PREVALENCE OF OPPORTUNISTIC INTESTINAL PARASITIC INFECTIONS AMONG HIV/AIDS PATIENTS ATTENDING OTHONA HOSPITAL, WOLAYITA SODO, SOUTHERN ETHIOPIA

M.Sc. Thesis

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PREVALENCE OF OPPORTUNISTIC INTESTINAL PARASITIC INFECTIONS AMONG HIV/AIDS PATIENTS ATTENDING OTHONA HOSPITAL, WOLAYITA SODO, SOUTHERN ETHIOPIA

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

By

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As thesis research advisors, we herby certify that we have read and evaluated this thesis prepared, under our guidance, by Menbereleul Mathewos, entitled "**Prevalence of Opportunistic Intestinal Parasitic Infections among HIV/AIDS Patients Attending Othona Hospital, Wolayita Sodo, Southern Ethiopia".**. We recommend that it can be submitted as fulfilling all the thesis requirements.

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

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DEDICATION

I dedicated this thesis manuscript to all my families and relatives for their continuous love, appreciation, encouragement, moral, and financial support during my studies.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my own original work and has not been presented for a degree in any University and that all sources of materials used for this thesis have been correctly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (M.Sc) degree at the Haramaya University and is deposited at the University Library to be made available to borrowers under rules of the Library.

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BIOGRAPHICAL SKETCH

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
ART	Anti Retroviral Therapy
CDC	Center for Disease control and prevention
CI	Confidence Interval
DALYs	Disability adjusted life years
DPPC	Disaster Prevention and Preparedness Commission
EDTA	Ethylendiaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
FACS	Florecence Activate Cell Scanning
FHAPCO	The Federal HIV and AIDS Prevention and Control Office
GI	Gastro Intestinal
HAART	Highly Active Anti Retroviral Therapy
HIV	Human Immunodeficency Virus
IP	Intestinal Parasites
MAB	Monoclonal Antibody
MOH	Ministry of Health
NCCLS	National Committee for Clinical Laboratory Standards
NMAABO	National Meteorological Agency Awassa, Branch Office
NSTC	National Science and Technology Council
OH	Othona Hospital
OIPI	Opportunistic Intestinal parasitic infection
OPD	Out Patient Department
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PLWHA	People Living With HIV/AIDS
RFLP	Restriction Fragment Length Polymorphism
SBO	Small bowel obstraction
SNNPR	Southern Nations Nationalities and Peoples' Region
SPSS	Statistical Package for Social Sciences
STHs	Soil Transmitted Helminths
TMP-SMX	Trimethoprim-Sulfamethoxazole
USAID	United States Agency for International Development
WFP	World Food Program

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ABSTRACT

It was well known that opportunistic intestinal parasitic infections among HIV/AIDS patients were high in developing countries compared with industrialized countries. Because of unhygienic conditions transmission of these parasites was more frequent in developing countries. The objective of this study was to determine the prevalence of opportunistic intestinal parasitic infections among HIV/AIDS patients with special emphasis on Cryptosporidium parvum, Isospora belli, Cyclospora cayetanensis and Blastocystis hominis infections as well as their associations with some socio-demographic risk factors and CD4+ T cell counts at different immunity levels. A hospital based cross sectional study and the five years retrospective analysis of clinical records was carried out from May-August, 2013 in Othona Hospital, Wolayita Sodo, Southern Ethiopia. Using serial sampling method, a total of 422 HIV/AIDS patients aged from 5-65 years old were included in this study. The samples were collected from all people living with HIV/AIDS until the sample size was reached. A pre-tested and structured questionnaire was employed to collect socio-demographic data and health record analysis of the patients. Stool samples were collected from 422 HIV/AIDS patients and were processed for opportunistic intestinal parasites using Modified Ziehl-Neelsen staining method, formol-ether concentration and by direct wet mount methods. Data was analyzed using the SPSS version-16. Chi-square and logistic regression were used to verify possible strength of opportunistic intestinal parasitic infection and exposure with different risk factors. Simultaneously, CD4+Tcell counts were recorded to assess the status of HIV/AIDS infection in relation to opportunistic parasitic infections. Out of 422 HIV/AIDS patients, opportunistic intestinal parasites were recorded from 268(63.5%) patients. Among these, C.parvum (14.2%), I. belli (8.5%), C.cayetanensis (2.8%) and B.hominis (2.6%) was predominant coccidian protozoan parasite identified from HIV/AIDS patients. In addition to opportunistic intestinal parasites, non-opportunistic intestinal protozoan parasites such as, Giardia lamblia (10.0%) and Entamoeba histolytica (9.0%) were also prevalent in HIV/AIDS patients. Among helminths, Strongloides stercolaris (10.2%) was the predominant helminthic parasite isolated in HIV/AIDS patients followed by Ascaris lumbricoides (2.0%) and Hooke worm (1.2%) among the study participants. In general, this study shows that the high prevalence of opportunistic intestinal parasitic infections among HIV/AIDS patients and its association with the lower CD4+ T cell counts (<200cells/μl). Therefore, regular monitoring of CD4+T cell counts and screening of these opportunistic agents in the HIV/AIDS patients will help to reduce the mortality and morbidity with administration of appropriate therapy.

Key words: *T-Lymphocyte cell counts, HIV/AIDS, Opportunistic intestinal parasites, Prevalence, Patients, Risk factors*

1. INTRODUCTION

Intestinal parasitic infections are among the most common infections world-wide where Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) is also prevalent. Sub-Saharan Africa is among the regions where intestinal parasitic infections are well established (WHO, 2002) and the largest burden of AIDS cases exist (UNAIDS/WHO, 2006). The HIV infection is a worldwide phenomenon and is a serious public health problem. It was estimated that 34 million people are living with HIV in the world. The rate of HIV infection was remarkably high in sub-Saharan Africa, where the majority of HIV and AIDS cases were concentrated (UNAIDS/WHO, 2002). In Ethiopia, the current estimated population living with HIV is 1.1 million (FHPCO, 2010). Ethiopia is one of the highly affected sub-Saharan countries (USAID/ETHIOPIA, 2010).

The incidence of intestinal parasitic infections is 50% in developed countries, whereas it reaches up to 95% in developing countries. It is estimated that at least more than one-quarter of the world's population is chronically infected with intestinal parasites and that most of these infected people live in developing countries (Fincham *et al*., 2003). However, intestinal parasites once considered to be controllable in developed countries, remain a major cause of morbidity and mortality worldwide.

Dramatic expansion of the HIV/AIDS pandemic has brought about a significant change in the fauna of intestinal parasites all over the world (Gomez *et al*., 1995). As a result, some intestinal parasites are among the main health problems in HIV/AIDS patients as opportunistic infections due to depleted immunity. The opportunistic intestinal parasites are the major problems in such group of patients (Goodgame, 1996). In immunocompetent individuals, opportunistic intestinal parasitic infection is usually self limiting but in immunocompromised hosts, particularly in AIDS patients with $CD4^+$ T cell counts below 200cell/mm^3, opportunistic intestinal parasitic infections can be life-threatening and must be treated properly (Baqai *et al*., 2005). The T-lymphocyte cellular responses are important in controlling infection, as evidenced by the increased disease severity in HIV-infected patients with CD4 counts less than 100 cells/µl (Karin *et al*., 2009). Several other factors also contribute to the expansion and reinvasion of newly emerging

intestinal parasites. Among these, increasing migration of people due to political instability, war, economical problems and travel to different countries are some of the main factors (Franzer and Muller, 1999). The public health importance of intestinal parasites as a major concern in most developing countries has been pronounced with the co-occurrence of malnutrition and HIV/AIDS. With HIV/AIDS pandemic, many intestinal parasites, previously considered being sporadic or zoonotic infections have become opportunistic parasites causing uncontrollable life threatening diarrhea (Muller, 1999).

Like in many other developing countries, intestinal parasites are widely distributed in Ethiopia largely due to the low level of environmental and personal hygiene, contamination of food and drinking water that results from improper disposal of human excreta (Kumie and Ali, 2005). More than half a million annual visits of the outpatient's services of the health institutions are due to intestinal parasitic infections (MOH, 1996). However, this report may be underestimated, because most of the health institutions lack appropriate diagnostic methods to detect low levels of parasite burden. In addition, some of the diagnostic methods for specific intestinal parasites, especially for the newly emerging opportunistic intestinal parasites, are not available in peripheral health institutions.

However, some of the investigators mainly focused on helminth parasites, whereas others on both intestinal protozoan and helminth parasites. After HIV infection and the development of AIDS, various kinds of opportunistic infections (OIs) develop in the patients that differ from country to country. Facilities required for the diagnosis of many OIs are not affordable in many of the countries with the highest burden of the disease. HIV infection has been shown to predispose the patients to many intracellular opportunistic intestinal parasites such as *Cryptosporidium parvum*, *Isospora belli* and *Cyclospora cayetanensis* (Goodgame, 1996).

Reports indicate that diarrhea occurs in 30-60% of AIDS patients in developed countries and in about 90% of AIDS patients in developing countries, especially in cases of persistent diarrhea (Wiwanitkit. 2001). Furthermore, the non- opportunistic helminth infections are also present, with an average prevalence of 10 % among HIV-infected patients (Singh, 1998). Although protozoan parasites remain important etiological agents of gastrointestinal infection among HIV-

infected persons around the world, most studies of helminth infection are reported in persons with HIV infection who reside in or are returned travelers or immigrants from less developed countries, especially in cases of persistent diarrhea (Wiwanitkit, 2001). Protozoan parasites especially coccidian parasites and other enteric parasites, like *Giardia lamblia* and *Entamoeba histolytica* account for a significant cases of diarrhea in HIV/AIDS patients (NGARVT, 2005).

A study condacted in selected Anti retroviral therapy centers in Adama, Afar and Dire-Dawa, reported that diarrhoea among HIV/AIDS patients was significantly associated with major intestinal protozoan parasites, such as *G. lamblia* and *E. histolytica* infections (Haileyesus and Beyene, 2009). Another study in Ethiopia also showed high prevalence of intestinal parasites like *Ascaris lumbricoides, E. histolytica, G. lamblia, C. parvum, I. belli* and *C. cayetanensis* among HIV positive individuals (W/Michael *et al*., 1999).

Information regarding the magnitude of opportunistic intestinal parasitic infections among HIV/AIDS patients is scarce in different parts of Ethiopia, particularly in the Southern Region of the country. Because of the shortage of clean water in many villages of rural parts of southern Ethiopia, the community was forced to use unprotected water. In such areas where people use water from different sources, the possibility of infection with water-borne diseases was expected to be extremely high. In addition, there was no information on the prevalence and magnitude of the intestinal parasite infections among HIV/AIDS patients in the present study area. Hence, the present study was conducted to fill the existing gap and enable stakeholders in understanding the prevalence of intestinal parasites among HIV patients; major risk factors that could predispose HIV patients to infection by intestinal parasites among HIV patients in Othona Hospital, Wolayita Sodo, Southern Ethiopia.

General objective

The main objective of this study was to determine the prevalence and patterns of opportunistic intestinal parasitic infections among HIV/AIDS patients in Othona Hospital, Wolayita Sodo, Southern Ethiopia.

Specific objectives

- To determine the prevalence of *Cryptosporidium parvum*, *Isospora belli*, *Blastocystis hominis* and *Cyclospora cayetanensis* infections among HIV/AIDS patients in Othona Hospital.
- To assess the trend and pattern of opportunistic intestinal parasitic infections among HIV/AIDS patients based on Health Record Analysis in Othona Hospital, Wolayita Sodo
- To determine the relationship between opportunistic intestinal parasitic infections among HIV/AIDS patients with their Socio-Demographic Characteristics and level of CD4+T Cell Counts.

2. LITERATURE REVIEW

2.1 Major Intestinal Protozoan Parasites and their Life cycles

Cryptosporidium-induced infections are considered to be one of the world's most commonly found causes of diarrheal illness in humans, especially infants and children (Bhan *et al.,* 1996), the elderly (Neill *et al*., 1996) and AIDS patients in particular (Poirot *et al*., 1996). In the last several years, however, because of improved diagnostic techniques and the rising tide of the AIDS epidemic, and immunosuppressed population in general, *Cryptosporidium* inhabits primarily microvillus brush border of intestinal epithelial cells (Bailey and Scott, 1998).

It is an intestinal pathogen having a zoonotic nature (Graczyk *et al*., 1997) responsible for clinical disease in mammalian species commonly infecting human beings. *Cryptosporidium parvum* has been reported in about 80 different animal species including cattle, pigs, horse, sheep and goats (Dubey *et al.,* 1990). The first case of human infection with *Cryptosporidium parvum* was reported in 1976 (Mosier and Oberst, 2000). Since then, it is an increasingly recognized agent of intestinal infection as a common cause of severe diarrhoea in immune-competent and immune-compromised humans and domestic animals (Hunter and Nicholes, 2002). Currently, there are thirteen species of *Cryptosporidium* categorized based on differences in host specificity, oocyst morphology, biochemical differences and site of infection, and most of them infect only one or a few groups of animals (Xiao *et al.,* 2004) (Table 1).

Table 1 *Cryptosporidium* species that Infect Humans and other Animals

Species	**Major Host**	**Minor Host**
C. andersoni	Cattle, Bactrian camels	Sheep
C. baileyi	Chicken, turkeys	Cockatiels, quails, ostriches
C. felis	Domestic cat	Humans, cattle
C. meleagridis	Turkey, humans	Parrots
C. muris	Rodent, Bactrian camels	Humans, rock hyrax, mountain goats
C. molnari	Fish	_
C. parvum	Cattle, sheep, goats, Humans	Deer, mice, pigs
C. saurophilum	Lizards	Snakes
C. serpentis	Snakes, lizards	_
C. wrairi	Guinea pigs	_
C. hominis	Humans, monkeys	Dugongs, sheep
C. canis	Dogs	Humans
C. galli	Finches, chicken, capercalles	_

Source: Xiao *et al.*, 2004.

Thirteen species of *Cryptosporidium* exist, with most human infections caused by *C. parvum* and less frequently by *C. hominis*, *C. meleagridis* and *C. canis* (Chen *et al.*, 2002). Moreover, immunodeficient patients are also known to be infected by other *Cryptosporidium* parasites, including *C. Suis*, *C. baileyi* and *C. muris* (Palmer *et al.*, 2003). To date eight valid *Cryptosporidium* species have been reported to be capable of infecting humans (Palmer *et al.*, 2003).

The life cycle of *Cryptosporidium* is monoxeous completed within the gastrointestinal tract of a single host (Figure 1) (Fayer *et al.*, 2000). The oocyst is spherical in shape measuring 3-6μm in diameter and it may be either thick or thin walled (Ramirez *et al*., 2004; Abhay *et al*., 2009). The resistant stage that is found usually in the environment is the thick walled oocyst excreted together with feces (Fayer *et al*., 2000).

Each oocyst has 4 infective sporozoites that come out from the oocyst using the suture at one side of the oocyst. The ileum is the preferable site of infection and the sporozoites penetrate epithelial cells of the ileum. *Cryptosporidium parvum* resides on the luminal surface of epithelial cells and it is used to be thought to reside extracellularly. However, ultra structural observations

have revealed that it is intracellular but extracytoplasmic, enclosed by a thin layer of host cell cytoplasm. Infection could possibly occur with ingestion of as few as 30 oocysts; some infection has also occurred with just a single oocyst (Fayer *et al*., 2000).

Cryptosporidium parvum can complete its life cycle in as short as 2 days and the infection may be short lived or may be persistent for months. Excystation of the oocyst is initiated by the body temperature, interaction with stomach acid and bile salt. The released sporozoites attach to epithelial cell and become enclosed with in parasitophorous vacuoles. The trophozoite stage then undergo asexual proliferation by merogony and two types of meronts are produced, Type I meronts and Type II meronts (O'donoghue, 1995; Fayer *et al*., 2000).

Type I meronts form 8 merozoites that are released from the parasitophorous vacuole when they mature. The merozoites then enter another brush border surface epithelium where they undergo another cycle of type I merogony (multiple fission or schizogony) or else they may develop into type II meronts (O'donoghue, 1995; Fayer *et al*., 2000).

The type II meronts give rise to 4 merozoites which do not undergo further merogony but produce gamonts, the sexual reproductive stages which fuse and form the only diploid stage in the life cycle, the zygote. A resistant oocyst wall is then formed around the zygote. The zygote undergoes asexual development (sporogony) and gives rise to copulated oocyst that contains 4 sporozoites (O'donoghue, 1995; Fayer *et al*., 2000). Two possible auto-infective cycles occur in *Cryptosporidium parvum*. The first is by the continuous recycling of Type I meronts and the second through sporozoites rupturing from thin-walled oocyst.

