium parvum</i>			<i>Entamoeba histolytica/dispar</i>		
			No. of positive	Prevalence (%)	P-value	No. of positive	Prevalence (%)	P-value	No. of positive	Prevalence (%)	P-value
Legedini	Dry	326	94	28.8	0.001*	11	3.4	0.00*	76	23.3	0.00*
	Wet	329	137	41.6		69	21.0		123	37.4	
Adada	Dry	315	106	33.7	0.00*	12	3.8	0.00*	90	28.6	0.00*
	Wet	313	154	49.2		55	17.6		142	45.4	
Legebira	Dry	309	92	29.8	0.00*	5	1.6	0.00*	79	25.6	0.00*
	Wet	302	136	45		73	24.2		129	42.7	

* represents significant difference (P<0.05)

P-value Comparing prevalence in dry and wet season

Table 5. Prevalence of giardiasis, amebiasis and cryptosporidiosis between dry and wet season sampling among children using protected and unprotected water sources in the selected sites of Dire-Dawa administrative region (November 2005 –May 2006).

Study Sites	Water source	sampling Season	No. of examined children	Parasite identified								
				<i>Giardia lamblia</i>			<i>Cryptosporidium parvum</i>			<i>Entamoeba histolytica/dispar</i>		
				No. of positive	Prev (%)	P-value	No. of positive	Prev (%)	P-value	No. of positive	Prev (%)	P-value
Legedini	protected	Dry	178	46	25.8	0.005*	5	2.8	0.00*	38	21.3	0.012*
		Wet	181	72	39.8		34	18.8		60	33.1	
	unprotected	Dry	148	48	32.4	0.042*	6	4.1	0.00*	38	25.7	
		Wet	148	65	43.9		35	23.6		63	42.6	
Adada	protected	Dry	150	41	27.3	0.014*	3	2	0.003*	35	23.3	0.034*
		Wet	123	51	41.5		13	10.6		43	35	
	unprotected	Dry	165	65	39.4	0.005*	9	5.5	0.00*	55	33.3	
		Wet	190	103	54.2		42	22.1		99	52.1	
Legebira	protected	Dry	199	62	31.2	0.005*	2	1	0.00*	51	25.6	0.00*
		Wet	182	82	45.1		42	23.1		79	43.4	
	Unprotected	Dry	110	30	27.3	0.005*	3	2.7	0.00*	28	25.5	
		Wet	120	54	45		31	25.8		50	41.7	

* represents significant difference (P< 0.05)

P- Value comparing prevalence in dry and wet seasons of the protected and unprotected water sources

Table 6. Prevalence of cryptosporidiosis, amebiasis and giardiasis between children using the protected and unprotected water sources in the three sites within Dire-Dawa administrative regions (November 2005 –May 2006).

Study sites	Water source	No. of examined children	Parasite identified								
			<i>Giardia lamblia</i>			<i>Cryptosporidium parvum</i>			<i>Entamoeba histolytica/dispar</i>		
			No. of positive	Prev. (%)	P-value	No. of positive	Prev. (%)	P-value	No. of positive	Prev. (%)	P-value
Legedini	Protected	359	118	32.9	0.157 ^{NS}	39	10.9	0.245 ^{NS}	98	27.3	0.059 ^{NS}
	Unprotected	296	113	38.2		41	13.9		101	34.1	
Adada	Protected	273	92	33.7	0.001*	16	5.9	0.001*	78	28.6	0.00*
	Unprotected	355	168	47.3		51	14.4		154	43.4	
Legebira	Protected	381	144	37.8	0.752 ^{NS}	44	11.5	0.246 ^{NS}	130	34.1	0.958 ^{NS}
	Unprotected	230	84	36.5		34	14.8		78	33.9	

* represents significant difference (P< 0.05)

NS represents non significant difference (P>0.05)

P- Value comparing prevalence in the protected and unprotected water sources

Table 7. Prevalence of giardiasis, cryptosporidiosis and amebiasis between children using protected and unprotected water sources in dry and wet season sampling in the three sites within Dire-Dawa Administrative region (November 2005 –May 2006).

Study Sites	Sampling season	Water source	No. of examined children	Parasite identified									
				<i>Giardia lamblia</i>			<i>Cryptosporidium parvum</i>			<i>Entamoeba histolytica/dispar</i>			
				No. of positive	Prev (%)	P-value	No. of positive	Prev (%)	P-value	No. of positive	Prev (%)	P-value	
Legedini	Dry	Protected	178	46	25.8	0.191 ^{NS}	5	2.8	0.535 ^{NS}	38	21.3	0.358 ^{NS}	
		unprotected	148	48	32.4		6	4.1		38	25.7		
	Wet	Protected	181	72	39.8	0.449 ^{NS}	34	18.8	0.281 ^{NS}	60	33.1		0.079 ^{NS}
		unprotected	148	65	43.9		35	23.6		63	42.6		
Adada	Dry	Protected	150	41	27.3	0.024*	3	2	0.048 *	35	23.3	0.05 *	
		unprotected	165	65	39.4		9	5.5		55	33.3		
	Wet	protected	123	51	41.5	0.028 *	13	10.6	0.009*	43	35		0.003 *
		unprotected	190	103	54.2		42	22.1		99	52.1		
Legebira	Dry	protected	199	62	31.2	0.475 ^{NS}	2	1	0.251 ^{NS}	51	25.6	0.973 ^{NS}	
		unprotected	110	30	27.3		3	2.7		28	25.5		
	Wet	protected	182	82	45.1	0.993 ^{NS}	42	23.1	0.584 ^{NS}	79	43.4		0.765 ^{NS}
		unprotected	120	54	45		31	25.8		50	41.7		

* represents significant difference (P< 0.05)

NS represents non significant difference (P>0.05)

P- Value comparing prevalence in the protected and unprotected water sources during wet and dry season sampling

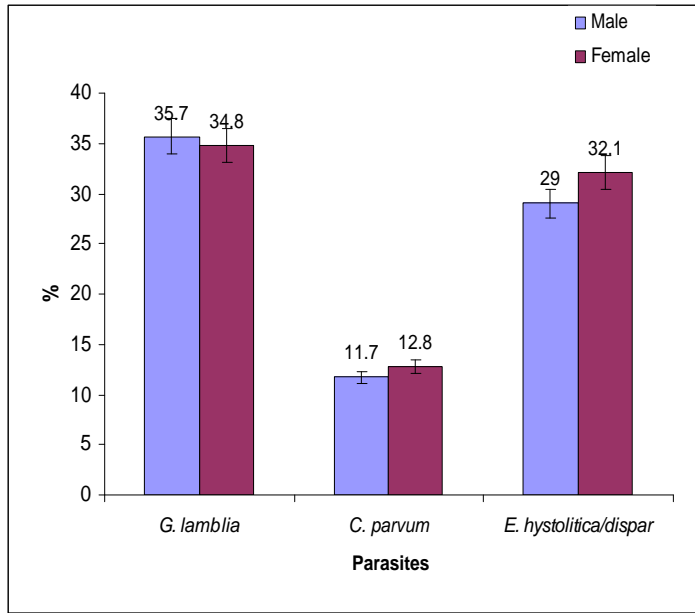


Figure 8. Prevalence of giardiasis, amebiasis and cryptosporidiosis by sex in Legedini (November 2005 – May 2006).

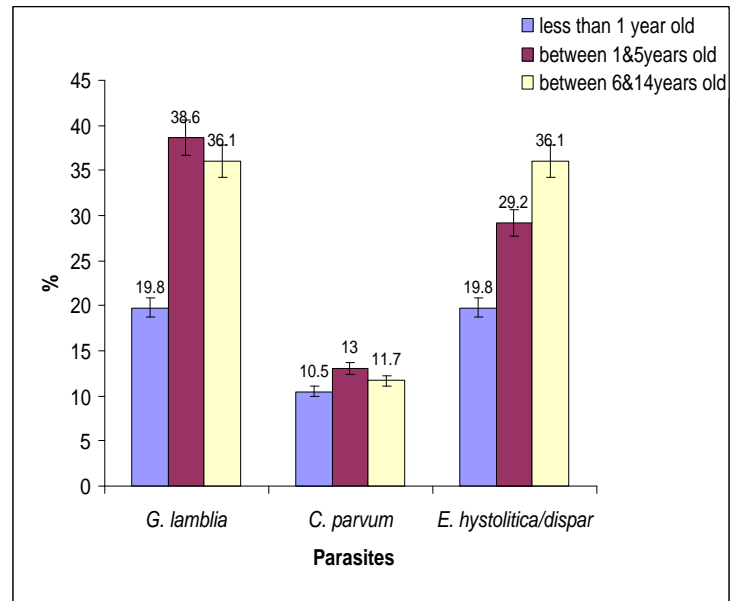


Figure 9. Prevalence of giardiasis, amebiasis and cryptosporidiosis among different age groups in Legedini (November 2005 – May 2006).

Table 8. Prevalence of giardiasis, cryptosporidiosis and amebiasis among villages in Legedini within Dire-Dawa administrative region (November 2005 – May 2006).

Villages in Legedini	No. of examined children	Giardiasis		Cryptosporidiosis		Amebiasis	
		No. of positive	% prevalence	No. of positive	% prevalence	No. of positive	% prevalence
Ajo	200	72	36	32	16	58	29
Kora	159	46	28.9	13	8.2	39	24.5
Total	359	118	32.9	45	12.5	97	27
Sellela	136	48	35.3	16	11.8	41	28.8
Hado Sere	160	65	40.6	28	17.5	46	30.1
Total	296	113	38.2	44	14.9	87	29

4.2.2. Adada

A total of 628 (315 in dry and 313 in wet season) fecal samples of children were examined during this study, of which 10.7% were found positive for cryptosporidiosis, 41.4% for giardiasis and 36.9% for amebiasis. The prevalence of giardiasis in dry and wet season sampling was 33.7 % and 49.2%, respectively and for cryptosporidiosis was 3.8% in dry and 17.6 in wet season sampling. Similarly the dry and wet season finding of amebiasis was 28.6% and 45.4% respectively. In all cases there was significant difference ($P<0.05$) between dry and wet season sampling (Table 4).