Experimentally infected animals have shown a prepatent period of 4 days but sometimes it could be 3 days in heavy infection. In humans when lower numbers of oocysts are probably ingested, the prepatent period is typically 4 to 6 days. The length of time in which oocysts are shed in feaces generally lasts 6 to 18days (4 to 10 days of diarrhea) in immunocompetent individuals but it may be prolonged in immunocompromised individuals. Some patients may discharge oocyst yet they appear asymptomatic (Fayer *et al*., 2000).

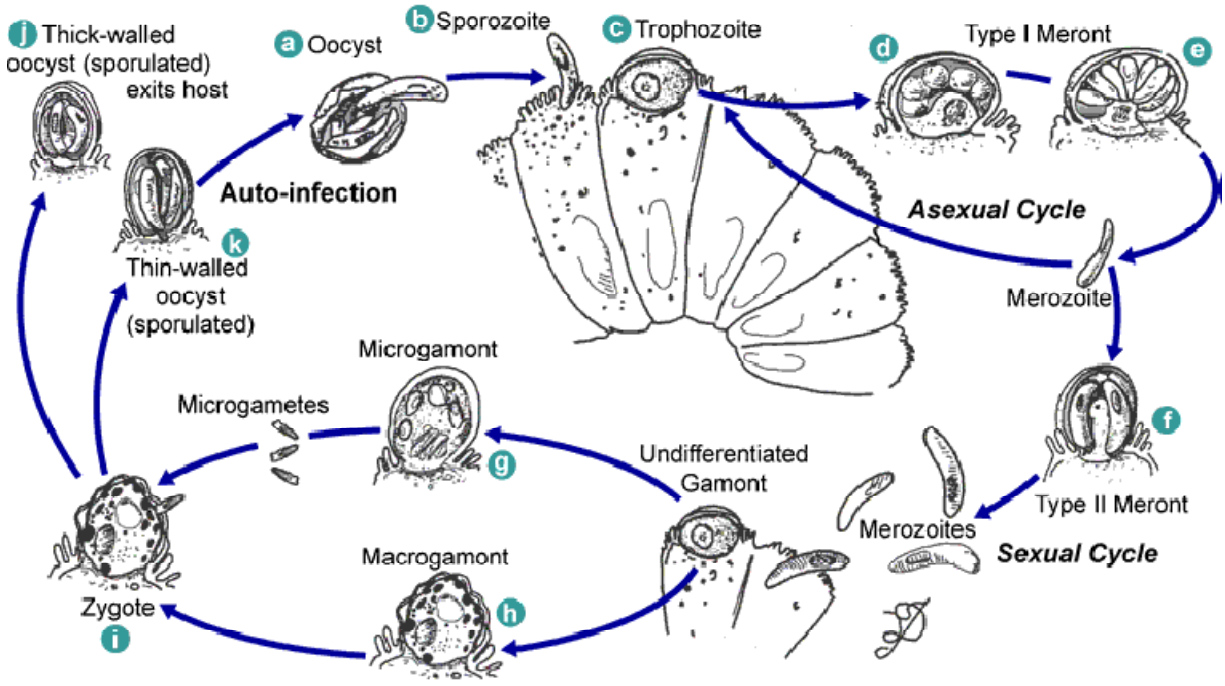

Figure 1 Life cycle of *Cryptosporidium* species (Source: PHIL 3386 - CDC/Alexander J. da Silva, PhD/Melanie Moser)

Developmental stages in duodenal and jejunal enterocysts, probably two generations of schizonts; following gamogony formation of spherical oocysts 8-10 µm in size. Prepotency about one week; oocysts are shed unsporulated in feces, then sporulate outside of host within five to 12 days to become infective. The sporulated oocysts contain two sporocysts with two sporozoites each (Fayer *et al*., 2000).

Isospora spp. is apicomplexan protozoan parasites that are taxonomically related to *Cryptosporidium*, *Toxoplasma*, and *Sarcocystis* spp., all members of the family Eimeriidae (suborder Eimeriina). *Isospora belli* is coccidian opportunistic intestinal parasite in HIV/AIDS patients and in some areas it is the cause of gastroenteritis. *I. belli* infections are essentially cosmopolitant in distribution but are more common in tropical and subtropical regions, especially Haiti, Mexico, Brazil, El Salvador, tropical Africa, the Middle East and South East Asia. In developed countries, immigrants are suspected as introducers of the disease (Curry and smith, 1998). Chronic infections are developed in some patients and oocysts are excreted for a

long duration, several months to years (Lindsay *et al,* 1997). This information has important implication in the era of HIV/AIDS pandemic.

The life cycle of *Isospora* is similar to that of other enteric coccidian parasites. It involves a multi step asexual stage (merogony), followed by sexual reproduction (gamogony) and the subsequent development of oocysts. Both the asexual and sexual stages develop in the intestinal cells of their hosts (several mammal species and humans), and produce an environmentally resistant cyst stage, the oocysts. The latter, when released into the environment, are usually unpopulated and non infective, requiring maturation to become infective (Lindsay *et al*., 1997). At time of excretion, the immature oocyst contains usually one sporoblast (more rarely two) (1).

In further maturation after excretion, the sporoblast divides in two (the oocyst now contains two sporoblasts); the sporoblasts secrete a cyst wall, thus becoming sporocysts; and the sporocysts divide twice to produce four sporozoites each(2). Infection occurs by ingestion of sporocysts-containing oocysts: the sporocysts excyst in the small intestine and release their sporozoites, which invade the epithelial cells and initiate schizogony (3). Upon rupture of the schizonts, the merozoites are released, invade new epithelial cells, and continue the cycle of asexual multiplication (4).Trophozoites develop into schizonts which contain multiple merozoites. After a minimum of one week, the sexual stage begins with the development of male and female gametocytes (5).Fertilization results in the development of oocysts that are excreted in the stool (1)(Figure 2).

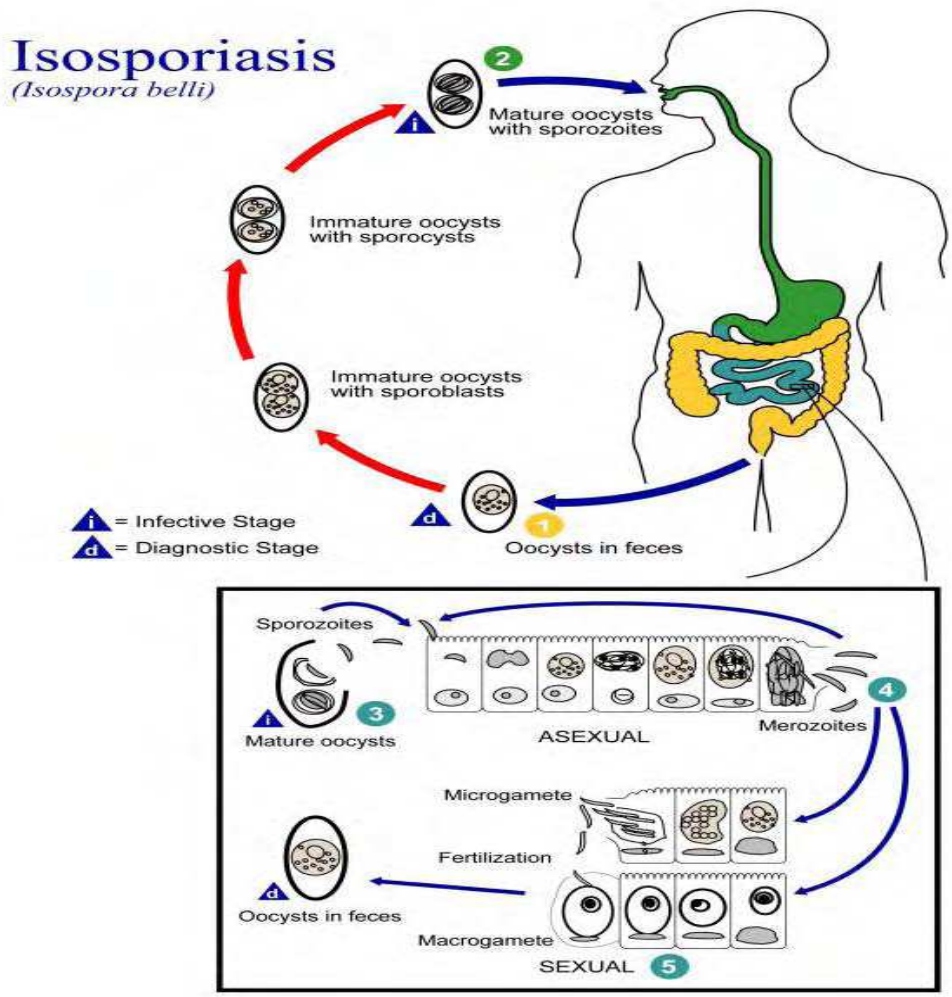

Figure 2 The life cycle of *Isospora belli* (Source: PHIL 3398 - CDC/Alexander J. da Silva, PhD/Melanie Moser).

Blastocystis hominis: Diarrhoeagenic intestinal parasites that were not recognized as such up to the recent past are emerging and increasing these days. The problem has become more serious with onset of HIV/AIDS pandemic. Among these the status of *Blastocystis hominis* as a cause of diarrhea is a controversial and not well-documented one (Jelinek *et al.*, 1997). Although *B. hominis* is often the most frequently reported from stool samples, its epidemiology is not clearly understood. The reason behind this could be lack of appropriate information about the epidemiology of the parasite, conflicting and paradoxical ideas on its classification and pathogenicity.

Based on ultrastuctural and structural evidences, *Blastocystis hominis* has now been classified under protozoa (Stenzel and Boreham, 1996). Some investigators have presented strong evidence for its pathogenicity, while others have considered it to be a commensal (Kaneda *et al*., 2002). Reports of asymptomatic and symptomatic *B. hominis* infections in humans are worldwide. *B. hominis* infections are predominantly reported from developing countries of tropical and subtropical regions. Travelers from the developed countries might be affected with *B. hominis* infection when they travel to these regions of the world (Jelinek *et al*., 1997). The infection rate has been reported to vary from 1.6% in industrialized countries to more than 50% in developing countries (Gericke *et al*., 1997). Infection with *B. hominis* has gained attention in case of immunocompromised individuals and HIV/AIDS patients (Escobedo and Nunez, 1997). The association of *B. hominis* with diarrhoea in immunosuppressed patients has been suggested in one study among Tanzanian children with chronic diarrhoea (Cegielski *et al*., 1993). Furthermore, molecular and immunological evidences have revealed that strain variation might be associated with pathogenic potentials (Kaneda *et al*., 2002).

In the most recent description of the *Blastocystis hominis* life cycle (Tan, 2004); infection in humans and animals is initiated when the fecal cysts are ingested. These develop into vacuolar forms in the large intestines, which subsequently reproduce via binary fission. Some vacuolar forms encysted and lose their surface coat during maturation. The environmentally resistant cyst is then transmitted to humans and animals via the fecal-oral route and the cycle is repeated.

Knowledge of the life cycle and transmission is still under investigation; therefore this is a proposed life cycle for *B. hominis*. The classic form found in human stools is the cyst, which varies tremendously in size from 6 to 40 μm (1).The thick-walled cyst present in the stools (1) is believed to be responsible for external transmission, possibly by the fecal-oral route through ingestion of contaminated water or food (2). The cysts infect epithelial cells of the digestive tract and multiply asexually (3, 4).Vacuolar forms of the parasite give origin to multi vacuolar (5a) and amoeboid (5b) forms. The multi-vacuolar develops into a pre-cyst (6a) that gives origin to a thin-walled cyst (7a), thought to be responsible for autoinfection. The amoeboid form gives origin to a pre-cyst (6a), which develops into thick-walled cyst by schizogony (7b).The thick-walled cyst is excreted in feces (1).

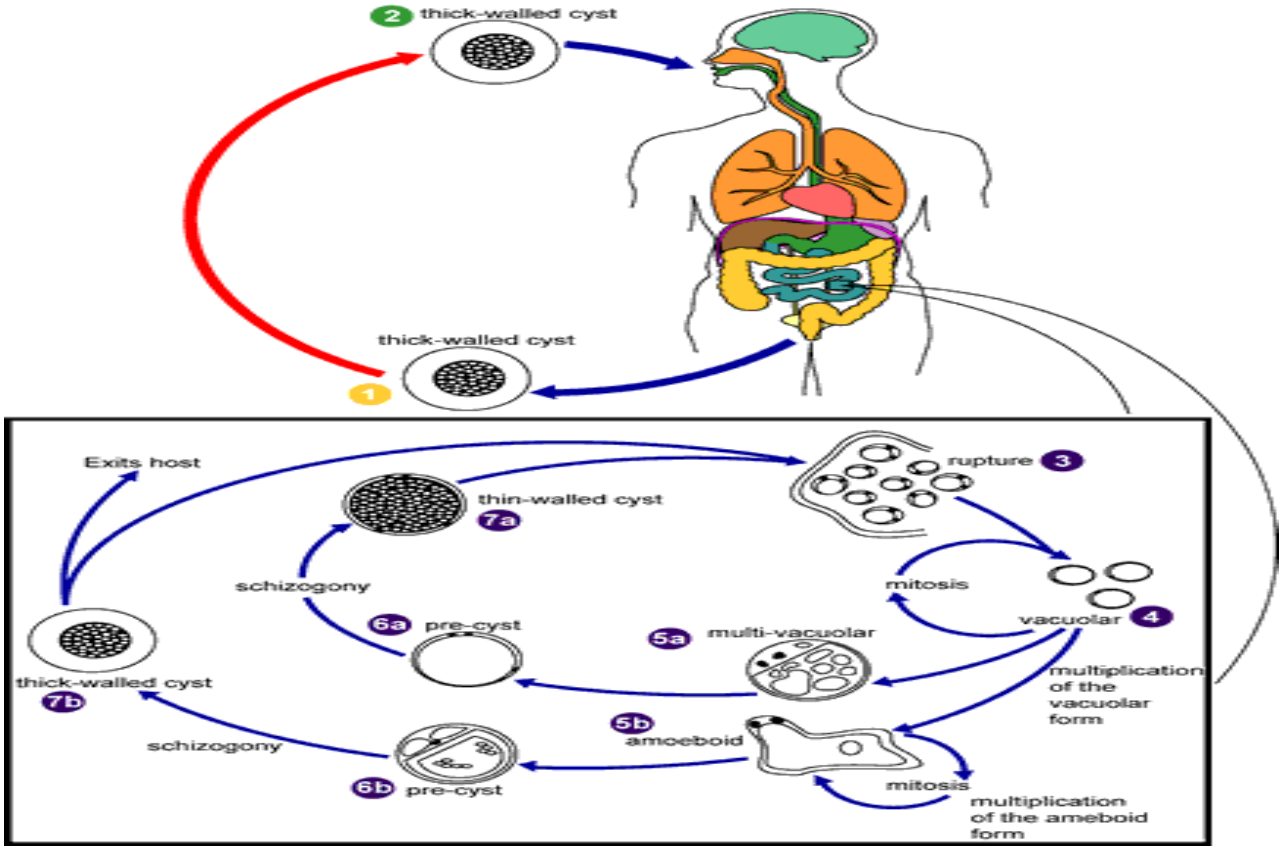

Figure 3 The life cycle of *Blastocystis hominis*. (Source: Parasite Res1995; 81:449)CDC.

Another newly defined coccidian opportunistic intestinal parasite in humans is *Cyclospora cayetanensis*. *Cyclospora* infections in humans have been documented since at least 1977. However, in the past 10 years or so, this pathogen has come into attention as the result of several major food borne outbreaks in USA and Canada, cases of prolonged gastrointestinal disease in travelers and expatriates associated with southeast Asia (Nepal, Pakistan), and the rise of the immunocompromised population (Mossimann *et al.*,1999; Herwaldt, 2000).

Recent phylogenetic analysis based on rRNA sequences suggested that *Cyclospora* was closely related to the Eimeria genus (Colombia *et al*., 1997). In humans, the habitat of *Cyclospora* is the enterocyte of the small intestine. All four asexual stages of Cyclospora (sporozoite, trophozoite, schizont, and merozoite) were observed within the parasitophorous vacuoles located in the apical

supranuclear region of the enterocytes (Sun *et al*., 1996). The term *Cyclospora cayetanensis* was first suggested in 1993 to define the infectious species in tropical and subtropical regions.

The life cycle of this organism is unknown; however environmental data suggest that *Cyclospora*, like *Cryptosporidium* species, is a water-borne parasite. The oocysts of *C. cayetanensis* are spherical, measuring 8-10μm in diameter and the mature oocyst contains 2 sporocysts. Oocysts of *C. cayetanensis*, are twice as large in comparison with *C. parvum* and are not sporulated (do not contain sporocysts - upon excretion). When freshly passed in stools, the oocyst is not infective (thus, direct fecal-oral transmission cannot occur; this differentiates *Cyclospora* from other important coccidian parasites).

In the environment, sporulation occurs after days or weeks at temperatures between 22°C to 32°C, resulting in division of the sporont into two sporocysts, each containing two elongate sporozoites. Fresh produce and water can serve as vehicles for transmission and the sporulated oocysts are ingested (in contaminated food or water). The oocysts excyst in the gastrointestinal tract, freeing the sporozoites which invade the epithelial cells of the small intestine. Inside the cells they undergo asexual multiplication and sexual development to mature into oocysts, which will be shed in stools. The potential mechanisms of contamination of food and water are still under investigation.

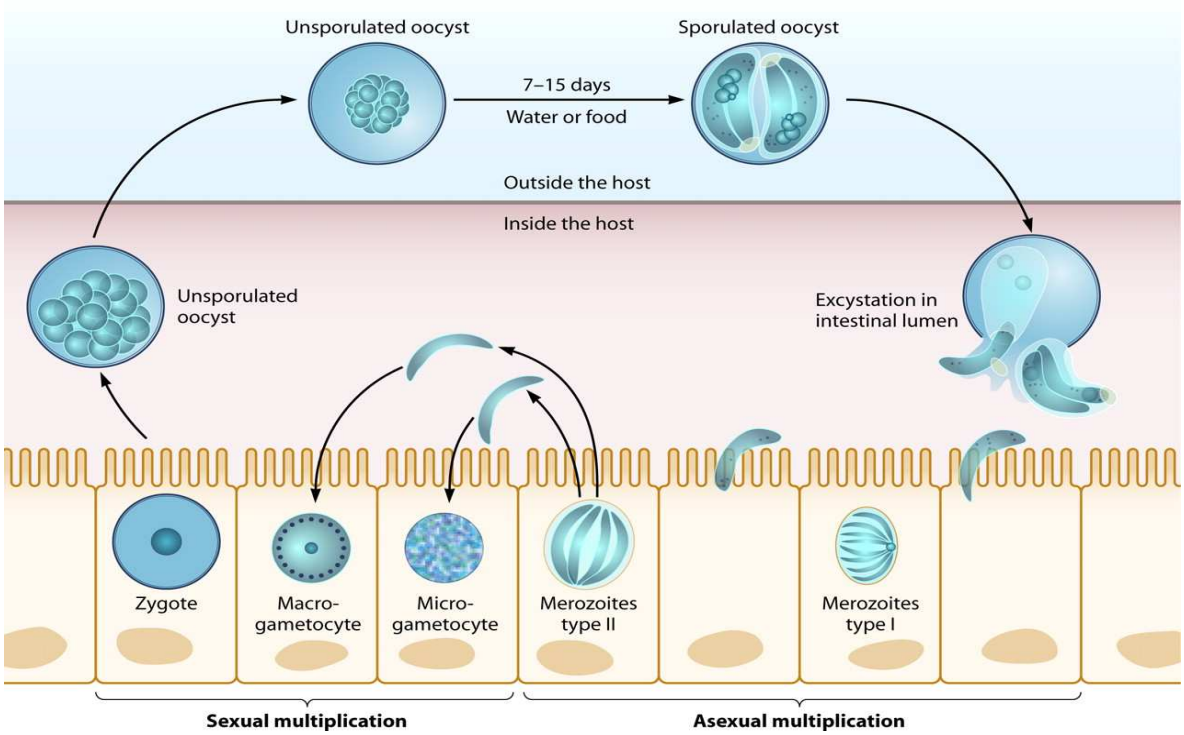

Figure 4 The life cycle of *Cyclospora cayetanensis* (source: CDC).

2.2. Major Intestinal Helminth Parasites and their Life Cycles

The Intestinal helminths vary greatly in size and female worms are larger than males (Despommier *et al*., 2005). After mating, each adult female produces thousands of eggs per day, which leave the body in the faces. People become infected with *Trichuris trichiura* and *Ascaris lumbricoides* by ingesting the fully developed eggs (Despommier *et al*., 2005). The common intestinal parasites such as *Ascaris lumbricoides*, *Trichuris trichiura*, Hookworm spp. and *Schistosoma mansoni* were very rare, and for each one of them the prevalence was below 2% in the HIV positives, and relatively high in the HIV negatives.over all the non opportunistic intestinal parasites such as *A. lumbricoides*, *Taenia* Spp. And *E. histolytica/dispar* was higher in HIV negatives than HIV positives (Endeshaw, 2004).

Among the helminths, disseminated strongyloidiasis caused by the nematode parasite. *Strongyloides stercoralis* has become increasingly recognized in immunocompromised humans

(Heyworth, 1996). It was commonly reported from patients with leukaemia, lymphoma including T cell types and those with organ transplantation that are put on long term corticosteroid chemotherapy (Schaffel *et al.*, 2001). The association between overwhelming strongyloidiasis and impaired cellular immunity would implicate that the depression of cell mediated immunity by HIV infection result in severe strongylodiasis in area where the parasite is endemic.

Ascaris lumbricoides is characterized by its great size. Males are 2–4mm in diameter and 15-31cm long. The males' posterior end is curved ventrally and has a bluntly pointed tail. Females are 3–6mm wide and 20–49cm long. The vulva is located in the anterior end and accounts for about a third of its body length. Uteri may contain up to 27 million eggs at a time with 200,000 being laid per day. Fertilized eggs are oval to round in shape and are 45-75 micrometers long and 35-50 micrometers wide with a thick outer shell. Unfertilized eggs measure 88-94 micrometers long and 44 micrometers wide (Roberts *et al.*, 2009).

Ascaris lumbricoides, or "roundworm", infections in humans occur when an ingested infective egg releases a larval worm that penetrates the wall of the duodenum and enters the blood stream. From here, it is carried to the liver and heart, and enters pulmonary circulation to break free in the alveoli, where it grows and molts. In 3 weeks, the larvae pass from the respiratory system to be coughed up, swallowed and thus returned to the small intestine, where they mature to adult male and female worms. Fertilization can now occur and the female produces as many as 200,000 eggs per day for a year. These fertilized eggs become infectious after 2 weeks in soil; they can persist in soil for 10 years or more (Murray *et al.*, 2005).

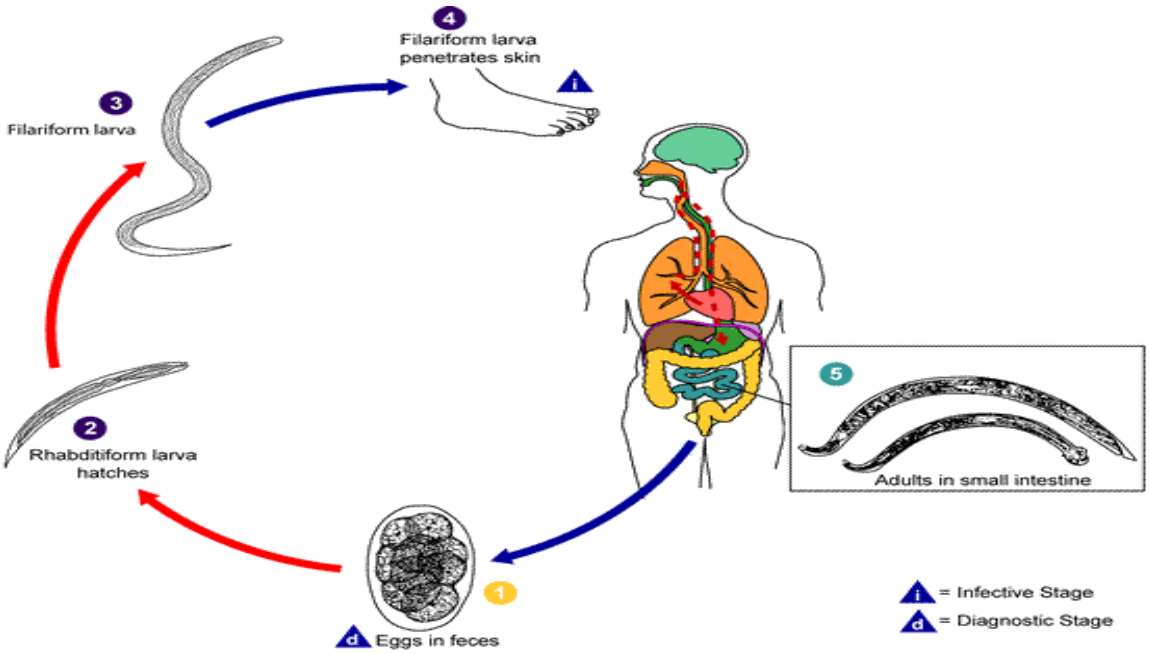

Figure 5The life cycle of hookworms (Source: CDC, 2007)

Strongyloides stercoralis: Chronic infection with *S. stercoralis* is most often asymptomatic. Hyperinfection describes a syndrome of accelerated autoinfection which results from immunosuppression. Gastrointestinal and pulmonary symptoms are common but non-specific, and include abdominal pain, diarrhea, vomiting, dynamic ileus, small bowel obstruction (SBO) and protein-losing enteropathy, as well as pneumonia (Keiser, 2004).

S. stercoralis has a complex dual life cycle that includes parasitic and free-living cycles.Infections in man arise when soil larvae penetrate the skin, pass in the blood to the lungs, and then ascend the tracheobronchial tree to be swallowed. In the duodenum and proximal jejunum female worms lay embryonated eggs that hatch internally (Grove, 1996). The resultant first-stage larvae (L1; rhabditiform larvae) are passed out in the feces and may develop directly into second (L2)–stage and third (L3; filariform larvae)–stage larvae may develop through 4 free-living larval stages to become free-living adult males and females. The free living adults reproduce sexually to produce L1, which also develop to L3. The L3 of either cycle can penetrate the skin of the human host, pass through the circulation to the lungs, enter the airways, be swallowed, and finally reach the intestine, where they mature into adult egg-laying females (Liu

and Weller, 1993). The massive larval tissue invasion causes an often fatal syndrome of diarrhoea, malabsorption, septicaemia, and encephalopathy.

The life cycles of most helminths follow the same pattern, adult hookworms of the genera *Necator* and *Ancylostoma* parasitise the upper part of the human small intestine, whereas *ascaris* round worm parasitise the entire small intestine and adult *trichuris* whipworms live in the large intestine, especially the caecum (Despommier *et al*., 2005). The parasites can live for several years in the human gastrointestinal tract. Human beings are regarded as the only major definitive host for these parasites, although in some cases *ascaris* infections can also be acquired from pigs (Crompton, 2001).

2.3. Pathogenesis and Clinical Manifestation of Human Intestinal Parasitic Infections

2.3.1. Human Intestinal Protozoan Parasite Infections

Cryptosporidium parvum can be found throughout the gastrointestinal tract; however it appears to have an affinity for epithelial cells in the jejunum, ileum and proximal colon. Cholangiocytes are also susceptible to infection, and apoptosis of these epithelial cells likely contributes to biliary tract disease. The respiratory tract also appears to be a site of infection in immunocompromised individuals. Epithelial cell death, by both apoptotic and necrotic mechanisms, has been noted in involved regions. There is evidence that infected epithelial cells can induce apoptosis in neighboring uninfected cells (Abhay *et al*., 2009).

In general, Good game (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 cryptosporidiosis. Immunocompetent and immunocompromised individuals differ greatly in their clinical symptoms. The most frequently observed clinical manifestations of cryptosporidiosis are profuse and watery diarrhea, often containing mucus but rarely blood or leucocytes and the symptoms include abdominal cramp, low grade fever, nausea and vomiting (Chen *et al*., 2002). The

reported incubation periods range from 3 to 22 days, for an average of 1 week (DuPont *et al.*, 1995). The severity and longevity of *Cryptosporidium* infections are directly related to the immune status of the host (Clark, 1999) lasting 3 to12 days in the immune-competent and up to several months in the immune-compromised host (Current and Garcia, 1991).

Clinical sign are generally more chronic and severe in the immuno-compromised and are not always confined to the gastrointestinal tract. Extra intestinal infection of the respiratory tract such as pancreatic duct, gallbladder and biliary tree has all been documented in human immunodeficiency virus infected patients (Hunter and Nichols, 2002). Mucus may be associated with diarrhoea, but blood or leukocytes are rarely reported in AIDS patients with cryptosporidiosis. About 2 to 7 liters per day (sometimes more) of diarrhoea have been reported (Manabe *et al.,* 1998). Most healthy hosts develop immunity after infection. In immunosuppressed hosts, however, like HIV infected humans, recovery is difficult and severe dehydration can lead to death (Hunter and Nichols, 2002).

Isospora belli, Cases of disseminated isosporiasis (lymph nodes, liver, and spleen) in AIDS patients have been reported (Bernard *et al.,* 1997) in order to assess the effect of the HIV epidemic on mortality from opportunistic infections in 1993 (Selik *et al.,* 1997). The overall results showed that the HIV epidemic had greatly increased the mortality from opportunistic infections; thus, the percentage of death with HIV as the underlying cause and the ratio of observed to predict death rate for cryptosporidiosis/isosporiasis were respectively, 90% and infinite (1.61/0.00).

Blastocystis hominis are diarrhoeagenic intestinal parasites that were not recognized as such up to the recent past are emerging and increasing these days. The problem has become more serious with onset of HIV/AIDS pandemic. Among these, the status of *Blastocystis hominis* as a cause of diarrhea is a controversial and not well-documented one (Jelinek *et al.,* 1997). Although *B. hominis* is often the most frequently reported from stool samples, its epidemiology is not clearly understood. The reason behind this could be lack of appropriate information about the epidemiology of the parasite, conflicting and paradoxical ideas on its classification and pathogencity.

Reports of asymptomatic and symptomatic *B. hominis* infections in humans are worldwide. *B. hominis* infections are predominantly reported from developing countries of tropical and subtropical regions. Well recognized clinical signs due to *B. hominis* in symptomatic individuals, include abdominal discomfort or pain, diarrhoea, nausea, vomiting, flatulence, gastroenteritis, colitis and other minor complaints (Stenzel and Boreham *et al*., 1996).

The most typical signs of *C. cayetanensis* disease include persistent, acute or protracted, relapsing watery, non bloody diarrhea that begins days or weeks after infection (Chambers *et al*., 1996). The onset may be abrupt or gradual with such symptoms as nausea, vomiting, anorexia, bloating, abdominal cramping, increased gas, watery diarrhea, fatigue, and malaise (Brennan *et al.*, 1996). Although *Cyclospora*-associated diarrhea may be acute or chronic, the latter appeared to occur more frequently (Sifuentes-Osornio *et al*., 1995).

Entamoeba histolytica/dispar/ is an intestinal parasite that characterized by possessing clear protoplasm which form pseudopodia. These pseudopodia are the means by which the organisms move and use for feeding purposes. The two species *Entamoeba hitolytica* and *Entamoeba dispar* are morphologically identical but pathologically distinct (WHO, 1997). However, only *E. hitolytica* is capable of causing disease (medically important).

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 about 10% of the world population is infected with *E. histolytica/dispar*/ (Gonin and Trudel, 2003), but most infections occur due to the noninvasive species (Petri and Singh, 1999). Asymptomatic infection with *E. histolytica* is defined as the presence of cysts in stools in the absence of colitis or extra intestinal infections. These healthy carriers may pass millions of cysts in the stool per day as the trophozoites multiply in the intestinal lumen (Petri and Singh, 1999; WHO, 1997). Approximately, 90% of all intestinal *E. histolytica* infections are asymptomatic. Clinical symptoms of acute intestinal amoebiasis include diarrhea, bloody stool that may contain necrotic mucosa, abdominal pain, tenderness and fever (Perti and Singh, 1999).

In Ethiopia, more than 60% of the diseases are caused due to poor environmental health conditions arising from unsafe and inadequate water supply and poor hygienic and sanitation practices. According to the Ministry of Health (1997), nearly 80% of rural and 20% of urban population have no access to safe water. A number of studies and routine diagnosis in Ethiopia indicate that amebiasis is one of the most widely distributed diseases.