The dry and wet season sampling prevalence of cryptosporidiosis among children using protected water sources was (2%) and (10.6%), respectively. The prevalence in children using unprotected water sources was correspondingly 5.5% for dry and 22.1% for wet seasons sampling. In both cases, the difference was statistically significant ($P<0.05$). On the other hand, for both children using the unprotected and protected water sources there was a statistical significant difference in infection prevalence of giardiasis and amebiasis between dry and wet season sampling ($P<0.05$) (Table 5).

In general the prevalence of giardiasis between children using protected and unprotected water sources was 33.7% and 47.3% respectively. Similarly, for children using the protected and unprotected water sources, the infection prevalence of cryptosporidiosis was 5.9% and 14.4% respectively and of amebiasis was 28.6% and 43.4% respectively. In all cases, a statistical significant difference was established (Table 6).

The research result in relation to water sources suggested that there was a significant difference ($P<0.05$) in infection prevalence of cryptosporidiosis, giardiasis and amebiasis between children using protected (2% for cryptosporidiosis, 27.3% for giardiasis and 23.3% for amebiasis) and unprotected (5.5% cryptosporidiosis, 39.4% for giardiasis and 33.3% for amebiasis) water sources during the dry season sampling. The wet season sampling prevalence of cryptosporidiosis in children using the protected (10.6%) and unprotected (22.1%) water sources showed significant difference ($P<0.05$). Similarly, the prevalence of giardiasis between children using protected (41.5%) and unprotected (54.2%) water sources, prevalence of

amebiasis among children using protected (35%) and unprotected (52.1%) water sources during the wet season sampling showed significant difference ($P < 0.05$) (Table 7).

The prevalence of cryptosporidiosis by age and by sex group showed no difference ($P > 0.05$). On the contrary there was a statistical difference ($P < 0.05$) in prevalence of giardiasis and amebiasis between age groups but not ($P > 0.05$) between sex group (Figure 10. and Figure 11).

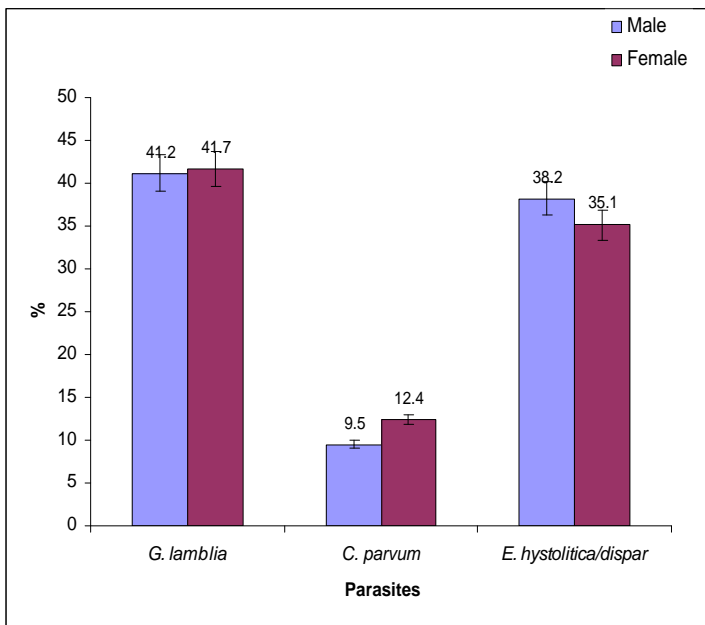


Figure 10. Prevalence of giardiasis, amebiasis and cryptosporidiosis by sex in Adada (November 2005 – May 2006).

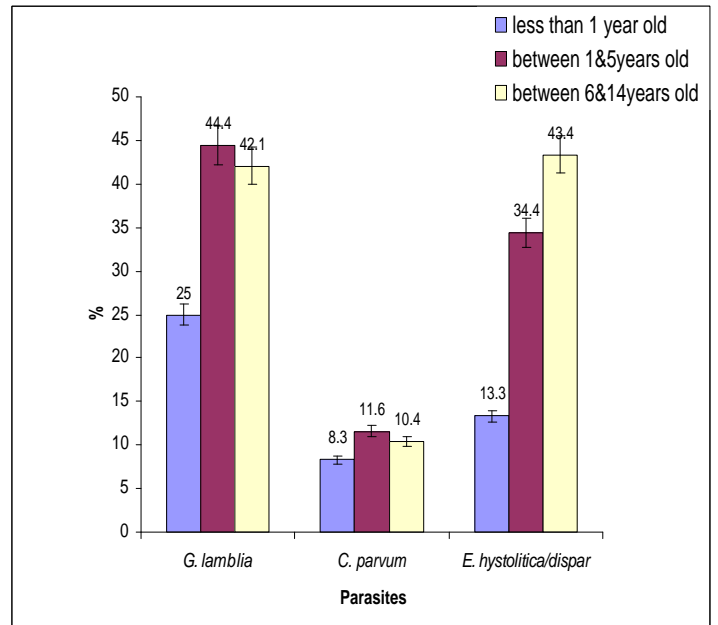


Figure 11. Prevalence of giardiasis, amebiasis and cryptosporidiosis among different age groups in Adada (November 2005 – May 2006).

4.2.3. Legebira

Based on the parasitological examinations of 611(dry, n=309; wet, n=302) stool specimens in Legebira, the prevalence of cryptosporidiosis giardiasis and amebiasis was 12.8%, 37.3% and 34%, respectively. The prevalence of cryptosporidiosis was 1.6% in dry and 24.2% in wet seasons sampling and for amebiasis the prevalence was 25.6% in dry and 42.7% in wet season sampling; similarly the infection prevalence of giardiasis was 29.8% in dry and 45% in wet seasons sampling. In all cases the infection prevalence showed statistical significant difference ($P<0.05$) between wet and dry seasons sampling (Table 4).

The prevalence of cryptosporidiosis between the dry (1%) and wet (23.1%) season sampling in children using protected water sources showed a significant difference ($P<0.05$). Similarly the infection prevalence among children using unprotected water source showed a highly significant difference ($P<0.05$) between dry (2.7%) and wet (25.8%) season sampling. In addition there was statistical significant difference ($P<0.05$) in prevalence of giardiasis and amebiasis between dry and wet season sampling among children using protected water sources as well as unprotected water sources (Table 5).

The prevalence of giardiasis between children using protected and unprotected water sources was 37.8% and 36.5%, respectively. Similarly for children using protected and unprotected water sources, the prevalence of cryptosporidiosis was 11.5% and 14.8%, respectively and the prevalence of amebiasis was 34.1% and 33.9% respectively. In all cases, no statistical significant difference was established ($P>0.05$) (Table 6).

Closer analysis of the infection prevalence of cryptosporidiosis between children using protected (1%) and unprotected (2.7%) water sources showed no difference ($P>0.05$) during the dry season sampling. The wet season sampling picture in children using protected (23.1%) and unprotected water sources (25.8%) also showed no significant difference ($P>0.05$). In addition the prevalence of giardiasis between children using protected and unprotected water sources was 31.2% and 27.3% respectively during the dry season sampling and 45.1% and 45% respectively during the wet season sampling. The result showed that there was no significant difference ($P>0.05$) between children using protected and unprotected water sources

in both during dry and wet season sampling. Similarly there was no significant difference ($P>0.05$) in prevalence of amebiasis between children using protected (25.6%) and unprotected (25.5%) water sources during the dry season sampling and between children using protected (43.4%) and unprotected (41.7%) water sources in wet season sampling (Table 7).

The prevalence of giardiasis, cryptosporidiosis and amebiasis in relation to sex group showed no difference ($P>0.05$). On the contrary there was a significant difference ($P<0.05$) among age categories in infection prevalence of giardiasis and amebiasis but there were no difference ($P>0.05$) in age categories of the prevalence of cryptosporidiosis (figure 12 and figure 13).

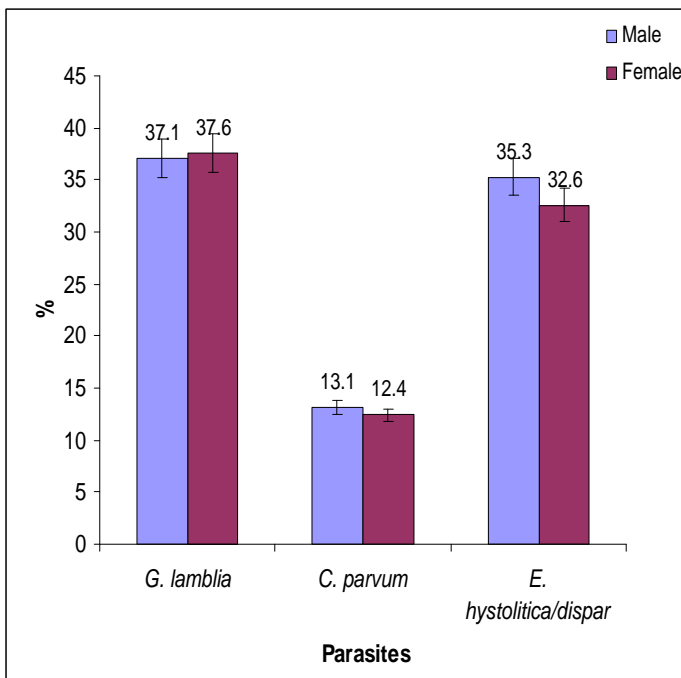


Figure 12. Prevalence of *Giardiasis*, amebiasis and Cryptosporidiosis by sex in Legebira (November 2005 – May 2006).