Giardia lamblia (also known as *Giardia duodenale or G. intestinalis)* is a unicellular flagellated intestinal protozoan parasite of humans isolated worldwide and ranked among the top 10 parasites of man. 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, clinical symptom of giardiasis includes diarrhea, epigastric pain, wasting and impaired absorptions (Orteg and Adam, 1997).

Giardia lamblia is the most protozoan intestinal parasites isolated world wide as causative agents of diarrhea. Epidemiological studies suggest that the parasite is responsible for about 5% of acute diarrhea and 20% of chronic diarrhea illness in the world. The incidence of diarrhea 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).

2.3.2. Human Intestinal Helminth Parasitic Infections

Parasitic helminths (worms) that infect humans belong to two phyla, Platyhelminths and Nematoda. The common intestinal helminths are trematodes (flukes) includes *Schistosoma mansoni*), nematodes (round worms) includes *Ascaris lumbricoides, Trichuris trichiura* and hook worms (*Necator americanus* and *Ancylostoma duodenale*) and cestodes (tape worms) includes *Hymenolepis nana, Taenia saginata* and *Taenia solium.* Helminthic infections are enhanced by poor socio-economic conditions, lack of sanitary facilitates, improper disposal of human feces, insufficient supplies of potable water, poor personal hygiene, poor housing conditions and lack

of education (WHO, 1996). According to Montresor *et al*. (1998), at global burden, over one billion of the world's population is estimated to be infected with helminthes parasites and over two billion people are at risk.

Ascaris lumbricoides is the largest of the intestinal nematodes found in man. Crompton (1999) reported that *A. lumbricoides* infects at least one-fourth of the world's population. Annual morbidity associated with the parasite has been estimated by WHO at 60,000 with another 250 million peoples said to be at risk for acquiring the infection (Montresor *et al.*, 1998). *A. lumbricoides* is a robust parasite.

Cestodes: cestodes are tapeworm, specialized flatworms, looking very much like a narrow piece of adhesive tape. Tapeworms are the largest, and among the oldest, of the intestinal parasites that have plagued humans and other animals since time began. The most important cestodes affecting humans and animals in Ethiopia are *Taenia saginata,* and *Hymenolepsis nana,* the former due to the custom of eating raw meat and the later due to unhygienic food consumption with contaminated hands and fingers that allow the ingestion of eggs from the faeces of an infected person (Belete and Kloos, 2006).

The adults of *Taenia saginata and Taenia solium* live in the intestine and are very large worms, i.e. several meters in length. Proglottids as well as eggs appear in faeces. The eggs of the two species are identical; they are round to oval in shape, measuring 35-43 μm in diameter and have a thick, radially-striated shell. The egg contains a 6-hooked embryo called an oncosphere or hexacanth. These eggs must be handled with extreme care because the egg of *Taenia solium* is infective to humans and produces cysticercosis (WHO, 2004).

2.4. Epidemiology of Human Intestinal Parasite Infection

2.4.1 Epidemiology of Intestinal Protozoan Parasite Infection

Cryptosporidium has a wide geographic distribution, though infection is more prevalent in regions of the world with poor sanitary conditions. Infection is more common during warm rainy

months. The reported prevalence of infection varies widely and is influenced by geographic region, age, immune status, local outbreaks and the range of sensitivities and specificities offered by different diagnostic modalities (Abhay *et al*., 2009). A major route of infection with *Cryptosporidium* is person-to-person transmission, as illustrated by outbreaks in day-care centers, persons with animal contact (e.g., farmers), or through the environment via contaminated drinking water or recreational water sources such as swimming pools and lakes (Abhay *et al*., 2009). The infection results from oral ingestion of as low a dose as approximately 30 infectious oocysts (DuPont *et al.*, 1995) invading the microvillous border of epithelial cells in the intestines. The infectivity, however, is affected by the type of infecting isolate and the immune status of the host (Teunis *et al.*, 2002).

Cryptosporidium parvum infection has been reported in 10 to 15% of children with diarrhea and 30 to 50% of AIDS patients with chronic diarrhea in the developing world (Colford, 1996). Infection principally occurs in the intestine of both immunocompetent and immunocompromised individuals, however, belier cryptosporidiosis has been reported in HIV infected patients, with 20-65% of the patients presenting with the so called AIDS cholangiopathy. In contrast when samples from asymptomatic individuals were examined, the prevalence ranged from 0 to 2% in developed nations compared with 0 to 9.8% in case of developing countries (O'Donoghue, 1995).

Most oocyts of *Isospora* are excreted unsporulated and undergo a developmental period (sporulation) outside the host and become infectious. Sporulated oocyst of *Isospora belli* are characterized by having two sporocysts and each sporocyst in turn containing four sporozoites. The oocysts of *Isospora belli* in humans measure 20-33 μm by 10-19 μm. They are elongated and ellipsoidal (Curry and smith, 1998). Transmission in humans occurs via faecal-oral route, mainly by ingestion of infectious oocysts from contaminated food and/or water.

Blastocystis hominis is transmitted by faecal-oral contamination, in a manner similar to other gastrointestinal protozoa (Leelayoova *et al.*, 2004). There are accumulating reports that *Blastocystis* is associated with intestinal disorders in individuals immunocompromised by HIV or immunosuppressive therapy (Cimerman *et al*., 2003; Hailemariam *et al*., 2004) suggesting that

Blastocystis is an opportunistic pathogen. The parasite is also common among food and animal handlers (Danchaivijitr *et al.,* 2005), although only one (Rajah Salim *et al.,* 1999) of these surveys included a comparison with a control population. However, it appears clear that cyclosporiasis can be contracted through consumption of fecally contaminated water supplies or food (Brennan *et al*., 1996). Some patients have been infected from accidental ingestion of aquarium water and from swimming in Lake Michigan. It seems likely that water supplies may be contaminated by bird droppings (Wurtz *et al.,* 1993). Person-to-person transmission is unlikely because excreted oocysts require days to weeks, under favorable environmental conditions, to sporulate and become infectious (Chambers *et al.,* 1996).

E. histolytica/dispar/ is transmitted as a result of the ingestion of infective cysts in food or water contaminated with sewage or from hands of persons contaminated with faeces. Lack of personal hygiene among cyst carriers contributes to the spread of the infection, enhanced by feeding on faeces containing the cysts and subsequently contaminated food (Cheesbrough, 1998). This protozoan parasite has a global distribution and an especially high prevalence in countries where poor socioeconomic and sanitary conditions predominate (Stanley, 2003). In developing countries, the infection occurs primarily among travelers to endemic regions, recent immigrants from endemic regions, homosexual males, immuno-compromised persons and institutionalized individuals (Swords and Canytey, 2002).

Giardia is the most commonly diagnosed intestinal parasite (Huang and White, 2006). Giardiasis is a typical fecal-oral disease with the cyst shed in the feces of the infected host contaminating food, water or fomites, ultimately leading to the ingestion of those cysts by the next host. Typically, giardiasis in humans occurs in areas where sanitation standards are low. Human outbreaks have been reported numerous times often associated with failure in a water-supply purification system, the lack of proper water purification, drinking from untreated/unfiltered surface water. *Giardia* and giardiasis are worldwide in distribution the prevalence appears greatest in the developing countries. While infants and young children seem to have the highest prevalence of infection, giardiasis occurs at all ages and apparently without gender preference normally (Thompson, 2002).

2.4.2 Epidemiology of Intestinal Helminth Parasite Infection

Intestinal helminth infections are widely distributed throughout the tropics and subtropics. Climate is an important determinant of transmission of these infections, with adequate moisture and warm temperature essential for larval development in the soil (Brooker *et al*., 2006). Equally important determinants are poverty and inadequate water supplies and sanitation (de Silva *et al*., 2003). In such conditions, soil transmitted helminth species are commonly co - endemic. There is evidence that individuals with many helminth infections have even heavier infections with soil transmitted helminths (Raso *et al*., 2004).

A study estimated that *Ascaris lumbricoides* infects 1,221 million people, *Trichuris trichiura* 795 million and hookworms infect 740 million (Brooker *et al*., 2006). Approximately, 85% of the Neglected Tropical Diseases (NTDs) burden results from STH infections (Hotez and Kamath, 2009). Moreover, it has been estimated that STH infections account for 12% of the total disease burden and about 20% of disability adjusted life years (DALYs) lost due to communicable diseases in children, globally (Awasthi *et al*., 2003).

The geographic distributions of *Ascaris* are worldwide in areas with warm, moist climates and are widely overlapping. Infection occurs worldwide and is most common in tropical and subtropical areas where sanitation and hygiene are poor (Bethony *et al*., 2006).

Human hookworm infection is caused by blood-feeding nematode parasites of the genus *Ancylostoma* and the species *Necator americanus*. Worldwide, *N. americanus* is the predominant etiology of human hookworm infection, whereas *A. duodenale* occurs in more scattered focal environments (Hotez *et al*., 2004). The worldwide number of cases of hookworm was recently estimated to be 740 million people (de Silva *et al*., 2003). The highest prevalence of hookworm occurs in sub-Saharan Africa and eastern Asia High transmission also occurs in other areas of rural poverty in the tropics, including southern China, the Indian subcontinent, and the Americas. In all regions, there is a striking relationship between hookworm prevalence and low socioeconomic status (de Silva *et al*., 2003).

2.5. Current Situation of Opportunistic Intestinal Parasite and HIV Infection in Ethiopia

Intestinal parasitic infections are among the major cause of diseases of public health problems in sub-Saharan Africa. Ethiopia is among the sub-Saharan countries with overlapping high rate of HIV and parasitic infections (Belete and Kloos, 2005). Several parasites have been implicated as major contributors to morbidity in HIV-infected persons living in Ethiopia, and the common intestinal protozoan parasites frequently encountered include: *Cryptosporidium spp*., *I.belli, B.hominis, Cyclospora, E. histolytica* and *Giardia lamblia* (Assefa *et al*., 1996).

C. parvum and *I. belli* is now becoming a common opportunistic intestinal protozoan parasite in Ethiopia. Reports from different parts of the country showed different prevalence rates of opportunistic parasitic infection. The prevalence of cryptosporidiosis in AIDS patients ranged from 8% in selected ART centers in Adama, Afar and Dire Dawa, 11% in South Western Ethiopia, 20.1% in Hawassa Referral Hospital, 25.9% in selected Addis Ababa Hospitals, 29% in Northwest Ethiopia and 40% in Addis Ababa Tikur Anbessa Teaching Hospital (Tadesse and Kassu, 2005; Assefa *et al.,* 2009).

The prevalence of *I.belli* in AIDS patients ranged from 2.4% in Gonder, 1.4% in Addis Ababa (Tadesse *et al*., 2005). The other like *B.hominis* and *C.cayetanensis* in HIV/AIDS pateints also reported from diferent parts of Ethiopia showed that 0.9% in Addis Ababa, 2.5% in Adama, Afar and Dire-Dawa, 10.5% in Bahir Dar, 14.1% in Jimma, 59% in Wonji and 3.7% in Jimma, respectively.

Although, the prevalence rates of giardiasis is different throughout the country. The prevalence of giardiasis in AIDS patients ranged from 11.2% in Hawassa Referral Hospital and 16% in selected ART centers in Adama, Afar and Dire-Dawa (Haileeyesus and Beyene *et al*., 2009). Amoebiasis is a common health problem and is prevalent in poor hygienic areas. Amoebiasis cases are most commonly found in rural areas due to poor water supply and lack of education (Sahih *et al*., 2011). Different parts of Ethiopia have different prevalence rates of amebiasis among HIV patients. The prevalence of amebiasis in AIDS patients ranged from 7.3% in North west Ethiopia, 10.3% in South Western Ethiopia, 13% in selected Antiretroviral Therapy (ART)

centers in Adama, Afar and Dire Dawa and 24.8% in Hawassa Teaching and Referral Hospital (Getachew *et al*., 2004; Assefa *et al.*, 2009; Huruy *et al*., 2011).

Intestinal helminth infections are endemic and cause considerable morbidity in Ethiopia. Low socio-economic status, cultural practice, low level of the environmental sanitation and livelihood requiring daily and frequent contact with contaminated water favor the transmission and wide distribution of helminths (Jeffrey *et al*., 2006). Various investigations conducted on geohelminths in Ethiopia have shown the far reaching implication of helminth infections to public health in many Ethiopian communities (Jemaneh, 2000). Difference in prevalence rates of opportunistic intestinal parasitic infection in different parts of Ethiopia among HIV patients may probably because of the low standard of environmental and personal hygiene, contamination of food and drinking water supplies that results from improper disposal of human excreta, and difference with immune status of study subjects, close contact with cattle, and living in overcrowded situations with many family members coupled with poor personal hygiene (Ayalew *et al.*, 2008).

2.6. Diagnosis of Intestinal Parasite Infections

Clinical laboratory diagnosis of *C.parvum* is primarily by stool examination and oocyst visualization (Stephen and Richard, 2001). Diagnostic testing for *Cryptosporidium* should be performed in HIV-infected patients who develop acute diarrhea, chronic diarrhea, or biliary tract disease, especially if their $CD4^+$ count is < 200 cells/mm^3. The easiest way to confirm the diagnosis is to identify oocysts with microscopic examination of stool or tissue. Routine ova and parasite testing does not detect *Cryptosporidium.* The modified acid-fast stain, which stains the organism red, is the most common method used to detect *Cryptosporidium* on stool microscopic examination (Chen *et al.*, 2002). *Cryptosporidium* can be differentiated from yeasts, which are similar size and shape but are not acid fast (Goodgame, 1996) and from other protozoan parasites, such as *I. belli* and *Cyclospora* species, based on size. Patients with mild disease may require repeat stool testing due to the high false negative rates with low organism burden. If modified acid fast staining is negative and clinical suspicion is high, direct immune-fluorescence microscopy and ELISA testing of stool have greater sensitivity and specificity (Chen *et al.*,

2002). Recently, new genetic methods of detecting *Cryptosporidium* have been developed, using polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), or other DNA-based detection methods (Coupe *et al.*, 2005).

Isospora belli can be diagnoised by examination of a fecal specimen for the oocysts is recommended. However, wet preparation examination of fresh material either as a direct smear or as concentrated material is recommended rather than the permanent stained smear (Garcia *et al.*,2007); National Committee for Clinical Laboratory Standards, 1997(Rigo and Franco, 2002). The oocysts are very pale and transparent and can easily be overlooked. They can also be very difficult to see if the concentration sediment is from polyvinyl-alcohol-preserved stool. These organisms are modified acid-fast positive and can also be demonstrated by using auramine-rhodamine stains. Organisms tentatively identified by using auramine-rhodamine stains should be confirmed by wet smear examination or modified acidfast stains, particularly if the stool contains other cells or excess artifact material (more normal stool consistency). Like *Cyclospora cayetanensis*, *I. belli* unstained oocysts will autofluoresce; they appear blue/violet under ultraviolet light and green under violet or blue/violet light. However, often the oocysts are seen in the concentration sediment wet preparation, and additional testing is not required for the diagnosis of isosporiasis.

Blastocystis poses considerable challenges to the diagnostic laboratory, for a number of reasons. The uncertain pathogenesis of the protozoan does not encourage microbiologists to look for the organism in specimens. The organism can be rather nondescript even in stained preparations, and can be confused as yeast, *Cyclospora* or fat globules. The pleomorphic nature of the parasite complicates identification. Lastly, the fecal cyst may predominate in cultures but are extremely difficult to identify without concentration methods due to their small size (3-5μm). *Blastocystis* is traditionally identified by looking for vacuolar forms in direct stool specimens (Andiran *et al.*, 2006), although this approach has been shown to be rather insensitive (Termmathurapoj *et al.*, 2004).

Present-day diagnostic laboratories should also include fecal cysts as an indicator of infection. These can be selectively concentrated by density-gradient approaches to increase sensitivity

(Zaman, 1996). In a recent report, it was observed that *in vitro* cultures of stool specimens were six and two times more sensitive when compared to simple smears and trichrome staining respectively (Termmathurapoj *et al.,* 2004). However, the same study revealed that the culture method did fail to detect some parasites that simple smears and trichrome staining did, indicating that not all *Blastocystis* isolates can be easily cultured *in vitro.*

Cyclospora cayetanensis, Cyclosporiasis has been diagnosed in immune-competent adults (Ooi *et al*., 1995), in children (Shlim *et a.,* 1991), immune-compromised patients (especially those with HIV infection) (Mossimann *et al*., 1999), patients with malignancies such as leukemia (Jayshree *et al*., 1998), and on rare occasions in asymptomatic carriers (Goodgame, 1996). Little is known about the host immune response to *C. cayetanensis* (Clarke *et al*., 1997). Its oocysts do not react with monoclonal antibody (MAb) specific for *Cryptosporidium parvum* (Ortega *et al*., 1993).

In the diagnosis of intestinal parasites, a wide variety of laboratory methods can be employed (Ahmadi *et al*., 2007). The choice of a particular technique for routine use is influenced by its affordability, simplicity, sensitivity and level of professionalism or technical skill involved (WHO, 2000). Stool microscopy using direct wet mounts, formol-ether concentration and the Kato-Katz technique offers many relative advantages over other diagnostic methods for detecting intestinal parasites (Bogoch *et al*., 2006). Since infections with multiple helminth species are norm rather than exception in the developing world, there is a need for well-trained laboratory technicians and quality control measures to ascertain accurate, specific diagnosis (Raso *et al.,* 2004).

2.7. Control and Prevention of Intestinal Parasite Infections

In the absence of effective and specific therapy against infection with *C. 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). The treatment of cryptosporidiosis is unsatisfactory (Abubakar *et al.,* 2007). There is no antimicrobial chemotherapeutic agent that will reliably eradicate the organism, 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). However, there are agents that appear to suppress infection. When highly active antiretroviral therapy reduces the HIV load, symptoms may resolve in patients with *Cryptosporidium* infection (Farthing, 2000). Treatment options of cryptosporidiosis depend largely on the immune status of the host, (Griffiths, 1998). Since the disease is self-limiting, in immune-competent 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 immune-compromised 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.*,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. 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.Recently,Nitazoxanide became an effective antibiotic against cryptosporidiosis in immunocompetent and probably in immune-compromised patients (Xian-Ming and LaRusso, 1999).

I. belli is thought to be the only species of *Isospora* that infects humans, and no other reservoir hosts are recognized for this infection. Transmission is through ingestion of water or food contaminated with mature, sporulated oocysts. Sexual transmission by direct oral contact with the anus or perineum has also been postulated, although this mode of transmission is probably

much less common. The oocysts are very resistant to environmental conditions and may remain viable for months if kept cool and moist; oocysts usually mature within 48h following stool evacuation and are then infectious (Frenkel *et al.,* 2003). It has been speculated that diagnostic methods for laboratory examinations may tend to miss the organisms when they are present. Since transmission is via the infective oocysts, prevention centers on improved personal hygiene measures and sanitary conditions to eliminate possible fecal-oral transmission from contaminated food, water, and possibly environmental surfaces (Certad *et al.,* 2003)

Effective eradication of the parasites has been achieved with cotrimoxazole, trimethoprim-sulfamethoxazole, pyrimethamine-sulfadiazine, primaquine phosphate-nitrofurantoin, and primaquine phosphate-chloroquine phosphate (Abramowicz, 1995). Other drugs proven to be ineffective include dithiazanine, tetracycline, metronidazole, phanquone, and quinacrine hydrochloride. The drug of choice is trimethoprimsulfamethoxazole, which is classified as investigational drugs for treatment of this infection.

In the most recent description of the *Blastocystis* life cycle (Tan, 2004); infection in humans and animals is initiated when the fecal cysts are ingested. These develop into vacuolar forms in the large intestines, which subsequently reproduce via binary fission. Some vacuolar forms encysted and lose their surface coat during maturation. The environmentally resistant cyst is then transmitted to humans and animals via the fecal-oral route and the cycle is repeated.

The need to treat individuals infected with *Blastocystis* has been equivocal, due to the uncertain pathogenesis of the organism and the observation that the disease is often mild and self-limiting. In cases where treatment is warranted, metronidazole (Flagyl) is the most commonly prescribed antibiotic (Nigro *et al.*, 2003). Various drug regimens for metronidazole have been prescribed ranging from 250–750 mg three times a day for 10 days (Moghaddam *et al.*, 2005), 1.5mg a day for 10 days (Cassano *et al*., 2005), or metronidazole may be used in combination with other drugs such as paromomycine (Pasqui *et al*., 2004) or co-trimoxazole (trimethoprim/ sulfamethoxazole) (Andiran *et al*., 2006).

Cyclosporiasis can be contracted through consumption of fecally contaminated water supplies or food (Brennan *et al.,* 1996). Some patients have been infected from accidental ingestion of aquarium water and from swimming in Lake Michigan. It seems likely that water supplies may be contaminated by bird droppings (Wurtz *et al.,* 1993). Person-to-person transmission is unlikely because excreted oocysts require days to weeks, under favorable environmental conditions, to sporulate and become infectious (Chambers *et al*., 1996). Strategies for prevention of *Cyclospora cayetanensis* infections are those usually recommended for avoiding any pathogen transmitted by the fecal oral route.

Treatment of cyclosporiasis consists of supportive care, maintenance of fluid and electrolyte status, symptomatic relief, and antibiotic therapy (Brown and Rotschafer, 1999). A number of antibiotics have been used in the treatment of cyclosporiasis, including metronidazole, norfloxacin, ciprofloxacin, quinacrine, nalidixic acid, tinidazole, diloxanide, spiramycin, and azithromycin, but without apparent benefit (Gascon *et al*.,1995).

The regulation of helminth populations is a complex process, influenced by host immunological and nutritional status, age and breed of the animal. The interaction between helminths infection and nutrition can be considered from two interrelated points of view: the influence of the helminth infections on the host's physiology and nutrition and the effect of host nutrition on the helminth populations, *i.e.* their establishment, persistence and reproductive capacity (Kaunas, 2007).

A well structured control strategy needs to be based on local and accurate data concerning the epidemiology, definition of appropriate chemotherapy and health education campaign sanitation monitoring and evaluation programmers (Albonico *et.al.,* 1999). All these component need to be integrated into the prevailing system of primary health care and must be based on multispectral collaboration (WHO, 1998), a goal often difficult to carry out in practice, which is why it is common to find control programs based on some of these elements and with limited results. The basic control programs, oriented for treating patients' chemotherapy, sanitation, oriented to stop or disconnect the exposure to the oral fecal transmission, and education, the key used as an instrument to implement the two ways of control programs. Control programs based on

sanitation aim to reduce or interrupt transmission, prevent re-infection and gradually reduce worm loads (Bundy, 1994). However, to be effective in a short period of time they need to be combined at their first stage with chemotherapy. Long term sanitary control programmers need to add elements to improve the economic conditions of a region, to ensure a reliable and permanent sanitation system and have permanent health education.

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

The development of effective helminth control is possible because of the availability of proven, cost-effective and logistically feasible intervention strategies. The choice of an appropriate anti-helminthic drug depends on its safety record; its therapeutic effect (cure rate or efficacy), its spectrum activity, local health policy, and financial considerations. A key issue for the optimal use of an anti-helmintic drug is to decide when and how frequently to treat the population of concern. Common drugs used are albendazole, pyrantel, mebendazole, tiabendazole and niclosamide. From an economic point of view, targated population chemotherapy programmes equal to half of the price of universal ones (O'Lorcain and Holand, 2000).

3. MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted at Othona Referral Hospital (ORH), Wolayita Sodo town, Wolayita Zone, SNNPR of Ethiopia from May-August, 2013(Figure 5). Wolayita Sodo is located between 6°36′ to 7°18′ north latitude and 37°12′ to 38°24′ east longitude (Figuer 5). The altitude ranges from 700-2940m above sea level. It is found in SNNPR, which is located at about 390km through Shashemane and 329km through Butajira-Hossana Southwest of Addis Ababa and 165km west of Hawassa. The annual rain fall ranges from 1200-1300mm. The mean annual temperature and humidity on average range from 17°C to 19°C and 50% to 60%, respectively (NMAABO, 2011).

According to the Central Statistical Agency (2007), the total population of Wolayita Sodo town is 102,929. Of these, 53,180 and 49,749 are males and females, respectively (Wolayita Sodo Town Finance and Economic Development Bureau, 2012). Wolayita Sodo town covers an area of about 24; 446km^2. It is divided in to 3- sub-towns (*kifile – ketemas*) and 11 *kebeles*. There are 20 private clinics (3 high clinics and 17 medium), 3 governmental health centers and two hospitals (one private and one governmental) in the town. Out of these health institutions, Othona Hospital was selected for this study due to accessibility of transportation, availability of parasite and HIV/AIDS related information and expected that greater number of HIV/AIDS patients visits Othona Hospital than other Health centers.

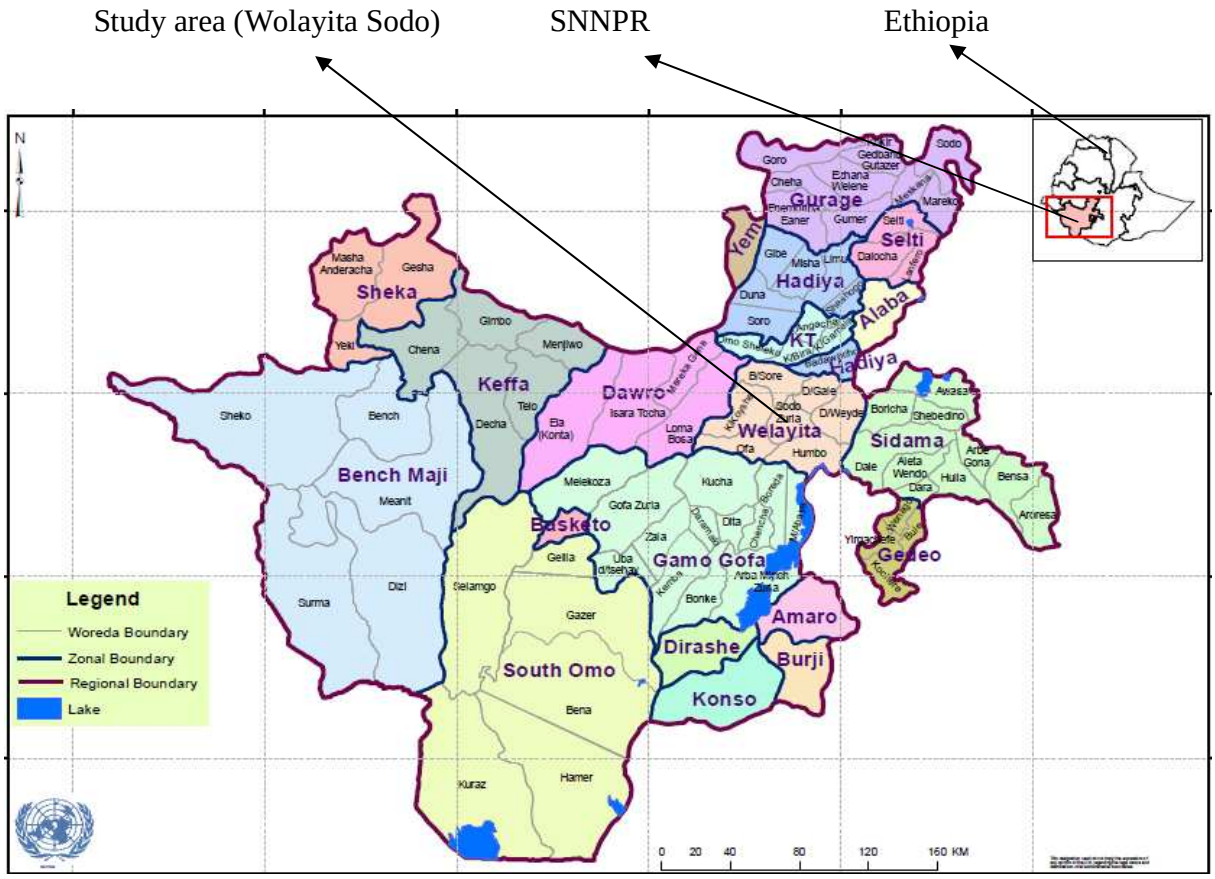

Figure 6 Map of Southern Nations, Nationalities, and People's Regional State showing the Study Area (WFP and DPPC, 2003).

3.2 Study Design

Hospital based cross-sectional survey study was conducted from May to August, 2013 in order to determine the prevalence of opportunistic intestinal parasitic infections among HIV/AIDS patients in the Othona Hospital, Wolayita Sodo town. Clinical, socio-demographic and anti-helminthic data were obtained through questionnaires and laboratory based diagnosis.

3.3 Target Study Population

Target population of the study was people living with HIV/AIDS (PLWHA) in the study area. The study population included PLWHA who came to Out Patient Department (OPD) of Othona

Hospital to monitor their disease status. People who were found to be HIV-positive at the hospital during the study period and referred from other health institutions for clinical and laboratory investigations were also considered in the study. The study did not include those who had taken drugs for any intestinal parasite for the past three months at the time of sample collection. In this study, patients less than 15 years of age were considered as children and their stool samples of them were brought with the help of their parents /care takers/.

3.4 Sample Size Determination

The sample size for this study was estimated by using the formula of Per Kish (1965) and assuming a prevalence of 50% since there was no study reported earlier in the study area.

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

$$\mathrm{n} = \frac{1.96^2 * 0.5(1 - 0.5)}{(0.05)^2} = 384$$

Where:

n= Total sample size;

P = prevalence of disease (50%)

d= Marginal error (0.05).

Z=1.96, where 95% confidence interval. Thus, considering 10% likelihood of non-compliance and non-responsive sample populations, four hundred twenty two (422) HIV/AIDS patients were included in the present study.

3.5 Sampling Method

Serial sampling technique was used to determine the prevalence of opportunistic intestinal parasites among HIV/AIDS patients attending Othona Hospital during the study time. Samples were collected from all in and out patient's people living with HIV/AIDS until the required sample size was reached.

3.6. Methods of Data Collection

3.6.1 Stool Sample Collection Procedure

Stool samples were collected by using serial sampling method from patients who came to Othona Hospital and who were eligible for this study as per the inclusion criteria mentioned earlier until the sample size was reached. Disposable plastic cups and applicator sticks were distributed to all sample population. Specific instruction for handling and avoidance of contamination of the stool samples were given to all study participants by the principal investigator and laboratory technician. They were also advised to bring stool samples about the size of a match stick head using cup and applicator sticks. The unique code of the study participants was labeled on the container. All stool samples for parasitological investigation were processed in the College of Health and Medicine laboratory of Wolayita Sodo University. All specimens were processed and analyzed within three hours of collection at the Othona Hospital Laboratory with the help of laboratory technicians.

3.6.2. Blood Sample Collection

Blood specimens were collected from opportunistic intestinal parasite positive patients aseptically by viepuncture into evacuate tubes containing EDTA anticoagulant, completely expanding the vacuum in the tubes. The blood specimens were mixed well with the anticoagulant to prevent clotting and were labeled.

3.6.3. Questionnaire Survey

All the 422 study participants were given a pre-tested structured questionnaire shown in Appendix II. The questionnaire was first developed in English and latter it was translated to Amharic language. The investigator also helped in reading the questionnaire for participants who cannot read and write. The purpose of the questionnaire was to collect data related to socio-demographic characteristics of the study subjects, major risk factors like source of drinking

water, practice of handling water, precaution during travel, source of food and feeding habit, type of occupation, presence of animals around home and contact with animals and their excreta. 422 questionnaires were dispatched and all of them were completely filled by respondents and returned to the principal investigator.

3.6.4. Health Record Analysis

Information related to HIV/AIDS patients who have been visiting the Othona Hospital for the last five years (from 2009 to August,2013) and opportunistic intestinal parasitic infections was collected by inspecting pateints' health records systematically using patients' health record review format developed by the principal invesigator.

3.6.5. Parasitological Examination Procedures

3.6.5.1. Direct Wet Mount

A direct wet mount of stool in normal saline (0.85% NaCl solution) was prepared and examined for the presence of motile intestinal parasites and trophozoites of *E.histolytica* and *G. lambilia*. Direct wet mount technique was employed as described in WHO (1997). With a marker the study identification number was written at one end of the slide and a drop of physiological saline was placed in the centre of the slide. Then, with a wooden applicator stick, a small portion of stool specimen (approximately 2 mg which was about the size of a match head) was picked and added to the drop of saline and thoroughly emulsified to make a thin uniform saline suspension not too thick that faecal debris may obscure organisms, and not too thin that blank spaces may be present. The suspension was carefully covered with a cover slip in a way as to avoid air bubbles. Finally the slide was placed on the microscope stage, and the preparation was examined systematically under the low power (10X) objective. When organisms or suspicious objects were seen, the high power (40X) objective was used to see more of the detailed morphology of the object for confirmation.