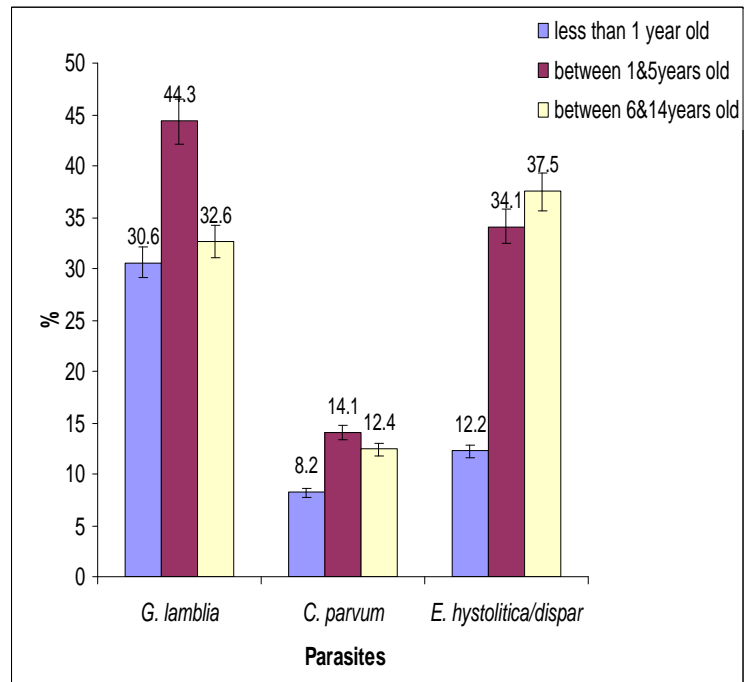


Figure 13. Prevalence of *Giardiasis*, amebiasis and Cryptosporidiosis among different age groups in Legebira (November 2005 – May 2006).

5. Discussion

According to the World Health Organization (1998), more than 33% of global deaths are due to parasitic diseases. Intestinal parasitic infections are among the most common infections in the world responsible for mortality and morbidity (WHO, 1991). Inadequate water supply and sanitation, polluted water or unavailability of water is largely responsible for many deaths in developing countries every year (Esrey *et al.*, 1989). In terms of pathogenic importance, *Giardia lamblia*, *Cryptosporidium parvum* and *Entamoeba histolytica/dispar* have been shown to be responsible for severe diarrhoeal episode especially in immunocompromised and younger children (Current and Garcia, 1991). This study examined the prevalence of *C. parvum*, *G. lamblia* and *E. histolytica/dispar* infections among children using different water sources in rural parts of Dire-Dawa using microscopic methods.

In the present study, a significant number of children were found harboring *Cryptosporidium* in Legedini, Adada and Legebira. Although the study subjects were living in different rural areas of Dire-Dawa, the prevalence on average was in agreement with the report obtained from Iqbal *et al.* (1999) where *C. parvum* infection among children is more prevalent in developing countries (5-10%). The proportion of *C. parvum* in this study was by far higher than that reported by Assefa *et al.* (1996) and Gebru and Girma (2000) in diarrhoeal children in Ethiopia. In the present study sites, the relatively higher *Cryptosporidium* infection in children might be not only from consumption of contaminated food and water but also from contact with farm animals since the community by large is agro-pastoralist and it also might be from the proximity of children with dogs and cats.

The rate of *Cryptosporidium* detected was similar with what was obtained in North-western Ethiopia by Mersha and Tiruneh (1992). The prevalence of Cryptosporidiosis in all study sites was much lower than what was reported from adult diarrhoeal AIDS patients (25.9%) from Addis Ababa hospitals (Fisseha *et al.*, 1999). Because of the intermittent nature of oocyst excretion of this parasite (Navin and Juranek, 1984), we believe that the prevalence of

Cryptosporidiosis in the present study could have been higher if more than one stool specimen had been collected from each child.

Similarly, higher level of giardiasis was observed in this study. This high prevalence might be correlated with variation in environmental and geographic settings of the study areas together with crowding condition and poor living standards of the society. The variation could be an indication of the development of giardiasis at early in life and high rate of carriage in all age groups and possibly of re-infection in the community. Unless prior infections were protective for disease, one would expect higher rate of giardiasis in developing countries but usually asymptomatic (Gilman *et al.*, 1988). Children with asymptomatic *Giardia* infection serve as unidentified carriers and may be responsible for transmission of infection (U.S. EPA, 1989). In a study conducted in pre school children (9.3%) (Seyoum *et al.*, 1981) and in the central and northern highlands of Ethiopia (3-23%) (McConnell and Armstrong, 1976), the prevalence of giardiasis was much lower than what was recorded in the present study.

In the overall prevalence of Cryptosporidiosis, giardiasis and amebiasis, significant variation between dry and wet seasons sampling was noted. Although we did not study prevalence every month, the present study suggests that there is a seasonal variation in the prevalence. This is in agreement with other studies reported elsewhere (Adegbola *et al.*, 1994; Enriquez *et al.*, 1997) as more intestinal parasitic infection is associated with increasing amount of rainfall. Other studies from Central America, South Africa, Kuwait and India also reported a high peak incidence during rainy season (Leach *et al.*, 2000; Iqbal *et al.*, 2001). High prevalence (33-43 %) during the wet seasons in the United States was reported (Amin, 2002), in England and Wales, 2-22% (Smerdon *et al.*, 2003), and around Guatemala City, 1% (Bern *et al.*, 2000). It is possible that the seasonal rains amplify the low level water source contamination by storm water runoff, containing faeces of infected humans and animals. This results in the higher numbers of cryptosporidiosis, giardiasis and amebiasis cases during high rainfall months until the return of the dry season when the number of oocysts again becomes low or negligible.