3.6.5.2. Formol-Ether Concentration Method

The concentration procedure recommended is the formalin-ether (or formalin-ethyl acetate) method. According to WHO (1997) standard procedure, protozoan cysts can be recovered by this method. Formalin–ether concentration technique was done at Othona Hospital. According to this method, using a wooden applicator stick, 1 gram of stool specimen was added to 10ml of 10% formalin in a small beaker and thoroughly emulsified, and brought into suspension. Next, the suspension was strained through a double layer of wet gauze directly into a 15 ml centrifuge tube. The gauze was then discarded, and more 10% formalin was added to the suspension in the tube to bring the total volume to 10 ml.

Then, 3ml of ether was added to the suspension in the tube, rubber stoppered and shaken vigorously for 10 seconds. The content was centrifuged at 2000rpm for 3minutes; the supernatant (comprising the top 3 layers) was discarded and iodine stain preparation was made using the sediment (Lindo *et al*., 1998). A few drops of the suspension was placed on microscope slide and covered with a cover slip. Finally, the preparation was examined microscopically using the low power (10 X) objective, and in a systematic manner as to observe the entire cover slip area. If an organism or suspicious objects are seen, the higher magnification (40 X) objective was used to observe its detailed morphology. Oocysts of *Isospora belli* can be demonstrated in feces after a formol ether concentration. *Cyclospora* oocysts from fresh stool wee fixed in 10% formalin and stained with modified acid-fast stain. Similarly, permanently stained preparations of fecal smears were used for identification of *Blastocystis hominis* (Lindo *et al*., 1998).

3.6.5.3. Modified Ziehl Neelsen Method

According to WHO (1997) Modified Ziehl- Neelsen method, Oocysts of *C. parvum* were determined by using the modified Ziehl-Neelsen staining method (fuschin followed by methylene blue); the oocysts were acid fast stained and observed characteristicaly spherical oocysts in fecal samples. According to this method a small portion of fresh stool sample was processed for detection of opportunistic intestinal parasites. Accordingly, a thin smear was prepared directly from fresh as well as from sediments of concentrated stool and allowed air dry.

The slides were fixed with methanol for 5 minutes and stained with carbolic fuchsine for 30 minutes. They were 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 observed under the light microscope with X1000 magnification (Asefa *et al*., 1996; Endeshaw *et al*., 2004).

3.6.6. CD4 Lymphocyte Counts

The $CD4^+$ T cell count were determined by using florescence activated cell scanning (FACS) analysis or flow cytometer (Becton Dickinson Immuno-cytometry system, and Jose, Calif., USA) in Othona Hospital. Briefly, 100 µl of whole blood was mixed with 10 µl of each monoclonal antibody combination in separate tubes and incubated at room temperature for 2 minutes. Red blood cells were lysed by adding 2ml of florescent activated cell sorter lysing solution (Becton Dickinson). Next, tubes were incubated in the dark at room temperature for 10 minutes and centrifuged at 300xg for 5minutes. The cell pellet was washed once with 2ml of isoton, resuspendesd in 500µl of isoton, and analyzed with simulset software (Becton Dickinson).

3.7. Data Analysis

The data collected from questionnaire survey in this study were included information related to socio-demographic characteristics. Frequency and cross tabulation was used to analyze those categorical data like age. The other major data collected were results of laboratory investigation i.e. parasite positivity, $CD4^+$ T cell count. Statistical analysis was performed by using SPSS version 16.0 software. Descriptive statistics were applied to state patterns of OIP in different immune categories as percentage and proportions. Chi-square statistics(X^2) and odd ratio (OR) was calculated to determaine/measure/ the degree of association between opportunistic intestinal parasitic infection and different risk factors. P-value ≤ 0.05 were considered statistically significant.

3.8 Data Quality Control

To ensure quality control, all the laboratory procedures including collection and handling of specimens were carried out in accordance with standard protocols (WHO, 1991). All the reagents were checked for contamination each time they were used. To ensure accurate identification of parasite species, bench aids for the diagnosis of intestinal parasites (WHO, 1994), and diagrams of various parasite ova and larvae from the parasitological were reviewed. Further, each slide was examined by two laboratory technicians and finally by the investigator.

3.9 Ethical Consideration

Ethical Clearance was obtained from University of Wolayita Sodo College of medicine and health science department of medical laboratory. The objectives and purpose of the study was explained to Othona Hospital administrative officials before the actual investigation.

4. RESULTS AND DISCUSSION

4.1. Socio-Demographic Characteristics of Study Participants

Socio-demographic characteristics of the study population are presented in Table 2. A total of 422 HIV/AIDS patients were examined for opportunistic intestinal parasites between May and August, 2013. Of these, 202(47.9%) were males and 220(52.1%) were females. The majoritiy of the participants were urban dewellers (70.9%), females (52.1%) and more than half of the study participants were aged between 36-50 years (Table 3).

Regarding their educational status, 113(26.8%) of the study participants were secondary school complete while 135(32%) were illiterate; 79(18.7%) of the study subjects had completed primary education and only 95(22.5%) of the study participants had a diploma and above level of education (Table 2). Three hundered one (71.1%) and one hundered twenty one (28.7%) of the sample population had Risky and non-risky occupational status. On the other hand, 8.3%, 39.1%, 42.7% and 10.0% of the study participants were single, married, divorced and widowed marital status, respectively (Table 2).

The majority of sample populations 233(52.8%) had animal contact and 228(54.0%) of the sample populations had poor sanitation practice. About (58.9%) of the sample populations were replied that they used protected water for drinking and domestic purposes. Whereas the other (41.1%) replied that they used unprotected water for drinking and domestic purposes. Regarding their latrine facility, 71.3%, 21.6% and 7.1% of sample populations was used toilet house, public and open field, respectively (Table 2).

Table 2Some Socio-Demographic charachterestics and association of risk factors with Intestinal Parasitic Infection of the Study Subjects in Wolayita Sodo Othona Hospital Southern, Ethiopia (May-August, 2013).

Risk factors	Frequency (%)	OIPI*			
		No.Posi (%)	OR(95%CI)	χ^2	p-value
Educational status					
Illiterate	135(32)	82(60.7)	0.85(0.822–0.889)	1.190	0.755
1^0 Educ.complete	79(18.7)	49(62.0)			
2^0 Educ.complete	113(26.8)	73(64.6)			
Diploma & Above	95(22.5)	64(67.4)			
Residence					
Rural	123(29.1)	84(68.3)	0.743(0.476-1.160)	1.715	0.190
Urban	299(70.9)	184(61.5)			
Latrine facilities					
In toilet home	301(71.3)	194(64.5)	0.000(0.000–0.007)	11.605	0.003
Field	30(7.1)	26(86.7)			
Public	91(21.6)	48(52.7)			
Source of drinking water					
Protected	245(58.9)	151(61.6)	1.214(0.811-1.818)	0.886	0.347
Unprotected	177(41.1)	117(66.1)			
Occupation					
Risky **	310(73.5)	206(63.8)	1.597(1.028-2.482)	4.370	0.037
Not risky***	112(28.7)	62(62.8)			
Sanitation practice****					
Good	194(46)	124(63.9)	0.687(0.643-0.731)	0.026	0.872
Poor	228(54)	144(63.2)			
Marital status					
Single	35(8.5)	27(77.1)	0.344(0.298-0.389)	3.580	0.311
Married	165(39.1)	106(64.2)			
Divorced	180(42.7)	110(61.1)			
Widowed	42(10)	25(59.5)			
Eating raw vegetables*****					
Yes	296(70.1)	181(61.1)	1.417(0.909-2.210)	2.379	0.123
No	126(29.9)	87(69.0)			
Animal contact					
Yes	223(52.8)	142(63.7)	0.985(0.662-1.464)	0.006	0.939
No	199(47.2)	126(63.3)			
Sex					
Male	202(47.9)	123(60.9)	1.242(0.835-1.847)	1.144	0.285
Female	220(52.1)	145(65.9)			
Age(yrs)					
5-17	5(1.2)	5(100)		-	-
18-35	39(9.2)	29(74.4)			
36-50	218(51.7)	131(60.1)	-	6.004	0.111
≥51	160(37.9)	103(64.4)			

*OIPI=opportunistic intestinal parasiticinfection**Risky = Farmers, housewives, janitors etc.
***Not risky = Teachers, office workers, students, etc. ****Sanitation status = household hygiene practices, sanitary homes, excreta disposal practices, Hand-

Washing practice, personal hygiene, etc. *****Eateing raw vegetable Habit = uncooked, unwashed, etc

4.2. Prevalence of Opportunistic Intestinal Parasitic Infections among HIV/AIDS Patients

A total of 422 fecal samples were collected from 422 HIV/AIDS patients. The prevalence of intestinal parasitic infections among HIV/AIDS patients in relation to sex and age was depicted in Table 3. The overall prevalence of intestinal parasites reported in this study (63.5%) was relatively high compared to that reported from Malaysia and South Western Ethiopia (Jimma) which were 36.5% (Huruy *et al*., 2011) and 37.9% (Akinbo *et al*., 2010), respectively. However, the prevalence rate of intestinal parasitic infection among HIV/AIDS patients in the current study (63.5%) was almost similar to the prevous study conducted from different parts of Ethiopia (62.5%) by Assefa *et al*. (2009) and Zelalem *et al*. (2008). In the present study, the overall prevalence of intestinal parasitic infections among HIV/AIDS patient was far lower than the rate detected at Bahir Dar GambiHigher Clinic (80.3%) among HIV/AIDS patients (Abebe, 2009).

As shown in Table 3, the prevalence of opportunistic intestinal parasitic infection among the age groups 5-17, 18-35, 36-50 and 51 and above years old in both sexes of the study participants was 100%, 74.4%, 60.1% and 64.4%, respectively. The age of study participants were catagoraized on the basis of risk groups with HIV/AIDS and parasite co-infections (WHO, 2010). The prevalence rate of intestinal parasitic infection was not statistically significant between male and female in all age groups (Table 3).

In this study, the prevalence rate of intestinal parasitic infection was relatively higher in females, 145(66%) than in males 123(60.9%). But, the result was not statistically significant (X^2=3.107; P=0.373). This did not agree with previous studies condacted in Adama, Afar and Dire-Dawa ART centers (Haileyesus and Beyene, 2009).

Table 3. Prevalence of Opportunistic Intestinal Parasitic Infections by age and sex among HIV/AIDS Patients (n=422) who were attending Othona Hospital from May-August, 2013.

Age group (years)	Male		Female		Both sexes		X^2	P-value
	No Examined	No Positive (%)	No. Examined	No Positive (%)	No Examined	No Positive (%)		
5-17	4	4(100)	1	1(100)	5	5(100)	1.875	0.392
18-35	19	16(84.2)	20	13(65)	39	29(74.4)	14.784	0.097
36-50	108	61(56.5)	110	70(63.6)	218	131(60.1)	8.653	0.565
≥51	71	42(59.1)	89	61(68.5)	160	103(64.4)	9.575	0.479
All age groups	202	123(60.9)	220	145(66)	422	268(63.5)	3.107	0.373

4.3. Major Intestinal Parasitic Species Identified among the Examined HIV/AIDS Patients in Othona Hospital

As shown in Table 4, in the present study the major intestinal parasitic species that were detected in stools of HIV/AIDS patients consisted of *C. parvum* (14.2%), *I.belli* (8.5%), *B.hominis* (2.6%), *C. cayetanensis* (2.8%), *G. lamblia* (10.0%) and *E. histolytica* (9.0%). In the present study, intestinal helminth parasite species were also detected. These were *S. stercoralis* (10.2%), *A.lumbricoides* (2.0%), hookworm spp (1.2%) and multiple infections (3.1%) of two and above were examined among HIV/AIDS patients (Table 4).

The prevalence of *C. parvum* in this investigation was 14.2%, which was quite high when compared with a study conducted at different countries in Africa, including Ethiopia. A study conducted in selected ART centers in Ethiopia and south western Ethiopia was reported 8% and 11%, respectively (Awole *et al*., 2003; Haileyesus and Beyene, 2009). Also it was quite higher than those reported from Nigeria (5.7%) and Ivory Coast (7.5%) (Okodua *et al*., 2003).

Generally, the variation in the prevalence of *C.parvum* among HIVAIDS patients could be related to the risk factors like exposure to food and water contaminated with *Cryptospordium* oocyst, close contact with animals and their feces, immune status of HIV/AIDS patients and probably due to use of more sensitive detection methods. Moreover, oocyst excretion is usually variable (Gupta *et al*., 2008).

The prevalence of *Isospora belli* (8.5%) infection observed in this study was comparable with the report from Zaire (8%). It was, however, quite higher than the reported prevalence from Ivory Coast (7.5%), Gondar (2.4%) and Addis Ababa (1.4%) (Tadesse *et al.*, 2005). On the other hand, this 8.5% prevalence of *I. belli* was lower as compared with a study from India, Ivory Coast and Zaire in which a high rate 16.6%, 17.9% and 19%, respectively(Mohandas *et al*, 2002). The above difference might be due to different geographical locations, factors like sanitation condition and water source of the study area (Gomez *et al.*, 1995).

Studies conducted on randomly selected cohort participants at Wonji had previously shown about 59% of *Blastocystis hominis* infection (Haileyesus and Beyene, 2009). This was in agreement with the rate recorded (>50%) in developing countries (Stenzel, 1996). However, in this investigation 2.6% of *B. hominis* among HIV/AIDS patients with diarrhea which was higher than compared with a study conducted at Addis Ababa (0.9%), but comparable with the study condacted at Adama, Afar and Dire-Dawa (2.5%). It was too low when compared to the study conducted at Bahir-Dar (10.5%), Jimma (14.1%) and Wonji (59%). The observed difference among HIV/AIDS patients reported so far from Ethiopia might be due to different factors, like the difference in the diagnostic method used,the endemicicity and variation in infective dose of the parasites, possibly the immune status of the patients, etc (Eriko *et al.*, 2010).

Cyclospora cayetanensis an emerging pathogen was also identified in 12(2.8%) of HIV/AIDS patients in the current study which is an emerging pathogen. There is no sufficient literature for comparison, but studies done at Jimma, Thailand and Cuba reported a prevalence of 3.7%, 2.2% and 3.0% among HIV/AIDS patients respectively, which was comparable with some of this invetigation (Mohammed *et al*., 2001).

The other protozoan infections (i.e., *G.lamblia* and *E.histolytica*) have not been found to be opportunistic in HIV/AIDS patients, because there was no evidence for infection prevalence as observed. According to this finding, diarrhea was one of the clinical manifestations in HIV/AIDS patients. This might be caused by various etiological agents. Among those etiological agents, coccidian parasites like *C. parvum* and *I.belli* are the causative agent of life threatening chronic watery diarrhea, weight loss and malabsorption.

With regard to helminths, the prevalence of *S. stercoralis* in the current study, which was (10.2%), was quite high when compared with a study conducted at Bahir Dar (1.2%) and Wondo Genet (0.69%) (Abebe, 2009; Eriko *et al.*, 2010). A study condacted by Mekonnen had reported higher prevalence of intestinal helminth infection among HIV/AIDS patients at the time of initiation of ART in patients whose CD4+ T cell count wsa less than 200 cells/μL than among non-ART HIV-positives who's CD4+ T cell count more than 200 cells/μL (Mekonnen, 2007). The difference observed between the prevalence reported in present study and earlier reports among HIV/AIDS patients from Ethiopia (Hailemariam *et al*, 2004) may be a reflection of the difference in the diagnostic methods used, the endemicity and variation in infective dose of the parasite in certain locality and possibly the immune status of the patients.

The other helminth infections (i.e., *A.lumbricoides* and Hookworm) have not been found to be opportunistic in HIVAIDS patients, because there was no evidence as opportunistic infections with the parasites in immunocompromised people (Singh, 1998).

Multiple infections were seen in 13(3.1%) of the total examined individuals (Table 4). In this study, multiple infections occurred in 13 individuals making 3.1% of the total examined subjects. This was lower than a study done in India portraying a polyparasitism of 36.2% prevalence among HIV/AIDS patients (Kaushal *et al*., 2007).

Table 4 Major Intestinal Parasitic Species Identified from Examined HIV/AIDS Patients Attending Othona Hospital from May-August, 2013.