There were no significant variations in prevalence of cryptosporidiosis, giardiasis and amebiasis in both dry and wet season sampling between the three sites. No significant difference was also observed among children using unprotected water sources between the

three sites and protected water sources between the three sites in wet season and dry season sampling. This implies that the three study sites have comparable water sources during the rainy season and dry season.

The prevalence of cryptosporidiosis, giardiasis and amebiasis between children using protected and unprotected water sources showed significant variations in Adada but not in Legedini and Legebira. The possible explanation for the absence of variations between the two water sources might be whatever water sources they used; the infection was not restricted to the water sources. It could also arise from other point of contamination before reaching to each household to drink. In addition, because of the scarcity and non- continuous supply of water, the people are forced to store water in containers for long period which allows the possibility of fecal contamination. Recently, there has been increased attention to the fact that drinking water, even if it is of good quality, can become contaminated between the point of collection and the home, and in the domestic environment. This is highlighted for example, when children dip their faecally contaminated hands in a household water container (Jensen *et al.*, 2004).

A case study presented to a learning workshop held in Awassa, Ethiopia in May 2006, disclosed that, contamination of water from tap to mouth was more than 60%. On top of this, the water tanks and pipe networks of protected water sources of all the study sites, from which the water is supplied to the community, were not cleaned in a regular basis after construction. In addition, no disinfectants were used in the study sites. Similar studies conducted in British Columbia showed that parasites including *Giardia* detected more frequently than *C. parvum* in drinking water using surface supplies (Isaac-Renton *et al.*, 1999). As it had been indicated by U.S. EPA (1989), providing high quality drinking water may not significantly reduce the incidence of giardiasis in developing countries. In spite of the fact that contaminated drinking water may be an important source of exposure in developing countries, the variety of other exposures including personal hygiene, food hygiene, and environmental factors may overwhelm the beneficial effect of protected drinking water. This could explain why there was no difference in infection among children using protected and unprotected and unprotected source of water in Legedini and Lgebira.

In Adada, the high activities of domestic animals and humans in the unprotected water sources might lead to repeated contamination of surface water, well, ponds with oocysts of *Cryptosporidium*. A similar observation was recorded in two outbreaks in the United State where wells had become contaminated with *C. parvum* (Craun *et al.*, 1998). In addition, a study conducted in British showed that large number of intestinal parasites including *C. parvum*, *G. lamblia* and *E. histolytica/dispar* were detected in drinking water samples from unprotected water sources where agricultural and human activities were high. On the other hand, in protected water with no agricultural activities and minimal human activities, the prevalence of cryptosporidiosis, giardiasis, amebiasis and other intestinal parasites were reduced (Isaac-Renton *et al.*, 1999). The low level of intestinal parasites among children using protected water sources in Adada highlights the importance of water development project in the rural parts of the country in reducing parasite burden. This is of course, in addition to either drinking good quality water or keeping personal hygiene and environmental sanitations.

Legebira, although statistically significant variation was not aobserved, the rate of *Giardia* and amoeba infection among children using protected water sources was higher than unprotected water sources. This might be due to two reasons: first some house holds probably use treatment such as filtration or boiling before consumption understanding the poor quality of unprotected drinking water, or second the water source might be inadequately protected. In agreement with the present findings, Craun *et al* (1998) explained the fact that in the United States, inadequately protected ground water sources cause twice as many waterborne parasites outbreak than did surface water.

Previous water quality analysis in Legedini indicated the presence of oocysts and cysts of *C. parvum* and *G. lamblia* in both protected and unprotected water sources. But the rate was higher in Sellela and Hado Sere well than Ajo tap water Kora spring (Scheelbeek, 2005). The present finding also strengthens the previous water quality reports of the area which showed lower prevalence rate in those children using water from protected sources (Ajo tap water Kora spring) and higher prevalence rate in those children using unprotected water sources (Sellela and Hado Sere well) irrespective of other contaminant factors.

Sex group and *Cryptosporidium* infection was not significantly associated in any of the three study sites indicating that the possibility of getting infection with *C. parvum* is similar for males and females. This is in agreement with a study in Mexico City where the gender of the children did not influence the rate at which *C. parvum* infection was detected (Enriquez *et al.*, 1997). Similarly Male and female individuals tested for cryptosporidiosis in Kwa Zulu-Natal population had similar positive rates (Jarmey-Swan *et al.*, 2001). But an opposite observation was reported from Guinea-Bissau by Molbak *et al* (1994) and by Fraser *et al* (1997) among Bedouin infants in Israel where the prevalence of *C. parvum* in males was higher than in the females. They suggested that as far as the immune competence is similar for male and female, there might be an unmeasured intra-familial factor functioning to expose infant boys or to protect infant girls in their study population. The same observation was made in a study conducted in hospitalized children in Delhi where *Cryptosporidium* infection was predominant in males than females (Mahajan *et al.*, 1992). In an isolated report, an increased prevalence was found for females in Nigeria (Okafor and Okunji, 1994)