Age group in yrs & sex	No.Examined	Protozoan parasite species					Helminth parasite species				Multiple Parasite*
		Cp	Ib	Bh	Cc	Eh	Gl	Al	Ss	Hw	
		No.Pos (%)	No.Pos (%)	No.Pos (%)	No.Pos (%)	No.Pos (%)	No.Pos (%)	No.Pos (%)	No.Pos (%)	No.Pos (%)	No.Pos (%)
5-17											
Male	4	2(50.0)	_	_	_	1(25)	1(25.0)	_	_	_	_
Female	1	_	_	_	_	1(100)	_	_	_	_	_
18-35											
Male	19	3(15.8)	2(10.5)	1(5.3)	_	2(10.5)	6(31.6)	1(5.3)	1(5.3)	_	_
Female	20	1(5.0)	3(15)	_	_	4(20.0)	_	_	2(10.0))	1(5.0)	2(10.0)
36-50											
Male	108	18(16.7)	5(4.6)	3(2.8)	3(2.8)	5(4.6)	8(7.4)	1(0.9)	12(11.1)	1(1.0)	5(5.0)
Female	110	15(13.6)	8(7.3)	2(1.8)	2(1.8)	10(9.1)	14(12.7)	3(2.7)	14(12.7)	_	2(1.8)
≥51											
Male	71	8(11.3)	10(14.1)	_	2(2.8)	7(9.9)	6(8.5)	1(1.4)	6(8.5)	_	2(2.8)
Female	89	13(14.6)	8(9.0)	5(5.6)	5(5.6)	8(9.0)	7(7.9)	2(2.2)	8(9.0)	3(3.4)	2(2.2)
Allage group											
Male	202	31(15.3)	17(8.4)	4(2.0)	5(2.5)	15(7.4)	21(10.4)	3(1.5)	19(9.4)	1(0.5)	7(3.5)
Female	220	29(13.2)	19(8.6)	7(3.2)	7(3.2)	23(10.5)	21(9.5)	5(2.3)	24(10.9)	4(1.8)	6(2.7)
Total	422	60(14.2)	36(8.5)	11(2.6)	12(2.8)	38(9.0)	42(10.0)	8(2.0)	43(10.2)	5(1.2)	13(3.1)

Cp =*Cryptosporidium parvum,* Ib= *Isospora belli,* Bh=*Blastocystis hominis,* Cc= *Cyclospora cayetanensis,* Eh=*Entameba histolytica/dispara/,* Gl= *Gardia lamblia,* Al=Ascaris *lumbricoides,* Ss= *Strongloides stercolaris,* Hw= Hookworm and pos=positive.
* = More than one species of parasitic infection.

4.4. Trends and Patterns of Opportunistic Intestinal Parasitic Infections among HIV/AIDS Patients in Othona Hospital from 2009 to April, 2013

Trends and patterns of opportunistic intestinal parasitic infections among HIV/AIDS patients were obtained from clinical records of Othona Hospital, Southern Ethiopia. The results revealed the presence of high cumulative annual prevalence of intestinal parasitic infection (27.3%) among HIV/AIDS patients over the five years (2009- April, 2013). Six types of intestinal parasites were identified at the Othona Hospital, These were; *C.parvum, E. histolytica/dispar/, A. lumbricoides, G. lamblia, S. stercoralis* and *Tenia* species from outpatient records. Of these intestinal parasitic infections, *G. lamblia* (8.6%) and *E. histolytica/dispar/* (8.5%) were the most frequent infection followed by *A. lumbricoides* 159(4.0%) and *Tenia* spp 32(1.0%) of helminths and Cestods, respectively (Table 6).

The degree of reduction in the prevalence of intestinal parasitic infection was not the same in all species of parasites (Table 5). However, the annual cumulative prevalence of intestinal parasitic infection between year 2009 (31.2%) and 2013-April (28.8%) shows slight difference among HIV/AIDS patients (Table 6 and Figuer 7).

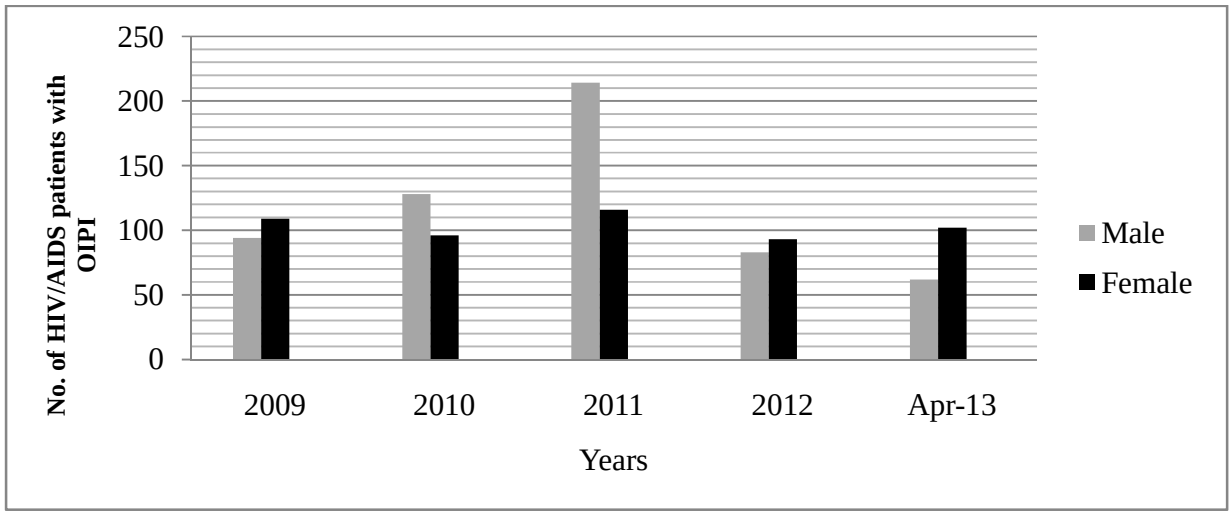

Figure 7 Distributions of HIV/AIDS Patients with One or More Opportunistic Intestinal Parasitic Infections (OIPI) by Sex and Year as per Records of the Othona Hospital from 2009 to April, 2013.

Table 5 comparision of some parasite speces between the current studies with prevous prevalence obtained from the retrospective study from 2009-April-2013.

Parasite species	5-yrs prevalence rate No (%)	Current prevalence rate No (%)
C.parvum	67(1.7)	60(14.2)
E. histolytica	343(8.5)	38(9.0)
G. lamblia	347(8.6)	42(10.0)
S. stercolaris	54(1.3)	43(10.2)
A.lumbricoides	159(4.0)	8(2.0)
Tenia spp	32(1.0)	ND*

*ND=not detected

However, among opportunistic intestinal parasitic infections, *C. parvum* 67(1.7%) and *S. stercolaris* 54(1.3%) were relatively low in the last five years than the current study [i.e. *C. parvum* (14.2) *and S. stercolaris* (10.2)] (Table 5). The prevalence rate of *E. histolytica*, *G. lamblia* and *A.lumbricoides* infections were relatively low in the last five year records, respectively (Table 5). This might be due to lack of appropriate diagnostic method for the past three years, unstructuered data recording systems of patients, awerness and attending habit of the people to the health centers in the study area may result in low prevalent rate.

Table 6The Prevalence of Intestinal Parasites by Species and Year from Outpatient Clinical Records of Othona Hospital Southern Ethiopia, 2009-April, 2013.

Year(GC) & Sex	Total No. Examined	Parasite species identified from out patient clinical records						
		protozoans			Helminths			
		CP*	Gl*	Eh*	Ss*	Al*	T.spp*	Total
		No (%)	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
2009								
Male	319	9(2.8)	29(9.0)	47(14.7)	6(2.0)	1(0.3)	2(0.6)	94(29.5)
Female	332	16(4.8)	36(10.8)	48(14.5)	3(1.0)	6(1.8)	0(0.0)	109(32.8)
2010								
Male	392	9(2.3)	55(14.0)	40(10.2)	8(2.0)	13(3.3)	3(1.0)	128(32.7)
Female	488	6(1.2)	40(8.2)	35(7.2)	8(1.6)	7(1.4)	1(0.2)	96(19.7)
2011								
Male	475	7(1.5)	39(8.2)	33(7.0)	2(0.4)	33(7.0)	4(1.0)	214(45.1)
Female	464	6(1.3)	56(12.1)	20(4.3)	4(1.0)	26(5.6)	4(1.0)	116(25)
2012								
Male	459	3(1.0)	21(4.6)	46(10.0)	3(1.0)	10(2.2)	0(0.0)	83(18.1)
Female	514	6(1.2)	25(4.9)	33(6.4)	6(1.2)	17(3.3)	6(1.2)	93(18.1)
April-2013								
Male	298	1(0.3)	11(3.7)	21(7.0)	5(1.7)	18(6.0)	6(2.0)	62(20.8)
Female	271	4(1.5)	35(12.9)	20(7.3)	9(3.3)	28(10.3)	6(2.2)	102(37.6)
All sex group								
Male	1943	29(1.5)	155(8.0)	187(10.0)	24(1.2)	75(3.9)	15(1.0)	581(30.0)
Female	2060	38(1.8)	192(9.3)	156(7.6)	30(1.5)	84(4.1)	17(1.0)	516(25.0)
Total	4012	67(1.7)	347(8.6)	343(8.5)	54(1.3)	159(4.0)	32(1.0)	1097(27.3)

**Cp=Cryptosporidium parvum, Eh=Entameba histolytica/dispara/, Gl= Gardia lamblia, Al=Ascaris lumbricoides, Ss= Strongloides stercolaris, T.spp= Tenia species*

4.5. Association of Opportunistic Intestinal Parasitic Infections and CD4+ T cell Counts of HIV/AIDS Patients who Attended Othona Hospital from May-August, 2013.

In this study from 422 HIV/AIDS patients and intestinal parasite co-infected study participants, 103(24.4%), 106(25.1%) and 213(50.5%) had CD4+ T cell count > 500cell/μL, between 200-500 cell/μL and < 200 cell/μL, respectively. Of these, the prevalence of intestinal parasitic infections identified among different immune levels were 57(21.3%), 69(25.7%) and 142(53.0%), respectively (Table 7).

The rate of parasitic infection increased with decreasing CD4 T-cell count among HIV infected individuals. The highest infection rate 142(50.5%) was at CD4+ T cell counts of less than 200cell/μL. Thus, the CD4+ T cell count is used as a marker of the ability of an indivuduals'

immune system to respond appropriately to opportunistic intestinal infections at the mucosal surface (McDonald, 2000).

The majority of opportunistic intestinal parasites such as, *C.parvum*, 46(76.7%); *I.belli*, 25(69.4%) and *S. stercolaris*, 32(74.4%) were associated with the lower CD4+ T cell counts (<200 cell/μL). The result showed stastical significance between opportunistic intestinal parasitic infections and lower level of CD4+ T cell counts ($p<0.05$) (Table 7). On the other hand, *B.hominis* 9(81.8%) and *C.cayetanensis* 32(66.7%) were did not show a significant association between infections and lower CD4+ cell counts ($p>0.05$) (Table 7). Other non-opportunistic intestinal parasite such as, *E.histolytica* 22(57.9%) and *G.lamblia* 25(59.5%) was highly prevalent with CD4+T Cell Counts >500 cell/μL. The result was not stastically significant ($p>0.05$) and the remaining intestinal parasites (i.e., *A.lumbricoides*, Hookworm and Multiple parasites) was associated with different level of $CD4^+$ T cell counts (Table 7). This investigation was in agreement with a study condacted at different parts of Ethiopia by Abebe (Abebe *et al.*, 2011).

4.6. Association of Opportunistic Intestinal Parasite Infection with Some Socio Demographic Risk factors of HIV/AIDS Patients who attended at Othona Hospital

The risk factors for acquiring Intestinal parasitic infections was assessed in all HIV/AIDS patients access for safe water source and water handling practices like boiling etc; sources of food and feeding habit like washing fruits with clean water before eating, precaution during travel, presence of animals around home and contact with animals, risky and non-risky occupations and educational status, were analyzed and presented in Table 2. As far as risk factors were concerned, in this study nine variables taken into consideration. Out of which two variables; Occupation and Latrine facilities were found to be significantly associated with acquisition of intestinal parasitic infections.

Table 7Association of Opportunistic Intestinal Parasitic Infections and CD4+T Cell Counts of HIV/AIDS Patients who attended at Othona Hospital during May-August, 2013.

Parasite species	No positive (%)	CD4+ T Cell Counts			OR(95%CI)	X^2	P-value
		< 200cell/ µl (N=213)	200-500 cells/µl (N=106)	>500 cells/µl (N=103)			
		No (%)	No (%)	No (%)			
Protozoans							
C.parvum	60(14.2)	46(76.7)	11(18.3)	3(5.0)	0.009(0.000-0.019)	20.955	0.000*
I.belli	36(8.5)	25(69.4)	11(30.6)	-	0.097(0.69-0.125)	5.515	0.043*
B.hominis	11(2.6)	9(81.8)	2(18.2)	-	-	0.244	0.621
C. cyastanensis	12(2.8)	8(66.7)	4(33.3)	-	-	0.300	0.584
E. histolytica	38(9.0)	3(7.9)	13(34..2)	22(57.9)	0.597(0.550-0.644)	3.203	0.524
G. lamblia	42(10.0	5(11.9)	12(28.6)	25(59.5)	0.472(0.424-0.519)	3.657	0.454
Helminths							
S. stercolaris	43(10.2)	32(74.4)	10(23.3)	1(2.3)	0.083(0.057-0.109)	10.780	0.029*
A.lumbricoides	8(2.0)	4(50.0)	3(37.5)	1(12.5)	0.052(0.031-0.073)	10.400	0.034*
Hookworm	5(1.2)	3(60.0)	-	2(40.0)	-	0.139	0.707
Multiple paras.	13(3.1)	7(53.8)	3(23.1)	3(23.10)	0.017(0.004-0.029)	13.029	0.011*
All OIPI	268(63.5)	142(53.0)	69(25.7)	57(21.3)	0.000(0.000-0.007)	1.243	0.000*

*P-value<0.05(result significant) with intestinal parasitic infection examined in HIV/AIDS patients and CD4+T Cell Counts.

Source of water and usage

Out of 422 study subjects, 245 participants responded that they had protected water supply (safe water sources and good handling practice like tap water supply, bottled water, filtered water and boiling water before use). Of these, 151(61.7%) was found to be positive for intestinal parasite infections. On the other hand, 177 of the study participants responded that they had unprotected (no safe water sources such as streams, rivers, etc, and had poor water handling practices). Of these, (66.1%) was positive for intestinal parasitic infection. The result show that there was no statistical significantce association between source of drinking water and parasitic infections (OR=1.21; P>0.05) (Table 2). This is may be due to the study participants were responded wrongly their source of water and usage habit.

Vegetable source and feeding habit

Vegetable source and feeding habit was the other risk factor assessed in the present study (Table 2). Out of 296 study subjects who have a clean and good feeding habit of vegetables 181(61.1%) was positive for intestinal parasitic infection, while from 126 study subjects, 87(69.0%) was found positive for intestinal parasitic infection. The relationship was not statistically significant (P>0.05). But it is advisable that HIV/AIDS patients should wash fruits and vegetables with clean or drinking water before eating. They should also use safe restaurants if they are not eating homemade foods. Most of opportunistic and non-opportunistic intestinal parasites are causes of water and food-borne diseases (Pozio, 2003; Dawson, 2005). These parasites could be transmitted to HIV/AIDS patients via contamination of food in the field or during harvesting and handling, the popular trend of eating raw vegetables and the contaminated hands and poor hygiene practices increase the risk of infection.

Contact with animals and their feces

Contact with animals and their feces was also analyzed were two hundered twenty three of the study subjects has contact with the animals and their feces, while 199 study subjects has no contact with animals and their feces. In this study, the rate of infection among those who had

contact with animals was 142(63.7%) while for those who had no contact with animals, the rate was 126(63.3%). The relationship was not statistically significant in this study(X^2=0.006, P=0.939) (Table 2). This study was agreed with the study carried by Akinbo (Akinbo *et al*. 2010) who reported that contact with animals was not a significant risk factor for acquiring intestinal parasitic infections. But, after asking the participants about their contact with animals, the majority of them reported a positive history of contact with animals, mainly caws, goats, sheep and other domestic animals. They had generally kept these animals as source of milk and meat within their house premises, and also had frequent contact with them for cleaning, milking etc. Moreover, on a study done at different parts of Ethiopia by Haileeyesus in 2010, concerning contact with animals showed significant for opportunistic intestinal parasitic infection. Touching farm animals was associated with infection by intestinal parasites (Hunter *et al*., 2004). Contact with cattle was also identified as risk factors for sporadic intestinal parasitic infection in the US, but no information was obtained on the causative species (Roy *et al*., 2004).

Occupation

Occupation was also analyzed as one of the risk factors in this study, because some occupations are more risky than others. Those who change dipper in the day care center, those who have close contact with animals like farmers, have increased risk of acquiring infection with intestinal parasites. On the other hand those, whose occupation is working in office and students, had relatively lower risk for infection with intestinal parasitic infection. From 310 study subjects having risky occupation, 206(63.8%) were positive for the intestinal parasites. While, from 112 study subjects with non-risky occupation 62(62.8%) were found to be positive for opportunistic intestinal parasitic infection. The result was statistically significant (OR=1.597; P=0.037) (Table 2). However, consistent to this study, (Assefa, *et al*. 2009) reported that farmers and merchants had a higher risk of parasitic infection than civil servants. Similarly, Akinbo *et al*. (2010) reported that artisans, farmers, and security officers were significantly affected while traders were least affected with intestinal protozoan parasitic infections among HIV/AIDS patients.

Level of education

The level of education among HIV/AIDS patient was considered by comparing those with illiterate, lower educational level and with those that have relatively higher educational level in present study. Of 422 study partyicipants, 135, 79, 113 and 95 of the study subjects have an educational level were illiterate, primary education complete, secondary education complete, diploma and above in the current study were depicted in table 8. From which 82(60.7%), 49(62%), 73(64.6%) and 64(67.4%) was positive for intestinal parasitic infection, respectively. The relationship was not statistically significant (P>0.05) (Table 2). However, contrary to this study, Östan *et al*. (2007), Hileyeesus and Beyene (2009) and Akinbo *et al*., (2010) reported that the parasitic infections declined with increased educational level of the study participants. In this study, as a level of education increases, the parasitic infection was also increased. This is may be due to negligence of study participants to pursue preventive measures of parasitic infections.

Availability of latrine

Three hundred one of the sample populations had latrine in toilet proximity to their homes, but 194(64.5%) of them was positive for intestinal parasites and the rest 30 and 91 study participants was not have latrine and they replied that they were defecated on open field or public laterine services. As result of this, 26(86.7%) and 48(52.7%) of the sample populations was` infected by intestinal parasites. According to Tellez *et al.,* (1997) living in “poor” conditions (high degree of crowding, low quality of water supply, improper disposal of excreta and unfinished or semi-finished type of floor) favors transmission of intestinal parasites. The association of intestinal parasitic infections with latrine availability was statistically significant (OR=0.000; P=0.003) (Table 2). This finding agreed with that of Akinbo *et al*. (2010) which reported that defecating in nearby bushes and type of toilet significantly affected the prevalence of intestinal parasitic infections with patient’s defecating in bushes having the highest prevalence of 50%.

Sanitation practice

Sanitation practice was considered by comparing those having good practice with those that have relatively poor practice. Out of 422 participants, 194 study participants responded that they had good practice (like good hygiene conditions, good household hygiene practices, sanitary homes, hygienic excreta disposal, hand-washing practice, clean nails, etc.). Of these 124(63.9%) were found to be positive for intestinal parasitic infection. On the other hand, 228 of the study participants responded that they had poor practice (poor hygiene conditions**,** poor household hygiene practices, unhygienic excreta disposal, unsanitary homes, dirty nails, etc.). Of these, 144(63.2%) was positive for intestinal parasitic infection which was not statistically significant (X^2=0.026, P=0.872) (Table 2). However, not in agreement with this study, a number of epidemiological studies have shown substantial reductions in intestinal parasitic infection by good practices such as hand-washing practice, sanitary homes, hygienic excreta disposal, etc (Humayun *et al*., 2002; WHO, 2005). In this study, substantial increases in intestinal parasitic infection by good practices were observed. This is may be due to the study participants were responded wrongly.

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. SUMMARY

Human immunodeficiency virus (HIV), causative agent in AIDS, is fast becoming a major threat in the world. Sub-Saharan Africa is among the regions where intestinal parasitic infections are well established (WHO, 2002) and the largest burden of AIDS cases exist (UNAIDS/WHO, 2006). Even though AIDS remains a global pandemic, HIV infection is a major medical problem in Ethiopia. It is estimated that 3.5 billion people are affected. The rate of infection is remarkably high in sub-Saharan Africa, where the majority of HIV and AIDS cases are concentrated (UNAIDS/WHO, 2002).

The progressive decline in immunological and mucosal defensive mechanisms predisposes HIV/AIDS patients to gastrointestinal infections, thus increasing susceptibility to a number of opportunistic intestinal pathogens, among which coccidian protozoan parasites like *Cryptosporidium* spp., *Isospora* spp., *Blastocystis* spp., and *Cyclospora* spp, are being frequently detected. The aim of this study was to determine the prevalence of opportunistic intestinal parasite infection among HIV/AIDS patients and their $CD4^+$ T Cell counts in Wolayita Sodo Othona Hospital Southern, Ethiopia. In the current study, HIV/AIDS patients with inclusion criteria were selected and some Socio-demographic information was collected.

Frequency and cross tabulation was used to analyze those categorical data like, age. The other major data collected were results of laboratory investigation i.e. parasite positivity, stool type, $CD4^+$ T cell count and outpatient clinical records. Statistical analysis was performed by using SPSS 16.0 software version. The association between variables was measured by using the Chi square(x^2). Data related to risk factors were analyzed using odds ratio (OR) at 95% confidence interval (CI).

The prevalence of any intestinal parasitic infection was significantly higher among HIV/AIDS patients. Specifically, rate of infection with *C.parvum,* and *I. belli,* were found in 14.2% and 8.5% of study participants, respectively. *B.hominis*, *C.cayetanensis, E.histolytica and G.lamblia*

were seen in 2.6%, 2.8%, 9.0% and 10.0% of all examined feces, respectively. Among helminth, *S. stercoralis* were found in 10.2% of HIV/AIDS patients was the highest in those with CD4+ T cell count less than 200 cells/μL. Immunodeficiency increased the risk of having opportunistic parasites and diarrhea. Therefore, improving patient immune status and screening at least for those treatable parasites is important.

5.2. CONCLUSION

Infection of *C. parvum, I. belli, C. cystanensis, B. hominis* and *S. stercoralis* was significantly higher among HIV/AIDS pateints, particularly in those with lower CD4+ T-cell counts. As no cure was available for opportunistic intestinal parasitic infections, people with HIV/AIDS should be advised on how to avoid infection, including the potential benefits of drinking boiled water and avoiding contact with animals. Screening of HIV/AIDS was also essential for early treatment of opportunistic intestinal parasite infection. Moreover, improving immune status of HIV infected patients with anti-retroviral therapy may help to reduce acquisition and/or proliferation of HIV associated parasitic infections and the likelihood of experiencing diarrhea.

This study was highlighted the importance of screening the HIV/AIDS patients and evaluating their absolute CD4+ T cell counts regularly. This highlights the need for early detection and treatment of such infections among the HIV/AIDS patients, to reduce the morbidity and the mortality.

5.3. RECOMMENDATION

The findings of the present study showed that opportunistic intestinal parasitic infection among HIV/AIDS patents was prevalent in Othona Hospital Wolayita Sodo. On the basis of the results obtained from the current study, the following recommendations are forwarded:

Public health measures should continue to emphasize the importance of environmental and personal hygiene.

- Health extension workers in each kebeles of Wolayita zone, should mobilize the community to improve the health situation through health education in their local languages related to environmental hygiene (like avoiding water and food contamination by subsequent preventive and control measures such as hygienic excreta disposal, hand washing after using the toilet and before handling food etc.)

- People engaged in HIV/AIDS consultancy service and ART nurses in Othona Hospital should provide advice for HIV patients on how to avoid infection, including the potential benefits of drinking treated water (by chemicals such as “wuha agar”) and boiled water, appropriate management of domestic animals and proper handling of their faeces and avoid eating contaminated food and raw or lightly cooked vegetables.

- Starting ART at early stage was a good way to prevent intestinal parasitic infections among HIV patients.
- Since the prevalence of opportunistic intestinal parasitic infection was higher in the current study, so that the Hospital should give more emphesis on management of those highly prevalent intestinal parasitic infections.

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7. APPENDICES

7.1. Appendix I: Written Consent Form

Consent Form

Code No----------

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

Name of Physician ---------- Health center ----------

I have been informed about a study that plans to investigate the "prevalence of Opportunistic Intestinal Parasite Infections among HIV/AIDS Patients Attending Othona Hospital in Wolaita Sodo Town, Southern Ethiopia" which helps in understanding the prevalence of OIPs among HIV/AIDS patients and at the same time it enables concerned bodies in designing a better control measures of Opportunistic Intestinal parasitic diseases in the study area.

For this study I was requested to give a stool sample for OIP infection identification. I was informed that I will get proper therapy if I found to be positive for any of intestinal parasites. The investigator has also briefed me that there would no major risks associated with the sampling procedure.

He also informed me that all laboratory results would be kept in secret. Moreover I was clearly informed that I have a right to withdraw from participating in this study and in so doing there will be no impact on the overall management of my conditions. I was given enough time to think over before I signed this informed consent. It is therefore; with full understanding of the situation that I gave informed consent and cooperate at my will in the course of the conduct of the study.

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

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

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

7.2. Appendix II: Questionnaire to be completed by Study Participants

Haramaya University School of Graduate studies Collage of Natural and Computational sciences Department of Biology

The purpose of this questioner is to get the relevant information about questioners for demographic and personal data among HIV/AIDS Patients attending Othona Hospital in Wolaita Sodo Town, Southern, Ethiopia. Kindly fill the questionnaire taking into consideration that this data will be employed only for scientific research; abiding by top confidentiality and privacy. Be informed that it is your special response that will help you to conduct the studies aimed to study the prevalence of opportunistic intestinal parasite infection at Othona Hospital. The questionnaire is especially designed to determine the major risk factors that predispose HIV/AIDS patients to infection by Opportunistic intestinal parasite among patients visiting Othona Hospital. So you are kindly requested to give your response and indicate your response by putting tick mark (√) on your response box□

Thank you in advance.

1. Sex Male □ Female □ age □
2. Art Started □ not started □
3. What is your educational status?

a)Illiterate □ b)10 –Education complet□ c)20-Education complet D)Diploma and Above

4. What is the source of your drinking water?

a) Tap water□ b) hand pump □ c) water tank □ d) others □

4.1. If you get it from one of the above source how you are going to use it?

a)Directly □ b) filtering □ c)treating with 'wuha agar'□ d)boiling □ e)other □

5. From where do you get your food?

a) Prepared at home □ b) from restaurant □ c)both □

6. How do you eat fruits and vegetables?

a)Directly, □ b)wash by drinking water, □ c)wash simply with water □

7. When you travel to another town or place do you consider the safety of water that you drink?

Yes □ No □

8. Are there animals in your residence area? Yes□ No □

8.1 If your answer to question no 8 is yes do you have a close contact with them or their feces?

Yes □ No □

9. What is your occupation?

a) Employed □ d) Housewife □

b)Daily-labor □ e) Farmers □

c) Students □ f) Others □

10. Where is your place of residence? Rural□ Urban□

11. What is your marital status? Single□ married □ divorced □ widowed□

12. What is your personal and environmental hygiene practice? Poor □ good□

13. Where do you get your latrine service? in toilet house □ Open field □ Public□

7.3. Appendix III: questionnaire to be completed by Laboratory Technician

The purpose of this questioner is to get the relevant information about Prevalence and pattern of Opportunistic Intestinal Parasite Infections and their CD4+ level among HIV/AIDS Patients attending Othona Hospital in Wolaita Sodo Town, Southern, Ethiopia. So you are kindly requested to give your response and indicate your response by putt ✓ tick mark on your response box .

Thank you in advance.

1. Date; dd ☐ mm ☐ yy ☐
2. Study code;
3. Technician;
4. Sample type; stool☐ blood ☐ sputum ☐
5. Stool appearance; loose☐ watery ☐ mucoid ☐ Bloody☐
6. Opportunistic parasite identified; C.parvum ☐ I.belli☐ B. hominis☐ C.cystanensis☐ others☐
7. Number of CD4+ cell and opportunistic parasite present;
   a) *C. parvum*; No of CD4+ cell---------- d) *C. cystanensis*; No of CD4+ cell----------
   b) *I. belli*; No of CD4+ cell ---------- e) others; No of CD4+ cell----------
   c) *B .hominis*; No of CD4+ cell----------
8. Opportunistic intestinal parasite identified in different patterns of HIV/AIDS pat
   a) *C. parvum*; acute ☐ chronic ☐ b) *I. belli*; acute ☐ chronic ☐ c*) B.hominis*; acute ☐ chronic ☐ d) *C.cyctanensis*; acute ☐ chronic ☐ e) others; acute------ chronic------

Amharic version Questionnaire

የዚህ መጠይቅ ዓላማ ከ ኤች.አይ.ቪ..ኤድስ ህሙማን ግለ-ታሪክ ና የአኗኗር ዘይቤ ዙሪያ መረጃ ለመሰብሰብ ነው። ይህ መረጃ ለጥናት ብቻ የሚዉል ሲሆን በእርስዎ ወይንም በቤተሰብ ላይ ምንም አይነት የጤና ችግርእንደማያስከትል ለማረጋገጥ እወዳለሁኝ። ለምርምር የሚያገለግል የደም አና የሰገራ ናሙና በመስጠት እንዲትተባበሩኝ በትህትና እጠይቆ q ለሁ።
ስለ ትብብረዎ አናመሰግናለን።

በአማረኛ የተፃፉ ጥያቄዎች
1. *ዕድሜ*------- 2. *ጸታ*--------
3) የኤች.አይ.ቪ. *መደሀንት ጀምረዋል* *ሀ)አዎን* *ለ)አልጀመረኩም*
4) *የመኖሪያ ቦታ* *ሀ).ገጠር* *ለ).ከተማ*
5. *የሰራ ሁኔታ* *ሀ). ገበሬ* *ለ). ነጋዴ* *ሐ).መንግስት ሰራተኛ* *መ). የቀን ሰራተኛ* *ሠ)ተማሪ* *ረ)ሌላ ካለ ይጥቀሱ*
6. *የትምህርት ደረጀ* *ሀ). የተማረ* *ለ). ያልተማረ*
7.በቡቶ አቅራቢያ የቤት እንስሳት አሉ? *ሀ)አዎን* *ለ)የለም*

መልሶ አዎን ከሆነ ከእንስሳት ጋር ግንኙነት አሎት? *ሀ)አዎን* ለ)የለም
8.*የ መፀዳጃ አገልግሎት የት ይጠቀማሉ? ሀ)በመፀዳጃ ቤት ለ). በሜዳ ላይ ሐ)በህዝብ መፀዳጃ ቤት*
9. *ለመጠጥ የምትጠቀሙት ዉሃ ፡ ሀ). ቧንቧ ለ).ከዉሀ ታንክ ሐ)የእጅ ፖ*ንፕ *መ)ሌላ ካሌ ይጥቀሱ----*

ከላይ የሚታገኙትን ዉሃ በምን መልኩ ይጠቀሙታል?
ሀ)በቀጥታ *ለ)በዉሃ አጋር አክመን* *ሐ)አፊልተን* *መ)በማንጠር* *ሠ)ሌላ ካለ ይጥቀሱ*
10. *ምግብ ከየት ይጠቀማሉ? ሀ)ከቤት* *ለ)ከሆቴል* *ሐ)ከሁለቱም*
11.*መንገድ ወይም ወደ ሌላ ሀገር ሲሄዱ የምግብ ና የዉሃ ንፅህና ይጠነቀቃሉ?*
ሀ)አዎን *ለ)አልጠነቀቅም*
12. *ፍራፍሬ ከመብላቶ በፊት አጥቦ የመጠቀም ልምድ አሎት?* *ሀ)አዎን* *ለ)የለኝም*
13.*የጋብቻ ሁኔታ* *ሀ)ያላገባ(ች)* *ለ)ያገባ(ች)* *ሐ)የፈታ(ች)* *መ)በሞት የተለየ(ች)*
14.*የግል ና የአከባብ ንፅህና የመጠበቅ ሁኔታ እንደት ነዉ?ሀ)ደካማ ነዉ ለ)ጥሩ ነዉ*