Sex related prevalence of *Giardia lamblia* and *Entamoeba histolytica/dispar* in children had similar patterns to that of *C. parvum* where the infection showed no significant difference in any of the three sites. On the contrary, Mahmud *et al* (1995) reported a higher prevalence of giardiasis among boys than girls in Egypt. Similar finding also showed that the prevalence of giardiasis was lower in Girls than boys in a study conducted in northern Jordan (Nimric, 1994). In a study conducted in Kenya, there were more male than females who contacted amebiasis (Chabalala and Mamo, 2001). In a countrywide survey of amebiasis in Ethiopia, the prevalence of amebiasis was more among females than males in a school children which shows significant variations but the difference was not statistically significant among non school communities (Erko *et al.*, 1995). However, in both genders, *E. histolytica/dispar* infection was equally distributed in a study conducted in Mexico (Acuna-Soto *et al.*, 2000). Because infection by *E. histolytica/dispar* is acquired by the consumption of food or drinking water contaminated with infective cysts, the probability of infection is the same for both genders. Similarly, in studies carried out in different parts of Iran, no significant difference was observed in prevalence between females and males (Sheiban and Rezaian, 1981). In the present study the possible explanation for the absence of variation among sex was, except infants all children irrespective

of their sex participate equally in social activities such as herding. Besides, the pattern of hygienic practices exhibited by male and female is also similar. In general, all activities done by male boys are equally engaged by females.

Based on the present study, the pattern of age difference in infection with *C. parvum* appears non significant in any of the three study sites ($P>0.05$) indicating that there was no significant variation in cryptosporidiosis. The reason for lack of variations between the three age groups might be associated with similar personal and environmental hygiene, poor water quality and stay of all age group children with farm animals. Although significant variations were not observed, the infections were slightly higher among 1 to 5 years old and lower among less than one year old. According to Casemore (1990), the peak incidence of cryptosporidiosis is among children aged 1-5 years which is in agreement with the present study. Similarly, in Manitoba, Canada, *C. parvum* was isolated much more frequently from faecal specimens collected from children under five years of age (Mann *et al.*, 1986). But this finding is dissimilar to the observation of Lindo *et al.* (1998) in Jamaica, Adegbola *et al.* (1994) and Tumwine *et al.* (2003) in sub-Saharan African countries including Uganda and Gambia, and Assefa *et al.* (1996) in Ethiopia where a difference in prevalence between age groups was observed. However, there was no significant variation among different age groups in this study, generally the prevalence of cryptosporidiosis among asymptomatic children less than 14 years old was higher than what was reported by Assefa *et al.* (1996) and Gebru and Girma (2000) (5.6%-9% in Addis Ababa and north-western Ethiopia) among children with diarrhoea in Ethiopia.

This study revealed differences in the prevalence of giardiasis among age group in all study sites. In all cases higher prevalence of giardiasis occurred in the 1 to 5-years-old age group and lower in age group less than 1 year old, indicating that infants might be protected from *Giardia* infections from the environment despite the contamination from their parents. In addition, the prevalence increases with age and with the development of immunity in older subjects the prevalence becomes reduced. This explanation is strengthened by the finding that giardiasis increased with the age of the children and was not clustered in a particular age group (Lindo *et al.*, 1998). In addition, Isaac-Renton and Philion (1992), Harter *et al.*, (1982) also found that the majority of *Giardia* infections were among children in the 1 to 5 year age group.

According to CDC (2000), the age-specific incidence of giardiasis was greatest in children aged 1-4 years followed by children aged 5-14 years in Los Angeles.

Significant difference in prevalence of amebiasis was observed among the three age group. The highest prevalence rate of amebiasis was seen at 6-14 age groups, and the lowest infection rate was seen at less than one age group. A similar age distribution of infection has been observed in studies in Iran (Sheiban and Rezaian, 1981). In endemic region, the highest infection rates have been seen in earlier age, for example in Mexico, 11% of the tested population aged 5 to 9 years was infected with *E. histolytica/dispar* (Caballero-Salcedo, *et.al.*, 1994). In another study conducted in Mexico, a lower prevalence of *Entamoeba histolytica/ dispar* was observed in breast-fed children below one year of age (Morales-Espinoza *et al.*, 2003). The reason for the low prevalence in this age group of the present study was children receive immunological protection through their mother's milk (Islam *et al.*, 1998) and at this stage of infancy the child interacts less with its surroundings.

Generally, the prevalence of giardiasis, cryptosporidiosis and amebiasis in the present study was lower in age group less than 1 year. Because, it has been known for a long time that babies who are breast-fed contract fewer infections than do those who are given formula. This is because breast milk actively helps newborns avoid disease in a variety of ways. This is particularly important during the first few months of life, when an infant often cannot mount an effective immune response against foreign organisms. In addition among breastfed children, the baby's own immune system develops more rapidly than does baby who is fed formula (Newman, 1995).

In this study some helminthes along with protozoans were also identified. Unlike other studies, the prevalence of intestinal helminthes in those study sites was very low and much less than what was reported by others (Mcconnel and Armstrong, 1976; Seyoum *et al.*, 1981; Ali *et al.*, 1999) in Ethiopia. The low prevalence of helminths in the present study although agreed with a previous report on a low prevalence of ascariasis in the low and dry areas of the country (Tedla and Ayele, 1986), the diagnostic methods applied for all parasites especially for helminthes did not provide the true picture of prevalence in the present study areas.

In addition the presence of the non-pathogenic intestinal parasites such as *Entamoeba coli*, *Endolimax nana*, *Iodoamoeba butschili* and *Chilomastic* could be an indication of fecal contamination of drinking water sources.

6. Conclusions and Recommendations

In the present study, higher prevalence of cryptosporidiosis, giardiasis and amebiasis has been found among children below 14 years old with an average prevalence rate of 11.9%, 38% and 33.7%, respectively. An increasing prevalence in this study was associated with factors such as scarcity of water for consumption, indiscriminate defecation by the inhabitants and their animals, geographical settings of an area (which is mountainous so whenever the rain comes it washes out waste products of human and other animals to their farm and surrounding), absence of toilet, keeping of livestock waste products in their backyards, living in overcrowded situations with many children per household coupled with poor sanitation, poor personal hygiene and high illiteracy. In addition a common practice of keeping certain domestic animals within or in close vicinity of house holds and many other factors contribute to the high prevalence of those parasites among children in Rural Dire-Dawa, Ethiopia.

This study also indicated that cryptosporidiosis, giardiasis and amebiasis were seasonal diseases in the sense that their prevalence was higher during wet seasons and lower during dry seasons.

Although significant difference was not obtained in the prevalence among children using protected and unprotected water sources in Legedini and Legebira, this does not mean that water development project does not have a role in reducing illness as a result of waterborne parasites; rather this water development should be conducted side by side with other discipline particularly with health sector. In addition untreated “protected” drinking water sources are not free of the waterborne parasitic pathogens.

It was estimated that the prevalence of *C. parvum* does not vary between infants below 1 year old and in children with age group between 1 and 5 and between 6 and 14, but *G. lamblia* and *Entamoeba histolytica* was seen to increase with age. However, for *Giardia*, the rate increased till some point and reduces as children become older. On the contrary sex related prevalence of giardiasis, cryptosporidiosis and amebiasis were not observed in this study, which implies male

and female children have equal chances of infection since they are engaged equally in all activities.

In light of the high proportion of giardiasis, cryptosporidiosis and amebiasis in the present study, we suggest that prevention, treatment and control of parasitic infections amongst children in these parts of Ethiopia are necessary before an outbreak occurs. So the decision makers should mobilize the community to improve health situations through:

- provision of adequate and safe water supply
- health education in their local languages related to personal hygiene such as hand washing after using the toilet and before handling food. Hygiene depends on the quantity of water that people have. In many areas, hand washing after defecation or before preparing food seems like a luxury when the water has to be fetched from a water point far away. However, washing hands with soap can reduce the risk of diarrhoeal diseases by 42 to 47 percent, and the promotion of hand washing might save a million lives per year (Curtis and Cairncross, 2003). Therefore Hand washing promotion should be one of an intervention method.
- environmental sanitation
- Cost effective water purification mechanisms such as boiling and chlorination.

Provisions such as listed above will help in enhancing the health and well-being of the community particularly that of children. In addition, the best way to prevent faecal-orally transmitted diseases is the sanitary disposal of human faeces in latrines or other improved sanitation facilities. Improved sanitation is also the only long-term sustainable option for controlling intestinal parasites. Improved sanitation has important additional benefits, especially to women. In many cultures, the only time when women or girls can defecate, if they have no toilet, is after dark. The walk to the defecation field, often in the dark, is when women run the greatest risk of sexual harassment and assault (www.lboro.ac.uk/well/resources).

- On top of this research should be carried out in the general population to have a clear picture on the epidemiology of intestinal parasites.
- Since the rate of amebiasis was higher among the study subjects, molecular techniques such as ELISA and PCR should be conducted to differentiate invasive (*E. histolytica*) with the non-invasive one (*E. dispar*).

7. References

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8. Appendix:

CONSENT FORM

Code No. -----

Name of study participant ----- Age----- Sex-----

Physician Name----- Site /Health center -----

I have been informed about a study that plans to investigate the “assessment of the association of *Giardia lamblia*, *Cryptosporidium parvum*, and *Entamoeba histolytica/dispar* infection prevalence with drinking water sources among children in Legedini, Adada and Legebira Dire Dawa, Eastern Ethiopia”, which will help in understanding the possible source of infections in relation to different water sources at different seasons and it also assists in designing a better control options of waterborne parasites in the area.

For this study I have been requested to give a stool sample for *Cryptosporidium* and *Giardia* identifications. I have been informed that I will get treatment if I will be positive for *Giardia* and other intestinal helminths and protozoan after giving the stool. The investigator has briefed me that there are no major risks associated with the sampling procedure. The investigator also informed me that all the laboratory results would be kept confidential. Moreover, I have also been well informed of my right to withdraw from participating in this project and that my actions will have no impact on the overall management of my conditions. I have been given enough time to think over before I signed this informed consent. It is therefore, with full understanding of the situation that I gave my informed consent and cooperates at my will in the course of the conduct of the study.

Name (participant) -----Signature -----Date -----

Name (investigator) -----Signature -----Date -----

Name (Witness) -----Signature -----Date -----

Declaration

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university and that all sources of materials used for the thesis have been correctly acknowledge

Name: Dawit Ayalew

Signature:

A handwritten signature in black ink, appearing to be 'Dawit Ayalew', written over a horizontal line.

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